Despite Similar Anxiolytic Potential, the 5-Hydroxytryptamine 2C Receptor Antagonist SB-242084 [6-Chloro-5-methyl-1-[2-(2-methylpyrid-3-yloxy)-pyrid-5-yl Carbamoyl] Indoline] and Chlordiazepoxide Produced Differential Effects on Electroencephalogram Power Spectra

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ABSTRACT

Serious efforts have been made to develop anxiolytics with improved clinical utility and reduced side effects. 5-Hydroxytryptamine (5-HT)2C receptor antagonists are potential anxiolytics; however, their effects on vigilance are not well characterized. To compare the effects of benzodiazepines and subtype-selective 5-HT2C receptor antagonists on anxiety, vigilance, and electroencephalogram (EEG) power density, social interaction test and polygraphic recordings were performed in male Sprague-Dawley rats after chlordiazepoxide (CDP; 4.0 mg/kg i.p.) and SB-242084 (6-chloro-5-methyl-1-[2-(2-methylpyrid-3-yloxy)-pyrid-5-yl carbamoyl] indoline) (0.1, 0.3, and 1.0 mg/kg i.p.) treatment. CDP and SB-242084 (0.3 and 1.0 mg/kg) had similar anxiolytic effects. Spectral analysis of EEG in wakefulness (W) and paradoxical sleep (PS) showed an opposite effect on θ activity (5–9 Hz); it decreased after CDP, whereas it increased after SB-242084 (even at 0.1 mg/kg). In addition, CDP significantly decreased slow-wave activity (0.5–4 Hz) in deep slow-wave sleep (SWS-2) and increased power at frequencies above 12 Hz mainly in W and PS. A markedly increased intermediate stage of sleep was also found after CDP treatment. At the highest dose, SB-242084 increased W and decreased SWS-2. In summary, low but potent anxiolytic doses of the subtype-selective 5-HT2C receptor antagonist SB-242084 did not affect vigilance states but caused an increased θ activity in W, raising the possibility of a cognitive-enhancing effect of the drug. In contrast, acute CDP administration, based on spectral analysis of the EEG, produced a more superficial sleep along with a decreased θ activity.

Efforts to improve the clinical utility and reduce the side effects of classic benzodiazepines have a long history (Haefely et al., 1992). Although the anxiolytic properties of these drugs are well established both clinically and experimentally (Argyropoulos and Nutt, 1999; File and Seth, 2003), the side effects like impaired short-term memory, vigilance, or psychomotor performance limit their clinical applicability (Kunsman et al., 1992; van Laar et al., 2001; Buffet-Jerrott and Stewart, 2002). On the other hand, these effects are often subtle when low doses are involved (Kunsman et al., 1992), and tolerance develops to the sedative and psychomotor effects with their continued use as anxiolytics (Lucki et al., 1986).

Pharmacological characterization of the effects of the...
5-HT$_2$ agonist 1-(3-chlorophenyl)piperazine (m-CPP) led to the hypothesis that activation of 5-HT$_{2C}$ receptors may mediate anxiety in humans and rodents although pharmacological tools used in these studies were not able to differentiate between 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (Kennett et al., 1989). The presence of the 5-HT$_{2C}$ receptor subtype in the rat frontal cortex, hippocampus, amygdala, nucleus accumbens, hypothalamus, periaqueductal gray matter, and septum provide an anatomical support for their role in the control of anxiety (Abramowski et al., 1995; Clemett et al., 2000). Recently, it has been suggested that increased anxiety in rodents, and possibly in humans, caused by acute administration of SSRI antidepressants or m-CPP, is mediated by activation of 5-HT$_{2C}$ receptors (Kennett et al., 1989; Dekney et al., 2000; Bagdy et al., 2001). The first subtype-selective 5-HT$_{2C}$ receptor antagonist SB-242084 has a high affinity ($pK_i = 9.0$) for the 5-HT$_{2C}$ receptor with 100-fold selectivity over the 5-HT$_{2B}$ receptor and at least 100-fold selectivity over all other receptors tested, including the 5-HT$_{2A}$ receptor subtype (Kennett et al., 1997; Bromidge et al., 2000). Besides, it has been demonstrated that SB-242084 has an anxiolytic potential in several tests of anxiety (Kennett et al., 1997; Bagdy et al., 2001; Martin et al., 2002).

The role of the 5-HT$_2$ receptor subtypes (5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$) in the regulation of the sleep-wakefulness cycle has been difficult to analyze due to the lack of receptor subtype-selective drugs (Portas et al., 2000). It is generally accepted that 5-HT$_2$ receptor antagonists like ritanserin or segerserin massively enhance slow-wave sleep (stages 3 and 4) in humans and increase deep slow-wave sleep (SWS-2) in rats (Detari et al., 1999; Portas et al., 2000; Kantor et al., 2002). In addition to the effects on SWS-2, ritanserin and segerserin increase low-frequency EEG activity administered at the beginning of the passive phase (inactive period) in rats (Borbely et al., 1988; Kantor et al., 2002) and humans (Dijk et al., 1989). Although ritanserin binds selectively to 5-HT$_2$ receptors, it cannot differentiate among subtypes, namely 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors of the 5-HT$_2$ receptor family (Bonnaus et al., 1997). Lately, it has been shown that the 5-HT$_{2B}$ receptor is implicated in the regulation of sleep-wake cycle (Kantor et al., 2004), but the role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in sleep and EEG processes has been controversial for a long time due to the lack of receptor subtype-selective drugs. In a recent study, the effects of the selective 5-HT$_{2C}$ receptor antagonist SB-243213 on the time of vigilance states were analyzed (Smith et al., 2002), but no EEG spectral analyses were done so far after selective blockade of these receptors.

To investigate the possible role of the 5-HT$_{2C}$ receptor in the regulation of sleep-wake cycle, vigilance states and EEG spectra in different vigilance states were analyzed in rats after blockade of this receptor by the subtype-selective 5-HT$_{2C}$ antagonist, SB-242084. In addition, we compared the effects of SB-242084 on vigilance and social behavior of rats with those produced by a widely used benzodiazepine anxiolytic drug, chloridrazepoxide (CDP), to get information about the possible sedative properties of the compounds at effective anxiolytic doses.

### Materials and Methods

#### Animal Maintenance and Surgery

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1978). Permission was given by the local ethical committee.

Male Sprague-Dawley rats (Crl:CD®BR; Charles River, Budapest, Hungary), weighing 230 to 250 g at implantation, were used in the experiments. Rats were kept in single cages with food and water available ad libitum, maintained in a 12-h light/dark cycle (lights from 9:00 AM to 9:00 PM, daylight-type fluorescent tubes, 18 W, approximately 300 lx), and at an ambient temperature of 21 ± 1°C.

Animals were equipped with EEG and EMG electrodes as described earlier (Kantor et al., 2004). Briefly, stainless steel screw electrodes were implanted epidurally over the left frontal cortex (L, 2.0 mm and A, 2 mm to bregma) and left parietal cortex (L, 2.0 mm and A, 2.0 mm to lambda) for fronto-parietal EEG recordings. The ground electrode was placed over the cerebellum. In addition, EMG electrodes (stainless steel spring electrodes embedded in silicon rubber, Plastics One Inc., Roanoke, VA) were placed in the muscles of the neck. Surgery was performed under halothane (2%) anesthesia (Fluotec 3) using a Kopf stereotaxic instrument. After a 10-day recovery period, the rats were attached to the polygraph by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement for the animals. To habituate the animals to the recording system, the rats were attached to the polygraph and received i.p. injections of physiological saline for 5 days before the experiments.

The animals remained connected to the recording cables throughout the study.

In a separate group of rats ($n = 10$) beside fronto-parietal electrodes hippocampal electrodes were implanted additionally to test whether changes in $\theta$ frequency range of cortical EEG reliably reflect changes in hippocampal activity. Surgery was carried out in a similar way as described above with the difference that a pair of bipolar electrodes (125-µTeflon insulated stainless steel, Medwire Corp., Mt. Vernon, NY) was inserted into hippocampus of animals (L, 2.0 mm and P, 2 mm to bregma on both sides). The deeper electrode reached the granular layer of the dentate gyrus, whereas the shorter was located in the oriens layer of the CA1 region. This arrangement yields to the largest amplitude signal because $\theta$ rhythm is in opposite phase in the two areas (Robinson, 1980).

#### Sleep Recording and Scoring

EEG, EMG and motor activity were recorded for 23 h after SB-242084 and for 6 h after CDP treatments, starting at light onset as described earlier (Kantor et al., 2004). Briefly, the signals were amplified (amplification factors approximately 5,000 for EEG and motor activity, 20,000 for EMG), conditioned by analog filters (filtering, below 0.53 Hz and above 30 Hz at 6 dB/octave), and subjected to analog to digital conversion with a sampling rate of 64 Hz/channel. The digitized signals were displayed on a monitor and stored on the computer for further analysis.

The vigilance states were scored visually for 4-5 periods over 1 to 4 h as follows: wakefulness (W), the EEG was characterized by low amplitude activity at $\beta$ (14–30 Hz) and $\alpha$ (8–13 Hz) frequencies accompanied with high EMG and motor activity; light slow wave sleep (SWS-1), high-voltage slow cortical waves (0.5–4 Hz) interrupted by low-voltage fast EEG activity (spindles, 6–15 Hz) accompanied with reduced EMG and motor activity; SWS-2, continuous high-amplitude slow cortical waves (0.5–4 Hz) with reduced EMG and motor activity; intermediate stage of sleep (IS), a short-lasting stage (mean, 3 s) just prior to paradoxical sleep and sometimes just after it was characterized by unusual association of high-amplitude spindles (mean 12.5 Hz) and low-frequency (5.4 Hz) $\theta$ rhythm; paradoxical sleep (PS), low amplitude and high frequency EEG activity with regular $\theta$ waves (5–9 Hz) accompanied by silent EMG and motor activity with occasional switching (Kantor et al., 2004). In addition to the visual analysis, the polygraphic recordings were classified by sleep analysis software (SleepSign for Animal; Kissei America, Inc., 2004).
Irvine, (CA), too. Results of manual and automatic analysis showed strong correlations for all vigilance states (Kantor et al., 2004).

EEG Power Spectral Analysis. EEG power spectra were computed for consecutive 4-s epochs in the frequency range 0.25 to 30 Hz (fast Fourier transformation routine, Hanning window; frequency resolution, 0.25 Hz). Epochs with artifacts were discarded on the basis of the polygraph records. Adjacent 0.25-Hz bins were summed into 1-Hz bins, and those above 30 Hz were omitted. Bins are marked by their upper limits, thus, 2 Hz refers to 1.25 to 2.00 Hz. The values of consecutive 4-s EEG epochs in W, SWS-1, and SWS-2, respectively, were averaged in the 1st h and in PS in the first 2 h after treatment to obtain the power density values for these vigilance states (Kantor et al., 2004). The drug-induced changes in EEG power spectra were calculated as the ratio of mean power spectra obtained following the injection of drug versus the mean power spectra obtained following administration of vehicle:

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\text{variations of mean power spectra (\%) = } \left( \frac{\text{EEG power following drug}}{\text{EEG power following vehicle}} \right) \times 100
\]

This procedure therefore allows for the change in EEG power, at each frequency, expressed as a percentage of the original power, induced by a drug, compared with the control, in the same animal (Sebban et al., 2002).

Comparison of Cortical and Hippocampal \(\theta\) Activity. Changes of \(\theta\) activity in recordings from cortical surface electrodes and from hippocampal deep electrodes were compared during audiogenic stress and CDP treatment. Stress was induced by a 2-kHz pure sinusoid tone applied for 5 min through two speakers attached to the top of the cages. Sound intensity was adjusted to 100 dB at the bottom of the cages. Vehicle or CDP (4 mg/kg) was injected i.p. at the beginning of the light phase. Tone was turned on after a 30-min waiting period. At least 3 days elapsed between the two treatments. Averaged power spectra were calculated from the cortical and hippocampal EEG signals for a 5-min period preceding the tone and for the duration of the tone. Relative changes in the integrated \(\theta\) (4–10 Hz) power and in the highest power value in this range were determined. The frequency belonging to the highest power value in the \(\theta\) range was also established before and during the tone. Vehicle and CDP effects were compared with two-tailed Student’s \(t\) test, adopting a significance level of \(P < 0.05\).

Behavioral Observations, Social Interaction Test. Another subset of experimentally naive animals were used in the social interaction test. Rats were kept four per cage with food and water available ad libitum and maintained on a 12-h light/dark cycle (lights from 6:00 AM to 6:00 PM), at an ambient temperature of 21 ± 1°C. Rats were housed in a room adjacent to the testing room at least for 2 weeks before the tests. All treatment groups consisted of 12 to 20 animals, and each rat was tested for social interaction with an unknown test partner that did not differ by more than 15 g in weight. The animals for the test were randomly assigned to treatments. The procedure was carried out as described earlier (Bagdy et al., 2001).

To test the anxiolytic properties of the drugs, we used the high-light (300 lx), unfamiliar arena test conditions, in which the animals were placed into the arena for first time during the test. Both members of a pair had the same prior familiarization experience and the same drug treatment. Each group of rats (60 cm × 60 cm × 40 cm) was placed in 10 × 10-cm compartments by lines on the floor.

The behavior of the animals was recorded on videotape. It was scored later by remote video monitoring and a computer program. The following behavior units were included in total social interaction: sniffing partner, anogenital sniffing, following, grooming partner, crawling under, climbing over, chasing, aggressive grooming, dominant posture, submissive posture, biting, boxing, kicking, pushing, and wrestling.

Drug Administration. All animals (21 rats) used in the sleep and EEG studies received vehicle and two doses of SB-242084 (0.1, 0.3, or 1.0 mg/kg i.p.) or vehicle and CDP (4.0 mg/kg i.p.) treatment in a random order (4 days between treatments). The solutions were injected in the first 5 min of light onset. SB-242084 (kindly provided by Dr. G. Kennett, Winnersh, Wokingham, UK) was dissolved in a solution of 25 mM citric acid and 10% (2-hydroxypropyl)-\(\beta\)-cyclodextrin (Fluka, Buchs, Switzerland) and injected i.p. in a volume of 1 ml/kg body weight. CDP (EGIS Pharmaceuticals Ltd., Budapest, Hungary) was dissolved in solution of 50 mM citric acid and 20% (2-hydroxypropyl)-\(\beta\)-cyclodextrin (Fluka) and injected i.p. in a volume of 1 ml/kg body weight.

Animals in the social interaction test of anxiety received vehicle or one of the drugs mentioned above. One animal received only one treatment (vehicle or one of the three different doses of SB-242084 or CDP). In the test situation, both members of the pair had the same treatment. Separate groups of animals were used for SB-242084 and CDP experiments in both tests.

Data Analysis. To statistically analyze the time course of changes in each vigilance state, data from each hour have been submitted to multivariate analysis of variance (MANOVA) for repeated measures with two main factors: treatment (vehicle and 0.1, 0.3, or 1.0 mg/kg SB-242084 or vehicle and 4.0 mg/kg CDP) and time (1–4 h). The EEG power spectra values were log-transformed before statistical analyses and were submitted to MANOVA for repeated measures with two main factors: treatment (vehicle and 0.1, 0.3, or 1.0 mg/kg SB-242084 or vehicle and 4.0 mg/kg CDP) and frequency bins (1–30 Hz). Once one-tailed statistical comparisons have been performed in the case of sleep and EEG studies, the differences were considered significant only if Tukey honest significant difference post hoc tests indicated statistical significance at \(P < 0.01\) after significant MANOVA. In social interaction test, data were analyzed using one-way analysis of variance. Tukey honest significant difference test was used for post hoc comparisons. Mean ±
S.E.M. data are given in the text and on the figures for the given number of animals/group.

**Results**

**Social Interaction Anxiety Test.** SB-242084 dose dependently increased the time of social interactions (64, 86, and 95% increase compared with the control at 0.1, 0.3, and 1.0 mg/kg i.p., respectively; Fig. 1) in social interaction test with high-light unfamiliar arena conditions. The drug was effective already at the 0.3 mg/kg i.p. dose (Fig. 1). Statistical data analysis showed significant treatment effect ($F_{3,85} = 4.215, P < 0.01$; $F_{3,85} = 4.215, P < 0.01$).

Similar to SB-242084, CDP (4.0 mg/kg i.p.) increased the time of total social interaction (104% increase compared with the control; Fig. 1). A significant treatment effect was also found ($F_{1,22} = 5.118; P < 0.05$).

**Vigilance States.** The duration of different vigilance states was not considerably affected by lower doses (0.1 and 0.3 mg/kg i.p.) of SB-242084. We did not find significant time by treatment interactions neither at 0.1 mg/kg i.p. (W, $F_{3,21} = 1.083, P = 0.378$; SWS-1, $F_{3,21} = 1.108, P = 0.368$; SWS-2, $F_{3,21} = 1.821, P = 0.174$; IS, $F_{3,21} = 1.847, P = 0.170$; PS, $F_{3,21} = 1.125, P = 0.361$) nor at 0.3 mg/kg i.p. (W, $F_{3,24} = 1.456; P = 0.252$; SWS-1, $F_{3,24} = 0.960, P = 0.427$; SWS-2, $F_{3,24} = 1.850, P = 0.165$; IS, $F_{3,24} = 1.476, P = 0.245$; PS, $F_{3,24} = 0.24, P = 0.995$) dose of SB-242084.

The highest dose (1.0 mg/kg i.p.) of SB-242084 increased W and decreased SWS-2 compared with vehicle in the 1st h (Figs. 2a and 3c). Later (in the 3rd and 4th h), a significantly increased SWS-1 was found (Fig. 3a). The time by treatment interactions were significant for W ($F_{3,15} = 12.063; P < 0.001$; Fig. 2a), SWS-1 ($F_{3,15} = 6.559; P < 0.01$; Fig. 3a), and SWS-2 ($F_{3,15} = 6.990; P < 0.01$; Fig. 3c).

CDP (4.0 mg/kg i.p.) caused a marked increase in IS in the 2nd h (Fig. 4b). A trend for decrease in PS in the 1st h (Fig. 4d) and a trend for increase in SWS-1 in the 2nd h (Fig. 3b) were also found. The time by treatment interactions were significant for W ($F_{3,18} = 4.851; P < 0.05$; Fig. 2b), IS ($F_{3,18} = 3.860; P < 0.05$; Fig. 4b), and PS ($F_{3,18} = 5.548; P < 0.01$; Fig. 4d). No significant changes were found in vigilance states at later time points (data not shown).

**EEG Power in Different Vigilance States.** Effects of drugs (SB-242084 and CDP) on EEG power density were analyzed separately in W, SWS-1, SWS-2, and PS. In W, SB-242084 increased EEG power density mainly in the θ band (5–9 Hz) compared with vehicle (Fig. 5, a–c). The treat-
ment by frequency interaction was significant for 0.1 and 1 mg/kg i.p. doses ($F_{29,203} = 2.647, P < 0.001; F_{29,146} = 2.844$, $P < 0.001$, respectively), and a strong tendency was found for 0.3 mg/kg i.p. dose ($F_{29,261} = 1.402; P = 0.089$). The most prominent increase was observed at 8 Hz, which was already present at the lowest dose (0.1 mg/kg i.p.) of the drug (Fig. 5a). SB-242084 did not change significantly the EEG spectra of different sleep stages (SWS-1, SWS-2, and PS) even at the highest dose (Figs. 5 and 6).

In contrast, CDP (4.0 mg/kg i.p.) produced marked variations of EEG spectra in all vigilance states relative to controls (Figs. 5 and 6). In W, CDP significantly decreased low-frequency EEG activity (1–4 and 7–9 Hz) and increased $\beta$ activity (14–30 Hz) compared with vehicle (Fig. 5d). Similar changes in EEG spectra were found in PS after CDP treatment. The drug decreased EEG power density in 8 Hz and increased mainly in the 12- to 24-Hz band (Fig. 5h). Spectral analysis of the EEG in SWS-2 after CDP treatment showed a marked decrease in power density in the 1- to 11-Hz and a slight increase in the 26- to 30-Hz band relative to controls (Fig. 6h). The changes in low-frequency EEG activity during SWS-1 produced by CDP were similar to those of SWS-2, but significant difference was found only at 8 Hz (Fig. 6d). In addition, the drug significantly increased EEG power density in 13 and 16 Hz during SWS-1 (Fig. 6d). Statistical data analysis showed significant treatment by frequency interactions ($F_{29,174} = 47.356, P < 0.001; F_{29,174} = 8.195, P < 0.001; F_{29,174} = 61.953, P < 0.001; F_{29,174} = 9.763, P < 0.001$) for W, PS, SWS-1, and SWS-2, respectively.

Changes during the Audiogenic Stress. The most obvious EEG effects induced by the audiogenic stress were behavioral inhibition and simultaneous $\theta$ oscillation in the hippocampus. This rhythmic activity was also obvious in the cortical recording. CDP (4 mg/kg) significantly decreased all indices of hippocampal activation both in the cortical and hippocampal EEG. Increase of integrated power was reduced after CDP by 13.4, 22.7, and 17.4% in the fronto-parietal and right and left hippocampal signals, respectively. The $\theta$ peak was found at $5.91 \pm 0.05$, $6.36 \pm 0.04$, and $6.40 \pm 0.05$ Hz in the three signals during tone application. CDP decreased peak frequency by 10.3, 7.39, and 7.80%, respectively. Peak power decreased by 12.3, 30.7, and 27.0%. All changes were significant at $P < 0.01$ ($n = 10$).

Discussion

Many of the laboratory procedures used in stress research bear little or no relation to natural stressors that can appear in natural environment of the animals. From a view, it seems that the social environment can be one of the most important
sources of stress (Koolhaas et al., 1997). Thus, the social interaction test of anxiety provides an ethologically based test that is sensitive to a number of environmental, physiological, and pharmacological factors that can affect anxiety (Guy and Gardner, 1985; File and Seth, 2003). In our studies, CDP caused significant anxiolysis at 4 mg/kg dose under high-light unfamiliar arena test condition associated with fear. SB-242084, even at the 0.3 mg/kg dose, proved to be an effective anxiolytic like CDP and did not show better anxiolytic properties at higher dose. Similar results were found previously after SB-242084 (Kennett et al., 1997; Bagdy et al., 2001) and CDP (Guy and Gardner, 1985; Kennett et al., 1997) treatment in the social interaction test of anxiety. In other tests of anxiety variable, usually higher doses of the 5-HT$_{2C}$ antagonist SB-242084 were required for a significant anxiolytic effect (Martin et al., 2002). To compare the sedative effects of the compounds, we used relatively low anxiolytic doses of the 5-HT$_{2C}$ antagonist SB-242084 and CDP. In addition to the standard scoring of vigilance states, we performed a spectral analysis of the EEG by means of fast Fourier transformation since this procedure allows a much more sensitive and detailed description of drug effects than conventional staging (Dijk et al., 1989; Sebban et al., 2002). Fourier analysis of EEG recordings allows more precise analysis of the frequency content of the recorded signal and yields data that may reveal alterations in integrated neuronal function induced by drug exposure (for review, see Eccles, 1988).

Spectral analysis of the EEG in W and PS after CDP and SB-242084 treatment showed an opposite effect of the drugs on neocortical $\theta$ activity (6–9 Hz). It has been demonstrated that the neocortical $\theta$ rhythm of rats is passively spread from the underlying hippocampus (Gerbrandt et al., 1978). $\theta$ rhythmicity of field potentials recorded in most cortical areas is thought to have a septo-hippocampal origin (Holsheimer and Feenstra, 1977). This is true also for the posterior cingulate cortex (Holsheimer and Feenstra, 1977; Talk et al., 2004), although the retrosplenial cortex may be an exception (Talk et al., 2004). Our supplementary experiments, in which virtually continuous $\theta$ activity was induced by an alarming tone, and the effect of CDP was evaluated, also confirmed that various measures of the $\theta$ oscillation change similarly in EEG signals recorded directly from the hippocampus and from the fronto-parietal leads. Therefore, it seems safe to suggest that opposite effects of CDP and SB-242084 treatment on neocortical $\theta$ activity reflect opposite changes in hippocampal activity. It is well established that the rhythm generators (i.e., pacemaker) of hippocampal-entorhinal $\theta$ oscillation are the rhythmically discharging cells of the medial septum (MS)/vertical limb of the diagonal band nucleus (DBv) that fire synchronously with the $\theta$ rhythm (Vertes and

![Fig. 4. Effect of SB-242084 (SB; 1.0 mg/kg i.p.; a and c) and CDP (4.0 mg/kg i.p.; b and d) on time of IS (a and b) and PS (c and d) in conscious, freely moving rats. Each point represents mean values (±S.E.M.) in each hour within the first 4 h after treatment. Inset, dose effect of SB (0.1, 0.3, or 1.0 mg/kg i.p.) and the effect of CDP (4.0 mg/kg i.p.) on time of IS (left) and PS (right) within the 1st h after treatment. *, significant effects ($P < 0.01$) compared with vehicle.](jpet.aspetjournals.org)
Fig. 5. Effects of CDP (4.0 mg/kg i.p.; d and h) and different doses of SB-242084 (0.1, 0.3, or 1.0 mg/kg i.p.; a–c and e–g) on EEG spectra (relative power density) in W and PS in conscious, freely moving rats in the 1st h (W) and in the first 2 h (PS) after treatment. Each point represents mean values (±S.E.M.) of 1-Hz bins in a 1-h recording period. The values are expressed for each bin as the percentage of the drug treatment values compared with vehicle treatment (=100%) of the same animal. * significant effects ($P < 0.01$) compared with vehicle.
Fig. 6. Effects of CDP (4.0 mg/kg i.p.; d and h) and different doses of SB-242084 (0.1, 0.3, or 1.0 mg/kg i.p.; a–c and e–g) on EEG spectra (relative power density) in SWS-1 and SWS-2 in conscious, freely moving rats in the 1st h after treatment. Each point represents mean values (±S.E.M.) of 1-Hz bins in a 1-h recording period. The values are expressed for each bin as the percentage of the drug treatment values compared with vehicle treatment (= 100%) of the same animal. *, significant effects (P < 0.01) compared with vehicle.
Kocsis, 1997). Both the MS and the hippocampus receive dense serotonergic innervations from the midbrain raphe nuclei (Asady et al., 1996). It has been demonstrated that inhibition of activity of midbrain raphe neurons evokes θ oscillation of septal neurons and θ wave activity in hippocampus (Vertes and Kocsis, 1997). Because serotonergic neurons have a tonic inhibitory effect on the septo-hippocampal activity via activation of GABAergic interneurons (Vertes and Kocsis, 1997; Leranth and Vertes, 1999), and the 5-HT<sub>2C</sub> receptor is present both in the MS/DBv and hippocampus located postsynaptically to the 5-HT nerve terminals (Pompiano et al., 1994; Clemett et al., 2000), the blockade of this receptor is expected to modulate the hippocampal θ activity. Correspondingly, we found a dose-dependent increase in neocortical θ activity after selective blockade of 5-HT<sub>2C</sub> receptor. Our result is in accord with a recent finding showing that SB-242084 induced or enhanced θ oscillation in MS/DBv and hippocampal neurons and θ wave activity of the hippocampus in anesthetized rats (Hajos et al., 2003). These data and our present findings demonstrate that 5-HT<sub>2C</sub> receptors, at least in part, mediate the tonic regulatory action of 5-HT system on the septo-hippocampal θ activity. A stage-dependent modulation of θ activity by the 5-HT<sub>2C</sub> receptor antagonist is intriguing, and the lack of action of the antagonist on θ during sleep could be explained by the decreased 5-HT activity/tone during this stage (Portas et al., 2000).

Studies focusing on the hippocampal θ rhythm in animals have provided good evidence that θ power is related to the encoding of new information (Klimesch, 1999; Buzsáki, 2002). Pharmacological manipulations demonstrated that drugs that decreased θ activity also blocked learning (Givens and Olton, 1995; Hasselmo et al., 2002), whereas drugs that promoted θ rhythm enhanced the induction of LTP and facilitated learning (Staubli and Xu, 1995; Wu et al., 2000). Thus, the increased θ activity after SB-242084 treatment raises the possibility of a cognitive-enhancing effect of the drug.

CDP, at a relatively low (4 mg/kg i.p.) but potent anxiolytic dose, had only mild to moderate effects on the duration of sleep and wakefulness. Similar results were found previously after 3 mg/kg p.o. dose of the drug in rats (Detari et al., 1999). On the other hand, CDP produced marked variations in EEG spectra in all vigilance states, namely a decreased lower frequency (<10 Hz) EEG power with reduced θ activity, and an increased power at frequencies above 12 Hz were found. Similar pharmaco-EEG profiles were obtained after CDP (Krijzer and van der Molen, 1987; Detari et al., 1999) or diazepam (Krijzer and van der Molen, 1987; Tobler et al., 2001) treatments. The reduced θ activity after benzodiazepines can explain the learning and memory impairment observed as a side effect after most types of benzodiazepines (Buffett-Jerrott and Stewart, 2002; Buzsáki, 2002).

It is widely accepted that slow-wave activity (SWA, EEG power in the 0.5–4.0-Hz band) is an indicator of sleep intensity (Achermann and Borbely, 2003). The reduced SWA after benzodiazepines (Borbely and Achermann, 1991; Tobler et al., 2001) reflects a more superficial sleep that can result an impaired daytime performance on the following day (Kusman et al., 1992; Roth, 2001; van Laar et al., 2001; Buffett-Jerrott and Stewart, 2002). In contrast, the 5-HT<sub>2C</sub> antagonists like ritalin-serin do not affect psychomotor performance and memory function (Danjou et al., 1992), whereas they induce a deeper or more intense sleep (Dijk et al., 1989; Borbely, 1995; Detari et al., 1999; Kantor et al., 2002) without affecting daytime performance or alertness on the following day (van Laar et al., 2001). In potent anxiolytic doses, the subtype-selective 5-HT<sub>2C</sub> antagonist SB-242084 did not affect significantly either the length of sleep stages or the intensity of sleep in our study. In contrast, CDP decreased SWA in SWS-2, resulting in reduced sleep intensity relative to controls.

Blockade of the 5-HT<sub>2C</sub> receptors by lower doses (0.1 and 0.3 mg/kg i.p.) of SB-242084 did not produce either marked sleep-waking effects or significant changes in sleep EEG; however, the drug had significant anxiolytic effect already at 0.3 mg/kg i.p. Although low doses of SB-242084 did not affect the sleep-wake cycle, we found an increased wakefulness and a decreased SWS-2 after the highest dose (1.0 mg/kg i.p.) of the drug. Targeted null mutation of the 5-HT<sub>2C</sub> receptor resulted in more wakefulness and less nonrapid eye movement sleep in mice (Frank et al., 2002). A similar, albeit not significant, increase in arousal was found in a recent study after 1 mg/kg p.o. dose of another 5-HT<sub>2C</sub> receptor antagonist, SB-243213 (Smith et al., 2002). SB-242084 and SB-243213 have a greater than 100-fold selectivity over 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptor subtypes, and it has been shown that SB-242084 reversed m-CPP-induced hypolocomotion by 50% at 2 mg/kg p.o. dose, whereas SB-243213 was effective already at 1 mg/kg p.o. (Bromidge et al., 2000). However, at higher dose (10 mg/kg p.o.), SB-243213 increased SWS-2 (Smith et al., 2002), which might otherwise be interpreted as a selective 5-HT<sub>2C</sub> receptor-mediated effect, e.g., loss of its selectivity for 5-HT<sub>2C</sub> receptor and of parallel blockade of 5-HT<sub>2A</sub> and/or other receptors. This is supported by the data that selective blockade of the 5-HT<sub>2A</sub> receptor by MDL 100907 increased EEG power only at 2 to 3 Hz (Sebban et al., 2002), and the increased low frequency activity is characteristic of SWS-2.

Recently, it has been reported that 5-HT<sub>2C</sub> antagonists increase extracellular noradrenaline and dopamine release in microdialysis experiments (Di Matteo et al., 2001; Millan, 2003). By double-label in situ hybridization, it has been demonstrated that expression of 5-HT<sub>2C</sub> receptor mRNA is restricted to GABAergic neurons in the substantia nigra of rats (Eberle-Wang et al., 1997). These data suggest that, via activation of GABAergic interneurons, 5-HT<sub>2C</sub> receptors exert an indirect tonic inhibitory influence upon the activity of mesocortical/mesolimbic dopaminergic and noradrenergic projections (Di Matteo et al., 2001, Millan, 2003). Since the sleep and EEG changes induced by the positive allosteric GABAA receptor modulator CDPs are opposite compared with those of SB-242084, it is interesting to speculate that SB-242084 caused a blockade of the 5-HT<sub>2C</sub> receptor-mediated activation of GABA neurons. Furthermore, pharmacological interventions causing a decrease of dopaminergic or noradrenergic transmission induce an increase of EEG spectral power, whereas an increase in dopaminergic or noradrenergic transmission induces a decrease in EEG spectral power, and these effects are usually characteristic at higher frequency ranges (Sebban et al., 2002). It is interesting that in the present studies the effects of the two compounds is markedly different at frequency ranges above 10 Hz. Based on this assumption, the parallel anxiolytic effect of the two compounds is intriguing, although 5-HT<sub>2C</sub> receptors could be localized in the central nervous system on neurons other than GABAergic, too.

In conclusion, our studies show that the subtype-selective 5-HT<sub>2C</sub> receptor antagonist SB-242084 at low but potent anxiolytic doses did not affect considerably either the length
of sleep stages or the intensity of sleep. The only effect was a significantly increased EEG power in the theta frequency range (peak power values at 5 and 8 Hz) in W and, at the highest dose, an increased W. The increased theta activity in W raises the possibility of a cognitive enhancing effect of this drug. In contrast, CDP resulted in decreased SWA in SWS-2; thus, a more superficial sleep and decreased theta activity in W and PS that may relate to the learning and memory impairments was observed as a side effect after most types of benzodiazepines.

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