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Pretreatment with 5-HT_{1A} receptor agonist flesinoxan attenuates Fos protein in rat hypothalamus

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Abstract

The 5-HT_{1A} receptor agonist flesinoxan has anxiolytic activity and concurrently enhances plasma corticosterone levels in rats. After a second injection of flesinoxan 24 h later, the corticosterone response disappears, but not the anxiolytic effects. Male rats received two injections with either flesinoxan or vehicle within 24 h. Flesinoxan challenge enhanced Fos immunoreactivity in the paraventricular nucleus of the hypothalamus, the central amygdala, and the dorsolateral part of the bed nucleus of the stria terminalis and plasma corticosterone levels in the vehicle-pretreated rats. Flesinoxan pretreatment resulted in an attenuated response of plasma corticosterone levels and Fos-positive neurons in the paraventricular nucleus of the hypothalamus, but not in the central amygdala and the bed nucleus after a flesinoxan challenge. The differential desensitization levels for both behaviour and neuroendocrine responses after flesinoxan treatment seem to correspond to different organization levels in the brain, like limbic system and hypothalamus.

Keywords: Immediate-early gene; Amygdala, central; Bed nucleus of the stria terminalis; Paraventricular nucleus of the hypothalamus; Corticosterone; CRH (corticotropin-releasing hormone)

1. Introduction

The serotonin (5-hydroxytryptamine, 5-HT) system is one of the most widespread neurotransmitter systems in the brain, involved in many types of physiological and behavioural processes (Jacobs and Azmitia, 1992), and known for its many different receptor subtypes (Hoyer et al., 1994; Peroutka, 1994). During the past decades accumulating evidence points to the involvement of 5-HT in anxiety-related behaviour (Griebel, 1995). Particularly, the 5-HT_{1A} receptor is thought to play a major role in this type of behaviour. 5-HT_{1A} receptor agonists show anxiolytic properties in human studies and in a variety of animal models (Deakin, 1993; Griebel, 1995). For instance, the selective and potent 5-HT_{1A} receptor agonist flesinoxan (Schipper et al., 1991; Ybema et al., 1994a,b), a phenylpiperazine derivate, which has been described in clinical studies as a promising antidepressant (Ansseau et al., 1993; Grof et al., 1993), is effective in the shock-probe burying paradigm in rats (Groenink et al., 1995b), the elevated plus-maze in mice (Rodgers et al., 1994), the stress-induced hyperthermia test in mice (Zethof et al., 1994), and the ultrasonic pup vocalization paradigm in rats (Mos and Olivier, 1989).

It is well known that the hypothalamic-pituitary-adrenocortical axis is activated by stress and during anxiety resulting in adrenal corticosterone release in rats (Axelrod and Reisine, 1984). Therefore, one might expect that anxiolytics, like 5-HT_{1A} receptor agonists, inhibit the hypothalamic-pituitary-adrenocortical axis and reduce corticosterone release. However, under basal conditions 5-HT_{1A}

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receptor stimulation by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Kelder and Ross, 1992) or flesinoxan (Groenink et al., 1995b) enhances the plasma levels of corticosterone and stress-induced rises in plasma corticosterone levels are not reduced by flesinoxan (Groenink et al., 1995b). Recently, we reported that 5-HT_{1A} receptor agonists activate Fos in the rat forebrain under basal conditions (Compaan et al., 1996; Coolen et al., 1995). The central amygdala, dorsolateral part of the bed nucleus of the stria terminalis, and corticotropin-releasing hormone (CRH)-containing neurons of the paraventricular nucleus of the hypothalamus show enhanced Fos immunoreactivity after flesinoxan treatment (Compaan et al., 1996). Accordingly, flesinoxan reduces anxiety and activates the hypothalamic-pituitary-adrenocortical axis under basal conditions. However, if animals have been pretreated with a 5-HT_{1A} receptor agonist, a desensitization to a challenge of a 5-HT_{1A} receptor agonist occurs, resulting in attenuation of the corticosterone response (Kelder and Ross, 1992), whereas the anxiolytic effects on behaviour are not attenuated (Groenink et al., 1996b). To elucidate the neural mechanisms underlying the differential effects of 5-HT_{1A} receptor agonists on anxiety and desensitization of the hypothalamic-pituitary-adrenocortical axis, we measured Fos immunoreactivity in brains of flesinoxan- or vehicle-pretreated rats. Fos was chosen as a marker for neural activation, because the immediate-early gene c-*fos* is rapidly and transiently expressed in neurons after various types of extracellular stimulation (Morgan and Curran, 1989, 1991; Sheng and Greenberg, 1990).

2. Material and methods

2.1. Animals

Male Wistar rats (U:WU-CPB, Utrecht, Netherlands) weighing approximately 200 g on arrival at the laboratory, were housed individually in clear Plexiglas cages (40 × 25 × 15 cm) on a sawdust bedding in animal rooms at controlled light/dark cycle (12L/12D; lights on at 7.00 a.m.), temperature (19–23°C), and relative humidity (50–60%). Standard lab chow and water were always available, except during the experiment when food was removed.

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-Duphar (Weesp, Netherlands) was used and dissolved in saline (0.9% NaCl, yielding pH 4.2). Saline adjusted to pH 4.2 was used as vehicle. The injections were administered subcutaneously in the flank, in a volume of 2 ml/kg body weight. For the present study we selected a dose of 3 mg/kg flesinoxan, because this dose clearly induces neuroendocrine and behavioural effects (Compaan et al., 1996; Groenink et al., 1995a,b).

2.3. Procedure

The effects of a flesinoxan challenge after pretreatment with flesinoxan on Fos immunoreactivity were studied in the rat brain. Male rats arrived 1 week prior to the experiment and were handled for 5 days. The animals were treated with one injection of flesinoxan (3.0 mg/kg) or vehicle at day 1 between 8.00 and 13.00 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. The rats were decapitated without anaesthesia 1 h after treatment, trunk blood was collected and the brains were removed and post-fixed. This procedure resulted in four groups ($n = 5$ each) of animals.

2.4. Fos immunocytochemistry

In the brains of male rats treated with flesinoxan or vehicle we studied Fos immunoreactivity as described previously (Compaan et al., 1996). The brains were post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48 h at 4°C. After 48 h incubation in 30% buffered sucrose solution for cryo-protection, the brains were cut on a cryostat microtome (coronal sections of 20 μm at −18°C). Thereafter the free-floating sections were rinsed in 0.01 M phosphate-buffered saline (PBS; pH 7.4) and stored in 0.01 M PBS with 0.1% sodium azide at 4°C until immunocytochemical staining was performed. For immunocytochemistry, the sections were rinsed with PBS, preincubated in 0.3% hydrogen peroxide to neutralize endogenous peroxidase, rinsed with PBS again, and preincubated in 5% normal donkey serum (Jackson ImmunoResearch Lab., USA) to prevent non-specific binding (30 min each). Thereafter the sections were exposed by successive incubations to the sheep polyclonal primary antibody against Fos (OA-11-824A, Cambridge Research Biochemicals, UK; 1:2000 for one overnight), 3% normal donkey serum (30 min), secondary antibody: biotinylated donkey anti-sheep immunoglobulin G (Jackson ImmunoResearch Lab., USA; 1:800; 90 min), and avidin DH: biotinylated horseradish peroxidase H complex (Vectastain ABC kit from Vector Lab., USA; 1:800; 90 min). During incubations with primary and secondary antibodies 0.3% Triton X-100 and 1% normal donkey serum were added. In between the incubations the sections were rinsed with 0.01 M PBS. All incubations were carried out at room temperature on a slow shaker. After rinsing with Tris buffer (pH 7.6), immunolabelling of Fos was visualized (black) by incubation in 0.03% 3,3′-diaminobenzidine tetrahydrochloride (DAB) + 0.3% nickel ammonium sulphate + 0.008% hydrogen peroxide in 0.05 M Tris for 10 min at room temperature. Thereafter, the sections were mounted on glass slides, air dried, and coverslipped.

2.5. Quantification of Fos-immunoreactive neurons

The number of Fos-immunoreactive neurons was measured in the paraventricular nucleus of the hypothalamus, the dorsolateral part of the bed nucleus of the stria terminalis, and the central amygdala, because these brain areas were affected after flesinoxan treatment in particular (Compaan et al., 1996). We used an automatic image analyzing system (Vidas, Kontron Elektronik, Germany) and measured at 100× magnification. Fos-immunoreactive neurons were counted in both left and right hemispheres. In the paraventricular nucleus of the hypothalamus, every fifth section was measured starting at 1.70 mm to 2.00 mm posterior to bregma (Paxinos and Watson, 1986). Accordingly, four sections with an interdistance of 100 μm have been measured throughout the paraventricular nucleus. In addition, Fos neurons were counted in one section of the dorsolateral part of the bed nucleus of the stria terminalis (−0.30 mm to bregma), and two sections of the central amygdala (−2.60 and −2.80 mm to bregma). The number of Fos neurons in each area represents the mean of the measured sections.

2.6. Corticosterone assay

Blood was collected in ice-cooled tubes containing 0.21 M EDTA (50 μl/ml blood). Plasma was separated by centrifugation (3000 rpm for 10 min at 4°C) and stored at −20°C until assayed. Plasma corticosterone concentrations were measured in duplicate using a standard radioimmunoassay (Diagnostic Products Corporation, Apeldoorn, Netherlands).

2.7. Statistics

For the number of Fos-immunoreactive neurons in each brain area significant treatment effects were determined using a multivariate analysis of variance (MANOVA; Pillais test according to Hand and Taylor, 1987) with pretreatment and challenge as between-subject factors and brain area as a repeated within-subject factor. For the plasma corticosterone levels, significant treatment effects were determined using a two-way analysis of variance (ANOVA) with pretreatment and challenge as between-subject factors. The analyses of variance were followed by post-hoc Duncan's multiple range test to determine further significant differences between groups.

3. Results

3.1. Fos immunoreactivity

As presented in Figs. 1 and 2, the number of Fos-positive neurons in the bed nucleus, paraventricular nucleus of the hypothalamus, and central amygdala has increased due to a flesinoxan challenge after vehicle pretreatment, as compared to vehicle-treated animals (Duncan's multiple range test: $P < 0.05$). However, after pretreatment with flesinoxan the Fos response to a flesinoxan challenge has reduced in the paraventricular nucleus ($P < 0.05$), but not in the amygdala and bed nucleus (Figs. 1 and 2). Flesinoxan pretreatment had no effect on basal Fos immunoreactivity in each brain area after vehicle challenge.

Thus, after statistical analysis, MANOVA revealed a significant flesinoxan challenge effect for Fos-immunoreactive neuron number in the bed nucleus of the stria terminalis ($F(1,16) = 24.97$; $P < 0.001$), central amygdala ($F(1,16) = 64.54$; $P < 0.001$), and paraventricular nucleus of the hypothalamus ($F(1,16) = 65.35$; $P < 0.001$). For the bed nucleus and amygdala only a flesinoxan challenge affected Fos neuron number independent of pretreatment (no interaction effect). However, in the paraventricular nucleus existed a significant flesinoxan pretreat-

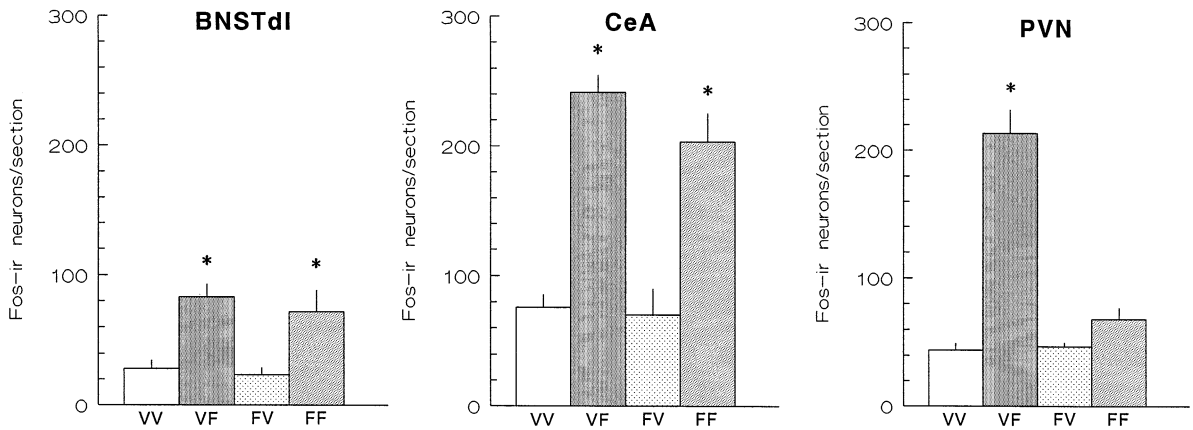
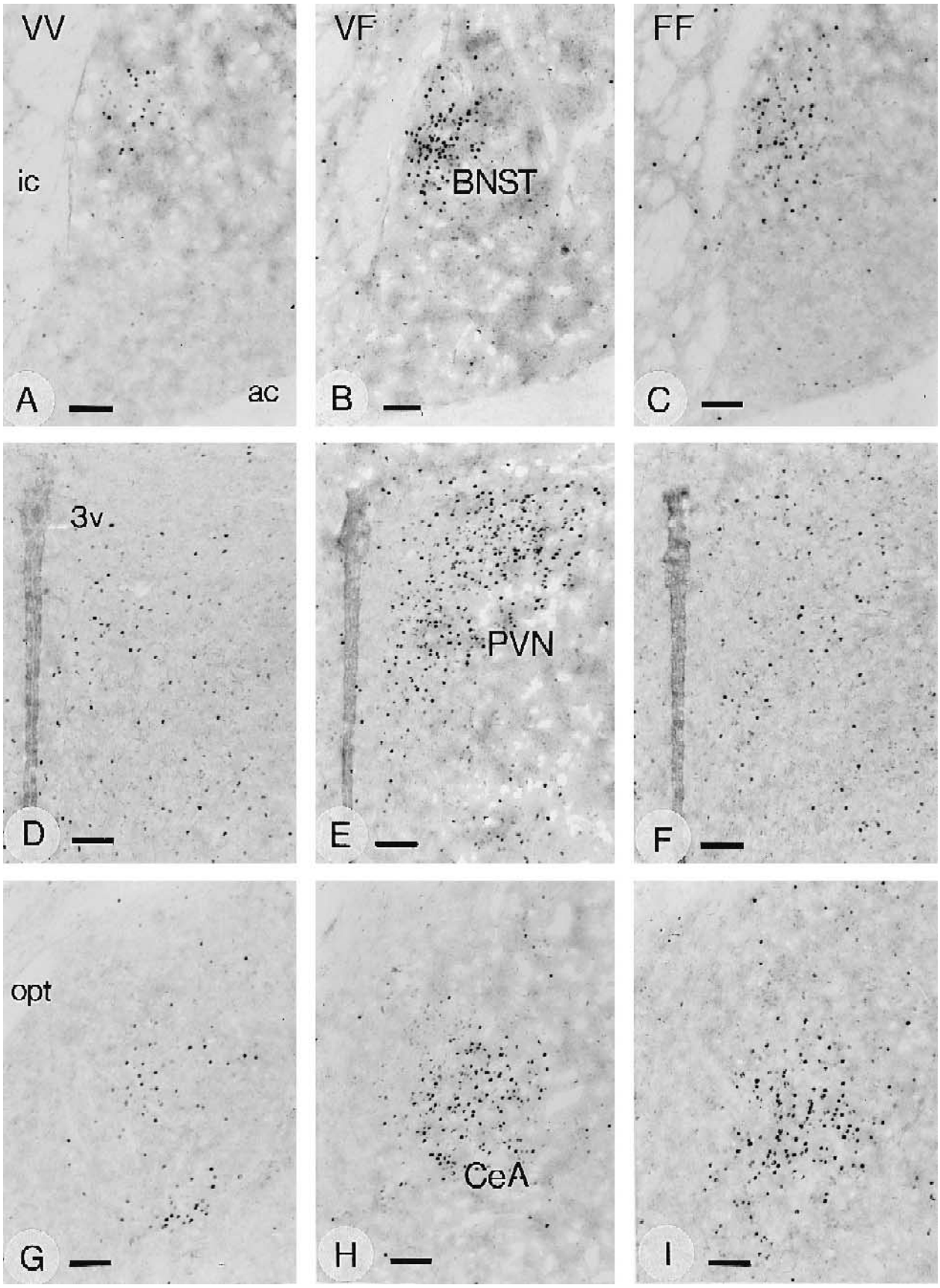


Fig. 1. Mean number of Fos-immunoreactive neurons per section in the dorsolateral part of the bed nucleus of the stria terminalis (BNSTdl), central amygdala (CeA), and paraventricular nucleus of the hypothalamus (PVN) after pretreatment and challenge with flesinoxan or vehicle in rat. Injections have been administered on two subsequent days after 24 h. * $P < 0.05$; Duncan's multiple range test; VV: vehicle pretreatment – vehicle challenge; VF: vehicle pretreatment – flesinoxan challenge; FV: flesinoxan pretreatment – vehicle challenge; FF: flesinoxan pretreatment – flesinoxan challenge.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ment effect on Fos immunoreactivity ($F(1,16) = 48.95$; $P < 0.001$) and an interaction effect between flesinoxan pretreatment and challenge ($F(1,16) = 46.95$; $P < 0.001$).

3.2. Plasma corticosterone levels

Rats pretreated with vehicle alone show enhanced (Duncan's multiple range test: $P < 0.05$) plasma corticosterone levels after flesinoxan challenge (47.72 ± 1.71 μg/dl), as compared to vehicle-challenged animals (1.12 ± 0.22 μg/dl). However, after flesinoxan pretreatment animals showed an attenuated corticosterone response to flesinoxan challenge 24 h later (29.18 ± 6.8 μg/dl; $P < 0.05$). This attenuated corticosterone response to a flesinoxan challenge is still significantly higher as compared to both vehicle-challenged groups (with and without flesinoxan pretreatment). Pretreatment with flesinoxan had no effect on basal plasma corticosterone levels measured 24 h later (0.90 ± 0.13 μg/dl). Thus, after statistical anlysis, ANOVA revealed a significant challenge ($F(1,16) = 114.57$; $P < 0.001$), pretreatment ($F(1,16) = 7.19$; $P = 0.016$), and an interaction effect ($F(1,16) = 6.86$; $P = 0.019$) on plasma corticosterone levels.

4. Discussion

In the present study we demonstrated again that the 5-HT_{1A} receptor agonist flesinoxan enhances Fos immunoreactivity in the paraventricular nucleus of the hypothalamus, central amygdala and bed nucleus of the stria terminalis (Compaan et al., 1996). In the paraventricular nucleus of the hypothalamus this Fos immunoreactivity and the corticosterone response is attenuated after pretreatment with flesinoxan, whereas Fos immunoreactivity in the central amygdala and bed nucleus remains unaffected. Accordingly, desensitization of the hypothalamic-pituitary-adrenocortical axis, i.e. attenuation of the corticosterone response, is reflected by a concurrent decrease of Fos immunoreactivity only in the paraventricular nucleus of the hypothalamus. Thus, the first injection with the 5-HT_{1A} receptor agonist flesinoxan causes alterations, resulting in an attenuation of the hypothalamic-pituitary-adrenocortical axis activation by a second flesinoxan administration as measured at the level of the hypothalamus and the adrenal cortex.

We found a reduction of Fos activation in the paraventricular nucleus of the hypothalamus, whereas the central amygdala and bed nucleus were not affected after repeated stimulation of the 5-HT_{1A} receptor, suggesting a differential mechanism or pathway. Moreover, the anxiolytic effects of flesinoxan do not desensitize, in contrast to the corticosterone response (Groenink et al., 1996b). Hence, this differential mechanism of flesinoxan-induced Fos in the brain might reflect the differential organization levels for hypothalamic-pituitary-adrenocortical axis regulation and anxiolytic effects by flesinoxan (Groenink et al., 1995b, 1996a).

Only one other report is available in which the effects of repeated serotonergic manipulation on Fos immunoreactivity have been studied (Li and Rowland, 1996). Repeated treatment with a lowe dose of dexfenfluramine, a serotonin releaser, produced tolerance to dexfenfluramine and reduced Fos immunoreactivity only in the paraventricular nucleus of the hypothalamus, whereas the central amygdala and bed nucleus were not affected. However, Fos immunoreactivity was attenuated in the central amygdala, but not in the bed nucleus, after dexfenfluramine pretreatment at an escalating dose regimen for several days. Dexfenfluramine releases serotonin at all nerve endings and thus affects all serotonergic receptor subtypes (Fuller et al., 1988), which differs from the specific 5-HT_{1A} receptor agonist flesinoxan. Nevertheless, both serotonergic drugs show the same differential mechanism underlying the activation of these brain areas.

A possible explanation for the effects of flesinoxan pretreatment on c-*fos* activation in the brain might be down-regulation of 5-HT_{1A} receptors, like receptor number, high/low affinity of receptor state, coupling to second messengers and G-proteins after the 5-HT_{1A} receptor. Somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus might be involved, because 5-HT neurons in the dorsal raphe nucleus project to CRH neurons in the paraventricular nucleus of the hypothalamus (Liposits et al., 1987; Sawchenko et al., 1983). Hence, the effects of flesinoxan could be mediated via stimulation of somatodendritic 5-HT_{1A} autoreceptors, resulting in a reduction of firing rate (Sprouse and Aghajanian, 1987) and consequently a reduced release at serotonergic terminals in the hypothalamus. This would provide the opposite effect of fenfluramine treatment, since the latter releases 5-HT from nerve endings (Fuller et al., 1988). However, both fenfluramine and flesinoxan treatment result in an activation of c-*fos* in the paraventricular nucleus of the hypothalamus and a rise in plasma corticosterone levels (Van de Kar et al., 1985). Hence, stimulation or desensitization of 5-HT_{1A}

Fig. 2. Fos immunoreactivity in the dorsolateral part of the bed nucleus of the stria terminalis (BNST; A–C), paraventricular nucleus of the hypothalamus (PVN; D–F), and central amygdala (CeA; G–I) of rats after vehicle pretreatment and vehicle (VV: A,D,G) or flesinoxan challenge (VF: B,E,H), and flesinoxan pretreatment and flesinoxan challenge (FF: C,F,I). The FV group is not shown, but is similar to the VV group. Injections have been administered on two subsequent days with a 24 h interval. Notice the increment of Fos-positive neurons after a flesinoxan challenge (VF) in the BNST, CeA, and PVN, as compared to vehicle (VV)-treated animals and the desensitization of Fos just in the PVN after FF treatment. ac: anterior commissure; ic: internal capsule; opt: optic tract; 3v: third ventricle; V: vehicle; F: flesinoxan. Scale bars: 13 μm.

autoreceptors might be overruled by post-synaptic effects of flesinoxan. Post-synaptic 5-HT_{1A} receptors are not present in the paraventricular nucleus of the hypothalamus, central amygdala and bed nucleus (Chalmers and Watson, 1991; Kia et al., 1996; Miquel et al., 1991; Pompeiano et al., 1992), suggesting that the post-synaptic effects of flesinoxan on attenuation of the Fos-immunoreactive response in the paraventricular nucleus of the hypothalamus are trans-synaptically mediated. Therefore, attenuation of activation by flesinoxan of this 5-HT_{1A} receptor-related pathway resulted in less Fos immunoreactivity in the hypothalamus.

The desensitization of the Fos response in the hypothalamic paraventricular nucleus might also be due to the initial activation of the hypothalamic-pituitary-adrenocortical axis after pretreatment with flesinoxan at day 1. This indirect desensitization mechanism might be possible via the initial rise of plasma corticosterone levels reducing (1) 5-HT_{1A} receptor mRNA expression post-synaptically in the hippocampus or (2) CRH synthesis at the level of the hypothalamus.

Firstly, the attenuated Fos response in the paraventricular nucleus of the hypothalamus might be due to a reduction of post-synaptic 5-HT_{1A} receptor synthesis in the hippocampus, caused by the initial plasma corticosterone rise (see for review Chaouloff, 1995). However, the effects of corticosterone administration on 5-HT_{1A} receptor mRNA are transient and last for only 15 h (Meijer and De Kloet, 1995). Anyway, in the present study reduced 5-HT_{1A} receptor mRNA might not be effective causing diminished number of 5-HT_{1A} receptors, because the turnover of the receptor takes longer than the flesinoxan injection interval of 24 h (Pinto and Battaglia, 1994).

Secondly, feedback mechanisms of corticosterone at the level of the hypothalamus might be involved (see for review Owens and Nemeroff, 1991). Due to the first injection of flesinoxan eventually CRH neurons in the hypothalamus are stimulated. Once released, CRH not only stimulates the release of adrenocorticotropin hormone (ACTH) at the level of the median eminence to activate the hypothalamic-pituitary-adrenocortical axis but also Fos synthesis in CRH neurons within the paraventricular nucleus of the hypothalamus (Arnold et al., 1992; Autelitano, 1994), probably via recurrent collaterals (Silverman et al., 1989). Subsequently, the initial rise in corticosterone due to flesinoxan pretreatment mediates its own future release by inhibition of CRH synthesis in the paraventricular nucleus of the hypothalamus via binding to glucocorticoid receptors (Rouquier et al., 1994). Indeed, pretreatment with dexamethasone blocks the 8-OH-DPAT-induced rise in corticosterone levels the next day (Kelder and Ross, 1992). This bound glucocorticoid receptor complex inhibits Fos synthesis as well (Karagianni and Tsawdaroglou, 1994). Furthermore, because Fos can act as a transcription factor by binding to specific sequences of DNA (Morgan and Curran, 1989), the transcription rate of genes encoding CRH might be altered (for review see Dragunow et al., 1989).

The effects on Fos immunoreactivity in the central amygdala and bed nucleus by the serotonergic system are probably mediated via peripheral effects (Groenink et al., data not shown). Lesioning of the lateral parabrachial nucleus results in attenuation of dexfenfluramine-induced Fos activation in the central amygdala and bed nucleus (Li et al., 1994), although vagotomy does not affect dexfenfluramine-induced Fos activation in the above-mentioned brain areas (Li and Rowland, 1995). Hence, to date a peripheral regulation of Fos via the serotonergic system is still possible and remains candidate for future research. Nevertheless, in our study no pretreatment effects on the level of amygdala/bed nucleus have been found, which suggest that these peripheral effects do not desensitize.

Finally, the desensitization of Fos in the hypothalamus is not due to flesinoxan metabolism or differences in kinetics after the second injection. Although a slight reduction in flesinoxan plasma levels does occur after two flesinoxan injections (Groenink et al., 1996b), this does not affect Fos immunoreactivity in the amygdala and bed nucleus. Moreover, the prolactin response even increased after flesinoxan pretreatment (Groenink et al., 1996b). The latter response might be due to activated oxytocin-producing neurons in the paraventricular nucleus of the hypothalamus (Coolen et al., 1995).

In conclusion, the differential effects of flesinoxan on the desensitization of the hypothalamic-pituitary-adrenocortical axis and maintenance of anxiolytic effects are reflected by a differential involvement of brain areas, respectively the paraventricular nucleus of the hypothalamus and the amygdala/bed nucleus. The desensitization of the Fos response in the paraventricular nucleus of the hypothalamus might be due to downregulation of post-synaptic 5-HT_{1A} receptors or 5-HT_{1A} receptor-related pathway, whereas negative feedback of glucocorticoids on CRH synthesis cannot be excluded. The flesinoxan-induced Fos immunoreactivity in the central amygdala and bed nucleus might be mediated via peripheral effects, which apparently do not desensitize.

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References

Ansseau, M., W. Pitchot, A.G. Moreno, J. Wauthy and P. Papart, 1993, Pilot study of flesinoxan, a 5-HT(1A) agonist, in major depression – effects on sleep REM latency and body temperature, Hum. Psychopharmacol. Clin. Exp. 8, 279.

Arnold, F.J.L., M. De Lucas Bueno, H. Shiers, D.C. Hancock, G.I. Evan and J. Herbert, 1992, Expression of c-fos in regions of the basal limbic forebrain following intra-cerebroventricular corticotropin-re-

leasing factor in unstressed or stressed male rats, Neuroscience 51, 377.

Autelitano, D.J., 1994, Glucocorticoid regulation of c-fos, c-jun and transcription factor AP-1 in the AtT-20 corticotrope cell, J. Neuroendocrinol. 6, 627.

Axelrod, J. and T.D. Reisine, 1984, Stress hormones: their interaction and regulation, Science 224, 452.

Chalmers, D.T. and S.J. Watson, 1991, Comparative anatomical distribution of 5-HT_{1A} receptor mRNA and 5-HT_{1A} binding in rat brain – a combined in situ hybridisation/in vitro receptor autoradiographic study, Brain Res. 561, 51.

Chaouloff, F., 1995, Regulation of 5-HT receptors by corticosteroids: where do we stand?, Fundam. Clin. Pharmacol. 9, 219.

Compaan, J.C., L. Groenink, J. Van der Gugten, R.A.A. Maes and B. Olivier, 1996, 5-HT_{1A} receptor agonist flesinoxan enhances Fos immunoreactivity in rat central amygdala, bed nucleus of the stria terminalis, and hypothalamus, Eur. J. Neurosci. 8, 2340.

Coolen, L.M., B. Olivier and J.G. Veening, 1995, Fos immunoreactivity in the male rat brain following 5-HT_{1A} agonist 8-OH-DPAT-induced ejaculation: possible role for oxytocin, 25th Annu. Meeting, Soc. Neurosci. Abstr. 21, 701.

Deakin, J.F.W., 1993, A review of clinical efficacy of 5-HT_{1A} agonists in anxiety and depression, J. Psychopharmacol. 7, 283.

Dragunow, M., R.W. Currie, R.L.M. Faull, H.A. Robertson and K. Jansen, 1989, Immediate-early genes, kindling and long-term potentiation, Neurosci. Biobehav. Rev. 13, 301.

Fuller, R.W., H.D. Snoddy and D.W. Robertson, 1988, Mechanisms of effects of *d*-fenfluramine on brain serotonin metabolism in rats: uptake inhibition versus release, Pharmacol. Biochem. Behav. 30, 715.

Griebel, G., 1995, 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research, Pharmacol. Ther. 65, 319.

Groenink, L., J. Van der Gugten, J. Mos, R.A. Maes and B. Olivier, 1995a, The corticosterone-enhancing effects of the 5-HT(1A) receptor antagonist, (*S*)-UH301, are not mediated by the 5-HT(1A) receptor, Eur. J. Pharmacol. 272, 177.

Groenink, L., J. Van der Gugten, P.M. Verdouw, R.A.A. Maes and B. Olivier, 1995b, The anxiolytic effects of flesinoxan, a 5-HT_{1A} receptor agonist, are not mediated by its neuroendocrine effects, Eur. J. Pharmacol. 280, 185.

Groenink, L., J. Mos, J. Van der Gugten and B. Olivier, 1996a, The 5-HT_{1A} receptor is not involved in emotional stress-induced rises in stress hormones, Pharmacol. Biochem. Behav. 55, 303.

Groenink, L., J. Van der Gugten, J.C. Compaan, R.A.A. Maes and B. Olivier, 1996b, Flesinoxan pretreatment differentially affects prolactin, corticosterone and behavioural responses to a flesinoxan challenge, Psychopharmacology (in press).

Grof, P., R. Joffe, S. Kennedy, E. Persad, J. Syrotiuk and D. Bradford, 1993, An open study of oral flesinoxan, a 5-HT_{1A} receptor agonist, in treatment-resistant depression, Int. Clin. Psychopharmacol. 8, 167.

Hand, D.J. and C.C. Taylor, 1987, Multivariate Analysis of Variance and Repeated Measures: A Practical Approach for Behavioural Scientists (Chapman & Hall, London).

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, 1994, International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46, 157.

Jacobs, B.L. and E.C. Azmitia, 1992, Structure and function of the brain serotonin system, Physiol. Rev. 72, 165.

Karagianni, N. and N. Tsawdaroglou, 1994, The c-fos serum response element (SRE) confers negative response to glucocorticoids, Oncogene 9, 2327.

Kelder, D. and S.B. Ross, 1992, Long lasting attenuation of 8-OH-DPAT-induced corticosterone secretion after a single injection of a 5-HT_{1A} receptor agonist, Arch. Pharmacol. 346, 121.

Kia, H.K., M.C. Miquel, M.J. Brisorgueil, G. Daval, M. Riad, S. El-mestikawy, M. Hamon and D. Verge, 1996, Immunocytochemical localization of serotonin(1A) receptors in the rat central nervous system, J. Comp. Neurol. 365, 289.

Li, B.H. and N.E. Rowland, 1995, Effects of vagotomy on cholecystokinin- and dexfenfluramine-induced Fos-like immunoreactivity in the rat brain, Brain Res. Bull. 37, 589.

Li, B.H. and N.E. Rowland, 1996, Effect of chronic dexfenfluramine on Fos in rat brain, Brain Res. 728, 188.

Li, B.H., A.C. Spector and N.E. Rowland, 1994, Reversal of dexfenfluramine-induced anorexia and c-Fos/c-Jun expression by lesion in the lateral parabrachial nucleus, Brain Res. 640, 255.

Liposits, Z., C. Phelix and W.K. Paull, 1987, Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat, Histochemistry 86, 541.

Meijer, O.C. and E.R. De Kloet, 1995, A role for the mineralcorticoid receptor in a rapid and transient suppression of hippocampal 5-HT_{1A} receptor mRNA by corticosterone, J. Neuroendocrinol. 7, 653.

Miquel, M.C., E. Doucet, C. Boni, S.E. Mestikawy, L. Matthiessen, G. Daval, D. Verge and M. Hamon, 1991, Central serotonin$_{1A}$ receptors: respective distributions of encoding mRNA, receptor protein and binding sites by in situ hybridization histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain, Neurochem. Int 19, 453.

Morgan, J.I. and T. Curran, 1989, Stimulus-transcription coupling in neurons: role of cellular immediate-early genes, Trends Neurosci. 12, 459.

Morgan, J.I. and T. Curran, 1991, Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun, Annu. Rev. Neurosci. 14, 421.

Mos, J. and B. Olivier, 1989, Ultrasonic vocalizations by rat pups as an animal model for anxiolytic activity: effects of serotonergic drugs, in: Behavioural Pharmacology of 5-HT, eds. P. Bevan, A.R. Cools and T. Archer (Erlbaum, Hillsdale, NJ) p. 361.

Owens, M.J. and C.B. Nemeroff, 1991, Physiology and pharmacology of corticotropin-releasing factor, Pharmacol. Rev. 43, 425.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, San Diego, CA).

Peroutka, S.J., 1994, Molecular biology of serotonin (5-HT) receptors, Synapse 18, 241.

Pinto, W. and G. Battaglia, 1994, Comparative recovery kinetics of 5-hydroxytryptamine 1A, 1B, and 2A receptor subtypes in rat cortex after receptor inactivation: evidence for differences in receptor production and degradation, Mol. Pharmacol. 46, 1111.

Pompeiano, M., J.M. Palacios and G. Mengod, 1992, Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding, J. Neurosci. 12, 440.

Rodgers, R.J., J.C. Cole and A. Davies, 1994, Antianxiety and behavioral suppressant actions of the novel 5-HT_{1A} receptor agonist, flesinoxan, Pharmacol. Biochem. Behav. 48, 959.

Rouquier, L., Y. Claustre and J. Benavides, 1994, Alpha(1)-adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: a microdialysis study, Eur. J. Pharmacol. 261, 59.

Sawchenko, P.E., L.W. Swanson, H.W.M. Steinbusch and A.A.J. Verhofstad, 1983, The distribution and cells of origin of serotonergic inputs to the paraventricular and supraoptic nuclei of the rat, Brain Res. 277, 355.

Schipper, J., T.M.T. Tulp, B. Berkelmans, J. Mos, J.A.M. Van der Heijden and B. Olivier, 1991, Preclinical pharmacology of flesinoxan: a potential anxiolytic and antidepressant drug, Hum. Psychopharmacol. Clin. Exp. 6, S53.

Sheng, M. and M.E. Greenberg, 1990, The regulation and function of c-fos and other immediate early genes in the nervous system, Neuron 4, 477.

Silverman, A.J., A. Hou-Yu and W.P. Chen, 1989, Corticotropin-releasing factor synapses within the paraventricular nucleus of the hypothalamus, Neuroendocrinology 49, 291.

Sprouse, J.C. and G.K. Aghajanian, 1987, Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists, Synapse 1, 3.

Van de Kar, L.D., J.H. Urba, K.D. Richardson and C.L. Bethea, 1985, Pharmacological studies on the serotonergic and nonserotonin-mediated stimulation of prolactin and corticosterone secretion by fenfluramine, Neuroendocrinology 41, 283.

Ybema, C.E., B. Olivier, J. Mos, M.T. Tulp and J.L. Slangen, 1994a, Adrenoceptors and dopamine receptors are not involved in the discriminative stimulus effect of the 5-HT_{1A} receptor agonist flesinoxan, Eur. J. Pharmacol. 256, 141.

Ybema, C.E., J.L. Slangen and B. Olivier, 1994b, Discriminative stimulus effect of flesinoxan – effects of 5-HT_{1A} antagonists and PCPA, Pharmacol. Biochem. Behav. 47, 957.

Zethof, T.J.J., J.A.M. Van der Heijden, J.T.B.M. Tolboom and B. Olivier, 1994, Stress-induced hyperthermia in mice: a methodological study, Physiol. Behav. 55, 109.