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European Journal of Pharmacology 404 (2000) 111–119

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Effects of flesinoxan on corticosteroid receptor expression in the rat hippocampus

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Received 13 April 2000; received in revised form 2 August 2000; accepted 8 August 2000

Abstract

Many agents that influence serotonergic neurotransmission modulate expression of hippocampal corticosteroid receptors. We have studied the effect of the specific 5-hydroxytryptamine, 5-HT_{1A}, receptor agonist flesinoxan on mRNA for glucocorticoid and mineralocorticoid receptors in the hippocampus and dorsal raphe nucleus. Since some responses to 5-HT_{1A} receptor stimulation show a strong desensitization, we studied the effect of a single and repeated injections of flesinoxan. Because of the close interrelationship between the serotonergic system and the hypothalamo-pituitary–adrenal axis, we also studied the possible involvement of corticosterone as a mediator of the effects of flesinoxan. We found that a single injection of flesinoxan (3 and 10 mg/kg subcutaneously, s.c.) after 3 h leads to a downregulation of glucocorticoid receptor mRNA in the hippocampus (dentate gyrus and CA1 areas) and dorsal raphe nucleus. This effect does not desensitize after a second treatment over 2 days. Mineralocorticoid receptor mRNA expression remained unaltered. The decrease in hippocampal glucocorticoid receptor mRNA expression occurs independently of circulating corticosterone since flesinoxan reduced glucocorticoid receptor mRNA in the hippocampus of adrenalectomized rats with or without corticosterone replacement. These data indicate that the 5-HT_{1A} receptor agonist flesinoxan alters glucocorticoid receptor expression via a direct pathway independently of corticosterone and argues for an intrinsic effect selective for hippocampal glucocorticoid receptor mRNA.

Keywords: 5-HT_{1A} receptor agonist; Flesinoxan; Glucocorticoid receptor; Mineralocorticoid receptor; Hippocampus; Dorsal raphe nucleus

1. Introduction

There is extensive evidence showing that corticosteroid receptors are regulated by multiple factors including serotonin and corticosteroids themselves. Adrenalectomy and corticosterone replacement studies have shown that corticosteroid receptors are upregulated in the absence of corticosterone and downregulated when it is present in excess (Reul et al., 1987, 1989, 1990; Herman et al., 1989). There are two types of corticosteroid receptors: the mineralocorticoid receptor and glucocorticoid receptor. Both receptor types are present in the hippocampus where they are co-localized (Van Steensel et al., 1996) but differ in their binding affinity to corticosterone. The affinity of mineralocorticoid receptor is approximately 10 times higher than the glucocorticoid receptor (Reul and De Kloet, 1985; Funder et al., 1988) and as a consequence of this, mineralocorticoid receptors are already activated with basal low levels of corticosterone, while glucocorticoid receptors are fully activated only when there are high levels of circulating corticosterone such as during stress or their circadian peak (Reul and De Kloet, 1985).

Serotonin (5-hydroxytryptamine, 5-HT) has been shown to have regulatory influences on neural corticosteroid receptors. 5-HT-depleting drugs, such as 6-hydroxydopamine (Weidenfeld et al., 1983), *p*-chloroamphetamine (Novotney and Lowy, 1995), methamphetamine (Lowy, 1990; Lowy and Novotney, 1994) and 5,7-dihydroxytryptamine (Seckl et al., 1990) reduced corticosteroid re-

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00605-1

ceptor mRNA expression and binding sites in the hippocampus. On the other hand, antidepressants, which are known to potentiate serotonergic actions, increase corticosteroid receptor levels (Peiffer et al., 1991; Seckl and Fink, 1992; Reul et al., 1993).

These effects on corticosteroid receptors brought about by changes in 5-HT levels are not surprising since corticosteroid receptors are localized in 5-HT neurons (Harfstrand et al., 1986) and there is a dense ascending serotonergic innervation from the median raphe nucleus, which projects to corticosterone-concentrating neurons of the dorsal hippocampus (Zhou and Azmitia, 1983; Azmitia et al., 1984). However, the mechanism underlying serotonergic modulation of corticosteroid receptor levels is still poorly understood. The 5-HT_{1A} receptor is a candidate to mediate effects of 5-HT on hippocampal neurons because of its high level of expression in the hippocampus (Chalmers and Watson, 1991; Pompeiano et al., 1992). Our previous data showed that acute injection of flesinoxan, a specific 5-HT_{1A} receptor agonist (Schipper et al., 1991), downregulates hippocampal 5-HT_{1A} receptor mRNA expression in the presence or absence of corticosterone (Sibug et al., 1998). In this paper, we extend these data to the regulation of corticosteroid receptor mRNA expression.

Flesinoxan has anxiolytic and antidepressant properties (Bradford, 1993) and activates the hypothalamo-pituitary–adrenal axis (Groenink et al., 1995). Repeated administration of flesinoxan or of other 5-HT_{1A} receptor agonists attenuate corticosterone response (Kelder and Ross, 1992; Groenink et al., 1997) and c-*fos* immunoreactivity in the paraventricular nucleus of the hypothalamus (Compaan et al., 1997). But other 5-HT_{1A} receptor-mediated effects, such as anxiolysis, do not show desensitization. In the case of corticosteroid receptors, we show here that glucocorticoid receptor mRNA expression in the dentate gyrus and CA1 is downregulated by flesinoxan, as measured by in situ hybridization histochemistry, and that this response is not desensitized after a second treatment of flesinoxan 24 h later.

Flesinoxan stimulates release of corticosterone (Koenig et al., 1987; Przegalinski et al., 1989; Kelder and Ross, 1992; Groenink et al., 1995) via a central action starting from the release of the corticotropin releasing hormone (CRH) in the hypothalamus (Korte et al., 1991; Compaan et al., 1996). Based on this, we hypothesized that flesinoxan may cause alterations only in rats with an intact hypothalamo-pituitary–adrenal axis. To test this hypothesis, we studied the effects of flesinoxan on mineralocorticoid and glucocorticoid receptor mRNA expression in the hippocampus of sham-operated, adrenalectomized and corticosterone-replaced adrenalectomized rats 3 h after its administration. Flesinoxan, on the contrary, downregulated glucocorticoid receptor mRNA expression in adrenalectomized rats in the absence or presence of exogenous corticosterone more robustly than in rats with an intact hypothalamo-pituitary–adrenal axis.

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 5 days before adrenalectomy. They were handled daily and had free access to food and water with a 12:12 dark/light cycle (lights on at 0800 h). 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 approved by the Animal Care Committee of the University of Leiden.

2.2. Drugs

Flesinoxan hydrochloride, (+)-*N*-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxan-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzamide-HCl, kindly donated by Solvay-Pharmaceuticals (Weesp, The Netherlands) was used and dissolved in saline (0.9% NaCl, pH 4.2). Saline (0.9%) adjusted to pH 4.2 was used as vehicle. The injections were administered subcutaneously (s.c.) in the dorsal flank, in a volume of 2 ml/kg body weight (bw) between 0800 and 1300 h.

2.3. Experimental design

2.3.1. Determination of the effects of repeated administration of flesinoxan on mineralocorticoid and glucocorticoid receptor mRNA expression

The rats were treated with one injection of flesinoxan (3.0 mg/kg) or vehicle at day 1 between 0900 and 1300 h. At day 2, the animals received a second injection of either flesinoxan (3.0 mg/kg) or vehicle during the same time period as the day before. This procedure resulted in four groups ($n = 6–7$ each) as follows: vehicle/vehicle, vehicle/flesinoxan, flesinoxan/vehicle and flesinoxan/flesinoxan.

2.3.2. Determination of the effects of acute administration of flesinoxan on mineralocorticoid and glucocorticoid mRNA expression

Three groups of rats, divided into two subgroups, were used: sham, adrenalectomized and adrenalectomized with implanted corticosterone pellet rats. The cholesterol (100 mg) and corticosterone (20 + 80 mg cholesterol) pellets were in tablet form and implanted s.c. at the dorsal back of the neck at the same time when adrenalectomy was done. From each group, one subgroup was injected with vehicle ($n = 6$) and the other subgroup with 10 mg flesinoxan/kg bw ($n = 5–6$ each) giving rise to a total of six subgroups. The injections were given s.c. in the morning 3 days after adrenalectomy.

All the rats from the experiments were decapitated 3 h after the last flesinoxan or vehicle injection. 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). The rats were observed during a 3-h period after drug or vehicle administration for the manifestation of the lower lip retraction behavior, a component of the 5-HT-behavioral syndrome, to check whether flesinoxan was efficiently administered.

2.4. Determination of plasma corticosterone levels

Total plasma corticosterone was determined using a radioimmunoassay (RIA) using 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

The antisense mineralocorticoid receptor probe was transcribed from a 513-bp rat brain cDNA fragment, which encodes for the last 30 amino acids at the C-terminus of mineralocorticoid receptor plus the adjacent highly specific 3′ untranslated region (courtesy of J.L. Arriza, USA). The antisense glucocorticoid receptor probe was transcribed from a 500-bp cDNA fragment (courtesy of M.C. Bohn, USA) and encoding for the N-terminal region of the glucocorticoid receptor molecule. In vitro transcription for the antisense and sense strands for both probes were generated with SP6 and T7 RNA polymerase, respectively, using a standard protocol (Boehringer, Mannheim).

2.6. In situ hybridization histochemistry

Cryostat sections (20 μm) of the dorsal hippocampus and dorsal raphe were cut at −20°C, thaw-mounted on 0.1% 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. They were then washed twice in PBS (5 min each), permeabilized with 0.01 N HCl for 10 min at room temperature, rinsed briefly with diethyl pyocarbonate-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 50% formamide, 10% dextran sulfate, 2× SSC, 1× Denhardt's solution, 10 mM dithiothreitol, 0.1 mg/ml yeast tRNA and 0.1 mg/ml poly A. Riboprobe (2.2×10^6 dpm) was added to aliquots of 1 ml and 100 μl of this mix was then pipetted on each slide containing five sections and were 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 45°C. The following morning, the coverslips were removed and the slides were washed thrice in 2× SSC at room temperature each time for 10 min and twice in 2× SSC/50% formamide at 55°C (15 min each). Thereafter, the slides were treated with RNAse A (2 mg/100 ml in 0.5 M NaCl, pH 7.5) at 37°C for 30 min, dipped shortly in 2× SSC at 37°C and washed thrice at 55°C in 2× SSC/50% formamide (15 min each) and finally twice in 2× SSC (5 min each). The slides were then dehydrated in a graded alcohol series, air-dried and exposed to X-OMAT AR film for 9 and 13–18 days for mineralocorticoid and glucocorticoid receptor, respectively.

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 pyramidal cell layer of areas 1 and 3 of Cornu Ammonis (CA), granule cell layer of the dentate gyrus and dorsal raphe nucleus were measured. The molecular layer between the CA2 and CA3 was measured for tissue background in the hippocampus because no specific labeling was observed in this layer while the region immediately outside the brain was used as background for the dorsal raphe nucleus.

2.8. Statistics

Optical density values of four sections for each cell field per animal were pooled and were analyzed for the different treatment groups. Two-way analysis of variance (ANOVA) was performed for the desensitization experiment in order to determine significant treatment effects on the corticosterone levels and the expression of mineralocorticoid receptor and glucocorticoid receptor mRNA expression in the hippocampus and glucocorticoid receptor mRNA expression in the dorsal raphe nucleus. The same test was done for the flesinoxan-acute-effects experiment to determine whether there was an interaction with treatment and adrenalectomy/corticosterone. The cortico-

Table 1
Plasma corticosterone levels after pretreatment with vehicle (0.9% NaCl) or flesinoxan and challenged 24 h later with vehicle or flesinoxan

Group	μg/dl
1. Vehicle + vehicle	0.5 ± 0.0
2. Vehicle + flesinoxan	5.4 ± 2.9[a]
3. Flesinoxan + vehicle	1.9 ± 0.8
4. Flesinoxan + flesinoxan	3.6 ± 1.0

Values are 3 h after last treatment.
[a] Statistically significant against vehicle + vehicle ($P < 0.05$).

Table 2
Plasma corticosterone levels 3 h after acute administration of vehicle (0.9% NaCl) or flesinoxan

Group	μg/dl
1. Sham + vehicle	1.2 ± 0.5
2. Sham + flesinoxan	10.4 ± 4.5[a]
3. ADX + vehicle	0.3 ± 0.0
4. ADX + flesinoxan	0.4 ± 0.0
5. ADX + B + vehicle	6.3 ± 0.6[a,b]
6. ADX + B + flesinoxan	7.5 ± 0.7

[a] Statistically significant against sham + vehicle ($P < 0.05$).
[b] Statistically significant against ADX + vehicle ($P < 0.05$).

sterone levels and receptor density for mineralocorticoid and glucocorticoid receptor were further analyzed using one-way ANOVA. Post-hoc comparisons were done using least significant different test and statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effects of flesinoxan on plasma corticosterone levels

Flesinoxan challenge enhanced plasma corticosterone levels in animals pretreated with vehicle in comparison with those challenged with vehicle ($P < 0.05$) (Table 1). However, the rats that were pretreated with flesinoxan did not show a significant increase in plasma corticosterone level after injection of flesinoxan.

In the second experiment, flesinoxan ($F(5,28) = 7.3$, $P < 0.02$) and adrenalectomy ($F(5,28) = 8.9$, $P < 0.02$) exerted main effects on the plasma corticosterone levels. Flesinoxan increased plasma corticosterone levels in sham animals ($P < 0.01$) and showed no effect on adrenalectomized and corticosterone-replaced adrenalectomized rats (Table 2). Between the two hormonally manipulated groups, the adrenalectomized group showed significantly lower levels of corticosterone in comparison with the adrenalectomized + corticosterone group ($P < 0.03$). The latter had also a significantly higher levels against the sham + vehicle group ($P < 0.05$). Two-way ANOVA showed an interaction between adrenalectomy/corticosterone and flesinoxan effect ($F(5,28) = 4.5$, $P < 0.04$).

3.2. Lower lip retraction

All rats injected with flesinoxan exhibited lower lip retraction and the qualitative manifestation of the behavior was not influenced by the hormonal status of the animal since no difference was observed among sham, adrenalectomized and adrenalectomized + corticosterone rats.

3.3. Effects of flesinoxan on glucocorticoid receptor mRNA expression

Repeated administration of flesinoxan decreased glucocorticoid receptor mRNA levels in the CA1 and dentate gyrus after vehicle pretreatment combined with a flesinoxan challenge in comparison with a vehicle challenge ($P < 0.01$ for both) (Fig. 1). Flesinoxan pretreatment had no influence on these challenge effects. Glucocorticoid receptor mRNA expression in the dorsal raphe nucleus of flesinoxan

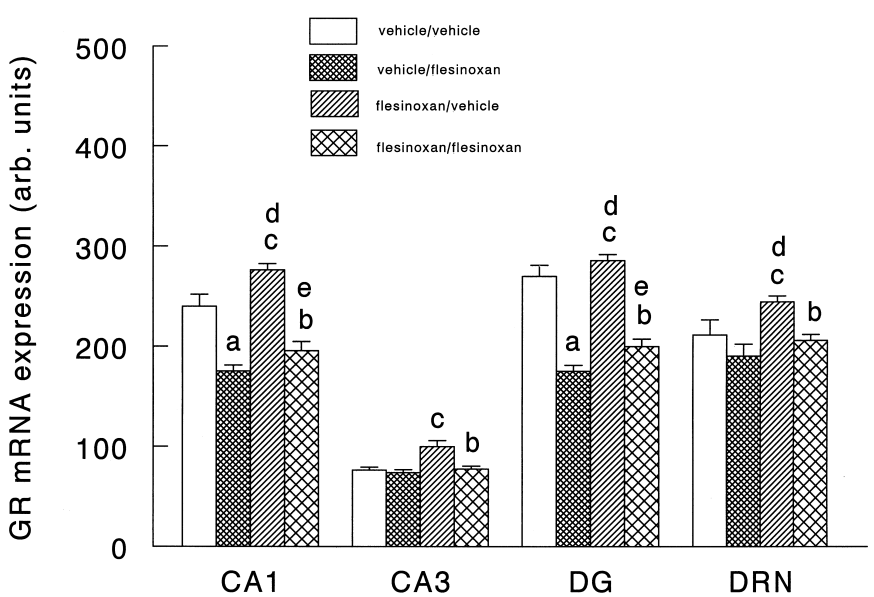

Fig. 1. Expression of glucocorticoid receptor (GR) mRNA in the rat hippocampus and dorsal raphe nucleus (drn) after pretreatment and challenged with flesinoxan or vehicle. Treatments have been administered on two subsequent days after 24 h and rats were killed 3 h after the last treatment. Each bar represents mean ± S.E.M. of GR mRNA expression. Significantly different ($P < 0.05$): (a) vehicle/vehicle vs. vehicle/flesinoxan; (b) flesinoxan/vehicle vs. flesinoxan/flesinoxan; (c) vehicle/vehicle vs. flesinoxan/vehicle; (d) vehicle/flesinoxan vs. flesinoxan/vehicle; (e) vehicle/vehicle vs. flesinoxan/flesinoxan.

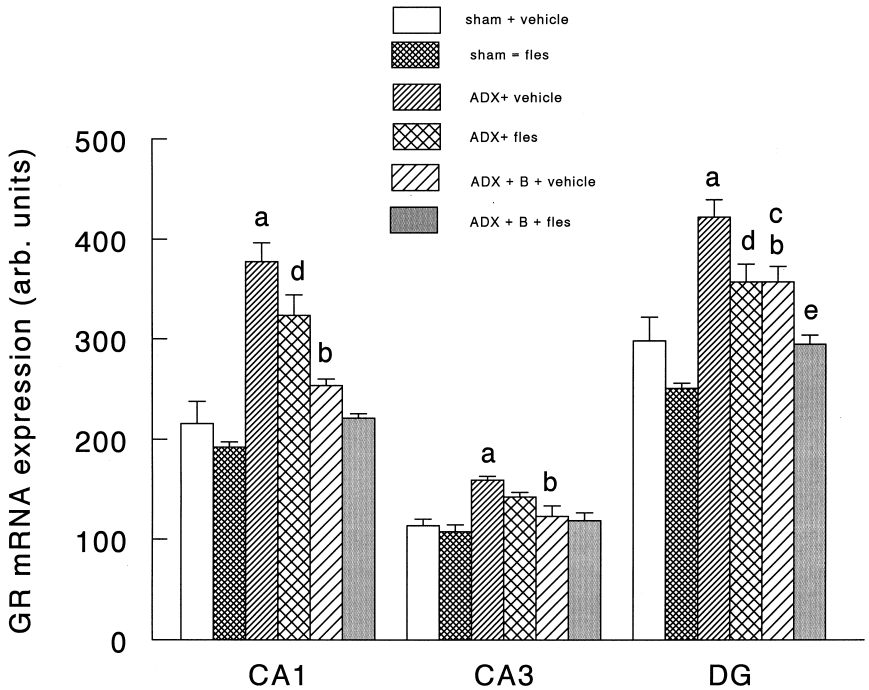

Fig. 2. Effects of flesinoxan (fles) on glucocorticoid receptor (GR) mRNA expression in the dorsal hippocampus of sham, adrenalectomized (ADX) and adrenalectomized + corticosterone (ADX + B) rats 3 h after acute injection. Each bar represents mean ± S.E.M. of GR mRNA expression. Significantly different ($P < 0.05$): (a) sham + vehicle vs. ADX + vehicle; (b) ADX + vehicle vs. ADX + B + vehicle; (c) sham + vehicle vs. ADX + B + vehicle; (d) ADX + vehicle vs. ADX + fles; and (e) ADX + B + vehicle vs. ADX + B + fles.

pretreated rats was reduced by a flesinoxan challenge treatment. The vehicle pretreated and flesinoxan challenged rats also showed a reduction but did not reach significance. ANOVA revealed a significant flesinoxan challenge effect for glucocorticoid receptor mRNA expression in the CA1 ($F(1,27) = 29.03$: $P < 0.01$) and dentate gyrus ($F(1,27) = 39.39$; $P < 0.01$) independent of pretreatment (no interaction effect).

In experiment 2, acute administration of flesinoxan exerted a main effect on glucocorticoid receptor mRNA

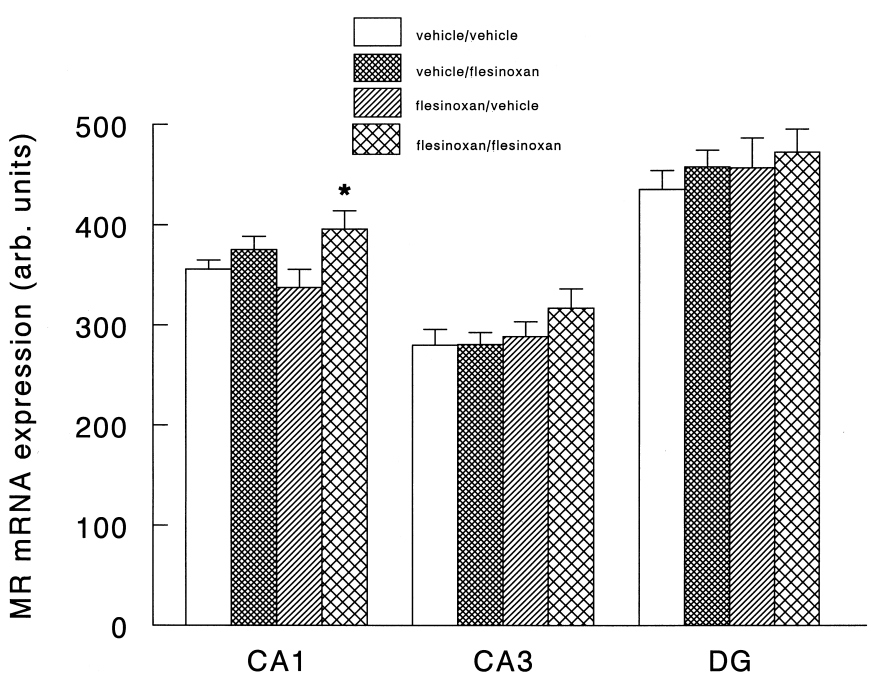

Fig. 3. Expression of mineralocorticoid receptor (MR) mRNA in the rat hippocampus after pretreatment and challenge with flesinoxan or vehicle. Treatments have been administered on two subsequent days after 24 h and rats were killed 3 h after the last treatment. Each bar represents mean ± S.E.M. of mineralocorticoid receptor mRNA expression. Significantly different ($P < 0.05$): * flesinoxan/vehicle vs. flesinoxan/flesinoxan.

levels in the CA1 ($F(5,26) = 8.7$, $P < 0.02$) and dentate gyrus ($F(5,26) = 19.2$, $P < 0.01$) (Fig. 2). Flesinoxan's reducing effect on glucocorticoid receptor expression was seen in the CA1 (14%) ($P < 0.03$) and in the dentate gyrus (15%) ($P < 0.02$) of the adrenalectomized group. Reduction took place also in the dentate gyrus of the adrenalectomized + corticosterone group (18%) ($P < 0.02$). Adrenalectomy/corticosterone exerted also a main effect in the CA1 ($F(5,26) = 50.8$, $P < 0.02$), CA3 ($F(5,26) = 16.4$, $P < 0.01$) and dentate gyrus ($F(5,26) = 24.8$, $P < 0.01$). A downregulating effect of corticosterone was observed in the CA1 of the vehicle-treated sham and adrenalectomized + corticosterone groups ($P < 0.01$ for both), CA3 ($P < 0.01$ for both) and dentate gyrus ($P < 0.01$, 0.02, respectively). Additionally, there is also a significant decrease of glucocorticoid receptor mRNA levels in the dentate gyrus of the vehicle-treated sham animals in comparison with the adrenalectomized + corticosterone group ($P < 0.03$).

3.4. Effects of flesinoxan on mineralocorticoid receptor mRNA expression

Repeated flesinoxan treatment did not affect mineralocorticoid receptor mRNA levels in the CA3 and dentate gyrus but caused an increase in the CA1 (Fig. 3, $P < 0.02$). Acute flexinoxan treatment did not exert an effect at all on the mineralocorticoid receptor mRNA levels in the hippocampus of sham, adrenalectomized and adrenalectomized + corticosterone-replaced rats (Fig. 4). However, adrenalectomy/corticosterone exerted a main effect in the CA1 ($F(5,28) = 12.0$, $P < 0.01$) CA3 ($F(5,28) = 3.2$, $P < 0.01$) and dentate gyrus ($F(5,28) = 6.2$, $P < 0.02$). Among the vehicle-treated groups, there is a significant reduction of mineralocorticoid receptor expression in the CA1 of the adrenalectomized + corticosterone group ($P < 0.02$) in comparison with adrenalectomized animals. Reduction was also observed in the CA3 of the sham ($P < 0.02$) and adrenalectomized + corticosterone ($P < 0.01$) groups against the adrenalectomized group.

4. Discussion.

The present study shows that flesinoxan exerts a direct downregulatory effect on glucocorticoid receptor mRNA expression in the hippocampus without the activation of the hypothalamo-pituitary–adrenal axis and receptor regulation through corticosterone. This is based on the following observations: firstly, acute flesinoxan treatment reduced glucocorticoid receptor mRNA levels and this effect persisted upon repeated stimulation by flesinoxan; and secondly, glucocorticoid receptor downregulation by flesinoxan persisted in the absence of corticosterone in adrenalectomized animals.

The flesinoxan effect is selective for glucocorticoid receptor mRNA expression in the hippocampus since no changes took place in the dorsal raphe nucleus and the mineralocorticoid receptor mRNA levels remained virtually unaffected. The downregulating effect of flesinoxan could be due to a reduced release of 5-HT in post-synaptic terminals. Raphe somatodendritic 5-HT_{1A} receptors are

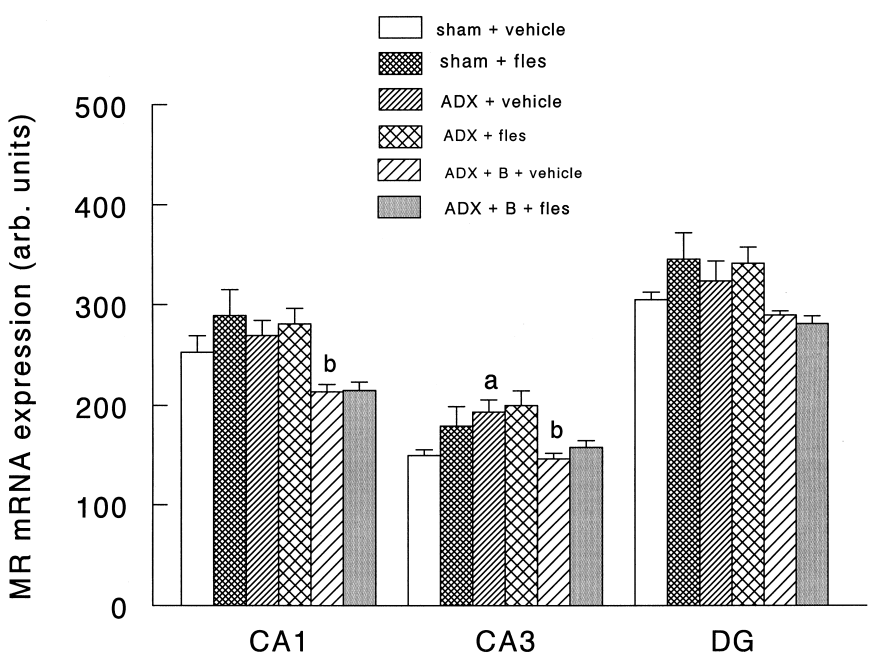

Fig. 4. Effects of flesinoxan (fles) on mineralocorticoid receptor (MR) mRNA expression in the dorsal hippocampus of sham, adrenalectomized (ADX) and adrenalectomized + corticosterone (ADX + B) rats 3 h after acute injection. Each bar represents of MR mRNA expression. Significantly different ($P < 0.05$): (a) sham + vehicle vs. ADX + vehicle; and (b) ADX + vehicle vs. ADX + B + vehicle.

activated after flesinoxan treatment leading to an inhibitory feedback. Indeed, several studies have shown that 5-HT levels affect hippocampal corticosteroid receptor levels. Administration of antidepressants, which facilitate serotonergic neurotransmission, elevates hippocampal glucocorticoid receptor and mineralocorticoid receptor mRNA expression (Peiffer et al., 1991; Seckl and Fink, 1992) and mineralocorticoid and glucocorticoid receptor binding (Reul et al., 1993), while treatment with the neurotoxin 5,7-dihyroxytryptamine decreases hippocampal glucocorticoid receptor and mineralocorticoid receptor mRNA expression (Seckl et al., 1990) and nuclear binding of corticosterone (Siegel et al., 1983). In addition, depletion of hippocampal 5-HT by acute administration of *p*-chloroamphetamine reduced both mineralocorticoid receptor and glucocorticoid receptor binding sites (Novotney and Lowy, 1995) while methamphetamine decreased hippocampal glucocorticoid receptor but not mineralocorticoid receptor binding sites (Lowy and Novotney, 1994). It should be noted that the severity of the lesion is also important since initial studies in this field demonstrated a reactive upregulation of soluble corticosteroid receptors after serotonergic denervation of the targets (Angelucci et al., 1982).

The downregulation of glucocorticoid receptor mRNA expression could have been mediated directly also through 5-HT_{1A} receptor activation in the hippocampus. Corticosteroid and 5-HT_{1A} receptors are co-localized in the hippocampus (Harfstrand et al., 1986) and ascending 5-HT nerve inputs from the dorsal raphe nucleus project directly to corticosterone-concentrating neurons in the hippocampus (Azmitia et al., 1984; Zhou and Azmitia, 1983). Genomic effects of 5-HT_{1A} receptor activation have been described for prodynorphin in spinal cord, via c-*fos* activation (Lucas et al., 1993). In the hippocampus, there is no direct proof of genomic responses but homologous downregulation of the receptor may occur (Sibug et al., 1998).

The corticosterone-independent effect shown by the adrenalectomized rats treated with the drug does not argue neither for a pre- or post-synaptic effect. 5-HT_{1A} receptor-mediated electrical responses in the dorsal raphe nucleus (Laaris et al., 1995) and hippocampus can be influenced by corticosterone (Hesen and Joels, 1996) but in the present data, we see no correlation of these electrophysiological findings at the level of the mRNA responses. Furthermore, this corticosterone-independent effect also rules out homologous regulation of glucocorticoid receptor through corticosteroids.

In the desensitization experiment, acute administration of flesinoxan significantly reduced glucocorticoid receptor mRNA expression in the CA1 and dentate gyrus of rats pretreated with vehicle (experiment 1), while only a decreasing trend was observed in sham-operated rats (experiment 2). This decreasing trend indicates, nonetheless, that receptor changes have taken place but these could have been counteracted by the effects of other factors (i.e. neurotransmitter systems) impinging on an intact hypothalamo-pituitary–adrenal axis. The hippocampus receives dense noradrenergic projections from the locus ceruleus (Loy et al., 1980) and noradrenergic mechanisms have been shown to be able to regulate glucocorticoid and mineralocorticoid receptors in the hippocampus. Neurotoxic lesions of the noradrenergic pathway by chronic 6-hydroxydopamine treatment reduced hippocampal corticosteroid binding sites (Weidenfeld et al., 1983) and decreased the binding affinity of glucocorticoid receptors (Maccari et al., 1992). Another possible candidate is the cholinergic system: medial septal cholinergic lesions increase hippocampal glucocorticoid receptor mRNA expression (Yau et al., 1992) while methamphetamine, which depletes brain dopamine and serotonin, exerted a dose-related reduction of hippocampal glucocorticoid receptor binding sites (Lowy and Novotney, 1994). However, the modulating effect of neurotransmitter systems on corticosteroid receptors' response to 5-HT_{1A} receptor agonists, like flesinoxan, remains to be clarified.

The observed effects of adrenalectomy and/or corticosterone on corticosteroid receptor expression agree with studies showing that mineralocorticoid and glucocorticoid receptor mRNA levels and binding sites are downregulated in the presence of either exogenous or endogenous corticosterone and upregulated in its absence (Reul et al., 1987, 1989, 1990; Herman et al., 1989). Furthermore, in agreement with reported data on the heterogenous and differential effects of corticosterone on these two types of receptors in the hippocampus (Reul et al., 1989; Herman et al., 1989), we observed that the anatomical sites where reduction took place differ for mineralocorticoid and glucocorticoid receptor expression.

Acute injection of flesinoxan increased plasma corticosterone levels significantly both in sham-operated rats (experiment 2) and intact vehicle pretreated rats (experiment 1). This stimulating effect of flesinoxan on plasma corticosterone levels is in accordance with studies in rats showing that systemic administration of 5-HT_{1A} receptor agonists like 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT) and flesinoxan enhance plasma corticosterone levels (Koenig et al., 1987; Przegalinski et al., 1989; Kelder and Ross, 1992; Groenink et al., 1995) via an increase in the circulating levels of the adrenocorticotropin hormone (Koenig et al., 1987; Gilbert et al., 1988; Bluet Pajot et al., 1995), through activation of CRH neurons in the hypothalamic paraventricular nucleus (Korte et al., 1991; Compaan et al., 1996). However, a second administration of flesinoxan, although not significant due to greater variability, caused a decrease in the corticosterone release in comparison with the acute effect. This desensitization effect is in line with data showing that repeated administration of 5-HT_{1A} receptor agonists causes a significant attenuation of the corticosterone response (Kelder and Ross, 1992; Groenink et al., 1995).

In summary, our study showed a downregulating effect of the 5-HT_{1A} receptor agonist flesinoxan on glucocorti-

coid receptor mRNA expression in the CA1 and dentate gyrus and indicates a direct effect independently of corticosterone. This finding might have impact in the treatment of 5-HT-related disorders using 5-HT_{1A} receptor agonists.

Acknowledgements

We would like to thank M.C. Fluttert, F. Romijn, P.M. Verdouw, W.E. Van der Wal and M.M.S. Naaijkens for excellent technical assistance. The support by NDRF Organon is also gratefully acknowledged.

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