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Flesinoxan treatment reduces 5-HT$_{1A}$ receptor mRNA in the dentate gyrus independently of high plasma corticosterone levels

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Abstract

Flesinoxan acts as a full 5-HT$_{1A}$ receptor agonist and displays anxiolytic and anti-depressant properties. 5-HT$_{1A}$ receptor agonists, including flesinoxan, increase corticosterone (B) levels in the blood and reduces 5-HT$_{1A}$ receptor mRNA expression in the hippocampus. In this study, we examined whether the 5-HT$_{1A}$ receptor downregulation induced by flesinoxan involves corticosterone control of 5-HT$_{1A}$ receptor gene transcription. In experiment I, intact male Wistar rats (180–200 g) were treated with flesinoxan (1.0, 3.0 and 10 mg/kg bw, sc) or vehicle and decapitated 3 h later. Flesinoxan administration resulted in a significant, dose-dependent downregulation of 5-HT$_{1A}$ receptor mRNA in the dentate gyrus and dorsal raphe nucleus. In experiment II, rats were sham-operated and implanted with a cholesterol pellet (100 mg) or were adrenalectomized and implanted with a corticosterone pellet (20 mg corticosterone + 80 mg cholesterol). Flesinoxan injection also caused a dose-dependent decrease of 5-HT$_{1A}$ mRNA in the dentate gyrus of adrenalectomized animals with corticosterone replacement. There was no effect in the dorsal raphe nucleus. In experiment III, adrenalectomized and adrenalectomized + corticosterone rats were sc injected with flesinoxan (10 mg/kg bw) or vehicle, and flesinoxan appeared to downregulate 5-HT$_{1A}$ receptor expression in the dentate gyrus independently of corticosterone as well. No significant effects were observed in the dorsal raphe nucleus. It is concluded that flesinoxan reduces 5-HT$_{1A}$ receptor expression in the dentate gyrus both through homologous downregulation and a corticosterone-mediated effect on the serotonergic (5-HT) system.

Keywords: Flesinoxan; Corticosterone; Dentate gyrus; Dorsal raphe nucleus; Homologous regulation

1. Introduction

Receptors for corticosteroid hormones and serotonin (5-HT), i.e., 5-HT$_{1A}$ receptors, are expressed abundantly in neurons of the hippocampus. The co-localization of these receptors suggests a reciprocal relationship between the raphe 5-HT system innervating the hippocampus and the hypothalamic–pituitary–adrenal axis, which regulates the secretion of corticosterone from the adrenal. The present study addressed the downregulation of 5-HT$_{1A}$ receptor expression in the hippocampus observed after administration of an agonist of this receptor.

It was found that 5-HT$_{1A}$ receptor agonists enhance the activity of the hypothalamic–pituitary–adrenal axis. Studies in the rat showed that systemic administration of the 5-HT$_{1A}$ receptor agonists, 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT), buspirone and flesinoxan, increase the circulating levels of adrenocorticotrophic hormone (ACTH) (Koenig et al., 1987; Gilbert et al., 1988; Bluet-Pajot et al., 1995) and corticosterone (Koenig et al., 1987; Przegalinski et al., 1989; Kelder and Ross, 1992) through activation of corticotrophic hormone (CRH) neurons in the hypothalamic paraventricular nucleus (Compaan et al., 1996). Alternatively, corticosterone reduces the 5-HT$_{1A}$ receptor mRNA level and 5-HT$_{1A}$ receptor binding capacity in regions of the hippocampus (Chalmers et al., 1993; Meijer and De Kloet, 1994; Zhong and Ciaranello, 1995) but not in the raphe nucleus (Holmes et al., 1995;

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Tejani-Butt and Labow, 1994). Thus, corticosterone replacement restored normal levels of 5-HT_{1A} receptor expression in the dentate gyrus and the CA1 pyramidal cell field, and this receptor expression was enhanced after adrenalectomy (Biegon et al., 1985; Mendelson and McEwen, 1992; Chalmers et al., 1993; Meijer and De Kloet, 1994). Corticosterone may repress 5-HT_{1A} receptor gene transcription either directly or indirectly through homologous downregulation by 5-HT, since the steroid enhances hippocampal 5-HT turnover (Korte-Bouws et al., 1996).

Flesinoxan acts as a selective full 5-HT_{1A} receptor agonist (Schipper et al., 1991) and displays anxiolytic and anti-depressant properties (Bradford, 1993). Administration of flesinoxan increases the plasma corticosterone level (Groenink et al., 1995) and reduces 5-HT_{1A} receptor mRNA expression in the hippocampus (Meijer, 1996). Moreover, flesinoxan is of particular interest because of its clinical relevance (Grof et al., 1993). The present study was, therefore, designed to determine whether flesinoxan downregulates 5-HT_{1A} receptors directly, or indirectly through corticosterone. For this purpose, we examined the effects of flesinoxan in the hippocampus and dorsal raphe nucleus of adrenalectomized rats with and without corticosterone replacement. We found that flesinoxan-induced downregulation of 5-HT_{1A} receptor expression in the dentate gyrus can occur independently of high plasma corticosterone levels.

2. Materials and methods

2.1. Animals and surgery

Male Wistar rats (180–200 g) from Charles River (Germany) were housed two per cage upon arrival and allowed to acclimatize for 5 days before adrenalectomy. They were handled daily and had free access to food and water with a 12:12 h dark:light cycle (lights on at 8:00 a.m.). Adrenalectomy was performed in the morning using the dorsal approach, under ether anesthesia, 3 days before the experiment. All animal experiments were in accordance with the governmental guidelines for care and use of laboratory animals and were approved by the Animal Care Committee of the University of Leiden.

2.2. Corticosterone replacement

The cholesterol (100 mg) and corticosterone (20 mg corticosterone + 80 mg cholesterol) pellets were in tablet form and were implanted sc at the dorsal back of the neck at the same time as when adrenalectomy was done. The injections were given sc early in the morning, 3 days after adrenalectomy. The rats were observed during a 3-h period after injection for the manifestation of the lower lip retraction behaviour, a component of the 5-HT-behavioural syndrome, to check whether flesinoxan was administered properly. The rats were decapitated 3 h after the injections. Trunk blood was collected for plasma corticosterone determination and the brain was dissected out and frozen in ethanol and dry ice cooled-isopentane (-40°C).

2.3. Experimental design

Experiment I was performed to determine whether flesinoxan exerts a dose-dependent effect on 5-HT_{1A} receptor mRNA expression. Intact rats were divided into four groups: group 1 ($n = 5$) was injected sc with 0.9% NaCl, group 2 ($n = 8$) with 1 mg/kg bw flesinoxan, group 3 ($n = 5$) with 3 mg/kg bw flesinoxan and group 4 ($n = 5$) with 10 mg/kg bw flesinoxan. Flesinoxan (Solvay Duphar, Weesp) was dissolved in 0.9% NaCl (pH 4.2) and injected in a volume of 200 μl/100 g bw.

Experiment II was designed to study whether the dose-dependent downregulation of 5-HT_{1A} receptor mRNA by flesinoxan occurs in the presence of a constant level of corticosterone. Five groups of rats were used. The first two groups were sham-operated and implanted with a cholesterol pellet and were then injected with saline ($n = 5$) or 10 mg/kg bw ($n = 5$) flesinoxan. The last three groups were adrenalectomized and at the same time implanted with a corticosterone pellet and treated with saline ($n = 6$), 1 mg/kg bw ($n = 5$) and 10 mg/kg bw ($n = 6$) flesinoxan.

Experiment III was performed to determine whether the reduction of 5-HT_{1A} receptor mRNA by flesinoxan is dependent on circulating corticosterone. Four groups of rats were used: the first two groups were adrenalectomized and injected with 0.9% NaCl ($n = 6$) and 10 mg flesinoxan/kg bw ($n = 6$) while the other two groups were adrenalectomized but with a corticosterone pellet implanted and were given parallel treatments as for the first two groups ($n = 6$ for each group).

2.4. Determination of plasma corticosterone levels

Total plasma corticosterone was determined using a radioimmunoassay (RIA) with an antibody against corticosterone-21-hemisuccinate as described elsewhere (Veldhuis et al., 1982). The detection limit of the RIA is 0.1 μg/dl.

2.5. Riboprobes

PCR 1000 plasmids, containing a 350 basepair insert of the rat 5-HT_{1A} receptor mRNA that encodes for the third intracytoplasmic loop of the receptor (amino acid 220–345 Albert et al., 1990), in two orientations (kindly provided by Organon, Oss, The Netherlands) were linearized with *Eco*RI. Sense and antisence [^{35}S]UTP labeled probes were generated by in vitro transcription using T7 polymerase according to a standard protocol (Boehringer, Mannheim).

2.6. In situ hybridization histochemistry

Cryostat sections (20 μm) of the dorsal hippocampus and dorsal raphe nucleus were cut at 20°C, thaw-mounted on 0.01% poly-L-lysine coated slides and stored at −80°C until hybridization. Sections were fixed in freshly prepared 4% paraformaldehyde in phosphate buffered saline (PBS) (pH 7.2) for 60 min at room temperature just before hybridization. The sections were then washed twice in PBS (5 min each), permeabilized by proteinase K treatment (100 μg/100 ml in 0.1 M Tris, pH 8.0) for 10 min at 37°C, rinsed briefly with diethylpyrocarbonate (DEPC)-treated water, acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0), washed with 2 × SSC (SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) for 10 min, dehydrated in an increasing graded ethanol series and then air-dried. The hybridization mix consisted of 70% formamide, 10% dextran sulfate, 2 × SSC, 1 × Denhardt's solution, 10 mM dithiothreitol, 0.1 mg/ml yeast tRNA and 0.1 mg/ml sheared salmon sperm DNA. Riboprobe (1.8×10^6 dpm) was added to 1-ml aliquots. A 100-μl portion of this mix was then pipetted on each slide containing five sections. The slides were then covered with 24 × 50 mm microscopic coverslips. Subsequently, the slides were stacked and sealed in slide boxes, placed inside a moist chamber and hybridized overnight at 53°C. The following morning the cover slips were removed and the slides were washed twice in 2 × SSC at room temperature each time for 10 min. Thereafter, the slides were treated with RNAse A (2 mg/100 ml in 0.5 M NaCl, pH 7.5) at 37°C for 10 min, washed successively at 55°C in 2 × SSC (10 min), 1 × SSC (10 min) and finally in 0.1 × SSC (10 min). The slides were then dehydrated in a graded alcohol series, air dried and exposed to X-OMAT AR film for 16–21 days.

2.7. Densitometric analysis

The autoradiograms were quantified using an Olympus image analysis system with the appropriate software (Paes Nederland, The Netherlands). A shading correction was first performed and the images were further corrected for film background. The optical density of the granule cell layer of the dentate gyrus and the dorsal raphe nucleus (−7.64 until −8.0 mm from the bregma, Paxinos and Watson, 1986) were measured. The molecular layer between the CA2 and CA3 was measured for tissue background because no specific labeling was observed in this layer. Since the in situ hybridization was performed for each experiment independently the absolute values were converted into percentages to allow us to compare the mRNA expression of the three experiments.

2.8. Statistics

Optical density values of five to eight sections for each cell field per animal were pooled and were analyzed for the different treatment groups. The corticosterone levels and 5-HT_{1A} receptor density were evaluated using One-way analysis of variance (ANOVA). Post-hoc comparisons were done using Student's *t*-test and statistical significance was accepted at $P < 0.05$. Two-way ANOVA was also performed to determine whether there was an interaction with the dose treatment and adrenalectomy.

3. Results

3.1. In situ hybridixation

The first experiment (Fig. 1) showed a significant dose-dependent downregulation of 5-HT_{1A} mRNA expression in the dentate gyrus 3 h after injection. The highest concentration of flesinoxan (10 mg/kg bw) (Fig. 2B) showed a reduction of 61%, 3 mg/kg bw flesinoxan, a reduction of 49%, and 1 mg/kg bw flesinoxan, one of 33% in comparison to those for rats treated only with 0.9% NaCl (Fig. 2A). A significant reduction in 5-HT_{1A} receptor mRNA expression was also observed in rats treated with 10 mg/kg bw flesinoxan in comparison with those treated with 1 mg/kg bw flesinoxan. In the dorsal raphe nucleus, the lowest dose of flesinoxan had no effect, while a significant downregulation was observed with 3 and 10 mg/kg bw flesinoxan. The magnitude of the reduction was similar with both doses.

In the second experiment (Fig. 3), all rats acutely treated with flesinoxan showed dose dependently decreased levels of 5-HT_{1A} receptor mRNA in the dentate gyrus. There was no significant difference in 5-HT_{1A} receptor expression between sham-operated rats and ADX + corticosterone rats injected with vehicle in the dentate gyrus. There was a reducing trend in the dorsal raphe

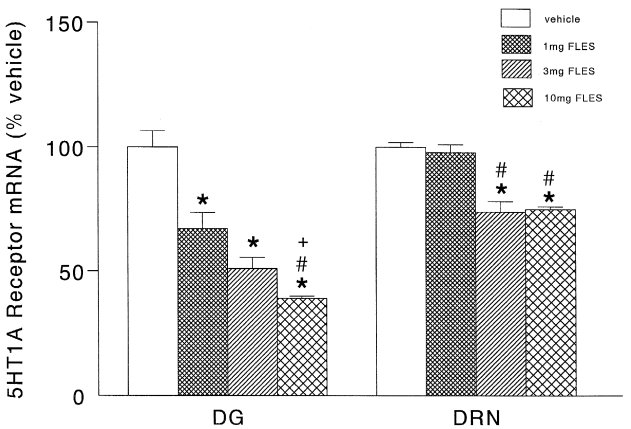

Fig. 1. Dose–response effects of acute sc injection of flesinoxan (FLES) on 5-HT_{1A} receptor expression in the dentate gyrus (DG) and dorsal raphe nucleus (DRN) of intact rats. Each bar represents the mean ± S.E.M. (% vehicle) of 5-HT_{1A} receptor mRNA expression. * Significantly different from vehicle, #significantly different from 1 mg flesinoxan, and + significantly different from 3 mg flesinoxan ($P < 0.05$).

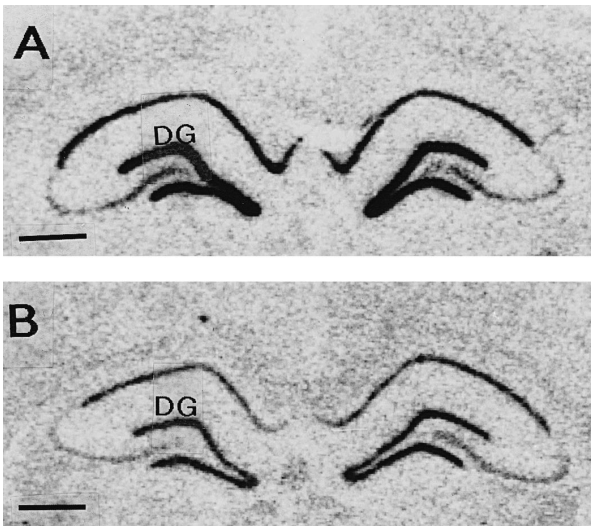

Fig. 2. Expression of 5-HT_{1A} receptor in the dentate gyrus (DG) of intact rats 3 h after an acute sc injection of saline (A) and 10 mg flesinoxan (B). A significant reduction of 5-HT_{1A} receptor expression can be seen in the dentate gyrus of the group treated with 10 mg flesinoxan. Scale bars: 70 μm.

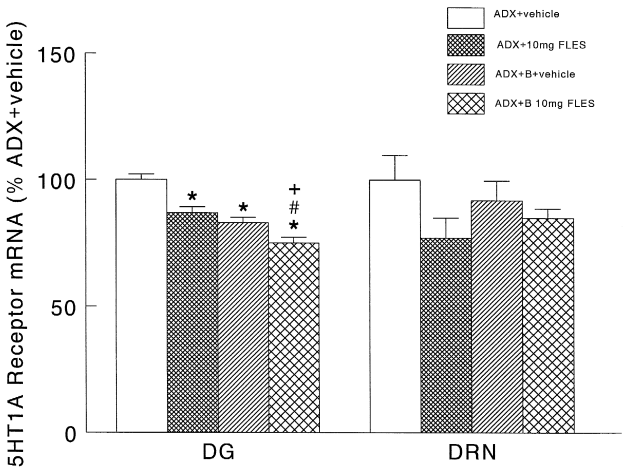

Fig. 4. Effects of acute sc injection of flesinoxan (FLES) on 5-HT_{1A} receptor expression in the dentate gyrus (DG) and dorsal raphe nucleus (DRN) of adrenalectomized (ADX) with and without corticosterone (B) (20 mg corticosterone + 80 mg cholesterol)-replaced rats. Each bar represents the mean ± S.E.M. (%ADX + vehicle) of 5-HT_{1A} receptor mRNA expression. ∗ Significantly different from ADX + vehicle, # significantly different from ADX + 10 mg flesinoxan, and + significantly different from ADX + B + vehicle ($P < 0.05$).

nucleus of the sham + flesinoxan group against the control but this failed to reach significance. Flesinoxan did not exert a downregulating effect in the dorsal raphe nucleus of adrenalectomized + corticosterone rats. Two-way ANOVA showed no interaction between adrenalectomy + corticosterone and flesinoxan dose effect in the two brain areas examined.

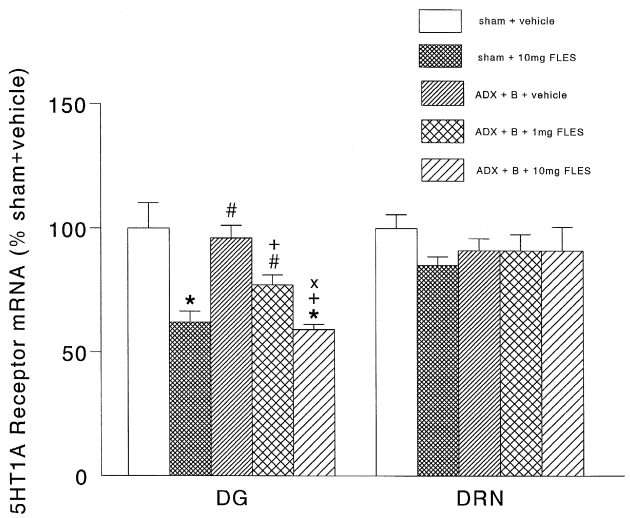

Fig. 3. Effects of acute sc injection of flesinoxan (FLES) on 5-HT_{1A} receptor expression in the dentate gyrus (DG) and dorsal raphe nucleus (DRN) of sham, adrenalectomized (ADX) + corticosterone (B) rats. Each bar represents the mean ± S.E.M. (% sham + vehicle) of 5-HT_{1A} receptor mRNA expression. ∗ Significantly different from sham + vehicle, # significantly different from sham + 10 mg FLES, + significantly different from ADX + B + vehicle, and × significantly different from ADX + B + 10 mg FLES ($P < 0.05$).

Results of the third experiment (Fig. 4) showed that flesinoxan significantly downregulated 5-HT_{1A} receptor mRNA expression in the dentate gyrus in both adrenalectomized and adrenalectomized + corticosterone rats. The magnitude of the reduction was in the following order: adrenalectomized + corticosterone + flesinoxan, adrenalectomized + corticosterone + vehicle and adrenalectomized + flesinoxan. In the dorsal raphe nucleus, flesinoxan only caused a reducing trend in adrenalectomized rats. No effect was observed in the adrenalectomized + corticosterone rats. There was no interaction between adrenalectomy/corticosterone and the flesinoxan dose effect.

In situ hybridization using the sense probe showed no specific labeling.

3.2. Determination of plasma corticosterone levels

The plasma corticosterone-levels in the different groups of intact rats in experiment I were not significantly different (Table 1A). The corticosterone values from rats treated with flesinoxan were highly variable. In groups treated with 1 mg and 3 mg, one rat from each group showed high concentrations, 7.02 and 14.62 (data not shown) respectively, and the animals treated with 10 mg/kg bw flesinoxan showed generally higher corticosterone levels than the other groups. In the second experiment, the plasma corticosterone levels in the sham-operated rats and the adrenalectomized + corticosterone rats were significantly different (Table 1B). Among the sham-operated rats, those injected with flesinoxan had significantly higher corticosterone levels than those injected with vehicle ($P = 0.05$). In the third experiment, the adrenalectomized groups

Table 1
Plasma corticosterone levels 3 h after flesinoxan or saline injection

Group	μg/dl
A. Plasma corticosterone levels in intact rats treated with 0.9% NaCl or flesinoxan (per kg bw)	
1. vehicle (0.9% NaCl)	0.91 ± 0.31
2. 1 mg flesinoxan	1.47 ± 0.92
3. 3 mg flesinoxan	3.24 ± 2.60
4. 10 mg flesinoxan	3.13 ± 0.77
B. Plasma corticosterone levels in sham and adrenalectomized (ADX) + corticosterone (B) rats treated with vehicle or flesinoxan (per kg bw)	
1. sham + vehicle	1.13 ± 0.39
2. sham + 10 mg flesinoxan	5.99 ± 1.66[a]
3. ADX + B + vehicle	5.71 ± 0.50[a]
4. ADX + B + 3 mg flesinoxan	6.70 ± 0.96[a]
5. ADX + B + 10 mg flesinoxan	7.10 ± 0.69[a]
C. Plasma corticosterone levels in adrenalectomized (ADX) and ADX + corticosterone (B) rats treated with vehicle or flesinoxan (per kg bw)	
1. ADX + vehicle	0.31 ± 0.10
2. ADX + 10 mg flesinoxan	0.35 ± 0.07
3. ADX + B + vehicle	6.31 ± 0.5[b,c]
4. ADX + B + 10 mg flesinoxan	7.47 ± 0.67[b,c]

[a] Significantly different from sham + vehicle ($P = 0.05$, 0.00 and 0.00, respectively).
[b] Significantly different from ADX + vehicle ($P = 0.00$ for both).
[c] Significantly different from ADX + 10 mg flesinoxan ($P = 0.00$ for both).

had significantly lower levels of corticosterone than did the adrenalectomized + corticosterone groups (Table 1C). Two-way ANOVA showed no interaction between adrenalectomy/corticosterone and the flesinoxan dose effect.

3.3. Lower lip retraction

All rats injected with flesinoxan exhibited lower lip retraction. The intensity and the onset of the behavior were dose-dependent. Rats treated with 1 mg/kg bw flesinoxan exhibited the behaviour about 30 min after injection and the intensity of protrusion was mild. Rats given 3 mg flesinoxan had a delay of approximately 15–20 min with moderate to strong protrusion and those given 10 mg flesinoxan had a delay of 5–10 min after injection with severe to very severe retraction. However, the qualitative manifestation of the behaviour was not influenced by the hormonal status of the animal since no differences were observed among intact, sham, adrenalectomized and adrenalectomized + corticosterone rats.

4. Discussion

The present study showed that flesinoxan downregulates 5-HT_{1A} receptor expression 3 h after its injection in a dose-dependent manner and with selectivity for the dentate gyrus of the hippocampus. The 5-HT_{1A} receptor mRNA downregulation induced by flesinoxan might involve corticosterone, since it is well documented that 5-HT_{1A} receptor agonists activate the hypothalamic–pituitary–adrenal axis (Koenig et al., 1987; Bluet-Pajot et al., 1995; Groenink et al., 1995). The subsequent rise in circulating corticosterone concentrations then would downregulate 5-HT_{1A} receptor expression in the dentate gyrus, since this also occurs after corticosterone administration (Chalmers et al., 1993; Meijer and De Kloet, 1994, 1995) or after chronic stress associated with elevated corticosterone levels (McKittrick et al., 1995). Alternatively, the results of experiment III with adrenalectomized rats clearly demonstrated that flesinoxan administration caused a significant reduction of 5-HT_{1A} receptor mRNA in the dentate gyrus in the absence of corticosterone also. Although this effect of flesinoxan in adrenalectomized animals was smaller than that after administration to the intact animals or to the adrenalectomized + corticosterone-treated rats, it argues for a direct flesinoxan control of 5-HT_{1A} receptor mRNA regulation. Accordingly, flesinoxan reduces dentate gyrus 5-HT_{1A} receptor expression by homologous, as well as by heterologous, downregulation involving corticosterone.

Groenink et al. (1995) showed that, in a similar dose range, flesinoxan causes an increase in plasma corticosterone levels, which attain a maximal increase at 90 min after sc injection. Unfortunately, their study covered only a 2-h period and at that time the plasma corticosterone levels had started to decline. Thus, killing of the rats after 3 h would have allowed plasma corticosterone to return towards its basal levels. The latter is supported by the plasma corticosterone levels which were still slightly increased in some rats, but with some variation. A time delay of 3 h certainly provided sufficient time for corticosterone to exert its suppression of 5-HT_{1A} receptor gene expression (Meijer and De Kloet, 1995). However, reduction of 5-HT_{1A} receptor expression can also take place in the presence of a constant circulating level of cortico-

sterone. Flesinoxan was also capable of reducing almost twofold 5-HT_{1A} receptor mRNA in the adrenalectomized corticosterone-replaced animals, while Two-way ANOVA showed no interaction between flesinoxan dose and corticosterone. The cumulative effect of flesinoxan in the presence of low corticosterone replacement was of the same magnitude as that observed after flesinoxan administration to intact animals which most likely had experienced exposure to high corticosterone. These data indicate that 5-HT_{1A} receptor downregulation can occur independently of a rise in plasma corticosterone level. Therefore, the effects of corticosterone and flesinoxan on dentate gyrus 5-HT_{1A} receptor regulation can be considered to be independent and additive.

Is the downregulation of 5-HT_{1A} receptor expression in the dentate gyrus mediated by mineralocorticoid receptors or by glucocorticoid receptors? The effectiveness of the low corticosterone pellet in ADX rats favors mineralocorticoid receptor mediation. Previous studies had shown that 5-HT_{1A} receptor mRNA and binding is induced by adrenalectomy and reversed by low doses of corticosterone (Mendelson and McEwen, 1992; Chalmers et al., 1993; Tejani-Butt and Labow, 1994; Meijer and De Kloet, 1995) or aldosterone (Kuroda et al., 1994) suggesting that this effect is mediated by the mineralocorticoid receptor. The fact that this corticosterone effect is blocked by the anti-mineralocorticoid RU28318 ([7.17 alpha]-17-hydroxy-3-*oxo*-7-propl-pregn-xene-21-carboxylic acid potassium) and not by the anti-glucocorticoid RU38486 (Meijer and De Kloet, 1995), further supports this latter possibility. Also the glucocorticoid receptor agonist RU28362 was not effective (Kuroda et al., 1994), but an effect of dexamethasone has been reported (Chalmers et al., 1994; Zhong and Ciaranello, 1995). Furthermore, in knockout mice homozygous for a disrupted glucocorticoid receptor gene, the 5-HT_{1A} receptor level in the hippocampus was not changed (Meijer et al., 1997). Since adrenalectomy still caused an increase in 5-HT_{1A} receptor expression in these mutants, this finding showed that occupancy of solely the mineralocorticoid receptors is sufficient to maintain the 5-HT_{1A} receptor level. Thus, the selectivity and mineralocorticoid receptor-mediation of the corticosterone effect on the dentate gyrus 5-HT_{1A} receptor expression is in agreement with results of most other studies.

The conclusions from most studies agree that the 5-HT_{1A} receptors in the presynaptic 5-HT_{1A} receptors in the dorsal raphe nucleus are quite resistant to corticosteroid-induced downregulation (Tejani-Butt and Labow, 1994; Holmes et al., 1995; Meijer and De Kloet, 1995). In the present study, at 3 days after adrenalectomy, we did not observe a corticosterone effect in the dorsal raphe nucleus. There is only one study which showed downregulation of 5-HT receptor number in the dorsal raphe, but this effect occurred 1 h after adrenalectomy (De Kloet et al., 1986) and probably reflected acute changes in 5-HT turnover rate (Van Loon et al., 1981, 1982; De Kloet et al., 1982). Although we did not measure the effect of 3-day adrenalectomy and corticosterone replacement on 5-HT release in the hippocampus and dorsal raphe area in the present study, the contribution of 5-HT autoregulation to 5-HT_{1A} receptor expression in the dorsal raphe nucleus was probably very limited. In line with this was our observation that corticosterone did not affect the intensity of flesinoxan-induced lower lip retraction, which is probably mediated by dorsal raphe nucleus presynaptic 5-HT_{1A} receptors (Berendsen et al., 1989, 1994).

Flesinoxan-induced 5-HT_{1A} receptor mRNA downregulation in adrenally intact and ADX corticosterone-replaced rats did not seem to be restricted in the dentate gyrus, but this effect in the dorsal raphe nucleus was only consistently observed with the highest dose range. This is an intriguing aspect of the study, since it is known that presynaptic receptors are activated at lower doses by 5-HT_{1A} receptor agonists (Dourish et al., 1985) and it was, therefore, expected that the most pronounced flesinoxan effects would have been in this region. Flesinoxan activation of presynaptic 5-HT_{1A} receptors would reduce the 5-HT nerve impulse flow in the raphe-hippocampal system and thus, the release of 5-HT from nerve terminals. Since such a presynaptic blockade of 5-HT release would instead lead to a reduced 5-HT release in the hippocampus, producing a compensatory rise in postsynaptic 5-HT_{1A} receptors, it is unlikely that it was the cause of the 5-HT_{1A} downregulation we now observed. The latter cannot proceed also indirectly through a flesinoxan-induced corticosterone effect on 5-HT turnover, since flesinoxan reduces 5-HT_{1A} receptors in ADX rats also.

The most likely explanation is that flesinoxan reduced 5-HT_{1A} receptor expression through homologous downregulation. Our data showing downregulation in the dentate gyrus in the absence of corticosterone strongly suggest such a direct activation of 5-HT_{1A} receptors. The suggestion that postsynaptic receptors are sensitive to this type of regulation stems from observations made after neurotoxic lesioning by central administration of 5,7-dihydroxytryptamine. This lesion has been reported to induce postsynaptic 5-HT_1 receptors in the dentate gyrus and other brain regions depleted of 5-HT (Sijbesma et al., 1991). Not all findings agree with this and some conflicting data can be ascribed to positioning of the lesion and to the time period allowed for the compensatory 5-HT receptor increase. The present study showed that the 5-HT_{1A} receptor mRNA downregulation in response to flesinoxan proceeds within 3 h. Desensitization of the presynaptic receptors might be involved in this regulation. Kennett et al. (1987) had shown that a single pretreatment with 8-OH-DPAT rapidly desensitizes 5-HT_{1A} presynaptic receptor-mediated responses. However, flesinoxan treatment did not alter the subsequent electrophysiological response to 5-HT (Hadrava et al., 1995). It is, therefore, still unclear whether changes in 5-HT release occur as a consequence of flesinoxan-induced desensitization.

In conclusion, our data clearly showed that acute flesinoxan injection exerts a dose-dependent effect on the reduction of 5-HT_{1A} receptor mRNA in the dentate gyrus but not in the dorsal raphe nucleus. This downregulation is probably brought about by two independent and additive effects: suppression via flesinoxan induced corticosterone secretion and a direct flesinoxan activation of hippocampal 5-HT_{1A} receptors. The latter effect probably underlies the desensitizing effect of repeated treatment with 5-HT_{1A} receptor agonists.

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