ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 815–823

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Alteration in 5-hydroxytryptamine agonist-induced behaviour following a corticosterone implant in adult rats

Kevin C.F. Fone*, Ian A. Topham

School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK

Received 15 April 2001; received in revised form 19 July 2001; accepted 3 August 2001

Abstract

Hypercortisolism and altered serotonergic function may account for the pathological symptoms seen in depression. This study examines the impact of 4 days continuous corticosterone treatment on 5-HT agonist-induced behaviour to delineate changes in 5-HT receptor function in the adult rat. The flat body posture, reciprocal forepaw treading, elevated corticosterone, hyperglycaemia, hypothermia and reduced hippocampal 5-HT induced by the 5-HT_{1A} agonist 8-OHDPAT (0.3 mg/kg ip) were all significantly attenuated by the corticosterone implant. The elevation in plasma corticosterone and back muscle contractions evoked by the 5-HT_{2A} agonist DOI (1 mg/kg ip) were attenuated, whilst wet-dog shakes were enhanced by corticosterone treatment. 5-HT_{2B} agonist-induced behaviour and the hypolocomotion and hypophagia induced by the 5-HT_{2C} agonist *m*-CPP (2.5 mg/kg ip) were unaltered but the mCPP-induced elevation in corticosterone was abolished by corticosterone treatment. Hypothalamic 5-HT receptors mediating corticosterone- and 5-HT_{1A} receptors, whether on serotonergic nerve terminals or postsynaptic neurones, were downregulated by corticosterone treatment. In contrast, 5-HT_{2A} receptors may be up- or downregulated dependent on whether they are on supraspinal or spinal neurones, respectively. A comparison of the brain region-dependent alteration in serotonergic function produced by hypercorticosterone in the rat with that seen in depression is discussed.

Keywords: 5-HT_{1A} receptor; 5-HT_{2A} receptor; 5-HT_{2B} receptor; 5-HT_{2C} receptor; Feeding behaviour; Corticosterone; HPA axis; Serotonin syndrome

1. Introduction

The neurobiology underlying depression is not fully understood, however, attenuated serotonergic neurotransmission may account for the majority of the core symptoms while hypercortisolism is one of the most consistent abnormalities seen in patients with major depression (Chaouloff, 2000; Nemeroff, 1998). Furthermore, there is a reciprocal interaction between serotonergic neurones and the hypothalamic–pituitary–adrenal (HPA) axis that may be dysfunctional in human depression (Nemeroff, 1998). In addition, one putative current mechanism of action of antidepressant drugs is restoration of synaptic 5-HT neurotransmission by downregulation of presynaptic 5-HT_{1A} and 5-HT_{1B} receptors, in particular by the serotonin selective reuptake inhibitors (Blier and de Montigny, 1998).

At the level of the paraventricular nucleus serotonin released from dorsal raphé nerve terminals acting on 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors augments adrenocorticotrophin hormone (ACTH) and thereafter corticosterone release (Bagdy, 1995). Conversely, several forms of stress, such as immobilisation, electric footshock or noxious stimuli, which activate the HPA axis increase the synthesis and release of 5-HT in several brain areas in experimental animals (Inoue et al., 1994). A sustained elevation of corticosterone levels in the rat could therefore be a useful model to investigate the change in 5-HT function accompanying human depression. The current study therefore examines the impact of infusion of corticosterone, using a subcutaneous slow release pellet, on a variety of behavioural and neurochemical changes elicited by activation of individual serotonin receptors by administration of receptor selective 5-HT agonists in adult rats.

Although previous groups (Bagdy et al., 1989; Berendsen et al., 1996; Dickinson et al., 1985; Takao et al., 1997; Young et al., 1992) have examined the impact of elevated corticosterone on 5-HT agonist responses, they have focused on the

* Corresponding author. Tel.: +44-115-970-9469; fax: +44-115-970-9259.

E-mail address: kevin.fone@nottingham.ac.uk (K.C.F. Fone).

0091-3057/02/$ – see front matter
PII: S0091-3057(01)00706-7

5-HT_{1A} and 5-HT_{2A} receptors, and little information is available on the functional change in other receptors, such as the 5-HT_{2B} and 5-HT_{2C}, which is also investigated herein.

2. Materials and methods

2.1. Animals

Adult male Lister hooded rats (284–348 g, Biomedical Services Unit, Nottingham University, UK) were housed in groups of four, or in individual cages if on a food restricted diet, in a temperature controlled (21 ± 2 °C) environment on a 12-h light/dark cycle (lights on 07:00–19:00 h). Unless otherwise stated, rats had free access to food (Beekay-Standard rat and mouse diet) and water and were weighed daily throughout the study.

Four days after implanting a subcutaneous corticosterone or control 100% cholesterol pellet under halothane anaesthesia, rectal temperature, open field behaviour and/or food intake were measured following injection of a selected 5-HT agonist or saline and postmortem hippocampal indoleamine levels were determined, as described below:

All experiments were performed in accordance with the UK animals (Scientific Procedures Act, 1986) legislation using a blind protocol. Between each behavioural test, all apparatus was cleaned with 20% (v/v) ethanol to remove odour cues.

2.2. Pellet construction and implantation

Pellets (500 mg cholesterol, control or 250 mg each of cholesterol and corticosterone, glucocorticoid treatment) were constructed in a 13-mm long pill mould by applying gentle heat until a liquid formed. Each resultant pellet was divided into half using a scalpel before being implanted subcutaneously above the scapula by making a small incision, under Halothane anaesthesia and asceptic conditions. The incision was sutured with sterile surgical thread and treated with plastic wound dressing (Nobecutane, AstraZeneca) and antiseptic spray (Betadine, Seaton Health Care), prior to allowing recovery from anaesthesia. Implants were performed 7 days after commencing the food restriction paradigm in those groups where food intake was to be monitored and remained in place for 4 days prior to performing the 5-HT agonist behavioural study.

2.3. Open field behaviour

Two types of behavioural study were performed dependent on the 5-HT agonist used; change in behaviour in free-feeding rats or change in behaviour and food intake in rats on a fixed feeding regimen. Separate saline groups ($n = 11$ and 12) were used as a control for each type of behavioural study. All behavioural experiments were performed by placing rats in individual activity monitor chambers (Medical Physics, Nottingham University) which recorded ambulation and rears and, in food intake studies, the rate of nose poking through a hole in the floor to reach finely crushed food flakes from a feeding tray. Each chamber consisted of a clear acrylic open field box, 40 × 20 × 25 cm high, with a wire mesh lid. Five parallel infrared beams (7.5 cm apart) crossed the chamber at three different heights. In feeding studies with the 5-HT agonists, *m*-chlorophenylpiperazine hydrochloride (*m*-CPP, $n = 12$) and α-methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine hydrochloride (BW723C86, $n = 12$) the lowest beams (3.5 cm below a 4-cm diameter hole in the floor) measured food retrieval rate. In all other studies, where a solid perspex floor was used, no activity was recorded from the lowest beams. In all studies, the middle layer of infrared beams (5 cm above the floor) assessed locomotion, while the upper beams (13 cm above the floor) counted the number of rears. To prevent false recording of locomotion by small movements such as grooming, a count was only generated from the middle row of detectors when two adjacent beams were broken simultaneously in a consecutive sequence, while each individual upper beam break was recorded as a rear. The number of counts from each layer in every chamber was cumulated separately in 4- or 5-min epochs using "Activity Monitor" software on an Apple Macintosh IIcx computer (Clemett et al., 1998).

In studies not monitoring food intake, each rat was placed in the box for 20 min prior to injection on the test day and behaviour was recorded for 20 min (20–40 min postinjection of (±)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide, 8-OHDPAT, $n = 12$, or (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride, DOI, $n = 12$).

While in the activity monitors, components of the serotonin behavioural syndrome were scored separately by an individual sat 1 m away, and screened, from the chamber. For 8-OHDPAT, reciprocal forepaw treading, lateral head weaving and flat body posture, were each rated (0 if absent, 1 if present for less than 10 s, 2 if present for more than 10 s and 3 if continuous) for a period of 20 s at 1-min intervals from 20- to 40-min postinjection. Thus for each behaviour, the maximum possible summated score was 60. For experiments with DOI (and saline control), the number of wet-dog shakes and back muscle contractions were counted separately using two tally counters (Fone et al., 1989).

In studies designed to examine the effect of the 5-HT agonist on food intake (*m*-CPP and BW723C86 studies) rats were individually housed and given access to 200 g of food from 09:00 to 16:00 h every day from 7 days prior to implanting the corticosterone pellet. This protocol allowed the rats to acclimatise to a constant food intake prior to 5-HT agonist study and did not cause any weight loss (Fone et al., 1998). Rats were habituated to the activity chamber for one hour with food present on the day prior to agonist injection. The next day immediately postinjection of *m*-CPP or BW723C86 each rat was placed in a chamber and activity

recorded for 1 h. Twenty minutes after injection on the test day, a tray containing 200 g crushed food pellets was placed under the centre of the hole in the floor of the activity box. The amount of food consumed was determined every 20 min by weighing the tray and any food spillage.

In all rats rectal temperature was recorded using a Portec thermocouple probe, immediately prior to it being placed in the activity box and again immediately after decapitation to prevent a stress-induced corticosterone elevation which would have resulted with restraint.

2.4. Plasma corticosterone measurement and HPLC of indoleamines

At the end of the behavioural measurement on the test day, rats were stunned and decapitated to allow collection of mixed arteriovenous trunk blood in heparinised vials (Sterilin Medical Products). Vials were centrifuged (5 min, room temperature, 2000 G) and plasma decanted and frozen on dry ice prior to storage at -20 °C for subsequent analysis of corticosterone. An aliquot of plasma was used prior to freezing to determine plasma glucose with a HemoCue β-glucose analyser (Angelholm, Sweden). For corticosterone determination, plasma was diluted 1:10 with sterile saline (0.154 M) and levels determined in duplicate from the linear portion of the standard curve using a radioimmunoassay kit (Gamma B 125I corticosterone double antibody, Immunodiagnostic Systems).

In the 8-OHDPAT group (and it's saline control), the hippocampus was rapidly removed, frozen in liquid nitrogen and stored at -80 °C for subsequent analysis by HPLC. 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine, DOPAC and homovanillic acid were separated from extracts of the hippocampus and measured by HPLC-ED using a hypersil 3 μm C18 column (10 cm × 4.6 mm I.D., Phenomenex). Samples were extracted in perchloric acid (1 ml, 0.1 M containing 1.6 mM sodium metabisulphite) by sonication on ice (30 s, Soniprep 150, MSE) followed by centrifugation (13,000 × G, for 10 min at 4 °C, Microfuge 12, Beckman, USA). The resultant supernatant was filtered (0.45 μm syringe filters, Millipore) and quantified by electrochemical detection using a mobile phase consisting of 0.15 M NaH_2PO_4, 0.1 mM EDTA, 0.5 mM 1-octanesulphonic acid and 13% (v/v) methanol at pH 3.8 and a potential of +0.7 V.

5-HT turnover was calculated as the 5-HIAA:5-HT ratio and levels were expressed against the original hippocampal wet weight.

2.5. Drugs

All 5-HT agonists were dissolved in 0.154 M saline which was used as control (1 ml/kg) and administered IP at the following doses; 8-OHDPAT (0.3 mg/kg), DOI (1 mg/kg), BW723C86 (5 mg/kg) and *m*-CPP (2.5 mg/kg). All agonists were obtained from Research Biochemical and cholesterol and corticosterone were purchased from Sigma. These agonists and the doses used were chosen on the basis of previous reports showing them to preferentially activate 5-HT_{1A} 8-OHDPAT (Bill et al., 1991), 5-HT_{2A} DOI (Kuroda et al., 1992), 5-HT_{2B} BW723C86 (Kennett et al., 1997) and 5-HT_{2C} *m*-CPP (Fone et al., 1998) receptors in vivo.

2.6. Statistics

Measures which were recorded objectively or timed, such as locomotor activity, feeding rate, rectal temperature, and indoleamine levels were analysed using parametric tests, while components of the serotonin behavioural syndrome which were rated subjectively were analysed using the Mann–Whitney *U* test following Kruskal Wallis. Where appropriate, two-way and one-way ANOVA followed by Duncan's new multiple range (behaviour) or Scheffe's *S* (indoleamine, glucose and corticosterone levels) post hoc test was used. In all cases $P < .05$ was considered significant.

3. Results

3.1. Impact of corticosterone pellets

In a preliminary group of rats ($n = 18$), the corticosterone implant was shown to slightly elevate the basal plasma corticosterone level both at the morning nadir and the evening zenith of the diurnal variation (Fig. 1), such that there was a main effect of treatment [ANOVA: $F(1,14) = 4.77$, $P = .047$]. However, there was no significant difference in plasma corticosterone at the mid day midpoint (on the fourth day after implantation) of either pellet (Fig. 2A), which was the reason for assessing the impact of pellet implantation on 5-HT agonist-induced corticosterone secretion at this time in all subsequent studies.

Although the body weight of rats with a corticosterone implant tended to be less than that of controls on the fourth

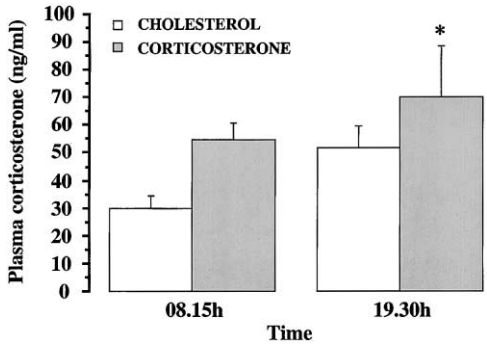

Fig. 1. Plasma corticosterone (ng/ml, mean ± S.E.M., $n = 5–6$) measured from mixed arteriovenous blood taken at 08:15 and 19:30 h on the fourth day after subcutaneous implantation of either a cholesterol (control) or corticosterone (125 mg) pellet. There was a main effect of treatment ANOVA $F(1,14) = 4.77$, $P = .047$ but no Treatment × Time interaction. * $P < .05$ from cholesterol at 08:15 h, Duncan's new multiple range.

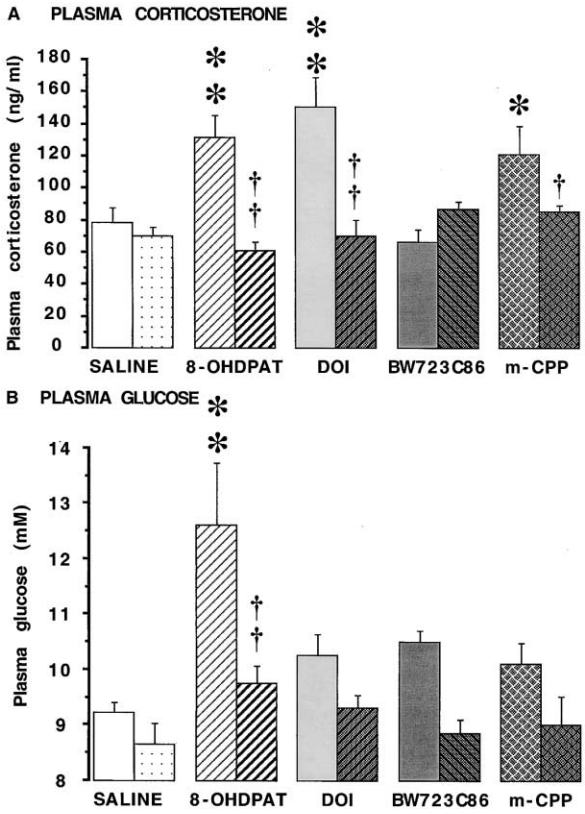

Fig. 2. Plasma levels (mean ± S.E.M.) of (A) corticosterone (ng/ml) and (B) glucose (mMol/l) after injection of a selected 5-HT agonist (as indicated, ip) or saline (1 ml/kg) 4 days after implantation of a subcutaneous cholesterol (light shaded left hand column in each pair, control) or corticosterone (right hand column) pellet. * $P<.05$ and ** $P<.01$ from saline in cholesterol controls and $^{\dagger}P<.05$ and $^{\dagger\dagger}P<.01$ from the same agonist given to cholesterol control, Scheffe's S test following ANOVA.

day of implantation this was not significant (data not shown). In addition, the corticosterone implant did not significantly alter either the plasma glucose level (Fig. 2B) or the core body temperature (Fig. 3) in rats treated with saline. The absence of any change in all these baseline measures by the dose of corticosterone used made this particularly suited to analyse the impact on 5-HT agonist challenge, as higher corticosterone doses have been shown to alter these baseline values (Akana et al., 1992).

3.2. Effect of 8-OHDPAT on rectal temperature, behaviour and neurochemistry

In rats with a control implant, 8-OHDPAT (0.5 mg/kg ip) elicited the expected behavioural components of the serotonin syndrome involving 5-HT_{1A} receptor activation; including extensive flat body posture, reciprocal forepaw treading and some lateral head weaving that was absent following saline injection (Table 1). However, 8-OHDPAT injection caused significantly less flat body posture ($P<.01$) and reciprocal forepaw treading ($P<.05$) in rats with a corticosterone than a control implant and the lateral head weaving showed a similar reduction, although the latter did not reach significance (Table 1). The locomotion and rear counts associated with 8-OHDPAT injection were comparable in corticosterone and control implant groups (data not shown). In control implanted rats (Fig. 2), 8-OHDPAT significantly elevated plasma corticosterone ($P<.01$) and glucose ($P<.01$) levels compared with that following saline injection, both of which were completely absent in rats with a corticosterone implant.

As shown in Table 1, when compared with saline, 8-OHDPAT significantly elevated the postmortem hippocampal 5-HT content ($P<.05$) in rats with a control but not those with a corticosterone implant. Thus there was a significant Implant × Agonist interaction [$F(1,18)=6.86$, $P<.017$] on hippocampal 5-HT levels. However, there was no significant main effect of either implant or agonist on either the 5-HIAA levels (data not shown) or the ratio of 5-HIAA:5-HT (Table 1).

3.3. Effect of DOI on corticosterone, glucose, temperature and behaviour

As shown in Fig. 2A, the acute significant ($P<.01$) rise in plasma corticosterone produced by DOI in rats with a control implant was absent in rats which had a corticosterone pellet. Although DOI tended to cause hyperglycaemia this was not significant from that in saline controls (Fig. 2B), neither was there any change in body temperature at the time measured (Fig. 3), irrespective of the implant given. However, the number of wet-dog shakes elicited by DOI was enhanced in corticosterone compared with that in control implanted rats, such that there was a main effect of both Implant [$F(1,100)=4.13$, $P=.045$] and Agonist [$F(1,100)=128.62$, $P=.0001$] but the Implant × Agonist interaction just failed to reach significance [$F(1,100)=2.91$, $P=.091$] (Fig. 4). In contrast, the

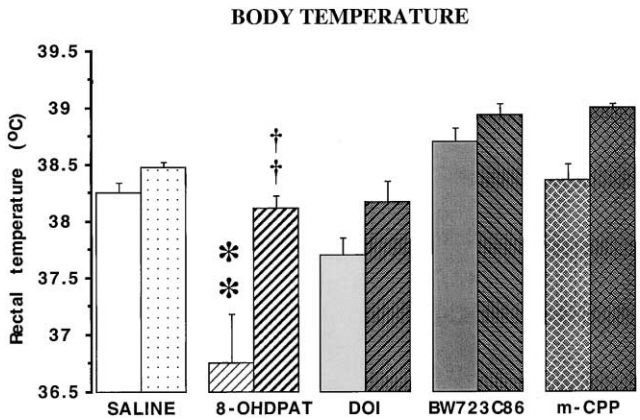

Fig. 3. Change in core body temperature (°C, mean ± S.E.M., $n=6$ each) produced by injection of a selected 5-HT agonist (as indicated, ip) or saline (1 ml/kg) 4 days after implantation of a subcutaneous cholesterol (light shaded left hand column in each pair) or corticosterone (right hand column) pellet. ** $P<.01$ from saline in cholesterol control and $^{\dagger\dagger}P<.01$ from the same agonist given in cholesterol control, Scheffe's S test following ANOVA.

Table 1
Comparison of the effect of the 5-HT_{1A} agonist, 8-OHDPAT (0.5 mg/kg ip) or saline (1 ml/kg) on stereotype behaviours (median (interquartile range)) and the hippocampal 5-HT level (pmol/mg wet weight) or 5-HIAA:5-HT ratio (mean ± S.E.M.)

	Saline		8-OHDPAT		
Measure	CHOL	CORT	CHOL	CORT	Statistics ANOVA
Reciprocal forepaw treading	0 (0)	0 (0)	7.5 (6)	3 (2)*	$H=18.27$, $P=.001$
Lateral head weaving	0 (0)	0 (0)	6.5 (5)	2 (2)	$H=16.04$, $P=.001$
Flat body posture	0 (0)	0 (0)	48 (7)	28 (16)**	$H=19.28$, $P=.001$
5-HT	4.176 ± 0.518	4.798 ± 1.165	8.395 ± 1.204†	3.725 ± 2.197*	$F(3,18)=4.79$, $P=.013$
5-HIAA:5-HT	1.20 ± 0.33	1.02 ± 0.73	0.61 ± 0.08	0.61 ± 0.08	$F(3,18)=1.51$, $P=.245$

* $P<.05$ Mann–Whitney U test (behaviour) or Scheffe's S test (5-HT) from 8-OHDPAT control implant.
** $P<.01$ Mann–Whitney U test (behaviour) or Scheffe's S test (5-HT) from 8-OHDPAT control implant.
† $P<.05$ from cholesterol/saline.

number of back muscle contractions produced by DOI was attenuated by the corticosterone implant, there being a main effect of Implant [$F(1,100)=4.20$, $P=.043$] and Agonist [$F(1,100)=114.79$, $P=.0001$] and a significant Implant × Agonist interaction [$F(1,100)=4.47$, $P=.037$]. In addition, in the case of back muscle contractions there was also an Agonist × Time Course interaction [$F(4,100)=3.55$, $P=.009$], the response being delayed in onset following DOI in corticosterone implanted animals.

3.4. Effect of BW723C86 on corticosterone and behaviour

At the dose used (5 mg/kg ip), the 5-HT_{2B} agonist BW723C86 failed to cause any significant alteration in plasma corticosterone or glucose or body temperature, irrespective of the implant given (Figs. 2 and 3), nor was there any change in locomotor activity or rears (data not shown). Similarly in rats on a restricted feeding time paradigm BW723C86 failed to significantly alter either the feeding rate or amount of food consumed, irrespective of the implant present, when compared with that in saline controls (Fig. 5), although the rate tended to increase in the control implanted rats.

3.5. Effect of m-CPP on corticosterone, glucose, temperature, locomotion and feeding

In rats with a control implant the 5-HT_{2C} agonist *m*-CPP caused a significant elevation in plasma corticosterone ($P<.05$) which was not observed in rats with a corticosterone implant (Fig. 2A). *m*-CPP failed to alter plasma

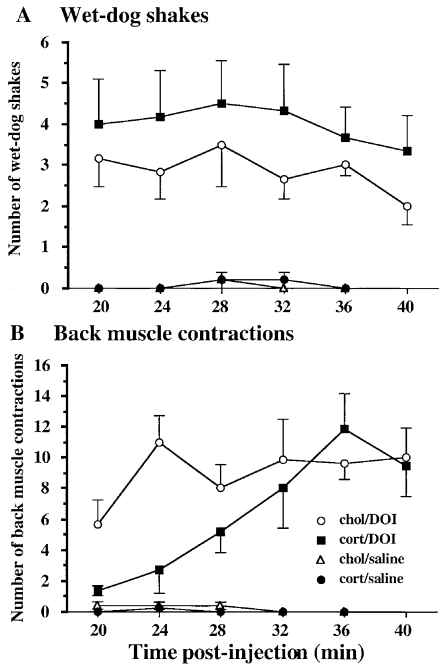

Fig. 4. Time course of change in the number (mean ± S.E.M. in 4 min epochs, $n=6$ each) of wet-dog shakes and back muscle contractions elicited by injection of the 5-HT_{2A} agonist, DOI (1 mg/kg ip) or saline 4 days after implantation of either a cholesterol or a corticosterone pellet.

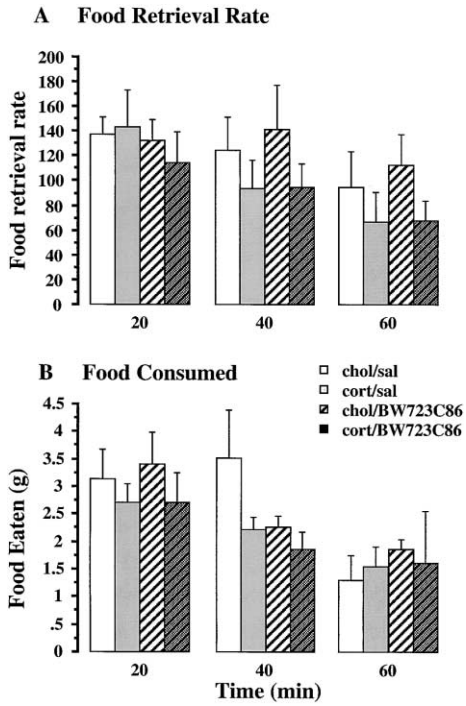

Fig. 5. (A) Food retrieval rate and (B) amount of food eaten (g) in 20-min intervals (mean ± S.E.M., $n=6$ each) commencing 20 min after injection of the 5-HT_{2B} agonist BW723C86 (5 mg/kg ip) or saline 4 days after implanting either a cholesterol or a corticosterone pellet (as indicated).

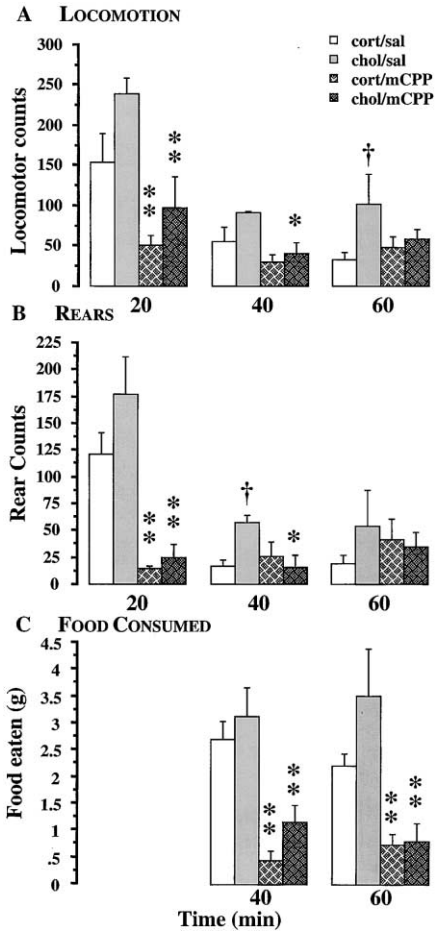

Fig. 6. Comparison of the (A) locomotor activity, (B) rears and (C) food eaten (g) in 20-min intervals (mean ± S.E.M., $n = 6$ each) commencing at the times indicated after injection of either the 5-HT_{2C} agonist *m*-CPP or saline, 4 days after implantation of either a cholesterol or a corticosterone pellet (as indicated). $^{*}P < .05$ and $^{**}P < .01$ from saline injection in rats implanted with the same type of pellet and $^{\dagger}P < .05$ from saline given to rats with a corticosterone implant, Duncan's new multiple range test following ANOVA.

glucose or body temperature irrespective of which pellet had been implanted (Figs. 2B and 3). When compared with saline, the very marked decrease in locomotion and rearing induced by injection of *m*-CPP was comparable in control and corticosterone implanted rats (Fig. 6A and B), being most apparent in the first 20 min after placement in an open field. At the same time, implantation of a corticosterone pellet did not alter the hypophagia evoked by *m*-CPP injection in rats which had been fasted overnight as part of a fixed feeding regime (Fig. 6C).

4. Discussion

In agreement with a previous corticosterone implant study (Meijer et al., 1997), the mid day mid circadian plasma corticosterone level was unaltered by the corticosterone implant but basal trough and, in the present study, peak levels of corticosterone appeared to be elevated. This agrees with previous data (Akana et al., 1992) showing that even an extremely small increase in corticosterone during the morning trough in rats adjusts the whole diurnal pattern of corticosterone secretion such that the average daily level remains unaltered. A similar elevation in plasma corticosterone, albeit throughout the diurnal rhythm but especially of the basal trough, is seen in approximately 50% of patients with severe depression (Rubin et al., 1987).

In the current study the corticosterone treatment markedly attenuated all of the effects mediated by the 5-HT_{1A} agonist 8-OHDPAT, including components of the serotonin syndrome and hippocampal 5-HT release which are mediated by activation of postsynaptic or presynaptic 5-HT_{1A} receptors, respectively. The marked hyperglycemia elicited by activation of central 5-HT_{1A} receptors and subsequently the adrenal medulla (Critchley et al., 1994) was also abolished by the corticosterone implant. Consistent with the current report, the hypothermia elicited by 8-OHDPAT (involving postsynaptic 5-HT_{1A} receptors in the rat (Bill et al., 1991) as in man) has previously been shown to be suppressed following corticosterone and enhanced by adrenalectomy in rats (Young et al., 1992, 1993). Attenuation of 8-OHDPAT hypothermia has been documented 14 but not 1 day after 50 mg/kg/day sc corticosterone (Takao et al., 1997) and 10 but not 3 days after 30 mg/kg/day (Young et al., 1992), a time course consistent with attenuation of receptor expression, probably in the hypothalamus.

Several previous studies have reported the profound impact of corticosterone or stress on 5-HT_{1A} receptor expression and function. For instance, adrenalectomy increases 5-HT_{1A} mRNA and receptor density in the dentate gyrus of the hippocampus (Burnet et al., 1992; Meijer and Dekloet, 1994; Tejani-Butt and Labow, 1994) which can be reversed by glucocorticoid restoration (Mendelson and McEwen, 1992; Tejani-Butt and Labow, 1994). Conversely, exposure of rodents to physical stress decreases 5-HT_{1A} binding in several hippocampal regions (Watanabe et al., 1993). However, change in 5-HT_{1A} receptor expression in the CA1 hippocampal subfield and the dorsal raphé nucleus may not occur with adrenalectomy (LeCorre et al., 1997) or at least may be resistant to reversal by corticosterone (Tejani-Butt and Labow, 1994). In addition, 5-HT_{1A} receptor change in these latter two areas is less sensitive to acute stress (Laaris et al., 1999) and may require a longer duration and more severe stress (Lopez et al., 1999) to elevate corticosterone to levels sufficient to occupy low affinity glucocorticoid receptors therein. Thus, in electrophysiological studies, mineralocorticoid receptor activation attenuates 5-HT_{1A}-mediated hyperpolarisation of hippocampal CA1 neurones (Joels et al., 1991; Meijer et al., 1997) due to transcription repression (Meijer et al., 2000), while glucocorticoid receptor activation on dorsal raphé neurones is largely responsible for 5-HT_{1A} autoreceptor desensitisation (Laaris et al., 1995). In contrast, direct administration of corticosterone to either adrenalectomised or adrenal-intact rats as in the current

study, reduces 5-HT_{1A} receptors in all hippocampal subfields (Maines et al., 1998; Mendelson and McEwen, 1992) and these autoreceptors on serotonergic raphé neurones, in most, but not all studies (Fernandes et al., 1997). The mechanism by which corticosterone attenuates 5-HT_{1A} receptor function is still unclear but could include attenuation at several points along the 5-HT_{1A} receptor-G protein-ion channel pathway, in addition to receptor downregulation and may also be brain region dependent (Meijer et al., 2000; Mueller and Beck, 2000). A similar reduction in 5-HT_{1A} mediated hypothermia and cortisol secretion occurs in depressed patients (Stahl, 1994; Shapira et al., 2000). Thus, the corticosterone-induced reduction in 5-HT_{1A} function in the rat seems to parallel that seen in human depression.

Acute immobilisation stress (Watanabe et al., 1993), maternal separation of rat pups (Vazquez et al., 2000) and direct elevation of corticosterone by implantation (Fernandes et al., 1997) increase cortical 5-HT_{2A} receptor density, consistent with the enhancement of DOI mediated wet-dog shakes following a corticosterone implant observed in this study. Kuroda et al. (1992) also reported that 10 days administration of ACTH (50 μg/day) enhanced DOI-induced wet-dog shakes and simultaneously increased [^{3}H]ketanserin receptor density in the neocortex of the rat forebrain (mainly 5-HT_{2A} sites), which was prevented by adrenalectomy. More recently, using a similar protocol to that herein, Takao et al. (1997) also reported augmentation of wet-dog shakes without alteration in the hyperthermia elicited by DOI following a 14-day corticosterone implant. In contrast, no previous group has examined the impact of corticosterone on the back muscle contractions that are elicited by activation of 5-HT_{2A} receptors on spinal neurones (Fone et al., 1989). Corticosterone treatment markedly attenuated DOI-induced back muscle contractions demonstrating a differential effect of corticosterone on spinal and supraspinal 5-HT_{2A} receptors, similar to the brain region-dependent variation in 5-HT_{1A} receptor response to corticosterone discussed earlier. Several postmortem studies in depressed patients have reported elevated 5-HT_{2A} binding in the prefrontal cortex and amygdala but not in the hippocampus (Hrdina et al., 1993) consistent with the brain region dependent pattern observed by elevated corticosterone in the rat.

Since the confirmation that the 5-HT_{2B} receptor is expressed in the rat brain (Duxon et al., 1997a), by using the 5-HT_{2B} agonist BW723C86 and more selective antagonists, it has been established that activation elicits anxiolysis (Duxon et al., 1997b) and mild hyperphagia (Kennett et al., 1997). As expression of the 5-HT_{2B} receptor is upregulated in the mesenteric artery of deoxycorticosterone-induced hypertensive rats (Watts et al., 1996) it was pertinent to establish whether corticosterone could also influence the CNS function of this receptor. BW723C86 failed to cause a significant hyperphagia or to alter body temperature or plasma corticosterone when given in cholesterol controls in the current study. Nonetheless, the lack of potentiation of any of these parameters following corticosterone suggests that there was no overt 5-HT_{2B} upregulation in the CNS akin to that seen in vasculature following cortiosterone.

Despite the wealth of evidence implicating the 5-HT_{2C} receptor in anxiety and depression (Cryan and Lucki, 2000; Fone et al., 1996) few previous studies have investigated the impact of elevated corticosterone on 5-HT_{2C} receptor function (Bagdy et al., 1989; Berendsen et al., 1996). In the current study *m*-CPP failed to enhance corticosterone release following corticosterone implantation, suggesting that downregulation or uncoupling of the 5-HT_{2C} receptor in the paraventricular nucleus which enhance CRF release may have occurred (Calogero et al., 1990). Emerging evidence suggests that a similar hypothalamic 5-HT_{2C} receptor dysfunction may occur in human depression, since a blunted prolactin response to intravenous m-CPP challenge has been reported in female patients with seasonal affective disorder (SAD) in the depressed state (Levitan et al., 1998). It is therefore at first sight paradoxical that rearing rats in social isolation produces supersensitivity to *m*-CPP-induced axiogenesis and enhanced corticosterone release (Fone et al., 1996), although it supports the proposal that 5-HT_{2C} receptor modulation occurs with long-term stress. Glucocorticoids generally downregulate hippocampal 5-HT_{2C} receptor mRNA. However, Holmes et al. (1997) showed that rats entrained to chronic food restriction, as used in this study, show marked intermittent corticosterone hypersecretion and an unaltered hippocampal 5-HT_{2C} receptor mRNA, even in the presence of an overt corticosterone hypersecretion. Although the hypolocomotion and hypophagia produced by *m*-CPP may involve limbic and hypothalamic neurones respectively (Fone et al., 1998), a similar multifactorial regulation in these areas could explain the resultant unaltered 5-HT_{2C} receptor function observed. An analogous 4-day corticosterone treatment attenuated the penile erections produced by the 5-HT_{2C} agonist MK 212 (Berendsen et al., 1996), although the site of the receptors mediating this response is unclear, lesions suggest that it is not the paraventricular nucleus (Bagdy and Makara, 1995).

Surprisingly, few studies have examined the ability of 5-HT agonists to increase plasma corticosterone following long-term elevation of this glucocorticoid, yet one of the most conspicuous observations herein was abolition of 5-HT-induced corticosterone release, irrespective of the agonist used. Lesions of the paraventricular nucleus of the hypothalamus attenuate corticosterone secretion by 5-HT agonists (Bagdy, 1995). Therefore downregulation or functional uncoupling of the 5-HT receptors therein or (for 5-HT_{1A} and 5-HT_2 receptors) also in the pituitary (Calogero et al., 1990), as a result of enhanced feedback inhibition by corticosterone, is a likely explanation for this effect.

In conclusion, implantation of a corticosterone pellet in adult rats with intact adrenal glands elevated the circadian trough and peak of plasma corticosterone and attenuated pre- and hippocampal and hypothalamic post-synaptic 5-HT_{1A} receptor function. With the exception of the hypo-

thalamus, supraspinal 5-HT_{2A} function was enhanced but that in the spinal cord attenuated. 5-HT_{2C} receptor activation of the HPA axis was also attenuated but not that involved in other functions. This profile of 5-HT receptor change appears similar to that in human depression (a blunted 5-HT_{1A} agonist-induced hypothermia and cortisol response (Mann, 1999), elevated prefrontal cortex 5-HT_{2A} receptors (Stahl, 1994) and attenuated 5-HT_{2C}-agonist mediated prolactin release in SAD (Levitan et al., 1998). The reciprocal change in 5-HT_{1A} and 5-HT_{2A} function is rapidly reversed following pellet removal in adult rats (Fernandes et al., 1997). However, the longer-term impact of elevating corticosterone in immature rats on the subsequent response to stress at adulthood is worthy of investigation as a potential animal model of depression.

References

Akana SF, Scribner KA, Bradbury MJ, Strack AM, Walker CD, Dallman MF. Feedback sensitivity of the rat hypothalamo–pituitary–adrenal axis and its capacity to adjust to exogenous corticosterone. Endocrinology 1992;131:585–94.

Bagdy G. Role of the hypothalamic paraventricular nucleus in 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptor-mediated oxytocin, prolactin and ACTH/corticosterone responses. Behav Brain Res 1995;73:277–80.

Bagdy G, Makara GB. Paraventricular nucleus controls 5-HT_{2C} receptor-mediated corticosterone and prolactin but not oxytocin and penile erection responses. Eur J Pharmacol 1995;275:301–5.

Bagdy G, Calogero AE, Aulakh CS, Szemeredi K, Murphy DL. Long-term cortisol treatment impairs behavioral and neuro-endocrine responses to 5-HT_1 agonists in the rat. Neuroendocrinology 1989;50:241–7.

Berendsen HHG, Kester RCH, Peeters B, Broekkamp CLE. Modulation of 5-HT receptor subtype-mediated behaviours by corticosterone. Eur J Pharmacol 1996;308:103–11.

Bill DJ, Knight M, Forster EA, Fletcher A. Direct evidence for an important species-difference in the mechanism of 8-OH-DPAT-induced hypothermia. Br J Pharmacol 1991;103:1857–64.

Blier P, de Montigny C. Possible serotonergic mechanisms underlying the antidepressant and anti-obsessive–compulsive disorder responses. Biol Psychiatry 1998;44:313–23.

Burnet PWJ, Mefford IN, Smith CC, Gold PW, Sternberg EM. Hippocampal 8-[*H*-3]hydroxy-2-(di-*n*-propylamino)tetralin binding-site densities, serotonin receptor (5-HT(1a)) messenger-ribonucleic-acid abundance, and serotonin levels parallel the activity of the hypothalamopituitary adrenal axis in rat. J Neurochem 1992;59:1062–70.

Calogero AE, Bagdy G, Szemeredi K, Tartaglia ME, Gold PW, Chrousos GP. Mechanisms of serotonin receptor agonist-induced activation of the hypothalamic–pituitary–adrenal axis in the rat. Endocrinology 1990; 126:1888–94.

Chaouloff F. Serotonin, stress and corticoids. J Psychopharmacol 2000; 14:139–51.

Clemett DA, Cockett MI, Marsden CA, Fone KC. Antisense oligonucleotide-induced reduction in 5-hydroxytryptamine7 receptors in the rat hypothalamus without alteration in exploratory behaviour or neuroendocrine function. J Neurochem 1998;71:1271–9.

Critchley DJP, Middlefell VC, Liddle CW, Foden ND, Dourish CT. Effects of the selective 5-HT_{1A} receptor antagonist WAY100135 and its enantiomers on 8-OH-DPAT-induced hyperglycemia in conscious rats. Eur J Pharmacol 1994;254:133–9.

Cryan JF, Lucki I. Antidepressant-like behavioral effects mediated by 5-hydroxytryptamine(2C) receptors. J Pharmacol Exp Ther 2000; 295:1120–6.

Dickinson SL, Kennett GA, Curzon G. Reduced 5-hydroxytryptamine-dependent behavior in rats following chronic corticosterone treatment. Brain Res 1985;345:10–8.

Duxon MS, Flanigan TP, Reavley AC, Baxter GS, Blackburn TP, Fone KC. Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. Neuroscience 1997a;76: 323–9.

Duxon MS, Kennett GA, Lightowler S, Blackburn TP, Fone KC. Activation of 5-HT_{2B} receptors in the medial amygdala causes anxiolysis in the social interaction test in the rat. Neuropharmacology 1997b;36:601–8.

Fernandes C, McKittrick CR, File SE, McEwen BS. Decreased 5-HT_{1A} and increased 5-HT_{2A} receptor binding after chronic corticosterone associated with a behavioural indication of depression but not anxiety. Psychoneuroendocrinology 1997;22:477–91.

Fone KCF, Johnson JV, Bennett GW, Marsden CA. Involvement of 5-HT_2 receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats. Br J Pharmacol 1989;96:599–608.

Fone KCF, Shalders K, Fox ZD, Arthur R, Marsden CA. Increased 5-HT_{2C} receptor responsiveness occurs on rearing rats in social isolation. Psychopharmacology 1996;123:346–52.

Fone KC, Austin RH, Topham IA, Kennett GA, Punhani T. Effect of chronic m-CPP on locomotion, hypophagia, plasma corticosterone and 5-HT_{2C} receptor levels in the rat. Br J Pharmacol 1998;123: 1707–15.

Holmes MC, French KL, Seckl JR. Dysregulation of diurnal rhythms of serotonin 5-HT_{2C} and corticosteroid receptor gene expression in the hippocampus with food restriction and glucocorticoids. J Neurosci 1997; 17:4056–65.

Hrdina PD, Demeter E, Vu TB, Sotonyi P, Palkovits M. 5-HT uptake sites and 5-HT_2 receptors in brain of antidepressant-free suicide victims depressives — increase in 5-HT_2 sites in cortex and amygdala. Brain Res 1993;614:37–44.

Inoue T, Tsuchiya K, Koyama T. Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat-brain. Pharmacol, Biochem Behav 1994;49:911–20.

Joels M, Hesen W, Dekloet ER. Mineralocorticoid hormones suppress serotonin-induced hyperpolarization of rat hippocampal CA1 neurons. J Neurosci 1991;11:2288–94.

Kennett GA, Ainsworth K, Trail B, Blackburn TP. BW 723C86, a 5-HT_{2B} receptor agonist, causes hyperphagia and reduced grooming in rats. Neuropharmacology 1997;36:233–9.

Kuroda Y, Mikuni M, Ogawa T, Takahashi K. Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT(2) receptor-binding sites in neocortex of rat forebrain and 5-HT(2) receptor-mediated wet-dog shake behaviors. Psychopharmacology 1992; 108:27–32.

Laaris N, Hajdahmane S, Hamon M, Lanfumey L. Glucocorticoid receptor-mediated inhibition by corticosterone of 5-HT_{1A} autoreceptor functioning in the rat dorsal raphe nucleus. Neuropharmacology 1995;34: 1201–10.

Laaris N, Le Poul E, Laporte AM, Hamon M, Lanfumey L. Differential effects of stress on presynaptic and postsynaptic 5-hydroxytryptamine-1A receptors in the rat brain: an in vitro electrophysiological study. Neuroscience 1999;91:947–58.

LeCorre S, Sharp T, Young AH, Harrison PJ. Increase of 5-HT_7 (serotonin-7) and 5-HT_{1A} (serotonin-1A) receptor mRNA expression in rat hippocampus after adrenalectomy. Psychopharmacology 1997;130: 368–74.

Levitan RD, Kaplan AS, Brown GM, Vaccarino FJ, Kennedy SH, Levitt AJ, Joffe RT. Hormonal and subjective responses to intravenous *m*-chlorophenylpiperazine in women with seasonal affective disorder. Arch Gen Psychiatry 1998;55:244–9.

Lopez JF, Liberzon I, Vazquez DM, Young EA, Watson SJ. Serotonin 1A receptor messenger RNA regulation in the hippocampus after acute stress. Biol Psychiatry 1999;45:934–7.

Maines LW, Keck BJ, Dugar A, Lakoski JM. Age-dependent loss of cor-

ticosterone modulation of central serotonin 5-HT_{1A} receptor binding sites. J Neurosci Res 1998;53:86–98.

Mann JJ. Role of serotonergic system in the pathogenesis of major depression and suicidal behaviour. Neuropsychopharmacology 1999;21: 99S–105S.

Meijer OC, Dekloet ER. Corticosterone suppresses the expression of 5-HT_{1A} receptor messenger-RNA in rat dentate gyrus. Eur J Pharmacol, Mol Pharmacol Sect 1994;266:255–61.

Meijer OC, VanOosten RV, DeKloet ER. Elevated basal trough levels of corticosterone suppress hippocampal 5-hydroxytryptamine$_{1A}$ receptor expression in adrenally intact rats: implication for the pathogenesis of depression. Neuroscience 1997;80:419–26.

Meijer OC, Williamson A, Dallman MF, Pearce D. Transcriptional repression of the 5-HT_{1A} receptor promoter by corticosterone via mineralocorticoid receptors depends on the cellular context. J Neuroendocrinol 2000;12:245–54.

Mendelson SD, McEwen BS. Autoradiographic analyses of the effects of adrenalectomy and corticosterone on 5-HT_{1A} and 5-HT_{1B} receptors in the dorsal hippocampus and cortex of the rat. Neuroendocrinology 1992;55:444–50.

Mueller NK, Beck SG. Corticosteroids alter the 5-HT_{1A} receptor-mediated response in CA1 hippocampal pyramidal cells. Neuropsychopharmacology 2000;23:419–27.

Nemeroff CB. Psychopharmacology of affective disorders in the 21st century. Biol Psychiatry 1998;44:517–25.

Rubin RT, Poland RE, Lesser IM, Winston RA, Blodgett ALN. Neuroendocrine aspects of primary endogenous depression. Arch Gen Psychiatry 1987;44:328–36.

Shapira B, Newman ME, Gelfin Y, Lerer B. Blunted temperature and cortisol responses to ipsapirone in major depression: lack of enhancement by electroconvulsive therapy. Psychoneuroendocrinology 2000; 25:421–38.

Stahl S. 5HT_{1A} receptors and pharmacotherapy. Psychopharmacol Bull 1994;30:39–43.

Takao K, Nagatani T, Kitamura Y, Yamawaki S. Effects of corticosterone on 5-HT_{1A} and 5-HT_2 receptor binding and on the receptor-mediated behavioral responses of rats. Eur J Pharmacol 1997;333:123–8.

Tejani-Butt SM, Labow DM. Time-course of the effects of adrenalectomy and corticosterone replacement on 5-HT_{1A} receptors and 5-HT uptake sites in the hippocampus and dorsal raphe nucleus of the rat-brain — an autoradiographic analysis. Psychopharmacology 1994;113:481–6.

Vazquez DM, Lopez JF, Van Hoers H, Watson SJ, Levine S. Maternal deprivation regulates serotonin 1A and 2A receptors in the infant rat. Brain Res 2000;855:76–82.

Watanabe Y, Sakai RR, McEwen BS, Mendelson S. Stress and antidepressant effects on hippocampal and cortical 5-HT(1A) and 5-HT(2) receptors and transport sites for serotonin. Brain Res 1993;615:87–94.

Watts SW, Baez M, Webb RC. The 5-hydroxytryptamine(2B) receptor and 5-HT receptor signal-transduction in mesenteric-arteries from deoxycorticosterone acetate salt hypertensive rats. J Pharmacol Exp Ther 1996; 277:1103–13.

Young AH, Macdonald LM, Stjohn H, Dick H, Goodwin GM. The effects of corticosterone on 5-HT receptor function in rodents. Neuropharmacology 1992;31:433–8.

Young AH, Dow RC, Goodwin GM, Fink G. The effects of adrenalectomy and ovariectomy on the behavioral and hypothermic responses of rats to 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT). Neuropharmacology 1993;32:653–7.