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ScienceDirect
Pharmacology, Biochemistry and Behavior 86 (2007) 79–85
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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ELB139 an agonist at the benzodiazepine binding site increases 5-HT in the striatum and prefrontal cortex of rats: a microdialysis study

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Received 9 June 2006; received in revised form 8 December 2006; accepted 15 December 2006
Available online 22 December 2006

Abstract

Benzodiazepines induce an immediate anxiolytic activity at the expense of side effects such as sedation, tolerance and withdrawal. In contrast, selective serotonin receptor uptake inhibitors (SSRIs) are known to offer long-term symptom improvement without inducing tolerance and withdrawal, but with a delayed onset of the anxiolytic effect. ELB139 is a novel agonist at the benzodiazepine binding site with pronounced anxiolytic and anticonvulsant activity without inducing tolerance to both effects after chronic administration. ELB139 shows a selectivity for alpha-3-subunit containing $GABA_A$ receptors. In the present study the effect of the compound on monoaminergic neurotransmitter levels were investigated by microdialysis. ELB139 induced a significant increase of extracellular 5-HT in the striatum and the medial prefrontal cortex of rats without affecting dopamine levels in these areas. The increase of 5-HT in the striatum was reversed by systemic and by local administration of the benzodiazepine antagonist flumazenil in the dorsal raphe nucleus by a microdialysis probe, suggesting that the increase in 5-HT was mediated by the activity of ELB139 at the benzodiazepine binding site. As the dorsal raphe nucleus is rich in alpha-3 subunits, this effect of ELB139 may be mediated by its subtype selectivity. Thus, ELB139 seems to combine effects seen with benzodiazepine agonists and SSRIs in one compound.

Keywords: Microdialysis; Serotonin; Striatum; Medial prefrontal cortex; Benzodiazepine; ELB139

1. Introduction

Benzodiazepines are still the most used anxiolytic compounds besides SSRIs (Atack, 2003). The fast onset of their anxiolytic activity and pronounced effects are appreciated. However, their use is affected by major side effects, like sedation, amnesia, muscle relaxation, tolerance and their abuse potential (Costa and Guidotti, 1996). In contrast, SSRIs lack a strong acute effect on symptoms but show a long-term improvement without inducing tolerance.

ELB139 (1-(4-chloro-phenyl)-4-piperidin-1-yl-1,5-dihydro-imidazol-2-one) is a new chemical entity emerging from a research and development programme for anticonvulsants based on *in vivo* screening in co-operation with the NIH (Rostock et al., 1998) and on pharmacophore modelling. In addition, the compound has been found to show strong anxiolytic activity, most likely due to its agonism at the benzodiazepine binding site (Langen et al., 2005). Whereas initial experiments revealed ELB139 to be a partial, low-affinity agonist at the benzodiazepine binding site, newer data indicate a functional subtype selectivity for the alpha-3-subunit containing $GABA_A$ receptor (Langen et al., 2005; Rabe et al., in press).

In the present study, the mechanism by which ELB139 induces these effects was investigated in a microdialysis study in rats. Microdialysates were collected from the striatum (STR) and the prefrontal cortex (mPFC), a brain area associated with anxiety-related behaviour (Graeff et al., 1996; Langen et al., 2002) and extracellular serotonin (5-HT), dopamine and their metabolite levels were determined by HPLC with electrochemical detection. In addition to the influence of the compound on these neurotransmitters, the reversibility of the effect by flumazenil was investigated.

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0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.12.010

2. Material and methods

2.1. Animals

Male Wistar rats (Crl: (WI) BR, Charles River, Germany) weighing 200–260 *g* were used. Prior to stereotactic implantation of the probe, the rats were housed in groups of two under standard conditions on a 12-h light/dark cycle (light on at 06:00 h) with *ad libitum* access to water and food (ssniff M/R 15, Spezialdiäten GmbH, Soest/Westfalen). After implanting the probe, the rats were housed singly in an opaque plastic box (32×25 cm, 50 cm high) with *ad libitum* food and water. Experiments were approved by the Committee on Animal Care and Use of the Federal State of Saxony and carried out following the German Law on the Protection of Animals.

2.2. Chemicals

ELB139 was obtained from elbion AG, Germany. Flumazenil was obtained from Tocris, UK. All other compounds were obtained from Merck Eurolab GmbH, Germany.

2.3. Drug administration

ELB139 was freshly prepared for each experiment as a suspension in 0.5% hydroxyethylcellulose and PEG300 (90/10%, v/v) and was administered intraperitoneally, in a volume of 0.5 ml/100 g. For intraperitoneal administration flumazenil was dissolved in Tween 80 and saline (0.6/99.4%, v/v) and was administered in a volume of 0.5 ml/100 *g*. When given by microdialysis probe, flumazenil at 10 μM was dissolved in Tween 80 and Ringers buffer (0.006/99.994%, v/v). This solution was perfused at a flowrate of 1 μl/min.

2.4. Surgery

Male rats were anaesthetised with chloral hydrate (3.6%, 1 ml/100 *g* i.p.) and placed in a stereotaxic frame for implantation of the microdialysis guide cannula (CMA/12, Carnegie Medicine, Sweden). The scalp was incised in the median anterior–posterior direction between lambda and bregma and a small hole was drilled into the skull. The stereotaxic coordinates were AP+1.0 mm, L-3.0 mm from bregma and 5.5 mm from the skull surface for STR, AP+3.2 mm, L 0.8 mm from bregma and 6.0 mm from the skull surface for mPFC and AP-7.6 mm, L 0.6 mm from bregma and 6.3 mm from the skull surface for dorsal raphe nucleus (DRN) according to the atlas of Paxinos and Watson (1986). Dental cement (Sinfony, ESPE Dental AG, Germany) was used to fix the guide cannula and anchor screws to the cranium. During the surgery rectal temperature was maintained at 37 °C using a heating blanket (CMA/150 temperature controller, Axel Semrau, Germany).

2.5. Microdialysis

The evening before the experiment the microdialysis probe (CMA/12, membrane length 4 or 2 mm for striatum or mPFC and dorsal raphe nucleus, respectively, Carnegie Medicin, Sweden) was inserted into the corresponding brain area through the guide cannula (about 17:00 h) and perfused with Ringer buffer (148 mM NaCl, 4 mM KCl, 2.4 mM $CaCl_2$, pH=7.4) at a flow rate of 0.1 μl/min. On the day of experiment, the flow rate was increased to 1 μl/min to allow for the collection of 20 μl samples every 20 min into microvials. When one probe was implanted microvials were stored in a fraction collector (CMA/170) at a temperature of 8 °C until analysed. Using a swivel joint, the perfusion arrangement allowed the animal to move freely within a hemispherical bowl. When two probes were implanted microvials were fixed directly to the tube with plasticine and exchanged every 20 min to avoid a twisting of the tubes. After an adjusting time of 1 h, three consecutive 20-min-fractions were collected to establish a basal level of neurotransmitter concentration.

Thereafter 30 mg/kg ELB139 or an equivalent volume of vehicle was administered i.p. The intraperitoneal administration of flumazenil or vehicle was performed 40 min afterwards. When flumazenil was infused by probe into the dorsal raphe nucleus, the infusion started 20 min after ELB139 administration. Then samples were collected for at least 180 min.

2.6. Analysis of samples

The dialysate was directly analysed by reverse-phase high-performance liquid chromatography with electrochemical detection (Coulochem II detector and Model 5014 B microdialysis cell, ESA, Germany). The samples were separated by a ZORBAX SB-Aq 2.1 mm ID×100 mm column (Agilent Technologies, Germany). Catecholamines were oxidised at 0.5 V. One μl 1% perchloric acid was added to the 20-minute-fraction and 10 μl of this mixture were injected into the HPLC-system.

The mobile phase contained 50 nM KH_2PO_4, 2.2 mM octan-1-sulfonic acid (OSA), 0.086 mM EDTA, 0.8% methanol, 1% acetonitril at pH=3.5. The flow rate was 0.23 ml/min, and the column temperature was 38 °C.

2.7. Statistical analysis

Interindividual differences in the basal levels of catecholomines required that we express the data as a percentage of mean basal level. For the mean basal level, data from three dialysates, collected before drug administration were averaged and the mean set at 100%; all individual values were calculated accordingly.

The results of microdialysis were analysed by two way analysis of variance (ANOVA) with time and drug as the two factors. Tukey test was used for individual comparison. $P<0.05$ was regarded as significant.

3. Results

3.1. Effect of ELB139 in the striatum

The mean±SEM of all basal levels of 5-HT, dopamine and their metabolites in the striatum was 0.057±0.015 nM for 5-HT,

2.29±0.29 nM for dopamine, 640.6±59.8 nM for homovanillic acid (HVA), 978.8±49.9 nM for 3,4-dihydroxyphenylacetic acid (dopac) and 289.4±18.6 nM for 5-hydroxy-3-indoleacetic acid (HIAA). The mean±SEM of all control values gained for 5-HT, dopamine and their metabolites in this brain area was 0.089±0.011 nM for 5-HT, 2.99±0.19 nM for dopamine, 756.7±39.6 nM for HVA, 981.7±26.7 nM for dopac and 313.5±13.7 nM for HIAA.

Administration of 30 mg/kg i.p. ELB139 induced a significant increase of 5-HT in the striatum of rats for 1 h and 40 min (F(compound×time)=10.87; p=0.001; n=5; Fig. 1) with a maximal increase of 379±108% 1 h after administration. The extracellular level of dopamine was not influenced by ELB139 at that dose (F(compound×time)=2.36; p=0.128; n=5; Fig. 2). There was a slight, but statistically significant, increase of HIAA (139±14%) observed predominantly when the 5-HT increase started to decline (F(compound×time)=19.84; p<0.001; n=5). There was no change of HVA and dopac concentration in the dialysate (F(compound×time)=16.04 (HVA), 1.36 (dopac); NS; n=5).

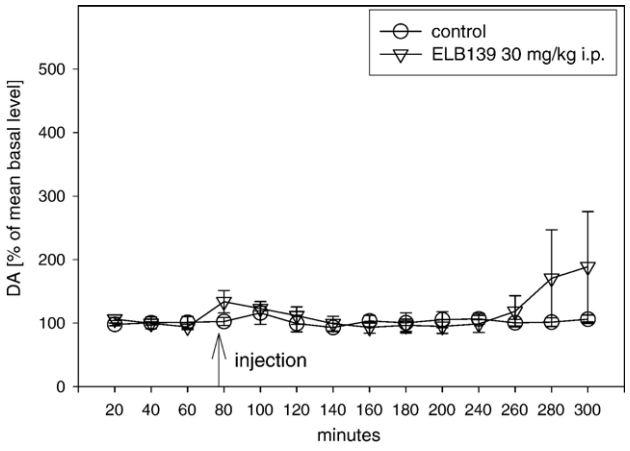

Fig. 2. Effect of intraperitoneal administration of 30 mg/kg ELB139 (n=5) in comparison to vehicle control (n=5) on dopamine level in the striatum. ELB139 had no effect on the dopamine concentration in the microdialysates of the striatum. Data are shown as mean±SEM. The mean basal level of dopamine was 2.29±0.29 nM.

3.2. Effect of ELB139 in the prefrontal cortex

The mean±SEM of all basal levels of 5-HT, dopamine and their metabolites in the prefrontal cortex was 0.047±0.005 nM for 5-HT, 0.32±0.03 nM for dopamine, 150.4±25.2 nM for HVA, 93.0±12.3 nM for dopac, 342.8±15.9 nM for HIAA and 1521.5±148.5 nM for noradrenaline (NA). The mean±SEM of all control values gained for 5-HT, dopamine and their metabolites in this brain area was 0.052±0.004 nM for 5-HT, 0.44±0.03 nM for dopamine, 247.4±21.5 nM for HVA, 117.8±8.7 nM for dopac, 361.2±8.3 nM for HIAA and 1681.7±65.9 nM for noradrenaline (NA).

In the prefrontal cortex, there was also an increase in the extracellular concentration of 5-HT, detectable after the administration of ELB139 at 30 mg/kg i.p.; but, the increase was lower than that seen in the striatum (F(compound×time)=10.02; p=0.002; n=6; Fig. 3). The peak effect was only 172±32% and was reached after 40 min. In addition, a significant increase of HIAA, the metabolite of 5-HT, (F(compound×time)=39.54; p<0.001; n=4) was detectable. The increase of HIAA started when the increase of 5-HT declined. The peak effect was 127±12%. Dopamine concentration (F(compound×time)=1.16; p=0.283; n=6) was unchanged. However, we observed a modest, but significant increase of dopac (F(compound×time)=30.45; p<0.001; n=6; Fig. 4) and of HVA (F(compound×time)=4.96; p=0.029; n=6). Dopac was maximally increased to 133±13%, the peak effect of HVA was 213±48%. Both peak effects were reached 100

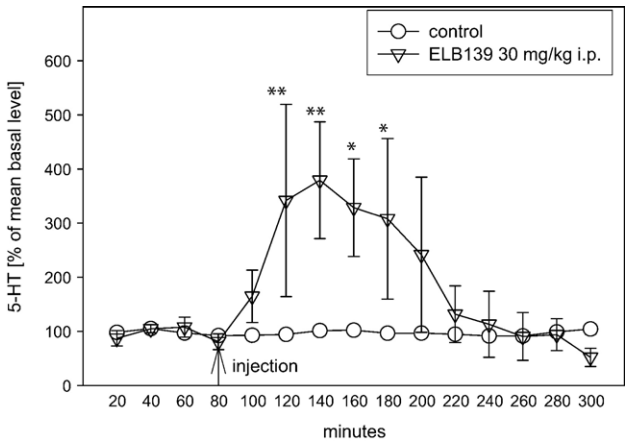

Fig. 1. Effect of intraperitoneal administration of 30 mg/kg ELB139 (n=5) in comparison to vehicle control (n=5) on 5-HT level in the striatum. Data are shown as mean±SEM. The mean basal level of 5-HT was 0.057±0.015 nM. ELB139 increases the 5-HT level in the striatum up to 379±108%. Statistically significantly different from control: $*p<0.05$, $**p<0.01$.

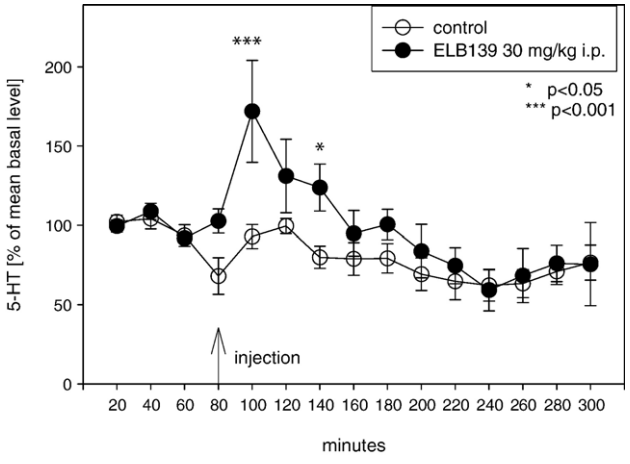

Fig. 3. Effect of intraperitoneal administration of 30 mg/kg ELB139 (n=6) in comparison to vehicle control (n=4) on 5-HT level in the mPFC. ELB139 induced a moderate but significant increase of extracellular 5-HT in the mPFC of rats. The mean basal level of 5-HT was 0.047±0.005 nM. ELB139 increases the 5-HT level in the mPFC up to 172±32% being a lower effect than in the striatum. Data are shown as mean±SEM. Statistically significantly different from control: $*p<0.05$, $***p<0.001$.

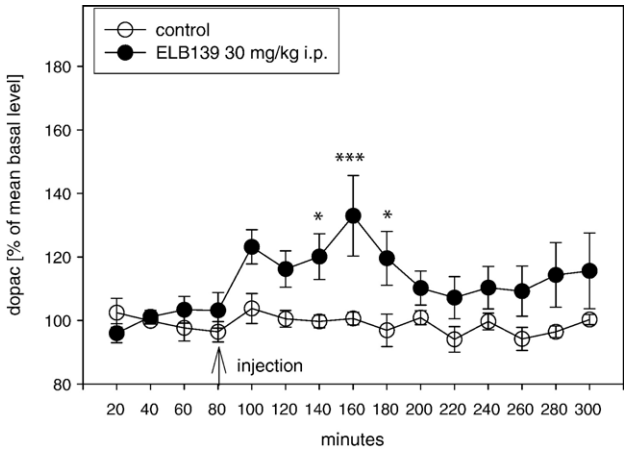

Fig. 4. Effect of intraperitoneal administration of 30 mg/kg ELB139 ($n=6$) in comparison to vehicle control ($n=4$) on dopac level in the mPFC of rats. ELB139 induced a moderate but significant increase of extracellular dopac in the mPFC of rats. The mean basal level of dopac was 93.0±12.3 nM. ELB139 increases the dopac level in the mPFC up to 133±13%. Data are shown as mean± SEM. Statistically significantly different from control: $*p<0.05$, $***p<0.001$.

min after the administration of ELB139. In addition, the levels of noradrenaline in the pre frontal cortex was determined (Fig. 5). Interestingly, the extracellular level of this neurotransmitter began to increase significantly up to 139±17% when the rise of 5-HT level started to diminish 2 h after administration of ELB139 (F(compound×time)= 55.10; p<0 0.001; n=3).

3.3. Reversal of the ELB139 effect by flumazenil

In order to evaluate whether the increase of 5-HT induced by ELB139 was mediated by its activity at the benzodiazepine binding site, flumazenil was co-administered with the compound. For that reason, flumazenil was administered either systemically (i.p.) or locally into the dorsal raphe nucleus by microdialysis probe.

3.4. Intraperitoneal administration of flumazenil

In this experiment, the mean±SEM of all basal levels of 5-HT in the striatum was only 0.023±0.004 nM, which was lower than the striatal basal level of 5-HT gained in the other experiments. The rats that received ELB139 alone showed a significant but only moderate and delayed increase of the 5-HT levels in the striatum in comparison to the basal level (F(compound×time)=100.38; p=0.008; n=5). The increase reached its peak level of 182±17% 100 min after the administration of ELB139. In the rats that received ELB139 and flumazenil, a slight increase of 5-HT was observed in the period between ELB139 and flumazenil administration that failed to achieve statistical significance. When flumazenil was administered at 5 mg /kg i.p. 40 min after ELB139, the increase of 5-HT was significantly suppressed below the basal level (F(compound×time)= 100.38; p<0.001; n=5; comparing ELB139 to ELB139+ flumazenil). Since ELB139 only moderately increased the 5-HT levels in this experiment, the results of this part of the study have to be interpreted cautiously. However, these data suggest that the increase of extracellular 5-HT may be mediated by the activity of ELB139 at the benzodiazepine binding site.

3.5. Administration of flumazenil into dorsal raphe nucleus by microdialysis probe

Further experiments were performed to learn more about the mechanism of the 5-HT increase. Thus, flumazenil was also administered by a second microdialysis probe directly into the dorsal raphe nucleus. Infusion of flumazenil or vehicle started 20 min after intraperitoneal administration of 30 mg/kg ELB139.

In these two-probe experiments, the mean±SEM of all basal levels of 5-HT in the striatum was 0.098±0.014 nM. The mean±SEM of all control values gained for 5-HT in this brain area was 0.068±0.015 nM. Thus, the 5-HT level of the control group decreased slightly during the experiment. The 5-HT levels of the rats that received flumazenil alone or ELB139 and flumazenil did not change during the recording time. The mean±SEM of all values of the rats that received flumazenil alone was 0.126±0.018 nM. The mean±SEM of all values of the rats that received ELB139 and flumazenil was 0.097± 0.015 nM.

In this experiment, ELB139 increased the 5-HT level up to 427±222% and 358±94% 20 and 40 min after compound administration, respectively.

When administering flumazenil by a microdialysis probe into the dorsal raphe nucleus, flumazenil was again able to significantly suppress the increase of extracellular 5-HT

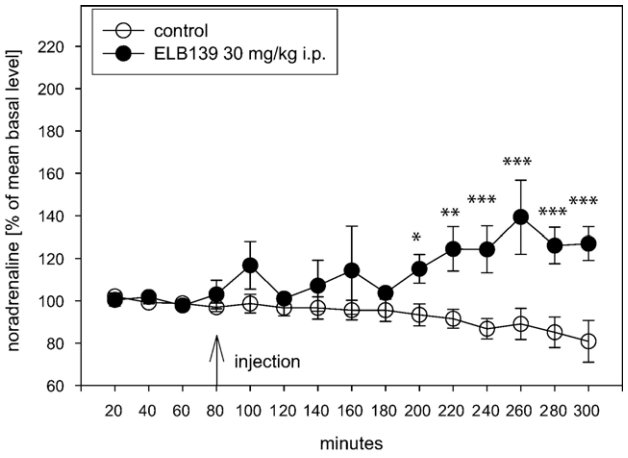

Fig. 5. Effect of intraperitoneal administration of 30 mg/kg ELB139 ($n=6$) in comparison to vehicle control ($n=4$) on extracellular noradrenaline in the mPFC of rats. After the decline of the 5-HT increase ELB139 induced a moderate but significant increase of the noradrenaline concentration in the microdialysate of the mPFC of rats. The mean basal level of noradrenaline was 1681.7±65.9 nM. ELB139 increases the noradrenaline level in the mPFC up to 139±17%. Data are shown as mean±SEM. Statistically significantly different from control: $*p<0.05$, $**p<0.01$, $***p<0.001$.

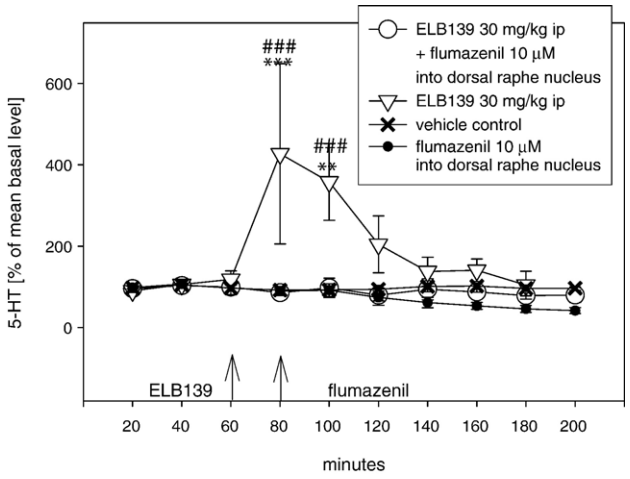

Fig. 6. Effect of locally administered flumazenil into the dorsal raphe nucleus on the increase of 5-HT level in the striatum induced by 30 mg/kg ELB139 i.p. Flumazenil at 10 µM was administered by a second microdialysis probe into the dorsal raphe nucleus. Infusion of flumazenil or vehicle started 20 min after the administration of ELB139. Data are shown as mean±SEM. The vehicle control group and the flumazenil-group contain $n=3$ rats, the ELB139±flumazenil-group contains $n=4$ rats and the ELB139-group contains $n=5$ rats. The mean basal level of 5-HT was 0.098±0.014 nM. ELB139 increased the 5-HT level up to 427±222% and 358±94% 20 and 40 min after ELB139 administration, respectively. Statistically significantly different from control: $**p<0.01$, $***p<0.001$; statistically significantly different from animals that received ELB139 and flumazenil: $###p<0.001$.

induced by ELB139 (F(compound×time)=9.84; p=0.003; n=5; comparing ELB139 to ELB139+flumazenil; Fig. 6).

4. Discussion

ELB139 is a novel benzodiazepine agonist with robust anxiolytic activity that in contrast to diazepam, is not susceptible to the development of tolerance (Langen, et al., 2005). Newer data indicate a functional selectivity for the alpha-3-subunit containing $GABA_A$ receptor (Rabe et al., in press). In comparison to diazepam, higher doses were needed to induce an anxiolytic effect. Interestingly, the anxiolytic effect of ELB139 in the elevated plus maze, the light-and-dark-box, and the Vogel conflict test, were at least as strong as the effect observed for diazepam, and in most cases tended to be stronger (Langen, et al., 2005). As the anxiolytic effects of ELB139 could be antagonised by flumazenil they are most likely mediated by its activity at the benzodiazepine binding site (Langen, et al., 2005). To get a better understanding of the mechanisms for this effect, measurement of monoaminergic neurotransmission using microdialysis techniques was performed.

ELB139 administered intraperitoneally was found to induce a significant increase of extracellular 5-HT in the striatum and the prefrontal cortex of rats that was followed by a moderate increase of HIAA, the metabolite of 5-HT (Figs. 1 and 3). The increase of 5-HT induced by ELB139 in the prefrontal cortex was lower and shorter in its duration than in the striatum. This increasing effect on 5-HT was unexpected since traditional benzodiazepines, such as diazepam and flurazepam, are reported to induce a decrease of this neurotransmitter in different brain areas (Millan, 2003; Pei et al., 1989; Wright et al., 1992), an observation that was also reproduced with diazepam in our laboratory (data not shown). It has been reported that the triazolo-benzodiazepines, such as alprazolam, can alter 5-HT levels in the brain in a more complex way. Thus, alprazolam is described to increase 5-HT levels in the CA1 region of the hippocampus, but to decrease 5-HT levels in the frontal cortex (Broderick et al., 1997, 1998; Millan, 2003). Because the effect of ELB139 on hippocampal 5-HT level was not determined, a direct comparison between alprazolam and ELB139 is not possible. However, ELB139 appears to have a slightly different pattern of 5-HT increase in the rat brain than the triazolo-benzodiazepines.

In accordance to the classical 5-HT hypothesis of anxiety, ELB139 would be expected to reduce extracellular 5-HT levels, at least in brain areas associated with anxiety-related behaviour such as the prefrontal cortex (Graeff et al., 1996). This hypothesis is supported by studies in rats, where lesions in the prefrontal cortex revealed a lower anxiety-related behaviour than sham-lesioned counterparts in the social interaction and the elevated plus maze test (Gonzalez et al., 2000). It is also supported by studies in humans demonstrating more severe decreases in cerebral blood flow to the prefrontal in less anxious people during anticipatory-anxiety than in more anxious people (Simpson et al., 2001). Additionally, microdialysis studies found that aversive conditions like the elevated plus maze test and the conditioned fear-induced freezing behaviour were accompanied by an increased 5-HT release in the prefrontal cortex, which can be reduced by anxiolytic agents like diazepam (Hashimoto et al., 1999; Langen et al., 2002; Rex et al., 1993; Yoshioka et al., 1995).

However, the association between 5-HT and anxiety cannot be reduced to a simple increase or decrease of the neurotransmitter as seen with the anxiolytic activity of SSRIs, which are know to induce an increase of extracellular 5-HT in the brain (Ohashi et al., 2003). Likewise, ipsapirone, a partial 5-HT1A agonist, does not change anxiety-related behaviour of rats in the elevated plus maze by decreasing 5-HT levels in the hippocampus (Wright et al., 1992).

The increase of extracellular 5-HT induced by ELB139 was less pronounced and shorter in duration than increase induced by SSRIs. Fluoxetine, for instance, increases 5-HT concentration in microdialysis fluid from striatum more than 4-fold (Perry et al., 1992, 1993). Nevertheless, ELB139 though primarily acting as a benzodiazepine agonist, may also affect the serotonergic neurotransmitter system in a way that is similar to SSRIs.

Treatment with ELB139 failed to alter dopamine levels in the striatum and in the prefrontal cortex in this study. However, a slight increase of HVA and dopac was detected in the prefrontal cortex, thus an influence on the dopaminergic neurotransmitter system cannot be excluded. Additionally, we were able to detect noradrenaline levels in the prefrontal cortex due to an improvement in the method (Fig. 5). There, an augmentation of noradrenaline levels was detected, which started after cessation of the increase in 5-HT and about 2 h after ELB139 administration. Because there is a delay in the increase, it is

likely not a direct effect of the compound, but by a secondary, perhaps regulatory mechanism.

Because ELB139 does not bind to 5-HT uptake sites (data not shown), we were interested in identifying the mechanism of action for its effect on 5-HT levels. The systemic administration of flumazenil partially reversed the ELB139-induced 5-HT increase in the striatum, indicating that the increase was associated, at least in part, to an interaction of the compound at the benzodiazepine binding site. Since classical benzodiazepines, are thought to decrease extracellular 5-HT levels in the examined brain areas, the mechanism was investigated more thoroughly. The striatum is innervated primarily by serotonergic neurons originating from the dorsal raphe nucleus (Kreiss and Lucki, 1994; Romero et al., 1994). Thus, flumazenil was administered directly into the dorsal raphe nucleus by a microdialysis probe. Locally administered flumazenil also reversed extracellular 5-HT increase in the striatum (Fig. 6). Thus, the increase of 5-HT in the striatum appears to be mediated by either a direct and indirect activity of ELB139 on serotonergic neurons in the dorsal raphe nucleus. There is a great deal of evidence that the activity of serotonin neurons in the dorsal raphe nucleus is influenced by a network of GABAergic neurons and that $GABA_A$ receptors are involved in this mechanism (Varga et al., 2001; Puig et al., 2005; Jankowski and Sesack, 2004). Flumazenil administered into the dorsal raphe nucleus did not distinctly reduce or increase the 5-HT level in the striatum, on its own. This suggests that the levels of striatal 5-HT were probably not influenced by the GABAergic tone in the dorsal raphe nucleus.

Serotonergic neurons in the dorsal raphe nucleus show a high labelling of alpha-3-subunit containing $GABA_A$ receptors, while there are only few neurons expressing the alpha-2-subunit (Rodriguez-Pallares et al., 2001). Additionally, Gao et al. (1993) found that the vast majority of serotonergic neurons in the raphe show strong alpha-3-subunit immunoreactivity but are devoid of alpha-1-subunit, whereas both subunits are present in GABAergic neurons of the raphe. We speculate that the dissimilar distribution of the $GABA_A$ receptor subunits plays a role in the unique effect of ELB139 compared to the classical benzodiazepines, like diazepam, on serotonergic neurotransmission. Consequently, the $GABA_A$ receptor alpha-3-subunit selectivity of ELB139 may modulate the GABAergic network in the dorsal raphe nucleus differently from classical benzodiazepines, which might explain the increase in 5-HT. However, the contrasting effect of ELB139 on 5-HT levels in comparison to other benzodiazepines was not completely elucidated and the involvement of other brain regions on the effects seen in this study cannot be excluded.

In summary, in this microdialysis study the novel benzodiazepine agonist ELB139 showed an atypical profile when compared to the classical benzodiazepines by inducing an increase of 5-HT in the striatum and the prefrontal cortex of rats. The selectivity for $GABA_A$ receptor subtypes containing alpha-3-subunits is proposed as possible mechanistic basis for this phenomenon, but additional effects cannot be excluded at this time. ELB139 appears to combine the effects seen with benzodiazepine agonists and SSRIs in one compound. Currently, ELB139, is undergoing a phase II clinical trials for anxiety.

Acknowledgements

The study was supported by the European Fund for Regional Development 2000–2006, sector technology support, and by the Federal State of Saxony (grant no. SAB 8093).

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