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DISSERTATION

**Pharmacological modulation of physiological and
pathophysiological neuronal network activities: special
emphasis on purinergic ATP receptors**

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Steffen Björn Schulz

aus Berlin

Gutachter: 1. Prof. Dr. Uwe Heinemann
2. Prof. Dr. med. A. Draguhn
3. Prof. Dr. med. Dr. h. c. P. Illes

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PHARMACOLOGICAL MODULATION OF PHYSIOLOGICAL AND
PATHOPHYSIOLOGICAL NEURONAL NETWORK ACTIVITIES: SPECIAL
EMPHASIS ON PURINERGIC ATP RECEPTORS

SUMMARY

ABSTRACT

The present studies (Schulz et al., 2012a, 2012b; Klafft et al., 2012) were designed to investigate the role of extracellular ATP and its receptors on physiological and pathophysiological activities of neuronal networks and to elucidate how the neuronal network is modulated by approved multi-target antipsychotics. Persistent gamma oscillations (30-100 Hz) were induced in the CA3 region of acute rat hippocampal slices by the application of either acetylcholine (ACh) or kainic acid (KA). ATP reduced the power of KA-induced gamma oscillations exclusively by activation of adenosine receptors after its degradation to adenosine. In contrast, ATP suppressed ACh-induced oscillations via both adenosine and P2 receptors. The latter were also activated by endogenous ATP since blockade of ATP-hydrolyzing enzymes also inhibited gamma oscillations. More specific antagonists revealed that ionotropic P2X2 and/or P2X4 receptors reduced the power of ACh-induced gamma oscillations whereas metabotropic P2Y₁ receptor increased it. Intracellular recordings from CA3 pyramidal cells suggest that adenosine receptors reduce the spiking rate and the synchrony of action potentials during gamma oscillations whereas P2 receptors only modulate the firing rate of the pyramidal cells. As a model of hypersynchronous pathophysiological network activity, we induced recurrent epileptiform discharges (REDs) in slices from naïve and pilocarpine-treated rats by elevating extracellular potassium concentration in combination with bicuculline. Application of ATP reversibly reduced the incidence of REDs in naïve and chronic epileptic slices via activation of adenosine A₁ receptors without discernible P2 receptor effects. In slices from naïve rats, the P2X7 receptor antagonist A 740003 slightly but significantly reduced the amplitude of slow field potentials of REDs. In slices from chronic epileptic rats, none of the P2 receptor antagonists affected the parameters of REDs. Because disturbances in neuronal network activities are also related to psychotic symptoms, we investigated the effects of first and second generation antipsychotics on ACh/Physo-induced gamma oscillations. Several antipsychotics inhibited the power of gamma oscillations and increased the bandwidth of the gamma band. To elucidate which receptors of these multi-target drugs are responsible for the alterations of gamma oscillations, the effects of the antipsychotics were correlated to their pK_i values for 19 receptors. We found that 5-HT₃ receptors may have an enhancing effect on gamma oscillations whereas D₃ receptors may inhibit them. This *in silico* predictions were confirmed by specific agonists.

In conclusion, we found that (a) adenosine receptors dampen neuronal network activity both in models of physiological gamma oscillations and pathophysiological recurrent epileptiform discharges, (b) P2Y₁ receptors support gamma oscillations, (c) P2X2 and P2X4 receptors suppress gamma activity, (d) P2X7 receptors show a minor proepileptic effect, and (e) antipsychotics influence gamma oscillations by interacting with 5-HT₃ and D₃ receptors.

INTRODUCTION

Synchrony of neuronal activity is essential within and between neuronal networks for information transfer and for higher cognitive functions such as sensory processing, attention, learning and memory (Buzsaki and Draguhn, 2004). Prominent example of such a neuronal synchrony are gamma oscillations, with a frequency range of 30-100 Hz. Disturbances in gamma oscillations during EEG recordings have been observed in schizophrenic patients (Kissler et al., 2000; Minzenberg et al., 2010). In more detail, negative symptoms such as social withdrawal or reduced attention correlate with reduced gamma oscillations while positive symptoms such as hallucinations go along with an enhanced gamma activity (Herrmann and Demiralp, 2005; Lee et al., 2010; Mulert et al., 2011). Another brain disorder which is characterized by altered synchrony (hypersynchrony) of neuronal networks is epilepsy (Scharfman, 2007). For both diseases the current pharmacological treatments have certain constraints like ineffectiveness towards a (sub-)group of symptoms, strong side effects or limitations in the number of patients responding adequately to the drug treatment (Dichter and Brodie, 1996; Erhart et al., 2006). Adenosine A₁ receptors are known for their reduction of neuronal network oscillation (Pietersen et al., 2009) and their anticonvulsive effects (Lee et al., 1984). However, little is so far known about the effects of its precursor molecule, ATP, and its receptors of the P2 family on neuronal networks. ATP has been only recently regarded as neuromodulator, whose effects, in contrast to systemic neuromodulators such as dopamine and serotonin, are locally restricted (Newman, 2003; Burnstock, 2008; Burnstock et al., 2011).

AIMS

The aim of the present work was to investigate the role of purinergic receptors on physiological and pathophysiological neuronal network activities. Therefore we chose two rat *in vitro* models: (a) epileptiform activity in the entorhinal cortex of naïve or pilocarpine-treated animals as an established model of temporal lobe epilepsy and (b) hippocampal gamma oscillations, induced either by acetylcholine or kainate. Dependent on the mode of induction, both gamma oscillations differ slightly in their mechanisms, but are reminiscent of physiological *in vivo* gamma oscillations (Bartos et al., 2007). Because disturbances in gamma oscillations are related to psychotic symptoms, we also tested a group of approved antipsychotics on that model. As these drugs target multiple receptors (Roth et al., 2004), we aimed to elucidate the receptors responsible for their effects on oscillations by correlation analyses. A short list of *in silico* candidate receptors was then investigated using specific agonists for validation.

METHODS

All experiments on animals were performed in accordance with the guidelines of the European Communities Council and the institutional guidelines approved by the Berlin Animal Ethics Committee (Landesamt für Gesundheit und Soziales Berlin, T0096/02, T0068/02, G0391/08). Wistar rats (*rattus norvegicus* f. *domestica*) were kept in a 12h/12h light/dark cycle and given food and water *ad libitum*. One group of animals received methylscopolamine (1 mg/kg) 30 min prior to a single injection of pilocarpine (340 mg/kg) followed by diazepam (10 mg/kg) to develop epileptogenesis and chronic epilepsy (pilocarpine-treated animals). All other animals were used without previous treatment (naïve animals).

Experiments were done on acute rat brain slices *in vitro*. Therefore, adult rats were anesthetized with isoflurane added to a 70:30 mix of N₂O and O₂, decapitated and their brains removed. These were subsequently cut into 400 µm thick horizontal combined entorhinal/hippocampal slices by a vibratome (DSK microslicer DTK-1000, Dosaka, Japan, or Campden Instruments Ltd., Leicester, UK). The slices were placed into custom-made interface chambers which are known for slower equilibration of slices with a given drug than in submerged chambers (Hájos et al., 2009), but reveal a better O₂ supply which is essential for fast neuronal network activity (Huchzermeyer et al., 2008). From the removal from the skull onwards, the brain tissue was bathed in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 129; KCl, 3; NaH₂PO₄, 1.25; NaHCO₃, 21; CaCl₂, 1.6; MgSO₄, 1.8; D-glucose, 10, saturated with 95 % O₂/ 5 % CO₂, either ice-cold (during the slicing) or at 36 ± 1 °C (in the interface chamber).

Field potentials (FP) were extracellularly recorded with glass pipettes filled with ACSF (resistance < 3 MΩ) and placed 80–120 µm below the cut surface of the slice. Network activity was induced in different ways. To obtain persistent gamma oscillations in the CA3 region of the hippocampus, either 100 nM kainic acid (KA) or 10 µM acetylcholine (ACh) combined with the ACh-esterase blocker physostigmin (Physo) were permanently applied to the slice. For recurrent epileptiform discharges (REDs), slices were bathed in ACSF containing elevated K⁺ (8 mM instead of 3 mM; NaCl was instead reduced from 129 to 124 mM) and 20 µM bicuculline. REDs were recorded in layers V/VI of the medial entorhinal cortex in slices from naïve and pilocarpine-treated rats with proven epileptic seizures of Racine's scale stage 5 during the days before the experiment.

Intracellular recordings were made from CA3b pyramidal cells with sharp glass microelectrodes filled with 2 M K⁺-acetate (resistance 60-100 MΩ) during gamma oscillations. The measurements were started after the stabilization of gamma oscillations but at least 20 min after penetration. Only cells with stable overshooting action potentials (APs) over the full period of the experiment were accepted for analysis.

Changes in the extracellular ATP concentration were detected using microelectrode electrochemical biosensors (Sarissa Biomedical, Coventry, UK). The function of the sensor is described in detail elsewhere (Llaudet et al., 2005). In short, the sensor consists of a platinum (Pt) wire coated with the enzymes glycerol kinase and glycerol-3-phosphatase oxidase. These enzymes degrade ATP by producing H₂O₂ which gets subsequently oxidized on the Pt wire, finally resulting in two free electrons per ATP molecule. To estimate the ATP concentration on basis of the measured current, a one-point-calibration with 10 µM ATP was done before and after each experiment (Frenguelli et al., 2007).

Drugs were applied after stabilization of the respective network activity, for a period of 20 – 60 min, followed by a wash-out. In most cases, the last 10 min of the drug application phase was used for analysis. Data were collected on hard disk using Spike2 (Cambridge Electronic Design, Cambridge, UK). Analysis was done semi-automated by custom-made scripts in Spike2. Treatment groups were compared using ANOVA followed by Fisher's LSD *post hoc* test and, where appropriate, unpaired or paired Student's t-test, respectively. For circular data obtained by intracellular recordings during gamma oscillations, appropriate statistical tests were used (Batschelet, 1981; Zar, 2010). Significance level was set at $p < 0.05$.

RESULTS

Applied ATP reduces gamma oscillations mainly via adenosine receptors

Application of ACh/Physo and of KA, respectively, reliably induced persistent gamma oscillations in the hippocampal region CA3. In the KA model, application of 300 µM ATP reversibly reduced the peak power of gamma oscillations (Fig. 1A). Since this effect disappeared when the adenosine receptor antagonist CGS-15943 (10 µM) was applied prior to ATP, it can be suggested that ATP does not act on P2 receptors but on adenosine receptors after degradation to adenosine by ectonucleotidases in the extracellular matrix. On the contrary, in the ACh/Physo gamma oscillation model, CGS-15943 only partially prevented the effect of 300 µM ATP, indicating that in the ACh/Physo model, both P2 and adenosine receptors are involved in the reducing effect of applied ATP.

ATP is quickly metabolized in the extracellular space

A confirmation that ATP acting directly on P2 receptors reduced ACh/Physo-induced gamma oscillations was shown by the co-application of ATP and the ecto-ATPase inhibitor ARL-67156 (50 µM). ARL-67156 itself already reduced the gamma peak power, most probably by accumulation of endogenously released ATP. When external ATP was additionally applied, the gamma peak power was stronger reduced than by ATP alone.

To prove whether ATP was indeed quickly metabolized extracellularly, we used ATP-sensitive biosensors, with which we could show that during application of 300 μM ATP, only 1.06 ± 0.56 μM ATP (geometric mean) can be measured in CA3 *stratum pyramidale*.

P2Y and P2X receptors differentially modulate ACh-induced gamma oscillations

To investigate which P2 receptors were responsible for the reducing effect of exogenous applied ATP on ACh/Physo-induced gamma oscillations, we next applied different P2 antagonists. While the broad band P2 antagonist PPADS strongly increased the gamma peak power, the usage of more specific antagonists revealed a more detailed view: TNP-ATP, an antagonist for P2X1, P2X2, P2X3, and P2X4, mimicked the effect of PPADS, i.e. it increased strongly gamma peak power. Other P2X antagonists did not show any effects (Fig. 1B). Among the P2Y receptor antagonists, only the selective P2Y1 receptor antagonist MRS 2179 affected gamma oscillations: it reduced the gamma peak power (Fig. 1C). These results show opposing effects of P2Y₁ and P2X2 and/or P2X4 receptors on gamma oscillations. The latter two receptors were not distinguishable from each other due to a lack of more specific antagonists.

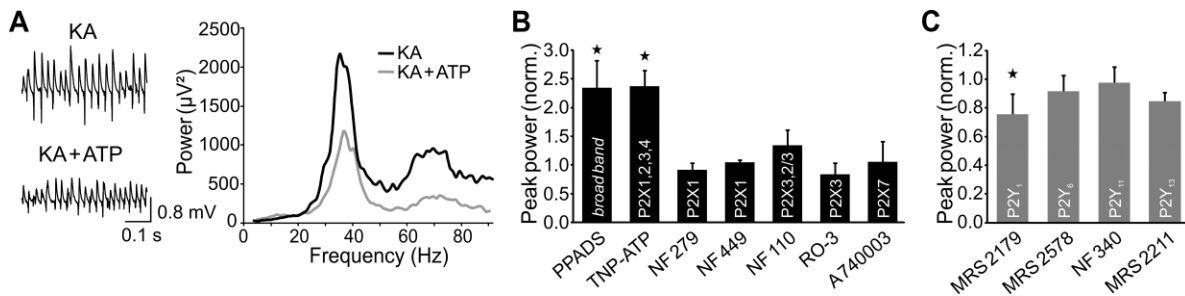


Fig. 1 (modified from Schulz et al., 2012a, Figs. 1, 4 and 5): (A) Kainate (KA)-induced gamma oscillations in the CA3 region of the hippocampus were inhibited by exogenous ATP. (B) Gamma oscillations induced by acetylcholine/physostigmin were enhanced by the P2 broad band antagonist PPADS and TNP-ATP, antagonist for P2X1, P2X2, P2X3 and P2X4. (C) Only the P2Y₁ antagonist MRS 2179 reduced the gamma peak power. * $p < 0.05$.

Blocking P2 receptors during ACh/Physo-induced gamma oscillations increases the firing rate of CA3b pyramidal neurons

Changes in gamma power can be caused by alterations of the firing rate of neurons or of the synchrony of synaptic and action potentials among cells. Therefore, to reveal which mechanism is responsible for the effects of P2 receptors we recorded intracellularly from CA3b pyramidal cells and related the spike timing to the simultaneously recorded ACh/Physo-induced gamma oscillations. Spike timing and accuracy were not altered after blockade of P2 receptors by PPADS, whereas the firing rate was significantly increased. Interestingly, application of the adenosine receptor antagonist CGS-15943, which amount for ~ 55 % of the reducing effect of ATP on ACh/Physo-induced gamma oscillations, did not result in any changes in the intracellular firing behavior (Fig. 2E).

Adenosine receptors reduce the rate and the accuracy of firing of CA3b pyramidal neurons during KA-induced gamma oscillations

Application of 300 μM ATP, which we have shown to inhibit KA-induced gamma oscillations exclusively via adenosine receptors, changed both spike timing and rate of pyramidal cells significantly. Spike timing was significantly altered by reducing the accuracy of spiking, whereas the preferred phase was not affected (Fig. 2A-C). In addition, the spike rate significantly and reversibly decreased in response to ATP (Fig. 2D).

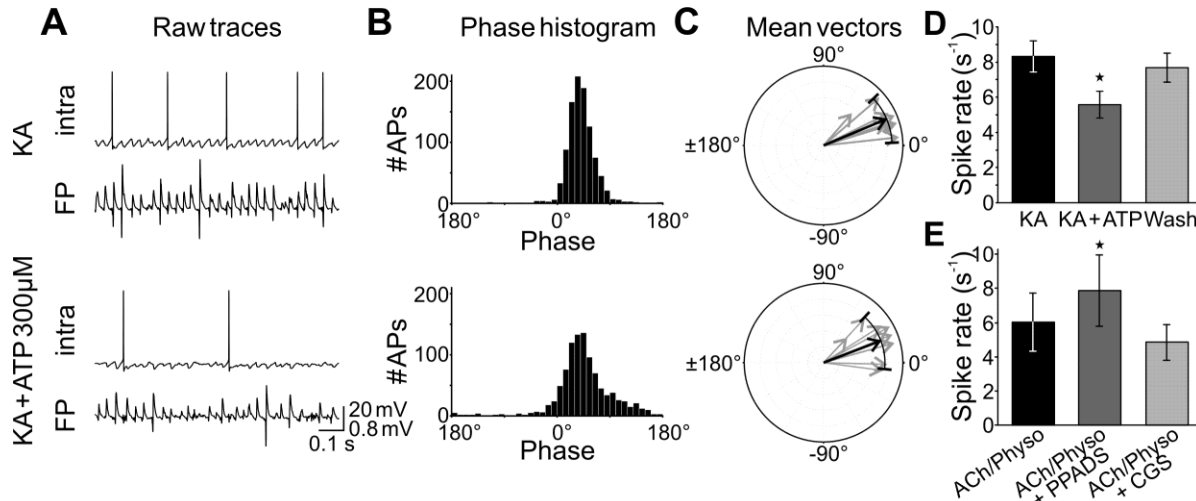


Fig. 2 (modified from Schulz et al., 2012a, Figs. 7 and 8): (A-D) ATP reduced the spiking rate and altered spike timing of CA3 pyramidal cells during kainate (KA)-induced gamma oscillations. All panels: top: control, bottom: application of 300 μM ATP. (A) Intracellular recording from a CA3 pyramidal cell and the corresponding field recording (FP). (B) Phase histograms of action potentials (APs) of the shown cell (bin size: 10°). (C) Mean vectors of each recorded cell (gray arrows, $n = 9$) and the mean vector \pm circular standard deviation of the whole population (black, bold arrow). 0° represents the troughs of the gamma cycles after low-pass filtering. (D) ATP reversibly reduced the spiking rate. (E) During gamma oscillations induced by acetylcholine/physostigmine (ACh/Physo), the P2 broad band antagonist PPADS increased, while the adenosine antagonist CGS-15943 did not affect the spiking rate.

Recurrent epileptiform discharges as a model of pathophysiological network activity

To investigate the role of purinergic receptors on recurrent epileptiform discharges as a model of hypersynchronous pathophysiological network activity, we applied elevated $[\text{K}^+]$ (8 mM) together with 20 μM bicuculline to induce REDs, as described earlier (Borck & Jefferys, 1999). RED incidence was significantly higher in slices from naïve rats than from pilocarpine-treated epileptic rats, while the mean duration of REDs was significantly shorter (Fig. 3A). There was no difference in the slow field potential amplitude.

Effects of adenosine receptors on recurrent epileptiform discharges

300 μM ATP significantly and reversibly reduced the incidence of REDs in both naïve slices and slices from pilocarpine-treated animals (Fig. 3B). The amplitude of the slow field potential was increased in chronic epileptic, but not in naïve slices. Duration of REDs was not affected in

either case. In both groups, application of the adenosine A₁ receptor antagonist DPCPX (1 μM) prior to ATP application prevented all observed effects of ATP. This indicates that the ATP effect is exclusively mediated by A₁ receptors which are activated after the degradation of ATP, comparable to KA-induced gamma oscillations. These results confirmed the known anticonvulsive effect of adenosine (Lee et al., 1984).

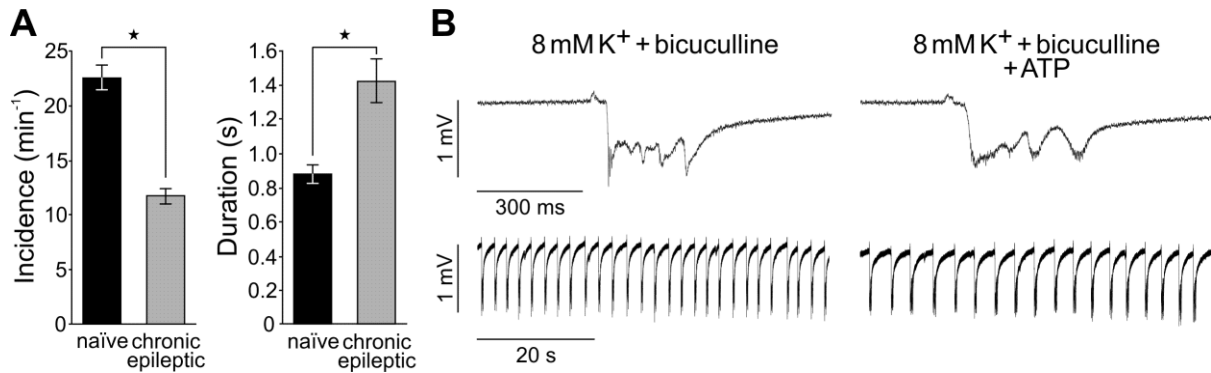


Fig. 3 (modified from Klafft et al., 2012, Figs. 1 and 2): (A) Properties of recurrent epileptiform discharges (REDs) in layer V/VI of medial entorhinal cortex of naïve and chronic epileptic rats. (B) ATP reduced the incidence of REDs, as shown here in a slice of a naïve rat. * $p < 0.05$.

Effects of P2 receptors on recurrent epileptiform discharges

Although the effect of exogenously applied ATP seems to be A₁ receptor-mediated, we aimed to test whether endogenously activated P2 receptors also have an effect on REDs. Therefore, we applied selective antagonists of these receptors and found that none of the broad-spectrum P2 antagonist PPADS, the P2X1, P2X2, P2X3 and P2X4 antagonist TNP-ATP and the selective P2Y1 antagonist MRS 2179 had an effect on any parameters of the REDs, neither in slices of naïve nor of chronic epileptic rats. In contrast, application of the specific P2X7 antagonist A 740003 reduced the amplitude of the slow field potentials in slices of naïve rats, whereas the incidence and duration of REDs were not changed. Since the slow field potential amplitude depends on the number of excited neurons, these results indicate a proepileptic effect of P2X7 receptors. Interestingly, the amplitude of slow field potentials was not affected by A 740003 in slices of chronic epileptic rats, indicating a different involvement of P2X7 receptors in REDs after epileptogenesis.

Effects of antipsychotics on gamma oscillations

Because disturbances in neuronal network activities are also related to psychotic symptoms (Kissler et al., 2000; Minzenberg et al., 2010), we next investigated the effect of eight approved antipsychotics on gamma oscillations. Out of them, three drugs (clozapine, haloperidol, risperidone) significantly reduced the gamma peak power of ACh/Physo-induced gamma oscillations, partially accompanied by an increase in the peak bandwidth, indicating reduced

oscillation coherence. Amisulpride, chlorprothixene, flupenthixol and ziprasidone had only a slight, but insignificant influence on the peak power. Chlorpromazine was the only investigated antipsychotic that tended to enhance the gamma peak power, but again, statistical significance was not achieved.

Correlation between pK_i values and changes in peak gamma power / bandwidth

All antipsychotics have a highly complex pharmacology with different affinities for a variety of receptors. To elucidate which receptors are responsible for their effects on gamma oscillations, we correlated the normalized inhibition in gamma peak power to the pK_i values of the individual antipsychotics for 19 different receptors. The same was done with the normalized increase in gamma peak bandwidth. For these analyses, it was assumed that the investigated antipsychotics act as competitive antagonists at the analyzed receptors. Several receptors showed a positive correlation with the power (e.g. the muscarinic receptors M_1 to M_5 , which can be explained by the cholinergic gamma induction), but only one of these correlations was significant: the serotonergic 5-HT₃ receptor (Fig 4A). A positive correlation indicates that drugs with high pK_i values (high affinity) for that receptor inhibited the power more effectively. This suggests that activation of 5-HT₃ receptors may enhance gamma power. Although 5-HT₃ receptors also showed the highest positive correlation of the pK_i values with the increase in gamma peak bandwidth, this did not reach statistical significance.

The dopamine D₃ receptor was the only receptor found to negatively correlate with the gamma power inhibition and the peak bandwidth increase (Fig. 4), suggesting that this receptor may reduce peak power and decrease the coherence of gamma oscillations.

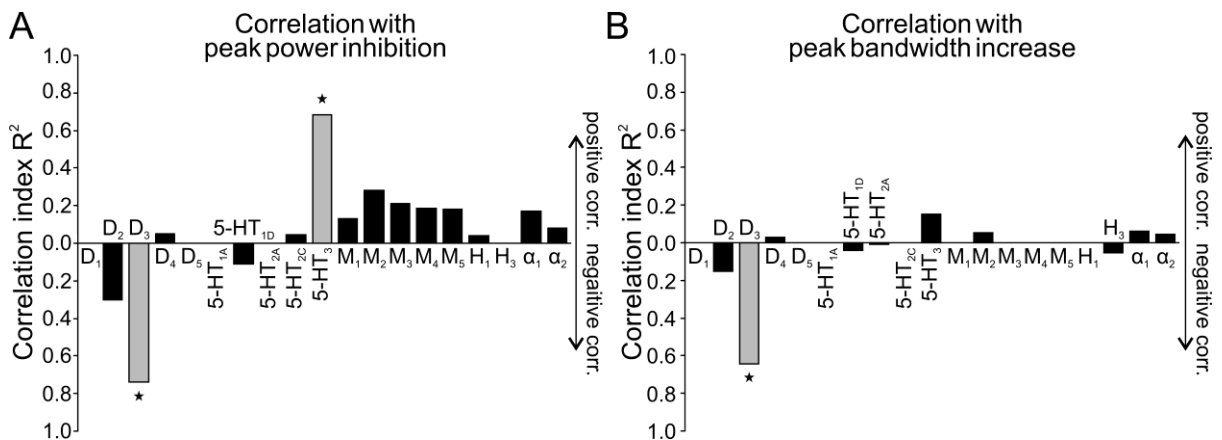


Fig. 4 (modified from Schulz et al., 2012b, Figs. 3 and 4): Correlations of the drug-induced inhibition of the peak power (A) and increase of peak bandwidth (B) with the drug-receptor pK_i values during ACh-induced gamma oscillations. The correlation index R^2 is shown for each receptor. Bars projecting downwards indicate a negative correlation, bars projecting upwards a positive correlation. * $p < 0.05$.

Effects of selective agonists of 5-HT₃, D₃ and 5-HT_{2C} receptors on gamma oscillations

To confirm these predictive calculations, we next applied selective agonists for 5-HT₃ and D₃ receptors and for 5-HT_{2C} receptors as an antipsychotic target without any correlation. As predicted, the 5-HT₃ agonist mCPBG (30 μM) increased the gamma peak power, without affecting the gamma peak bandwidth. In contrast, but in line with our predictions, the D₃ agonist PD 128907 (10 μM) decreased the gamma peak power and increased the gamma peak bandwidth. As expected, CP 809101 (30 μM), a 5-HT_{2C} receptor agonist, did not affect gamma oscillations. In conclusion, these data confirm the correlation data and the prediction for the receptor subtypes involved in the modulation of gamma oscillations by antipsychotics.

DISCUSSION

In the present studies we found that (a) activation of adenosine receptors dampen neuronal network activity both in models of physiological gamma oscillations and pathophysiological recurrent epileptiform discharges, (b) P2Y₁ receptors support gamma oscillations, (c) P2X2 and P2X4 receptors suppress gamma activity, (d) P2X7 receptors show a minor proepileptic effect, and (e) antipsychotics influence gamma oscillations by interacting with 5-HT₃ and D₃ receptors.

P2X7 receptors are Ca²⁺ permeable cation channels which have been described to mediate Ca²⁺ influx and glutamate release from neurons (Sperlágh et al., 2002). Moreover, P2X7 receptors seem to be involved in propagation of astrocytic Ca²⁺ waves and glutamate release (Suadicani et al., 2006). Blocking P2X7 receptors might therefore reduce the amount of released transmitters and limit the activation of neurons and astrocytes during REDs (Engel et al., 2012). In ACh-induced gamma oscillations, however, the total amount of released glutamate does not necessarily determine the power of oscillations because of the tonic activation and depolarization of neurons by ACh.

Rather, gamma oscillations are driven by a precisely timed perisomatic feedback inhibition onto pyramidal cells, mainly by parvalbumin (PV)-positive basket cells (Bartos et al., 2007; Gulyás et al., 2010). P2Y₁ receptors are functionally expressed on CA3 interneurons but not on pyramidal cells (Bowser and Khakh, 2004; Kawamura et al., 2004). Their stimulation may increase the precisely timed GABA release from PV-positive interneurons which may lead to increased synchronization of pyramidal cell firing and subsequently to enhanced oscillation power (Mann and Paulsen, 2007).

Neuronal P2X channels seem to be restricted to the axons of CA3 pyramidal cells (Khakh et al., 2003; Rodrigues et al., 2005; Khakh, 2009). Ionotropic receptors expressed on axon terminals or along the axons of CA3 pyramidal cells can modify network activity by altering synaptic transmission (Schicker et al., 2008) or axonal signal conduction by shunting the action potentials (Sasaki et al., 2011). Since generation of ectopic spikes in CA3 pyramidal cell axons

may be essential for the development of gamma oscillations (Traub et al., 2000, 2004), their inhibition by P2X2 and P2X4 receptors can explain the inhibitory effect of these receptors on gamma oscillations. Support for this hypothesis are our findings showing that a blockade of P2X channels increases the firing rate of pyramidal cells, possibly due to higher phasic excitatory input via the recurrent collaterals in the CA3 hippocampal region.

While PV-positive fast-spiking interneurons are most likely the generator of gamma oscillations (Gulyás et al., 2010), cholecystokinin (CCK)-containing regular spiking basket cells seem to have a rather modulatory role on the synchrony of neuronal populations, especially due to their potential to excite PV-positive cells by releasing CCK and subsequent postsynaptic activation of CCK2 receptors (Freund, 2003). Unlike PV-positive basket cells, CCK-containing interneurons express a number of neuromodulatory receptors such as serotonergic 5-HT₃ receptors (McMahon and Kauer 1997). Thus it can be expected that activation of 5-HT₃ receptors increase the power of gamma oscillations by stimulation of these cells. D₃ receptors have been shown to inhibit the amplitude of IPSCs in CA1 pyramidal cells possibly by causing internalization of GABA_A receptors on the postsynaptic site (Hammad and Wagner, 2006; Swant et al., 2008). This inhibition of inhibitory inputs onto hippocampal pyramidal cells may represent a possible mechanism how D₃ receptors are able to suppress the power and coherence of hippocampal gamma oscillations.

We found that ATP concentrations reached levels ~300 times lower in the slice than in the bath. This rapid degradation of ATP might elucidate why P2 receptors are hardly involved in effects evoked by applied ATP. In epileptic tissue, the degradation may be even faster, because ectonucleotidase activity is permanently raised in different epilepsy models (Bonan et al., 2000; Vianna et al., 2005). This can explain why the endogenous proepileptic effects of ATP via P2X7 receptors were attenuated in the chronic epileptic brain. However, endogenous ATP at concentrations in the range of a few hundred nanomolar to few micromolar can obviously finely tilt the balance between excitation and inhibition in neuronal networks via the activation of P2Y, P2X and adenosine receptors.

Our findings suggest new potential therapeutic targets for schizophrenia and epilepsy. We also found target receptors by which standard antipsychotics might improve the disturbed gamma oscillations in schizophrenia patients. Our results implicate that besides adenosine receptors (Lee et al., 1984, Pietersen et al., 2009), dopamine D₃ receptors and the purinergic P2Y₁, P2X2 and/or P2X4 might be new targets to ameliorate the symptoms of schizophrenia. Our data also suggest that correlation of receptor affinities with the biological effects can be used as a reliable approach to predict the targets responsible for the pharmacological effects of multi-target drugs.

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Die **Anteilserklärung** muss den Anteil des Promovenden/der Promovendin an den Publikationen ausweisen und von ihm/ihr und dem betreuenden Hochschullehrer/der betreuenden Hochschullehrerin unterschrieben sein:

Steffen Björn Schulz hatte folgenden Anteil an den vorgelegten Publikationen:

Publikation 1: Steffen B. Schulz*, Zin-Juan Klafft*, Anton R. Rösler, Uwe Heinemann, Zoltan Gerevich: Purinergic P2X, P2Y and adenosine receptors differentially modulate hippocampal gamma oscillations. *Neuropharmacology* 62, 914-924; 2012
40 Prozent

Beitrag im Einzelnen: Mitplanung der Experimente, Durchführung und Auswertung von ca. der Hälfte der Experimente, Mitarbeit am Manuskript.

Publikation 2: Steffen B. Schulz, Karin E. Heidmann, Arpad Mike, Zin-Juan Klafft, Uwe Heinemann, Zoltan Gerevich: First and second generation antipsychotics modulate hippocampal gamma oscillations by interactions with 5-HT₃ and D₃ receptors. *British Journal of Pharmacology* 167, 1480-1491; 2012
40 Prozent

Beitrag im Einzelnen: Mitplanung der Experimente, Durchführung der Mehrheit der Experimente, Auswertung von ca. der Hälfte der Experimente, Mitarbeit am Manuskript.

Publikation 3: Zin-Juan Klafft, Steffen B. Schulz, Anna Maslarova, Siegrun Gabriel, Uwe Heinemann, Zoltan Gerevich: Extracellular ATP differentially affects epileptiform activity via purinergic P2X7 and adenosine A₁ receptors in naïve and chronic epileptic rats. *Epilepsia* 53, 1978-1986; 2012
30 Prozent

Beitrag im Einzelnen: Mitplanung der Experimente, Durchführung von ca. der Hälfte der Experimente, Mitarbeit am Manuskript.

Unterschrift Promovend
Steffen Schulz

Unterschrift Betreuender Hochschullehrer
Prof. Dr. Uwe Heinemann

Publication 1: Steffen B. Schulz*, Zin-Juan Klafit*, Anton R. Rösler, Uwe Heinemann, Zoltan Gerevich (2012): Purinergic P2X, P2Y and adenosine receptors differentially modulate hippocampal gamma oscillations.
Neuropharmacology 62:914-924
DOI: 10.1016/j.neuropharm.2011.09.024

Publication 2: Steffen B. Schulz, Karin E. Heidmann, Arpad Mike, Zin-Juan Klaf, Uwe Heinemann, Zoltan Gerevich (2012): First and second generation antipsychotics modulate hippocampal gamma oscillations by interactions with 5-HT₃ and D₃ receptors.
British Journal of Pharmacology 167:1480-1491
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Publication 3: Zin-Juan Klafft, Steffen B. Schulz, Anna Maslarova, Siegrun Gabriel, Uwe Heinemann, Zoltan Gerevich (2012): Extracellular ATP differentially affects epileptiform activity via purinergic P2X7 and adenosine A₁ receptors in naïve and chronic epileptic rats. *Epilepsia* 53:1978-1986
DOI: 10.1111/j.1528-1167.2012.03724.x

Lebenslauf

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

List of own publications

Journals:

Klaft ZJ, **Schulz SB**, Maslarova A, Gabriel S, Heinemann U, Gerevich Z (2012). Extracellular ATP differentially affects epileptiform activity via purinergic P2X7 and adenosine A₁ receptors in naïve and chronic epileptic rats. *Epilepsia* 53(11):1978-1986

Schulz SB, Heidmann KE, Klaft ZJ, Mike A, Heinemann U, Gerevich Z (2012). First and second generation antipsychotics influence hippocampal gamma oscillations by interactions with 5-HT₃ and D₃ receptors. *British Journal of Pharmacology* 167(7):1480-1491

Schulz SB*, Klaft ZJ*, Rösler AR, Heinemann U, Gerevich Z (2012). Purinergic P2X, P2Y and adenosine receptors differentially modulate hippocampal gamma oscillations. *Neuropharmacology* 62(2):914-24

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Posters:

Klaft ZJ, Sandow N, Kim S, Päsler D, Leite Antonio L, Kovacs R, Kann O, Horn P, Vajkoczy P, Meencke HJ, Cavalheiro EA, **Schulz SB**, Gerevich Z, Gabriel S, Lehmann TN, Heinemann U (2012). Epileptiform activity in human neocortex slices: Induction, Pharmacoresistance and purinergic modulation. Gordon Conference, Waterville Valley, NH

Klaft ZJ, **Schulz SB**, Maslarova A, Gabriel S, Heinemann U & Gerevich Z (2012). Endogenously activated P2X₄, P2X₇ and P2Y₁ receptors have minor proconvulsive effects on epileptiform activity in rat medial entorhinal cortex. 8th FENS Forum of Neuroscience, Barcelona

Klaft ZJ, **Schulz SB**, Maslarova A, Gabriel S, Heinemann U, Gerevich Z (2012). Endogenously activated P2 receptors mediate minor modulatory effects on epileptiform activity in rat medial entorhinal cortex. Berlin Neuroscience Forum, Bad Liebenwalde

Mike A, Pesti K, Sas B, **Schulz SB** (2012). Unique type of sodium channel inhibition by riluzole. 56th Annual Meeting of the Biophysical Society, San Diego

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Klaft ZJ, **Schulz SB**, Rösler AR, Heinemann U, Gerevich Z (2010). ATP-activated P2X and P2Y receptors differentially modulate gamma network oscillations in the rat hippocampus. 7th FENS Forum of Neuroscience, Amsterdam

Schulz SB*, Klaft ZJ*, Rösler AR, Heinemann U, Gerevich Z (2010). Transiently decreased extracellular ATP concentration during the onset of hippocampal gamma network oscillations which are differentially modulated by ATP-activated P2X and P2Y receptors. EMBO Workshop “GABA Signaling and Brain Networks”, Amsterdam

Schulz SB, Klaft ZJ, Heinemann U, Gerevich Z (2010): Transiently decreased extracellular ATP concentration during the onset of hippocampal gamma network oscillations. Berlin Neuroscience Forum, Bad Liebenwalde

Klaft ZJ, **Schulz SB**, Rösler A, Heinemann U, Gerevich Z (2010). Differential modulation of gamma oscillations via ATP-activated P2X and P2Y receptors in the rat hippocampus. Berlin Neuroscience Forum, Bad Liebenwalde

Schulz SB, Klaft ZJ, Heinemann U, Gerevich Z (2009). Effect of ATP-activated P2 receptors on hippocampal gamma network oscillations. Berlin Brain Days 2009, Berlin

Schulz SB, Rochefort C, Scharff C (2008): Role of FoxP2 in proliferation and neurogenesis in the striatal ventricular zone, giving rise to Area X neurons in zebra finches. Berlin Neuroscience Forum, Bad Liebenwalde

* equally contributed

Erklärung

„Ich, Steffen Björn Schulz, erkläre, dass ich die vorgelegte Dissertation mit dem Thema:

Pharmacological modulation of physiological and pathophysiological neuronal network activities: special emphasis on purinergic ATP receptors

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

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