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The anxiolytic effects of flesinoxan, a 5-HT_{1A} receptor agonist, are not related to its neuroendocrine effects

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Abstract

The effects of flesinoxan, a selective 5-HT_{1A} receptor agonist, were studied under basal non-stress conditions and in the shock-probe burying paradigm. Flesinoxan (1 and 3 mg/kg s.c.) significantly reduced burying and freezing behaviour, indicating clear anxiolytic properties. Under non-stress conditions, injection of 3 mg/kg flesinoxan significantly enhanced plasma corticosterone and glucose levels, whereas prolactin secretion was significantly enhanced after both 1 mg/kg and 3 mg/kg flesinoxan. Flesinoxan (1 and 3 mg/kg) did not suppress shock-probe stress-induced rises in plasma corticosterone and glucose levels. The enhanced plasma prolactin levels induced by flesinoxan were not further affected by shock-probe exposure. Our data show that the anxiolytic effects of flesinoxan in the shock-probe burying paradigm are not related to increases in plasma corticosterone and glucose levels.

Keywords: 5-HT_{1A} receptor; Corticosterone; Glucose; Prolactin; Flesinoxan; Defensive burying; Anxiety

1. Introduction

The involvement of 5-HT_{1A} receptors in the pathogenesis of anxiety and depression has been demonstrated in several clinical and animal studies (for review see Deakin, 1993). This research was largely instigated because of the fact that buspirone, a partial 5-HT_{1A} receptor agonist, appeared to be effective in the treatment of generalized anxiety (for review see Murphy et al., 1991). Clinical studies involving the selective and potent 5-HT_{1A} receptor agonist flesinoxan also demonstrate promising results with regard to generalized anxiety disorder and depression (Ansseau et al., 1993; Deakin, 1993; Graf et al., 1993). In various animal models of anxiety 5-HT_{1A} receptor agonists show an anxiolytic profile, although in some paradigms these compounds do not exert anxiolytic activity (for review see Barrett, 1991). In classical punishment and conflict tests 5-HT_{1A} receptor agonists are not or only marginally active (Barrett, 1991), whereas they are potently anxiolytic in other animal models of anxiety, viz. ultrasonic distress vocalization in rat pups (Mos and Olivier, 1989), stress-induced hyperthermia in mice (Lecci et al., 1990) and conditioned ultrasonic vocalization in adult rats (Molewijk et al., 1995).

Apart from the anxiolytic and antidepressant effects, 5-HT_{1A} receptor agonists also affect neuroendocrine systems which are involved in stress regulation. 5-HT_{1A} receptor agonists stimulate the hypothalamic-pituitary-adrenal and the sympathoadrenal axis and enhance plasma prolactin levels in humans (Yatham and Steiner, 1993) and animals (Chaouloff, 1993; Levy and Van de Kar, 1992). 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a prototypic full 5-HT_{1A} receptor agonist, increases plasma adrenocorticotropin hormone (ACTH) (Calogero et al., 1990), corticosterone (Koenig et al., 1987), adrenaline (Bagdy et al., 1989), glucose (Chaouloff et al., 1990a), as well as plasma prolactin levels in the rat (Kellar et al.,

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1992). Partial 5-HT_{1A} receptor agonists such as buspirone and ipsapirone also induce rises in plasma ACTH (Gilbert et al., 1988), corticosterone (Matheson et al., 1988), adrenaline and noradrenaline levels (Chaouloff et al., 1990b; De Boer et al., 1991a), as well as in plasma prolactin levels (Urban et al., 1986). These stress hormone-enhancing effects in combination with the anxiolytic effects of 5-HT_{1A} receptor agonists seem contradictory, as anxiolytics in general have been proposed to block the stress hormone response to stress (Le Fur et al., 1979).

In the present study, the effects of the full 5-HT_{1A} receptor agonist, flesinoxan (Schipper et al., 1991; Ybema et al., 1990), on plasma corticosterone, glucose and prolactin levels were investigated under basal non-stress conditions and in the shock-probe defensive burying paradigm. This anxiety paradigm was introduced by Pinel and Treit (1978) and is based on a typical avoidance response of rats, characterized by pushing and spraying bedding material with forepaws and snout (burying) towards an object previously associated with an aversive stimulus. During the test, an electrified probe is inserted through a wall of the test cage. Rats tend to explore this probe, but after receiving a shock they react by either burying the probe or by immobility at a distance from the probe. Benzodiazepines as well as 5-HT_{1A} receptor agonists are able to suppress burying behaviour whereas anxiogenic drugs such as yohimbine and benzodiazepine-inverse agonists increase burying behaviour (for review see De Boer et al., 1991b).

Simultaneous measurement of anxiety and stress hormone concentrations in the shock-probe paradigm enabled us to assess whether the neuroendocrine effects of flesinoxan are related to its putative anxiolytic effects.

2. Materials and methods

2.1. Animals

Male Wistar rats (Harlan-CPB, Zeist, Netherlands), weighing approximately 300 g on arrival in the laboratory, were housed individually in clear Plexiglas cages under non-reversed 12-h light/12-h dark cycle conditions (lights on from 7:00 a.m. to 7:00 p.m.). The animals were housed at constant room temperature ($21 \pm 2°$ C) and relative humidity ($55 \pm 5\%$) with free access to water and standard food (Hope Farms), unless stated otherwise.

2.2. Surgery and blood sampling

The rats were equipped with cannulas in the jugular vein according to the technique described by De Boer et al. (1991a), using Hypnorm (5–10 mg/kg fluanisone and 0.15–0.30 mg/kg fentanylcitrate) and Dormicum (5 mg/kg midazolam-hydrochloride) as anaesthetic after premedication with atropine (1 mg/kg). This technique allows frequent blood sampling in freely moving animals, without disturbing them behaviourally or physiologically. The animals were allowed to recover for at least one week prior to the start of the experiments. During this period, the animals were handled daily and accustomed to the blood sampling procedure. Before the start of an experiment, bedding material was replaced and food was removed from the cages. Subsequently, the rats were connected to polyethylene blood sampling tubing and given at least a 1-h rest before testing was started. During the experiment blood samples of 0.35 ml were withdrawn. Immediately after each blood sample an equal volume of saline was transfused through the cannula. At the end of an experiment the jugular vein cannula was filled with 0.9% NaCl containing 500 IU heparin/ml and 60% polyvinylpyrrolidone (Merck) and closed with a small polyethylene plug.

2.3. Chemical determinations

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 (RIA), with an antiserum raised against corticosterone-21-hemisuccinate bovine serum albumin as described earlier (Van Oers et al., 1992). Free [^{3}H]corticosterone was counted after precipitation of the bound fraction with a second antibody. Intra- and inter-assay variabilities were 4% and 7%, respectively.

Plasma glucose levels were measured in duplicate using a commercially available hexokinase UV test (Hoffmann-LaRoche, Diagnostica, Mijdrecht, Netherlands). Plasma prolactin levels were measured in duplicate using a standard RIA for rat prolactin (Amersham, Netherlands). Bound [^{125}I]prolactin was counted after precipitation with a second antibody. Intra- and inter-assay variabilities were 3.2% and 8.1%, respectively.

2.4. Shock-probe defensive burying paradigm

We used the shock-probe device as described by De Boer et al. (1990). Thirty minutes after drug administration, the animals were placed in a new cage (similar to the home cage: clear Plexiglas, $25 \times 25 \times 30$ cm) in which a shock-probe (length = 6.5 cm; diameter = 1 cm) was inserted through the wall. Whenever the probe was touched by the rat, the rat received a 2 mA shock.

During the entire 15-min test period the shock circuit was left on, according to the so-called 'repeated-shock probe test' (Treit and Fundytus, 1988). The cage floor was covered with a 2-cm layer of bedding material. Video registrations were made during the test and behaviour was analysed afterwards using specially designed software (Noldus, Wageningen, Netherlands). Burying behaviour (shoveling bedding material toward or over the probe with forepaws and/or snout) and freezing (immobilized, crouched posture) were used as indices of anxiety. Other behavioural characteristics were also scored and subdivided into five main groups: inactivity (sitting, lying), exploration (locomotion/rearing/sniffing), probe-directed activities (sniffing, gnawing or approaching the probe), eating/drinking and attentive behaviour (attention, scanning). The duration of behaviours was scored during a 10-min period, which started immediately after the first shock.

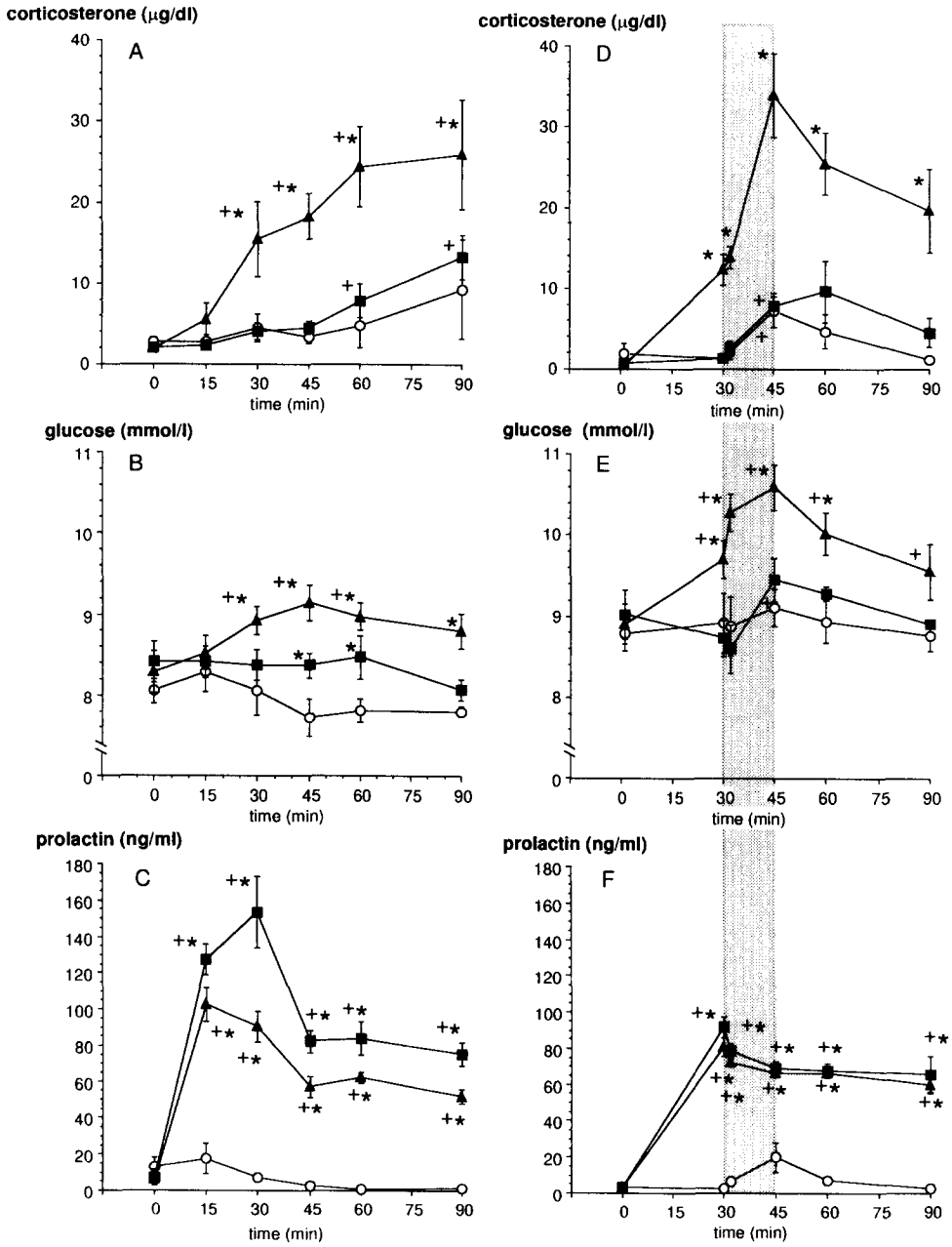

Fig. 1. Time course of plasma corticosterone, glucose and prolactin levels after subcutaneous administration of vehicle (○) or flesinoxan (■ = 1 mg/kg, ▲ = 3 mg/kg) under basal non-stress conditions (A, B, C resp.) or in the shock-probe burying paradigm (D, E, F resp.). Each time point represents the mean (±S.E.M.) from 4–7 rats. * $P < 0.05$ as compared to the value for vehicle at the corresponding time point (Duncan's multiple range test); + $P < 0.05$ as compared to $t = 0$ min (paired Student's t-test).

2.5. Drugs

Flesinoxan-hydrochloride (*R*(+)-*N*-[2[4-(2,3-dihydro-2-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazine]ethyl]-4-fluorobenzoamide), synthesized by Solvay Duphar (Weesp, Netherlands), was dissolved in saline (vehicle). Flesinoxan solution (pH 4.2) or vehicle (saline, adjusted to pH 4.2 using hydrochloric acid) was administered subcutaneously in the flank, in a volume of 2 ml/kg body weight.

2.6. Experiments

Two separate experiments were performed. In the first, a dose-response study of flesinoxan-induced rises in plasma corticosterone, glucose and prolactin levels was performed under basal, non-stress conditions. The rats were tested in the home cage and injected subcutaneously with vehicle, 1 or 3 mg/kg flesinoxan (n = 5–7 per dose) immediately after withdrawal of a control blood sample at $t = 0$ min. Subsequent blood samples were taken at $t = 15$, 30, 45, 60 and 90 min post-injection, respectively. The animals were tested once, with conditions randomized over 4 test days. In the second experiment the effects of vehicle or flesinoxan (1 or 3 mg/kg s.c.; $n = 4$–7 per dose) on defensive burying behaviour were measured together with the effects on plasma corticosterone, glucose and prolactin levels. The rats were injected at $t = 0$ min, immediately after the collection of a control blood sample. Thirty minutes after the injection another blood sample was taken and subsequently the rats were placed in a new cage with a shock-probe for 15 min. Two minutes after the first shock (approximately 32 min post-injection) as well as directly before returning the animals to their home cage ($t = 45$ min post-injection) blood samples were collected. Two additional blood samples were taken in the home cage, 60 and 90 min after injection. The animals were tested once, with conditions randomized over 5 successive days.

2.7. Statistics

For plasma corticosterone, glucose and prolactin levels, statistical comparisons were performed using a repeated measures analysis of variance (ANOVA) with sampling time as a repeated within-subject factor and with flesinoxan treatment as between-subject factor. Further analyses were performed using a paired Student's t-test (within-group comparisons) or Duncan's multiple range test (between-group comparisons) in order to determine the source of significance detected in the ANOVA. The Kruskall-Wallis test was used to analyse overall effects on behavioural parameters and if significant was followed by the Mann-Whitney U-test (2-tailed) to determine the source of the detected significance. Significance was set at a level of $P < 0.05$.

3. Results

3.1. Basal non-stress condition

Flesinoxan (Fig. 1A) significantly and dose dependently increased plasma corticosterone ($F(16,2) = 8.01$, $P = 0.004$) under non-stress conditions. Injection of 1 mg/kg s.c. flesinoxan only slightly enhanced plasma corticosterone levels at 60 and 90 min, whereas administration of 3 mg/kg s.c. flesinoxan markedly increased plasma corticosterone concentrations from 30 min up to 90 min after injection as compared to vehicle.

Plasma glucose levels (Fig. 1B) were enhanced following flesinoxan administration ($F(17,2) = 6.72$, $P = 0.007$). In the 1 mg/kg s.c. flesinoxan-treated group no change was found in plasma glucose levels compared to basal levels at $t = 0$ min. In the 3 mg/kg flesinoxan group plasma glucose concentrations were enhanced from 30 min up to 90 min after injection.

Flesinoxan significantly enhanced plasma prolactin concentrations ($F(18,2) = 89.3$, $P < 0.0001$); Fig. 1C), although not dose dependently. Maximal increases in plasma prolactin levels were already found after 15 (3 mg/kg) to 30 min (1 mg/kg). Subsequently, prolactin levels declined rapidly but remained elevated up to 90 min after drug administration as compared to those of control-treated rats. From 30 min up to 90 min post-injection, plasma prolactin levels in the 1 mg/kg-treated rats were significantly higher than in the 3 mg/kg-treated animals (at all time points $P < 0.0001$; significance not indicated in Fig. 1C).

3.2. Shock-probe

After exposure to the shock-probe (Fig. 1D), the vehicle-treated group showed a small but significant increase in plasma corticosterone concentration at t = 45 min ($P = 0.045$) as compared to $t = 0$ min. The rats treated with 1 mg/kg s.c. flesinoxan showed a corticosterone enhancement comparable to that of the vehicle rats following probe exposure ($P = 0.005$ at $t = 45$ min compared to the corresponding $t = 0$ min). Injection of 3 mg/kg s.c. flesinoxan significantly enhanced plasma corticosterone at $t = 30$ min post-injection, similar to what was found in experiment 1. After exposure of the rats to the shock-probe, plasma corticosterone concentrations sharply increased further from 12.3 μg/dl at $t = 30$ min to 33.9 μg/dl at $t = 45$ min. In these 3 mg/kg flesinonan-treated rats corticosterone levels were still significantly enhanced 90 min after injection as was also found under basal non-stress test conditions (experiment 1). The increase in plasma cortico-

sterone levels between $t = 30$ min and $t = 45$ min was significant in both the 1 mg/kg and the 3 mg/kg flesinoxan-treated groups, whereas the changes in plasma corticosterone levels during this time period were not significant under basal conditions.

In the vehicle-control group, plasma glucose levels (Fig. 1E) were slightly but significantly enhanced after exposure of the rats to the probe ($t = 45$ min, $P = 0.019$) as compared to the levels at $t = 0$ min. Plasma glucose levels were not significantly elevated in the 1 mg/kg flesinoxan group (as compared to vehicle and corresponding $t = 0$ min) following probe exposure. Injection of 3 mg/kg s.c. flesinoxan significantly enhanced plasma glucose concentrations at $t = 30$ min (similar to experiment 1). After the rats were confronted with the shock-probe glucose levels sharply increased further from 9.7 mmol/l at $t = 30$ min to 10.6 mmol/l at $t = 45$ min. Plasma glucose concentrations remained significantly enhanced up to 60 min after injection of 3 mg/kg flesinoxan.

In control animals, plasma prolactin levels increased following exposure to the shock-probe ($t = 45$ min), but not significantly. The plasma prolactin levels declined to their baseline values (Fig. 1F, $t = 60$ min) after termination of the stressor. Plasma prolactin levels were significantly enhanced 30 min after injection of either 1 or 3 mg/kg flesinoxan, but shock-probe exposure had no additional effect on plasma prolactin levels in flesinoxan-treated animals. In fact, plasma prolactin concentrations declined somewhat during shock-probe exposure (from 30 to 45 min), but remained elevated up to 90 min after flesinoxan administration. Both under basal and shock-probe stress conditions the decrease in plasma prolactin levels between 30 min and 45 min was significant in the flesinoxan-treated rats. The time course of plasma prolactin levels following flesinoxan administration and shock-probe exposure was comparable to that after flesinoxan administration under basal conditions (experiment 1) as was also the absence of a dose-dependent effect.

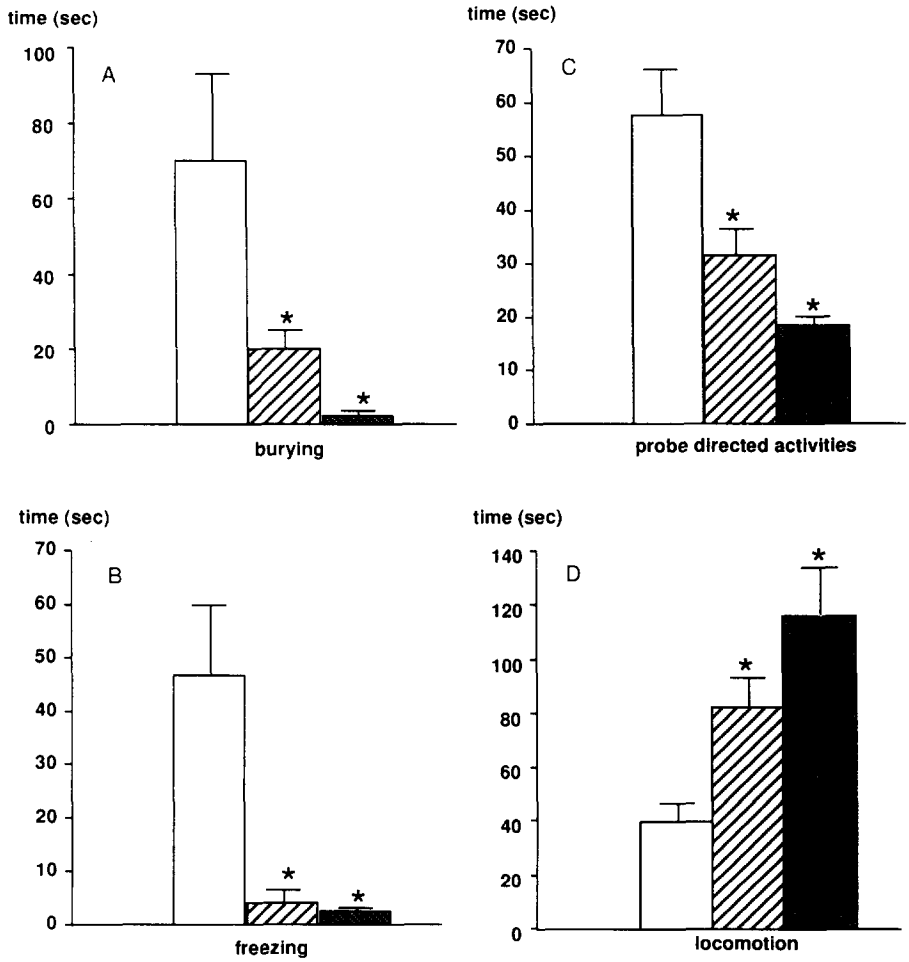

Fig. 2. Effects of subcutaneous administration of vehicle (open bars) or flesinoxan (hatched bars = 1 mg/kg, dark bars = 3 mg/kg) on shock-probe behaviour. Each bar represents the mean (± S.E.M.) from seven rats. * $P < 0.05$ as compared to vehicle-treated animals (Duncan's multiple range test).

In the shock-probe burying test, flesinoxan dose dependently reduced the time spent burying (Fig. 2A, $H = 10.7$, $P = 0.005$) and freezing (Fig. 2B, $H = 11.8$, $P = 0.007$) compared to the time of the vehicle-treated rats. The latency to burying ($H = 7.2$, $P = 0.028$) and the duration of probe-directed behaviour (Fig. 2C, $H = 11.6$, $P = 0.003$) and rearing ($H = 12.1$, $P = 0.002$) were also dose dependently reduced, whereas the duration of locomotion (Fig. 2D, $H = 12.1$, $P = 0.002$), eating bedding material ($H = 9.2$, $P = 0.01$) and inactivity ($H = 13.9$, $P = 0.001$) was increased following flesinoxan administration. Flesinoxan did not affect other behavioural characteristics such as attentive behaviour, grooming and chewing (data not shown).

4. Discussion

Flesinoxan exerts a clear anxiolytic profile in the shock-probe burying paradigm as indicated by a decreased duration of burying, freezing and probe-directed activities as well as a decrease in latency to burying the probe in flesinoxan-treated rats. The increased time spent on locomotion found in the flesinoxan-treated groups shows that flesinoxan does not simply suppress all behavioural activities. The anxiolytic properties of flesinoxan are comparable with the effects described for 8-OH-DPAT, another full 5-HT_{1A} receptor agonist (Treit et al., 1993), for the partial 5-HT_{1A} receptor agonists buspirone (Treit and Fundytus, 1988) and ipsapirone (Korte and Bohus, 1990) and for the prototypical anxiolytics diazepam and chlordiazepoxide (Tsuda et al., 1988; Treit and Fundytus, 1988). These data indicate that the shock-probe burying paradigm is suitable to detect the anxiolytic effects of 5-HT_{1A} receptor agonists, as has also been postulated by Treit and Fundytus (1988).

The anxiolytic effects of flesinoxan in the shock-probe paradigm are not reflected in plasma corticosterone and glucose levels measured during the shock-probe test. Exposure of animals to the shock-probe enhances plasma corticosterone concentrations (De Boer et al., 1990), plasma glucose levels (this study) and plasma adrenaline and noradrenaline concentrations (De Boer et al., 1990). Flesinoxan did not reduce the increase in plasma corticosterone and glucose levels induced by exposure to the shock-probe, despite its clear anxiolytic effects on shock-probe behaviour. In fact, the responses seem to be additive after the highest dose of flesinoxan. A similar dissociation of shock-probe behaviour and neuroendocrine parameters has been reported by Korte et al. (1992) for the partial 5-HT_{1A} receptor agonist, ipsapirone. Since both doses of flesinoxan significantly reduced burying and freezing but only the 3 mg/kg flesinoxan-treated group differed from control animals with regard to plasma corticosterone and glucose levels after exposure to the shock-probe, anxiolytic behavioural effects seem not to depend on, be paralleled by or related to changes in plasma corticosterone and glucose levels. Therefore, changes in plasma corticosterone and glucose levels seem not to be indicative for the anxiolytic activity of 5-HT_{1A} receptor agonists.

The anxiolytic effects of 5-HT_{1A} receptor agonists may be mediated by the presynaptically located 5-HT_{1A} receptor (Carli et al., 1989; Higgens et al., 1992), although the literature is not conclusive on this (Kostowski et al., 1989; Kataoka et al., 1991). The neuroendocrine effects of flesinoxan and other 5-HT_{1A} receptor agonists are mediated postsynaptically (Gilbert et al., 1988; Przegalinski et al., 1989). The possible involvement of presynaptic 5-HT_{1A} receptors in anxiolysis and the role of postsynaptic 5-HT_{1A} receptors in the neuroendocrine effects of flesinoxan may contribute to the absence of a relation between the anxiolytic and neuroendocrine effects of flesinoxan.

It has been suggested that corticosterone itself has anxiolytic properties (File et al., 1979). However, our findings show that the anxiolytic effects of flesinoxan in the shock-probe test are not mediated via its corticosterone enhancing effects as the 1 mg/kg dose of flesinoxan did not enhance plasma corticosterone levels but exerted clear anxiolytic effects.

The inability of flesinoxan (and other 5-HT_{1A} receptor agonists) to reduce stress-induced rises in plasma corticosterone and glucose concentrations may be due to its intrinsic effects on these parameters. Flesinoxan enhances both plasma corticosterone and glucose levels under basal conditions. These intrinsic effects may mask possible stress hormone-reducing effects of flesinoxan, as was also suggested by Urban et al. (1986). There are at least two possibilities to explain this absence of suppression. It is possible that stress-induced activation of the hypothalamic-pituitary-adrenal axis is mediated by the same receptor as the effect of flesinoxan, namely the 5-HT_{1A} receptor. Serotonin is involved in the central control of the hypothalamic-pituitary-adrenal axis and has also been suggested to participate in the mediation of stress-induced activation of the hypothalamic-pituitary-adrenal axis (for review see Chaouloff, 1993). Although the involvement of serotonin seems to depend on the type of stressor used, several authors contribute a stimulatory role to serotonin in stress-induced hypothalamic-pituitary-adrenal axis activation (for review see Chaouloff, 1993). This physiological role of serotonin may be mediated by 5-HT_{1A} receptors. If this hypothesis is true, flesinoxan and shock-probe stress both activate the hypothalamic-pituitary-adrenal axis by stimulation of 5-HT_{1A} receptors, which excludes a suppression of stress-induced corticosterone secretion by flesinoxan and other 5-HT_{1A} receptor agonists. This would be in

accordance with our findings for the 1 mg/kg flesinoxan-treated group as well as with the findings of Urban et al. (1986) for the partial 5-HT_{1A} receptor agonist buspirone. Doses of flesinoxan and buspirone which had no intrinsic effect on corticosterone were not able to suppress stress-induced corticosterone secretion compared to that of control stressed rats (this study; Urban et al., 1986). If the 5-HT_{1A} receptor mediates shock-probe stress-induced corticosterone secretion, it should be possible to block this effect with a 5-HT_{1A} receptor antagonist devoid of agonistic effects, such as WAY100,635 (Fletcher et al., 1994).

As mentioned before, the involvement of 5-HT in stress-induced hypothalamic-pituitary-adrenal axis activation seems to depend on the stressor used (Chaouloff, 1993). Therefore, as a second possibility, it could be suggested that the corticosterone-enhancing effects induced by flesinoxan and other 5-HT_{1A} receptor agonists are mediated by a mechanism other than the emotional shock-probe stress-induced corticosterone secretion. The possible involvement of two independent corticosterone-enhancing mechanisms is supported by our finding that the increases in plasma corticosterone levels found following shock-probe exposure in the 3 mg/kg flesinoxan group seemed more pronounced than the rises induced by administration of 3 mg/kg flesinoxan under basal conditions. Urban et al. (1986) reported similar effects using a high dose of buspirone. These data suggest that the stress-induced changes in plasma corticosterone levels could be mediated by a (serotonergic) mechanism other than the intrinsic corticosterone effects of 5-HT_{1A} receptor agonists. Considering the role of serotonin in stress, 5-HT_{2A}, 5-HT_{2C} or 5-HT_3 receptors may mediate the stress-induced secretion of corticosterone, as activation of these receptors also results in enhanced plasma corticosterone levels (Aulakh et al., 1994; Bagdy et al., 1989; Saphier and Welch, 1994).

Hyperglycaemia is thought to be mainly induced by circulating adrenaline released from the adrenal medulla (Smythe et al., 1989; Steffens et al., 1984) and thus may well reflect sympathoadrenal medullary activity. 5-HT_{1A} receptor agonists enhance plasma adrenaline levels (Chaouloff et al., 1990a,b; De Boer et al., 1991a) and induce hyperglycaemia (this study, Critchley et al., 1994; Chaouloff and Jeanrenaud, 1987). The intrinsic effect of flesinoxan on plasma glucose levels, as found in this study, is in accordance with these findings. The additional effect of shock-probe exposure over the intrinsic effect of flesinoxan suggests the involvement of two independent mechanisms which regulate the hyperglycaemia. The effect of flesinoxan on plasma glucose is likely to be mediated by 5-HT_{1A} receptors, as this effect could be blocked by (*S*)-UH301, a selective 5-HT_{1A} receptor antagonist (Groenink et al., 1995). The stress-induced changes in plasma adrenaline levels are thought to be mediated by α_2-adrenoceptors (Bialik et al., 1988). These data together could explain the inability of flesinoxan to suppress the hyperglycaemia induced by shock-probe stress. However, it should be noted that corticotropin-releasing factor (CRF) stimulates sympathetic tone to the adrenal medulla, thereby increasing circulating adrenaline levels (Fisher, 1989). Therefore, the effects found on plasma glucose levels may also have been influenced by the interaction between the hypothalamic-pituitary-adrenal and the sympathoadrenal medullary system.

Flesinoxan significantly enhanced plasma prolactin levels, but not dose dependently. The absence of a dose-reponse relationship after administration of another 5-HT_{1A} receptor agonist (8-OH-DPAT) has been reported before (Simonovic et al., 1984; Aulakh et al., 1988) and is probably due to the fact that maximum prolactin responses are found at relatively low drug doses, obscuring dose-response relations (Kellar et al., 1992).

Shock-probe stress induced a small increase in plasma prolactin levels which quickly disappeared after termination of the stressor. Such a small prolactin reponse to stress has also been reported by others (Urban et al., 1986; Gala, 1990; Rittenhouse et al., 1992). The time course of changes in plasma prolactin concentrations after shock-probe stress in flesinoxan-treated rats was largely similar to that found in flesinoxan-treated rats under basal conditions. These results may suggest that flesinoxan blocks the prolactin response to stress, although the increase in plasma prolactin levels induced by the shock-probe stress was not significant. Urban et al. (1986) reported that buspirone, another 5-HT_{1A} receptor agonist, effectively blocked the prolactin response to stress. Such a blockade could be mediated by the corticosterone secretion induced by 5-HT_{1A} receptor agonists, as corticosterone has been found to suppress prolactin secretion (Gala, 1990; Drago and Scapagnini, 1984). In our experiments, however, 1 mg/kg flesinoxan-treated animals did not differ from control animals in their corticosterone response to stress, whereas their prolactin response was markedly different. Therefore, it is not likely that the effects of flesinoxan on stress-induced prolactin secretion are mediated via an enhancement of corticosterone secretion. The apparent suppression of prolactin secretion by flesinoxan may also be due to other factors. Flesinoxan may have induced maximal prolactin levels, so that a further increase in prolactin concentrations becomes impossible, or increased plasma prolactin levels may inhibit further prolactin secretion, as suggested by Milenkovic et al. (1990). This seems in line with the fact that the prolactin response to stress depends on pre-stress prolactin levels (Gala, 1990). Several studies have demonstrated that serotonin is involved in the release of prolactin (Flores et

al., 1992; Jorgensen et al., 1992), but whether serotonin also mediates stress-induced prolactin secretion is still unclear.

In conclusion, our data clearly show that the anxiolytic effect of flesinoxan in the shock-probe test is not related to the simultaneous increase in plasma corticosterone and glucose levels found under these conditions. Although flesinoxan basically enhances plasma prolactin levels, these are not further affected by shock-probe stress in flesinoxan-treated rats. From our results it cannot be concluded whether stress-induced and flesinoxan-induced secretion of corticosterone, glucose and prolactin are mediated by similar (5-HT_{1A} receptor mediated) or different mechanisms. The use of selective 5-HT_{1A} receptor antagonists in future studies may answer this question.

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