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DISSERTATION

Effects of a 15 mg single-dose eplivanserin on respiratory function and sleep structure in patients with mild to moderate chronic obstructive pulmonary disease (COPD)

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Table of Contents

List of Tables

List of Figures

List of Abbreviations and Definition of Terms

ABG	Arterial blood gas
AE	Adverse event
AFS	Ease of awakening following sleep
AHI	Apnea Hypopnea Index
ALT	Alanine aminotransferase
ATS	American Thoracic Society
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (German Health Authorities)
BFW	Behavior following wakefulness
BMI	Body mass index
CMH	Cochran-Mantel-Haenszel
CNS	Central nervous system
COPD	Chronic Obstructive Pulmonary Disease
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
ERS	European Respiratory Society
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GAW	Airways conductance
GCP	Good Clinical Practice

GTS	Ease of getting to sleep
HBs	Hepatitis B surface
β-HCG	Beta-Human Choriogonadotropin
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
5-HT	5-Hydroxytryptamine
ICH	International Conference on Harmonization
IMP	Investigational Medical Product
LPS	Latency to persistent sleep
LSEQ	Leeds Sleep Evaluation Questionnaire
MedDRA	Medical Dictionary for Regulatory Activities
NTEAE	Non Treatment Emergent Adverse Event
OSA	Obstructive sleep apnea syndrome
$PaCO_2$	Carbon dioxide partial pressure
PaO_2	Oxygen partial pressure
PFT	Pulmonary Function Testing
PLMS	Periodic leg movement syndrome
PO_2	Arterial and venous oxygen tension
PSG	Polysomnography
QOS	Quality of sleep
RAW	Airways resistance
REM	Rapid-Eye-Movement
RHb	Deoxygenated "reduced" hemoglobin
RIP	Respiratory Inductance Plethysmography
RN	Raphe Nuclei
RSL	REM sleep latency

SAE	Serious Adverse Event
SaO2	Oxygen saturation
SOC	System Organ Class
SOL	Sleep onset latency
SPT	Sleep period time
SSRIs	Selective serotonin reuptake inhibitors
SWS	Slow wave sleep
TEAE	Treatment Emergent Adverse Event
TIB	Time spent in bed
TST	Total sleep time
VAS	Visual Analog Scale
VC	Vital Capacity
VTG	Thoracic gas volume
WASO	Wake after sleep onset

1 Introduction

1.1 Background

Chronic obstructive pulmonary disease (COPD), a common medical ailment, results in significant morbidity and mortality. An estimated 9−10% of people around the globe suffer from the disease in both developed and less developed countries, and it ranks fourth among causes of death in the U.S. According to experts, it will become the third leading cause of death worldwide by 2020 as people continue to smoke and as the global population ages (Bellia et al.2003).

COPD is characterized by airflow obstruction; its breath-related symptoms include wheezing, exertional dyspnea, chronic cough, and expectoration (Rennard, 1998). These symptoms, which may present in conjunction with hyper-responsiveness of the airway, are partly reversible. Mannino writes: "COPD is a non-specific term referring to a set of conditions that develop progressively as a result of a number of different disease processes. It mostly refers to chronic bronchitis and emphysema and to a subset of patients with asthma. These conditions can be present with or without significant physical impairment (2001)." Patients often complain of dyspnea due to the disease's chronic and symptomatic nature.

Co-morbidities accompanying COPD include cardiovascular disease, unexplained weight loss, weakness in peripheral muscles, anxiety, depression, cognitive impairment, and sleep disorders (Watz, et al. 2006 and Urbano, et al. 2006). Around half of all patients with severe COPD suffer from insomnia, sleep-related hypoxia, or other sleep disorders (Klink et al. 1994).

There are different causes for sleep disorders in COPD patients. Some causes arise from symptoms of the disease, such as respiratory distress, cough and in some cases co-morbidity with depression and anxiety or side effects from some drug treatments. In addition, the physiological response to hypercapnia could also play a role in sleep disturbance. The difficulties of patients with COPD during daytime is well known. However, their condition at night, during sleep, has not been adequately investigated. The normal decrease in breathing at night causes mild, clinically insignificant hypoventilation. However, COPD patients show abnormalities in gas exchange at night that can lead to serious hypoxemia and such consequences as arrhythmias, pulmonary hypertension and potentially even death, although this has not been proved conclusively (Doghramji, 2008).

Subjective complaints of sleep disturbance appear to be associated with respiratory symptoms common in patients with COPD: dyspnea, cough, or sputum production. Among COPD patients

older than 65 years, 38% complain of morning tiredness, and 35% complain of early awakenings (Bellia et al., 2003).

More than 50% of COPD patients report frequent nighttime awakenings, long latency of sleep, and/or insomnia in general (George, 2000). COPD patients, suffering from nocturnal hypercapnia and hypoxia, awake more often and suffer greater disruptions to sleep as they seek to improve their respiration (Roth, 2009). This leads not only to disruption of sleep but also to chronic insomnia among vulnerable individuals (Urbino, 2006). Nocturnal hypoxia – that is, a sleep time lack of oxygen -- occurs during REM sleep, when breathing is shallowest. When COPD grows worse, many patients experience problems in falling or staying asleep. They report poorer quality of sleep than healthy subjects due to sleep fragmentation related to desaturation. Sleep disturbance ranges from difficulty in falling asleep to frequent awakenings followed by daytime drowsiness. Klink and his co-workers note that "insomnia may be transient (less than a week), short term (a week to a month) or chronic (more than a month) (1987)." Analysis of a large database of COPD patients revealed that 21.4% had been diagnosed with and treated for insomnia; this compared to only 7.2% of non- COPD patients (Vallarino et al., 2005).

The causes of sleep-related complaints and objective sleep disturbances in patients with COPD are not fully understood. Normal changes in respiration associated with sleep can have a negative impact on COPD patients; they can lead to reduced gas exchange and potentially hypoventilation, hypoxemia and hypercapnia. This is especially the case during REM sleep, when muscle atonia is typical. A further decrease in the diaphragm's contractility and ventilatory responsiveness may result from hypercapnia. (George and Bayliff, 2003 and Douglas, 2005).

Hypoxia may provoke an arousal response although this effect varies widely. In COPD patients, the impaired activity of the respiratory muscles also affects breathing during sleep. Increases in airway resistance may cause exaggerated bronchial constriction that may be clinically significant. Reduced intercostal muscle activity also affects breathing during sleep. In patients with impaired respiratory function, there is likely to be a relationship between hypoxemia and hypercapnia. Some of the possible etiological factors are summarized below:

1.2 Disease-specific Factors

According to Doghramji, following factors play a role:

- "Excessive mucus production and cough and the increased work of breathing associated with airflow limitation," which cause inspiratory resistance and increased ventilatory

effort and, in turn, raise the frequency of sleep-time arousals among normal subjects. These factors, exacerbated by lying in the supine position, are likely to contribute to arousals in patients with COPD (Doghramji, 2008).

- Nocturnal dyspnea, also enhanced by the supine position (Doghramji, 2008).
- Oxyhemoglobin desaturation during sleep, which is exaggerated during REM sleep when postural muscle tone is normally at its lowest point.

  The response to hypoxemia and/or hypercapnia is characterized by an increase in ventilation and respiratory effort which, in turn, leads to sleep-related arousals. There are many reasons for oxyhemoglobin desaturation during sleep including (but not limited to):

  Primary reasons:
    - Sleep-related hypoventilation
    - Reduction in functional residual capacity
    - Increased ventilation-perfusion mismatch

  Secondary reasons:
    - Depression and anxiety
    - Medications used to treat COPD
    - Obstructive sleep apnea syndrome (OSA). There is a high correlation between COPD and OSA (Doghramji, 2008). Initial research has suggested that among patients with OSA, the prevalence of COPD is higher (11 to 14%) than among the general population (Bradley et al. 1986, Chaouat et al. 1995). Chaouat and co-workers found that for the same degree of bronchial obstruction in COPD, those patients also suffering from OSA have more significant sleep-related oxygen desaturation.

1.3 Co-morbid Factors:

The National Sleep Foundation has noted: “There is a high likelihood of co-morbidity of COPD and other medical and psychiatric conditions, and the likelihood of reporting insomnia increases in proportion to the number of coexisting conditions (2003).” Therefore, insomnia can present as a co-morbid condition whose etiology is unrelated to the process of COPD itself, but can arise from other medical and psychological conditions that are co-morbid with COPD. Insomnia can also be a primary condition without any relation to any other underlying disorders. Primary insomnia is considered a disorder of hyperarousal present throughout the entire day (Doghramji,

2008). During the day, this hyperarousal may display itself as a state of hypervigilance, and during the night, as difficulty in initiating and maintaining sleep. Both cognitive and physiological models of insomnia explain this hyperarousal (Roth et al., 2007).

Several polysomnography studies in COPD patients have shown that their sleep architecture is significantly undermined through such disturbances as decrease in the proportions of REM and slow wave sleep, decreases in sleep efficiency and overall sleep time, and an increase in awakenings and arousals beyond normal values (Stege et al., 2008). Although the long-term effects of sleep disturbance have not been investigated so far, it seems that these additional effects also have a negative influence upon lung function in COPD patients. It is well established that insomnia patients develop more psychiatric disorders in comparison to healthy people. Breslau et al. studied 1000 subjects from 21 to 30 years with follow-ups over the course of three years. He could show that insomnia patients had a substantially higher risk of developing such psychiatric disorders as depression, substance abuse or anxiety disorders than others (Breslau et al., 1996).

Anxiety and depression are two of the most common co-morbidities in patients with COPD (Maurer et al., 2008). Maurer and co-workers reported: “In stable COPD, the prevalence of clinical depression ranges between 10% and 42%, while that of anxiety ranges between 10% and 19%. The risk of depression is higher in patients with severe COPD compared to control subjects, with the highest rates, up to 62%, found in oxygen dependent patients” (Maurer et.al., 2008). It is well known that insomnia patients often develop such psychiatric disorders and that such disorders could negatively affect the life quality of COPD patients.

1.4 Effects of Chronic Obstructive Pulmonary Disease Treatment on Sleep

Several medications used to treat COPD, such as corticosteroids and theophylline, have been reported to cause sleep problems (Tiak et al. 2009).

Theophylline is a member of the methylxanthine group of biochemicals like caffeine. Side effects of theophylline include restlessness or caffeine-type jitters or insomnia. Patients taking theophylline often complain about disturbed sleep (Raghu et al. 2009). Roehrs et al. studied the short term effects of low dose (3mg/kg) and high dose (6mg/kg) theophylline on sleep and alertness in 24 young volunteers in a double blind study. Among the group given high dose at night there was an increase in sleep latency and awakenings, and a decrease in total sleep duration. The stages of sleep were not affected. In the group receiving day time theophylline the

nap studies showed that the mean sleep latency increased from 11 minutes in placebo group to 17 minutes in the high dose theophylline group. Raghu et al. reviewed different studies which were performed in COPD or asthma patients to investigate the role of theophylline of sleep disturbance and found out that in summary, theophylline increases latency to sleep and arousals, and decreases quality of sleep (Raghu et al. 2009).

Although studies regarding the effects of most beta-2 agonists on sleep are not available, salbutamol does not appear to affect sleep quality adversely in patients with asthma and COPD (Veale et al.1994). Calverley et al. studied in the large multicenter trial of inhaled salmeterol in COPD patients the effect of salmeterol of sleep. The 1542 subjects included in the salmeterol arm of the study did not report insomnia or other sleep disturbances as one of the side effects (Calverley et al. 2007).

The anticholinergic drug ipratropium resulted in an improved subjective sleep quality and an increase in REM sleep time in patients with moderate to severe COPD (Martin et al., 1999). However, in another study, the long-acting anticholinergic drug tiotropium did not show an effect on sleep quality (Tashkin et al., 2008 and McNicholas et al., 2004).

The effect of inhaled steroids on sleep disturbance is not well known. In the large multicenter trial of inhaled fluticasone in COPD patients (TORCH Study), over 1500 patients included in the fluticasone arm had no sleep disturbances as one of the side effects reported (Calverley et al. 2007 and Raghu et al. 2009). The manufactures of Budesonide and Mometasone list insomnia as a side effect at a rate of 1% to 3% during the clinical trials, but the manufactures of Fluticasone, Beclomethasone and Triamcinolone do not list any sleep disturbance as known side effects (Raghu et al. 2009). In conclusion, sleep disturbance due to inhaled steroids is not well studied so far.

1.5 Management of Insomnia in Chronic Obstructive Pulmonary Disease Patients

Clinical studies support the role of many agents for the treatment of insomnia in COPD patients, but they do not always provide assurance that these therapies can be applied safely, especially in this population. However, medication management is one of the major approaches to the treatment of insomnia in COPD patients.

The poor quality of sleep among COPD patients can lead to decreased functioning in daytime due to excess drowsiness, decreased psychomotor vigilance, and altered neurocognition. The management of sleep problems in COPD patients should focus first on optimizing the patient's overall respiratory condition. Measures for improving the quality of sleep should center on

minimizing such symptoms as dyspnea and cough that can disturb sleep patterns through arousal. Many traditional sedatives and hypnotics have been used to treat COPD patients: benzodiazepines, pyrazolopyrimidines, imidazopyridines and, less often, phenothiazines and antidepressants.

Benzodiazepines, which can be used for the effective treatment of many medical and psychiatric conditions, remain the most commonly used agents. Nevertheless, they should be used with caution, given their depression effect on respiration. This is especially the case for patients with COPD. Many case reports and series have described adverse pulmonary events in patients taking benzodiazepines.

Comparing non-benzodiazepines with benzodiazepines, non-benzodiazepines have proved to be much safer, especially in cases of overdose. They also have a lesser tendency to induce dependence and addiction, although both can still become a problem in cases of extended use (Neubauer, 2006, Najib, 2006, Lieberman, 2007).

Zopiclone, zolpidem and zaleplon are the first three non-benzodiazepine sedatives that were brought into the market. All three drugs are strong sedatives that have been used exclusively for the treatment of insomnia. However, these drugs also have disadvantages; all three compounds have side effects, including in some cases pronounced amnesia and much less often hallucinations (Stone et al., 2007; Toner et al., 2000).

Overall, the most common drugs used to treat insomnia in COPD patients can potentially cause respiratory problems. Many of the traditional sedatives and hypnotics used in treating the COPD population have shown an increased number of adverse pulmonary events. Over the past 30 years, benzodiazepines have been the preferred drugs for treating insomnia. Benzodiazepines act non-selectively at two central receptor sites -- omega (1) and omega (2) – which are located in different parts of the central nervous system (CNS). According to Terzano and co-workers, “the sedative action of benzodiazepines is related to omega (1) receptors, whereas omega (2) receptors are responsible for their effects on memory and cognitive functioning” (Terzano et al., 2003). Due in part to rebound, some people have difficulties in discontinuing these drugs.

Some antidepressants, especially from the tricyclic class, along with antipsychotics, have been used for years to treat insomnia. Selective serotonin reuptake inhibitors (SSRIs) generally disrupt sleep early during the course of treatment. Sedative antidepressants such as trazodone can offset this impact on alertness; this is probably due to the fact that they block 5-HT_2 receptors, which become over-stimulated by an increase in 5-HT (Kaynak et al., 2004). Recently, new drug

treatments have become available after a relatively stagnant period since the 1990s, when the Z-drugs were developed.

1.6 The Serotonergic System

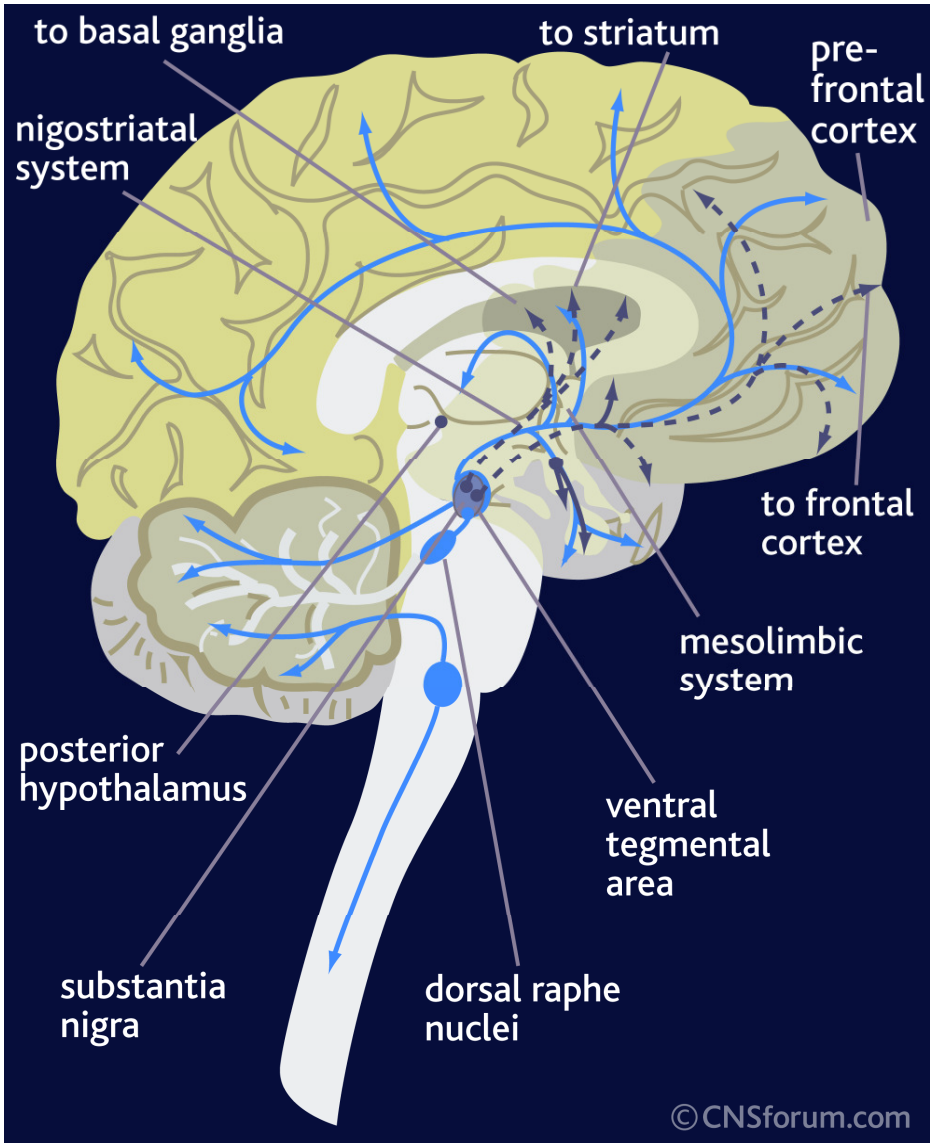
5-Hydroxytryptamine (5-HT), the first hypothesized sleep-promoting substance in modern neuroscience, is a widely-distributed neurotransmitter in the CNS of mammals. The multiplicity of 5-HT receptor subtypes and their complex pharmacology has hampered our understanding of 5-HT's physiological role (Bradley et al., 1986).

1.6.1 Overview

The majority of serotonergic cell bodies that innervate the brain are situated in the raphe nuclei (RN) in the brainstem (the median raphe and the dorsal raphe nuclei) (Figure 1). Several brain structures like amygdale or ventral hippocampus are innervated by the dorsal raphe as well as the median raphe (Jacobs et al., 1992 and Pineyro and Blier, 1999).

Figure 1: Serotonergic System

Source:http://www.cnsforum.com/content/pictures/imagebank/hirespng/Neuro_path_DA_SCH.png).

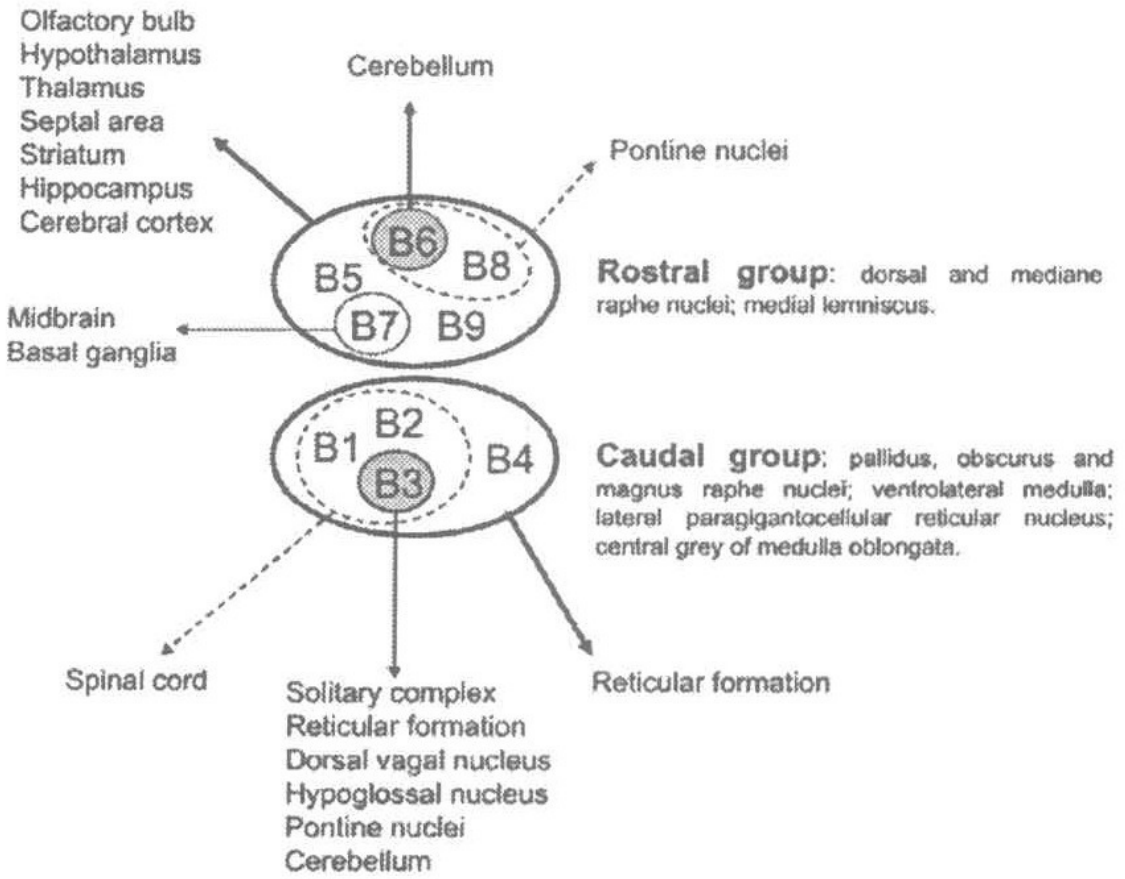


Serotonergic neurons of the CNS are situated in clusters within the raphe nuclei, central gray and reticular formation (Jacobs et al., 1992) and have been classified into nine groups from B1 to B9. According to Ciranna, "nerve fibers arising from the caudal groups of serotonergic neurons (B1-B4) form a descending system directed to the spinal cord and also project to cerebellum, pontine and midbrain structures, whereas ascending fibers originate from the rostral groups of

serotonergic neurons (B5-B9) and innervate almost all brain areas" (Ciranna, 2006). Figure 2 shows the principal groups of serotonergic neurons in the CNS, along with their projection sites.

Figure 2: Principal Groups of Serotonergic Neurons

Source: Ciranna. 2006



To date, fourteen subtypes of serotonin receptors with different physiological functions have been cloned. Ciranna writes: "With the exception of the 5-HT3 receptor, which is a ligand-gated ion channel, all the other 5-HT receptor subtypes are metabotropic G-protein-coupled receptors and modulate an intracellular second messenger system. 5-HT receptors have been grouped into seven principal classes, named 5-HT1 to 5-HT7; for each subtype, Table 1 indicates the pharmacological characteristics, the localization, the intracellular action mechanism and final effect on neuronal excitability, the physiological function in which the receptor is involved and the pathologies deriving from its malfunctioning (Ciranna, 2006)."

Table 1: Serotonin Receptor Subtypes

Receptor	Localization	Agonists	Antagonists	Physiological function	Malfunctioning pathologies
5-HT_{1A}	Dorsal raphe;Hippocampus	8-OH-DPAT bspirone Gepirone	NAN 190 MDL 73005EF WAY 100635	Autoreceptor; Modulation of Release of other Neurotransmitters Modulation of anxiety	Anxiety; Depression
5-HT_{1B}	Hippocampus; Striatum; Substabtia nigra; Raphe nuclei; Cerebellum; Frontal cortex; Cerebral arteries	Sumatriptan	GR5562 S216641 SB272183	Nerve terminal autoreceptor; Modulation of Release of other Neurotransmitters	Migraine
5-HT_{1D}	Dorsal raphe; Human heart	Sumatriptan PNU 109291	BRL 15572	Autoreceptor	Migraine
5-HT_{2A}	Cortex; Basal ganglia; Peripheral tissues	DOI DOB	Ketanserin MDL 100907 Cinanserin Mianserin Methysergide	Possible role in learning and memory	Psychiatric disorders
5-HT_{2B}	Cerebellum; Lateral septum; Hypothalamus; Amigdala; Cardiac valves	BW 723C86	SB 200646 SB204741	Food intake; Behaviour	Anxiety; Feeding disorders; Cardiac valvulopathies
5-HT-_{2C}	Choroid plexus; Hippocampus Habenula Substantia nigra Raphe nuclei	Ro 600175	Mianserin Methysergide Mesulergine	Food intake; Neuroendocrine regulation	Feeding disorders; Cognitive impairment
5-HT_{3}	Olfactory bulb Cerebral cortex Hippocampus Amygdala Hypothalamus	2-methyl-5-HT SR 57227	ICS 205930 Zacopride Ondanserton Graniserton Tropiserton	Pre-synaptic Modulation of transmitter Release	Anxiety; Schizophrenia; Cognitive Impairment

Receptor	Localization	Agonists	Antagonists	Physiological function	Malfunctioning pathologies
5-HT_4	Solitary tract Nucleus Colliculi Hippocampus Peripheral tissues	Renzapride BIMU 8 RS 67506 ML 10302	GR 113808 SB 204070	Modulation of transmitter release Memory enhancement	Neurodegenerative disease; Caediac arrhythmia
5-HT_6	Striatum Amygdala N. accumbens Hippocampus Cortex Olfactory tubercle		RO 630563 SB 271046 SB 357134	Modulation of acetylcholine transmission	Cognitive disfunctions (Alzheimer)
5-HT_7	Cerebral cortex; Thalmic nulei; Hypothalamus; Limbic structures	8-OH-DPAT	SB 258719 SB 269970	Control of circardian rhythms; Thermoregulation Mood and Behavior	Affective disorders; Migraine; Nociception

Source: Ciranna 2006

Ciranna notes: "In many brain regions 5-HT receptors have been localized on neurons that do not receive direct serotonergic innervations; in parallel, 5-HT fibers often lack typical synaptic contacts with post-synaptic neurons (Ciranna, 2006; also see Descarries et al., 1990; Umbriaco et al., 1995)."

Neuronal serotonin plays an essential role in various physiological functions including sex, aggressive behavior, feeding, thermoregulation, endocrine regulation, pain modulation, motor activity, memory and learning, mood, anxiety and sleep regulation (Kahn et al.1991, Barnes et al. 1999). The subfamily of 5-HT_2 receptors consists of three members: 5-HT_{2A}-5HT_{2c}.The distribution of 5-HT_{2A} receptors in the CNS has been characterized extensively. Receptor autoradiography studies have demonstrated high levels of 5-HT_{2A} receptor binding sites in numerous forebrain regions, with lower levels in the basal ganglia and hippocampus (Lopez-Gimenez, et al. 1997). The serotonergic pathway is one of the key pathways that contribute to wakefulness (Abrams et al. 2005, Sharpley et al. 1994). Several studies have shown that serotonin plays key roles in sleep-wakefulness regulation. Some atypical SSRIs have been shown

to increase slow wave sleep (SWS), particularly those that bind to the serotonin 5-HT_{2A} receptor (Idzikowski et al. 1986, Sharpley et al. 2005).

1.6.2 The 5-HT_{2A} Receptor

The 5-HT_{2A} receptor plays an essential role in mediating a large number of physiological processes in CNS and in the periphery. It is now assumed that 5-HT, which is released at maximum during wakefulness, promotes wakefulness per se through actions on 5-HT_2 receptors and later triggers sleep by stimulating sleep-promoting systems (Jouvet, 1999).

In addition, it has been suggested that increasing SWS and reducing arousals and stage shifts may improve sleep maintenance (Monti et al. 2006, Sharpley et al. 1994).

De Martinis and Winokur noted in 2007: "Data to support a role for enhanced 5-HT neurotransmission in promoting the onset of sleep include results from studies involving administration of the 5-HT biosynthesis precursors L-tryptophan and 5-hydroxytryptophan, both of which have been reported to hasten the onset of sleep. In contrast, depletion of 5-HT, by administration of reserpine or induction of electrolytic or neurotoxic lesions, has been reported to produce states of profound insomnia. On the other hand, activation of various 5-HT receptor subtypes has been shown to inhibit specific sleep stages. For example, 5-HT1A receptor agonists produce a decrease in REM sleep as well as an increase in wakefulness. Agonists at the 5-HT1D receptor (also know as the 5-HT1B receptor in non-humans) have been reported to produce similar sleep-related effects, including inhibition of REM sleep and an increase of wakefulness. 5-HT2 receptor agonists, in contrast, have been reported to inhibit SWS, whereas 5-HT2 antagonists markedly increase SWS (De Martinis et al., 2007)." Midbrain 5-HT neurons represent a part of the system opposing rapid eye movement (REM). Investigations making use of receptor-specific pharmacological treatments and knock-out mice have shown that 5-HT_{1A} and 5 HT_{1B} receptors play a role in regulating REM sleep (Adrien et al., 2004; Boutrel et al., 1999 and 2002). 5-HT_{2A} receptor has been implicated in serotonergic neurons' regulation of arousal. Graham and co-workers write, "5HT_{2A} receptor activation is likely to lead to enhancement of arousal, as shown by the sedative property of 5HT_{2A} receptor antagonists, such as ketanserin (Graham et al., 2002)." Several 5-HT_{2A} receptor antagonists (e.g., eplivanserin, pruvanserin, M-100907), along with an inverse agonist (APD125), have been in development as potential hypnotic agents to treat insomnia (Becker et al., 2006; Gerschell et al., 2006).

1.6.3 5-HT_{2A} Receptor Antagonist (Eplivanserin)

There is a clinical need for effective hypnotic agents that are safer and well-tolerated. Eplivanserin was a new chemical entity that is a selective $5HT_{2A}$ receptor antagonist. It was developed for the treatment of insomnia with sleep maintenance.

In vitro, eplivanserin showed a potent antagonistic effect on $5HT_2$ receptor-mediated contractions in several preparations of isolated smooth muscle (rat jugular vein, rat caudal artery, rat uterus, guinea-pig trachea, rabbit thoracic aorta). Under the same experimental conditions, eplivanserin in doses up to 0.1 μM had no effect on serotonin-induced contraction of rat stomach fundus and guinea pig ileum, which involve $5HT_{2B}$ and $5HT_3$ receptor activation, respectively (Rinaldi-Carmona et al. 1992).

Eplivanserin delayed the onset of REM sleep in rats following of administration from 0.85 mg/kg body weight upwards (Rinaldi-Carmonna et al., 1992). The duration of wakefulness and slow-wave sleep was not markedly affected. Eplivanserin modified the EEG spectral density by increasing the power in low frequencies and reducing it in middle and high frequencies. This corresponds to a slowing of the EEG and to loss of sleep spindles and fast activities. These data are consistent with the reported effects of other $5HT_2$ receptor antagonists, such as ritanserin, in rats.

In vitro, in brain stem-spinal cord preparations from newborn rats, when tested for its ability to antagonize serotonin-induced respiratory modifications, eplivanserin (50mM) altered neither resting respiratory frequency nor the increase in frequency($5HT_2$ receptor-mediated). In contrast, eplivanserin reduced in a dose-dependent manner (5 to 50 mM) both the tonic discharge of cervical motoneurons and the decrease of inspiratory hypoglossal activity by serotonin and other $5HT_2$ receptor agonists (Monteau et al. 1994).

The development of specific $5HT_2$ receptor antagonists has provided new tools for investigating the role of the serotonergic system in the regulation of sleep.

1.7 Aims of this Work

Among COPD patients, sleep disturbance tends to become more severe as the disease advances and substantially worsens COPD patients´ quality of life. Cormick and co-workers note: “Subjective complaints of sleep disturbance appear to be associated with the presence of respiratory symptoms of cough, dyspnea, wheezing or sputum production. Many COPD patients

report use of hypnotics (28% compared to controls 10%) to combat these sleep disturbances (1986).” When a sedative is prescribed, extra caution is necessary for patients with greater risk of adverse respiratory effects. This is the case, for example, for patients with advanced respiratory disease or with hypercarbia. Certain medications are known to affect respiratory drive. In patients with COPD, narcotics, benzodiazepines, and barbiturates can aggravate respiratory failure (George & Bayliff, 2003).

Clinical studies support the role of many different agents in the treatment of insomnia in COPD patients, but they do not always provide assurance that these therapies can be safely used, especially in this population.

Eplivanserin was a new drug class for this indication, which was effective in the treatment of insomnia and may deliver safety advantages compared to current sedative hypnotic drugs in COPD patients.

The primary aim of this study was to investigate the effects of eplivanserin 15 mg single dose versus placebo on respiratory function in COPD patients with mild to moderate intensity. To investigate the specific role of eplivanserin in the modulation of respiratory control, we analyzed the pharmacodynamic effects of eplivanserin 15 mg single dose versus placebo on respiratory function as measured by mean overnight oxygen saturation (SaO_2). Additionally we investigated the pharmacodynamic effects of eplivanserin on respiratory function (air flows, lung volumes, airways resistance and conductance via spirometry and body plethysmography).

The secondary aim was to investigate the pharmacodynamic effects of eplivanserin on night polysomnography (PSG) including apnea hypopnea index (AHI), mood and alertness.

We have also assessed the clinical and laboratory safety of eplivanserin during this study.

2 Material and Methods

To investigate the effects of eplivanserin twenty-eight patients with mild to moderate COPD were recruited for the clinical study and received a single dose of either active drug (eplivanserin 15 mg) or placebo in a double-blind, randomized, crossover study.

The study protocol describing the main objectives, tasks and requirements of this study was approved by the Ethics Committee of Berlin, Germany, and also by the *Bundesinstitut für Arzneimittel und Medizinprodukte* (BfArM, the Federal Institute for Drugs and Medicinal Products) of the Federal Republic of Germany. The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice (GCP), as defined by the International Conference on Harmonization (ICH) and the applicable regulatory requirements.

In compliance with the Declaration of Helsinki, the patients, prior to their inclusion in the study, were provided with full verbal and written information on the nature, objectives, significance, expected benefits, potential risks and consequences of the study. Every patient provided freely-signed written informed consent prior to participation in the clinical study.

2.1 Patient Selection, Study Conduct and Study Populations

Male and non-pregnant females aged between 20 and 75 years with mild to moderate COPD were included. Patients were recruited for this study from the PAREXEL GmbH data base and also by advertisement.

Patients who had given their written informed consent and met all inclusion criteria and none of the exclusion criteria stated below were randomly assigned to one of the following two treatment sequences:

- Sequence 1: Treatment A : 15 mg eplivanserin (3x5mg eplivanserin tablets), followed by Period 2: Treatment B: placebo (3 placebo tablets)
- Sequence 2: Treatment B : placebo (3 placebo tablets), followed by Treatment A: 15 mg eplivanserin (3 x 5 mg eplivanserin tablets)

They received a single dose of the first investigational medical product (IMP) in period 1 and after a washout of 14 days (which is considerably longer than the necessary duration of 5 times of

the half life 6-8h as reported from Landolt and Wehrle, 2009) in period 2 a single dose of the second IMP in a crossover study as foreseen in the treatment sequence to which he/she was randomized.

Seventy-two patients were screened to enroll at least 28 eligible patients as necessary for the study analysis (see section 3). The most frequent reasons for the exclusion were the criteria for respiratory function (subjects with severe COPD or instable), the ECG (subjects with borderline QTC), vital signs (subjects with hypertension) or the laboratory criteria (subjects with abnormal liver enzymes).

Only the patients who signed the informed consent and were able to understand and follow the study-specific restriction were screened between Day -21 and Day -2. Each patient underwent an adaptation night in the sleep laboratory before dosing in each treatment period.

For the analyses of this study two different study populations were defined:

All patients who were randomized in the study took at least one dose of the IMP and were therefore used for all PD and safety analyses.

The inclusion and exclusion criteria for this study are described in detail in the next two subsections.

2.1.1 Inclusion Criteria:

- Male or female, between 20 and 75 years of age, inclusive
- Clinical history of mild to moderate COPD for at least 3 years, according to GOLD guidelines (see below)
- The diagnosis of stable, mild to moderate COPD has to be confirmed by pulmonary function testing (PFT) at screening (see below)
- With vital signs after 10 minutes resting in supine position:
    - 95mmHg<systolic blood pressure<160mmHg
    - 45mmHg<diastolic blood pressure<90mmHg
    - 40bpm<hearth rate<100bpm
- Normal 12-lead ECG; 120ms<PR<220ms, QRS<120ms, QTc≤430ms if male, ≤450ms if female.

- Laboratory parameters within the normal range, unless the investigator considers an abnormality to be clinically irrelevant for COPD patient; however, serum creatinine and hepatic enzymes (Aspartate Aminotransferase [AST] and Alanine Aminotransferase [ALT]) should be strictly below the upper laboratory norm.
- If female, menopause definition: over the age of 60 years, or between 45 and 60 years being amenorrheic for at least 2 years with plasma FSH level > 30 UI/L
- If pre-menopausal female, the patient should have been surgically sterilized, or a double contraception method was requested during the whole study, meeting the criteria for a highly effective method of birth control according to the Note for Guidance on non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals and according to the following double contraception algorithm.
- Patients who were eligible for the study on the basis of a pre-study physical examination, medical history, vital signs, ECG and the result of safety clinical laboratory tests.
- Patient had to be able to read and understand the subject information sheet.
- Patient was to give written informed consent prior to any procedure related to the study.
- Established clinical history of COPD in accordance with the following definition by the American Thoracic Society/European Respiratory Society (ATS/ERS): COPD is a preventable and treatable disease characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. “Although COPD affects the lungs, it also produces significant systemic consequences (ERS Guidelines, 2004).”
- Patient diagnosed with COPD, as defined by the ERS Consensus Statement: “Chronic obstructive pulmonary disease (COPD) is a disorder characterized by reduced maximum expiratory flow and slow forced emptying of the lungs; features which do not change markedly over several months. Most of the airflow limitation is slowly progressive and irreversible. The airflow limitation is due to varying combinations of airways disease and emphysema; the relative contribution of the two processes is difficult to define in vivo. The airway component consists mainly of decreased luminal diameters due to various combinations of increased wall thickening, increased intra luminal mucus, and changes in

the lining fluid of the small airways (ERS Guidelines, 2004)."

- The diagnosis of stable, mild to moderate, COPD condition was to be confirmed at screening by Pulmonary Function Test (PFT). Mild airflow limitation was defined as:
    - Computerized spirometry test: post-bronchodilator: FEV1*>50% predicted.
    - GOLD severity criteria of COPD post-bronchodilator:
        - Stage I (mild) COPD:
            - Post-bronchodilator FEV1/FVC**≤ 0.7
            - FEV1 predicted ≥ 80%
        - Stage II (moderate) COPD:
            - Post- bronchodilator FEV1/FVC≤ 0.7
            - FEV1 predicted: 50-80%

        * FEV1= forced expiratory volume in one second

        ** FVC: forced vital capacity
- Finger pulse oximetry at rest, in supine position > 85%
- Stable physical health for at least 2 weeks prior to entering the study
- Smoking status:
    - Patient was to have a personal history of smoking of at least 10 packs year (1 pack year = 20 cigarettes smoked per day for 1 year or equivalent)
    - At study entry, patient could have been either non-smoker, or current smoker
    - If current smoker, patient (no smoking will be allowed in the 30 minutes prior PFT)
    - NB: specific wash-out periods for the screening spirometry: 6 hours for short-acting bronchodilator, and 48 hours for long-acting bronchodilators and tiotropium.

2.1.2 Exclusion criteria:

- Presence of any acute, evaluative, and/or unstable clinically relevant disease or any

condition that may interfere with the interpretation of study data in the investigator's judgment.

- Blood donation within one month before study entry, or intent to donate blood within one month after study completion.
- Patient tested positive at screening and/or at baseline, for alcohol and/or drug of abuse (opiates, cocaine, amphetamines, and cannabinoids).
- Presence of drug or alcohol abuse (alcohol consumption greater than 21 units per week for males and greater than 14 units per week for females) within one year before inclusion (an alcohol unit is defined as 250ml of beer/lager or 100ml of wine or 25ml of spirits).
- History of drug abuse within the last 6 months, or history of substance abuse if deemed significant according to the investigator.
- Excessive consumption of beverages with xanthine bases (i.e. tea, coffee, or cola > 5 cups or glasses/day)
- If female, pregnancy (defined as positive ß-HCG blood test), or breast- feeding.
- Use of any over-the-counter including tryptophan, valerian root (*Valeriana officinalis*), Kava (*Piper methysticum forst*), melatonin, St. John's Wort (*Hypericum perforatum*), Alluna (herbal sleep supplement with valerian root) or prescription sleep medication, including hypnotics, sedatives, or anxiolytics within one week or five half-lives (whichever is longer) prior to screening.
- Use of any substance with psychotropic effects or properties known to affect sleep/wake, including, but not limited to: neuroleptics, morphine/opioid derivates, sedative antihistamines, stimulants, antidepressants, clonidine, within one week or five half-lives (whichever is longer) prior to screening.
- Positive results on urine drug screen for drugs known to alter sleep (amphetamine/methamphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, benzodiazepines, phencyclidine, propoxyphen and alcohol).
- Patient has taken any investigational product within 1 month prior to dosing.
- Positive reaction to any of the following tests: hepatitis B surface (HBs) antigen, anti-

hepatitis C virus (HCV) antibodies, anti-human immunodeficiency virus 1 (HIV 1) antibodies, anti-human immunodeficiency virus 2 (HIV 2) antibodies.

- Patient who, in the judgment of the investigator, was likely to be non-compliant with the obligations inherent to the study participation, or unable to cooperate because of a language problem or poor mental development.
- Inability to understand the nature, purpose, scope, and possible consequences of participating in the study.
- Patient was unable to complete the study questionnaires and scales.
- Patient in exclusion period of a previous study according to applicable regulations, patient who cannot be contacted in case of emergency.
- Subject was the investigator or any sub-investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in the conduct of the protocol.

The use of any concomitant medication except hormonal contraception (for females) was forbidden during the study, specially the use of any drugs affecting sleep patterns and/or respiration.

COPD medications were allowed, and were to be administrated at stable dosage over the 4 weeks prior to study entry, with specific washout periods before the screening spirometry:

- Short-acting bronchodilator: 6 hours;
- Long-acting bronchodilator: 48 hours;
- Tiotropium: 48 hours;
- Inhaled corticoids were allowed, and were to remain at a stable dosage over the past 4 weeks, but in case of combination with a long-acting bronchodilator, the patient was to be switched to a short-acting bronchodilator and corticosteroids alone;
- Oral corticosteroids: last intake was to be at least 1 month prior to screening;
- Theophyllin: 48 hours.
- Salbutamol as rescue medication was allowed if the patient’s respiratory condition worsened during the washout as needed.

2.2 Tests Performed During the Study

During the study the following tests were performed to estimate the patients with regard to COPD and other PD parameters especially with focus on quality of sleep, mood and alertness.

2.2.1 Spirometry

Common Terminology of Spirometry Tests:

- **VC-Vital Capacity** - The amount of air that can be forcibly exhaled from the lungs after a full inhalation.
- **FVC-Forced Vital Capacity** - The amount of air which can be forcibly exhaled from the lungs after taking the deepest breath possible.
- **FEV1-Forced Expiratory Volume in One Second** - The amount of air which can be forcibly exhaled from the lungs in the first second of a forced exhalation.
- **FEV1/FVC-FEV1-Percent (FEV1%)** - The ratio of FEV1 to FVC tells what percentage of the total amount of air is exhaled from the lungs during the first second of forced exhalation.

Spirometry was performed while the patient was in a sitting position. All lung function tests were repeated, until three technically acceptable measurements were made. All three measurements as well as the highest values for FEV1 and FVC were recorded in the subject's file. The FEV1/FVC was determined using the highest FEV1 and the highest FVC values. A screening spirometry was performed pre and post bronchodilator.

2.2.2 Body plethysmography

Body plethysmography is one of many pulmonary function tests which determines how much air is present in the lungs when a patient takes a deep breath and how much air is left in the lungs after the patient exhales as much as he/she can. Patients were required to sit in an enclosed plastic box and then, wearing a nosepiece, they were instructed on how to breathe through a mouthpiece. In COPD patients, the amount of air remaining in the lungs while breathing is greater than normal.

The most common measurements made using the body plethysmograph are VTG (thoracic gas volume) and R_{aw} (airways resistance) (DuBois et al., 1956). Airways conductance (G_{aw}) is also commonly calculated as the reciprocal of R_{aw}. Specific airways conductance (i.e. conductance/unit of lung volume) is routinely reported as sG_{aw}.

- "VTG is expressed in liters (BTPS, or body temperature and pressure saturated) and is the volume of gas in the lung when the mouth shutter is closed. In plethysmographic studies, it is commonly used to represent the functional residual capacity (FRC) (AARC Clinical Practice Guidelines, 2001)."
- R_{aw} is reported in cm H_2O/L/s (i.e. cm $H_2O \cdot L^{-1} \cdot s^{-1}$).
- sG_{aw} is reported in L/s/cm H_2O (i.e. $L \cdot s^{-1} \cdot$ cm H_2O^{-1}) and is the reciprocal of the R_{aw} ($1/R_{aw}$) divided by the lung volume at which the resistance measurement is made.

Patients rested in the body box for at least 30 seconds prior to any assessments. Plethysmography was performed with an appropriately maintained and calibrated body plethysmograph as follows:

- Click on body plethysmography icon
- The plethysmograph was closed and the patient was asked to wait for 30 seconds until the temperature within the box stabilized.
- The patient then placed the mouthpiece in his mouth and the nose clips on his nose and breathed gently. After a few moments, the patient was instructed to increase the breathing frequency to between 30-40 breaths per minute. This had the effect of increasing the lung volume, the panting phase was continued until the subject's lung volume no longer rose. The last 5-10 pressure volume curves generated prior to the occlusion was captured and analyzed.
- At this point the airflow to the subject was occluded and the subject was instructed to make respiratory efforts to counteract the occlusion.
- The measurement was saved and a short break of approximately 30 seconds was allowed for the patient. A total of three tests were performed.
- Each panting session generated 5-10 sG_{aw} measures; the closest to the mean of these was recorded.

2.2.3 Finger Pulse Oximetry

Finger pulse oximetry was performed as an additional safety parameter. Pulse oximetry is widely used to rapidly monitor arterial oxygen saturation (SaO_2) (Mc Carthy et al. 1993). It has many of the characteristics of an ideal monitoring technique: portability, non-invasiveness, ease of use (calibration is not required) and the capability for continuous on-line monitoring of SaO_2. The measurement was made with calibrated devices (Critikon Dinamap pro 100). The measurement

was carried out by means of an oxygen-saturation sensor (finger clip or adhesive sensor) on a fingertip. In order to clearly distinguish between the measured value of this non-invasive, indirect determination of oxygen saturation and arterial oxygen saturation, and the direct determination by blood gas analysis, the subscript p was used to indicate the pulse-oximetric measuring method. Functional Saturation (S_aO_2) is defined as:

$$S_aO_2 = HbO_2/ (HbO_2+ RHb)$$

Where HbO_2 is the concentration of the oxygenated hemoglobin and
RHb the concentration of deoxygenated "reduced" hemoglobin (Yao et al. 2008).

In addition to the saturation, the pulse in the smallest blood vessels (capillaries) was also measured by the clip sensor or the adhesive sensor.

There are two light sources with defined infrared ranges on one side of the sensor; on the other side a photo sensor is situated. Due to the different absorption pattern of hemoglobin and oxygenated hemoglobin in the 660nm range, in the 940nm range, and with ambient light in order to tare, the two forms of hemoglobin can be differentiated. The pulse oximeter measures only the pulsatile blood flow in order to exclude tissue or vessel bias. The percentage of oxygenated red blood cells was calculated by the device. The reference range for healthy people lies between 96% and 100%. The finger clip was fitted to any of the individual fingers of the subject. After a few seconds, the oxygen saturation and the heart rate were read from the device. An alarm was sounded if there was no clear signal due to movement or accidental removal of the device.

Only patients who met all the inclusion criteria, did not fall under the exclusion criteria, and were in a fasting state were invited to the clinic on the morning of Day -1. The following tests were performed to ensure that the patient still met the inclusion criteria to participate in the study:

- Physical examination, vital signs, clinical laboratory tests including urine drug /alcohol, finger pulse oximetry, current medical history and concomitant medication.
- Blood gas analysis on ear lobe: Arterial blood gas (ABG) analysis is useful in evaluating the clinical condition of COPD patients; however, arterial puncture or insertion of an arterial catheter may sometimes be difficult and cause many complications. Arterialized ear lobe blood samples have been described as adequate for gauging gas exchange in COPD patients.

Arterial blood gas is based on the assumptions that sufficient vasodilatation can be achieved locally by means of the topical application of a vasoactive cream on the earlobe; that arterial and venous oxygen tension (PO_2) in the earlobe tends to converge, and that the arterialized earlobe PO_2 resembles the arterial PO_2. After aseptic cleaning, the lateral distal portion of the earlobe was salved with a thermo salve (Finalgon^R Salve) and after ca. 10 minutes was punctured with a scalpel blade. The blood gas sample was obtained by "contact" with the capillary tube's tip. The tube was squeezed shut with fingers in order to avoid air bubbles. The arterialized samples were collected in heparinized glass capillaries and immediately introduced into the blood gas analyzer (iStat, Abbot), and within two minutes, it provided PH, PO_2, PCO_2 and HCO_3 readings, which were then recorded.

2.2.4 Leeds Sleep Evaluation Questionnaire (LSEQ)

Leeds Sleep Evaluation Questionnaire is a standardized instrument to measure sleep difficulties in the context of clinical investigation (Parott et al., 1980). It is a retrospective instrument in which the patients are asked to compare current aspects of sleep with those prior to the study in which they are enrolled. In this study, the German validated translation by MAPI research institute was used to assess the subjective changes in aspects of sleep and morning awakening. The 10 questions were chosen to reflect four aspects of sleep and early morning behavior:

- Ease of getting to sleep (GTS): A: How would you compare getting to sleep now with getting to sleep normally?
    1. Harder than usual/easier than usual
    2. Slower than usual/quicker than usual
    3. Felt less drowsy than usual/felt more drowsy than usual
- Quality of sleep (QOS): How would you compare the quality of sleep now with your usual sleep?
    4. More restless than usual/ more restful than usual
    5. More periods of wakefulness than usual/ fewer periods of wakefulness
- Ease of awakening following sleep (AFS): How did your awakening compare with your usual pattern of awakening?
    6. More difficult than usual/easier than usual
    7. Took longer than usual/took shorter than usual
- Behavior following wakefulness (BFW): How did you feel on waking?
    8. Tired/alert

- How do you feel now?
    9. Tired/alert
- How was your sense of balance and coordination upon getting up?
    10. More clumsy than usual/less clumsy than usual

A 100-unit line separates the two halves of each question. The questionnaire instructions were: "Each question is answered by placing a vertical mark on the answer line. If no change was experienced then place your mark in the middle of the line. If a change was experienced then the position of your mark will indicate the nature and extent of the change, i.e. large changes near the end of the line, small changes near the middle."

2.2.5 Visual Analog Scale (VAS) of Mood and Alertness (Bond A. and Lader M., 1974)

We used the German validated translation produced by the MAPI research institute. The patients were asked to place a mark at that point on the line that represented their current state along that continuum. The VAS score is the distance (measured in mm) between the subject's mark and one or the other end of the line. The 16-analog scale is based on three factors that assess changes in self-rated alertness, self-rated calmness, and self-rated contentment. The individual analogs are listed below:

1. Alert/Drowsy
2. Calm/Excited
3. Strong/Feeble
4. Muzzy/Clear-Headed
5. Well Coordinated/ Clumsy
6. Lethargic/Energetic
7. Contented/Discontented
8. Troubled/Tranquil
9. Mentally Slow/Quick-Witted
10. Tense/Relaxed
11. Attentive/Dreamy
12. Incompetent/Proficient
13. Happy/Sad
14. Antagonistic/Amicable
15. Interested/Bored

16. Withdrawn/Gregarious

2.2.6 Polysomnography (PSG)

Patients who continued to satisfy all eligibility criteria (i.e. meet all the inclusion criteria and none of the exclusion criteria) remained in the clinical unit overnight, and underwent the first polysomnographic recording (habituation). The SIESTA-Amplifier from the Compumedics Company with the sleep-software PSG profusion was used for the PSG recording. Final inclusion and randomization was done before the Investigational Product administration on day 1 of period 1. Night polysomnography consisted of:

- Electroencephalogram (EEG)
- Electrooculogram(EOG)
- Electromyogram (EMG)
- Two-lead ECG
- Respiratory effort
- Naso-oral airflow
- Pulse oximetry

The PSG was performed at PAREXEL and the data was sent to the Competence Center of Sleep Medicine at the Center of Neurology, Neurosurgery and Psychiatry at the Charité University of medicine, Campus Eschenallee, for analysis. A Manual of Operations for PSG was written by PAREXEL staff in cooperation with Prof. Danker Hopfe and her co-worker to ensure maximum harmonization in the recording and transfer of data to and from the corresponding sleep lab.
The Manual of Operations for PSG contained the following chapters:

2.2.7 Polysomnographic Recording and Montage

This section described all the important steps in polysomnographic recording, as well as the procedures for transferring data to the corresponding sleep lab.
To evaluate the influence of a drug on CNS activity during the night as well as on specific sleep parameters and to determine sleep quality and sleep efficiency in an objective manner, PSG is typically performed for several (consecutive) nights (adaptation, baseline, and one or more nights post-administration). In the majority of clinical trials including PSG, the primary polysomnographic parameter of interest was sleep efficiency, which was analyzed using a mixed

model containing fixed factors for treatment, period and sequence together with a random term for subject nested within sequence.

PSG could also be beneficial in order to assess incidence and dimension of various types of sleep disorders and other related syndromes, e.g. obstructive sleep apnea syndrome (OSA), periodic leg movement syndrome (PLMS) and narcolepsy. Therefore, PSG is excellently suitable for screening procedures.

The montage consisted of the following channels:

EEG

The EEG electrodes had gold cups. Impedances for all the EEG electrodes were not to exceed 5000 Ohms (5 kΩ).

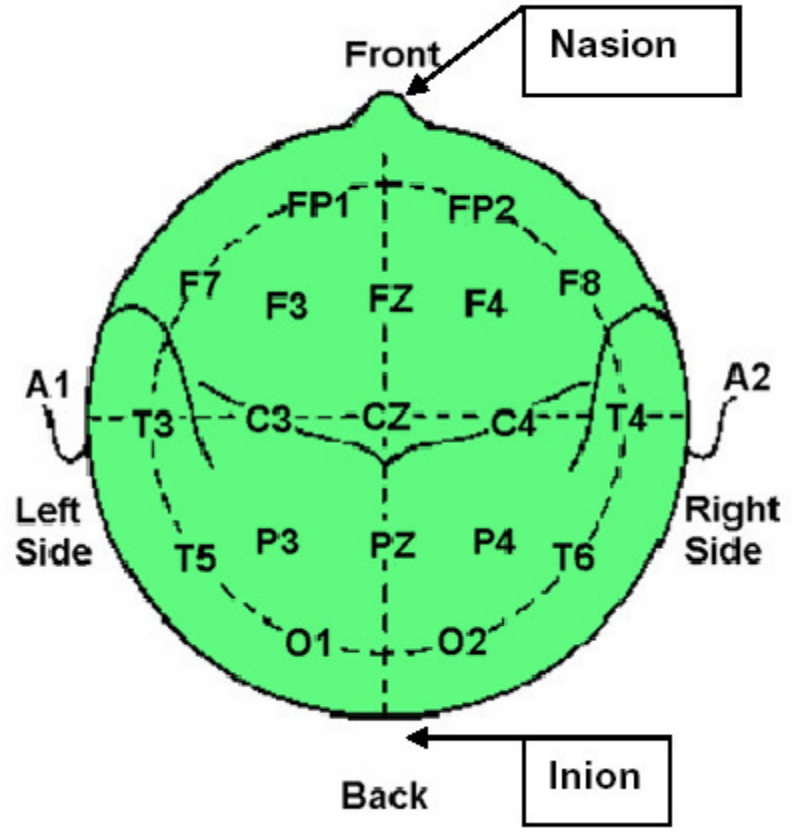
- C3-A2/M2 (i.e. C3 referenced against A2)
- C4-A1/M1
- O1-A2/M2
- O2-A1/M1

In order to allow for scoring according to the new guidelines (Silber et al., 2007), if subsequently desired, additional signals were recorded on separate channels:

- F3-A2/M2
- F4-A1/M1

These channels refer to positions of the international 10/20 system depicted below:

Figure 3: EEG-Recordings



EOG

For the registration of the electrooculogram (EOG), one electrode each was set at the outer corner of the eye (epicanthus)

EMG:

<u>EMG mental and sub-mental:</u>

To register the muscle activity (EMG) and to have a backup registration, the mental and sub-mental regions were selected.

- Mental: the mental electrodes were placed left and right in the middle of each muscle hill at the chin
- Sub-mental: The sub-mental electrode was set under the chin in the middle of the transition from the lower jaw bone to the muscles (male patients not wearing a beard should be wet shaved)

The EOG and EMG electrodes were fixed with a connecting head band and connected to the acquisition module together with the EEG electrodes on top of the head.

EMG tibialis:

Due to the fact that the diagnosis of leg movements (e.g. periodic leg movements) was not a target parameter, signals were not recorded from either leg.

Two-lead ECG:

A modified two-lead ECG was used to monitor rate and rhythm of the heart. Electrodes were placed in the right arm (RA) and left leg (LL) positions on the thorax.

Respiratory effort:

Respiratory effort was assessed using respiratory inductance plethysmography (RIP) for both thorax and abdomen. This method was used in preference to piezo strains due to fewer artifacts and interfaced with the system to allow for visual identification of apneas and hypopneas.

Thoracic respiration: the breath belt is set on the 4^{th} intercostal region.

Abdominal respiration: the breath belt is set at 2 cm above the upper border of the hip bone.

Nasal and oral respiration: assessed using a nasal cannula in preference to a thermistor.

Snoring microphone: For diagnosing apneas and hypopneas snoring noise was also captured. The snoring microphone was affixed next to the trachea using adhesive tape. The cable was directed through the connecting band for relief.

Finger pulse oximetry:

Arterial oxygen saturation (SaO_2) was continually monitored using a pulse oximeter (finger sensor) (see Section 2.2.3).

Body position:

Body position was continually monitored using a mercury switch.

Preparation of Recording

The patients went to bed around 22:45h and turned their lights off at 23:00h. The following morning the patients were awakened around 7:00h.

Recording

Lights out and lights on time, and all disturbing events which might have influenced the sleep of a patient were recorded in the Events Log / Tech Notes sheet.

Converting the Recording into EDF

For evaluation the polysomnographic recording, e.g. sleep staging, the recording was divided

into 30 seconds segments. Sleep staging was done following the guidelines of Rechtschaffen and Kales, 1968. The result of the visual evaluation was archived as data file for statistical evaluation on corresponding storage media (e.g. CD-ROM, DVD).

From the polysomnographic monitoring measurements the following parameters were determined (Table 2):

Table 2: Sleep Parameters

Parameter	Description
Time spent in bed (TIB)	Time from "lights off" to "lights on", 480 minutes as predefined
Sleep efficiency index (SEI)	TST: TIB (expressed in %)
REM sleep	The time spent in the REM sleep stage (in minutes) in TIB
REM episodes	Number of time a subject falls into REM sleep in TIB
Sleep period time (SPT)	Time from sleep onset (3 continuous sleep stage 1 epochs) or from first epoch NREM2, 3, 4 or REM (whatever occurs first), to last epoch of sleep
Total sleep time (TST)	Total time spent in the different sleep stages (1,2,3,4 Non-REM and REM) in TIB
Slow wave sleep (SWS_1)	Percent of SPT that are stage 3 and 4 sleep
Slow wave sleep (SWS_2)	Percent of TST that are stage 3 and 4 sleep
Wake after sleep onset (WASO)	The number of minutes of wake after the initiation of persistent sleep (20 consecutive epochs)
Wake during SPT	Time spent awake during SPT
REM sleep latency (RSL)	Time from sleep onset to 1st epoch REM
Sleep onset latency (SOL)	Time from lights off to sleep onset (3 continuous sleep stage 1 epochs) or to first epoch NREM2, 3, 4 or REM, whatever occurs first
Apnea Hypopnea Index (AHI)	The number of apnea/ hypopnea episodes per hour of sleep (TST) (apnea: respiration suspends for at least 10 seconds hypopnea: change of amplitude for at least 50 % without desaturation)
Latency to persistent sleep (LPS)	Number of minutes from lights off to the 1st epoch of continuous 20 epochs of non-wake

2.3 Procedures and Assessments on Study Visits

2.3.1 Screening Visit

Before any screening-related procedures were performed, the patients gave signed written informed consent.

The following procedures were performed during the screening visit:

Every patient was asked at screening about his/her medical history, current COPD condition, smoking history, drug use and concomitant therapy/medication.

The physical examination consisted of general appearance, examination of head, neck, heart, skin, lymph nodes, extremities and lung.

Measurement of height and weigh, BMI (Body Mass Index) = weight (in kg)/height (in cm) ^{2}

12- lead ECG using the electronic cardio-base system after patient rested for 10 minutes.

Vital sign measurements (heart rate, systolic and diastolic blood pressure) were performed using the Dinamap Pro Care® automatic calculating device after 10 minutes' resting. The standing blood pressure was measured after 3 minutes' standing.

The laboratory tests comprised hematology (red blood cell count, hematocrit, hemoglobin, white blood cell count with differential: neutrophils, eosinophils, basophiles, monocytes and lymphocytes, platelets); biochemistry (plasma electrocytes: sodium, potassium, chloride, calcium, magnesium); liver function (AST, ALT, alkaline phosphatases, gamma-glutamyl transferase, total and conjugated bilirubin); renal function (creatinine); metabolism (glucose, albumin, total proteins, total cholesterol, triglycerides); muscle (creatine phosphokinase); and urinalysis (proteins, glucose, erythrocytes, leucocytes, ketone bodies, and PH).

A ß-HCG blood test and a FSH plasma test were performed only in female subjects.

Serology test in serum: hepatitis B antigen, hepatitis C antibodies, anti-HIV1 and anti-HIV2 antibodies.

Urine drug screening: amphetamines/methamphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, propoxyphene, phencyclidine.

In order to evaluate the current COPD status according to GOLD criteria, a computerized

spirometry was performed pre and post bronchodilator. A triple measurement was performed at every scheduled time point. The best reading was chosen for the analysis. Spirometry required the patient, after all air had been expelled, to inhale deeply. This was followed by a rapid exhalation so that all the air was exhausted from the lungs. In accordance with the ERS Guidelines, "COPD causes the air in the lungs to be exhaled at a slower rate and in a smaller amount compared to a normal, healthy person (2004)." The amount of air in the lungs will not be readily exhaled due to either a physical obstruction (such as with mucus production) or a narrowing of the airway caused by chronic inflammation.

2.3.2 Experimental Part

The eligible patients entered the clinic on Day -1 in the morning. To ensure that they still met the inclusion criteria, the safety parameters were checked in all of the subjects: ECG, vital signs, spirometry, clinical laboratory test, update of medical history and current medication, LSEQ, VAS and physical examination. Polysomnography was performed on Day-1 for adaption reasons. LSEQ and VAS were performed on day 1 after patients had slept for 8 hours. Capillary blood gas analysis, spirometry and plethysmography were performed in the morning at awakening and 2 hours after awakening. Patients were randomized in one of the sequences shortly before the drug administration in the evening. The study drug was administrated five minutes after the patient started dinner (approximately at 7:00pm). The second polysomnography recording was performed from approximately 11:00pm (4 hours post dose) until 7:00 am (12 hours post dose). On day 2 patients were discharged after blood sampling for pharmacokinetic, capillary blood gas analysis, Bond & Lader VAS, LSEQ, vital signs, body plethysmography and spirometry in the morning after awakening. After a washout period of at least 14 days, the patients entered the clinic for the second period. For safety reasons, a follow-up visit was made between 14 and 17 days after the end of period 2. The end-of-study visit included a physical examination, an ECG examination, a check of vital signs, safety laboratory tests and an update of the medical history and current medication. After examining all results, patients were safely released from the study.

Period two of the study was of the same design as period one. Patients who received a placebo in period one received 15 mg of eplivanserin in period two and those who received 15 mg of eplivanserin in period one, received a placebo in period two.

On Day 17, after the safety data was reviewed, patients were discharged from the clinic. A follow-up visit was scheduled between Day 30 and Day 33.

2.3.3 Follow-up Visit

The following procedures were performed during the follow-up visit: Physical examination including measurement of weigh, vital signs, 12 lead- ECG and clinical laboratory tests. The patients were discharged from the study if all the safety measurement were within the normal range for COPD patients. The total duration of study participation for each patient was from 5 to 8 weeks (from screening visit to end of study visit).

3 Statistics

The sample size calculation for this study was based on estimation of the within-subject standard deviation from the literature (Steens et al.1993). For this 2-fold-crossover design trial the sample size calculation was performed for within-subject standard deviations of 1, 1.5, 2 and 2.5. For a within-subject standard deviation of 1.5% the total number N of subjects required to have 90% power to detect a mean difference between eplivanserin and placebo of 1.5 in oxygen percent saturation (SaO_2) was 24 subjects, i.e. 12 subjects per sequence group using a two group t-test (Crossover ANOVA) with a 0.050 two-sided significance level. The assumed difference of 1.5% is in accordance with the effect observed with flunitrazepam vs zolpidem in the study “acute effect of zolpidem, triazolam and flunitrazepam on arterial blood gases and control of breathing in severe COPD” (Murciano et al.1993 and Midgren et al. 1989). In all 28 patients were enrolled into the study to ensure to have completed at least 24 subjects for the statistical analysis.

Individual data were listed. Data for each parameter were summarized using descriptive statistics (n, mean, sd, sem, minimum, median, maximum). Continuous variables (age, height, weight, BMI) were summarized by means of descriptive statistics. Qualitative variables (gender, race) were summarized in frequency tables.

For the primary analysis an analysis of variance (ANOVA) with fixed terms for treatment, sequence, period and random term for patient within sequence was performed. The model was fitted by estimated generalized least squares (GLS) with restricted maximum likelihood (REML) estimates of random effects, using SAS® version 9.1.3 (SP 4) PROC MIXED. The estimate and 95% confidence interval of the difference in means between active treatment and placebo were calculated within the mixed model framework.

If not otherwise stated exploratory analyses for additional parameters were performed with the same model (ANOVA) as described for the primary analysis.

If a corresponding baseline value (last reading before treatment) was taken into account, this value was implemented as covariate (ANCOVA) in the corresponding model.

The effect of gender and COPD status was also analyzed using the models as already described above, but taking into account these variables as additional fixed effects in the corresponding analysis (ANOVA/ANCOVA).

Safety data, subjective evaluation of the quality of sleep, self rating of mood and vigilance, and residual effects on the following day were summarized descriptively.

4 Results

4.1 Demography and Other Baseline Conditions

Altogether, 28 patients with mild to moderate COPD were randomized in order to receive complete data for 24 patients with at least 30% of each gender and also 30% of each severity. As no patient dropped out, data of all 28 patients enrolled (14 patients with mild/14 patients with moderate COPD) were analyzed. The demographic data obtained for the patients, stratified by COPD status, are included in Table 3.

Table 3: Summary of Demographic Data

	Mild COPD (N=14)	Moderate COPD (N=14)	All (N=28)
Sex [n (%)]			
Male	6 (42.9%)	7 (50.0%)	13 (46.4%)
Female	8 (57.1%)	7 (50.0%)	15 (53.6%)
Race [n (%)]			
Caucasian/white	14 (100%)	14 (100%)	28 (100%)
Age (yrs)			
Mean (SD)	55.1 (8.4)	54.4 (9.3)	54.8 (8.7)
Median	53.0	53.5	53.5
Min, Max	41, 70	39, 72	39, 72
Height (cm)			
Mean (SD)	170.7 (11.5)	168.9 (8.4)	169.8 (9.9)
Median	168.5	170.0	170.0
Min, Max	155, 190	152, 183	152, 190
Weight (kg)			
Mean (SD)	78.29 (14.43)	76.96 (13.81)	77.63 (13.88)
Median	78.25	75.25	76.05
Min, Max	53.4, 105.0	55.1, 111.8	53.4, 111.8
BMI (kg/m^2)			
Mean (SD)	26.74 (3.06)	26.93 (4.25)	26.83 (3.63)
Median	26.95	25.75	26.45
Min, Max	19.4, 32.3	21.5, 35.3	19.4, 35.3

N=number of all subjects, SD=standard deviation, BMI=body mass index
Note: Number corresponds to the count of patients with non-missing data used for the calculation of percentages.

COPD status was evenly distributed between the two sequence groups (p=0.4579 Cochran-

Mantel-Haenzel test (CMH test), but gender differences were observed (eplivanserin 15 mg/placebo: 4 female, 10 male; placebo/eplivanserin 15 mg: 11 female, 3 male; p=0.0092 CMH test). Therefore, analyses stratified by gender have to be taken into account for the interpretation of the study results in connection with COPD status as well and the imbalance has to be taken into account for the interpretation of related results.

The summary of demographic data showed similar results for patients with mild and moderate COPD (Table 3). No statistical tests for demographics were performed to compare patients with mild/moderate COPD for these parameters.

The medical history of none of the patients interfered with the assessment of the study. Previous medications had to be stopped before study started.

For the COPD treatment with salbutamol and inhalative corticosteroid was allowed during the trial if required. Only hormonal replacement therapy was used in two female patients as concomitant medication during the whole trial. Two of the patients took paracetamol during the study for headache; one patient took a combination of acetylsalicylic acid and paracetamol for headache and one patient a combination of acetylsalicylic acid, paracetamol and caffeine. One patient reported having a toothache and was treated with valeriana extract and lidocain injection. One patient was treated with enoxacin for bacteriuria and tamusulosin for prostate adenoma. The severity of the COPD (mild or moderate) was assessed at baseline according to the GOLD criteria (Global Initiative for Chronic Obstructive Lung Disease, 2006) (Table 4).

Table 4: Descriptive Statistics for Spirometry Parameters at Baseline

	Mild COPD (N=14)	Moderate COPD (N=14)	All (N=28)
Forced expiratory volume (Liters)			
Number	14	14	28
Mean (SD)	2.25 (0.76)	1.59 (0.37)	1.92 (0.68)
Min, Max	0.8, 3.4	1.1, 2.4	0.8, 3.4
Forced vital capacity (L)			
Number	14	14	28
Mean (SD)	3.91 (1.34)	3.16 (0.76)	3.53 (1.13)
Min, Max	2.1, 7.2	2.0, 4.8	2.0, 7.2
Peak expiratory flow (L/s)			
Number	14	14	28
Mean (SD)	4.78 (1.66)	3.76 (1.01)	4.27 (1.45)
Min, Max	1.6, 8.3	2.2, 5.8	1.6, 8.3

COPD=Chronic obstructive pulmonary disease, SD=Standard deviation

4.2 Analysis of Efficacy

4.2.1 Mean Overnight Oxygen Saturation (SaO_2)

The oxygen percent saturation (SaO_2) measured during the sleep for the entire night was the primary endpoint of the study. Treatment difference estimates for the primary objective (ANOVA of SaO_2 during time in bed) are summarized below (Table 5).

Table 5: Treatment Difference Estimates for SaO_2 (%) During Time in Bed (ANOVA)

SaO_2 (%) during	Comparison	LSM	p-value	95% CI [Lower; Upper]
Time in bed	Eplivanserin 15 mg – Placebo	-0.30	0.2499	[-0.83;0.23]

LMS=Least square mean, CI= Confidence interval

For the test of fixed effects in the ANOVA, no significant p-values of fixed effects were obtained

for the default model (treatment: p=0.2499; sequence: p=0.9277; period: p=0.7438) or for the model including gender and COPD-status as fixed effects (treatment: p=0.2540; sequence: p=0.7564; period: p=0.7362; gender: p=0.6626; COPD: p=0.8455).

The observed means (SD) of SaO_2 during time in bed raw data were 93.77% (1.29) and 93.45% (1.66) for placebo and eplivanserin 15 mg, respectively (Table 6).

A single dose of eplivanserin 15 mg did not alter SaO_2 during time in bed in the study population.

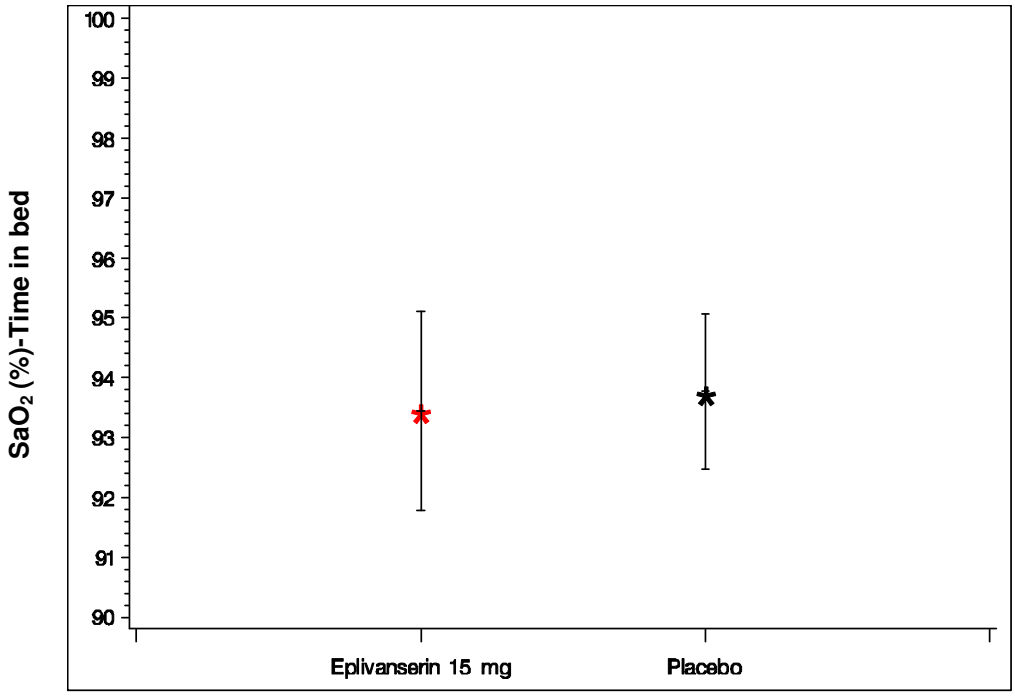
Table 6: Descriptive statistics for SaO_2 (%) During Time in Bed

Treatment	N	Mean	SD	Min	Median	Max
Eplivanserin 15 mg	28	93.45	1.66	89.34	93.53	97.12
Placebo	27	93.77	1.29	91.44	93.43	96.02

SD=standard deviation, SEM=standard error of the mean, min=minimum; max=maximum

A mean plot (+/-SD) for SaO_2 during time in bed is shown in the Figure 4 below:

Figure 4: $Sa0_2$ (%, Mean +/- SD) Measured During Time in Bed



Treatment difference estimates for SaO2% during different sleep stages are summarized below (Table 7).

Table 7: Treatment Difference Estimates for SaO_2 (%) During Sleep Stages (ANOVA)

SaO_2 (%) during sleep stage	**Comparison**	**LSM**	**95% CI [Lower; Upper]**
NREM 1	Eplivanserin 15 mg – Placebo	-0.26	[-0.80; 0.28]
NREM 2	Eplivanserin 15 mg – Placebo	-0.31	[-0.84; 0.23]
SWS	Eplivanserin 15 mg – Placebo	-0.38	[-0.99; 0.23]
REM	Eplivanserin 15 mg – Placebo	-0.11	[-0.72; 0.50]
WAKE	Eplivanserin 15 mg – Placebo	-0.07	[-0.62; 0.49]

NREM=non-rapid eye movement; SWS=slow wave sleep; REM=rapid eye movement; LMS=least square mean, CI=confidence interval

SaO_2 (%) showed no significant differences between eplivanserin 15 mg and placebo during the different sleep stages. The test of fixed effects showed no significant results for the default model or the model including gender and COPD status (Tables 8 and 9).

Table 8: Test of Fixed Effects for the ANOVA of SaO_2 (%) During Different Sleep Stages

p-values (ANOVA) of Fixed Effects	Sleep stage				
	NREM1	NREM2	SWS	REM	WAKE
Treatment	0.3309	0.2509	0.2106	0.7077	0.8022
Sequence	0.6936	0.9210	0.5524	0.9861	0.4762
Period	0.7688	0.5546	0.2651	0.8320	0.6777

NREM=non-rapid eye movement; SWS=slow wave sleep; REM= rapid eye movement

Table 9: Test of Fixed Effects Including Gender and COPD Status for the ANOVA of SaO_2 (%) During Different Sleep Stages

p-values (ANOVA) of Fixed Effects	Sleep stage				
	NREM1	NREM2	SWS	REM	WAKE
Treatment	0.3343	0.2552	0.2175	0.7148	0.8059
Sequence	0.5682	0.8618	0.5074	0.6563	0.4016
Period	0.7641	0.5478	0.2618	0.8243	0.6744
Gender	0.6069	0.9248	0.6574	0.4036	0.5706
COPD	0.9858	0.6897	0.4301	0.9221	0.8311

NREM= non- rapid eye movement; SWS=slow wave sleep; REM= rapid eye movement

Descriptive statistics of SaO_2 (%) during different sleep stages are shown in Table 10.

Table 10: Descriptive Statistics of SaO_2 (%) During Different Sleep Stages

SaO2 (%) during sleep stage	Treatment	N	Mean	SD	Min	Median	Max
NREM1	Eplivanserin 15 mg	28	93.78	1.69	89.20	93.71	97.35
	Placebo	27	94.05	1.22	92.14	93.75	96.41
NREM2	Eplivanserin 15 mg	28	93.29	1.59	89.83	93.24	97.24
	Placebo	27	93.61	1.30	90.36	93.39	95.98
SWS	Eplivanserin 15 mg	26	93.10	1.64	89.63	93.03	96.92
	Placebo	26	93.54	1.43	90.06	93.26	96.09
REM	Eplivanserin 15 mg	28	93.13	2.30	85.99	93.37	97.03
	Placebo	27	93.27	2.00	87.43	93.10	96.24
WAKE	Eplivanserin 15 mg	28	94.37	1.56	90.13	94.52	97.64
	Placebo	27	94.45	1.20	92.11	94.31	96.46

NREM=non-rapid eye movement; SWS=slow wave sleep; REM= rapid eye movement

Mean plots (+/- SD) for SaO_2% during the different sleep stages are shown below (Figures 5-9).

Figure 5: SaO_2 (%, Mean +/- SD) During Stage 1 NREM sleep

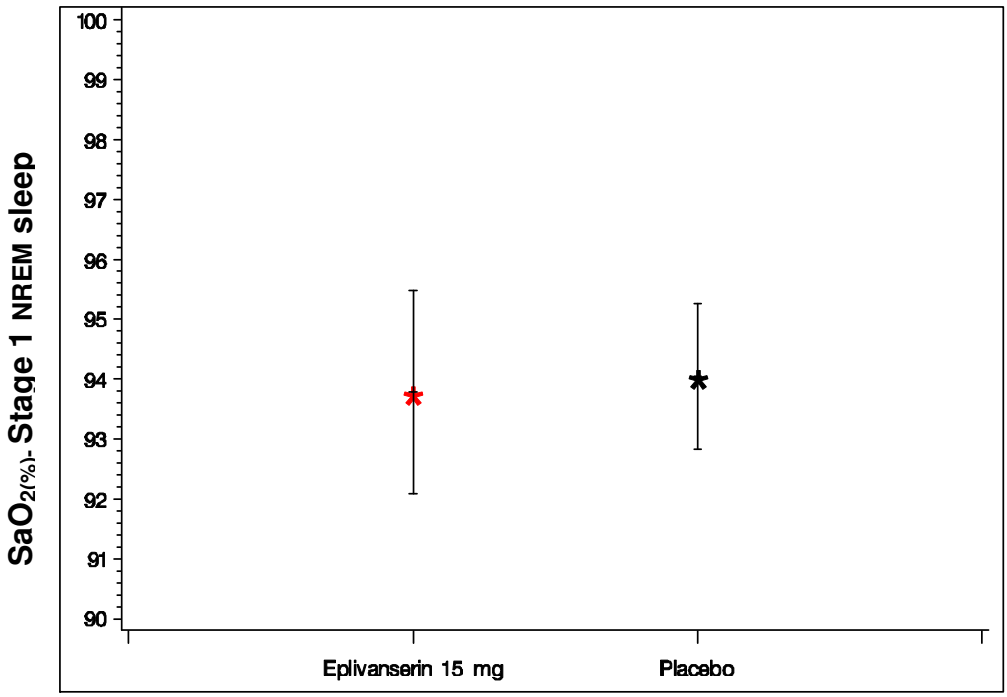


Figure 6: SaO_2 (%, Mean +/- SD) During Stage 2 NREM sleep

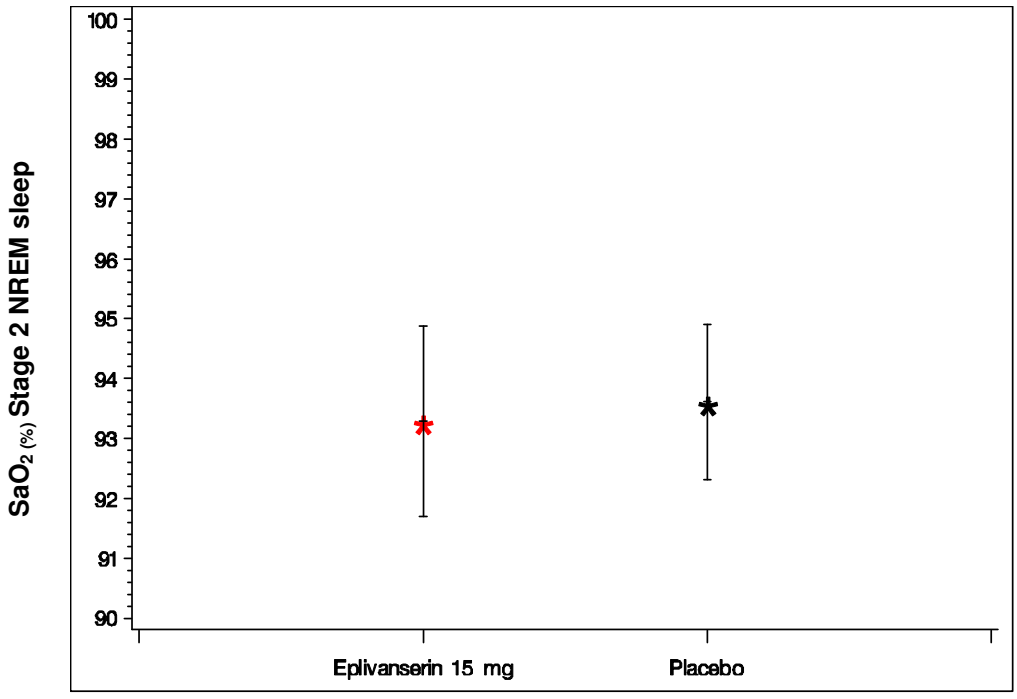


Figure 7: SaO_2 (%, Mean +/- SD) During Stage SWS

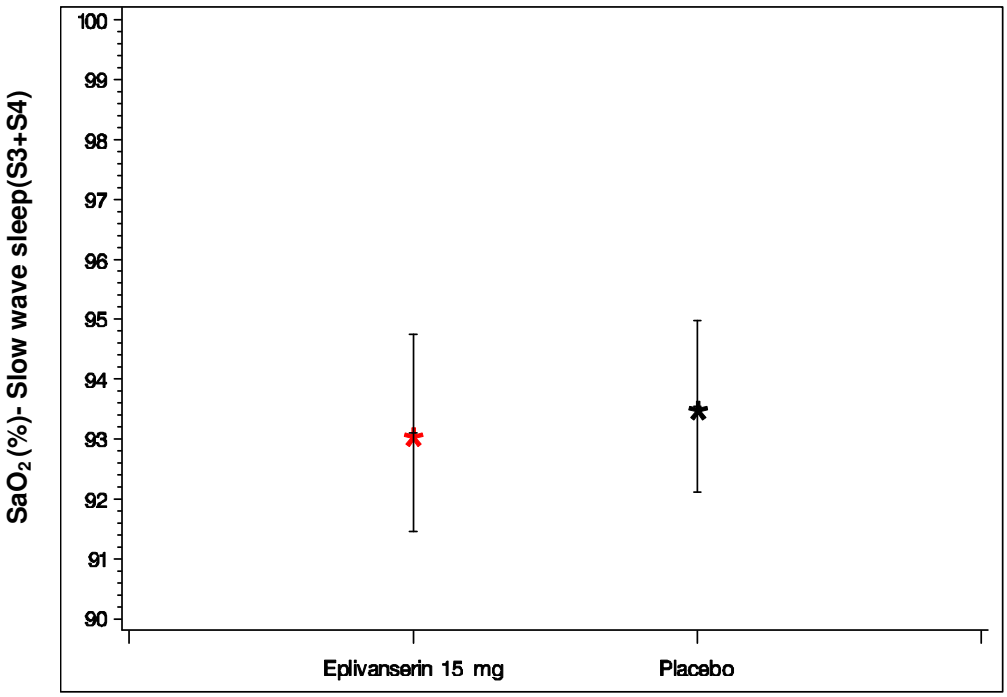


Figure 8: SaO_2 (%, Mean +/- SD) During Stage REM sleep

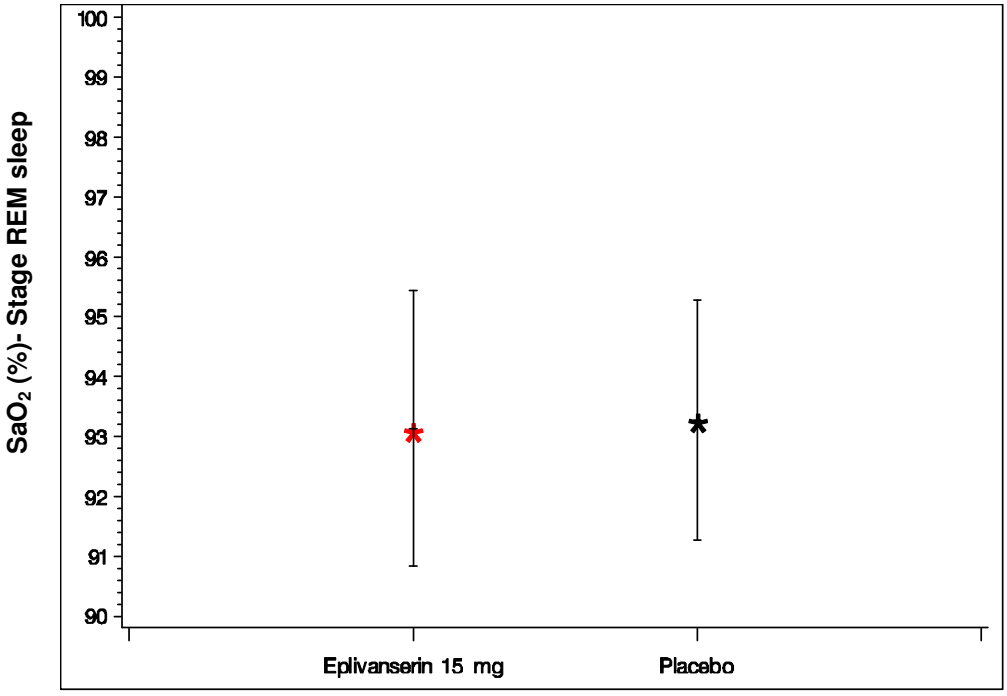
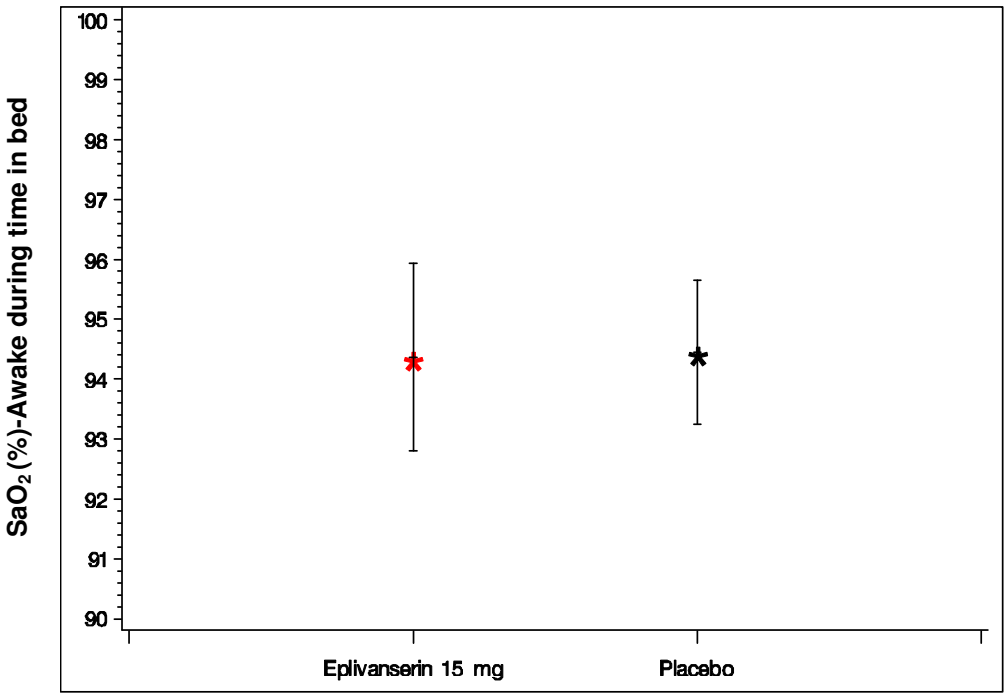


Figure 9: SaO_2 (%, Mean +/- SD) Awake During Time in Bed



Overall, oxygen saturation SaO_2(%) showed no significant differences between active drug (eplivanserin 15 mg) and placebo for the overall comparison or for any analyses stratified by sleep stage.

4.2.2 Spirometry

The observed data showed that a single dose of eplivanserin 15 mg had no significant effect on the spirometry parameters in the study population. Treatment difference estimates for spirometry are summarized below (Table 11):

Table 11: Treatment Difference Estimates (ANOVA) for Spirometry Parameters

	Comparison	LSM	95% CI [Lower; Upper]
FEV1 (L)	Eplivanserin 15 mg – Placebo	-0.03	[-0.14;0.07]
FVC (L)	Eplivanserin 15 mg – Placebo	-0.12	[-0.28;0.04]
PEF (L/s)	Eplivanserin 15 mg – Placebo	-0.05	[-0.26;0.17]

FEV1=Forced expiratory volume, FVC=Forced vital capacity, PEF= Peak expiratory flow,
LSM=Least square mean, CI=Confidence interval

None of the spirometry parameters measured during treatment (14 h values) showed any significant differences between eplivanserin 15 mg and placebo. All tests (ANOVA) of fixed effects showed significant results for sequence as fixed effect with the default model (Table 12).

Table 12: Test of Fixed Effects (ANOVA) of Spirometry Parameters

p-values (ANOVA) of Fixed Effects	FEV1	FVC	PEF
Treatment	0.5289	0.1411	0.6683
Sequence	**0.0106**	**0.0479**	**0.0233**
Period	0.7269	0.4268	0.4032

FEV1=Forced expiratory volume, FVC=Forced vital capacity, PEF=Peak expiratory flow

As expected, the test of fixed effects for the model including gender and COPD status showed significant results based only on COPD status and gender. The FEV1, FVC, and PEF values for patients with moderate versus mild COPD were lower, as were those of women in comparison to men, given the generally smaller lung capacity of women. The treatment difference was also non significant for all spirometry parameters (Table 13).

Table 13: Test of Fixed Effects Including Gender and COPD Status (ANOVA) of Spirometry Parameters

p-values (ANOVA) of Fixed Effects	FEV1	FVC	PEF
Treatment	0.5289	0.1411	0.6683
Sequence	0.4349	0.9183	0.9891
Period	0.7269	0.4268	0.4032
Gender	**0.0006**	**0.0004**	**<0.0001**
COPD	**0.0004**	**0.0185**	**0.0086**

FEV1=Forced expiratory volume, FVC=Forced vital capacity, PEF=Peak expiratory flow

As anticipated, the fixed effects reflected the differences based on COPD status and gender at baseline. That is, patients with moderate COPD had lower FEV1, FVC and PEF values than those with mild COPD both before and after treatment, and the same was true of women in comparison to men both before and after treatment. Moreover, the differences versus baseline for moderate versus mild COPD patients and for men versus women were statistically insignificant. Thus, treatment with eplivanserin had no effect on FEV1, FVC, or PEF values across gender or on the basis of mild versus moderate COPD.

Descriptive statistics of spirometry parameters is given in Table 14.

Table 14: Descriptive Statistics of Spirometry Parameters

Spirometry Parameters	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
FEV1 (L)	Eplivanserin 15 mg	-10:00	28	1.90	0.69	0.78	1.69	3.38
		14:00	28	1.92	0.71	0.67	1.74	3.36
	Placebo	-10:00	28	1.91	0.66	1.08	1.71	3.24
		14:00	28	1.95	0.76	0.96	1.78	3.76
FVC (L)	Eplivanserin 15 mg	-10:00	28	3.51	1.13	1.72	3.34	7.20
		14:00	28	3.47	1.12	1.64	3.35	6.93
	Placebo	-10:00	28	3.58	1.07	2.00	3.32	6.50
		14:00	28	3.58	1.19	2.17	3.28	7.26
PEF (L/s)	Eplivanserin 15 mg	-10:00	28	4.23	1.49	1.46	4.03	8.34
		14:00	28	4.32	1.43	1.60	4.16	7.75
	Placebo	-10:00	28	4.34	1.46	2.21	4.03	8.84
		14:00	28	4.37	1.48	2.14	3.85	7.79

FEV1=Forced expiratory volume, FVC=Forced vital capacity, PEF=Peak expiratory flow

Spirometry parameters are shown in Figures 10-12.

Figure 10: Spirometry - Forced Expiratory Volume: FEV1 (L, Mean +/- SD)

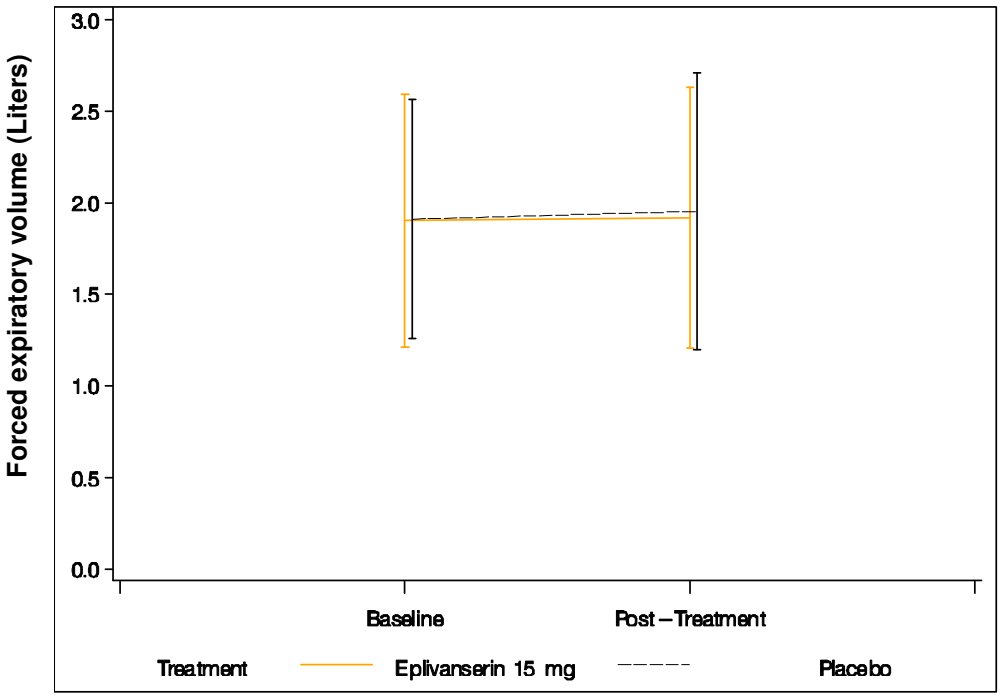


Figure 11: Spirometry - Forced Vital Capacity: FVC (L, Mean +/- SD)

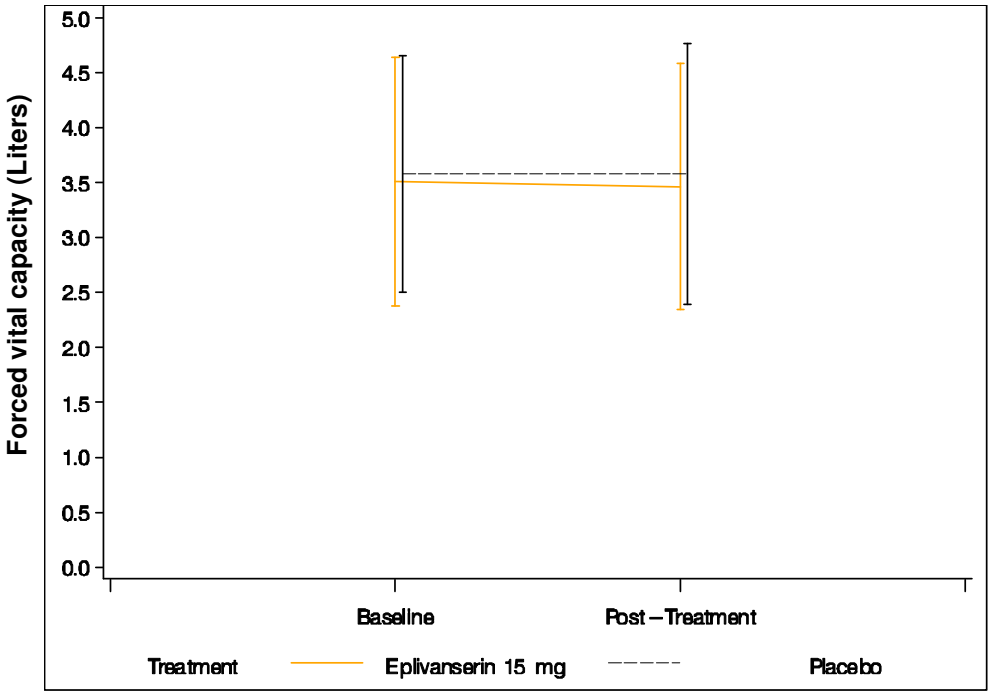
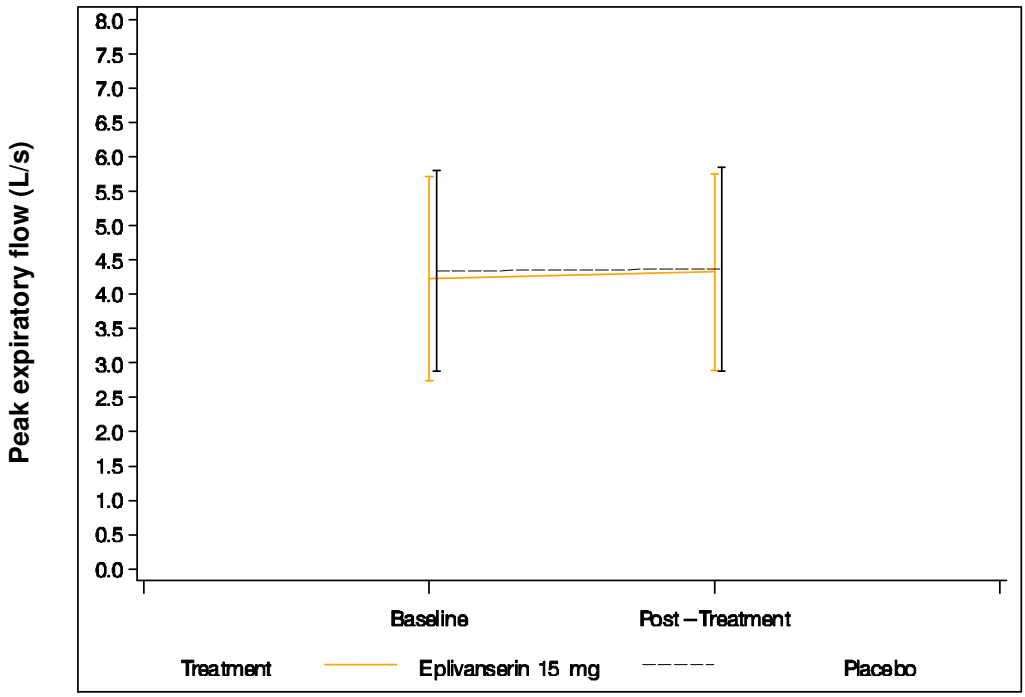


Figure 12: Spirometry - Peak Expiratory Flow: PEF (L/s, Mean +/- SD)



4.2.3 Body Plethysmography

No significant effect on plethysmography parameters were observed in the study population after a single dose of eplivanserin 15 mg. Treatment difference estimates for body plethysmography parameters are summarized below (Table 15):

Table 15: Treatment Difference Estimates (ANOVA) of Body Plethysmography Parameters

	Comparison	LSM	p-value	95% CI
Airways resistance (KPa * s/l)	Eplivanserin 15 mg – Placebo	0.02	0.4880	[-0.04;0.08]
Airways conductance (1/KPa * s)	Eplivanserin 15 mg – Placebo	-0.01	0.6642	[-0.05;0.03]

LSM=Least Square mean; CI=Confidence interval

Descriptive statistics of plethysmography parameters are presented in Table 16.

Table 16: Descriptive Statistics of Plethysmography Parameters

Spirometry Parameters	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
Airways resistance (KPa * s/l)	Eplivanserin 15 mg	-10:00	28	0.49	0.22	0.21	0.46	1.02
		14:00	28	0.50	0.24	0.19	0.47	1.17
	Placebo	-10:00	28	0.51	0.22	0.21	0.50	1.02
		14:00	28	0.48	0.20	0.20	0.49	0.99
Airways conductance (1/KPa * s)	Eplivanserin 15 mg	-10:00	28	0.49	0.24	0.19	0.39	1.02
		14:00	28	0.49	0.27	0.16	0.41	1.32
	Placebo	-10:00	28	0.46	0.22	0.18	0.40	0.93
		14:00	28	0.50	0.26	0.18	0.41	1.17

Plethysmography parameters are shown in Figures 13+14.

Figure 13: Plethysmography - Airways Resistance (RAW, Mean +/-SD)

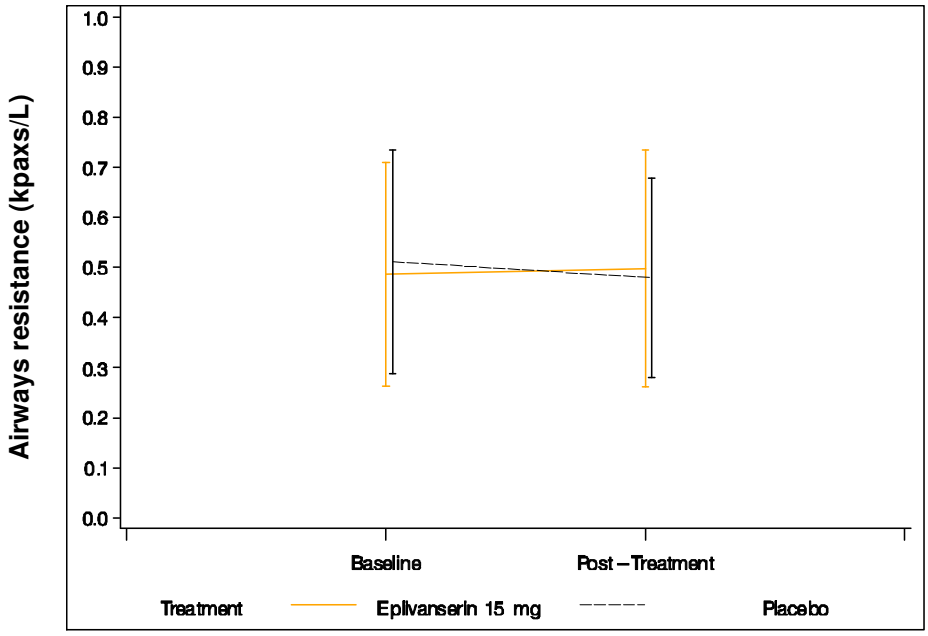
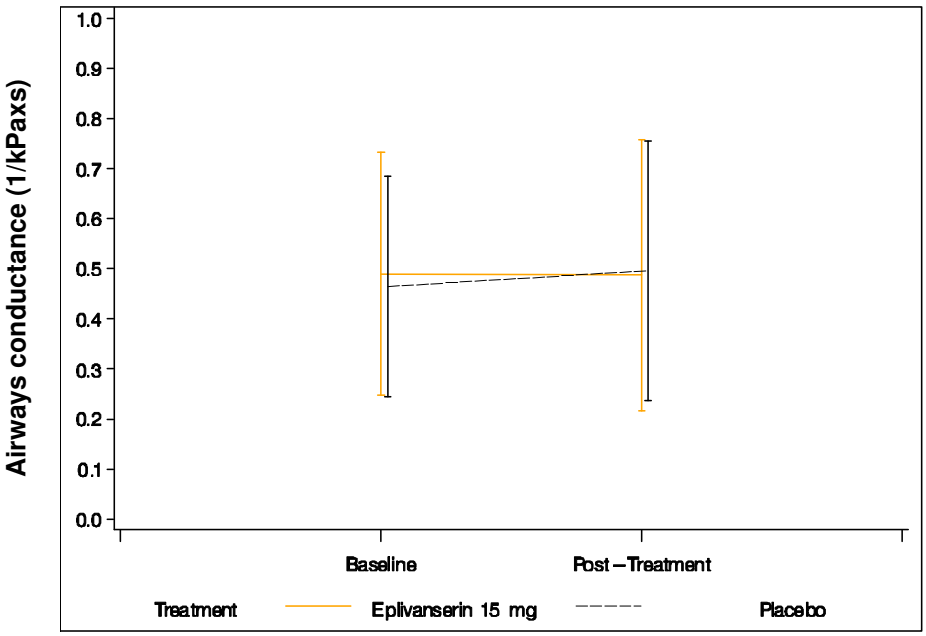


Figure 14: Plethysmography - Airways Conductance (SGAW, Mean +/-SD)



4.2.4 Polysomnography

Three polysomnography parameters (wake after persistent sleep onset, total sleep time, number of awakenings) showed significant differences between eplivanserin 15 mg and placebo (Table 17).

Table 17: Treatment Difference Estimates (ANOVA) for Polysomnography Parameters

Polysomnography Parameter	Comparison	LSM	95% CI [Lower; Upper]
Apnea Hypopnea Index (AHI)	Eplivanserin 15 mg – Placebo	-0.63	[-1.70; 0.44]
Wake after Persistent Sleep Onset (min)	Eplivanserin 15 mg – Placebo	**-19.4**	**[-35.6; -3.2]**
Total Sleep Time (min)	Eplivanserin 15 mg – Placebo	**16.4**	**[0.29; 32.4]**
Number of Awakenings	Eplivanserin 15 mg – Placebo	**-2.6**	**[-4.0; -1.2]**
Latency to Persistent Sleep (min)	Eplivanserin 15 mg – Placebo	1.5	[-4.0; 7.0]

The test of fixed effects showed significant results for these three parameters (wake after persistent sleep onset, total sleep time, number of awakenings) for both the default model and the model including gender and COPD status (Tables 18 and 19).

Table 18: Test of Fixed Effects (ANOVA) of Polysomnography Parameters

	Polysomnography Parameters				
p-values (ANOVA) of Fixed Effects	Apnea Hypopnea Index (AHI)	Wake after Persistent Sleep Onset (min)	Total Sleep Time (min)	Number of Awakenings	Latency to Persistent Sleep (min)
Treatment	0.2338	**0.0212**	**0.0462**	**0.0010**	0.5755
Sequence	0.4165	0.2197	0.2880	0.2381	0.9332
Period	0.9161	0.4362	0.2881	0.8953	0.4656

Table 19: Test of Fixed Effects Including Gender and COPD Status (ANOVA) of Polysomnography Parameters

	Polysomnography Parameters				
p-values (ANOVA) of Fixed Effects	Apnea Hypopnea Index (AHI)	Wake after Persistent Sleep Onset (min)	Total Sleep Time (min)	Number of Awakenings	Latency to Persistent Sleep (min)
Treatment	0.2341	**0.0228**	**0.0476**	**0.0010**	0.5593
Sequence	0.3550	0.7735	0.9902	0.5784	0.5225
Period	0.9165	0.4532	0.2933	0.9075	0.4852
Gender	0.6200	0.2084	0.0986	0.5789	0.1306
COPD	0.9619	0.0987	0.1987	0.2146	0.2945

Descriptive statistics of polysomnography parameters are displayed in Table 20.

Table 20: Descriptive Statistics of Polysomnography

Polysomnography Parameter	Treatment	N	Mean	SD	Min	Median	Max
Apnea Hypopnea Index (AHI)	Eplivanserin 15 mg	28	2.50	2.36	0.00	2.00	10.70
	Placebo	27	3.19	3.91	0.20	1.80	14.10
Wake after Persistent Sleep Onset (min)	Eplivanserin 15 mg	28	43.64	29.77	8.50	29.75	116.00
	Placebo	27	63.35	39.93	14.00	55.00	177.00
Total Sleep Time (min)	Eplivanserin 15 mg	28	424.95	29.97	367.50	429.00	468.50
	Placebo	27	408.85	42.67	294.50	422.00	455.50
Number of Awakenings	Eplivanserin 15 mg	28	7.50	3.14	3.00	7.00	15.00
	Placebo	27	10.11	3.70	4.00	10.00	21.00
Latency to Persistent Sleep (min)	Eplivanserin 15 mg	28	18.21	16.82	0.50	12.25	80.00
	Placebo	27	16.41	8.74	0.50	14.50	40.00

A single dose of eplivanserin 15 mg compared to placebo had the following effects on polysomnographic parameters:

- A non significant reduction of the apnea hypopnea index (AHI, mean [SD]) from 3.19[3.91] to 2.50[2.36] episodes of apnea hypopnea/hour with eplivanserin 15 mg compared with placebo, respectively.
- The mean [SD] of wake duration after persistent sleep onset (mean wake duration after persistent sleep onset, min) shortened significantly from 63.4 [39.9] to 43.6 [29.8] minutes with eplivanserin 15 mg compared with placebo, respectively).
- Total sleep time (TST, mean [SD]) was significantly prolonged from 408.9 [42.7] to 425.0 [30.0] minutes with eplivanserin 15 mg compared with placebo, respectively).

- Number of awakenings after sleep onset (mean [SD] number of awakenings after sleep onset reduced significantly from 10.1 [3.7] to 7.5 [3.1] with eplivanserin 15 mg compared with placebo, respectively).
- No significant change in latency to persistent sleep (mean LPS was 16.4 [8.7] and 18.2 [16.8] minutes with eplivanserin 15 mg compared with placebo, respectively).

Polysomnograph parameters are displayed in Figures 15-19 below.

Figure 15: Polysomnography - Apnea Hypopnea Index (AHI, Mean +/-SD)

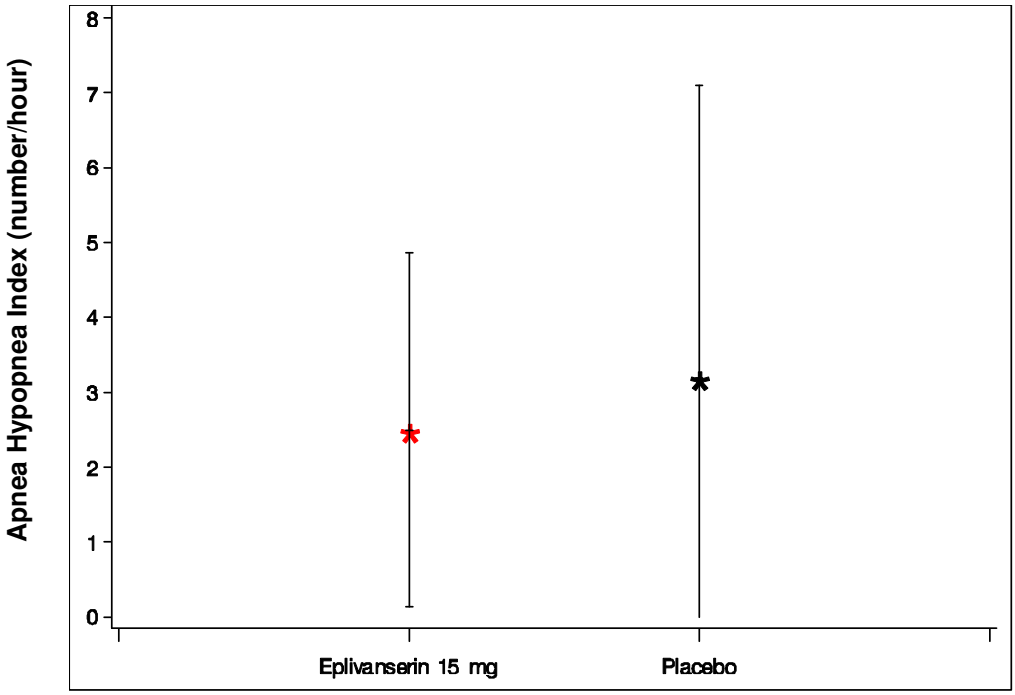


Figure 16: Polysomnography - Wake after Persistent Sleep Onset (min, Mean +/-SD)

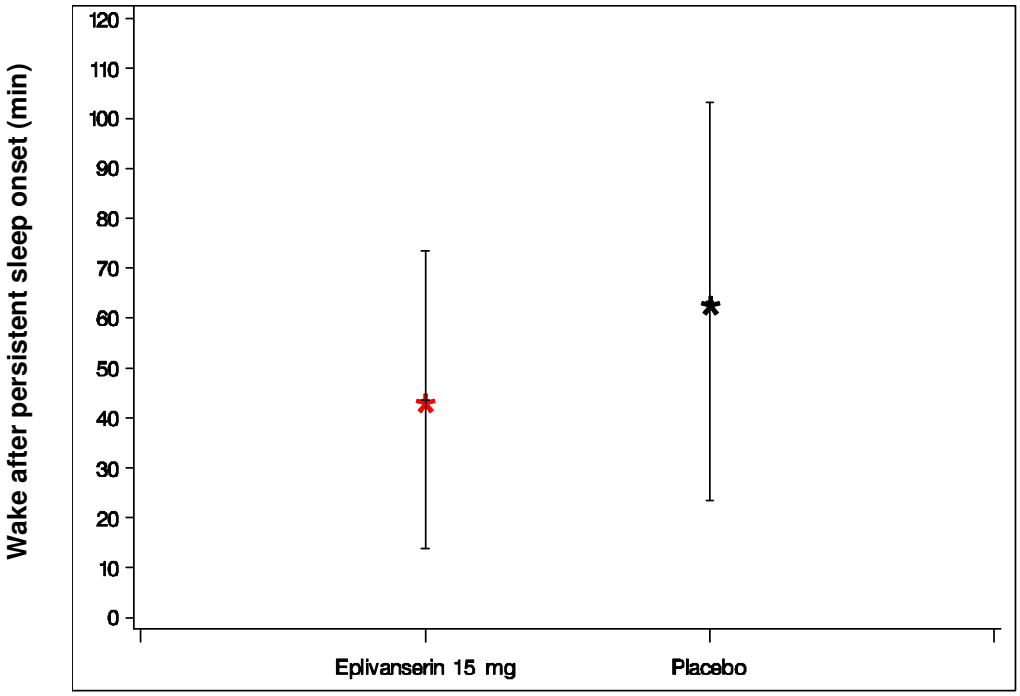
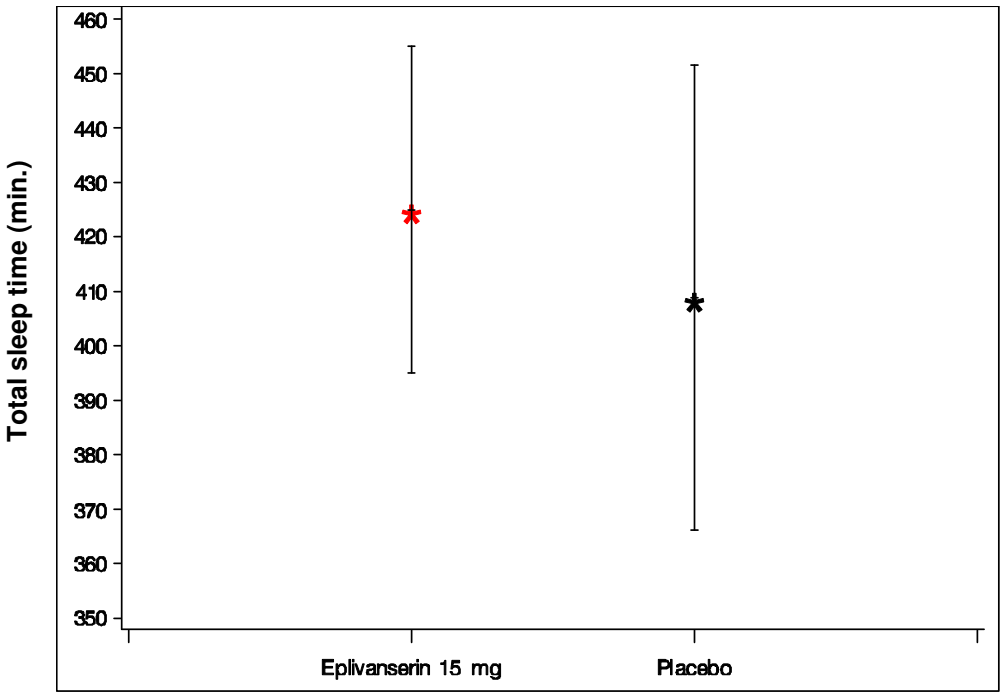


Figure 17: Polysomnography - Total Sleep Time (min, Mean +/-SD)



Placebo Eplivanserin 15 mg

Figure 18: Polysomnography - Number of Awakenings after Sleep Onset

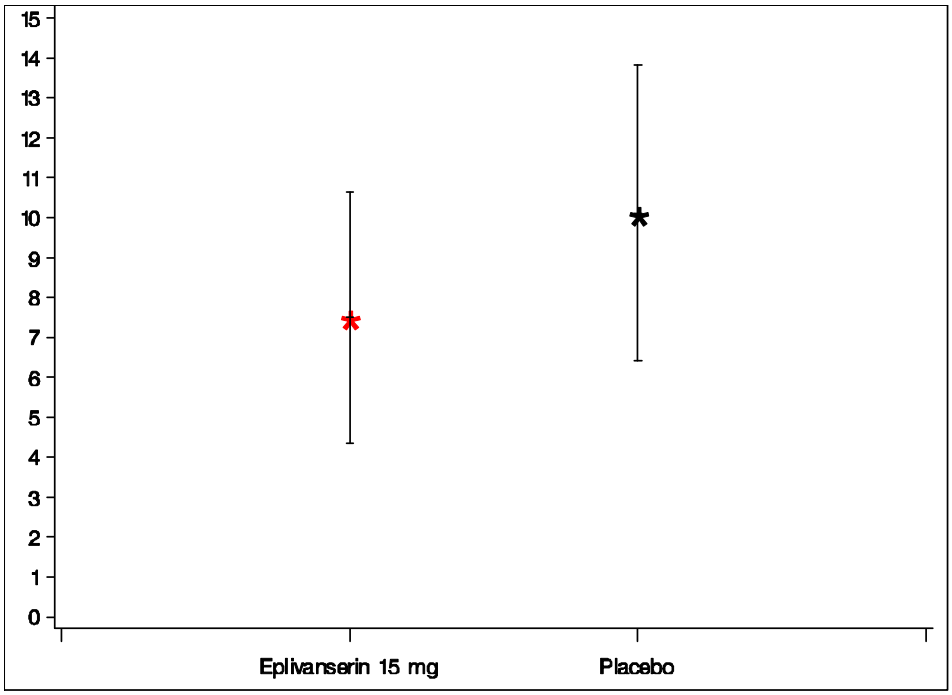
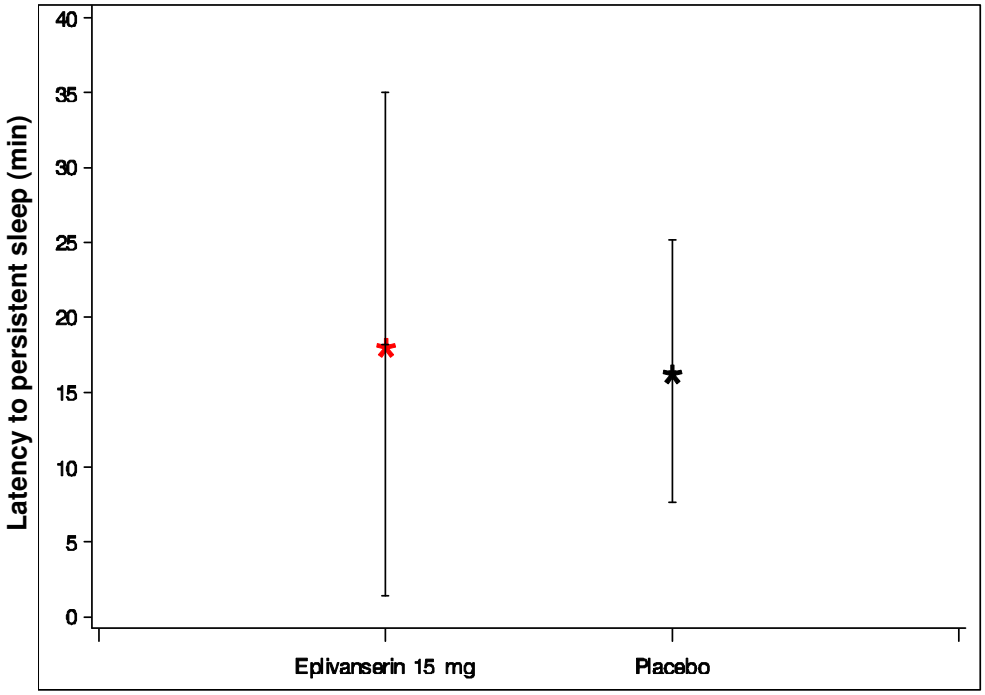


Figure 19: Polysomnography - Latency to Persistent Sleep (min, Mean +/-SD)



4.2.5 Capillary Blood Gas Parameters

Blood gas parameters were measured two times before and at 12.5h and 14h post treatment. A covariance analysis (ANCOVA) of the 12.5h post treatment blood gas parameter values was performed using the fixed effects of the standard model and the corresponding last pre-treatment value as covariate.

Table 21: Treatment Difference Estimates (ANCOVA) for Capillary Blood Gas Parameters

Capillary Blood Gas Parameter	Comparison	LSM	95% CI [Lower; Upper]
Blood Bicarbonates (MMOL/L)	Eplivanserin 15 mg – Placebo	-0.488	[-1.137; 0.162]
Carbon Dioxide Partial Pressure (mmHg)	Eplivanserin 15 mg – Placebo	-0.49	[-3.36; 2.38]
Blood pH	Eplivanserin 15 mg – Placebo	**-0.04**	**[-0.07; -0.01]**
Partial Pressure of Oxygen (mmHg)	Eplivanserin 15 mg – Placebo	-1.92	[-5.53; 1.68]

A single dose of eplivanserin 15 mg compared to placebo had no effect on blood bicarbonate, the partial pressure of oxygen and carbon dioxide. Only a slightly reduced pH (p=0.0132) without clinical meaningfulness was observed (Table 21 and 22).

Table 22: Test of Fixed Effects (ANCOVA) of Capillary Blood Gas Parameters

p-values (ANCOVA) of Fixed Effects	Capillary Blood Gas Parameters			
	Blood Bicarbonates (MMOL/L)	Carbon Dioxide Partial Pressure (mmHg)	Blood pH	Partial Pressure of Oxygen (mmHg)
Baseline value	**0.0158**	**0.0065**	0.2936	**0.0225**
Treatment	0.1330	0.7249	**0.0132**	0.2813
Sequence	0.1943	0.3197	0.1548	0.5521
Period	0.6796	0.4637	0.5957	0.6667

The other parameters showed only a significant baseline effect showing that these mainly influenced the differences between the subjects (see Table 23).

Table 23: Descriptive Statistics of Capillary Blood Gas Parameters

Polysomno-grapy parameter	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
Blood Bicarbonates (MMOL/L)	Eplivanserin 15 mg	-11:30	27	24.00	1.54	20.00	24.00	27.00
		-10:00	28	24.11	1.73	21.00	24.00	29.00
		12:30	28	24.36	1.52	20.00	25.00	26.00
		14:00	28	24.07	1.36	21.00	24.50	26.00
	Placebo	-11:30	28	24.57	1.29	23.00	25.00	28.00
		-10:00	27	24.26	1.40	22.00	24.00	27.00
		12:30	28	24.89	1.64	23.00	24.50	28.00
		14:00	28	24.39	1.50	21.00	24.50	27.00
Carbon Dioxide Partial Pressure (mmHg)	Eplivanserin 15 mg	-11:30	27	35.86	3.20	29.50	35.50	43.70
		-10:00	28	36.27	2.52	31.20	37.05	41.40
		12:30	28	36.89	3.35	32.70	36.20	48.00
		14:00	28	36.45	2.44	33.50	36.20	43.90
	Placebo	-11:30	28	36.56	2.75	30.70	36.60	41.90
		-10:00	27	35.98	2.28	30.90	36.30	40.00
		12:30	28	37.12	6.37	31.10	36.05	67.00
		14:00	28	35.94	3.64	22.50	35.65	42.10
Blood pH	Eplivanserin 15 mg	-11:30	27	7.43	0.05	7.40	7.40	7.50
		-10:00	28	7.43	0.05	7.40	7.40	7.50
		12:30	28	7.41	0.04	7.30	7.40	7.50

Polysomno-grapy parameter	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
		14:00	28	7.42	0.04	7.40	7.40	7.50
	Placebo	-11:30	28	7.43	0.04	7.40	7.40	7.50
		-10:00	27	7.42	0.04	7.40	7.40	7.50
		12:30	28	7.45	0.05	7.40	7.45	7.50
		14:00	28	7.43	0.07	7.40	7.40	7.70
Partial Pressure of Oxygen (mmHg)	Eplivanserin 15 mg	-11:30	27	73.22	7.82	61.00	72.00	91.00
		-10:00	28	74.82	11.62	61.00	71.50	115.00
		12:30	28	69.32	7.66	52.00	69.00	90.00
		14:00	28	72.79	7.36	60.00	74.00	90.00
	Placebo	-11:30	28	73.50	8.70	60.00	71.50	97.00
		-10:00	27	77.74	10.10	63.00	76.00	102.00
		12:30	28	72.79	8.57	60.00	72.00	100.00
		14:00	28	78.14	16.21	61.00	72.50	143.00

The summarized data are shown in the Figures 20-23 below.

Figure 20: Capillary Blood Gas Parameters - Blood Bicarbonates (MMOL/L, Mean +/-SD)

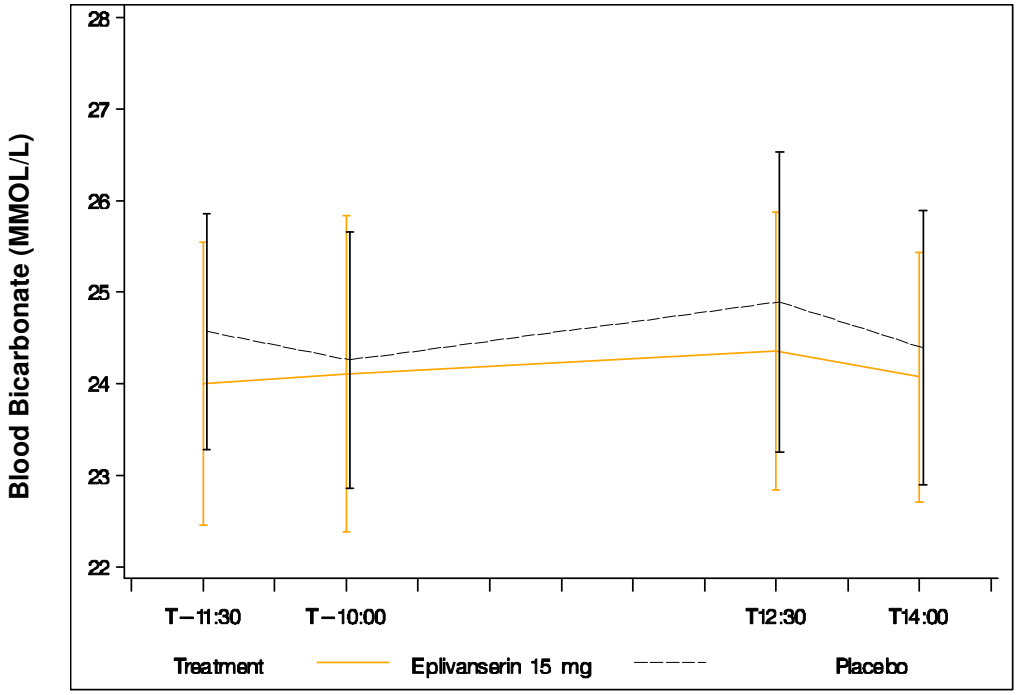


Figure 21: Capillary Blood Gas Parameters - Carbon Dioxide Partial Pressure (mmHg, Mean +/-SD)

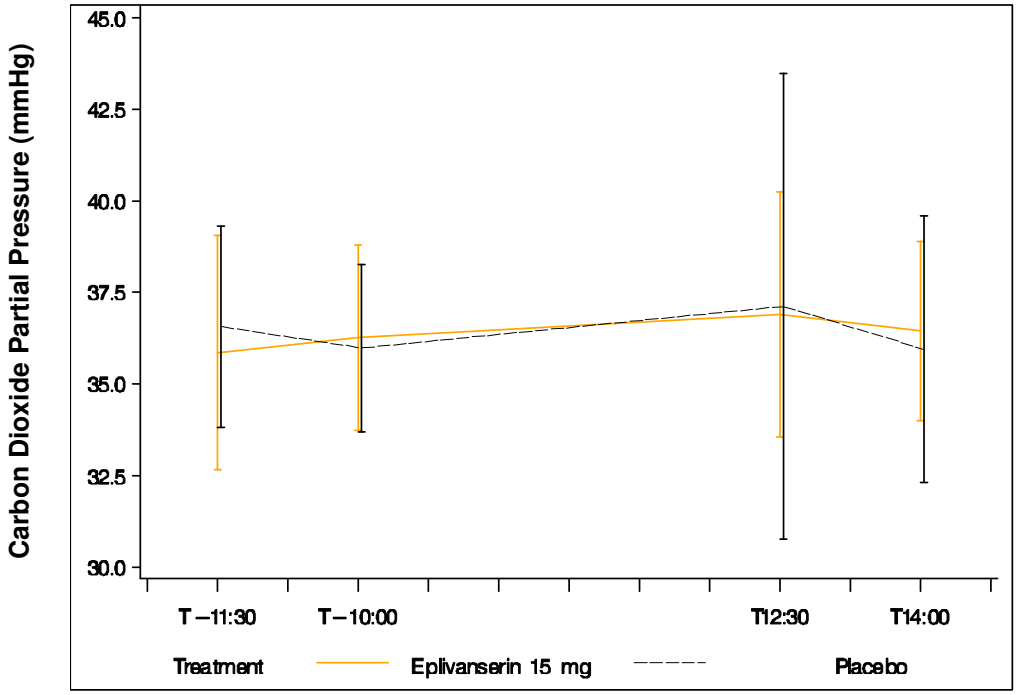


Figure 22: Capillary Blood Gas Parameters - Blood pH (Mean +/-SD)

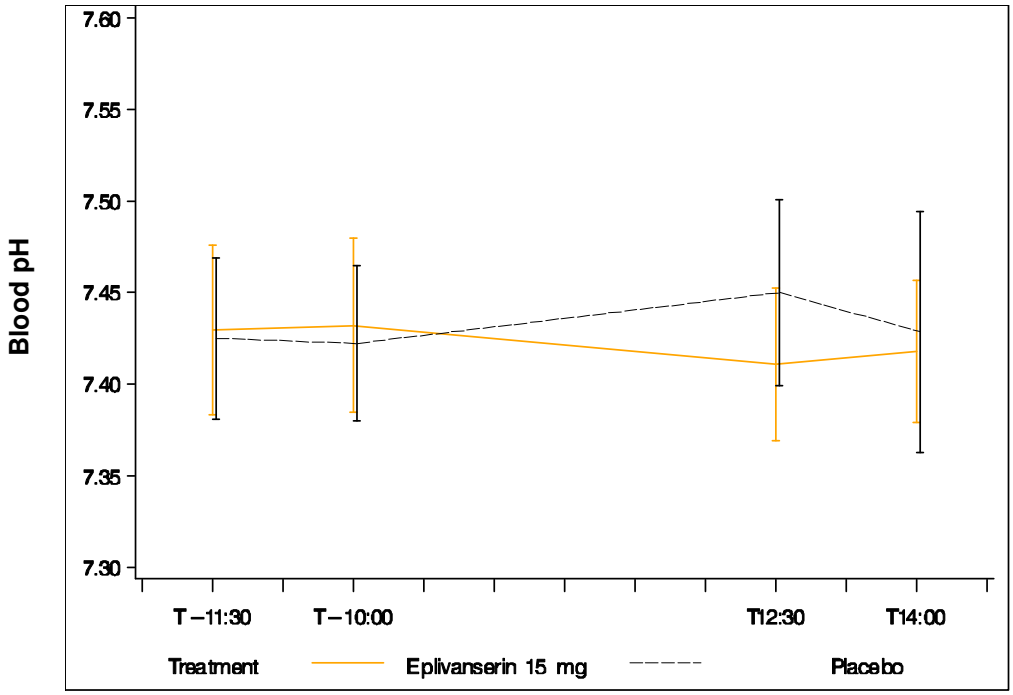
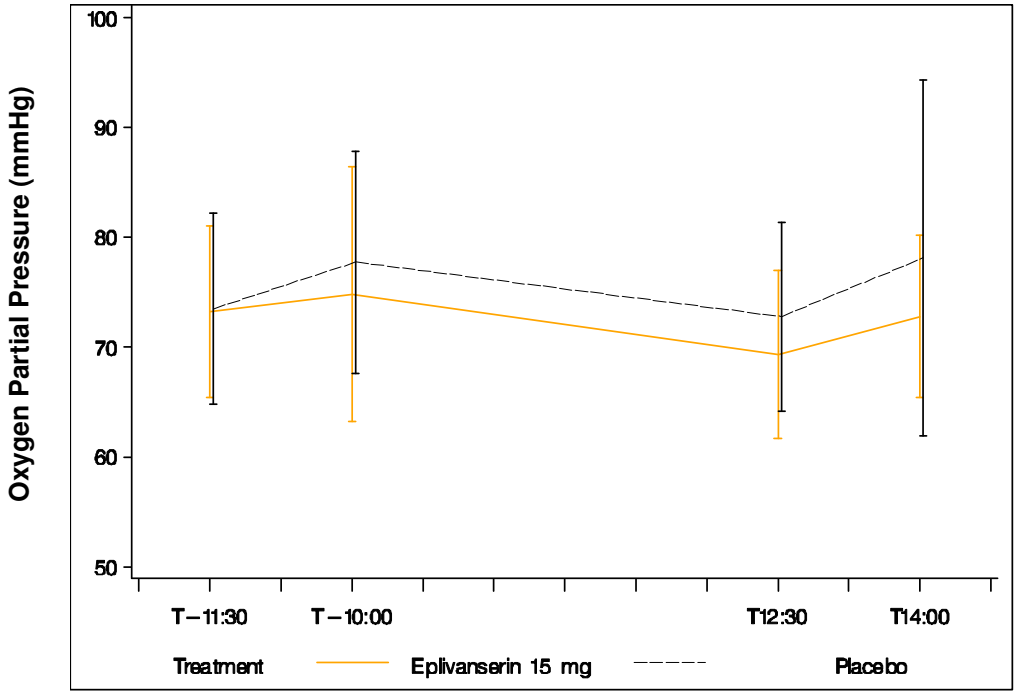


Figure 23: Capillary Blood Gas Parameters - Partial Pressure of Oxygen (mmHg, Mean +/-SD)



4.2.6 Leeds Sleep Evaluation Questionnaire (LSEQ)

A single dose of eplivanserin 15 mg compared to placebo had the following effects (post-treatment compared to baseline) on LSEQ scores:

- No effect on ease of getting to sleep (mean change from baseline is -2.1 [22.1] and -4.9 [28.7] for placebo and eplivanserin 15 mg, respectively).
- Slight decrease in quality of sleep (mean change from baseline was -0.9 [19.7] and -10.5 [27.2] for placebo and eplivanserin 15 mg, respectively) which was not statistically significant.
- No change on ease of awakening from sleep (mean change from baseline is 1.6 [17.8] and -3.1 [7.8] for placebo and eplivanserin 15 mg, respectively).
- Slight but statistically not significant decrease on behavior (alertness) following wakefulness (mean change from baseline is 0.1 [18.0] and -8.5 [23.6] for placebo and eplivanserin 15 mg, respectively).

Leeds Sleep Evaluation Questionnaire (LSEQ) parameters were measured before and at 12.5h post treatment. A covariance analysis (ANCOVA) of the 12.5h post treatment parameter values was performed using the fixed effects of the standard model including gender and COPD status and the corresponding last pre-treatment value as covariate.

Table 24: Treatment Difference Estimates (ANCOVA) for LSEQ Parameters

LSEQ Parameter	Comparison	LSM	95% CI [Lower; Upper]
Ease of Getting to Sleep Score	Eplivanserin 15 mg – Placebo	2.48	[-7.21; 12.17]
Behavior following Wakefulness	Eplivanserin 15 mg – Placebo	-6.47	[-16.12; 3.18]
Ease of Awakening from Sleep Score	Eplivanserin 15 mg – Placebo	-2.35	[-9.21; 4.50]
Quality of Sleep Score	Eplivanserin 15 mg – Placebo	-7.19	[-20.61; 6.22]

Table 25: Test of Fixed Effects (ANCOVA) of LSEQ Parameters

p-values (ANCOVA) of Fixed Effects	LSEQ Parameter			
	Ease of Getting to Sleep Score	Behavior Following Wakefulness	Ease of Awakening from Sleep Score	Quality of Sleep Score
Baseline value	0.6216	**<0.0001**	**0.0084**	**0.0004**
Treatment	0.6013	0.1800	0.4855	0.2803
Sequence	0.5523	0.1444	0.0733	0.1194
Period	0.4869	0.6528	0.2375	0.5033
Gender	0.8616	0.2741	0.1661	0.7054
COPD	0.1713	0.9406	0.3637	0.2600

The covariance analysis showed no significant treatment effect of eplivanserin 15 mg compared to placebo but the results showed a significant effect of the baseline value for three parameters: behavior following wakefulness, ease of awakening from sleep and quality of sleep, showing that for these LSEQ parameters the status which was already present before study treatment was the main effect for the differences between the patients. The ease of getting to sleep score was not influenced at all. Descriptive statistics of LSEQ score parameters is given in Table 24. Considering the wide dispersion of results for each parameter, the clinical meaningfulness of the change is doubtful.

Table 26: Descriptive Statistics of LSEQ Score Parameters

LSEQ Score Parameter	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
Ease of Getting to Sleep Score	Eplivanserin 15 mg	-11:30	28	151.93	17.20	123.00	150.00	213.00
		12:30	28	147.07	26.46	85.00	150.00	224.00
	Placebo	-11:30	28	146.21	16.92	116.00	150.00	193.00
		12:30	28	144.11	20.01	94.00	148.50	186.00
Behavior following Wakefulness	Eplivanserin 15 mg	-11:30	28	164.86	26.85	109.00	160.00	244.00
		12:30	28	156.39	32.66	78.00	150.00	228.00
	Placebo	-11:30	28	156.61	27.84	88.00	150.00	224.00
		12:30	28	156.75	29.94	103.00	150.00	228.00
Ease of Awakening from Sleep Score	Eplivanserin 15 mg	-11:30	28	101.64	9.03	82.00	100.00	124.00
		12:30	28	98.57	8.07	73.00	100.00	112.00
	Placebo	-11:30	28	97.75	13.10	58.00	100.00	127.00
		12:30	28	99.32	17.20	54.00	100.00	161.00
Quality of Sleep Score	Eplivanserin 15 mg	-11:30	28	99.11	10.92	75.00	100.00	126.00
		12:30	28	88.64	24.67	19.00	100.00	123.00
	Placebo	-11:30	28	93.43	23.49	37.00	100.00	144.00
		12:30	28	92.50	21.67	45.00	100.00	156.00

The effects of a single dose of Eplivanserin 15 mg compared to placebo are shown in Figures 24-27 below.

Figure 24: Leeds Sleep Evaluation Questionnaire - Ease of Getting to Sleep Score (Mean +/- SD)

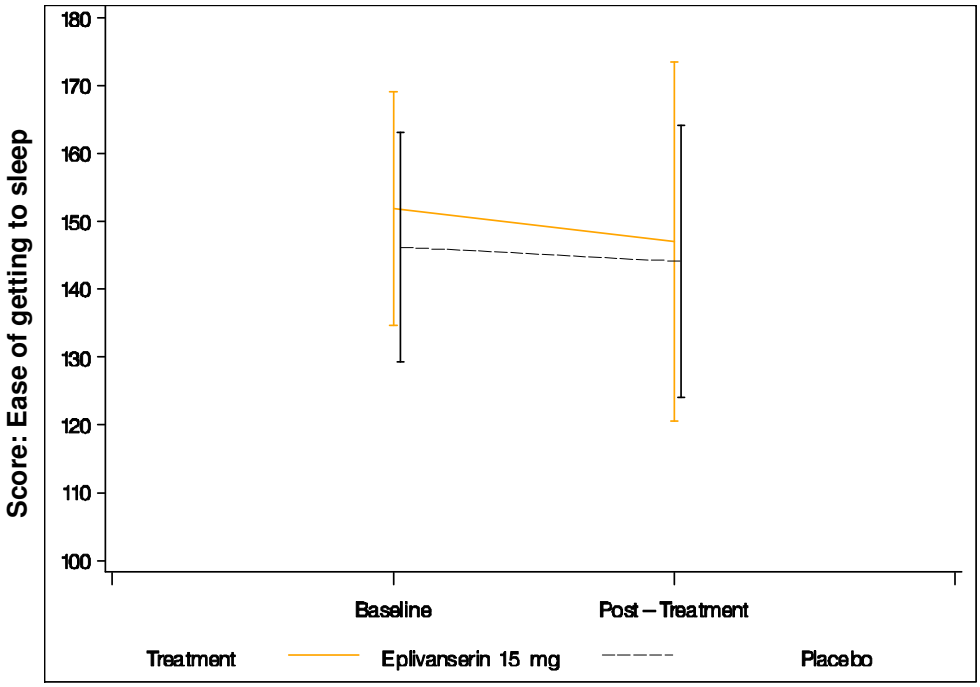


Figure 25: Leeds Sleep Evaluation Questionnaire - Quality of Sleep Score (Mean +/- SD)

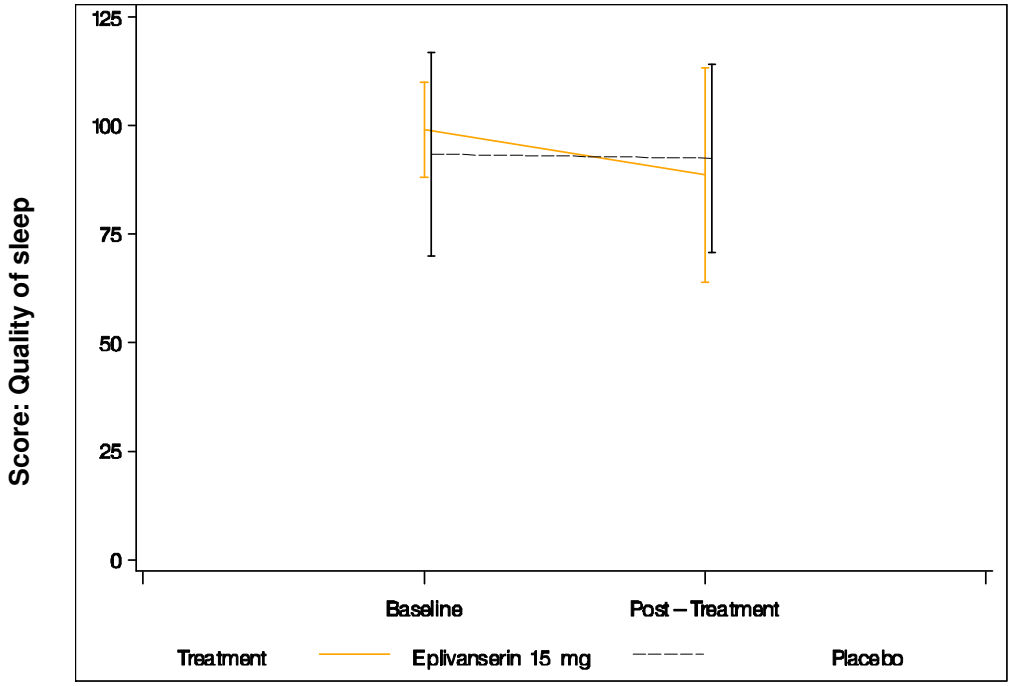


Figure 26: Leeds Sleep Evaluation Questionnaire - Awakening from Sleep Score (Mean +/- SD)

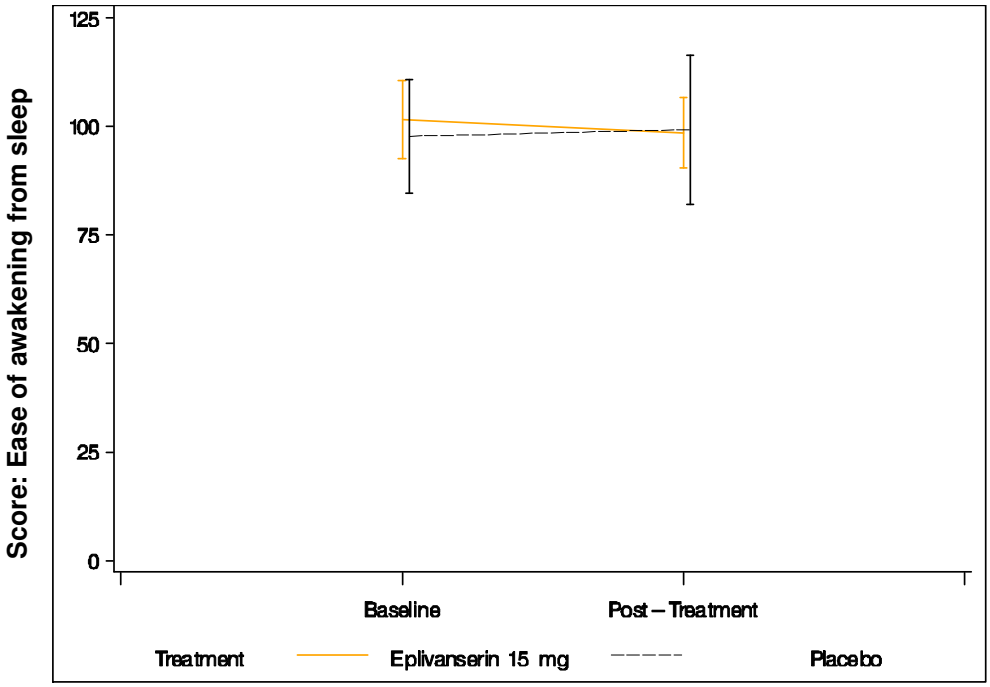
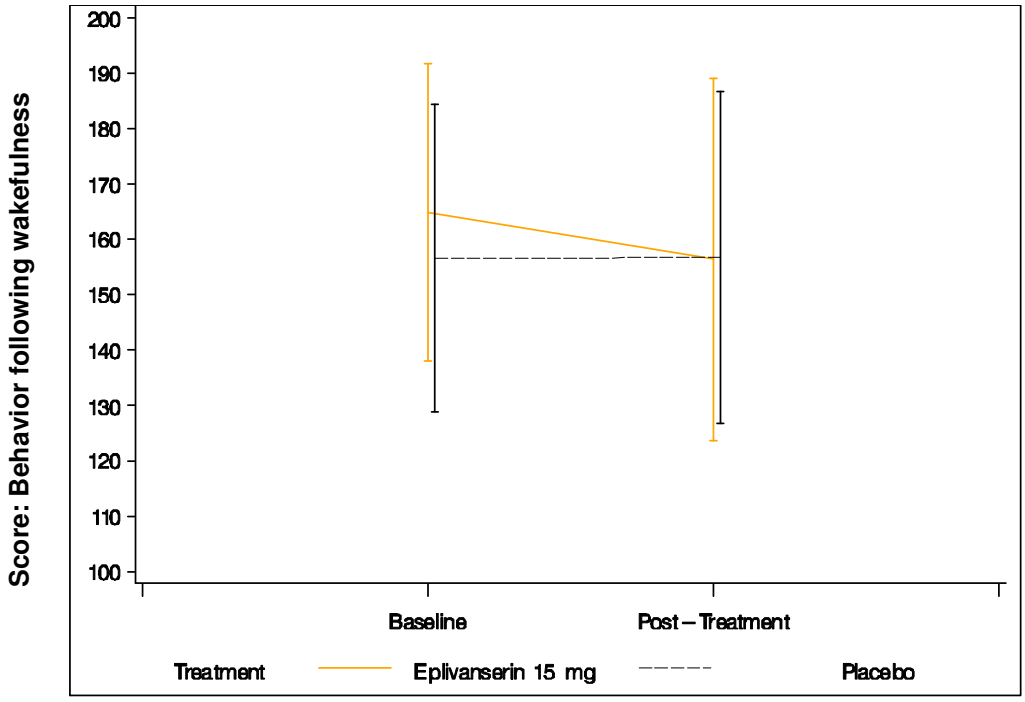


Figure 27: Leeds Sleep Evaluation Questionnaire - Behavior Following Wakefulness Score (Mean +/- SD)



4.2.7 Bond and Lader Visual Analog Scale

Bond and Lader Visual Analog Scale (VAS) parameters were measured two times before and at 12.5h and 14h post treatment. A covariance analysis (ANCOVA) of the 12.5h post treatment parameter values was performed using the fixed effects of the standard model including gender and COPD status and the corresponding last pre-treatment value as covariate.

Table 27: Treatment Difference Estimates (ANCOVA) for Bond and Lader VAS Parameters

Bond and Lader VAS Parameter	**Comparison**	**LSM**	**95% CI [Lower; Upper]**
Alertness Score	Eplivanserin 15 mg – Placebo	-0.73	[-4.10; 2.63]
Contentedness Score	Eplivanserin 15 mg – Placebo	0.30	[-2.65; 3.25]
Calmness Score	Eplivanserin 15 mg – Placebo	1.80	[-1.54; 5.15]

Table 28: Test of Fixed Effects (ANCOVA) of Bond and Lader VAS parameters

p-values (ANCOVA) of Fixed Effects	Bond and Lader VAS parameter		
	Alertness Score	Contentedness Score	Calmness Score
Baseline value	**<0.0001**	**<0.0001**	**<0.0001**
Treatment	0.6578	0.8364	0.2772
Sequence	0.0831	0.5670	0.2825
Period	0.7333	0.6256	0.0501
Gender	0.0595	0.6947	0.1600
COPD	0.4764	0.8005	0.6530

The covariance analysis showed no significant treatment effect of eplivanserin 15 mg compared to placebo. Descriptive statistics of VAS score parameters are given in Table 29. Also the Bond and Lader VAS results (alertness, contentedness, and calmness) showed only a significant effect of the baseline value, showing that also for these parameters the status which was already present before study treatment was the main effect for the differences between the subjects.

Table 29: Descriptive Statistics of Bond and Lader VAS parameters

Polysomnography parameter	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
Alertness Score	Eplivanserin 15 mg	-11:30	28	57.55	10.73	40.60	55.25	86.30
		-10:00	28	64.15	11.44	45.70	63.90	88.30
		12:30	28	57.05	12.03	36.90	56.65	85.10
		14:00	28	62.11	12.53	49.70	58.55	96.20
	Placebo	-11:30	28	55.86	8.46	38.80	53.55	75.80
		-10:00	28	63.40	13.37	49.00	59.25	94.10
		12:30	28	57.34	10.04	44.40	54.20	80.70
		14:00	28	61.21	12.15	50.00	55.25	93.60
Contentedness Score	Eplivanserin 15 mg	-11:30	28	59.16	9.61	44.80	56.90	82.60
		-10:00	28	64.26	12.21	46.80	62.90	93.80
		12:30	28	58.83	12.50	27.80	55.70	84.80
		14:00	28	62.13	14.52	40.20	56.70	96.40
	Placebo	-11:30	28	56.86	9.03	48.40	53.80	83.80
		-10:00	28	62.54	13.71	47.00	57.50	94.80
		12:30	28	57.65	9.56	49.20	54.40	82.20
		14:00	28	59.56	13.22	36.20	53.70	93.00
Calmness Score	Eplivanserin 15 mg	-11:30	28	57.16	10.16	37.50	55.75	86.00
		-10:00	28	61.27	14.00	36.50	58.75	89.50
		12:30	28	57.39	12.56	20.00	56.00	83.50
		14:00	28	60.98	15.26	35.00	56.00	96.00
	Placebo	-11:30	28	56.48	9.69	39.00	54.00	78.00
		-10:00	28	59.61	11.28	43.50	56.25	91.00
		12:30	28	54.61	9.81	35.50	50.50	79.00

Polysomnography parameter	Treatment	Time [hh:mm]	N	Mean	SD	Min	Median	Max
		14:00	28	58.73	13.02	39.50	54.00	94.00

A single dose of eplivanserin 15 mg compared to placebo had no significant effect on alertness, contentedness or calmness VAS score. The VAS measurements are displayed in Figures 28-30.

Figure 28: Bond and Lader Visual Analog Scale - Alertness Score (Mean +/- SD)

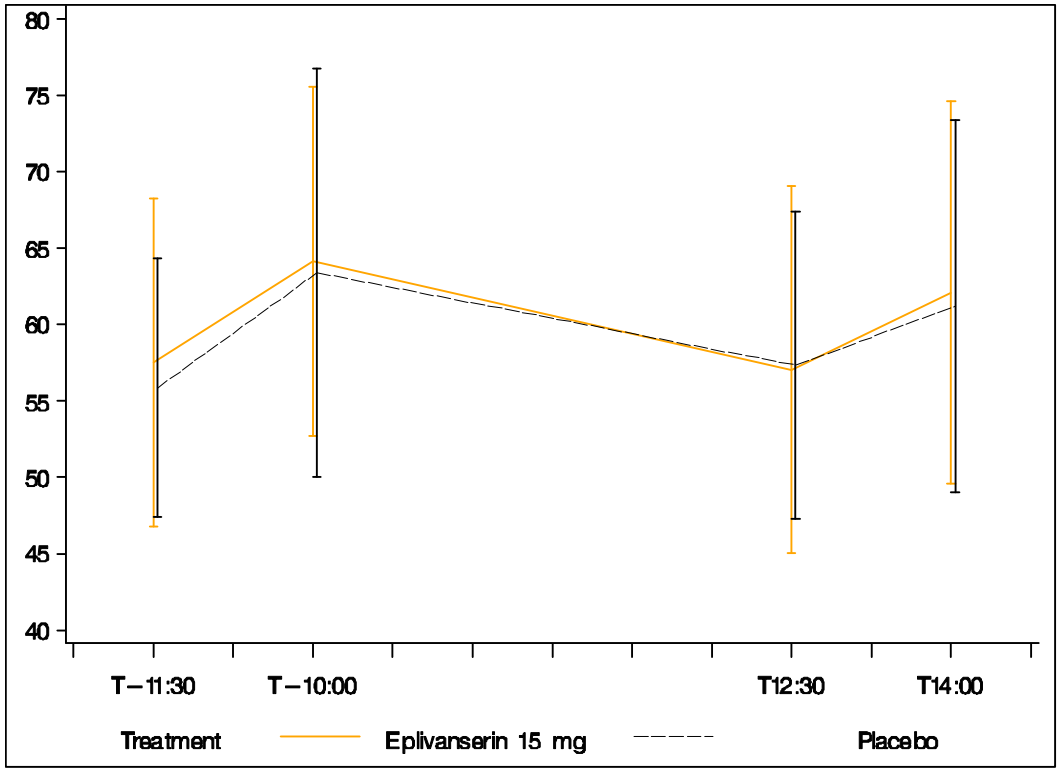


Figure 29: Bond and Lader Visual Analog Scale - Contentedness Score

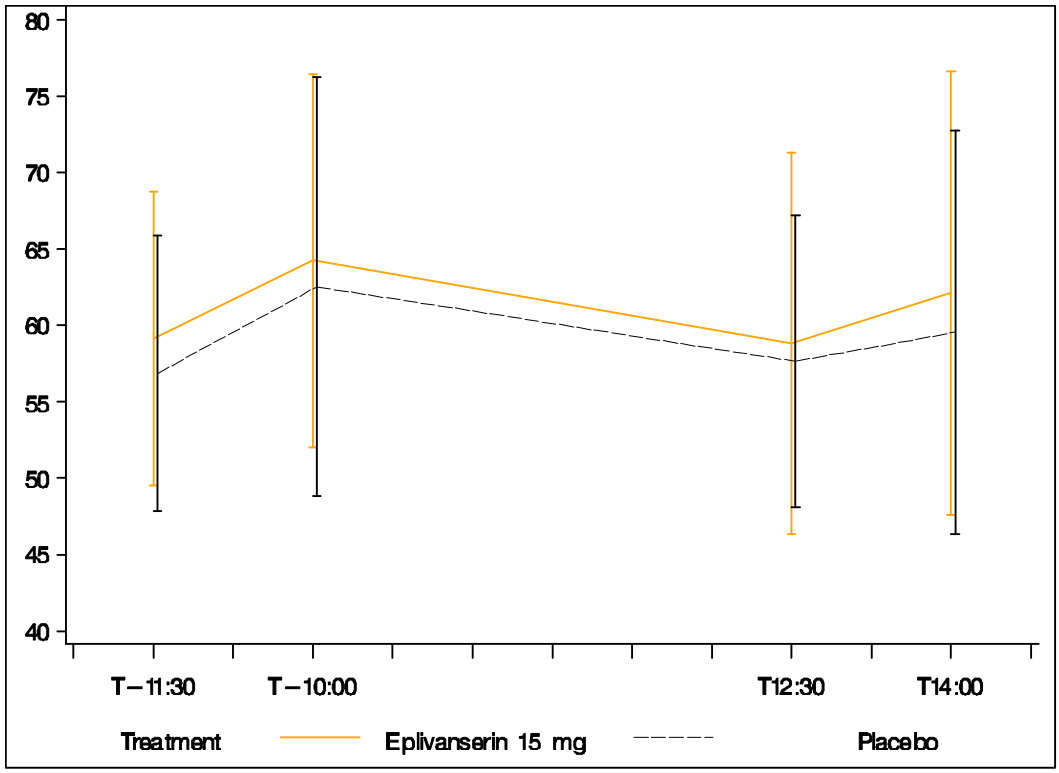
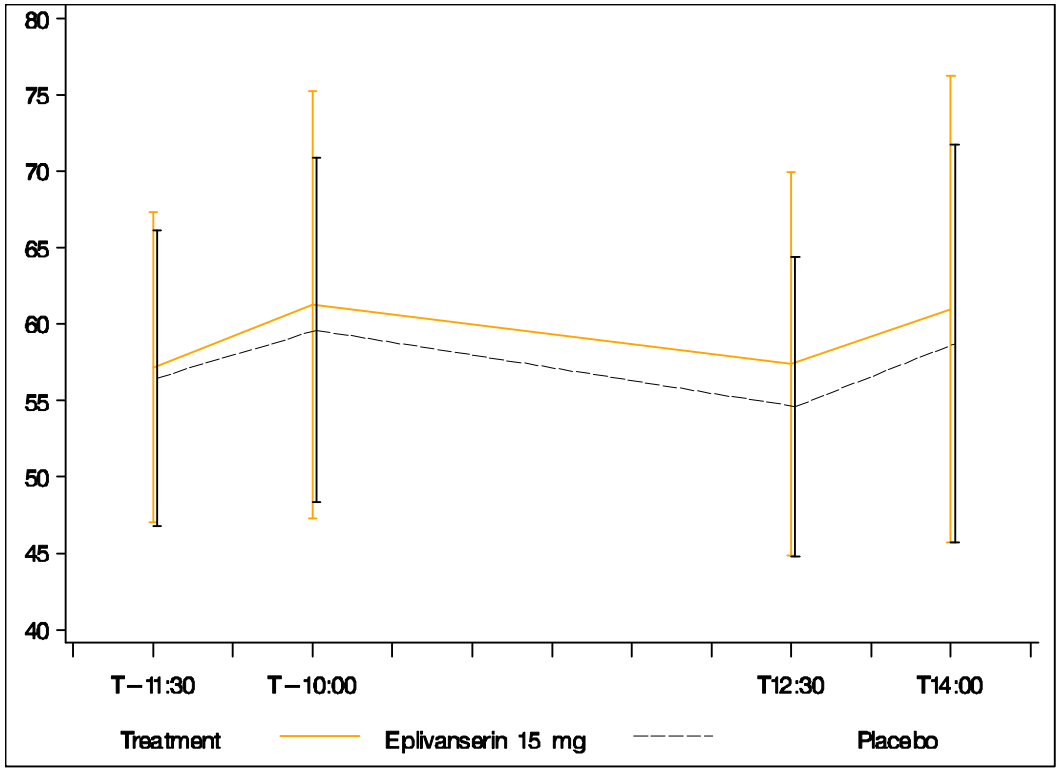


Figure 30: Bond and Lader Visual Analog Scale - Calmness Score



4.3 Analysis of Safety Parameters

The safety evaluation was based upon the review of the individual values and descriptive statistics (summary tables).

For all safety data, the observation period was divided into the following treatment periods:

- The pre-treatment period was defined as the time from the patients' informed consent until the administration on Period 1 Day 1.
- The on-treatment period was defined for each period as the time from the administration up to either 14 days (included) after dosing or the administration in the next period if done before the 14 days.
- The post-treatment period, defined for each period as the time starting 14 days after dosing up to either the administration of the next period or the end of the study.

4.3.1 Adverse Events

All the adverse events recorded during the study were coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 10.1) which assigns each term to a preferred term (PT) then to a primary system organ class (SOC). They were classified into predefined standard categories according to chronological criteria for each period:

4.3.1.1 Treatment Emergent Adverse Events (TEAE):

These are adverse events that occurred or worsened during the treatment periods, irrespective of their relationship to drug. TEAE were assigned to the treatment received (placebo or eplivanserin 15 mg) at the time of the adverse event onset.

4.3.1.2 Non-Treatment Emergent Adverse Events (NTEAE):

Non-treatment adverse events (NTEAE) are adverse events that occurred or worsened during the pretreatment period or the post-treatment periods.

All adverse events reported in the study were listed, sorted by patient, onset date and time.

The overview of TEAEs is summarized in the table below: There were no deaths, no serious adverse events and no discontinuations due to a TEAE during the study.

Table 30: Overview of Treatment Emergent Adverse Events – Safety Population

	Placebo		Eplivanserin 15 mg	
	(N=28)	**n (%)**	**(N=28)**	**n (%)**
Any TEAE	**7**	**25.0%**	**8**	**28.6%**
Any severe TEAE	**0**	**0**	**0**	**0**
Any serious TEAE	**0**	**0**	**0**	**0**
Any TEAE leading to permanent treatment discontinuation	**0**	**0**	**0**	**0**

N=Number of patients exposed; TEAE=Treatment Emergent Adverse Events; n (%)=Percentage of patients with at least one TEAE in each category

All adverse events reported during the study are summarized in the table below:

Table 31: Number (%) of Patients with TEAEs by System Organ Class and Preferred Term – Safety Population

Primary System Organ Class **Preferred Term**	**Placebo**		**Eplivanserin 15 mg**	
	(N=28)	n (%)	(N=28)	n (%)
Any class	7	25.0%	8	28.6%
Infections and infestations	2	7.1 %	2	7.1 %
Nasopharyngitis	1	3.6%	2	7.1%
Asymptomatic bacteriuria	1	3.6%	0	0%
Psychiatric disorders	0	0%	1	3.6%
Anxiety	0	0%	1	3.6%
Nervous system disorders	5	17.9%	3	10.7%
Headache	4	14.3%	2	7.1%
Dizziness	1	3.6%	1	3.6%

Primary System Organ Class Preferred Term	Placebo (N=28)	 n (%)	Eplivanserin 15 mg (N=28)	 n (%)
Respiratory, thoracic and mediastinal disorders	1	3.6%	0	0%
Dyspnea	1	3.6%	0	0%
Gastrointestinal disorders	1	3.6%	2	7.1%
Diarrhea	0	0%	1	3.6%
Toothache	0	0%	1	3.6%
Vomiting	0	0%	1	3.6%
Nausea	1	3.6%	0	0%
Reproductive system and breast disorders	1	3.6%	0	0%
Menopausal symptoms	1	3.6%	0	0%
General disorders and administration site conditions	1	3.6%	0	0%
Application site irritation	1	3.6%	0	0%
Investigations	0	0%	1	3.6%
Body temperature increased	0	0%	1	3.6%

TEAE=Treatment Emergent Adverse Event; for treatment emergence, elapsed time since last administration is 14 days.

MedDRA version: 10.1; N=number of patients exposed, n (%) = number and percentage of patients with at least one TEAE.

4.3.2 Analysis of Adverse Events

The incidence of patients with at least one TEAE was similar under both the eplivanserin 15 mg and the placebo treatments. All TEAEs were of mild or moderate intensity.

The following TEAEs were reported only after eplivanserin 15 mg treatment, each in 1 patient: anxiety, diarrhea, toothache, vomiting, and body temperature increased. TEAEs reported in more than one patient included nasopharyngitis (2 patients on eplivanserin 15 mg treatment and 1 patient on placebo treatment) and headache (2 patients on eplivanserin 15 mg treatment and 4 patients on placebo treatment).

Among those TEAEs, only anxiety was considered as related to the study drug. It occurred in one patient. This female patient (mild COPD) aged 58 years was dosed with eplivanserin 15 mg

at 06:45 PM in Period 1. The PSG period started at 10:46 PM.

The patient was asked to sleep in the PSG laboratory until wake up. About 3 hours after the light was turned off, 6 hours and 25 minutes after drug intake, the subject rang the bell to alert the nurses. When the staff opened the door, the patient reported feeling anxiety in the dark room, where every door and window was closed without any noises. It was decided to stay at her bedside until she felt comfortable. After 20 minutes, she was tired, did not feel any anxiety anymore and wanted to continue with PSG. This finding was observed only during Period 1. The patient reported that she never had such a problem before. This finding was assessed as possibly drug related.

4.3.3 Deaths, Serious Adverse Events and Other Significant Adverse Events

None of the subjects died during the study and no SAE of significant AE occurred during the study. None of the AEs led to withdrawal of any of the subjects.

4.3.4 Safety Conclusion

- The percentage of patients who experienced any TEAEs was similar between eplivanserin 15 mg (8/28 patients - 28.6%) and placebo (7/28 patients - 25%) groups. All TEAEs were of mild to moderate intensity.

- There were no laboratory, vital sign or ECG results of clinical concern.

Altogether, a single dose of eplivanserin 15 mg in patients with mild or moderate COPD was well tolerated.

5 Discussion

Twenty-eight patients with mild to moderate COPD who fulfilled all of the inclusion and none of the exclusion criteria were enrolled in the study. We investigated the effect of a single dose of eplivanserin 15 mg on respiratory function and sleep structure compared to placebo after an overnight stay in a PSG laboratory in cross-over design. The observed data were statistically analyzed with ANOVA for the primary/secondary parameters with fixed terms for treatment, sequence, and period. Descriptive statistics were collected and analyzed for subjective evaluation of the quality of sleep, self rating of mood and vigilance and next-day residual effects.

We were able to show that a single dose of eplivanserin 15 mg compared to placebo did not change significantly the value of SaO_2 measured during sleep (Tables 5 and 6). Nor could any significant changes in SaO_2 be detected in different sleep stages as compared to placebo. Spirometry and body plethysmography, used during the day to test lung function, also did not show any significant difference between placebo and eplivanserin 15 mg in any of these parameters (Tables 11-16).

In addition, blood gas analysis was used to determine gas-exchange levels in the blood related to lung function after the administration of eplivanserin 15 mg compared to placebo. The data collected by capillary blood gas analysis showed no appreciable effect on blood bicarbonate or partial pressure of carbon dioxide or oxygen. The slight decrease in pH was not considered clinically important.

Descriptive statistics were collected and ANOVA was performed to analyze the effect of a single dose of eplivanserin 15 mg compared to placebo on polysomnography parameters. A significant increase (ANOVA) in total sleep time was found after the administration of a single dose of eplivanserin 15 mg as compared to placebo – a desired effect for a substance used to treat insomnia. The wake duration after persistent sleep onset and number of awakenings decreased significantly in comparison to placebo – another desired effect for a substance used to treat insomnia.

The LSEQ and Bond and Lader Visual Analog Scale were used in addition to polysomnography to assess the subjective feeling of the subjects after awakening. The analysis of the results showed a wide dispersion for each parameter. Therefore, it is doubtful that the changes observed were clinically meaningful.

The aim of the present study was to investigate the effect of a single dose of a new 5-HT_{2A} receptor antagonist, eplivanserin, a member of a new substance class for the treatment of insomnia, on respiratory function and sleep parameters.

Several 5-HT_{2A} receptor antagonists (e.g., eplivanserin, pruvanserin, M-100907), along with an inverse agonist (APD125), underwent development as potential hypnotic agents to treat insomnia (Becker et al. 2006; Gerschell et al., 2006). In recent years, numerous studies have demonstrated the role of serotonin and especially of the 5-HT_{2A} receptor in the sleep-wake system. In view of the sleep problems experienced by COPD patients and the potential worsening of gas exchange when a hypnotic is used (Guilleminault et al., 1990), the safety and efficacy of new agents for the treatment of insomnia in COPD patients must be ascertained.

However, up to now, only a few studies have evaluated the effect of 5-HT_{2A} receptor antagonists on respiratory function during wake and sleep in this special population. We shall discuss our results in detail below.

5.1 Effects on Respiration during Sleep

It is well known that the COPD patients complain about breathing difficulties during the day and night. Mohsenin writes: “There are several mechanisms underlying non-apneic oxygen desaturation during sleep in COPD patients. They include decreased functional residual capacity, diminished ventilatory response to hypoxia and hypercapnia, impaired respiratory mechanical effectiveness, diminished arousal responses, respiratory muscle fatigue, diminished nonchemical respiratory drive, increase in upper airway resistance, and the position of baseline saturation values on the oxyhemoglobin dissociation curve” (Mohsenin, 2005).

Usually the most marked reductions in SaO_2 occur during REM sleep (Cohn et al, 1984). The measurement of oxygen saturation with a pulse oximeter in a group of patients with severe COPD demonstrated a nearly 20% decrease in SaO_2 during non-REM sleep and a nearly 40% decrease in oxygenation during REM sleep as compared with wakefulness. The changes resulted primarily from reduced tidal volume (Becker et al. 1999).

We measured the oxygen percent saturation during sleep using finger pulse oximetry. No significant differences in $\%SaO_2$ value compared to placebo could be found during the time in bed (tables 5, 6). In addition, the SaO_2 value, which was analyzed during the different stages of sleep, showed no significant differences between placebo and single dose 15 mg eplivanserin. Furthermore, we wanted to know if there was a difference based on gender and COPD status. No

significant difference was present in relation to gender or COPD status between placebo and eplivanserin treatment (Table 9).

Few studies had been previously performed by other investigators into the effect of various insomnia drugs on respiratory function in COPD patients during sleep.

Rodney (1993) and his co-worker investigated the effect of zolpidem and triazolam on sleep and respiration in mild to moderate COPD patients. Twenty-four patients with insomnia and mild to moderate COPD were studied in a double blind, randomized, single-dose, placebo and active drug controlled study. They investigated in each period the effect of a single dose of triazolam 0.25mg, zolpidem 5mg, zolpidem 10mg and placebo. Patients were assigned randomly to one of the treatment groups. During the night, arterial oxygen saturation was measured. The respiratory assessment showed in this study that the mean arterial oxygen saturation in REM sleep was significantly lower than that of NREM for each sequence. Their results showed that no statistically significant difference could be found in SaO_2 between different sequences.

These results correlate to the results that we observed in the present study. Kryger et al. (2008) investigated the use of ramelteon (selective melatonin receptor agonist) in 26 patients with mild to moderate COPD. The patients received either ramelteon 16mg or placebo in a cross-over study. In the study, arterial oxygen saturation was monitored overnight. There were no significant differences between treatment groups for any of the assessed respiratory parameters (Kryger et al. 2008). The results of the studies by Rodney and Kryger showed that the use of a single dose of zoplidem5mg, 10mg, triazolam 0.25 and ramelteon 16mg is safe in patients with mild to moderate COPD. We could demonstrate that a single dose of eplivanserin 15 mg is also safe in patients with mild to moderate COPD.

In contrast, years before (1976), Geddes and his co-workers investigated the effect of nitrazepam and flurazepam on the ventilatory response to carbon dioxide. Both drugs were often prescribed as members of the benzodiazepine family at that time. The authors showed in their study that flurazepam significantly depressed CO_2 sensitivity. This was the first unequivocal evidence of central depression of respiration by a benzodiazepine (Geddes et al. 1976). George and Bayliff (2003) and Stege and his co-worker (2008) showed that benzodiazepine altered the activity of nerves that innervate upper-airway muscles and thus decrease the central respiratory drive and increase upper airway resistance. Such central depressions of respiration increase the risks of benzodiazepine for COPD patients.

Altogether, it seems that the non-benzodiazepine and ramelteon have better safety profiles than

benzodiazepines. Our results showed that this is also true for a single dose of $5HT_{2A}$-receptor antagonists like eplivanserin. They also do not have any depressor effects on central control of breathing in patients with mild to moderate COPD as measured by pulse oximetry.

Still, it must be remembered that this was a single dose study; caution should be exercised in extrapolating the results to multiple dosing over longer periods. However, it seems unlikely that adverse respiratory effects due to mechanisms of action of this drug group would be more prevalent with repeated use. In contrast to benzodiazepines, which depress the central respiration (Stege et al., 2008), to my knowledge, no effects on central respiratory regulation were observed in $5HT_{2A}$-receptor antagonists to date; therefore, it seems unlikely that multiple dosing would cause more adverse respiratory effects.

5.2 Effects on Respiratory Function (Spirometry, Body Plethysmography)

Hypoventilation appears to be the major mechanism underlying hypoxemia during sleep in COPD patients, but ventilation–perfusion mismatch and reduction in functional residual capacity during REM sleep may also be a contributory factor (Flenley et al., 1989).

More marked hypoventilation, resulting in severe arterial oxygen desaturation, may occur when hypnotics are used in COPD patients (Guilleminault et al., 1990). For the diagnosis, monitoring and assessment of COPD, spirometry represents the gold standard because it is the most standardized, reproducible, and objective way to measure airflow limitation.

We performed spirometry and body plethysmography at screening for the diagnosis and during the study on day 1 pre dose and on day 2 post dose. Fourteen patients with mild COPD and fourteen patients with moderate COPD participated in the study. The data that we collected through spirometry measurements showed no significant difference between a single dose of eplivanserin in comparison to placebo. In addition, we analyzed the fixed effects of gender and COPD status in relation to spirometry parameters. As expected, the tests showed significant differences based on gender or COPD status. The treatment difference was non-significant for all spirometry parameters (Tables 11-14).

Similar results were found through body plethysmography (Tables 15-16).

The effect of hypnotic drugs on respiratory parameters as measured by spirometry and body plethysmography in COPD patients has not been investigated much. A few studies have investigated the effect of various hypnotic drugs on this sub-population (COPD patients) using spirometry. In one small study (nine patients with stable COPD), Jolly and co-workers (Jolly et al., 1996) investigated the effect of a single dose of 1.5 to 2 mg lorazepam on the respiratory

function; the authors noted a 20% decrease in minute ventilation due to decreased tidal volume and a small increase in $PaCO_2$. This could be explained by the effects of benzodiazepines on the central nervous system. Skeletal muscle strength and endurance decreased significantly (22 and 50% respectively). This was to be expected, given the previously-reported muscular actions of this class of drugs. Respiratory muscle function parameters showed significant reductions (10 to 20%), as was the case with diaphragmatic function. The study concluded that a single dose of lorazepam reduces the strength and endurance of respiratory muscles in stable chronic COPD patients. After a single dose, they also observed a reduction in respiratory muscle function parameters of 10-15%, along with negative impacts on diaphragmatic endurance (Jolly et al., 1996).

Other studies found similar results with regard to other benzodiazepines. Cohn and co-workers (Cohn et al. 1992) studied the effect of a single dose of 2 mg of estazolam and of a single dose of 30 mg of flurazepam on cardiopulmonary functioning in patients with COPD. They demonstrated that acute administration of flurazepam decreased the tidal volume and raised inspiratory flow.

Unlike benzodiazepines, single doses of zolpidem (10mg) and zopiclone (7.5 to 10mg) have been found to have no significant impact on central control of breathing and ventilatory drive in normal subjects or in patients with mild to moderate COPD (Beaupré et al. 1988, Ranlov et al. 1987, Cohn et al. 1993). These results are comparable to the results that were observed in this study.

5.3 Effect during Different Sleep Periods

To investigate the efficacy of hypnotics, different variables can be measured. Sleep maintenance and induction are often measured by PSG, while sleep quality is often measured by self- rating. As a standard for sleep induction, sleep latency is often used, and for sleep maintenance, the number of awakenings after sleep onset. Sleep induction and sleep maintenance are also reflected in total sleep time and in sleep efficiency, which can be measured by PSG. There is no established PSG metric for the measurement of sleep quality; therefore, various self-rating questionnaires are used.

Although the aim of this study was not to investigate the efficacy of eplivanserin in preventing sleep disturbance, PSG data were collected and analyzed.

The results showed a significant difference between the effects of a single dose of eplivanserin 15 mg in comparison to placebo on wake after sleep onset, total sleep time and number of

awakenings. Insomnia patients often report excessive wake duration after the onset of persistent sleep. The aim of all drugs used to treat insomnia is to reduce the wake after sleep onset (WASO) and to increase the total sleep time (TST). A single dose of eplivanserin 15 mg reduced significantly the number of awakenings after sleep onset in comparison to placebo. Total sleep time was prolonged significantly from 408.9 to 425.0 minutes in comparison to placebo. The mean wake duration after persistence sleep onset was shortened from 63.4 to 43.6 minutes. No differences were found based on gender or mild/moderate COPD status (Tables 17-20). This finding corresponds to the previous testing of the $5HT_{2A}$ receptor antagonist in rats (Dugovic et al. 1989).

Dugovic and his co-workers demonstrated that selective $5HT_{2A}$ receptor antagonists enhance slow wave sleep (SWS) and delta power during non- rapid eye movement (NREM) sleep and decrease the number of awakenings without significant effect on REM sleep in rats (Dugovic et al. 1989).

In 2008, Morairty et al. investigated the effects of a single dose of 1.0, 3.0 and 10mg/kg of RO4368554 ($5HT_6$ receptor antagonist) and a single dose of 0.1, 1.0 and 3.0mg/kg of MDL100907 ($5HT_{2A}$ receptor antagonist) relative to a single dose of 10mg/kg of zolpidem in rats. The results showed that these two ligands selectively inhibit either $5HT_6$ or $5HT_{2A}$ receptors, which affect "sleep and wake and associated physiologic parameters during the active phase of the rodent circadian cycle. The effects of these compounds were compared with zolpidem, a hypnotic medication that acts as an agonist at the type I benzodiazepine (ω1) binding site on the $GABA_A$ receptor." The results of Morairty et al. supported "5-HT_{2A} receptor involvement in NREM sleep." Each of the three compounds that they used increased NREM sleep and reduced wakefulness – however, over different courses of time and to a greater or lesser extent. Although all three varying doses of MDL reduced sleep latency, this effect was weaker than that of zolpidem 10 mg. The increased NREM sleep and reduced wakefulness induced by MDL were not in evidence until the second hour following treatment (Morairty et al., 2008).

In 2010, Vanover and Davis reviewed various studies regarding the role of $5HT_{2A}$ receptor antagonists in the treatment of insomnia in animals and humans. They concluded that the polysomnographic data in all the studies support the thesis that treatment with $5HT_{2A}$ receptor antagonists increases slow-wave sleep and decreases wake after sleep onset.

The studies, they reported, support the thesis that eplivanserin and other $5HT_{2A}$ receptor antagonists are involved and effective in maintaining sleep (Vanover and Davis, 2010). These

findings correspond to our results with regard to the effect of a single dose of 15 mg eplivanserin on different sleep stages.

In contrast, Ashton found that "benzodiazepines not only affect sleep duration, but also the distribution and the composition of the different stages of sleep" (Ashton 1995). Repeated administration of BDZs drastically reduces SWS both in normal sleepers and in insomniacs, and normal sleep is restored only slowly after treatment is discontinued. Moreover, a diminution of delta-band frequencies appears immediately after medication with BDZs, continues during intermediate- and long-term administration, and persists even after withdrawal. Short-, intermediate, and long-acting BDZ compounds all depress SWS (Parrino et al. 1996).

The new class of $5HT_{2A}$ receptor antagonists, as well as the new hypnotics without a BDZ molecular structure, represent more "natural" agents; they induce sleep without changing its normal structure (Vanover and Davis, 2010).

5.4 Effects of Capillary Blood Gas Analysis

Arterial blood gas analysis is a simple way of assessing overall control of breathing. Roth (2009) writes: "The changes in arterial blood gases that occur in normal subjects during sleep are exacerbated in patients with COPD." When awake, patients with COPD also experience varying levels of change in arterial blood gas values. Those COPD patients who demonstrate even mildly hypoxic levels of diurnal arterial oxygen tension (PaO_2) tend to develop substantial oxygen desaturation at night, particularly during REM sleep (Douglas 1998).

Capillary blood gas analysis was performed in this study during wakefulness (directly after awaking and 2 hour after awaking). It is not possible to perform it during sleep because it would wake the subjects. Therefore, no information can be provided based on capillary blood gas analysis during sleep. Nevertheless, this study found no appreciable effect after treatment with a single dose of 15 mg eplivanserin on blood bicarbonate and the partial pressure of carbon dioxide in comparison to placebo during wakefulness. The pH value and partial pressure of oxygen decreased slightly, but without any clinical significance (Tables 21-23). To my knowledge, no previous studies have been performed that have made use of capillary blood gas analysis in COPD patients after the administration of substances for the treatment of insomnia that could support the current study's results.

5.5 Leeds Sleep Evaluation Questionnaires (LSEQ) and Visual Analog Scale (VAS Sleep Quality Scale)

Hang-over effect is the major problem in the treatment of insomnia with hypnotic drugs. Various symptoms have been reported after taking hypnotic drugs, including impairment of cognitive functions, drowsiness and reduced psychomotor performance in the morning after and memory impairment during the day.

The LSEQ has been used to generate standardized data on the relative efficacy of different compounds in terms of inducing sleep and improving its perceived quality. The LSEQ also provides comparative data on subjective hangover effects, such as difficulties in awakening, or a residual feeling of sedation the following morning. Thus, the LSEQ has been used repeatedly to provide serial measurements for evaluating drugs and is the only instrument that has been developed specifically and validated to measure medication effects (Spielman et al. 2000).

Self-evaluation of sleep is a common method to evaluate the effect of psychopharmacological drugs. Studies have shown that this measurement can estimate subjective differences in affectivity between the active drug and placebo. Although the LSEQ cannot be used for investigating objective sleep changes, it can nevertheless provide useful information regarding subjectively detected changes in sleep and early morning behavior, with an acceptable rate of failure and an acceptable degree of validity.

In this study the LSEQ evaluation questionnaire was used to assess the subjective changes on aspects of sleep and morning awakening. No significant treatment effect of eplivanserin 15 mg compared to placebo was found. No effect was found on ease of getting to sleep or on ease of awakening from sleep between baseline and post-dose. Only a slight, statistically insignificant decrease in quality of sleep was observed (Figures 23-26).

In 1993, Kamali et al. investigated the potential development of withdrawal symptoms after the abrupt discontinuation of 10mg/daily ritanserin ($5HT_{2A}$ and $5HT_{2C}$ antagonist) for 8 weeks. At the start of the study and at various intervals throughout, psychological assessments were conducted. They showed that the discontinuation of ritanserin, a member of the $5HT_{2A}$ receptor antagonist family, did not appear to be associated with symptoms of withdrawal (Kamali et al., 1993).

In comparison, withdrawal symptoms from benzodiazepines and GABA-A mediated drugs are

well known and can be a safety issue (Vanover and Davis 2010).

However, it is difficult to assess the withdrawal symptoms after the administration of a single dose of a drug. Moreover, the LSEQ score parameters obtained in the current study varied widely and thus the clinical meaningfulness of the changes observed is doubtful.

The visual analog scale (VAS) was used to measure aspects of sleep and daytime functioning and the effects of therapeutic interventions on them. No significant changes were found for a single dose eplivanserin 15 mg in comparison to placebo. These results are in agreement with the findings of Kamali and his co-workers, who found an absence of withdrawal effects for ritanserin. Because their study was based on multiple doses, they are better qualified to evaluate withdrawal symptoms than our single-dose study.

In contrast to $5HT_{2A}$ antagonists, several residual impairments that negatively affect the quality of life of insomnia patients have been reported the day after taking benzodiazepines, including impaired work performance, waking mood and accident risks.

Overall, the data from the current study confirm that selective blockage of the $5HT_{2A}$ pathway can significantly improve sleep maintenance parameters without suppression of respiratory parameters in patients with mild to moderate COPD. In addition, the current results support further investigation into $5HT_{2A}$ receptor antagonist as a potential treatment for sleep maintenance insomnia and for safe use with COPD patients co-morbid with insomnia.

Although the results of this study were positive, none of the $5HT_{2A}$ receptor antagonists have yet reached the efficacy requirement for marketing approval from regulatory agencies. Pharmaceutical companies have, at least for the time being, discontinued development of these drugs. Vanover and co-workers have offered an explanation: “it appears that many $5HT_{2A}$ receptor antagonists have lacked a sufficient benefit to risk ratio, whether it be due to lack of efficacy or safety concerns.” However, safety concerns, Vanover and Davis add, “do not appear to be necessarily target-mediated” (Vanover and Davis, 2010).

By better understanding the mechanism by which this class of drugs works, better drugs can be developed for the treatment of insomnia. Based on this study, the various antagonists of the $5HT_{2A}$ receptor could play an important role in the future treatment and therapy of insomnia.

5.6 Conclusion

Insomnia represents the most common sleep disorder; it affects millions of people as either a primary or co-morbid condition. In a survey conducted by the National Sleep Foundation (NSF) in 2004-2005, around 21% of adults 18 or over responded that they had a sleep problem, and 24% said that sleep problems had a negative impact upon their daily lives. Among individuals suffering from insomnia, 42% reported problems sleeping almost every night, and 88% had experienced difficulties sleeping for more than one year. The lack of effective management of insomnia can have serious consequences for affected individuals in terms of reduced quality of daily life. The impairment of cognitive performance and diminished concentration resulting from insomnia can lead to difficulties at work, relationship problems, and decreased enjoyment of family and social life (National Sleep Foundation, 2005).

In general, patients with COPD suffer more often from insomnia than the general population. Nocturnal respiratory disorders are often associated with COPD (Fleetham et al., 1982). COPD patients sleep poorly and demonstrate more marked arterial oxygen desaturation during sleep than normal persons (Flenley et al., 1989). In addition, many symptoms associated with COPD – including frequent urination at night, chest pain, and coughing – also contribute to disturbed sleep.

Hypnotics, the treatment of choice for insomnia, are widely taken on a regular basis in the general population. Hypnotic Benzodiazepines, widely used for this indication, are well known for their depressor effect on the central control of breathing and their myorelexant effect. They decrease the central respiratory drive and increase upper airway resistance by altering the activity of nerves that innervate muscles in the upper airways (George et al. 2003 and Stege et al. 2008). These two effects can therefore easily aggravate and/or contribute to the occurrence of nocturnal respiratory disorders, especially obstructive apnea and nocturnal desaturation. These adverse effects have been clearly demonstrated in COPD patients (Stege et al., 2008; Murciano et al., 1993; Block et al., 1984; Timms et al., 1988).

As hypnotics are widely prescribed, it is important that those drugs in use should have as little effect on respiratory events as possible. Especially the sub-population of COPD patients with insomnia could benefit from the use of new agents for the treatment of insomnia that do not attack the respiratory function.

Eplivanserin, a selective 5-HT$_{2A}$ receptor antagonist, was a member of a new therapeutic class

under clinical investigation for the treatment of insomnia. Unlike the GABAA agonist, the 5-HT2A antagonists are non-hypnotic and non-sedative. Rosenberg and co-workers write, "Psychomotor impairment and somnolence are not observed, even at peak drug levels (2008)." They promote sleep maintenance by decreasing the number of awakenings, sleep stage shifts, and arousals (Idzikowski et al., 1986; Luthringer et al., 2006; Landolt et al., 1999). Eplivanserin's pharmacological properties made it of special interest in high risk patients, such as those with COPD.

This study was designed to evaluate the effects of 15 mg eplivanserin compared with placebo on respiratory function and sleep parameters in patients with COPD of mild to moderate intensity. It showed that a single dose of 15 mg eplivanserin compared with placebo had no significant effects on respiratory function as measured by spirometry or body plethysmography or on different sleep stages as measured by Polysomnography (PSG) and finger-pulse oximetry. Moreover, no significant effects were found in capillary blood gas analysis.

Polysomnography showed a reduction in the number of awakenings after sleep onset, a reduction of the apnea-hypopnea index and of wake duration after persistent sleep onset, and an increase in total sleep time. PSG measurements showed no changes in latency to persistent sleep.

The Bond and Lader VAS score and LSEQ, performed after treatment, did not show any significant changes compared with placebo.

The performed descriptive statistics did not show any significant differences based on gender or between mild and moderate COPD patients.

No significant changes were observed in any of the safety parameters (ECG, laboratory results, vital signs or reported adverse events). The data collected confirmed that a single dose of eplivanserin 15 mg was well tolerated in mild to moderate COPD patients without any worsening in their respiratory condition.

Because this was a single dose study, its results should be extrapolated only with caution to a situation in which this drug or other $5HT_{2A}$ receptor antagonists will be used for longer periods. More research is needed to evaluate the long-term effects of treatment and the most appropriate management strategy for COPD patients with chronic insomnia.

Although the development of most $5HT_{2A}$ receptor antagonists has currently been discontinued and they have not cleared final approval by regulatory agencies, future research should focus on (1) the effects of hypnotics on sleep in insomniacs with co-morbid illness and on the status of the co-morbid condition (independent from sleep); (2) the effects of hypnotics and $5HT_{2A}$ receptor antagonists on specific populations (i.e., ethnic and racial groups, older adults, etc.); and (3) the

impact of hypnotics and $5HT_{2A}$ receptor antagonists upon such dimensions of waking life as cognition, occupational performance, healthcare utilization, and general quality of life. Such studies should constitute part of the evaluation of all drugs used or proposed for the treatment of insomnia. If one of the existing $5HT_{2A}$ receptor antagonists is further developed and approved by regulatory agencies, it will undoubtedly play an important role in the development of improved treatments for insomnia.

6 Zusammenfassung

In Rahmen einer doppelblinden, randomisierten, placebokontrollierten Überkreuzstudie wurde die Wirkung einer 15 mg Einzeldosis von Eplivanserin auf die Atemfunktion und Schlafstruktur bei Patienten mit leichter bis mittelschwerer chronisch obstruktiver Lungenerkrankung (COPD) untersucht. Eplivanserin gehört zu einer neuen Arzneimittelklasse, den $5HT_{2A}$ Rezeptor Antagonisten, die zur Behandlung von Schlaflosigkeit eingesetzt werden könnten.

Insgesamt 28 Patienten in Alter von 20 bis 75 Jahren, die seit mindestens drei Jahren an COPD litten, wurden in die Studie eingeschlossen. Die Patienten wurden in jeder Periode eingeladen, zur Eingewöhnung eine Nacht in Schlaflabor zu verbringen. Alle Teilnehmer erhielten sowohl 15 mg Eplivanserin als auch Placebo in zwei Behandlungsperioden, 14 Patienten in der Reihenfolge Eplivanserin – Placebo und 14 Patienten Placebo – Eplivanserin. Zur Beurteilung der Atemfunktion wurde die Sauerstoffsättigung (SaO_2) über Nacht während der Polysomnographie-Aufzeichnung gemessen. Während des Tages wurden Spirometrien, Bodyplethysmographien sowie Kapillarblutgasanalysen zu Beurteilung der Lungenfunktion durchgeführt.

Die Ergebnisse der Studie zeigen, dass eine Einzeldosis von 15 mg Eplivanserin die Atemfunktion in Patienten mit leichter bis mittelschwerer COPD im Vergleich zu Placebo nicht negativ beeinflusst.

Es konnten keine wesentlichen Unterschiede zwischen 15 mg Eplivanserin in Vergleich zu Placebo, weder in den SaO2 Messungen während der Nacht noch bei der Spirometrie bzw. Bodyplethysmographie während des Tages, beobachtet werden. Die Polysomnographie zeigte eine Reduktion des Apnea-hypopnea-Indexes und die Dauer der Wachphasen nach dem Anfang des durchgehendem Schlafes (wake duration after persistence sleep onset), eine Erhöhung der Gesamtschlafzeit, eine Reduktion der Anzahl von Aufwachsphasen nach dem Einschlafen und keine Änderung in der Latenzzeit bis zu durchgehenden Schlaf.

Die Untersuchungen mit der visuellen Analogskala (VAS) sowie des Schlaf-Fragebogens (LSEQ) zur Beurteilung der Schlafqualität zeigten minimale bzw. keine Veränderungen im Vergleich zur zum Ausgangswert vor der Dosierung.

Insgesamt konnte gezeigt werden, dass ein Einzel Dosierung von 15 mg Eplivanserin in Patienten mit leichter bis mittelschwerer COPD gut verträglich ist und insbesondere ihre Lungenfunktion nicht negativ beeinflusst.

7 References

1. AARC Clinical Practice Guideline Body Plethysmography: 2001 Revision & Update: Respir Care 2001; 46(5):506–513.
2. Abrams JK, Johnson PL, Hay-Schmidt A, Mikkelsen JD, Shekhar A, Lowry CA. Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. Neuroscience 2005; 133:983-97.
3. Adrien J, Alexandre c, Boutrel B, Popa D(2004) Contribution of the "Knock-out" technology to understanding the rule of serotonin in sleep regulations.Arch Ital Biol 142:369-377.
4. Ashton H. Toxicity and adverse consequences of benzodiazepine use. Psychiatric Annals 1995b; 25:158-65.
5. Barnes, N.M., Sharp, T, A review of centeral 5-HT receptors and their function. Neuropharmacology 38:1083-1152; 1999.
6. Beaupré A, Soucy R, Phillips R, Bourgouin J. Respiratory center output following zopiclone or diazepam administration in patients with pulmonary disease; Respiration. 1988; 54(4):235-40.
7. Becker HF, Piper AJ, Flynn WE, et al. Breathing during sleep in patient with nocturnal desaturation. Am J Respir Crit Care Med 1999; 159 (1):112-118.
8. Becker JR, Thomas S. Future Treatment for Depression, Anxiety, Sleep Disorders, Ps Psychosis;andADHD.[23January2006]http://www.neurotransmitter.net/newdrugs.html, updated July 20, 2009.
9. Bellia V, Catalano F, Scichiolone N, et al. Sleep disorders in the elderly with and without chronic air flow obstruction: the SARA study. Sleep, Vol.26, No3, 2003:318-323.
10. Block AJ, Dolly FR, Slayton PC. Does flurazepam affect breathing on oxygenation during sleep in patients with chronic lung disease?Am Rev Respir Dis 1984; 129(2): 230- 33.
11. Bond A., and Lader M.: The use of analog scales in rating subjective feeling Br. J. med. Psychol. (1974), 47, 211-218.

12. Boutrel B, Franc B., Hen R, Hamon M, Adrien J.: Key role of 5-HT ^{1B} receptors in the regulation of paradoxical sleep as evidenced in 5-HT^{1B} receptors in the regulation of paradoxical sleep as evidenced in 5-HT^{1B} knock-out mice.1999; J Neurosci 19(8): 3204-3212.
13. Boutrel B, Monaca C, Hen R, Hamon M, Adrien J.: Involvement of 5-HT$_{1A}$ receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT$_{1A}$ knock-out mice.2002 J Neurosci 22(11): 4686–4692.
14. Bradley PB. Engel G., Feniuk W., et al.: Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine.Neuropharmacology 1986, 25(6): 563-576.
15. Bradley TD, Rutherford R, Lue F, et al. Role of diffuse airway obstruction in the hypercapnia of obstructive sleep apnea. Am rev respire Dis 1986; 134:920-4.
16. Breslau N, Roth T, Rosenthal L, Andreski P. Sleep disturbance and psychiatric disorders: a longitudinal epidemiological study of young adults. Biol Psychiatry. 1996; 39:411-418.
17. Calverley PM, Anderson JA, Celli B, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 2007; Vol, 356, No.8:775-89.
18. Chaouat A, Weitzenblum E, Krieger J, Ifoundza T, Oswald M, Kessler R.Association of chronic obstructive pulmonary disease and sleep apnea syndrome. Am J Respir Crit Care Med 1995; Vol., 151, No 1:82-6.
19. Ciranna L. “Serotonin as a Modulator of Glutamate- and GABA-Mediated Neurotransmission: Implications in Physiological Functions and in Pathology; current Neuropharmacology 2006, 4, 101-114.
20. Cohn MA, Starz K, Nay KN, Gazeroglu h, Belsiot A, Sackner MA, Effects of hypnotics on breathing during sleep in chronic obstructive lung disease, Chest 1984; 86 (suppl): 32.
21. Cohn MA, Morris DD, and Juan D. Effects of estazolam and flurazepam on cardiopulmonary function in patients with chronic obstructive pulmonary disease, Drug Saf 1992; 7(2): 152-8.
22. Cohn MA. Effects of Zolpidem, codeine phosphate and placebo on respiration. A double- blind, crossover study in volunteers. Drug Saf 1993;9(4): 312-9.

23. Cormick W, Olson LG, Hensley MJ, Saunders NA. Nocturnal hypoxemia and quality of sleep in patients with chronic obstructive lung disease. Thorax 1986; 41:846-54.
24. De Martinis, Nicholas A., Winokur, Andrew, Department of Psychiatry, university of Connecticut School of Medicine, Effects of Psychiatric Medications on Sleep and Sleep Disorders, CNS & Neurological Disorder-Drug Targets, 2007, 6, 17-29.
25. Descarries, L., Audet, M.A., Doucet, G., Garcia, S., Pleskevich, S., Seguela, P., Soghomonian, J.J., Watkins, K.C.(1990): Morphology of Central serotonin neurons, Brief review of quantified aspects of their distribution and ultra structural realationships. Ann.N.Y. Acad.Sci.600, 81-92.
26. Dogrhramji, K.: insomnia and Comborid Chronic Obstructive Pulmonary Disease; Medascape Neurology & Neurosurgery; 05/30/2008.
27. Douglas NJ. Sleep in patients with chronic obstructive pulmonary disease. Clin Chest Med. 1998; 19(1):115–125.
28. Douglas NJ. Respiratory physiology: control of ventilation. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine. 4th ed. Philadelphia, Pa: Elsevier Inc.; 2005:224-231.
29. DuBois AB, Botelho SY, Comroe JH. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease. J Clin Invest 1956; 35:327.
30. Dugovic C, Wauquier A, Leysen JE, Marrannes R, Janssen PA. Functional role of 5-HT2 receptors in the regulation of sleep and wakefulness in the rat. Psychopharmacology (Berl) 1989; 97:436-42.
31. ERS Guidelines: B.R. Celli, W. MacNee, and committee members: Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004; 23: 932–946.
32. Fleetham J, West P, Mezon B, et al. Sleep arousals and oxygen desaturation in chronic obstructive pulmonary disease. AM Review Respir Dis 1982; 126: 429-33.
33. Flenley DC, Chronic obstructive pulmonary disease. In: Kryger MH, Roth T, Dement WC, eds. Principles and practice of sleep medicine. Philadelphia: Saunders, 1989: 601-10.
34. Geddes, D. M., Rudolf, M., and Saunders, K. B: Effect of nitrazepam and flurazepam on the ventilatory response to carbon dioxide (1976). Thorax, 31, 548-551.
35. George CF, Perspectives on the management of insomnia in petients with chronic

respiratory disorders. Sleep 2000; 23(Suppl. 1):S31-S35 (discussion S6-8).

36. George CF, Bayliff CD. Management of insomnia in patients with chronic obstructive pulmonary disease. Drugs. 2003; 63:379-387.
37. Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2006: http://goldcopd.org/Guidelineitem.azsp?11=2&12=1&intId=996.
38. Graham SJ, Langley RW, Balboa VA, Bradshaw CM, Szabadi E.: Effects of ketanserin and haloperidol on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man.J.Psychopharmakology.2002 Mar; 16(1):15-22.
39. Guilleminault C. Benzodiazepines, breathing, and sleep. AM J Med 1990; (Suppl 3A): 25S-28S.
40. Idzikowski C, Mills FJ, Glennard R. 5-Hydroxytryptamine-2 antagonist increases human slow wave sleep. Brain Res 1986; 378:164-8.
41. Jacobs, B.L. and E.C. Azimitia (1992). "Structure and the function of the brain serotonin system." Physiol Rev 72(1): 165-229.
42. Jolly E, Aguirre L, Jorge E, Luna C. Acute effect of lorazepam on respiratory muscles in stable patients with chronic obstructive pulmonary disease. Medicina (B Aires) 1996; 56(5Pt.1):472-8.
43. Jouvet M. Sleep and serotonin: an unfinished story. Neuropsychopharmacology 1999; 21:24s-27s.
44. Kahn, R.S.; Wetzer, S. m-Chlorophenylpiperazine as a probe of serotonin function. Biol. Psychiatry 30:1139-1166; 1991.
45. Kamali F, Stansfield SC, Ashton CH, Hammond GL, emanuel MB, Rawlins MD. Absence of withdrawal effects of ritanserin following chronic dosing in healthy volunteers. Psychopharmacology.1993; 108:213-217.
46. Kaynak, H., Kaynak, D., Gozukirmizi, E., et al (2004). The effects of trazodone on sleep in patients treated with stimulant antidepressants. Sleep Medicine, 5, 15-20.
47. Klink ME. Dodge R, Quan SF. The relation of sleep complaints to respiratory symptoms in a general population. Chest 1994; 105: 151-154.
48. Klink ME, Quan SF. Prevalence of reported sleep disturbances in a general adult population and their relationship to obstructive airways diseases. Chest. 1987; 91:540-546.

49. Kryger M, Wang-Weigand S, Zhang J, Roth T. Effect of ramelteon, a selective MT_1/MT_2 –receptor agonist, on respiration during sleep in mild to moderate COPD, Sleep Breath 2008;12:243-250.
50. Landolt HP, Meier V, Burgess HJ, et al. Serotonin-2 receptors and human sleep: effect of a selective antagonist on EEG power spectra. Neuropsychopharmacology 1999; 21:455-66.
51. Lanolt HP and Wehrle R. Antagonism of Serotonergic 5-$HT_{2A/2C}$ receptors: mutual improvement of sleep, cognition and mood? European Journal of Neuriscience, Vol.29, PP.1795-1809, 2009.
52. Lieberman JA (2007). "Update on the safety considerations in the management of insomnia with hypnotics: incorporating modified-release formulations into primary care". Primary Care Companion to the Journal of Clinical Psychiatry 9(1):25-31. PMID 17599165.
53. Lopez-Gimenez, J. F., G. Mengod, et al. (1997). "Selective visualization of rat brain 5- HT2A receptors by autoradiography with [3H] MDL 100,907." Naunyn Schmiedebergs Arch Pharmacol 356(4): 446-54.
54. Luthringer R, Prosser W, Arnal MA, et al. Pharmacokinetic and pharmacodynamic effects of the selective 5HT2A inverse agonist APD125 in healthy adults. Sleep 2006; 29:A39.
55. Mannino DM, MD, FCCP: Clinicl review Article: Chronic Obstructive Pulmonary Disease: Epidemology and Evauation, Oct.2001 Hospital physician,page 22.
56. Martin RJ, Bartelson BL, Smith P, et al. Effect of ipratropium bromide treatment on oxygen saturation and sleep quality in COPD. Chest. 1999; 115:1338-1345.
57. Maurer J. et al., Anxiety and Depression in COPD: Current understanding, unanswered questions, and research needs, CHEST / 134 / 4 / OCTOBER, 2008, 43S-56S
58. McNicholas WT, Calverley PM, Lee A, Edwards JC. Long-acting inhaled anticholinergic therapy improves sleeping oxygen saturation in COPD. Eur Respir J. 2004; 23:825-831.
59. Midgren B, Hansson L et.al. The effects of nitrazepam and flunitrazepam on oxygen desaturation during sleep in patients with stable hypoxemic nonhypercapnic COPD, Chest, 1989; 95; 765-768.

60. Mohsenin, V. Sleep in Chronic Obstructive Pulmonary Disease. Seminars in Respiratory and Critical Care Medicine, 2005; 26/1: 109-116.
61. Monti JM, Jantos H. Effects of the serotonin 5-$HT_{2A/2C}$ receptor agonist DOI and of the selective 5-HT_{2A} or 5-HT_{2C} receptor antagonists EMD 281014 and SB-243213, respectively, on sleep and waking in the rat. Eur J Pharmacol 2006; 553: 163-70.
62. Monteau R. et al. Further evidence that various 5-HT receptor subtypes modulate central respiratory activity: in vitro studies with SR46349B. Eur J Pharmacol 1994; 259(1):71-4.
63. Morairty Stephen R.; Linda Hedley; Judith Flores; Renee Martin, ; Thomas S. Kilduff, "Selective $5HT_{2A}$ and $5HT_6$ Receptor Antagonists Promote Sleep in Rats SLEEP, Vol. 31, No. 1, 2008; 34-44.
64. Murciano D, Armengaud MH, Cramer PH, et al, Acute effects of Zolpidem, triazolam, flunitrazolam on arterial blood gases and contol of breathing in severe COPD.EUR Respir J 1993; 6: 625-29.
65. Najib J (2006)."Eszopiclone, a nonbenzodiazepine sedative-hypnotic agent for the treatment of transient and chronic insmnia"Clinical Therapeutics 28(4):491-516. doi:10.1016/j.clinthera.2006.014.PMID 16750462.
66. National Institutes of Health. NIH State-of-the-Science Conference Statement on Manifestations and Management of Chronic Insomnia in Adults. NIH Consens State Sci Stateatioments. 2005; 22:1–30.
67. National Sleep Foundation. 2003 Sleep in America Poll. National Sleep Foundation, Washington, 2003 Available at : http://www.sleepfoundation.org/site/c.huIXKjM0IxF/b.2417365/k.1460/2003_Sleep_in_America_Poll.htm Accessed April 9, 2008.
68. National Sleep Foundation. Summary of Findings: 2005 sleep in America poll. March 2005. Available at: www.kintera.org/atf/cf/{F6BF2668-A1B4-4FE8-8D1A-A5D39340D9CB}/2005_summary_of_find-ings.pdf. Accessed August 14, 2007.
69. Neubauer DN (2006). "New approaches in managing chronic insomnia".CNS spectrum 11(8 Suppl 8):1-13.PMID 16871130.
70. Parrino L, Terzano Mario G, Polysomnography effect of hypnotic drugs, Psychophyrmacology(1996) 126: 1-16.
71. Parott C and Hindmarch I. , The Leeds Sleep Evaluation Questionnaire in Psychopharmacological investigations- a Review, Psychophamacology 1980, 71,

173-179.

72. Pineyro, G. and P. Blier (1999):" Autoregulation of serotonin neurons: role in antidepressant drug action." Pharmacol Rev 51(3): 533-91.
73. Raghu M. Shyamsunder Subramanian; Sleep Disturbance Due to Pulmonary Medications: current Respiratory Medicine reviews, 2009, 5,225-229.
74. Ranlov PJ, Nielson SP, Effect of zopicolone and diazepam on ventilatory response in normal human subjects. Sleep 1987; 10(Suppl.1): 40-7.
75. Rechtschaffen A, Kales A, eds. A manual of standardized terminology, techniques and scoring system of sleep stages in human subjects. Los Angeles: Brain information Service/Brain Research Institute, University of California, 1968.
76. Rennard SI. COPD: overview of definitions, epidemiology, and factors influencing its development (review).Chest 1998; 113(4 Suppl):235S-41S.
77. Rinaldi-Carmona M, Congy C, Santucci V et al. biochemical and pharmacological properties of SR46349, a new potent and selective 5-hydroxytryptamine 2 receptor antagonist. J Pharmacol Exp Ther 1992; 262(2):759-68.
78. Rodney D. Steens, Zoe Pouliot, Thomas W. Millar, Meier H. Kryger and Charles F. George. Effects of Zolpidem and Triazolam on Sleep and Respiration in Mild to Moderate Chronic Obstructive Pulmonary Disease; 1993 American Sleep Disorders Association and Sleep Research Society; Sleep, 16(4):318-326.
79. Roehrs T, Merlotti L, Halpin D, Rosenthal L, Roth T, Effects of Theophylline on noctural sleep and daytime sleepiness/alertness. Chest 1995; 108:382-7.
80. Rosenberg R. and co-worker, APD125, a Selective Serotonin 5-HT2A Receptor Inverse Agonist, Significantly Improves Sleep Maintenance in Primary Insomnia: Sleep. 2008 December 1; 31(12): 1663–1671.
81. Roth T, Hypnotic use for insomnia management in chronic obstructive pulmonary disease: Sleep Medicine 10(2009) 19-25.
82. Roth T, Roehrs T, Pies R. Insomnia: pathophysiology and implications for treatment. Sleep Med Rev. 2007; 11:71-79.
83. Sharpley AL, Elliott JM, Attenburrow MJ, Cowen PJ. Slow wave sleep in humans: role of 5-HT2A and 5-HT2C receptors. Neuropharmacology 1994; 33:467-71.
84. Sharpley AL, Attenburrow ME, Hafizi S, Cowen PJ. Olanzapine increases slow wave sleep and sleep continuity in SSRI-resistant depressed patients. J Clin Psychiatry 2005; 66:450-4.

85. Spielman A, Yang C, Glovinsky P. Assessment techniques for insomnia In: Kryger M, Roth T, Dement W, eds. Principles and practice of sleep medicinePhiladelphia: Saunders Co., 2000. 1239–1250.
86. Steens RD; Pouliot Z; Millar TW; et al. Effects of Zolpidem and triazolam on sleep 1993 Jun; 16(4):318-26.
87. Stege G, Vos PJ, van den Elshout FJ, et al. Sleep, hypnotics and chronic obstructive pulmonary disease. Respir Med. 2008; 102:801-814.
88. Stone JR, Zorick TS, Tsuang J (2007).” Dose-related illusions and hallucinations with zaleplon”. Clin Toxicol (Philadelphia): 1-2.doi:10.1080/15563650701517442.PMID 17852167.
89. Tashkin DP, Celli B, Senn S. et al. A 4- year trial of tiotropium in chronic obstructive pulmonary disease. N Engl J Med 2008,359:1543-54.
90. Terzano MG, Rossi M, Palomba V, Smerieri A, Parrino L.” New drugs for insomnia: comparative tolerability of zopiclone, zolpidem and zaleplon “.Drug Saf. 2003;26(4):261-82.
91. Tiak K. Verma, MD, MBA, Medical Director of the Sleep Health Center of Cumberland Sleep Health Centers Newsletter, 2009, Sleep and Chronic Obstructive Pulmonary Disease, I-877-SLEEPHC,I-877-753-3742.
92. Timms R, Dawson A, Hajdukovic RM, et-al. Effects of triazolam on sleep and arterial oxygen saturation in patient with chronic obstructive pulmonary disease Arch Intern Med 1988; 148: 2159-63.
93. Toner LC, Tsambiras BM, Catalano g, Catalono MC, cooper Ds (2000).” Central nervous system side effects associate with zolpidem treatment”. Clin Neuropharmacol 23(1):54-8. PMID 10682233.
94. Umbriaco, D., Garcia, S., Beaulieu, C., Descarries, L.(1995) Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). Hippocampus5(6),605-20.
95. Urbano F, Mohsenin V. Chronic obstructive pulmonary disease and sleep; the interaction. Panminerva Med 2006;48(4):223-30.
96. Vallarino CR, Mini L. Prevalence of insomnia in patients with chronic obstructive pulmonary disease in a large database. Value Health 2005; 8(3):322.
97. Vanover Kimberly E.and Davis Robert E., Role of $5HT_{2A}$ antagonists in the treatment of insomnia, Nature and Sience of Sleep 2010:2, 139-150.

98. Veale D, Cooper BG, Griffiths CJ, Corris PA, Gibson GJ. The effect of controlled-release salbutamol on sleep and nocturnal oxygenation in patients with asthma and chronic obstructive pulmonary disease. Respir Med. 1994; 88:121-124.
99. Watz H, Magnussen H. Comorbidities of COPD. Internist (Berl) 2006 Sep; 47(9):895-6.898-900.
100. World Health Organization. The GOLD global strategy for the management and prevention of COPD. Available at http://www.goldcopd.com. Accessed 13 August 2001.
101. Yao & Artusio´s anesthesiology: Problem oriented patient management; Fun-Sun F. Yao, Manuel L. fonts, Vinod Malhorta; 2008, page 1181.

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Erklärung

„Ich, Sara Armani, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: Effects of a 15mg single-dose eplivanserin on respiratory function and sleep structure in patients with mild to moderate chronic obstructive pulmonary disease (COPD), selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe. “

Datum Unterschrift

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.