6. SUMMARY

Anxiety related behaviour of rats: strain and stock differences

Anxiety disorders represent one of the most common emotional diseases and consequently the development of a suitable and lasting therapy is of important public interest. One major area of neuropharmacology is the investigation of the central mechanisms which are responsible for the creation of anxiety disorders, and subsequently is the development of new anxiolytics. Benzodiazepines, which are used as standard anxiolytics, have a wide therapeutical span, but they also have certain side effects. Hence, there is a necessity to develop solely anxiolytic pharmacotherapeutics. A major problem in this development are the conflicting effects of serotonergic drugs, which are observed with animal models of anxiety. Anxiolytic, anxiogenic, or no effects were found even when identical tests were used. Reasons for these contradictions could be, besides varying conditions of the experiments, differences in the animals being used and above all, strain or stock differences have an enormous influence on anxiety related behaviour. These uncharacterised differences in the animals used for such tests could underlie the different effects observed with newly developed anxiolytics.
The aim of this study was to investigate in more detail the influence of strain or stock differences on the baseline anxiety related behaviour of rats. The effects different anxiety levels have on the application of various anxiolytics and the differences between strains and stocks in the metabolism of these anxiolytics were investigated. Furthermore, the question of whether there are differences in the central serotonergic system was explored, because serotonin plays a major role in the creation of anxiety disorders.

To get an overall picture of the influence of breeding conditions on anxiety related behaviour, three stocks of Fischer rats (Winkelmann, Schönwalde and Charles River) bred in our animal unit, were compared to Fischer rats received directly from the same breeders. For these experiments three wide-spread animal models of anxiety were chosen: the elevated plus maze-, the black and white box- and the modified open field-test. The results show that breeding conditions really influence the anxiety related behaviour of rats. Raised under our breeding conditions, Fischer/Schönwalde rats became less anxious, whereas Fischer/Winkelmann rats became more anxious compared to the directly received animals.

The pharmacological investigations were carried out on two different Wistar stocks (Winkelmann and BgVV) and one Fischer strain from Winkelmann. For the experiments the same animal models were used as described above. The anxiolytic substances, diazepam, 8-OH-DPAT and ritanserin, were chosen.
In all animal models the Wistar/Winkelmann rats proved to be less anxious than the Fischer/Winkelmann and Wistar/BgVV rats. In the elevated plus maze-test the Wistar/BgVV rats were slightly more anxious than the Fischer/Winkelmann rats, whereas in the other two tests they behaved in a similar fashion.
Diazepam has an anxiolytic effect even in low doses on the more anxious Fischer/Winkelmann rats. In comparison, diazepam could produce no anxiolytic effect on the less anxious Wistar/Winkelmann rats. With the more anxious Wistar/BgVV rats diazepam had a tendency for an anxiolysis.
In all animal tests 8-OH-DPAT had no effect on the anxiety related behaviour of all groups of rats. Also ritanserin, which was examined only in the elevated plus maze-test, was ineffective.

Because there were distinct differences in the effect of diazepam between rat strains and stocks, blood plasma concentrations of diazepam and three of its metabolites, n-desmethyldiazepam, temazepam and oxazepam, were measured at the time the behavioural tests took place. The concentrations of these four substances were significantly higher in the Fischer/Winkelmann rats in comparison to both Wistar stocks. There were no differences between the two stocks of Wistar rats. N-desmethyldiazepam and temazepam were equally abundant in the Fischer/Winkelmann rats, whereas in both stocks of Wistar rats temazepam was proportional lower.

To investigate differences in the central serotonergic system, the levels of serotonin were measured in the prefrontal cortex, the hippocampus and in the median/dorsal raphe.
There were clear differences between the rat strains and stocks. In the more anxious Fischer/Winkelmann rats, the level of serotonin was significantly higher in the prefrontal cortex as well as in the hippocampus compared to both Wistar stocks. The Wistar/BgVV rats were at an intermediate level. Here, the levels in the hippocampus and the median/dorsal raphe were higher than in the Wistar/Winkelmann rats.
The application of diazepam had a different effect on the central levels of serotonin. Whereas diazepam produced a significant decrease of the serotonin levels in the prefrontal cortex and the hippocampus in the more anxious Fischer/Winkelmann and Wistar/BgVV rats, there was

shown to be only a small reduction in the hippocampus in the less anxious Wistar/Winkelmann rats. The level in the raphe nuclei was not influenced in any of the three groups tested by the application of diazepam.

Therefore, these results show that there is a large impact of breeding conditions as well as strain and stock differences on anxiety related behaviour. Differences in the baseline behaviour might have an influence on the effects of pharmacotherapeutics and could produce both false positive or negative results.
Additionally, it appears that the different effects of diazepam can be traced back to a different metabolism.
Differences in the central serotonergic system seem to reflect a difference in the anxiety related behaviour of the examined rat strains and stocks. Also, the effect of diazepam on the central serotonergic systems seems to depend on the rat strain being used.

Furthermore, strain and stock differences should be taken into consideration in future investigations and it is recommended to cite exactly in the description of a method the source of the animals.