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Modulation of 5-HT receptor subtype-mediated behaviours by corticosterone

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Abstract

Malfunction of the serotonergic system and dysregulation of the hypothalamo-pituitary-adrenocortical axis have been implicated in the pathophysiology of depression. Several studies provide evidence for reciprocal influences between glucocorticoids and 5-HT receptors. The effect of repeated treatment with a high dose of corticosterone (50 mg/kg s.c. twice daily for 4 days) on 5-HT receptor subtype-mediated behaviours was studied. It was found that in rats that were repeatedly treated with corticosterone the number of 2-chloro-6-(1-piperazinyl)pyrazine HCl (MK 212)-induced, 5-HT_{2C} receptor-mediated penile erections were reduced, whereas both MK 212 and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced 5-HT_{2A} receptor-mediated head shakes were increased. The (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)-induced lower lip retraction mediated by presynaptic 5-HT_{1A} receptors was unchanged, whereas the open field activity induced by 8-OH-DPAT was enhanced in corticosterone pretreated rats. These changes in 5-HT receptor subtype-mediated behaviours were not seen after a single injection with corticosterone given 24 h or 5 days before. The results suggest that 5-HT_{2A}, 5-HT_{2C} and postsynaptic 5-HT_{1A} receptor-mediated behaviour can be modulated by repeated treatment with a high dose of corticosterone.

Keywords: Corticosterone; 5-HT receptor subtype; Lower lip retraction; Penile erection; Head shake; Open field behavior

1. Introduction

Malfunction of the serotonergic system has been implicated in the pathophysiology of depression. Increased or decreased 5-HT_{2A} and 5-HT_{1A} receptor numbers in patients suffering from major depressive disorders, atypical depression or seasonal affective disorder respectively, was found by several (reviewed in Berendsen, 1995) but not all authors (Lowther et al., 1994). A direct correlation between increased 5-HT_{2A} receptor activity and the severity of depression seems to exist (Biegon et al., 1990). The functional interactions between 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors that have been shown to take place in animals and men has led to the hypothesis that a disturbed balance between the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors may cause or contribute to the symptomatology of depression in man (Berendsen, 1995). Also disturbances in the regulation of the hypothalamic-pituitary-adrenocortical axis are often observed in depression. The most important disturbances include elevated secretion of cortisol and elevated corticotropin releasing factor (CRF) levels in cerebrospinal fluid (reviewed in Peeters and Broekkamp, 1994). Numerous studies have described reciprocal influences between the 5-hydroxytryptamine (5-HT, serotonin) system and changes in the hypothalamic-pituitary-adrenocortical axis. The hippocampus, the paraventricular nucleus of the hypothalamus and other brain regions involved in hypothalamic-pituitary-adrenocortical axis regulation are densely innervated by 5-HT-containing fibers originating from the raphe nuclei (Jacobs and Azmitia, 1992; Törk, 1990). 5-HT nerve terminals make synaptic contact with CRF containing neurons in rat hypothalamus and 5-HT and 5-HT receptor agonists stimulate CRF release from isolated rat hypothalamus in vitro (Fuller, 1990).

Evidence that serotonin is involved in the regulation of the hypothalamic-pituitary-adrenocortical axis comes from studies in laboratory animals in which increased plasma levels of adrenocorticotropin hormone (ACTH) and corticosterone levels have been measured after administration of 5-HT receptor agonists, releasers, precursors or reuptake inhibitors (Bruni et al., 1982; Fuller, 1981; Fuller

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et al., 1976, 1978; Naumenko, 1968, 1969; Okada et al., 1972; Petraglia et al., 1984; Schettini et al., 1979). It was shown that activation of 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C}, but not 5-HT_3 receptors results in elevated plasma corticosterone levels (Aulakh et al., 1988, 1992; Haleem et al., 1989; Owens et al., 1990; Koenig et al., 1987, 1988; Korte et al., 1992; Levy et al., 1993). Some evidence that the 5-HT receptor subtypes that mediate the corticosterone increase are located in the brain comes from studies with 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and quipazine. 8-OH-DPAT increases corticosterone plasma levels when applied locally to the paraventricular nucleus of the hypothalamus (Haleem et al., 1989) and an increase of corticosterone by quipazine could only be prevented by centrally acting 5-HT_{2A}/5-HT_{2C} receptor antagonists (Fuller et al., 1986).

Glucocorticoid administration on the other hand has been demonstrated to affect 5-HT-mediated behaviour. Repeated treatment with relatively high doses of corticosterone (50 mg/kg twice daily for 4 days) has been shown to reduce the behavioural response to parachloroamphetamine, 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) and 8-OH-DPAT (Dickinson et al., 1985; Haleem, 1992), and 7 days treatment with cortisol (25 mg/kg/day) attenuated the suppressant effect of *m*-chlorophenylpiperazine (mCPP) on food intake, leaving this response to 8-OH-DPAT unchanged (Bagdy et al., 1989).

At present a number of behavioural responses are known to be the result of selective activation of one of the 5-HT receptor subtypes. For example, it has been shown that lower lip retraction in rats is the reflection of selective activation of presynaptic somatodendritic 5-HT1A autoreceptors probably within the median raphe nucleus (Berendsen et al., 1989, 1994), induction of penile erections in male rats is the result of selective activation of postsynaptic 5-HT_{2C} receptors (Berendsen et al., 1990) (formerly named 5-HT_{1C} receptors; Hoyer et al., 1994) and head shakes in rats are seen after activation of 5-HT_{2A} receptors (Peroutka et al., 1981; Yap and Taylor, 1983) (formerly named 5-HT_2 receptors; Hoyer et al., 1994). In the present experiments these receptor subtype selective behaviours were used to study the effect of repeated treatment with 50 mg/kg of corticosterone on the functions of the 5-HT_{1A}, 5-HT_{2C} and 5-HT_{2A} receptors. Also the effect of repeated corticosterone treatment on open field behaviour of rats and on changes in open field behaviour induced by activation of 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors were studied.

2. Materials and methods

2.1. Animals

Naive male Wistar rats (Cpb; WU, Harlan Sprague Dawley, Zeist, Netherlands) weighing 200–250 g were used. The animals were housed in white PVC cages (40 × 40 × 18 cm) with a wire mesh lid, five animals per cage, under controlled 12 h light-12 h dark cycle, with lights on at 06:00 a.m. The rats were allowed free access to standard food pellets and tap water. Rats were used only once. The experiments were scored blind with respect to the different treatments.

2.2. Corticosterone treatment

In the experiments in which corticosterone pretreated rats were used, corticosteron 50 mg/kg was injected subcutaneously twice daily (around 8.30 h and around 17.00 h) for 4 days. The experiments were done between 17 h and 20 h after the last corticosterone injection. In control experiments corticosterone was injected once 20 h before or 5 days before the actual experiment.

2.3. Behavioural procedures

2.3.1. Lower lip retraction

Lower lip retraction was scored as described before (Berendsen et al., 1989). Rats were injected with 8-OH-DPAT and placed individually in clear macrolon observation cages (23 × 17 × 14 cm) with a grid floor. Lower lip retraction was scored each 5 min from 10 till 40 min after injection of 8-OH-DPAT. Scores were as follows: 0 = lower incisors not or hardly visible (not different from untreated rats), 0.5 = lower incisors partly visible, and 1 = lower incisors completely visible. The scores per rat were summed, thus a maximum score of 7 is possible. The test was run in blocks with a maximum of ten animals per block. The treatments were randomized over the animals in the experiment with the restriction that each treatment was present in each block at least once. Dose groups and the placebo groups each consisted eight rats.

2.3.2. Penile erections

Penile erections were scored for a 30 min period following injection of MK 212 as described before (Berendsen et al., 1990). Groups of five rats were observed simultaneously. Immediately after injection of MK 212 the rats were placed individually in small Perspex observation cages (7.5 × 18 × 30 cm). A mirror was placed behind the cages to allow all-round vision of the rats. A penile erection is defined as previously described (Berendsen and Gower, 1986): repeated pelvic thrusts immediately followed by an upright position presenting an emerging, engorged penis which the rat proceeds to lick, eating the ejaculate if present. Each treatment was, at random, present in each group of five rats that were observed simultaneously.

2.3.3. Head shakes

Head shakes were induced by subcutaneous injections with DOI. Immediately after treatment with DOI the rats were placed individually in the same observation cages as

used for measuring penile erections. During 30 min hereafter the number of head shakes was counted. Five animals were observed at the same time. Each of the treatments was present in these five rats in a random order.

2.3.4. Open field behaviour

Open field observations were made using EthoVision (Video Tracking and Motion Analysis System; Noldus Information Technology, Wageningen, Netherlands). 64 rats were used in this experiment, eight rats for each treatment group. Half of the animals was pretreated with corticosterone, the other half with placebo. After dosing the rats with an agonist or placebo on the test day, they were placed in a waiting cage during 5 min and then placed in the open field for 10 min. The open field consists of a square black arena 100 × 100 cm with black walls 50 cm high. The floor is divided into several areas, e.g. corners, edges and innerfield (see dotted lines in Fig. 4). Corners are defined as the 25 × 25 cm squares in the corners of the open field, edges are the remaining 25 cm wide strips along the sites of the open field and the innerfield is the remaining 50 × 50 cm square in the middle. The number of times the rat changed from area (= total visits), the number of times the rat entered the innerfield (= visits innerfield) and the total distance run by the rat (= travelled distance) were measured. The total number of faecal pellets in the waiting cage and in the open field for each rat was also counted. With the aid of an X-Y plotter the walking pattern of the rats in the open field arena was recorded. Upon removal of each animal from the open field the arena was carefully cleaned with a damp cloth.

2.4. Statistics

Lower lip retraction was scored seven times (each 5 min from 10 till 40 min after injection with 8-OH-DPAT) and the scores were summed for each rat. Thus a total maximal score of 7 could be reached for each animal. The final results are expressed as the mean score per group ± standard error of the mean (S.E.M.). The results of the penile erection and head shake tests are also expressed as the mean score per group ± S.E.M. The statistical significance of the drug effects on lower lip retraction, penile erections and head shakes were evaluated with the Mann-Whitney *U*-test, corrected for ties. Open field parameters are expressed as mean number of visits to the innerfield, mean number of total visits and the mean travelled distance per treatment group ± S.E.M. The statistical significance of the drug effects on these parameters were evaluated with a two factor analysis of variance (ANOVA).

2.5. Drugs and solutions

The following drugs were used in these studies: (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) from Research Biochemicals (RBI); 2-chloro-6-(1-piperazinyl)pyrazine monohydrochloride (MK 212; Merck, Sharpe and Dohme) and corticosterone (Organon). Corticosterone was suspended in a solution of 5% mulgofen (EL 719, GAF) in saline. The other compounds were dissolved in sterile saline solution. All drug solutions and suspensions were freshly prepared and were injected subcutaneously into the loose skin at the back of the neck. A dose volume of 5 ml/kg body weight was used. Control animals were injected with an equivalent volume of vehicle.

3. Results

3.1. General

The rats treated during 4 days with corticosterone 50 mg/kg twice daily behaved normally but lost body weight. Over these 4 days the mean weight increase of placebo treated rats that were used in the open field experiment was 24.8 ± 1.4 g, whereas the body weight of corticosterone treated rats was reduced by 18.1 ± 1.3 g ($n = 32$ for each group). Rats treated with DOI 0.32 mg/kg were very reactive upon touching after the 10 min open field observation period was completed. They reacted with running, jumping and squeaking.

3.2. Effect of repeated corticosterone administration on 8-OH-DPAT induced lower lip retraction

Injection of 8-OH-DPAT dose dependently induced lower lip retraction in placebo pretreated rats, with a maximum score after 0.22 mg/kg. This induction of lower lip retraction was not affected by corticosterone pretreatment (Fig. 1). During the observation of the first series of animals in this experiment it was observed that some rats yawned a lot. In the next blocks these yawns were counted. Thus the number of yawns of 6 animals in each group

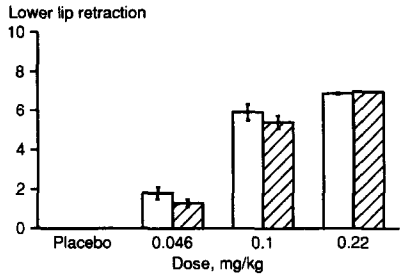

Fig. 1. Effect of corticosterone 50 mg/kg injected twice daily for 4 days on 8-OH-DPAT induced lower lip retraction. Bars represent mean lower lip retraction score ± S.E.M. Lower lip retraction was scored each 5 min from 10 till 40 min after s.c. injection with 8-OH-DPAT. Open bars represent placebo pretreated rats, hatched bars represent corticosterone pretreated rats. Number of animals per group = 8.

were scored. The number of yawns in the corticosterone pretreated group followed by placebo treatment on the test day was significantly higher ($P < 0.05$) than that of placebo pretreated rats followed by placebo treatment on test day (mean scores ± S.E.M. were 14.7 ± 3.2 and 0.5 ± 0.5 respectively for corticosterone and placebo pretreated rats). Treatment with 8-OH-DPAT had no effect on this corticosterone induced yawning.

A single injection with corticosterone 50 mg/kg s.c. given 20 h or 5 days before the actual experiment also had no effect on lower lip retraction induced by 8-OH-DPAT.

3.3. Effect of repeated corticosterone administration on DOI induced head shakes

Injection of DOI dose dependently induced head shakes in placebo pretreated rats (Fig. 2). After 0.46 mg/kg of DOI a mean number ± S.E.M. of 19.5 ± 1.8 was reached. A highest dose of 0.46 mg/kg was chosen because in former experiments it was shown that this dose of DOI induced an almost maximal number of head shakes (Berendsen and Broekkamp, 1991). The number of head shakes of placebo treated rats was 2.4 ± 0.8 ($P < 0.001$). The number of head shakes induced by 0.22 mg/kg of DOI was significantly increased in rats that were pretreated with corticosterone ($P < 0.05$).

3.4. Effect of repeated corticosterone administration on MK 212 induced penile erections

In control animals, injection with MK 212 causes a dose dependent increase of the number of penile erections without changing the number of head shakes in these animals (Fig. 3). Corticosterone pretreatment caused significant reduction of the number of penile erections induced by MK 212, whereas the number of head shakes in the same animals was increased. This increased number of head shakes was statistically significant after 0.46 and 1.0 mg/kg of MK 212. A single injection with corticosterone

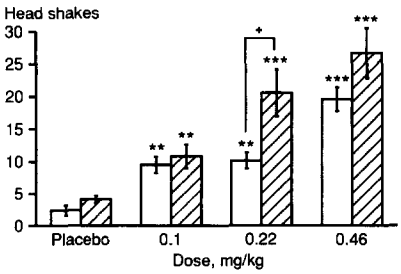

Fig. 2. Effect of corticosterone 50 mg/kg injected twice daily for 4 days on DOI induced head shakes. Bars represent mean number of head shakes ± S.E.M. counted during 30 min after s.c. injection of DOI. ★★, $P < 0.01$; ★★★, $P < 0.001$ if compared to placebo groups. +, $P < 0.05$ if compared to DOI treated group without corticosterone. Open bars represent placebo pretreated rats, hatched bars represent corticosterone pretreated rats. Number of animals per group = 8.

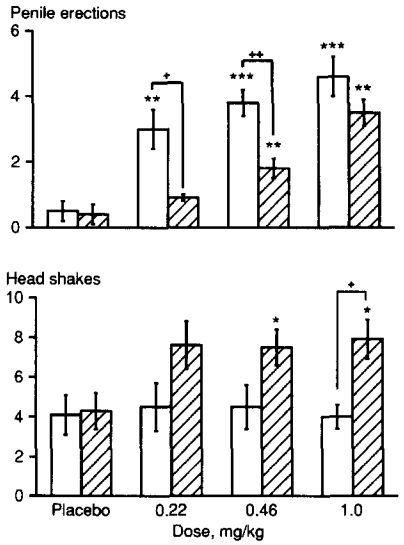

Fig. 3. Effect of corticosterone 50 mg/kg injected twice daily for 4 days on penile erections and head shakes induced by MK 212. Bars represent mean number of penile erections (upper panel) or mean number of head shakes (lower panel) ± S.E.M. counted during 30 min after injection of MK 212. ★, $P < 0.05$; ★★, $P < 0.01$; ★★★, $P < 0.001$ if compared to placebo pretreated group. +, $P < 0.05$; ++, $P < 0.01$ if compared to the relevant corticosterone pretreated group. Open bars represent placebo pretreated groups, hatched bars represent corticosterone pretreated rats. Number of animals per group = 8.

20 h or 5 days before the actual experiment had no effect on the induction of penile erections or head shakes by MK 212 (results not shown).

3.5. Effect of repeated corticosterone administration on open field behaviour

Fig. 4 shows the walking patterns of the rats in the open field after the various treatments. In Fig. 5 the effects of the various treatments on the total number of visits (number of changes from areas in the open field: $V_t \times 10$), the number of visits to the innerfield (V_i) and the total distance ($T_d \times 5$ m) run by the rats in the open field are shown. Fig. 5 (top) shows the effect of repeated corticosterone treatment. This treatment caused an overall reduction of open field behaviour. This reduction is significant for the number of total visits and total distance run. The walking pattern of the animals was not affected by repeated corticosterone treatment (Fig. 4).

Injection with 8-OH-DPAT caused a peculiar behaviour of the rats in the open field (Fig. 4). The rats walked in circles along the walls of the open field, avoiding the innerfield and as a consequence the number of innerfield visits is strongly reduced, whereas the total number of visits and the total distance is not changed (open columns of Fig. 5 (second from above) compared to open columns of Fig. 5 (top)). The rats showed a strong flat body posture and hind limb abduction, they walked like a crocodile in the open field arena. 8-OH-DPAT treatment also caused a

strong increase in the number of faecal pellets produced by the rats: mean numbers ± S.E.M. were 8.3 ± 0.5 and 2.6 ± 0.8 for 8-OH-DPAT and placebo treated rats, respectively ($P < 0.01$). In corticosterone pretreated rats, the total number of visits and the total distance were significantly increased after 8-OH-DPAT (Fig. 5 (second from above) hatched columns compared to hatched columns Fig. 5 (top)). The number of visits to the innerfield and the

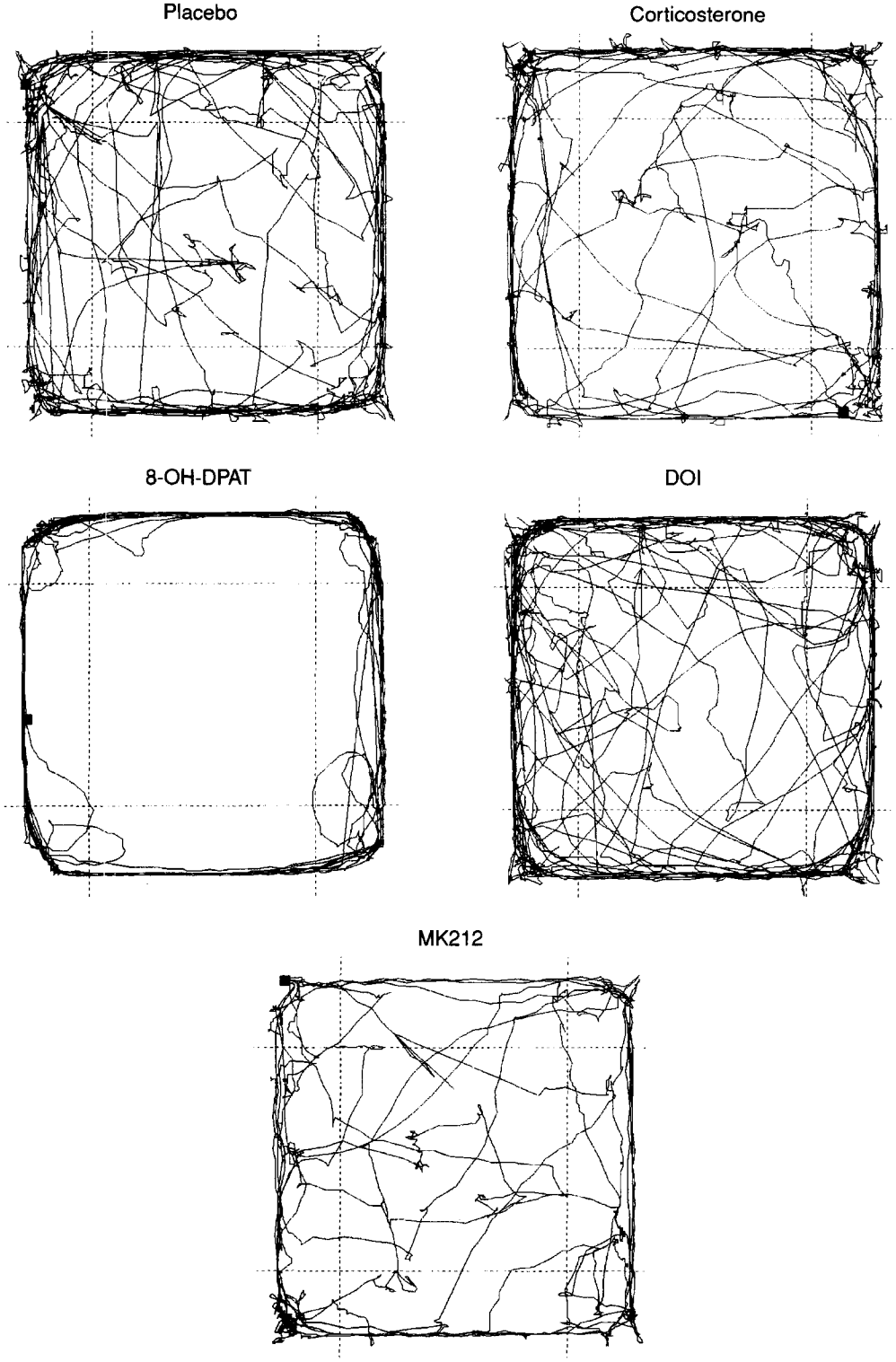

Fig. 4. Representing examples of the walking patterns of rats in the open field arena after treatment with placebo, a single injection with 0.32 mg/kg of 8-OH-DPAT, 0.32 mg/kg of DOI, 0.32 mg/kg of MK 212 measured from 10 to 20 min after s.c. injection and after corticosterone 50 mg/kg injected twice daily for 4 days measured ±20 h after the last injection.

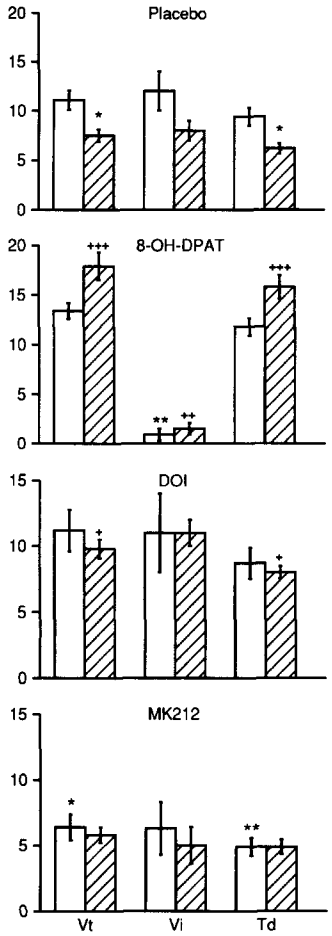

Fig. 5. Effect of corticosterone 50 mg/kg injected twice daily for 4 days on open field behaviour of rats after treatment with placebo (top), 8-OH-DPAT 0.32 mg/kg (second from above), DOI 0.32 mg/kg (second from down) or MK 212 0.32 mg/kg (bottom). Compounds were injected s.c. ±20 h after last injection with corticosterone and 10 min before open field behaviour was measured. Bars represent the mean number of total visits ($V_t \times 10$), number of visits to the innerfield (V_i) and total distance run by the animals in the open field ($T_d \times 5$ m). Open bars represent placebo pretreated groups, hatched bars represent corticosterone pretreated groups. ★ $P < 0.05$; ★★ $P < 0.01$ if compared to the placebo pretreated group presented in top figure. +, $P < 0.05$; + +, $P < 0.01$; + + +, $P < 0.001$ if compared to the corticosterone pretreated group presented in top figure.

walking pattern was not affected by corticosterone pretreatment. The mean numbers of faecal pellets ± S.E.M. in these groups were 5.8 ± 0.6 and 3.3 ± 0.8 for corticosterone plus 8-OH-DPAT and corticosterone plus placebo treated rats respectively ($P < 0.05$).

DOI 0.32 mg/kg had no effect on open field behaviour or walking pattern of rats that were placebo pretreated (Fig. 5 (second from down) open columns; Fig. 4). In corticosterone pretreated rats, DOI attenuated the corticosterone induced reduction of total visits and total distance significantly (hatched columns of Fig. 5 (second from down) compared to hatched columns of Fig. 5 (top)). The number of faecal pellets after DOI in placebo or corticosterone pretreated rats was not different from placebo (mean numbers ± S.E.M. were 2.6 ± 0.7 and 3.3 ± 0.8 respectively after corticosterone plus DOI and corticosterone plus placebo treatment).

MK 212 0.32 mg/kg caused an overall reduction of open field behaviour without changing the walking pattern (open columns of Fig. 5 (bottom) compared to open columns of Fig. 5 (top); Fig. 4). This reduction reached the level of significance for the total number of visits and the total distance. The reduced activity of corticosterone pretreated rats was not changed by MK 212 (hatched columns of Fig. 5 (bottom) compared to hatched columns of Fig. 5 (top)). The number of fecal pellets in the corticosterone plus MK 212 treated rats was significantly lower than that in the corticosterone plus placebo treated rats (mean numbers ± S.E.M. were 0.5 ± 0.5 and 3.3 ± 0.8 respectively after corticosterone plus MK 212 and corticosterone plus placebo treatment; $P < 0.05$).

Pretreatment of the rats with a single injection with corticosterone 50 mg/kg given 20 h before the actual experiment did not change open field behaviour. Open field behaviour induced by 8-OH-DPAT, DOI and MK 212 was also not affected by a single corticosterone injection 20 h before (results not shown).

4. Discussion

The relatively high dose of 50 mg/kg of corticosterone was chosen for these studies because this dose has been shown to cause plasma levels comparable to those seen in animals stressed by acute immobilisation (Hodges and Jones, 1963; Dickinson et al., 1985). In agreement with earlier studies this dose, given twice daily for 4 days, caused a reduction in body weight and reduced open field activity (Haleem, 1992; Dickinson et al., 1985). The present experiments show that the same treatment regimen reduced the number of penile erections and increased the number of head shakes in response to injection of the preferential 5-HT_{2C} receptor agonist MK 212. The head shake response to the 5-HT_{2A} receptor agonist DOI is enhanced in corticosterone pretreated rats. The lower lip retraction response to the 5-HT_{1A} receptor agonist 8-OH-DPAT was not changed, whereas in the open field the number of total visits and the total distance run by the animals was increased in corticosterone pretreated rats. These changes were not seen 24 h or 5 days after a single treatment with corticosterone. Thus only repeated exposure of rats to this dose of corticosterone changes the behaviour mediated by 5-HT receptor subtypes.

The enhanced head shake response to DOI in corticosterone pretreated rats is in agreement with the study of Kuroda et al. (1992). They found this response enhanced after 10 days treatment with ACTH. They also found the density of 5-HT_2 receptors increased after 10 days treatment with 20 and 50 mg/kg corticosterone which suggests

that the enhanced head shake response may be due to this increased 5-HT_2 receptor density.

In the present experiments the compounds MK 212 and DOI were used as agonists for the 5-HT_{2C} and 5-HT_{2A} receptors respectively. MK 212 shows a 25-fold selectivity for 5-HT_{2C} receptors in binding (Hoyer, 1988a). Injected in male rats at low doses (up to 1 mg/kg) the compound induces penile erections (Berendsen et al., 1990). After higher doses head shakes are seen and no penile erections. This suggests that at lower doses the compound activates only 5-HT_{2C} receptors. DOI binds with about equal affinity to 5-HT_{2C} and 5-HT_{2A} receptors (Hoyer, 1988b). After injection of this compound in rats, the 5-HT_{2A} receptor-mediated head shakes were seen and no penile erections. But in rats in which the 5-HT_{2A} receptors were blocked, the 5-HT_{2C} receptor-mediated penile erections were seen (Berendsen et al., 1990).

The reduction of penile erections induced by MK212 in corticosterone treated rats may not be an independent effect of corticosterone on 5-HT_{2C} receptor-mediated behaviour. The opposite interdependence of 5-HT_{2C} and 5-HT_{2A} receptor-mediated responses, shown previously (Berendsen and Broekkamp, 1990; Berendsen, 1995), may play a role in this reduced response to MK 212. The number of 5-HT_{2A} receptor-mediated head shakes was increased both after MK 212 and DOI in corticosterone pretreated rats. This increased head shake response suggests that the balance between 5-HT_{2A} and 5-HT_{2C} receptor-mediated behavioural responses is changed in favor of the 5-HT_{2A} receptor-mediated response thereby reducing 5-HT_{2C} receptor-mediated behaviour. However other mechanisms by which the expression of 5-HT_{2C} receptor-mediated behaviour is affected, such as down regulation of 5-HT_{2C} receptors, can not be excluded. In agreement with the reduced induction of penile erections after activation of 5-HT_{2C} receptors, Bagdy et al., 1989 showed that plasma prolactin, norepinephrine, epinephrine and the food intake response to the 5HT_{2C} receptor agonist mCPP were reduced after 7 days treatment with 25 mg/kg/day of cortisol.

Lower lip retraction is the result of selective activation of the presynaptic somatodendritic 5-HT_{1A} autoreceptors probably in the median raphe nucleus (Berendsen et al., 1989, 1994). Repeated corticosterone treatment could not change this response induced by the selective 5-HT_{1A} receptor agonist 8-OH-DPAT. Hyperphagia induced by 8-OH-DPAT, another effect mediated by 5-HT_{1A} autoreceptors, as well as decreased plasma norepinephrine and epinephrine response to this compound were also found to be unchanged after seven days of cortisol treatment (Bagdy et al., 1989). This suggests that the sensitivity of the presynaptic 5-HT_{1A} receptor is not changed by repeated glucocorticoid treatment.

In agreement with Dickinson et al. (1985) we found that corticosterone treatment caused an overall reduction of open field behaviour. This reduction was completely attenuated and changed into a hyperactivity by 8-OH-DPAT. Thus in contrast to the effect of corticosterone on a pre-synaptic 5-HT_{1A} receptor-mediated effect, this treatment changes post-synaptic 5-HT_{1A} receptor-mediated effects. However, since 8-OH-DPAT has also appreciable affinity for the 5-HT_7 receptor (Tsou et al., 1994) an influence of this receptor on the effect of 8-OH-DPAT can not be excluded. The altered walking pattern induced by 8-OH-DPAT was not affected by corticosterone pretreatment. Our findings in the open field are different from the effects found on other 5-HT_{1A} receptor-mediated behaviours. Reduced forepaw treading and hind limb abduction were found after 5-MeODMT and parachloroamphetamine (Dickinson et al., 1985). The non-selectivity of these compounds, 5-MeODMT also binds to 5-HT_{2C} and to 5-HT_{2A} receptors (Hoyer, 1988b) and parachloroamphetamine has 5-HT releasing properties, might play a role in this respect. However forepaw treading after the selective 5-HT_{1A} receptor agonist 8-OH-DPAT was also found to be reduced in corticosterone pretreated rats (Haleem, 1992).

As explained for the head shake response, the attenuation of the corticosterone-reduced open field activity after DOI may be due to an increased response to activation of 5-HT_{2A} receptors in corticosterone pretreated rats. In placebo pretreated rats DOI had no effect on open field behaviour, whereas MK 212 caused a strong reduction of this behaviour. This suggests that reduced open field behaviour is due to 5-HT_{2C} receptor activation. DOI, which binds with equal affinity to 5-HT_{2A} and 5-HT_{2C} receptors, does not change open field behaviour. Thus the reduced open field activity induced by 5-HT_{2C} receptor activation might be attenuated by concomitant activation of 5-HT_{2A} receptors. An increased influence of 5-HT_{2A} receptors in the effect of DOI (as seen in the head shake response) may also reduce the hypoactivity in the corticosterone pretreated rats.

As yet it can not be excluded that the changes in the behavioural responses after activation of 5-HT receptor subtypes, are due to changes in the metabolism of the used compounds after corticosterone pretreatment. However, a changed metabolism of 8-OH-DPAT is unlikely since the effect of 8-OH-DPAT is only changed for open field behaviour and not for lower lip retraction. A changed metabolism of MK 212 is also unlikely. The number of penile erections after MK 212 is reduced after corticosterone pretreatment but in the same animals the number of head shakes was found to be increased.

In these studies we have seen that repeated treatment with corticosterone modifies the behaviour mediated by 5-HT_{2A}, 5-HT_{2C} and post synaptic 5-HT_{1A} receptors. It is tempting to speculate that these changes in behaviour reflect changes of the sensitivity of at least some of these receptor subtypes, such as 5-HT_{2A} and postsynaptic 5-HT_{1A} receptors. This is the more likely since Kuroda et al. (1992) found that 10 days administration with 20 and 50

mg/kg of corticosterone increased 5-HT_2 receptor density in the neocortex of rat forebrain.

Increased plasma levels of corticosterone were seen after stress and several changes in 5-HT receptor subtype-mediated behaviours were reported after stress. These changes are not always the same as those observed in the present study after repeated corticosterone treatment. Stress induced by repeated immobilisation and long term isolation caused enhanced forepaw treading induced by 8-OH-DPAT and 5-MeODMT (Keller et al., 1994; Wright et al., 1991) whereas the same response to 8-OH-DPAT was found to be reduced in rats that were exposed to cold stress (Zamfir et al., 1992). Both decreased and increased 5-HT_{2C} receptor-mediated penile erections were found after stress. Increased penile erections in response to mCPP were found after chronic unpredictable mild stress whereas decreased penile erections were found after stress induced by 72 h sleep deprivation (Moreau et al., 1993). It might be possible that the different stressors cause different increases in plasma corticosteroid levels or have no effect thereon. For instance Zamfir et al. (1992) found no change of plasma corticosterone levels after cold stress. It can also not be excluded that stress changes the response of 5-HT receptor subtype-mediated behaviour via other mechanisms than corticosterone release.

After chronic stress and consequently chronic activation of the hypothalamic-pituitary-adrenocortical axis changes in central monoamines in certain susceptible people are seen. Therefore it has been suggested that monoaminergic abnormalities with consequent affective disorders, are secondary to an overdrive of the hypothalamic-pituitary-adrenocortical axis (Dinan, 1994). On the other hand (De Villiers et al., 1989) found that levels of plasma cortisol in adolescents with major depressive disorder did not differ from those in a group of healthy adolescents. They conclude that the duration of the illness may be important in determining the full expression of the biochemical abnormalities in major depression. In our studies repeated but not single treatment with corticosterone was able to change the 5-HT receptor subtype-mediated behavioural responses. Moreover activation of either 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors results in activation of the hypothalamic-pituitary-adrenocortical axis and increased corticosteroid levels, whereas in return repeated treatment with high doses of corticosterone only changes the behaviour mediated by some of these receptor subtypes. It might thus be suggested that an elevated corticosteroid level could be secondary to a disturbed balance between 5-HT receptor subtypes, in particular a dysbalance between 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors.

References

Aulakh, C.S., J.L. Hill and D.L. Murphy, 1992, Effects of various serotonin receptor subtype-selective antagonists alone and on *m*-chlorophenylpiperazine-induced neuroendocrine changes in rats, J. Pharmacol. Exp. Ther., 263, 588.

Aulakh, C.S., J. Zohar, K.M. Wozniak, J.L. Hill and D.L. Murphy, 1988, Clorgyline treatment differentially affects *m*-chlorophenylpiperazine-induced neuroendocrine changes, Eur. J. Pharmacol. 150, 239.

Bagdy, G., A.E. Calogero, C.S. Aulakh, K. Szemeredi and D.L. Murphy, 1989, Long-term cortisol treatment impairs behavioural and neuroendocrine responses to 5-HT1 agonists in the rat, Neuroendocrinology 50, 241.

Berendsen, H.H.G., 1995, Interactions between 5-hydroxytryptamine receptor subtypes: is a disturbed receptor balance contributing to the symptomatology of depression in humans?, Pharmacol. Ther. 66, 17.

Berendsen, H.H.G. and C.L.E. Broekkamp, 1990, Behavioural evidence for functional interactions between 5-HT-receptor subtypes in rats and mice, Br. J. Pharmacol. 101, 667.

Berendsen, H.H.G. and C.L.E. Broekkamp, 1991, Attenuation of 5-HT_{1A} and 5-HT_2 but not 5-HT_{1C} receptor mediated behaviour in rats following chronic treatment with 5-HT receptor agonists, antagonists or anti-depressants, Psychopharmacology 105, 219.

Berendsen, H.H.G. and A.J. Gower, 1986, Opiate-androgen interactions in drug-induced yawning and penile erections in rats, Eur. J. Pharmacol. 122, 239.

Berendsen, H.H.G., F. Jenck and C.L.E. Broekkamp, 1989, Selective activation of 5-HT_{1A} receptors induces lower lip retraction in the rat, Pharmacol. Biochem. Behav. 33, 821.

Berendsen, H.H.G., F. Jenck and C.L.E. Broekkamp, 1990, Involvement of 5-HT_{1C}-receptors in drug-induced penile erections in rats, Psychopharmacology, 101, 57.

Berendsen, H.H.G., F.G.M. Bourgondien and C.L.E. Broekkamp, 1994, Role of dorsal and median raphe nuclei in lower lip retraction in rats, Eur. J. Pharmacol. 263, 315.

Biegon, A., A. Grinspoon, B. Blumenfeld, A. Bleich, A. Apter and R. Mester, 1990, Increased serotonin 5-HT_2 receptor binding on blood platelets of suicidal men, Psychopharmacology 100, 165.

Bruni, J.F., R.L. Hawkins and S.S.C. Yen, 1982, Serotonergic mechanism in the control of ß-endorphin and ACTH release in male rats, Life Sci. 30, 1247.

De Villiers, A.S., V.A. Russell, M.E. Carstens, J.A. Searson, A.M. Van Zyl, C.J. Lombard and J.J.F. Taljaard, 1989, Noradrenergic function and hypothalamic-pituitary-adrenal axis activity in adolescents with major depressive disorder. Psychiatr. Res. 27, 101.

Dickinson, S.L., G.A. Kennett and G. Curzon, 1985, Reduced 5-hydroxytryptamine-dependent behaviour in rats folowing chronic corticosterone treatment, Brain Res. 345, 10.

Dinan, T.G., 1994, Glucocorticoids and the genesis of depressive illness a psychobiological model, Br. J. Psychiatr. 164, 365.

Fuller, R.W., 1981, Serotonergic stimulation of pituitary-adrenocortical function in rats, Neuroendocrinology 32, 118.

Fuller, R.W., 1990, Serotonin receptors and neuroendocrine responses, Neuropsychopharmacology 3, 495.

Fuller, R.W., H.D. Snoddy and B.B. Molloy, 1976, Pharmacologic evidence for a serotonin neural pathway involved in hypothalamus-pituitary-adrenal function in rats, Life Sci. 19, 337.

Fuller, R.W., H.D. Snoddy and J.A. Clemens, 1978, The effect of quipazine, a serotonin receptor agonist, on serum corticosterone concentration in rats, Endocr. Res. Commun. 5, 161.

Fuller, R.W., K.D. Kurz, N.R. Mason and M.L. Cohen, 1986, Antagonism of a peripheral vascular but not an apparently central serotonergic response by xylamidine and BW501C67, Eur. J. Pharmacol. 125, 71.

Haleem, D.J., 1992, Repeated corticosterone treatment attenuates behavioural and neuroendocrine responses to 8-hydroxy-2-(di-*n*-propylamino) tetralin in rats, Life Sci. 51, 225.

Haleem, D.J., G.A. Kennett, P.S. Whitton and G. Curzon, 1989, 8-OH-DPAT increases corticosterone but not other 5-HT_{1A} receptor-dependent responses more in females, Eur. J. Pharmacol. 164, 435.

Hodges, J.R. and M.T. Jones, 1963, The effect of injected corticosterone on the release of adrenocorticotrophic hormone in rats exposed to acute stress, J. Physiol. 167, 30.

Hoyer, D., 1988a, Functional correlates of serotonin 5-HT_1 recognition sites, J. Recept. Res. 8, 59.

Hoyer, D., 1988b, Molecular pharmacology of 5-HT_{1C} receptors, Trends in Pharmacol. Sci. 9, 89.

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.

Keller, E.A., L.M. Cancela, V.A. Molina and O.A. Orsingher, 1994, Lack of adaptive changes in 5-HT sites in perinatally undernourished rats after chronic stress: opioid influence, Pharmacol. Biochem. Behav. 47, 789.

Koenig, J.I., G.A. Gudelsky and H.Y. Meltzer, 1987, Stimulation of corticosterone and β-endorphin secretion in the rat by selective 5-HT receptor subtype activation, Eur. J. Pharmacol. 137, 1.

Koenig, J.I., H.Y. Meltzer and G.A. Gudelsky, 1988, 5-Hydroxytryptamine1A receptor-mediated effects of buspirone, gepirone and ipsapirone, Pharmacol. Biochem. Behav. 29, 711.

Korte, S.M., G.A. Bouws and B. Bohus, 1992, Adrenal hormones in rats before and after stress-experience: effects of ipsapirone, Physiol. Behav. 51, 1129.

Kuroda, Y., M. Mikuni, T. Ogawa and K. Takahashi, 1992, Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT2 receptor binding sites in neocortex of rat forebrain and 5-HT2 receptor-mediated wet-dog shake behaviours, Psychopharmacology 108, 27.

Levy, A.D., Q. Li, P.A. Rittenhouse and L.D. Van de Kar, 1993, Investigation of the role of 5-HT_3 receptors in the secretion of prolactin, ACTH and renin, Neuroendocrinol. 58, 65.

Lowther, S., F. De Paermentier, M.R. Crompton, C.L.E. Katona and R.W. Horton, 1994, Brain 5-HT_2 receptors in suicide victims: violence death, depression and effects of antidepressant treatment, Brain Res. 642, 281.

Moreau, J-L., F. Jenck, J.R. Martin, S. Perrin and W.E. Haefely, 1993, Effects of repeated mild stress and two antidepressant treatments on the behavioural response to 5-HT_{1C} receptor activation in rats, Psychopharmacology 110, 140.

Naumenko, E.V., 1968, Hypothalamic chemoreactive structures and the regulation of pituitary-adrenal function. Effects of local injections of norepinephrine carbachol and serotonin into the brain of guinea pigs intact brains and after mesencephalic transection, Brain Res. 11, 1.

Naumenko, E.V., 1969, Effect of local injection of 5-hydroxytryptamine into rhinencephalic and mesencephalic structures onthr pituitary-adrenal function in guinea pigs, Neuroendocrinology 5, 81.

Okada, F., Y. Saito, T. Fujieda and I. Yamashita, 1972, Monoamine changes in the brain of rats injected with L-5-hydroxytryptophan, Nature 238, 355.

Owens, M.J., E. Edwards, C.B. Nemeroff, 1990, Effects of 5-HT_{1A} receptor agonists on hypothalamo-pituitary-adrenal axis activity and corticotropin-releasing factor containing neurons in the rat brain, Eur. J. Pharmacol. 190, 113.

Peeters, B.W.M.M. and C.L.E. Broekkamp, 1994, Involvement of corticosteroids in the processing of stressful life-events. A possible implication for the development of depression, J. Steroid. Biochem. Mol. Biol. 49, 417.

Peroutka, S.J., R.M. Lebovitz and S.H. Snyder, 1981, Two distinct central serotonin receptors with different physiological functions, Science 210, 827.

Petraglia, F., F. Facchinetti, E. Martignoni, G. Nappi, A. Volpe and A.R. Genazzani, 1984, Serotonergic agonists increase plasma levels of ß-endorphin and ß-lipotropin in humans, J. Clin. Endocrinol. Metab. 59, 1138.

Schettini, G., A. Quattrone, D.R. Gianfranco and P. Preziosi, 1979, Effect of selective degeneration of brain serotonin-containing neurons on plasma corticosterone levels: studies with *d*-fenfluramine, Pharmacol. Res. Commun. 11, 545.

Törk, I., 1990, Anatomy of the serotonergis system. In: The neuropharmacology of Serotonin, eds. P.M. Whitaker-Azmitia and S.J. Peroutka, Ann. NY Acad. Sci. 600, 9.

Tsou, A., A. Kosaka, C. Bach, P. Zuppan, C. Yee, L. Tom, R. Alvarez, S. Ramsey, D.W. Bonhaus, E. Stefanich, L. Jakeman, R.M. Eglen and H.W. Chan, 1994, Cloning and expression of a 5-hydroxytryptamine$_7$ receptor positively coupled to adenylyl cyclase, J. Neurochem. 63, 456.

Wright, I.K., H. Ismail, N. Upton and C.A. Marsden, 1991, Effect of isolation rearing on 5-HT agonist-induced responses in the rat, Psychopharmacology 105, 259.

Yap, C.Y. and D.A. Taylor,1983, Involvement of 5-HT_2 receptors in the wet-dog shake behaviour induced by 5-hydroxytryptophan in the rat, Neuropharmacology 22, 801.

Zamfir, O., P. Broqua, V. Baudrie and F. Chaouloff, 1992, Effects of cold stress on some 5-HT_{1A}, 5-HT_{1C} and 5-HT_2 receptor-mediated responses, Eur. J. Pharmacol. 219, 261.