IMIPRAMINE AND CITALOPRAM REVERSE CORTICOSTERONE-INDUCED ALTERATIONS IN THE EFFECTS OF THE ACTIVATION OF 5-HT₁A AND 5-HT₂ RECEPTORS IN RAT FRONTAL CORTEX

Using extracellular recording we studied changes in the reactivity of rat frontal cortical slices to the 5-HT₁A, 5-HT₂ and 5-HT₄ receptor agonists, (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphtalene hydrobromide (8-OH-DPAT), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) and zacopride, respectively, induced by an earlier treatment of animals with corticosterone lasting 1 or 3 weeks. Spontaneous bursting activity was recorded in ex vivo slices incubated in a medium devoid of Mg²⁺ ions and containing picrotoxin (30 µM). Repetitive, but not single, corticosterone administration resulted in an attenuation of the effect of the activation of 5-HT₁A receptors and in an enhancement of the effect related to 5-HT₂ receptors. The effect of 5-HT₄ receptor activation remained unchanged. In separate two sets of experiments rats were treated with corticosterone for 3 weeks and additionally with imipramine or citalopram, beginning on the eighth day of corticosterone administration. In the corticosterone plus imipramine as well as corticosterone plus citalopram groups the effects of 8-OH-DPAT and DOI were not different from control indicating that corticosterone-induced functional modifications in the reactivity of 5-HT₁A and 5-HT₂ receptors were reversed by antidepressant treatments.

Key words: corticosterone, imipramine, citalopram, 5-HT₁A receptors, 5-HT₂ receptors, 5-HT₄ receptors, cortical slice
INTRODUCTION

Chronic stress and related to it, prolonged hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis and chronic exposure to high level of glucocorticoids in the circulatory system, have been linked to the pathophysiology of depressive disorders (1, 2, reviewed in: 3, 4). Depressive disorders are associated with both structural and functional abnormalities in a number of brain structures including frontal cortical areas (5). Frontal cortex is involved in the control of mood, cognition and motor behavior, which are impaired in the course of depressive disorders, possibly due to dysfunctional monoaminergic transmission, including the serotonergic one (6). Changes in frontal cortical 5-HT\textsubscript{2} as well as 5-HT\textsubscript{1A} receptor density have been implicated in depression and in antidepressant treatments (e.g. 7, 8, 9).

In rats, the mRNAs for serotonin receptor subtypes found in frontal cortical neurons include 5-HT\textsubscript{1A/B/D}, 5-HT\textsubscript{2A/C}, 5-HT\textsubscript{3}, 5-HT\textsubscript{4}, 5-HT\textsubscript{6} and 5-HT\textsubscript{7} (10), however, the most abundant are 5-HT\textsubscript{2A} and 5-HT\textsubscript{1A} subtypes (11, 12, 13, 14). We have previously shown that the synchronized network activity in rat frontal cortical slices represents a sensitive model to assess the modulatory effects of 5-HT receptor agonists (15). Repeated administration of imipramine or citalopram induces selective changes in the reactivity of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors in rat frontal cortex (16). Repeated corticosterone administration has been suggested to represent one animal model to study the role of stress in depression (17). We have recently demonstrated that treatment with a tricyclic antidepressant, imipramine, ameliorates alterations in the effects of the activation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{4} receptors induced in the CA1 area of rat hippocampus by repeated corticosterone administration (18).

The present study was aimed at [1] determining the effects of treatment with corticosterone on the responsiveness of rat frontal cortical synchronized network activity to the activation of 5-HT\textsubscript{1A}, 5-HT\textsubscript{2} and 5-HT\textsubscript{4} receptors and [2] determining whether repeated administration of imipramine, a tricyclic antidepressant which blocks 5-HT and norepinephrine reuptake, or citalopram, a selective 5-HT reuptake inhibitor, would result in the reversal of corticosterone-induced effects.

MATERIALS AND METHODS

Animals

Experiments, which were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences, were performed on male Wistar rats, weighing initially approx. 100 g. Rats were housed under a 12 h light/darkness cycle and had free access to standard food and tap water. The following experimental groups were studied: [1] single injection of corticosterone; [2] corticosterone treatment lasting 7 days; [3] corticosterone treatment lasting 21 days; [4] corticosterone plus imipramine group and [5] corticosterone plus citalopram group. In the instances no. 4 and no. 5, rats received corticosterone for 21 days and since the day 8\textsuperscript{th} of corticosterone treatment, they additionally received imipramine (group 4) or citalopram (group 5)
for 14 days. 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 (except the group no. 1). Control animals received 1% Tween 80. Imipramine or citalopram, dissolved in water, were administrered per os (dose: 10 mg/kg, volume: 2 ml/kg) twice daily. Control rats received the same amount of water.

**Slice preparation and recording**

Rats were killed two days after the last drug administration, approximately two hours after the beginning of the light phase of the light/darkness rhythm. Their brains were removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (130), KCl (5), CaCl₂ (2.5), MgSO₄ (1.3), KH₂PO₄ (1.25), NaHCO₃ (26) and glucose (10), bubbled with a mixture of 95% O₂/5% CO₂, pH 7.4. Coronal slices (450 µm) were cut through the frontal cortex (2-3 mm anterior to bregma) using a vibrating microtome. After a recovery for at least 1 hr at room temperature a single slice was transferred to the recording chamber of a submerged type and superfused at 1.5 ml/min with warmed (32 ± 0.5°C) modified ACSF in which [NaCl] was raised to 132 mM and [KCl] was lowered to 2 mM, devoid of Mg²⁺ ions and containing 30 µM picrotoxin. For recording, glass micropipette filled with 2M NaCl (1-4 MΩ) was positioned approx. 2 mm lateral to the midline and approx. 0.3 mm below the pial surface (layer II/III). The signal was band-pass filtered (1 - 1000 Hz), recorded using 1401 interface and SIGAVG software (CED, UK) and displayed using a chart recorder (Gould, USA).

**Chemicals**

After stabilization of spontaneous bursting activity patterns, slices were superfused with a modified ACSF containing one of the following 5-HT receptor agonists: (±)-2-dipropyloamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT; Sigma), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride ((±)-DOI hydrochloride, Sigma) and 4-amino-5-chloro-2-methoxy-substituted benzamide ((R,S)zacopride, generously donated by Delalande, France). Imipramine was purchased from Polfa, Poland and citalopram was generously donated by Lundbeck, Denmark.

The results are expressed as means ± SEM. Statistical analysis was carried out using t-test.

**RESULTS**

Spontaneous bursting activity patterns recorded in the present study (Fig. 1A₁) were similar to those reported previously (15, 16). A single burst (Fig. 1A₂) consisted of an initial negative field potential followed by a slower, negative-going waveform and field potential oscillations of variable duration. It has previously been shown that bath application of 8-OH-DPAT (2 µM) for 9 min results in a 5-HT₁A receptor-mediated decrease of bursting frequency, while both the 5-HT₂ receptor agonist DOI (1µM), applied for 9 min, and 5-HT₄ agonist, zacopride (5µM), applied for 15 min, produce an increase of bursting rate (15, 16).

Neither single nor repetitive administration of corticosterone resulted in changes in the basal frequency of epileptiform discharges (Table 1). However, repetitive but not single (Fig. 1B), administration of corticosterone resulted in significant changes in the reactivity of the slices to the agonists of 5-HT receptor
subtypes. As illustrated in Fig. 1C, corticosterone treatment lasting 7 days induced an attenuation of the inhibitory effect of 8-OH-DPAT on bursting frequency (\(P<0.01, t=-3.882, df=12\)) and an enhancement of the excitatory effect of DOI (\(P<0.001, t=-6.353, df=18\)) while the excitatory effect of zacopride remained unchanged (\(P=0.166, t=-1.437, df=21\)). Fig. 1D shows that after repetitive corticosterone administration lasting 21 days (D) resulted in changes in the reactivity to the application of 8-OH-DPAT and DOI but not zacopride. In this and in the following figure: white bars - control, black bars - slices obtained from corticosterone-treated animals. Numbers on bars indicate numbers of slices in each group. \(*P<0.01, t\)-test.
Table 1. The basal frequency of epileptiform bursts in ex vivo slices. Veh - vehicle, Cort - corticosterone, Imi - imipramine, Cit - citalopram. *P<0.05, t-test.

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<th></th>
<th>Veh 1d</th>
<th>Cort 1d</th>
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<th>Con 21d + Imi 14d</th>
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<td>0.0095</td>
<td>0.021</td>
<td>0.014</td>
<td>0.0089 0.012</td>
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<td>frequency ± SEM</td>
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<td>± 0.0017</td>
<td>± 0.0013</td>
<td>± 0.0018</td>
<td>± 0.0003</td>
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![Graph A](image1)

**A**

Fig. 2. The effects of treatment with corticosterone plus imipramine (A) and with corticosterone plus citalopram (B) on 8-OH-DPAT - induced decrease and DOI - induced increase of spontaneous bursting frequency. The differences between slices obtained from treated and control animals are not significant.
It has previously been demonstrated in naive rats that repetitive administration of imipramine or citalopram, lasting 14 days, induced adaptive modifications in 5-HT$_{1A}$ and 5-HT$_{2}$ receptor-mediated modulatory effects on bursting frequency (16). In order to check whether imipramine treatment would reverse adaptive modification induced by repetitive corticosterone administration, the effects of conjoint administration of corticosterone and antidepressant were investigated. As illustrated in Fig. 2 A, in slices prepared from animals treated conjointly with corticosterone and imipramine, 5-HT$_{1A}$ and 5-HT$_{2}$ receptor-mediated effects were not different from those induced in brain slices taken from control rats (for 5-HT$_{1A}$ receptors: $P=0.531$, $t=0.635$, $df=26$; for 5-HT$_{2}$ receptors: $P=0.946$, $t=0.069$, $df=20$). Similarly, Fig. 2B shows that in slices prepared from brains of animals treated conjointly with corticosterone and citalopram, 5-HT$_{1A}$ and 5-HT$_{2}$ receptor-mediated effects were not different from those induced in slices obtained from control rats (for 5-HT$_{1A}$ receptors: $P=0.218$, $t=1.26$, $df=28$; for 5-HT$_{2}$ receptors: $P=0.193$, $t=-1.337$, $df=27$). It should be noted that the basal bursting frequency was significantly lower in slices prepared from brains of rats treated concurrently with corticosterone and imipramine than in the control group (Table 1). Such an effect did not occur in the group of animals treated with corticosterone and citalopram.

**DISCUSSION**

The pattern of spontaneous activity demonstrated by frontal cortical slices used in this study results from a combination of two phenomena: an enhancement of NMDA receptor-mediated, mono- and polysynaptic excitatory postsynaptic potentials due to incubation in Mg$^{2+}$-free ACSF and blockade of fast GABA$_A$ receptor-mediated inhibitory transmission by picrotoxin (16, 17, 19, 20). Spontaneous bursts are initiated in a small group of interconnected neurons and are dependent on intrinsic properties of pyramidal cells (20, 21, 22). It has previously been demonstrated that spontaneous epileptiform activity in frontal cortical slices could be modulated by certain 5-HT receptor agonists. While the activation of 5-HT$_{1A}$ receptors results in a reversible decrease in bursting frequency, the activation of 5-HT$_{2}$ and 5-HT$_{4}$ receptors - in an increased frequency (16, 17). 8-OH-DPAT is an agonist of 5-HT$_{1A}$ and 5-HT$_{7}$ receptors, however, the contribution of 8-OH-DPAT-mediated activation of the 5-HT$_{2}$ receptor to the observed effects is unlikely since the level of this receptor in the neocortex of adult rats is low (23). Thus, the observed effect of 8-OH-DPAT could be attributed to the activation of 5-HT$_{1A}$ receptors. The influence of 5-HT receptor agonists on bursting rate may be interpreted as a result of a reduction (by 8-OH-DPAT) and an increase (by DOI, zacopride) of the excitability of the neuronal pacemaker for spontaneous epileptiform activity. 5-HT$_{1A}$ receptors reduce the excitability of pyramidal cells by inducing membrane hyperpolarization and a decrease in the input resistance through opening of inwardly rectifying potassium channels (GIRKs; 24). 5-HT$_{2}$
receptors increase the excitability of pyramidal neurons by inducing membrane depolarization, a decrease of spike frequency accommodation and an occurrence of the slow afterdepolarization which follows the burst of spikes (25, 26). Similarly, 5-HT4 receptors increase pyramidal cell excitability by depolarization as well as by a decrease of the slow afterhyperpolarization and spike frequency adaptation (27) due to a closure of potassium channels. Moreover, in the cortex, activation of 5-HT2A receptors enhances spontaneous EPSPs (28).

Repeated corticosterone administration has been used as an animal model to study the role of stress in depression. It has been shown that repetitive corticosterone injections for 20-21 days result in an increased percentage of time immobile and smaller percentage of time swimming during the forced swim test, commonly regarded as a depression-like behavior in rats (17, 29). Corticosterone-treated animals did not express changes in activity levels or anxiety in the open-field or social interaction tests. Adrenal glucocorticoids, secreted into the circulatory system, interact with nerve cells through binding to two types of intracellular receptors: the high-affinity mineralocorticoid receptors and the lower-affinity glucocorticoid receptors, whose activation may alter expression of at least 70 genes (30). In the present study, repeated, but not single, corticosterone administration lasting 7 and 21 days altered the modulatory influence of 8-OH-DPAT and DOI on the frequency of spontaneous bursting. This result suggests that the observed effects represent adaptive modifications in the reactivity to the two 5-HT receptor agonists resulting from prolonged elevation of corticosterone level in the circulating blood. Prolonged corticosterone treatment (7 days) has been reported to decrease the number of 5-HT1A receptors (31) as well as the level of G protein (32) in rat frontal cortex. On the other hand, long-term elevation of corticosterone for 1 or 3 weeks is not related to changes in the expression of 5-HT1A receptor mRNA in the CA1 area of rat hippocampus (33), although decreased 5-HT1A receptor binding in the dentate gyrus was reported, following chronic corticosterone treatment (34). In CA3 pyramidal cells, corticosterone treatment results in a decrease of membrane conductance elicited by guanosine 5'-O-13-thiotriphosphate, which might be attributed to a reduction in G protein function or to reduced coupling between the G protein and potassium channel (35). Corticosterone treatment-induced increase in the reaction to the activation of 5-HT5 receptors is likely to result from an increased number of these receptors in the cortex (34, 36). Interestingly, the present data demonstrate that the reactivity of rat frontal cortical 5-HT4 receptors remains unchanged after chronic corticosterone administration, in contrast to the CA1 area of the hippocampus (18).

It has previously been shown that both repeated imipramine and citalopram enhanced the effect of the activation of 5-HT1A receptors on spontaneous bursting in frontal cortical slices obtained from naive rats and attenuated the effect related to 5-HT3 receptor activation, which seemed opposite to the outcome of repeated corticosterone administration, evident in this study, while the effect of the activation of 5-HT4 receptor remained unchanged (16). Treatments with imipramine or citalopram result in a reduction of 5-HT2 receptor density in rat frontal cortex (37,
Either a decrease (37, 41) or no change in the density of 5-HT\textsubscript{1A} receptors in rat brain (42, 43) have been found to occur after chronic imipramine. A lack of changes in the level of mRNA encoding 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors after imipramine or citalopram treatments has been reported (44, 45, 46). Thus, the available data suggest that the outcome of an antidepressant treatment may involve both a modification of the functional effects of the activation of certain 5-HT receptors on the activity of a neuronal network and a change in the number of these receptors. Treatment of rats with antidepressants induces an enhancement of cellular effector systems uncorrelated with changes in 5-HT\textsubscript{1A} receptor binding in the hippocampus, which may involve modifications in the capacity of the receptor to activate G protein (43). It has recently been reported that increases in postsynaptic 5-HT\textsubscript{1A} receptor agonist-stimulated [35S]GTP\textsubscript{γ}S binding occur in rat hippocampus after imipramine and fluoxetine treatments, indicative of a modification of the initial, activation step of receptor/G protein coupling (47).

In the present study, repetitive corticosterone administration did not influence basal bursting frequency. Thus, it may be concluded that corticosterone treatment does not influence the excitability of frontal cortical neurons in \textit{ex vivo} slice preparations. However, the frequency of discharges was lower in slices prepared from animals receiving concurrently corticosterone and imipramine but not corticosterone and citalopram, in comparison to controls. We have noted previously that treatment of rats with imipramine, but not with citalopram, resulted in a reduction of the mean discharge rate in frontal cortical slices (16). Reduced bursting rate after imipramine treatment could conceivably be related to antidepressant-related impairment of glutamatergic transmission. We have shown that repetitive imipramine administration for 2 weeks profoundly decreases the amplitude of stimulation-evoked field potentials (48). The decrease of field responses after citalopram treatment although significant was, however, much smaller (48) which could explain a lack of a difference in the frequency of spontaneous bursting after corticosterone plus citalopram treatment, seen in the present study.

These results indicate that chronic exposure to high levels of corticosterone, comparable to that occurring during chronic stress, results in an selective, adaptive modifications of the function of selected postsynaptic 5-HT receptor subtypes in rat frontal cortex. Corticosterone-induced modifications in the reactivity to the activation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors are likely to exert general effects on the function of the frontal cortex, since these modifications result in a net increase of the excitatory action of 5-HT on frontal cortical neurons. The effects of two antidepressants, imipramine and citalopram, which were administered concurrently with corticosterone, beginning at the time-point when corticosterone-induced modification had already been pronounced, include the normalization of the function of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} receptors. The mechanism by which treatment with corticosterone influences functions of selected 5-HT receptors and its reversal by antidepressants remains to be established.
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