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# *In vivo*/*ex vivo* and behavioural study on central effects of 5-$HT_{1B/1D}$ and 5-$HT_{1A}$ antagonists in guinea pigs

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**Abstract**

Serotonin 5-$HT_{1A}$ and 5-$HT_{1B/1D}$ receptors control serotonin (5-HT) release and are targets for the pharmacological treatment of psychiatric disorders. We investigated effects of the 5-$HT_{1B/1D}$ antagonist GR127935, the 5-$HT_{1A}$ antagonist WAY 100635 and a combination of both in guinea pigs on the behaviour in the forced swimming test and on extracellular 5-HT in the hippocampus and the prefrontal cortex using *in vivo* microdialysis. Tissue content of 5-HT, 5-HIAA and 5-HT turnover (ratio 5-HIAA/5-HT) were determined in a sample containing i) the median and dorsal raphe nuclei, ii) the frontal cortex, or iii) the ventral hippocampus *ex vivo*.

*Behaviour:* Administration of WAY 100635 (0.3–3.0 mg/kg, i.p.) or GR127935 (1.0–10.0 mg/kg, i.p.) or the combination of both delayed immobility in the forced swim test.

*Microdialysis:* Systemic administration of WAY 100635 (1 mg/kg i.p.), perfusion with GR127935 (10 μM perfused into the frontal cortex) in the medial prefrontal cortex or the combination of both treatments had no significant effect on extracellular 5-HT.

*5-HT tissue content and 5-HT turnover in the tissue:* Compared to controls, WAY 100635, GR127935 and the combination thereof, decreased cortical 5-HT (−30%), increased 5-HIAA and consequently 5-HT turnover in the cortex threefold and the raphe nuclei twofold. WAY 100635 decreased 5-HT in the hippocampus (−40%), too. WAY 100635 and GR127935 and their combination increased hippocampal 5-HIAA and 5-HT turnover twofold, compared to controls.

The results suggest that both 5-$HT_1$ antagonists have subtle effects on 5-HT function under resting conditions; combined treatment has no superior effects compared to solitary treatment.


*Keywords:* 5-$HT_{1A}$ antagonist; 5-$HT_{1B/1D}$ antagonist; Cortex; Forced swim test guinea pig; Hippocampus; Microdialysis; Serotonin; Tissue content

## 1. Introduction

Serotonin receptors have been implicated to play an important role in the pathophysiology of depressive disorders and the mechanism of action of antidepressants.

Recently it has been shown that the dynamic modulation of 5-$HT_{1B}$ receptor function by the protein p11, which translocates the receptor to the membrane, is involved in molecular adaptations occurring in neuronal networks being dysfunctional in depression (Svenningsson et al., 2006; Svenningsson and Greengard, 2007). Further, evaluations of the pharmacological and endocrine actions of triptanes as 5-$HT_{1B/1D}$ receptor agonists indicate a reduced 5-$HT_{1B/1D}$ receptor function in depression (Cleare et al., 1998; Whale et al., 2001).

On the other hand, it has also been confirmed that the 5-$HT_{1A}$ receptor plays a key role in depression and response to antidepressants: During depressive episodes 5-$HT_{1A}$ receptor binding potential is increased in antidepressant naïve depressed patients compared to healthy volunteers and previously treated depressed subjects (Parsey et al., 2005) possibly due to a genetic variation of the 5-$HT_{1A}$ receptor gene in the dorsal raphe nucleus resulting in higher 5-$HT_{1A}$ receptor binding (Lemonde et al., 2004). Additionally, it is suggested that higher 5-$HT_{1A}$ binding may be associated with poorer response to antidepressants (Parsey et al., 2006; Serretti et al., 2004).

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Drawbacks of antidepressant therapy at present are the late onset of antidepressant effects and the relatively high percentage of non-responders to treatment. Therefore, numerous attempts have been undertaken to introduce new therapeutic strategies for depressive patients and multi-target strategies with adjunctive drug combinations are discussed to enhance the therapeutic effectiveness (Millan, 2006). Increased interest is directed towards the effects of antagonists at the 5-$HT_{1A}$ receptors and antagonists at 5-$HT_{1B/1D}$ receptors as an approach to augment or hasten the onset of therapeutic effect of selective reuptake inhibitors or tricyclic antidepressants (Hughes et al., 2007). Another attempt to improve the antidepressant therapy is to combine antagonists at 5-$HT_{1A}$ and 5-$HT_{1B/1D}$ receptors (Gobert et al., 2000; Hughes and Dawson, 2004).

In the present study we examined first whether or not 5-$HT_{1B/1D}$ receptors have an impact on behaviour of laboratory animals in a test for antidepressants, the Porsolt swim test. To test for an additive or superadditive effect of the combination of a 5-$HT_{1B/1D}$ antagonist with a 5-$HT_{1A}$ antagonist the behavioural effects of the 5-$HT_{1B/1D}$ antagonist GR127935 and the 5-$HT_{1A}$ antagonist WAY 100635, singly and in combination, were studied. Guinea pigs were chosen for our study, since it has been shown that in humans and guinea pigs both 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors are expressed in a similar pattern (Bidmon et al., 2001), whereas the expression in rats differs especially in the cortex (Varnas et al., 2005). Additionally, previous studies have shown that the effects of 5-$HT_{1B/1D}$ antagonists on extracellular 5-HT levels are diminished in rats in comparison to guinea pigs (Hervas et al., 2000; Hughes and Dawson, 2004).

In a second part of the study we characterised the effects of the 5-$HT_{1B/1D}$ receptor antagonist, the 5-$HT_{1A}$ receptor antagonist and their combination on 5-HT transmission in cortex and hippocampus, brain structures with relevance for depression-like symptoms and the action of antidepressants. Intracerebral microdialysis studies in freely moving guinea pigs in their home cages and an *ex vivo* assay for determination of tissue contents of 5-HT and 5-HIAA and consequently the 5-HT turnover were carried out. Previous studies of our group and pilot experiments preceding this study could not demonstrate any effect of GR127935 on extracellular 5-HT levels in cortex of guinea pigs in the familiar environment of the home cage (Rex et al., 1996). These previous data implicate that extracellular 5-HT levels following systemic administration of the 5-$HT_{1B/1D}$ antagonist GR127935 are controlled by both the differently located 5-$HT_{1B/1D}$ autoreceptors, the one on serotonergic nerve endings in several terminal regions and the other as autoreceptor on varicosities within the raphe nuclei in an opposite manner. It has been shown that intra-raphe perfusion with a 5-$HT_{1B/1D}$ agonist decreases serotonin release in the raphe region (Davidson and Stamford, 1995) and consequently increases serotonin release in projection areas. However, 5-$HT_{1B/1D}$ agonists acting primarily at presynaptic receptors on the nerve endings in the projection areas have been demonstrated to induce an opposite functional effect, i.e. a decrease in serotonin release in terminal areas (Adell et al., 2001). Therefore, it is conceivable that conflicting results have been obtained regarding the effects of 5-$HT_{1B/1D}$ antagonists after systemic administration on *in vivo* serotonin release in several brain regions of rodents: Either an increasing (Rollema et al., 1996; Stenfors et al., 2004) or no effect on extracellular 5-HT levels (Rex et al., 1996; Rollema et al., 1996; Stenfors et al., 1999) or decreased 5-HT levels in freely moving animals (de Groote et al., 2003; Roberts et al., 1999; Skingle et al., 1995). Since in our previous experiments GR127935 failed to influence extracellular 5-HT levels in the cortex of guinea pigs in home cage, in the present microdialysis study the substance was directly infused in the cortex to avoid the counteracting influence via dendritic 5-$HT_{1B/1D}$ receptors in the raphe region. It was hypothesised that thereby the antagonistic effect of GR127935 on presynaptic receptors in terminal structures will be uncovered, inducing a local increase in cortical extracellular 5-HT at least. It was postulated that then a combined effect of both antagonists might be demonstrated.

Similar to the 5-$HT_{1B/1D}$ receptors the 5-$HT_{1A}$ receptors are widely distributed in the brain and serve as somatodendritic autoreceptors on serotonergic raphe neurons and as postsynaptic heteroreceptors in many brain regions. It is generally accepted that a stimulation of the somatodendritic 5-$HT_{1A}$ autoreceptors inhibits serotonin release in the projection areas. When studying the effects of 5-$HT_{1A}$ antagonists differing results have been observed, too: Some studies showed a stimulation of serotonin release following systemic administration (Mundey et al., 1996; Stenfors et al., 1999), while others revealed no effect on 5-HT release (Assie and Koek, 1996; Hughes and Dawson, 2004). This discrepancy especially regarding the action of the 5-$HT_{1A}$ antagonists is explained by the low endogenous tone of the serotonergic transmission in the raphe region and consequently at 5-$HT_{1A}$ receptors under resting conditions and the limited sensitivity of detection methods for 5-HT.

When summarizing the above mentioned pharmacological actions of 5-$HT_{1A}$ antagonists and 5-$HT_{1B/1D}$ antagonists the following can be hypothesised: Blocking simultaneously both 5-$HT_{1A}$ somatodendritic autoreceptors and 5-$HT_{1B/1D}$ presynaptic autoreceptors in projection areas is expected to enhance serotonergic neurotransmission by abolishing the inhibition of i) neuronal firing through 5-$HT_{1A}$ receptors and ii) of terminal release through 5-$HT_{1B/1D}$ receptors.

Surprisingly, there are only few studies determining serotonin release in the CNS following simultaneous blockade of the 5-$HT_{1A}$ and the 5-$HT_{1B/1D}$ receptors with differing results and only following systemic drug administration (Hervas et al., 2000; Hughes and Dawson, 2004; Stenfors et al., 1999).

Blockade of the terminal 5-$HT_{1B/1D}$ autoreceptors increases release of 5-HT into the extracellular space. Reuptake mechanisms transport most of the released 5-HT back into the terminals, where it is either deaminated to 5-hydroxyindoleacetic acid (5-HIAA) or stored in the vesicles. The ratio of 5-HIAA/5-HT is been used as a measure of 5-HT turnover (Ross and Stenfors, 1997).

It has been shown earlier, that drugs may affect the amount of stored 5-HT without changes in 5-HT release (Rex et al., 2003; Schaechter and Wurtman, 1989). Therefore, beside the *in vivo* microdialysis studies, we have determined *ex vivo* the content of 5-HT, its main metabolite 5-hydroxy-3-indoleacetic acid (5-

HIAA) and calculated subsequently the 5-HT turnover (5-HIAA/5-HT ratio) in a region containing both median and dorsal raphe nuclei, the frontal cortex and the ventral hippocampus.

## 2. Experimental procedures

### 2.1. Animals

Female Dunkin Hartley Guinea Pigs (Charles River, Kisslegg, Germany), 370±20 g were housed in groups of 4 under a 12/12 h light/dark schedule. Water and food were freely available. All experiments were carried out between 8:00 a.m. and 4:00 p.m.

The animals were aged between 50 and 70 days at use, which is before sexual maturity.

### 2.2. Drugs

5-$HT_{1B/1D}$ antagonist GR127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-95-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide) (Sigma Aldrich), 5-$HT_{1A}$ antagonist WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexane carboxamide) or vehicle (saline+1% Cremophor EL). The injection volume was 1 ml/kg for the i.p. injections and 1 µl/min for the reversed microdialysis, respectively.

### 2.3. Porsolt swim test

The procedure of the test was similar to the procedure of the rat forced swim test introduced by Porsolt and is described in detail by Wicke et al. (in press). Briefly, the guinea pig forced swim test consists of a training session and the scoring session in the water tank: Twenty-four hours before the scoring session, animals were placed gently into a cylinder (height 40 cm, inner diameter 28 cm, water depth: 21 cm) filled with warm water (30±1 °C) for a training swim session of 5 min. Afterwards the animals were allowed to dry under a bowl-fire before they received an i.p. injection of GR127935 (1.0, 3.0, 10.0 mg/kg), WAY 100635 (0.3, 1.0, 3.0 mg/kg), a combination of GR127935 (3.0 mg/kg) and WAY 100635 (1.0 mg/kg) or vehicle and were returned into their home cage. According to the classic Porsolt regimen the guinea pigs received the next day two identical drug injections at 4 h and 30 min before the scoring session. For investigation of swimming behaviour they were transferred into the cylinder for a 5-min observation period. The latency to become immobile was determined (Wicke et al., in press). This was defined as the time at which the guinea pig first initiated a stationary posture. This parameter has been established already in the rat and mice forced swim test (Carlezon et al., 2002; Pliakas et al., 2001). For each experiment fresh water was used.

### 2.4. Microdialysis

Seven days prior to testing the animals received a neuroleptanalgesia by an i.m. injection of a freshly prepared mixture of fentanyl (0.05 mg/kg)+midazolam (2.0 mg/kg)+xylazine (2.0 mg/kg) (Henke and Erhardt, 2004). Two microdialysis guide cannulae were implanted unilaterally into the hippocampus (AP=−5.6 mm, ML=4.7 mm from bregma and depth=7.5 mm from brain surface) and into the prefrontal cortex (AP=+4.0 mm, ML=3.5 mm from bregma and depth=3.0 mm from brain surface) (Rössner, 1965) and fixed to the skull with acrylic cement. Following surgery the animals were placed in individual cages with water and food (Altromin 3230®) ad libitum and allowed to recover.

Twenty-four hours before the experiment a microdialysis probe (CMA 12; CMA/Microdialysis AB, Sweden, 2.0 mm dialysis membrane) was inserted into each guide cannula and perfused with artificial cerebrospinal fluid (CSF, 0.3 µl/min) containing: 5 mM glucose, 125 mM NaCl, 27 mM $NaHCO_3$, 2.5 mM KCl, 0.5 mM $NaH_2PO_4$, 1.2 mM $Na_2HPO_4$, 0.5 mM $Na_2SO_4$, 1 mM $MgCl_2$, 1 mM $CaCl_2$. Two hours before testing the probe flow rate was increased (1 µl/min). Microdialysis samples (20 µl) were taken at 20-min intervals and analysed immediately using HPLC with electrochemical detection (for details see: Rex et al., 1999).

At first, samples were collected every 20 min until a stable basal 5-HT level could be determined. Then the resting animals received the 5-$HT_{1B/1D}$ antagonist GR127935 (10 µM perfused into the frontal cortex by reversed microdialysis for the next 120 min) or vehicle via the cortical microdialysis probes followed by the intraperitoneal administration of WAY100635 (1 mg/kg i.p.) or vehicle 60 min after the start of the GR127935 perfusion. Dialysis samples were collected for the following 140 min in the home cage. The concentration of GR127935 was chosen on the basis of the *in vitro* recovery of GR127935 (13±4%) in our lab and the GR127935 mediated effects on 5-HT efflux shown previously (de Groote et al., 2003; Hallbus et al., 1997; Hervas et al., 1998; Roberts et al., 1997).

### 2.5. 5-HT+5-HIAA content and 5-HT turnover

Following a seven day wash out period the animals were randomly assigned to a treatment identical to the microdialysis experiments with none of the animals receiving exactly the same treatment as in the first experiment. Forty minutes after the second administration the animals were briefly sedated with isoflurane (Forene®, Abbott GmbH, Germany) decapitated, and the brains carefully removed and immediately frozen in liquid nitrogen. Subsequently, sagittal sections (1 mm thick) of the prefrontal cortex (+3.2 mm relative to bregma), the hippocampus (−5.8 relative to bregma) and a region containing the median/dorsal raphe nucleus (−7.8 mm relative to bregma; Rössner, 1965) were prepared and the regions of interest dissected using a tissue punch cannula (inner diameter 1 mm, Hauptner, Germany). The samples were weighed, immersed in ice cold 0.1 M perchloric acid (600 µl), homogenised and centrifuged for 10 min (14,000 rpm at 5 °C). The concentrations of 5-HT and 5-HIAA in the supernatant were determined by HPLC with electrochemical detection (Bert et al., 2001). Concentrations of 5-HT and 5-HIAA were indicated in ng/mg wet tissue and pg/mg wet tissue, respectively.

Table 1
Effects of the 5-$HT_{1A}$ antagonist WAY100635 (i.p.) and the 5-$HT_{1B/1D}$ antagonist GR127935 (i.p.), administered alone and combined, on the latency to immobility in the forced swim test

| Treatment | Dose [mg/kg] | Latency to immobility [s] |
|---|---|---|
| Vehicle | | 77.7±9.5 |
| WAY100635 | 0.3 | 102.5±15.0 |
| | 1.0 | 128.0±13.2* |
| | 3.0 | 135.8±7.8* |
| GR127935 | 1.0 | 92.4±11.7 |
| | 3.0 | 121.7±13.8* |
| | 10.0 | 136.6±38.4* |
| WAY100635+GR127935 | 1.0+3.0 | 142.8±32.0* |

The data are presented as mean±SEM and were analysed using a One Way ANOVA followed by the Holm-Sidak-Test. ($n$=6–7, *$p$<0.05).

The ratio of 5-HIAA and 5-HT concentrations was calculated and, as a marker for serotonin degradation, used as a measure of the 5-HT turnover.

All experiments were performed according to the principles of laboratory animal care and the German Law on the Protection of Animals.

### 2.6. Statistics

In the Porsolt swim test for each animal receiving a drug one control animal, which had received vehicle, was tested at the same time. Data of the Porsolt swim test were analysed using a One Way ANOVA followed by an all pairwise multiple comparison with the Holm-Sidak-Test. All microdialysis data are expressed as a percentage of basal levels. Statistical analysis of the microdialysis data was performed with a Two Way Repeated Measures ANOVA with time and treatment as factors followed by the Holm-Sidak method. The data from the *ex vivo* experiments were analysed using a One Way ANOVA followed by all pairwise multiple procedures (Holm-Sidak method). All data are presented as means±SEM. Differences of the means $P$<0.05 were considered as statistically significant. Group size for the microdialysis experiments and the Porsolt swim test was $n$=6–10 and for the determination of 5-HT tissue levels $n$=6. SigmaStat® V3.0.1 was used for statistical analysis.

## 3. Results

### 3.1. Porsolt swim test

The 5-$HT_{1A}$ antagonist WAY 100635 and the 5-$HT_{1B/1D}$ antagonist GR127935, respectively, significantly increased the latency to immobility in a dose-related manner [$F$(7, 58)=3.682, $p$=0.02]. The lowest significantly effective doses were 1.0 mg/kg for WAY 100635 ($p$=0.005) and 10 mg/kg for the 5-$HT_{1B/1D}$ antagonist GR127935 ($p$=0.001). Treatment with a combination of both drugs had no additive effects ($p$=0.736 vs. GR127935, $p$=0.421 vs. WAY 100635) (Table 1) on the behaviour of the guinea pigs in the forced swim test.

### 3.2. Microdialysis

The following basal levels of 5-HT and 5-HIAA were determined by microdialysis in the prefrontal cortex: 5-HT 10.2±0.75 fmol/20 µl, 5-HIAA 1.6±0.12 pmol/20 µl and in the ventral hippocampus: 5-HT 11.7±1.02 fmol/20 µl, 5-HIAA 1.7±0.16 pmol/20 µl.

In the prefrontal cortex the 5-$HT_{1B/1D}$ antagonist GR127935 perfused into the cortex and the 5-$HT_{1A}$ antagonist WAY 100635 ($p$=0.619), given systemically, had no significant effect on the 5-HT efflux at any time following administration [$F$(3, 30)=1.626, $p$=0.447]. However, following perfusion with GR127935 the 5-HT levels determined with microdialysis tended to decrease ($p$=0.073). The combined treatment with both drugs also had no significant effect on the 5-HT efflux in the cortex ($p$=0.447; Fig. 1).

In the ventral hippocampus neither perfusion with the 5-$HT_{1B/1D}$ antagonist GR127935 ($p$=0.429) nor the i.p. administration of the 5-$HT_{1A}$ antagonist WAY 100635 ($p$=0.092) had a significant effect on the 5-HT efflux at any single time point following administration [$F$(3, 30)=1.994, $p$=0,134]. The combined treatment with both drugs also had no significant effect on the 5-HT efflux in the cortex ($p$=0.447; Fig. 1) and in the ventral hippocampus ($p$=0.188; Fig. 2).

The efflux of 5-HIAA in the prefrontal cortex [$F$(3, 30)=1.224, $p$=0,310] and in the ventral hippocampus [$F$(3, 30)=

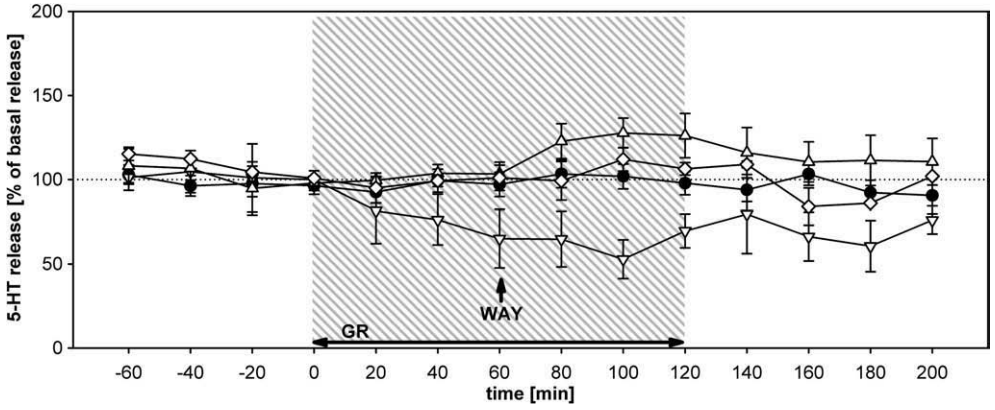


Fig. 1. GR127935, (10 µM perfused into the frontal cortex for 120 min [shaded area], ▽, $n$=6), WAY 100635, 1.0 mg/kg i.p., (△, $n$=6) and both drugs co-administered (◇, $n$=6) had no sustained effect on the 5-HT release in the prefrontal cortex compared to vehicle treated controls (●, $n$=6). The data are presented as mean±SEM and were analysed using a Two Way Repeated Measures ANOVA with time and treatment as factors, followed by the post hoc Holm-Sidak-test.

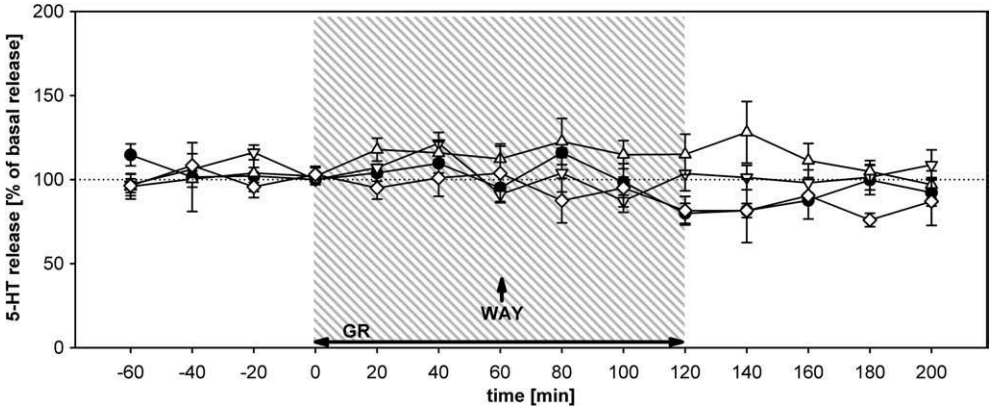


Fig. 2. Impact of GR127935, (10 μM perfused into the frontal cortex for 120 min [shaded area],▽, $n=6$), WAY 100635, 1.0 mg/kg i.p., (△, $n=6$) and both drugs co-administered (◇, $n=6$) on the 5-HT release in the ventral hippocampus compared to vehicle treated controls (●, $n=6$). The data are presented as mean±SEM and were analysed using a Two Way Repeated Measures ANOVA with time and treatment as factors, followed by the post hoc Holm-Sidak-test.

0.910, $p=0{,}447$] did not change significantly during the experiment. No obvious variations in motor activity were observed in the guinea pigs during the experiments.

### 3.3. 5-HT + 5-HIAA content and 5-HT turnover

The tissue concentrations of 5-HT and the main metabolite 5-HIAA were determined in discrete brain areas, relevant for the central serotonergic neurotransmission system. The analysis revealed noticeable effects of WAY 100635 (1 mg/kg, given systemically) and GR127935 (10 μM, perfused into the cortex) alone or in combination on the tissue concentrations of 5-HT in the prefrontal cortex [$F(3, 35)=7.253$, $p=0.002$] and decreased tissue contents of 5-HT caused by WAY 100635 only in the ventral hippocampus [$F(3, 35)=11.150$, $p<0.001$]. Neither WAY 100635, nor GR127935, nor the combination thereof

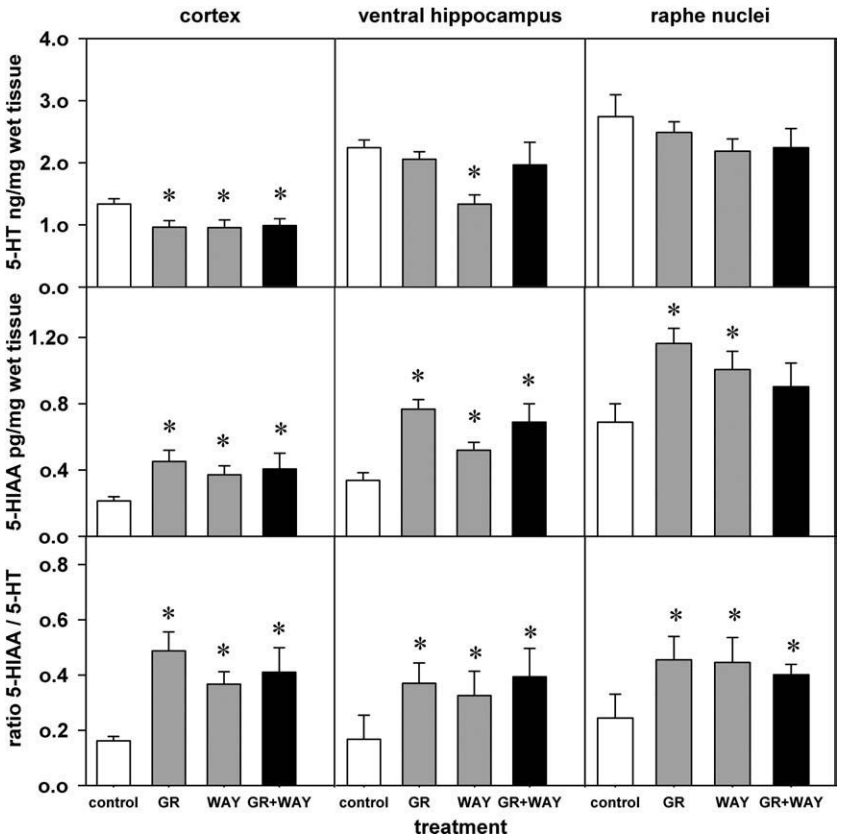


Fig. 3. Effect of GR127935, (10 μM perfused into the frontal cortex for 20 min, GR $n=6$), WAY 100635, 1.0 mg/kg i.p., (WAY, $n=6$) and both drugs co-administered (GR + WAY, $n=6$) on the 5-HT tissue content, the 5-HIAA tissue content and the 5-HT turnover (5-HIAA/5-HT ratio) in the tissue in the prefrontal cortex, the ventral hippocampus and a region containing the dorsal and the median raphe nuclei compared to vehicle treated controls ($n=6$). The data are presented as mean±SEM and were analysed using a One Way ANOVA followed by the post hoc Holm-Sidak-test. *$p<0.05$ compared to vehicle treated controls. ($n=6$).

changed the tissue content of 5-HT in the median and dorsal raphe nuclei (Fig. 3).

Determination of 5-HIAA contents in homogenates of the frontal cortex, the ventral hippocampus and the brain region containing the dorsal and the median raphe nuclei showed drug induced increases in the 5-HIAA concentration (Fig. 3). Calculating the ratio of 5-HIAA and 5-HT, we found that WAY 100635, GR127935 as well as the combined treatment of both drugs increased the metabolism of 5-HT in the cortex approximately threefold [$F(3, 35)=7.072$, $p<0.001$] and in the hippocampus twofold [$F(3, 35)=13.617$, $p<0.001$] as well as in the raphe nuclei [$F(3, 35)=7.150$, $p<0.05$], where no significant effect on the 5-HT tissue content could be seen (Fig. 3).

## 4. Discussion

Our results show that antagonism at the 5-$HT_{1B/1D}$ receptors prolongs the latency to immobility in guinea pigs in the forced swim test, a parameter associated with antidepressant-like activity not only in rats (Carlezon et al., 2002; Pliakas et al., 2001) but also guinea pigs (Wicke et al., in press). This is in line with recently reported effects of the $5HT_{1B}$ receptor antagonist SB-616234 exhibiting anxiolytic/antidepressant-like activity in the forced swim test in mice and ultrasonic vocalisation test in guinea pigs and rats (Dawson et al., 2006). The 5-$HT_{1A}$ antagonist WAY 100635 dose-dependently delayed the onset of immobility in the guinea pig. While WAY 100635 had not shown antidepressant-like action in rats on its own, it intensifies the antidepressant-like effect of other antidepressants in the rat (Tatarczynska et al., 2004). On the other hand, the forced swim test is used to detect antidepressants, i.e. SSRIs and other drugs, which increase 5-HT release. WAY 100635 blocking somatodendritic 5-HT receptors in the raphe nuclei antagonises the autoinhibition of 5-HT and consequently, increases the firing rate of 5-HT neurons causing a stimulated 5-HT release in projection areas (Liu et al., 2005; Mlinar et al., 2005). Based on this principle, the observed antidepressant-like effect of WAY 100635 in the guinea pig is plausible.

However, unexpectedly, the combination of both drugs did not induce a more pronounced effect than each antagonist alone. Since our data demonstrate behavioural effects of both drugs in the Porsolt test in guinea pigs a neurochemical characterisation of the drug actions was worthwhile. Additionally, we evaluated the effects of the drug combination on 5-HT transmission in the neurochemical studies.

It is known that 5-$HT_{1B/1D}$ receptors are not only located in projection areas of the serotonergic system, but also in the raphe nuclei. Most of the previous work was directed toward the function of the 5-$HT_{1B/1D}$ autoreceptors focussed on the role of the raphe nuclei in regulation of local 5-HT release. In only two studies the effects of local and systemic administration of 5-$HT_{1B}$ agonists and antagonists in rat or guinea pig brain regions, but not in the hippocampus, were determined, showing different drug effects in respect to the region examined and the route of drug administration, and wide variations in drug effects (Adell et al., 2001; Roberts et al., 1997).

Electrophysiological and neurochemical studies indicate that 5-$HT_{1B/1D}$ receptor antagonists lead to opposite effects in the raphe region and in the projection areas: in raphe nuclei these drugs induce an increase of the local release (Hopwood and Stamford, 2001; Roberts and Price, 2001), consequently decreasing the 5-HT release in projection areas by autoinhibition, whereas in the projection areas 5-HT release is increased by antagonist action directly at the local presynaptic 5-$HT_{1B/1D}$ receptors.

In a previous microdialysis study of our group in awake guinea pigs and in pilot experiments to this study we could not demonstrate an effect of GR127935 given systemically on extracellular 5-HT in frontal cortex, suggesting an interference of drug actions at both receptor locations.

To avoid a direct antagonist action at the 5-$HT_{1B/1D}$ receptors located in the dorsal and median raphe nuclei (Adell et al., 2001) and to assess solely the effects of a blockade of the terminal autoreceptors in the serotonergic projection areas we used the reverse microdialysis technique to administer GR127935 into the frontal cortex.

Our *in vivo* microdialysis study revealed no significant effects on the extracellular 5-HT concentration following administration of the 5-$HT_{1B/1D}$ antagonist GR127935 or the 5-$HT_{1A}$ antagonist WAY100635 either given alone or in combination, despite the fact that the doses used had effects on 5-HT efflux reported in the literature (Roberts et al., 1997), despite the fact that the doses of GR127935 and WAY 100635 used were effective in other studies.

In our opinion, the results confirm the idea of a low tonic activity of the serotonergic raphe neurons under resting conditions in calm and non-stressed animals. However, the 5-$HT_{1A}$ antagonist as well as the 5-$HT_{1B/1D}$ antagonist seem to show more efficacy if extracellular 5-HT is increased and the somatodendritic autoreceptors are stimulated, e.g. by administration of a serotonin re-uptake inhibitor (Jongsma et al., 2005; Roberts et al., 1999). Discrepancies to the results of other groups, where local application of the 5-$HT_{1B/1D}$ antagonist GR127935 into the cortex in a similar dose induced an increase in cortical 5-HT efflux (Roberts et al., 1997) could be caused by a higher endogenous 5-HT tone at the terminal 5-$HT_{1B/1D}$ autoreceptors, compared to our animals. Other authors reported that WAY 100635 increases serotonergic function only when serotonergic neuronal activity was high but not when activity was low (Johnson et al., 2002). Additionally, strain differences, as known from rats, have to be taken into account, too. Genetic differences, e.g. polymorphisms of the 5-HT transporters, could also contribute to the divergent effects of 5-$HT_1$ antagonists on extracellular 5-HT. A more effective re-uptake mechanism raises the possibility that although 5-HT release might be amplified after administration of 5-$HT_1$ antagonists, excess extracellular 5-HT might be swiftly removed (Johnson et al., 2002).

Other authors described a decrease in cortical 5-HT efflux following local perfusion at lower doses (Skingle et al., 1995). Looking at our results showing no effect of GR127935, it might be considered, that GR127935 has been reported to possess also partial agonist properties (de Groote et al., 2003; Roberts et al., 1997), which could lead to a direct interference with WAY 100635 in our experiments.

The present results are in line with studies in guinea pigs and rats, where WAY 100635 reversed the effects of the 5-$HT_{1A}$ agonist 8-OH-DPAT but had no apparent effect alone (Assie et al., 2005; Fletcher et al., 1996).

It has also taken into account the principle of the *in vivo* microdialysis. The microdialysis determines "only" compounds that are released in larger quantities overflowing the synaptic cleft and seeping into the extracellular space. Dialysate concentrations represent only a fraction of actual concentrations in the extracellular space surrounding the microdialysis probe. So using the microdialysis changes in transmitter release of a smaller magnitude might be missed. As an additional reason for the absence of change in extracellular 5-HT after treatment with either a 5-$HT_{1A}$ antagonist and/or a 5-$HT_{1B/1D}$ receptor antagonist has been suggested an effective reuptake of 5-HT, preventing 5-HT molecules to overflow the extracellular space in a larger area (Pineyro and Blier, 1999).

*In vivo* microdialysis as well as the determination of the tissue levels *ex vivo* assesses different facets of the serotonergic neurotransmission. Since it is known that drugs may lower the tissue levels 5-HT without affecting the release of 5-HT (Rex et al., 2003; Schaechter and Wurtman, 1989), we determined *ex vivo* 5-HT and its major metabolite 5-HIAA in tissue samples from the same projection areas as used in the *in vivo* microdialysis experiments as well as in a region containing the dorsal and the median raphe nuclei.

Our *ex vivo* experiments revealed a fairly uniform pattern of changes in the 5-HT turnover. In the *ex vivo* experiments we could show a marked increase in the 5-HIAA/5-HT ratio suggesting an accelerated turnover of 5-HT following administration of WAY 100635 or GR127935 as well as the combination of both drugs.

Tissue concentrations of 5-HT were decreased by the 5-$HT_{1A}$ antagonist WAY 100635 in the ventral hippocampus and the frontal cortex, both regions with high density of 5-$HT_{1A}$ receptors, demonstrating an effect on serotonergic function not seen in the microdialysis experiments. The 5-$HT_{1B/1D}$ antagonist GR127935 reduced the 5-HT concentration only in the frontal cortex, the perfusion site.

While the 5-$HT_{1A}$ antagonist WAY 100635 alone and in combination with GR127935 tended to decrease the 5-HT content in the raphe nuclei, the 5-$HT_{1B/1D}$ antagonist alone had no effect on the 5-HT concentration in the raphe region. Both results suggest that the feed back loop mediated through postsynaptic 5-$HT_{1A}$ receptors and somatodendritic autoreceptors is of secondary importance. In addition, we cannot exclude the possibility that the techniques available and used in our study are not sensitive enough to pick up smaller effects caused by this feed back loop.

It is well known that most of the 5-HT released into the synaptic cleft will be transported into the presynapse by the 5-HT transporters, and either degraded to 5-HIAA or stored in the vesicles. An increased ratio of 5-HIAA and 5-HT in the tissue is an indication for an augmented degradation of 5-HT, previously re-uptaken from the synaptic cleft. Interestingly administration of WAY 100635 and GR127935 increased the turnover of 5-HT in the frontal cortex and in the ventral hippocampus, inferring an increased re-uptake of 5-HT from the synaptic cleft, preceded by an enhanced release of 5-HT. Since GR127935 was infused in the cortex region for a longer period, diffusion of the drug towards the hippocampus could explain the concomitant effect in this brain region. In own previous experiments extensive diffusion of drugs microinjected intracerebrally could be seen starting at 20 min after injection (personal communication Dr. Fink).

The co-administration of the 5-$HT_{1B/1D}$ antagonist GR127935 with the selective 5-$HT_{1A}$ antagonist WAY 100635 had no additive or superior effect on the 5-HT turnover in the cortex, in the ventral hippocampus or in the region containing the raphe nuclei. This in accordance with behavioural effects showing also neither a summation nor a potentiating effect of the drug combination in comparison to the single drug effects.

In conclusion, the present microdialysis data in freely moving and non-stressed guinea pigs suggest that in our study and under resting conditions both 5-$HT_{1A}$ and 5-$HT_{1B/1D}$ autoreceptors apparently are under a rather marginal tonic control and antagonists may have therefore only small or no effects. This has also been suggested in *in vitro* electrophysiological studies (Johnson et al., 2002). Additionally, an avid reuptake mechanism may also dampen release-enhancing effects of the 5-$HT_1$ autoreceptor antagonists.

However, the *in vitro* tests showing region-specific decreases in 5-HT and the concomitant increase in local 5-HT turnover suggest that both 5-$HT_1$ antagonists have subtle effects on 5-HT function, even under resting conditions. However, the expected additive effect of the drug combination did not occur.

In our opinion chances are that in normal non-stressed animals the functional impact of 5-$HT_{1A/1B/1D}$ antagonists is less noticeable but more pronounced in animals with malfunction of serotonergic neurotransmission.

Further work is required to fully characterise the relative roles of 5-$HT_{1A}$ and 5-$HT_{1B/1D}$ autoreceptors in the regulation of 5-HT efflux particularly during behavioural activation.

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