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IMIPRAMINE COUNTERACTS CORTICOSTERONE-INDUCED ALTERATIONS IN THE EFFECTS OF THE ACTIVATION OF 5-HT$_7$ RECEPTORS IN RAT HIPPOCAMPUS

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Using extracellular recording we studied changes in the reactivity of rat hippocampal slices to an agonist of the 5-HT$_7$ 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)-1piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635; 2 μM), an antagonist of the 5-HT$_{1A}$ receptor, in the medium devoid of Mg^{2+} ions. Saturation binding assays were performed using [^{3}H]-(2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride (SB 269970), a specific antagonist of the 5-HT$_7$ receptor. Repetitive, but not single, corticosterone administration lasting 7 and 21 days, resulted in an enhancement of the effect related to the 5-HT$_7$ 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-HT$_7$ receptor were reversed and further weakened by imipramine treatment. This effect was accompanied by a reduction in the density of [^{3}H]-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-HT$_7$ receptor.

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

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-HT$_{1A}$ receptor (9-11) and an enhancement of 5-HT$_4$ receptor-mediated response (10). High corticosterone-induced adaptive changes in the reactivity of rat hippocampal 5-HT$_{1A}$ and 5-HT$_4$ 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-HT$_7$ receptor, may be involved in the pathomechanism of depression and the action of antidepressant drugs (reviewed in: 13, 14). The 5-HT$_7$ 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-HT$_7$ 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-HT$_7$ 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 CA3 circuitry to the activation of 5-HT$_7$ receptors (22). Since those experiments have been conducted in naive animals, in the present study we set out to evaluate whether imipramine administration would normalize changes in the reactivity of 5-HT$_7$ receptors, induced by repetitive corticosterone administration. Moreover, we investigated the density of 5-HT$_7$ receptors as well as the affinity of these receptors to radiolabelled selective antagonist [^{3}H]-SB 269970.

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 8th 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 administrered *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-HT$_7$ 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), $CaCl_2$ (2.5), $MgSO_4$ (1.3), KH_2PO_4 (1.25), $NaHCO_3$ (24) and D-glucose (10), which was bubbled with the mixture of 95% O_2/5% CO_2. 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 submerged 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 Mg^{2+} ions and it contained 2 µM WAY 100635, to block 5-HT$_{1A}$ 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) - induced effects were assessed by comparing the average frequency over 6-10 min after beginning of 5-CT application to baseline values (22, 23). Dose-response data were fitted to the Hill equation using the Sigma Plot software (SPSS Inc., USA) and compared using two-way ANOVA followed by post hoc LSD Fisher's test. Data from treated and control rats were compared using paired *t*-test.

Membrane preparation and saturation analysis

Since electrophysiological experiments demonstrated that effects of the 5-HT$_7$ receptor activation after corticosterone treatment lasting 7 and 21 days were similar (*Fig. 1A, B*), only rats of the experimental groups 2, 3 and 4 (see above) were subjected to the saturation analysis. Rats were decapitated two days after the last substance administration. Their hippocampi were removed, frozen immediately on dry ice and stored at -80°C. The membranes were prepared according to the method described previously (24) by homogenizing (Ultra Turrax) the tissue in 20 volumes (based on wet weight) of 50 mM Tris-HCl (pH=7.4 at 37°C). Following centrifugation (50000xg, 12 min, 4°C) pellets were resuspended in the same medium and incubated at 37°C for 15 min. After three further centrifugation and resuspension steps, pellets were stored at -80°C for further analysis.

The saturation binding assays were performed using the method of Thomas *et al*. (25). On the day of experiment membranes (approx. 15 mg tissue/tube) were defrosted, suspended in Tris-HCl buffer (50 mM, pH=7.4 at 37°C) containing $CaCl_2$ (4 mM), pargyline (0.1 mM) and ascorbic acid (1 mM) and incubated with [^{3}H]-SB 269970 (eight concentrations within the range: 0.2-11 nM) for 60 min at 37°C. Non-specific binding was determined using 10 µM 5-HT. Incubation was terminated by filtration through Whatman GF/B filters followed by immediate washing with ice-cold Tris-HCl buffer. Bound radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy (Beckman L SM 6500). All assays were performed in triplicate in three separate experiments. Binding data were analysed using the non-linear regression (GraphPad Software Inc., San Diego, USA) generating K_d and B_{max} values.

Drugs

5-carboxamidotryptamine maleate (5-CT) was purchased from Tocris, N-[2-[4-(2-methoxyphenyl)-1piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635) and imipramine - from Sigma and corticosterone - from MP Biomedicals. [^{3}H]-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 Mg^{2+}-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-HT$_7$ 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 (K_d) of 5-HT$_7$ receptors to [^{3}H]-SB 269970, a selective 5-HT$_7$ receptor antagonist (25) nor their maximum density (B_{max}, *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 [^{3}H]-SB-269970 to 5-HT$_7$ receptors (K_d), however, it decreased the maximum density (B_{max}) of these receptors (*Fig. 2*).

We have previously shown that repetitive administration of imipramine for 14 days resulted in a significant attenuation of

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.

cort 7 d		cort 21 d		cort 21d + imi 14 d	
treated (Hz)	vehicle (Hz)	treated (Hz)	vehicle (Hz)	treated (Hz)	vehicle (Hz)
0.141±0.007 *n*=23	0.146±0.007 *n*=24	0.188±0.012 *n*=27	0.203±0.017 *n*=25	0.166±0.011 * *n*=23	0.204±0.017 *n*=25

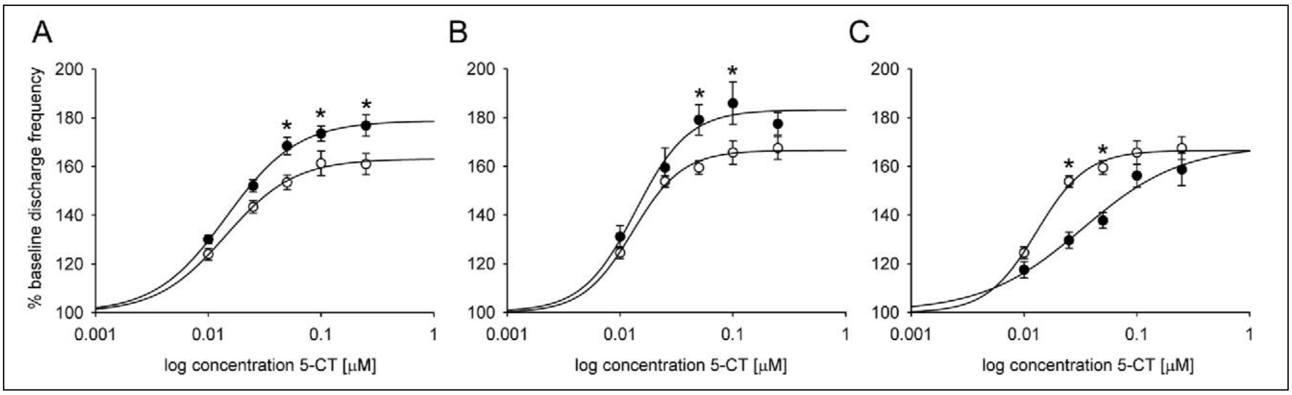

Fig. 1. Dose-response curves for the effect of 5-CT on the bursting activity in *ex vivo* slices. In each graph filled circles denote mean values (±SEM) for slices prepared from treated animals and open circles - from controls, receiving vehicle. The solid lines are fits to the Hill equation. (A) In slices obtained from rats treated with corticosterone for 7 days the analysis yielded EC_{50} 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. EC_{50} 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 8_{th} of corticosterone treatment, rats additionally received imipramine for 14 days. EC_{50} 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.

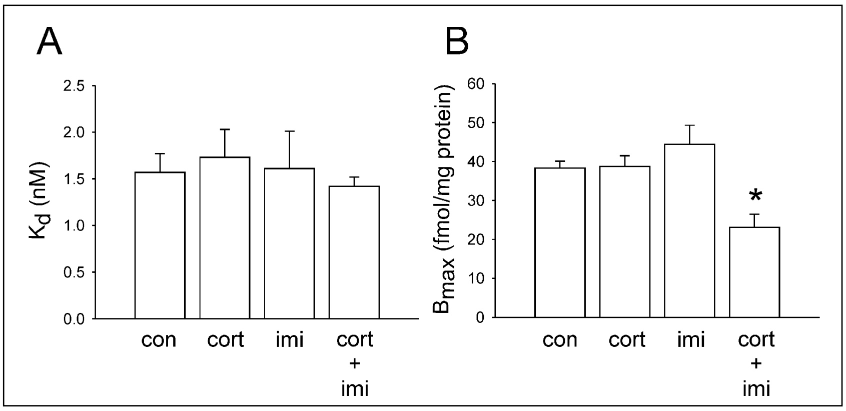

Fig 2. The effects on [^{3}H]-SB 269970 binding of repetitive corticosterone administration (cort) for 21 days, imipramine (imi) for 14 days and corticosterone plus imipramine (cort+imi) for 21 and 14 days since the day 8^{th} of corticosterone treatment, respectively. Con: the control group, * $P<0.05$.

the excitatory effect of 5-CT, which manifested itself as a decrease in the EC_{50} 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 K_d and B_{max} 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-HT_7 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 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-HT$_7$ receptor-mediated increase of the excitability of hippocampal pyramidal cells results from a reduction of the slow afterhyperpolarization (sAHP) due to a reversible blockade of the Ca^{2+}-activated K^+ channel (33-35) and an increase of the hyperpolarization-activated nonselective cation current, I_h (36). Therefore, 5-HT$_7$ receptors enhance spontaneous bursting in the CA3 area in Mg^{2+}-free aCSF (22, 34).

The present data demonstrate that repeated corticosterone administration, lasting 7 and 21 days, results in an enhancement 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 EC_{50}. No change in binding properties of [^{3}H]-SB 269970 in hippocampal homogenates occurred after corticosterone treatment lasting 21 days. Since activation of the 5-HT$_7$ receptor stimulates $G\alpha_s$ protein-mediated signal transduction pathway, these results are consistent with an increase in the level of $G\alpha_s$ 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-HT$_7$ receptor 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-HT$_7$ receptor mRNA expression in the CA3 area (39). After 7 days of chronic unpredictable stress the expression of 5-HT$_7$ 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-HT$_{1A}$ receptor. It has been shown in CA3 pyramidal neurons that chronic, high level of corticosterone alters 5-HT$_{1A}$ 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-HT$_{1A}$ receptor mRNA in the CA1 area, however in this case, contrary to the 5-HT$_7$ receptor-mediated effects, the electrophysiological manifestations of the 5-HT$_{1A}$ 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-HT$_7$ receptors in the CA3 area (22). Imipramine treatment resulted in an increase in the EC_{50} 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 [^{3}H]-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 effect of the 5-HT$_{1A}$ receptor activation on the excitability of rat CA3 (43, 44) and CA1 pyramidal neurons (45), which are not accompanied by changes in 5-HT$_{1A}$ receptor binding in the CA1 area (45). As with the corticosterone treatment, imipramine-induced adaptive modifications regarding the 5-HT$_{1A}$ and the 5-HT$_7$ receptor reactivity were opposite. These results suggest that imipramine treatment modifies signalling cascades downstream of the receptor. These modifications are likely to involve changes in the capacity of the receptor to activate G protein and/or changes in G protein expression or phosphorylation (46, 47). An attenuation of the 5-HT$_{1A}$ receptor function, measured at the level of receptor-G protein interaction without changes in receptor number, has been reported in BDNF knockout mice (48). Thus, the effects of imipramine treatment, seen in the present study, might be related to an elevation of BDNF mRNA level found to occur in the hippocampus after repetitive administration of the drug (49). In contrast, downregulation of the 5-HT$_7$ receptor, induced by a chronic treatment with a variety of antidepressants including imipramine, has previously been demonstrated in the suprachiasmatic nucleus of rat hypothalamus (16, 17), where the treatment induced a reduction in 5-HT$_7$ receptor density by approx. 30%, without changing receptor affinity. Thus, adaptive effects of imipramine treatment seem to differ, depending on a particular part of the brain. The imipramine effect may, at least partially, relate to the fact that the drug directly interacts with the 5-HT$_7$ receptor and produces functional Fos immunoreactivity (17).

We have previously demonstrated that repetitive administration of imipramine for 14 days to rats receiving daily corticosterone injections, with doses and in a regime identical to those used in the present study, normalized the responses of hippocampal pyramidal cells to agonists of 5-HT$_{1A}$ and 5-HT$_4$ receptors (12). In the present study imipramine treatment also reduced the 5-HT$_7$ 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-HT$_7$ receptors was reduced below control levels. Decreased basal discharge frequency, evident in this group, is unlikely to account for the reduced reactivity of 5-HT$_7$ 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 EC_{50} value for the 5-HT$_7$ receptor agonist 5-CT. The change was accompanied by a reduction in the B_{max} value obtained from the saturation binding assay without a change in the affinity of [^{3}H]-SB-269970 to 5-HT$_7$ 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-HT$_7$ 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-HT$_{1A}$ receptors and enhances the effect related to the activation of 5-HT$_2$ 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-HT$_7$ 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-HT$_7$ receptors remains under control of glucocorticoid receptors. Exposition of astrocyte cultures to dexamethazone resulted in a decreased the expression of the 5-HT_7 receptor gene (53). Conversely, stimulation of 5-HT_7 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-HT_7 receptors.

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