Moderate differences in circulating corticosterone alter receptor-mediated regulation of 5-HT neuronal activity.

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This work was supported by a Wellcome Trust project grant.
Abstract

Circulating glucocorticoid levels vary with stress and psychiatric illness and play a potentially important role in regulating transmitter systems that regulate mood. To determine whether chronic variation in corticosterone levels within the normal diurnal range altered the control of 5-HT neuronal activity, male rats were adrenalectomized and implanted with either a 2 % or 70 % corticosterone/cholesterol pellet (100 mg). Two weeks later the regulation of 5-HT neuronal activity in the dorsal raphe nucleus was studied by in vitro electrophysiology. At this time serum corticosterone levels approximated the low-point (2 %) and mid-point (70%) of the diurnal range. The excitatory response of 5-HT neurones to the α₁-adrenoceptor agonist phenylephrine (1-11 μM) was significantly greater in the 2 % group than the 70 % group. In contrast, the inhibitory response to 5-HT (10-50 μM) was significantly lower in the 2 % group than the 70% group. Thus, chronic variation in circulating corticosterone over a narrow part of the normal diurnal range causes a shift in the balance of positive and negative regulation of 5-HT neurones, with increased α₁-adrenoceptor-mediated excitation and reduced 5-HT-mediated autoinhibition at lower corticosterone levels. This shift would have a major impact on control of 5-HT neuronal activity.

Keywords: 5-HT₁A autoreceptor, α₁-adrenoceptor, adrenalectomy, dorsal raphe nucleus, electrophysiology, rat
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Introduction

Corticosterone is the major glucocorticoid hormone of rodents and is analogous to cortisol, the major glucocorticoid in man. Circulating levels of corticosterone vary considerably in life. In particular they have distinct diurnal and ultradian rhythms (Gallo & Weinberg, 1981; Windle et al., 1998; 2001), which change with development and ageing (Meaney et al., 1992), and which show changes with stressors (Nolan et al., 2000; Windle et al., 2001). Glucocorticoids have many effects in the brain mediated by intracellular glucocorticoid and mineralocorticoid receptors (GRs and MRs), which are widely (but differentially) expressed in the brain (Reul & de Kloet, 1985). The activation of GRs can promote or repress the transcription of genes, which possess glucocorticoid response elements (GREs) within their promoter region (Reichardt & Schutz, 1998).

The midbrain dorsal raphe nucleus (DRN) contains the cell bodies of the majority of 5-hydroxytryptamine (5-HT) neurones that innervate the forebrain (Tork, 1985). In situ hybridization studies of the DRN have revealed the presence of GR mRNA (Morimoto et al., 1996), but not MR mRNA (R. McQuade personal communication). Furthermore, immunocytochemical studies have shown that GR protein is present in the DRN (Morimoto et al., 1996) and GRs, have been found to be expressed within 5-HT cell bodies in the DRN (Härflstrand et al., 1986). Thus, circulating glucocorticoids have the potential to regulate gene expression in 5-HT neurones.

One of the major regulators of 5-HT neuronal activity in the DRN is noradrenaline, which causes excitation through α1B-adrenoceptors (Gallager & Aghajanian, 1976; Sakai & Crochet, 2000). The α1B-adrenoceptor gene has been shown to include a
GRE (Gao & Kunos, 1993) and hence may be sensitive to changes in glucocorticoids. 5-HT neuronal activity is also subject to 5-HT$_{1A}$ receptor-mediated autoinhibition (Blier et al., 1998). Previous studies have indicated that 5-HT$_{1A}$ receptors may be sensitive to (albeit gross changes) in corticosterone (Tejani-Butt & Lablow 1994; Takao et al., 1997).

The aim of the present study was to determine whether circulating corticosterone within the normal diurnal range can alter the sensitivity of the major regulators of 5-HT neuronal firing and so influence the activity of 5-HT neurones. In order to address this question groups of animals with serum corticosterone clamped at two different levels within the normal diurnal range were compared. The sensitivity of 5-HT neurones to the $\alpha_1$-adrenoceptor agonist phenylephrine and to 5-HT was examined by in vitro electrophysiology using in DRN slices prepared from these two groups. A preliminary account of these results has been published in abstract form (Judge et al., 2003).
Methods and Materials

Animals

All experiments were carried out in accordance with the UK Animals (Scientific procedures) Act of 1986 and the European Community Council Directive of 24th November 1986 (86/609/EEC). Male hooded Lister rats (Charles River, Kent) were housed in groups of four under controlled conditions of temperature and humidity in a 12 h light/12 h dark cycle (lights on 7 am). All animals were allowed to acclimatise to the holding facilities for one week before interventions were made.

Corticosterone pellets

Corticosterone pellets containing either 2% or 70% corticosterone in cholesterol were made by mixing corticosterone (Sigma) and cholesterol (Sigma) in the appropriate (w/w) proportions and heating gently over a Bunsen burner flame until molten. The liquid was rapidly poured into a cubic pill mould. Once hardened, the pellets weighing 105-120 mg were trimmed with a scalpel blade to 100 mg.

Surgery

Animals undergoing surgery for adrenalectomy and corticosterone pellet implantation were anaesthetised with isoflurane (5% induction, 2% maintenance). A single midline incision was made in the back and the muscles on each side of the spine were parted by blunt dissection to allow access to the rear of the abdomen. The adrenal glands were removed and the muscles sutured. Before closing the skin wound, a subcutaneous pocket was made by blunt dissection and a pellet was implanted.
No untreated (adrenally intact) or adrenalectomy only group was included in this study as the comparison was between groups with constant steroid levels within the normal diurnal range. Animals were allowed to recover from the anaesthetic and were group housed in warm conditions (25°C) for 1-2 days before being returned to the normal holding facilities. All animals received peri-surgical analgesia with a non-steroidal anti-inflammatory drug (meloxicam or carprofen) as deemed appropriate by the veterinarian. After surgery, all animals were allowed \textit{ad libitum} access to 0.9 % saline in addition to tap water. Animals were weighed daily and their general health assessed by visual inspection.

At the time of surgery rats destined for implantation with 2 \% pellets weighed 205 ± 2 g (n = 9) and those receiving 70 \% pellets weighed 209 ± 2 g (n = 9). Although rats implanted with a 70 \% pellet had lower weight gain immediately after surgery relative to 2 \% group, at the time of the electrophysiological experiments there was no significant weight difference between the groups (267 ± 5 g (n = 9) vs 257 ± 4 g (n = 9)). All animals appeared healthy throughout.

\textit{Collection of tissues}

For the determination of serum corticosterone levels, naïve (intact) or surgically prepared rats were moved to a quiet room adjacent to the experimental room at least 12 h before sacrifice. Naïve animals were handled briefly twice a day for at least 3 days prior to sacrifice and groups were sacrificed at 8 am and 7 pm. Adrenalectomized animals were handled during the course of daily weighing and were sacrificed between 8 and 9 am on either day 13, 14, 15 or 16 post-surgery. In order to avoid leaving animals alone in the cage, the first and second rats from each
cage of four were used on separate days, and the third and fourth rat were always sacrificed on the same day within 20 min of one another. Animals were sacrificed by guillotine and trunk blood collected. Blood was allowed to clot on ice before samples were centrifuged and the resulting serum removed. Serum samples were stored frozen at -20˚C until assay for corticosterone using a commercially available radioimmunoassay kit (‘Coat-a-Count’, Diagnostic Products Corporation, Los Angeles, CA).

Electrophysiology
Following decapitation the brain was rapidly removed from the skull and submerged in ice cold oxygenated artificial cerebrospinal fluid (aCSF) (composition (mM): NaCl: 124, MgSO₄ 2.4, KH₂PO₄ 1.25, KCl 3.25, NaHCO₃ 26, CaCl₂ 1, D-glucose 10, pH 7.4). The frontal part of the brain was removed and the mid- and hindbrain mounted on a metal block using cyanoacrylate glue. Coronal slices of the midbrain (400 μm thick) containing the DRN were cut in cold aCSF using a vibrating microtome (Vibratome 1000). Slices were trimmed and mounted on lens tissue in an interface perfusion chamber. Two or three slices were mounted in each chamber, and were perfused with aCSF warmed to 36˚C. The surface of the slices was exposed to an atmosphere of warmed humidified 95 % O₂/5 % CO₂. Following an equilibration period of around 1 h, the perfusion medium was changed to one containing α₁-adrenoceptor agonist phenylephrine at 1 μM (control medium) and the slices perfused for at least 30 min before recordings began. All perfusion media were oxygenated by constantly bubbling through 95 % O₂/5 % CO₂ and were pumped at 0.5 ml/min.
Extracellular recordings were made from neurones in the DRN using glass microelectrodes (1.5 mm OD, Clarke Electromedical; 1-3 Ω *in vitro* impedance) filled with 2 M NaCl. Signals were amplified (x1000), notch filtered (50Hz) and were displayed on an oscilloscope and fed to an audio amplifier/speaker. Unit activity was discriminated by means of a pulse discriminator and the TTL pulses generated were fed to a PC via a computer interface (1401 or micro1401, CED, UK). Data were collected using Spike2 software (version 4, CED, UK). Neurones were identified as putative 5-HT neurones on the basis of their location, basal electrophysiological characteristics (spike shape, firing rate and pattern, VanderMaelen & Aghajanian, 1983) and their inhibitory response to 5-HT. Between one and eight neurones were recorded from each animal.

Following a period of recording of “basal” firing activity (i.e. in 1 μM phenylephrine), drugs were applied to the slice. To apply drugs, the perfusion medium was changed to one containing either higher concentrations of phenylephrine (3.5, 6 or 11 μM) or one containing 5-HT (10, 25 or 50 μM plus 1 μM phenylephrine). Drugs were applied for 2 min. In most cases drugs were applied at increasing concentrations and the order of 5-HT and phenylephrine application was semi-randomised. Some neurones were tested with both 5-HT and phenylephrine although, because of limitations in the length of stable recording, others were tested with only one drug. Although neurones were exposed to multiple drug applications we verified that this did not modify subsequent responses to phenylephrine or 5-HT.

**Drugs**
All drug solutions were made freshly on the day of the experiment. A stock solution of phenylephrine (Sigma) was prepared and used to make up a 1 µM solution in aCSF. A stock solution of 5-HT (Sigma) in this 1 µM phenylephrine/aCSF solution was prepared. All final working dilutions of 5-HT and phenylephrine were also prepared in the 1 µM phenylephrine/aCSF solution.

Electrophysiological data analysis and statistics

Electrophysiological data were analysed off-line from the firing rate data collected using Spike 2. Basal firing was determined in a 120 s period at the start of the recording during perfusion with the control medium (1 µM phenylephrine). Responses to drug applications were determined as the firing rate in a 120 s period covering the maximum change from baseline. As activity induced by 1 µM phenylephrine was recorded following long-term continuous perfusion, it was considered separately from the increases in firing rate in response to higher concentrations of phenylephrine applied for 2 minutes. Basal firing rate (1 µM phenylephrine) and responses to increased concentrations of phenylephrine, were expressed as absolute firing rate (Hz) as firing is believed to be almost entirely α1-adrenoceptor dependent (VanderMaelen & Aghajanian, 1983). Responses to 5-HT were expressed as a percentage change (decrease) relative to the firing rate in control medium recorded immediately before application of each concentration of 5-HT. As animals were treated in vivo, the rat was considered to be the experimental unit. Therefore, average responses for each animal were calculated for basal firing rate and responses to drug applications in the 1-8 neurones from that individual. A group mean response was then calculated from the average values for individual animals. We also examined the variability of values within animals and present the data from individual
neurones. Data presented are individual examples, responses in individual neurones, or the group mean ± SEM.

Statistical analysis was by unpaired Student’s t-test or two-way repeated measures analysis of variance (ANOVA), as appropriate. The significance of correlations was assessed using Pearson’s product moment (r).
Results

Serum corticosterone

The normal diurnal range of corticosterone levels was determined by measuring plasma corticosterone levels in naïve rats. In trunk blood taken at 8 am serum corticosterone was 12.6 ± 4.7 ng/ml (range: 1.3-54.5 ng/ml, n = 12) and in trunk blood taken at 7 pm was 156.2 ± 23.7 ng/ml (range: 58.2-268.6 ng/ml, n = 12) (Fig. 1). In a 12 h light/12 h dark cycle with lights on at 7am, these timings are representative of the trough and peak, respectively, of the corticosterone rhythm (e.g. Ishikawa et al., 1995).

To clamp the plasma corticosterone levels within the normal diurnal range, rats were adrenalectomized and implanted with corticosterone/cholesterol pellets. Between 8 and 9 am 13-16 days following surgery, serum corticosterone in trunk blood from animals implanted with a 2 % corticosterone pellet was 19.8 ± 4.7 ng/ml (range: 6.8-56.0 ng/ml, n = 11). Animals implanted with a 70 % pellet had significantly higher mean serum corticosterone levels (64.5 ± 6.4 ng/ml; range: 34.6-89.3 ng/ml, n = 9; p<0.001, t-test) than in the 2 % group (Fig. 1).

Effect of corticosterone on the modulation of neuronal firing by phenylephrine

The control perfusion medium contained the α1-adrenergic agonist phenylephrine (1 μM) in order to evoke activity in otherwise silent 5-HT neurones. Under these conditions, presumed 5-HT neurones in DRN slices from both treatment groups fired regularly and at a slow rate (range: 0.22-2.8 Hz). Although there was considerable variation in the basal firing rate of individual neurones within animals (Fig. 2), the
mean basal firing rate was significantly higher in the 2 % group (1.41 ± 0.07 Hz) than in the 70 % group (1.03 ± 0.16 Hz; p<0.05, unpaired t-test) (Fig. 2).

Application of higher concentrations of phenylephrine (3.5, 6 and 11 μM) caused a concentration-dependent increase in firing rate in neurones from both groups of animals which reversed readily on washout of the drug (Fig. 3). However, sensitivity to phenylephrine was greater in the 2 % group than in the 70 % group (Fig. 3A,B) with the mean firing rate following application of phenylephrine being higher in the 2 % group than the 70 % group for all concentrations of the drug (Fig. 3C). Two-way ANOVA revealed that there was a significant difference between the treatment groups (significant main effect of treatment: F1,18 = 6.1; p<0.05), and a significant main effect of drug concentration: F2,36 = 31.5; p<0.001). Furthermore, there were significant negative correlations between the serum corticosterone level and mean firing rate, for individual animals at baseline and following all three higher concentrations of phenylephrine (Fig. 3D, Table 1).

Effect of corticosterone on the modulation of neuronal firing by 5-HT
Application of 5-HT inhibited the firing of 5-HT neurones in DRN slices from both treatment groups in a concentration-dependent manner. However, the inhibitory response to 5-HT was smaller in the 2 % group than in the 70 % group (e.g. Fig. 4A,B) with the mean percentage inhibition of firing being much lower in animals implanted with 2 % than 70 % pellets for all concentrations of the drug (Fig. 4C). Two-way ANOVA of these data revealed a significant difference between the two treatment groups (significant main effect of treatment: F1,17 = 4.7, p < 0.05) and a significant effect of concentration (F2,34 = 148; p<0.001). In contrast to the responses

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to phenylephrine, there were no significant correlations between serum corticosterone levels in individual animals and the mean % inhibition of firing for any of the concentrations of 5-HT applied (data not shown).
Discussion

The present study examined the effects of differing corticosterone levels within the normal diurnal range on the major excitatory and inhibitory regulators of 5-HT neuronal firing. Implantation of 100 mg pellets containing 2% or 70% corticosterone into adrenalectomized rats clamped corticosterone levels at the bottom and mid points, respectively, of the normal diurnal range. This relatively small change, approximating to only 30% of the normal diurnal range, had very marked effects on the responses of DRN 5-HT neurones. Thus, responses to phenylephrine were markedly increased and responses to 5-HT were markedly reduced in the group exposed to lower corticosterone levels compared to those exposed to the higher corticosterone levels. This shift from excitatory to inhibitory responses has important implications for physiological and pathophysiological regulation of 5-HT neuronal activity.

Corticosterone levels

The present study aimed to modulate corticosterone levels within the normal diurnal range and to examine the effects of these modulations on the regulators of 5-HT neuronal firing. In line with the observations of ourselves and others, in naïve animals a marked difference in corticosterone levels was found between the peak and trough of the normal diurnal rhythm (Gallo & Weinberg, 1981; Leitch et al., 2003). Adrenalectomy and implantation of corticosterone/cholesterol pellets was used to clamp serum corticosterone levels at two points within this range. Pellets were employed to overcome some of the limitations of other methodologies (e.g. steroid injections) which arise from the short half-life and negative-feedback regulation of corticosterone (Gomez et al., 1998; Windle et al., 1998). Furthermore, experiments
were conducted following two weeks of corticosterone treatment as previous data indicate that some effects of corticosterone may be subject to a considerable time-lag (e.g. Day et al., 1999). Animals implanted with a 70% corticosterone pellet had serum corticosterone levels in the mid diurnal range. Given that the normal diurnal rhythm is symmetrical about this mid point (Gallo & Weinberg, 1981), the overall exposure to corticosterone in these animals would be expected to be normal, though of course in the treated animals there would be no diurnal rhythm. In animals implanted with a 2% pellet, corticosterone levels were similar to those at the trough of the normal diurnal rhythm and thus overall exposure could be considered to be hypo-physiological. Thus, importantly this study has examined effects occurring within a narrow part of the normal diurnal range (approximately 30%) in contrast to other studies that have used treatments resulting in steroid levels below or well above this range. Although corticosterone levels were measured only on the morning of the electrophysiology experiment, previous studies using similar corticosterone/cholesterol pellets have shown that corticosterone levels remain fairly constant after a short period of elevated levels in the first few days post-implantation (Meyer et al., 1979; Akana et al., 1985).

Effects of corticosterone on 5-HT neuronal responses to phenylephrine

Noradrenaline provides the major excitatory drive to the DRN (Baraban and Aghajanian, 1980) and the excitatory action of phenylephrine on 5-HT neurones is inhibited by the α1-adrenoceptor antagonist prazosin (VanderMaelen & Aghajanian, 1983; and own unpublished data) indicating the involvement of α1-adrenoceptors. Indeed, in situ hybridization histochemical analysis has shown that in the DRN, the α1-adrenoceptors are predominantly of the α1B subtype (Pieribone et al., 1994). Here
we found that the excitatory response of 5-HT neurones to phenylephrine was significantly greater in animals treated with a 2 % pellet than in animals with a 70 % pellet. This was evidenced by both a higher mean basal firing rate (i.e. driven by 1 μm phenylephrine) and higher firing rates following brief application of increasing concentrations of the agonist. This relationship with corticosterone levels was further evidenced by the highly significant negative correlations between serum corticosterone levels in individual animals and firing rate for all concentrations of phenylephrine, indicating that 5-HT neurones in the DRN are more sensitive to α₁B-adrenoceptor-activation at lower corticosterone levels.

The effects of corticosterone on the functional sensitivity of α₁B-adrenoceptors in vivo have not previously been examined. Day et al. (1999), found that the expression of α₁B-adrenoceptor mRNA in the hypothalamic paraventricular nucleus was increased by adrenalectomy and decreased by corticosterone administration. Indeed α₁B-adrenoceptor mRNA was higher after corticosterone replacement which produced levels in the mid diurnal range, than after corticosterone replacement leading to levels in the high diurnal range (Day et al. 1999). Here we found that α₁B-adrenoceptor sensitivity was higher in animals with low corticosterone than in those with higher corticosterone. From the present experiment it is unclear whether corticosterone modulates α₁-adrenoceptor-sensitivity directly or indirectly. Thus, although the spontaneous discharge of noradrenergic locus coeruleus neurones has been reported to increase following adrenalectomy (Pavcovich & Valentino, 1997), this would be expected to lead to a desensitization of α₁B-adrenoceptors rather than the apparent increase in sensitivity seen in the present study. An alternative mechanism by which corticosterone may alter α₁B-adrenoceptor sensitivity is by a direct effect on the
transcription of the receptor gene. Indeed, the gene encoding the $\alpha_{1B}$-adrenoceptor has been reported to contain a GRE (Gao & Kunos, 1993) although, Day et al. (1999) reported that adrenalectomy was without effect on $\alpha_{1B}$-adrenoceptor mRNA in the DRN. Finally, several components of the phosphatidyl inositol signaling pathway, to which $\alpha_{1B}$-adrenoceptors are linked, have been shown to be sensitive to changes in corticosterone (Dwivedi et al., 2000). Hence, it is possible that corticosterone alters $\alpha_{1B}$-adrenoceptor sensitivity by acting on downstream elements of the receptor-effector complex.

Effects of corticosterone on 5-HT neuronal responses to 5-HT

The firing activity of 5-HT neurones of the DRN is sensitive to autoinhibition (Sprouse & Aghajanian, 1987). It is established that this autoinhibition is mediated by 5-HT$_{1A}$ receptors located on the cell bodies and dendrites of 5-HT neurones (Riad et al., 2000). In line with this we have previously shown that the inhibitory response to application of 5-HT is completely blocked by the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (Johnson et al., 2002; Fairchild et al., 2003). Here, as in previous studies, we found that 5-HT inhibits firing. The present study showed that this inhibitory response was significantly smaller in animals treated with a 2 % pellet than in animals with a 70 % pellet. This inhibitory response to the endogenous agonist 5-HT is contributed to, not only by 5-HT$_{1A}$ receptors, but also by the activity of the 5-HT transporter (Johnson et al., 2002) and potentially by 5-HT$_{1B}$ receptors (Davidson and Stamford, 2000). Thus, the attenuated response to 5-HT may be explained by an increase in 5-HT reuptake at the lower corticosterone level. However, this is unlikely as adrenalectomy, with or without corticosterone pellet implantation, has been shown to be without effect on 5-HT transporter binding (Kulikov et al.,...
1997). It is also possible that the response to 5-HT may be altered by a change in the functional activity of 5-HT\textsubscript{1B} receptors in the DRN. However, evidence indicates that these receptors control 5-HT release in the cell body region and do not directly affect firing rate (Adell \textit{et al.}, 2001).

The most plausible mechanism underlying this change in response to 5-HT is a reduced functional activation of 5-HT\textsubscript{1A} receptors at lower levels of corticosterone. Indeed, several previous studies have shown that the function of 5-HT\textsubscript{1A} autoreceptors is corticosterone sensitive (McAllister-Williams \textit{et al.}, 1999, Man \textit{et al.}, 2002, Leitch \textit{et al.}, 2003), although some studies have shown no effect of corticosterone manipulations (Gur \textit{et al.}, 2001). Thus, although depletion of corticosterone by adrenalectomy is without effect (Laaris \textit{et al.}, 1995, McAllister-Williams \textit{et al.}, 1999), administration of high doses of corticosterone to adrenally intact animals has been reported to result in a desensitization of somatodendritic 5-HT\textsubscript{1A} receptors (Man \textit{et al.}, 2002). Similarly, we have recently observed that administration of corticosterone to intact rats by pellet implantation results in a reduction in the sensitivity of 5-HT neurones to somatodendritic 5-HT\textsubscript{1A} receptor-activation (Leitch \textit{et al.}, 2003). Although these reports appear contrary to the present findings with corticosterone administration decreasing 5-HT\textsubscript{1A} sensitivity, it is notable that, administration of corticosterone by pellet causes a decrease in the diurnal peak of plasma corticosterone without any increase in mean daily level (Leitch \textit{et al.}, 2003). Administration of corticosterone by single daily injection may also decrease the normal diurnal peak by negative feedback (Gomez \textit{et al.}, 1998). Thus, in each of the different steroid treatments (Man \textit{et al.}, 2002, Leitch \textit{et al.}, 2003, and the present data) reduced 5-HT\textsubscript{1A} autoreceptor function is associated with a relative decrease in the

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It has been reported that the 5-HT$_{1A}$ receptor gene includes a GRE (Ou et al., 2001) however, ligand binding and in situ hybridization studies have shown no effect of corticosterone on either 5-HT$_{1A}$ receptor mRNA or binding to the 5-HT$_{1A}$ receptor in the DRN, even where there was evidence of functional desensitization (Laaris et al., 1995; Fairchild et al., 2003). A more likely possibility is that elements of the receptor-effector coupling downstream of the receptor protein itself are modulated by corticosterone.

Impact of corticosterone on 5-HT neuronal activity in vivo

Noradrenergic excitation mediated by $\alpha_1$-adrenoceptors is the major stimulatory influence on the spontaneous activity of 5-HT neurones in the anaesthetized rat (Baraban & Aghajanian, 1980; Gartside et al., 1997). In non-anaesthetized animals the role of $\alpha_1$-adrenoceptor mediated excitation is controversial. Thus, whilst Heym and colleagues (1984) presented data indicating that $\alpha_1$-adrenoceptors play only a small role in controlling 5-HT firing in the non-anaesthetized cat, more recently Sakai & Crochet, (2000) concluded that $\alpha_1$-adrenoceptor activation is a major regulator of 5-HT neuronal activity during quiet waking and sleep. Furthermore, microdialysis studies showing that $\alpha_1$-adrenoceptor blockade markedly reduces extracellular 5-HT in the forebrain of both non-anaesthetized (de Boer et al., 1996) and anaesthetized (Rouquier et al., 1994) rats, supports $\alpha_1$-adrenoceptor-mediated excitation being a major stimulatory influence on 5-HT activity. Taken together, these data suggest that, the alteration in sensitivity of 5-HT neurones in the DRN to $\alpha_1$-adrenoceptor-activation observed in the present study following moderate changes in corticosterone, would influence both the spontaneous firing activity of DRN 5-HT neurones and the forebrain release of 5-HT.
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The impact of changing the sensitivity to 5-HT-mediated autoinhibition is less clear since the role of autoinhibition in the physiological control of 5-HT neuronal firing is uncertain. Thus, some in vivo electrophysiological studies have reported that 5-HT1A receptor blockade increases 5-HT firing, suggesting that activation of the 5-HT1A autoreceptor plays a significant role in restraining 5-HT neuronal activity (Hajós et al., 2001; Bjorvatn et al., 1998). However, studies in anaesthetized animals have more consistently shown no effect of 5-HT1A receptor blockade (Gartside et al., 1995; Sprouse et al., 2000 but see Hajós et al., 2001). Finally, in vitro evidence from our own laboratory (Johnson et al., 2002) would argue that 5-HT1A-mediated autoinhibition does not function under conditions in which 5-HT firing is in the normal physiological range (0.5-5 Hz) and is driven exclusively by α1-adrenoceptors.

The present data indicate that moderate but persistent changes in circulating corticosterone within the normal diurnal range can significantly influence the sensitivity of neurotransmitter receptors regulating 5-HT neuronal activity, with a shift to excitatory drive at lower steroid levels. This is important because circulating levels of corticosterone show persistent changes within this range in response to psychological and physical stressors, and as a consequence of the aging process (Meaney et al., 1992). Moderate changes in circulating glucocorticoids have also been reported in several psychiatric disorders (Weber et al., 2000; Yehuda, 2002), as well as in chronic fatigue syndrome (Cleare, 2003). Thus, in post-traumatic stress disorder and chronic fatigue syndrome peak glucocorticoid levels are lowered and the rhythm flattened (Yehuda, 2002; Cleare, 2003), whilst in depression the pattern is one of flattened rhythm with raised trough (Weber et al., 2000). Given that dysfunction of
5-HT systems is implicated in mood and anxiety disorders (for reviews see Deakin, 1998; Nutt, 2002), our findings that moderate changes in glucocorticoid levels markedly alters the regulation of 5-HT neuronal activity, suggests that glucocorticoid abnormalities might in part underlie the pathophysiology of these conditions.

Acknowledgements

This work was supported by a project grant from The Wellcome Trust.
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References


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Table 1. Correlations between serum corticosterone and response to phenylephrine.

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<th>Significance of the correlation</th>
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Data are Pearson product moment ($r$) coefficients and were calculated from responses in both groups of corticosterone implanted animals (n=20).
Figure 1. Corticosterone levels in adrenalectomized rats implanted with 2 % and 70 % corticosterone pellets compared to the normal diurnal range. Bars show mean ± SEM serum corticosterone levels in trunk blood collected from 2 % (n = 11) and 70 % (n = 9) groups between 8 and 9 am on day 13-16 post-surgery. Horizontal lines and shaded areas are the mean and SEM of serum corticosterone levels in trunk blood taken from naïve animals at 8 am (AM: n = 12) and 7 pm (PM: n = 12).
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Figure 2. Basal firing rates of DRN 5-HT neurones from adrenalectomized rats implanted with 2 % and 70 % corticosterone pellets. Dots represent the basal firing rate of individual 5-HT neurones recorded from slices taken from animals implanted with 2 % (closed symbols) and 70 % (open symbols) corticosterone pellets. Neurones from the same animal are joined by a vertical line. Columns and bars show the mean + SEM group firing rates calculated from the average firing rates in individual animals in the 2 % group (shaded bar, n = 11) and the 70 % group (open bar, n = 9) (* = p<0.05, unpaired t-test).
Figure 3. Excitatory responses to phenylephrine of 5-HT neurones. (A,B) Examples of recordings showing responses to increasing concentrations of phenylephrine of individual 5-HT neurones in slices obtained from adrenalectomized rats implanted with either a 2% (A) or a 70% (B) corticosterone pellet. Basal activity was recorded...
in the continuous presence of 1 μM phenylephrine. Higher concentrations of phenylephrine (3.5 μM, 6 μM and 11 μM) were applied for periods of 2 min as indicated by the bars. Note that recordings have not been corrected for the perfusion lag (approximately 2 min). (C) Concentration-response relationships for excitatory responses to phenylephrine in neurones from animals implanted with a 2 % pellet (closed symbols, n = 11) and 70% pellet (open symbols, n = 9). Note the responses to phenylephrine are expressed as absolute firing rate (Hz) as firing is believed to be almost entirely α1-adrenoceptor dependent (VanderMaelen & Aghajanian, 1983). Data shown are mean ± SEM. (D) Correlation between serum corticosterone and firing rate following application of 6 μM phenylephrine in neurones from animals implanted with 2 % (closed symbols) and 70 % (open symbols) corticosterone pellets. The regression line (y=1.8-0.0075x) is also shown. See Table 1 for statistical analysis.
Figure 4. Inhibitory responses of 5-HT neurones to 5-HT (A,B) Examples of recordings showing inhibitory responses to increasing concentrations of 5-HT in individual neurones recorded from slices taken from adrenalectomized rats implanted with either a 2% (A) or a 70% (B) corticosterone pellet. Recordings were made in the continuous presence of 1 μM phenylephrine and increasing concentrations of 5-
HT (10 μM, 25 μM and 50 μM) were applied for 2 min as indicated by the bars. Note that the recordings have not been corrected for the lag-time of the perfusion system (approximately 2 min). (C) Concentration-dependent inhibitory responses to 5-HT in neurones from animals implanted with a 2 % corticosterone pellet (open symbols, n = 11) and a 70 % pellet (closed symbols, n = 9). Data shown are mean ± SEM.