INTRODUCTION

Since a prolonged elevation of the plasma corticosteroid level often occurs in the course of depressive disorders, it has widely been accepted that long-lasting alterations in the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis constitute a risk factor for the precipitation of the disease (1, 2, reviewed in: 3). Adrenal glucocorticoids interact with nerve cells through binding to two types of intracellular receptors: the high-affinity mineralocorticoid receptors (MRs) and the lower-affinity glucocorticoid receptors (GRs), whose activation alters expression of more than 200 genes (4-6). The activity of hippocampal neurons is modulated by the midbrain serotonergic (5-hydroxytryptamine, 5-HT) projection (7). Dysfunctional 5-HT neurotransmission has been implicated in the pathomechanism of depression (2, 8). Long-lasting exposure to high corticosterone levels results in an attenuation of responses of rat hippocampal neurons to the activation of the 5-HT1A receptor (9-11) and an enhancement of 5-HT4 receptor-mediated responses (10). High corticosterone-induced adaptive changes in the reactivity of rat hippocampal 5-HT1A and 5-HT4 receptors could be reversed by repetitive administration of a tricyclic antidepressant, imipramine (12).

It has recently been suggested that another subtype of serotonergic receptors, namely the 5-HT7 receptor, may be involved in the pathomechanism of depression and the action of antidepressant drugs (reviewed in: 13, 14). The 5-HT7 receptor knockout mice show decreased immobility in behavioral tests, resembling the effect which occurs after administration of antidepressants to normal animals (15). Downregulation of the 5-HT7 receptor has been found to occur in rat suprachiasmatic nucleus of the hypothalamus after chronic treatment with tricyclic antidepressants, including imipramine (16, 17). The selective 5-HT7 receptor antagonist SB 269970 has antidepressant-like activity (18) and enhances the action of antidepressant drugs (19, 20, reviewed in: 21).

We have previously shown that repetitive imipramine administration decreases the responsiveness of rat hippocampal slices to an agonist of the 5-HT7 receptor, 5-carboxamidotryptamine (5-CT; 0.025-1 µM), induced by an earlier treatment of animals with corticosterone. Using extracellular recording we studied changes in the reactivity of rat hippocampal slices to an agonist of the 5-HT7 receptor, 5-carboxamidotryptamine (5-CT; 0.025-1 µM), induced by an earlier treatment of animals with corticosterone. Spontaneous bursting activity was recorded in ex vivo slices incubated in the presence of 2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylecyclohexanecarboxamide (WAY 100635; 2 µM), an antagonist of the 5-HT1A receptor, in the medium devoid of Mg2+ ions. Saturation binding assays were performed using [3H]-[(2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[4-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride (SB 269970), a specific antagonist of the 5-HT7 receptor. Repetitive, but not single, corticosterone administration lasting 7 and 21 days, resulted in an enhancement of the effect related to the 5-HT7 receptor activation without changes in its binding properties. In a separate set of experiments rats were treated with corticosterone for 3 weeks and additionally with imipramine, beginning on the eighth day of corticosterone administration. In the corticosterone plus imipramine group the excitatory effect of 5-CT was weaker than in the corticosterone group, indicating that corticosterone-induced functional modifications in the reactivity of the 5-HT7 receptor were reversed and further weakened by imipramine treatment. This effect was accompanied by a reduction in the density of [3H]-SB 269970 binding sites. Thus, imipramine treatment counteracts the corticosterone-induced increase in the reactivity of the hippocampal circuitry to the activation of the 5-HT7 receptor.

Key words: 5-carboxamidotryptamine, adaptive changes, epileptiform activity, hippocampal slice, WAY 100635

MATERIALS AND METHODS

Treatment of animals

Experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology and were...
carried out in accordance with the European Community guidelines and national law. Male Wistar rats, weighing approx. 80 g at the beginning of the experiment, were housed under a controlled light/darkness cycle (light on: 7:00-19:00) and had free access to standard food and tap water. The following experimental groups were studied: (1) corticosterone treatment lasting 7 days; (2) corticosterone treatment lasting 21 days; (3) imipramine treatment lasting 14 days and (4) corticosterone plus imipramine group. In the last instance, rats received corticosterone for 21 days and since the day 8° of corticosterone treatment, they additionally received imipramine for 14 days (12). Each treated group had a matched control group, receiving vehicle, but otherwise handled identically and investigated concurrently with treated animals.

Corticosterone, suspended in 1% solution of Tween 80 in water, was injected subcutaneously (dose: 10 mg/kg; volume: 1 ml/kg) twice daily. Control animals received 1% Tween 80. Imipramine, dissolved in water, was administered per os (dose: 10 mg/kg; volume: 2 ml/kg) twice daily. Control rats received the same amount of water.

**Slice preparation, electrophysiological recording and data analysis**

Since the effects of imipramine on the reactivity of 5-HT7 receptors have been described previously (22), only rats of the experimental groups 1, 2, and 4 (see above) were subjected to *ex vivo* electrophysiological experiments. Rats were decapitated two days after the last substance administration. Their brains were rapidly removed and immersed in an ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (124), KCl (5), CaCl2 (2.5), MgSO4 (1.3), KH2PO4 (1.25), NaHCO3 (24) and D-glucose (10), which was bubbled with the mixture of 95% O2/5% CO2. After dissection, the hippocampus was cut into transverse slices (450 µm thick) using a vibrating microtome (Vibratome, USA). Slices were kept in a holding chamber at room temperature for 1-6 h. Recording was performed in the chamber of a submersed type. Slices were superfused at 32±0.5°C (2.5 ml/min) with a modified aCSF, in which [NaCl] was raised to 132 mM and [KCl] was lowered to 3 mM. Modified aCSF was devoid of Mg2+ ions and it contained 2 µM WAY 100635, to block 5-HT1A receptors. Glass micropipettes filled with 0.9% NaCl (2-4 MΩ) were inserted in the pyramidal layer of the CA3 area. Spontaneous epileptiform bursts were amplified (Axoprobe 2, Axon Instruments, USA), band-pass filtered (1 Hz-10 kHz), A/D converted (micro1401 interface with Signal 2 software, CED, UK) and analysed off-line. Activity was also displayed on a chart recorder (TA240, Gould, USA).

Bursting frequency was determined as a number of events per 1 min bins. 5-carboxamidotryptamine maleate (5-CT) was purchased from Tocris, N-[2-(2-methoxyphenyl)-1-piperazinyl][ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635) and imipramine - from Sigma and corticosterone - from MP Biomedicals. [3H]-SB-269970 was purchased from Amersham.

**RESULTS**

Epileptiform bursting of a regular frequency occurred within 15-20 min after placement of slices in a nominally Mg2+-free, modified aCSF. Individual bursting events consisted of an initial, population spike-like waveform (3-4 mV in amplitude) which was followed by a slower, positive-going wave with superimposed series of spikes (see examples in: 22, 23). Application of 5-CT resulted in a dose-dependent, 5-HT7 receptor-mediated increase in the bursting frequency which reached maximum between 6 and 10 min after the beginning of 5-CT application (22, 23). Repeated administration of corticosterone for 7 and 21 days did not change the mean baseline bursting frequency, which was not different from that recorded in slices obtained from control groups of animals, receiving vehicle (Table 1). However, the 5-CT-induced increase in the bursting frequency was significantly enhanced in slices prepared from animals treated repeatedly with corticosterone for 7 days (Fig. 1A) as well as for 21 days (Fig. 1B). In contrast, single administration of corticosterone did not modify the effect of 5-CT application (data not shown). Repeated administration of corticosterone for 21 days changed neither the affinity (Kd) of 5-HT7 receptors to [3H]-SB 269970, a selective 5-HT7 receptor antagonist (25) nor their maximum density (Bmax, Fig. 2).

In slices obtained from rats which received corticosterone for 21 days and since the eighth day of corticosterone treatment, additionally, imipramine for 14 days, the 5-CT-induced increase in the bursting frequency was significantly weaker than in the control group, receiving vehicle (Fig. 1C). In these slices a decrease in the mean basal bursting frequency was evident (Table 1). The treatment did not result in a change in the affinity of [3H]-SB-269970 to 5-HT7 receptors (Kd), however, it decreased the maximum density (Bmax) of these receptors (Fig. 2).

We have previously shown that repetitive administration of imipramine for 14 days resulted in a significant attenuation of
the excitatory effect of 5-CT, which manifested itself as a decrease in the EC50 value (3 nM and 18 nM for the control and imipramine-treated group, respectively) without a change in the maximum discharge frequency (22). As illustrated in Fig. 2, administration of imipramine for 14 days did not result in changes in Kd and Bmax values.

**DISCUSSION**

The results of the present study demonstrate that imipramine treatment counteracts the corticosterone administration-induced increase in the reactivity of rat CA3 hippocampal circuitry to the activation of the 5-HT7 receptor. Repeated corticosterone administration has often been used as an animal model to study the role of stress in depression, and it has been shown that corticosterone injections (40 mg/kg) for 21 days resulted in an increased percentage of time immobile and a smaller percentage of time swimming during the forced swim test, commonly regarded as a depression-like behavior in rats (26). In the present work the daily dose of corticosterone was lower (20 mg/kg), however, this amount has also been shown by other investigators to increase immobility time in the forced swim test, when administered repetitively for 20 days (27). It has been established that prolonged corticosterone treatment results in shrinking of apical, but not basal, dendritic tree of rat CA3

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**Table 1.** The effect of repetitive administration of corticosterone (cort) for 7 and 21 days (d), and corticosterone plus imipramine (cort+imi) for 21/14 days, on the mean (±SEM) baseline discharge frequency. *P*<0.05, *t*-test.

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**Fig. 1.** Dose-response curves for the effect of 5-CT on the bursting activity in *ex vivo* slices. In each graph filled circles denote mean values (±SEM) for slices prepared from treated animals and open circles - from controls, receiving vehicle. The solid lines are fits to the Hill equation. (A) In slices obtained from rats treated with corticosterone for 7 days the analysis yielded EC50 values of 14 nM for both the experimental and the control group. Calculated values of maximum discharge frequency are 177% and 162% of baseline values, respectively. (B) Corticosterone treatment lasting 21 days. EC50 values: 14 nM and 13 nM for the experimental and the control group, respectively. Calculated values of maximum discharge frequency are 183% and 162%, respectively. (C) Corticosterone plus imipramine group. Corticosterone was administered for 21 days and since the day 8th of corticosterone treatment, rats additionally received imipramine for 14 days. EC50 values: 33 nM and 13 nM for the experimental and the control group, respectively. Calculated values of maximum discharge frequency are 160% and 166%, respectively. For each point: n=9 to 17. *P*<0.05, ANOVA.

**Fig. 2.** The effects on [3H]-SB 269970 binding of repetitive corticosterone administration (cort) for 21 days, imipramine (imi) for 14 days and corticosterone plus imipramine (cort+imi) for 21 and 14 days since the day 8th of corticosterone treatment, respectively. Con: the control group, *P*<0.05. **Table 2.** The effect of repetitive administration of corticosterone (cort) for 7 and 21 days (d), and corticosterone plus imipramine (cort+imi) for 21/14 days, on the mean (±SEM) baseline discharge frequency. *P*<0.05, *t*-test.
pyramidal cells (28, 29) and that this changes resemble the effects of chronic stress (30). The data regarding the effects of prolonged elevation of the corticosterone level on electrophysiological properties of rat CA3 pyramidal neurons are scarce, but it has been reported that 2 weeks of corticosterone administration increases the ratio of nonbursting to bursting cells in the CA3 area (31). Noteworthy, three weeks of restraint stress increased selectively the magnitude of NMDA receptor-mediated postsynaptic currents in CA3 pyramidal cells (32). In the present study, however, we did not notice any corticosterone treatment-related effects on baseline epileptiform activity patterns in ex vivo slices, which were prepared two days after the last corticosterone administration to minimize any acute effects of the corticosteroid.

The 5-HT₇ receptor-mediated increase of the excitability of hippocampal pyramidal cells results from a reduction of the slow afterhyperpolarization (aHP) due to a reversible blockade of the Ca²⁺-activated K⁺ channel (33-35) and an increase of the hyperpolarization-activated nonselective cation current, iₜ (36). Therefore, 5-HT₇ receptors enhance spontaneous bursting in the CA3 area in Mg²⁺-free aCSF (22, 34).

The present data demonstrate that repeated corticosterone administration, lasting 7 and 21 days, results in the changes of the excitatory effect of 5-CT on epileptiform activity due to an increase in the mean discharge frequency for a given 5-CT concentration, and without a change in the EC₅₀. No change in binding properties of [³H]-SB 269970 in hippocampal homogenates occurred after corticosterone treatment lasting 21 days. Since activation of the 5-HT₇ receptor stimulates Gₛ, protein-mediated signal transduction pathway, these results are consistent with an increase in the level of Gₛ protein which has been demonstrated in CA3 pyramidal cells in rats subjected to prolonged corticosterone treatment (37). Earlier work has demonstrated that the blockade of corticosterone synthesis increases 5-HT₇ receptors mRNA expression in the CA3 area of rat hippocampus and this effect is reversed by corticosterone replacement at a dose producing full occupation of MRs and a partial occupation of GRs (38). Interestingly, also acute restraint stress increases 5-HT₇ receptor mRNA expression in the CA3 area (39). After 7 days of chronic unpredictable stress the expression of 5-HT₇ receptor mRNA was still elevated by 9%, however, plasma corticosterone level was not significantly increased at that time (39). More data are available regarding adaptive effects of corticosterone on rat hippocampal 5-HT₄ receptors. It has been shown in CA3 pyramidal neurons that chronic, high level of corticosterone alters 5-HT₄ receptor-mediated response on the level of of cellular effector systems (40). Moreover, prolonged elevation of corticosterone level for up to 3 weeks did not alter the expression of 5-HT₁₈ receptor mRNA in the CA1 area, however in this case, contrary to the 5-HT₇ receptor-mediated effects, the electrophysiological manifestations of the 5-HT₁₈ receptor activation were reduced (9, 11, 41).

We have previously shown that repetitive administration of imipramine, lasting 14 days, results in a decreased responsiveness of 5-HT₇ receptors in the CA3 area (22). Imipramine treatment resulted in an increase in the EC₅₀ without change in maximum discharge frequency. The present data extend this finding in showing that in rats not treated with corticosterone the imipramine-induced effect is not accompanied by changes in binding properties of [³H]-SB 269970. In line, earlier work has shown that while repeated administration of imipramine does not modify the basal synaptic transmission in the CA1 area (42), it alters the basal synaptic transmission and the excitatory effect of 5-HT₇ receptors (12). In the present study imipramine treatment also reduced the 5-HT₇ receptor-mediated effect, which had previously been enhanced due to repetitive administration of corticosterone. However, in slices obtained from corticosterone plus imipramine-treated rats the reactivity of 5-HT₇ receptors was reduced below control levels. Decreased basal discharge frequency, evident in this group, is unlikely to account for the reduced reactivity of 5-HT₇ receptors, since in each experiment the effects of 5-CT application were normalized relative to the basal frequency. We have previously observed a similar reduction in the frequency of epileptiform discharges in neocortical ex vivo slices obtained from rats receiving corticosterone and imipramine (50). In the present study this treatment resulted in an increase in the EC₅₀ value for the 5-HT₇ receptor agonist 5-CT. The change was accompanied by a reduction in the Bₘ₅₀ value obtained from the saturation binding assay without a change in the affinity of [³H]-SB269970 to 5-HT₇ receptors. Thus, neither imipramine nor corticosterone, applied separately, induced a reduction in radioligand binding, but the combination of both resulted in a downregulation of 5-HT₇ receptors.

In ex vivo frontal cortical slices we have previously shown that repetitive corticosterone administration for 21 days attenuates the effect of the activation of 5-HT₁₈ receptors and enhances the effect related to the activation of 5-HT₇ receptors (50), which colocalize in a majority of cortical pyramidal cells (51). As in the hippocampus, imipramine treatment reversed corticosterone-induced functional modifications in the reactivity of these receptors. Since imipramine administration results in an increase in the amount of available serotonin (and noradrenaline) it is conceivable that the biological relevance of its effect might be related to the dampening of the excessive excitatory effect of serotonin, on the excitability of hippocampal neurons, acting via 5-HT₇ receptors whose reactivity had already been elevated by an earlier treatment with corticosterone. Imipramine may inhibit corticosterone-induced gene transcription in cell cultures (52), but it is not known whether the expression of hippocampal 5-HT₇ receptors remains under control of glucocorticoid receptors. Exposition of astrocyte cultures to dexamethasone resulted in a
decreased the expression of the 5-HT₁ receptor gene (53). Conversely, stimulation of 5-HT₃ receptors increased the expression of glucocorticoid receptors in hippocampal neurons (54). It has recently been shown that acute blockade of MEK-ERK signaling produces a depressive-like phenotype in mice and blocks the effects of antidepressants, including a tricyclic drug, desipramine (55). Moreover, long-term corticosterone treatment of mice resulted in a reduction in the level of phosphorylated ERK1/2 and this deficit was reversed by subsequent administration of amitryptiline, another tricyclic antidepressant (56). Thus, one possibility is that the blockade of MEK-ERK signaling might reveal changes in the expression of hippocampal 5-HT₃ receptors.

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REFERENCES

47. Hensler JG. Regulation of 5-HT_1A receptor function in brain following agonist or antidepressant administration. *Life Sci* 2003; 72: 1665-1682.

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