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Activation of lumbosacral 5-HT_{2C} receptors induces bursts of rhythmic activity in sympathetic nerves to the vas deferens in male rats

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1 We previously demonstrated that *p*-chloroamphetamine (PCA) intravenously (i.v.) evokes a specific patterned bursting response in the vas deferens nerve (VDN) of anaesthetised male rats that is associated with contraction of the vas deferens, and ejaculation and contraction of the bulbospongiosus muscles. The present study used selective 5-HT agonists to induce similar rhythmic bursting responses in the VDN in order to reveal the 5-HT receptor subtypes involved.

2 The 5-HT_{2C} receptor agonist (1.0 mg kg^{-1} Ro600175 i.v.) evoked the characteristic bursting pattern responses in the VDN. The 5-HT_{1A} receptor agonist (1.0 mg kg^{-1} 8-OH-DPAT i.v.) failed to elicit any responses. However, 8-OH-DPAT coadministered in combination with Ro600175 induced a potentiation of the responses.

3 Responses were also evoked in rats with a mid-thoracic spinalisation, with a more predictable response being observed following the combination of agonists. This suggests an action of both agonists in the lumbosacral spinal cord.

4 Responses were blocked by 0.5 mg kg^{-1} SB206553 i.v. (5-$HT_{2B/C}$ receptor antagonist) or 0.5 mg kg^{-1} WAY100635 i.v. (5-HT_{1A} receptor antagonist), but not 0.1 or 1.0 mg kg^{-1} SB269970 i.v. (5-HT_7 receptor antagonist).

5 We suggest that activation of 5-HT_{2C} and 5-HT_{1A} receptor subtypes synergistically elicits contraction of the vas deferens through the activation of sympathetic preganglionic neurones in the spinal cord.

6 These data support the idea of a proejaculatory action of 5-HT_{2C} receptors in the lumbosacral spinal cord, suggesting a descending 5-HT excitatory pathway in addition to a 5-HT inhibitory pathway. An excitatory action of 8-OH-DPAT at lumbosacral sites is also evident.
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Abbreviations: o.d., outside diameter; PCA, *p*-chloroamphetamine; PCPA, *p*-chlorophenylalanine; VDN, vas deferens nerve

Introduction

We have previously described and characterised a series of responses associated with emission and ejaculation evoked by an intravenous administration of *p*-chloroamphetamine (PCA) (Stafford *et al*., 2003; 2006a). These in part consisted of a highly repeatable bursting pattern of intense sympathetic activity in the vas deferens nerve (VDN), a branch of the hypogastric nerve innervating the vas deferens. The responses were shown to be linked to emission and always associated with other ejaculatory events such as rhythmic contraction of the bulbospongiosus muscles and expulsion of seminal fluid, events occurring during sexual behaviour. These proejaculatory actions of PCA were not unexpected as several groups have reported that PCA given either intravenously (i.v.) or intraperitoneally can evoke the motor responses associated with sexual behaviour (Humphries *et al*., 1980; 1981; Rényi, 1985; Yonezawa *et al*., 2000). The novelty of our previous investigation was the characterisation of robust measurable responses in a sympathetic nerve that could be studied in an anaesthetised animal. In order to further investigate the central regulation of emission and ejaculation, we are interested in determining the neurotransmitters and receptor subtypes involved in generating this response. Here this model has now been used to address the question of how PCA could have induced the increased patterned activity in the VDN.

PCA interacts with 5-HT transporters, preventing reuptake of 5-HT into the axon terminal, and inhibits tryptophan hydroxylase, an enzyme used in the degradation of 5-HT (Gobbi *et al*., 2002), causing an acute rise in the extracellular concentration of 5-HT. It has been reported that at high concentrations PCA also causes the release of the catecholamines: noradrenaline and dopamine (Steranka & Sanders-Bush, 1977). We can therefore presume that the ejaculatory responses triggered by PCA may be as a result of activation of a certain receptor, or receptors, belonging to the 5-HT, dopaminergic or noradrenergic families.

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However, several studies into the action of PCA-induced ejaculation have suggested its main influence is *via* a 5-HT effect (Humphries *et al*., 1980; Renyi, 1985; Yonezawa *et al*., 2000). Pretreatment of rats using *p*-chlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, completely abolished ejaculations in both the conscious and urethane-anaesthetised rats (Humphries *et al*., 1980; Rényi, 1985; Yonezawa *et al*., 2000). Additionally, several 5-HT receptor antagonists (metitepine, methergoline, kentanserin and pirenperone) significantly reduced ejaculate weight (Rényi, 1985).

Our knowledge of the membrane receptor mediating the actions of 5-HT on the central pathways controlling ejaculation is presently unclear. On the one hand, a 5-HT$_2$ receptor mediation is suggested by showing that 5-methoxy-dimethyltryptamine, a 5-HT$_2$ receptor agonist evokes ejaculatory responses in the absence of PCA, with similar characteristics to the PCA-induced ejaculations (Renyi, 1986a, b). 5-HT$_2$ receptor activation has also been reported to cause seminal emission in *ex-copula* reflex tests (Mas *et al*., 1985). In addition to effects upon ejaculation, 5-HT$_2$ receptors, in particular 5-HT$_{2C}$ receptors, have been implicated in the regulation and elicitation of erection in the rat (Steers & De Groat, 1989; Berendsen *et al*., 1990; Millan *et al*., 1997). Furthermore, it may be significant that *m*-chlorophenylpiperazine (a 5-HT$_2$ agonist) induces responses in the cavernous nerve associated with erection (Steers & De Groat, 1989), which upon close inspection are very similar to the PCA responses in the VDN previously reported by us (Stafford *et al*., 2003; 2006a).

On the other hand, 5-HT$_{1A}$ receptor activation by 8-OH-DPAT significantly reduced ejaculatory latency during copulation and restored copulatory efficiency in castrated rats (Ahlenius *et al*., 1981). The facilitatory effects of 8-OH-DPAT on ejaculation *in-copula* were later shown to be blocked by pindolol (Ahlenius & Larsson, 1989), and by the more selective 5-HT$_{1A}$ receptor antagonists WAY100635 (Ahlenius & Larsson, 1998; Carro-Juárez & Rodríguez-Manzo, 2001) or NAD-299 (Hillegaart & Ahlenius, 1998). Administration of 8-OH-DPAT has also been shown to reverse coital reflex exhaustion *ex-copula* (Carro-Juárez & Rodríguez-Manzo, 2001) and decrease the ejaculation latency in 'sluggish' ejaculators (Sura *et al*., 2001; Pattij *et al*., 2003). However, a 5-HT receptor effect of 8-OH-DPAT is not without question as it has been reported to increase dopaminergic transmission in the medial preoptic area of the brain by activation of D$_2$-like receptors (Lorrain *et al*., 1998; Matuszewich *et al*., 1999; Clement *et al*., 2006), which we have recently shown can induce rhythmic activity in the VDN (Stafford & Coote, 2006b).

Nonetheless, 5-HT appears to be able to initiate or facilitate copulation and ejaculation *via* two 5-HT receptor subtypes. How this might be is so far not explained. It may be that the 5-HT receptor subtypes are on different neurones in the central sympathetic network controlling ejaculation as such studies do not reveal where in the CNS PCA or 5-HT agonists and antagonists are acting. Additionally, the ejaculatory response to agonists may be secondary to other components of sexual processes and the methods used to date would not reveal this. This critisism can be overcome by the use of the preparation we have described (Stafford *et al*., 2006a) in which recordings are made from the VDN in anaesthetised rats with intact CNS and in which the lumbosacral spinal cord can be isolated by transection. Furthermore, the preparation provides predictable precisely measurable responses. Therefore, in this investigation, experiments were performed using selective 5-HT receptor agonists and antagonists that mimic the responses to PCA, with the aim of clarifying the roles of different receptor subtypes in the emission phase of ejaculation in rats.

Methods

The methods used here are similar to those previously described for PCA experiments (Stafford *et al*., 2003; 2006a, c) and are briefly described here.

Animal preparation

In a total of 48 male Wistar rats, weighing 280–320 g, anaesthesia was induced using gaseous 5% enflurane, 95% oxygen. The right femoral vein was cannulated using 0.96 mm (outside diameter (o.d.)) polythene tubing. Urethane was then administered as required to invoke and sustain deep anaesthesia (1.75–2.25 g kg^{-1} i.v.). The right femoral artery was cannulated using 0.96 mm (o.d.) polythene tubing filled with heparinised saline (20 U ml^{-1}). The arterial cannula was connected to a pressure transducer (Capto SP 844, ADInstruments, Oxfordshire, U.K.), Bridge Amp and Powerlab (ADInstruments) to monitor and record blood pressure and heart rate using Chart v4.1.1 (ADInstruments). Blood pressure and heart rate of rats (except spinal rats, see below) before drug adminstration were 114 ± 4 mmHg and 416 ± 4 beats min^{-1}. The trachea was intubated using a shortened 6F (yellow) luer cannula (Portexn Ltd, Kent, U.K.) to maintain a clear airway. The animal was placed on a heating blanket (Harvard Instruments), and temperature monitored using a rectal probe, to maintain temperature at 37°C.

A ventral midline incision was made from the base of the sternum as far as the corpus penis, to access the abdomen. The abdomen was held open by securing the abdominal wall to a metal oval frame. The colon, ileum, seminal vesicles and bladder were secured aside in order to clearly access the VDN. The nerve was dissected away from the surrounding tissue and mounted onto 0.35 mm silver bipolar hook electrodes and the abdominal cavity filled with warm (37°C) liquid paraffin to provide electrical isolation and prevent the nerve from drying out.

VDN activity was recorded, amplified (Neurolog, Digitimer Ltd, Welwyn Garden City, U.K.), filtered at 50 Hz low frequency and 3 kHz high frequency (Neurolog, Digitimer Ltd) and displayed on an oscilloscope (Gould, Ilford, U.K.). The signal was also passed through a Powerlab 8SP system (ADInstruments) for analysis on a Power Mac G4 using Chart v4.1.1 (ADInstruments). Raw nerve activity was converted to firing frequency using a window discriminator in the Chart software set to remove electrical noise, which was confirmed by nerve crush at the end of experiment. All recordings were conducted with the rat in the supine position. Animals were left undisturbed for around 1 h before the administration of compounds.

Animal groups

The following three groups of animals were prepared: the first to investigate the effect of 5-HT$_{2C}$ and 5-HT$_{1A}$ receptor activation in the CNS-intact rat, the second to confirm the

receptor subtypes using antagonists, and the third to investigate the CNS site of action of the agonists.

Group 1a: Effect of 5-HT$_{2C}$ receptor agonists

In four animals, an administration of 1.0 mg kg^{-1} Ro600175 i.v. was given while recording nerve activity in the VDN. A period of 1 h after the initial dose was left before a subsequent administration of the same dose.

Group 1b: Effect of 5-HT$_{1A}$ receptor agonist

In four animals, 1.0 mg kg^{-1} 8-OH-DPAT i.v. was administered while recording VDN activity. A second administration of the same dose was given 1 h after the initial administration.

Group 1c: Effect of combination of receptor agonists

A cocktail containing an equal concentration of 8-OH-DPAT and Ro600175 was prepared. In 12 animals, this cocktail was administered i.v. *via* the femoral vein. Doses (1.0 ml kg^{-1}) containing either 0.1, 0.5 or 1.0 mg ml^{-1} of each agonist were administered to four separate animals while recording VDN activity. A period of 1 h after the initial dose was left before a subsequent administration of the combination of agonists at the same dose.

Group 2: Preadministration of antagonists

In each of four animals (12 in total), 0.5 mg kg^{-1} SB206553 (a 5-HT$_{2B/C}$ antagonist), 0.1 mg kg^{-1} WAY100635 (a 5-HT$_{1A}$ antagonist) or 0.1 mg kg^{-1} SB269970 (a 5-HT$_7$ antagonist) was administered i.v., 2 min before the administration of the combination of 0.5 mg kg^{-1} 8-OH-DPAT and 0.5 mg kg^{-1} Ro600175. Two hours later, the agonist combination was administered again but without further administration of the antagonists to test for recovery. The exception was rats given SB269970, in which the second cocktail dose was preceded by a higher dose of 1.0 mg kg^{-1} SB269970. VDN activity was recorded as for the other groups.

Group 3: Spinal animals

In 16 anaesthetised rats, following the initial placement of cannulae, a laminectomy and mid-thoracic (T8/9) complete spinal cord section was performed. Separation was ensured by placing small ($\sim$1 mm) cotton wool balls between the cut ends. Blood pressure and heart rate during this period was 91 ± 4 mmHg and 385 ± 12 beats min^{-1}. Two or more hours later, recordings of VDN activity were commenced and the experiment was then carried out as for the CNS-intact animals measuring VDN activity after a single dose of either 1.0 mg kg^{-1} 8-OH-DPAT ($n=4$ rats), 1.0 mg kg^{-1} Ro600175 ($n=4$ rats), the combination of 0.5 mg kg^{-1} 8-OH-DPAT and 0.5 mg kg^{-1} Ro600175 ($n=4$ rats) or the combination of 1.0 mg kg^{-1} 8-OH-DPAT and 1.0 mg kg^{-1} Ro600175 ($n=4$ rats). A subsequent administration at the same dose was given 1 h after the initial administration.

All experiments conformed to the U.K. Animals Scientific Procedures Act 1986. Rats were killed at the end of the experiments using an overdose of anaesthetic.

Compounds

All compounds were obtained from Tocris (Bristol, U.K.), dissolved in physiological saline (0.9% (w v^{-1})) and administered i.v. into the femoral vein at a volume of 1.0 ml kg^{-1} and washed in with 0.1 ml saline.

Statistical analysis

Data are presented as mean $\pm$ s.e.m. Comparisons were made at the 5% significance level using: a one-way ANOVA with Bonferroni's *post hoc* test for dose comparisons of group 1 animals, a paired *t*-test for comparisons between first and second administrations in all groups, and an unpaired *t*-test for other comparisons. Where no responses were obtained in any of the animals, comparisons of numbers of responses were made using a one-sample *t*-test to a hypothetical value of zero.

Results

Group 1a: Effect of 5-HT$_{2C}$ receptor agonist

Following the initial preparation, the animal was left undisturbed for up to 1 h during which the baseline VDN activity remained stable. Administration of Ro600175 (1.0 mg kg^{-1} i.v.) caused a slow increase in baseline activity after a delay of 1–2 min, which culminated in a sequence of up to six intense synchronised bursts of activity, before decreasing rapidly to a tonic plateau level of 36 ± 22 spikes s^{-1} above baseline (Figure 1a). In two rats, burst pattern responses in the VDN occurred following the first administration of Ro600175, while in one of these two rats and a third rat a response was observed following the second administration given 1 h after the first.

The injection of the 5-HT$_{2C}$ receptor agonist caused an immediate rapid and transient increase in blood pressure of 33 ± 4 mmHg and heart rate of 37 ± 8 beats min^{-1}, which were not associated with any alterations in VDN activity.

Group 1b: Effect of 5-HT$_{1A}$ receptor agonist

In four rats, administration of 8-OH-DPAT (1.0 mg kg^{-1} i.v.) increased tonic activity in VDN to around 13 ± 3 spikes s^{-1} above baseline. However, in three animals neither a first or second administration of the agonist resulted in a typical sequence of synchronised bursting activity like that observed with Ro600175 (Figure 1b). In the fourth rat, two such responses were elicited but only after a second dose of 8-OH-DPAT.

The administration of 8-OH-DPAT caused a rapid and transient decrease of blood pressure of 34 ± 4 mmHg and increase of heart rate of 67 ± 20 beats min^{-1}. These changes were not associated with any alterations in VDN activity.

Group 1c: Effect of combination of receptor agonists

Combining the agonists in a 'cocktail' in doses of 0.1, 0.5 or 1.0 mg kg^{-1} for each agonist resulted in robust generation of repeated burst pattern responses (Figure 1c), so that the number of responses per drug treatment was markedly increased (Figure 2a). The mean number of responses to a

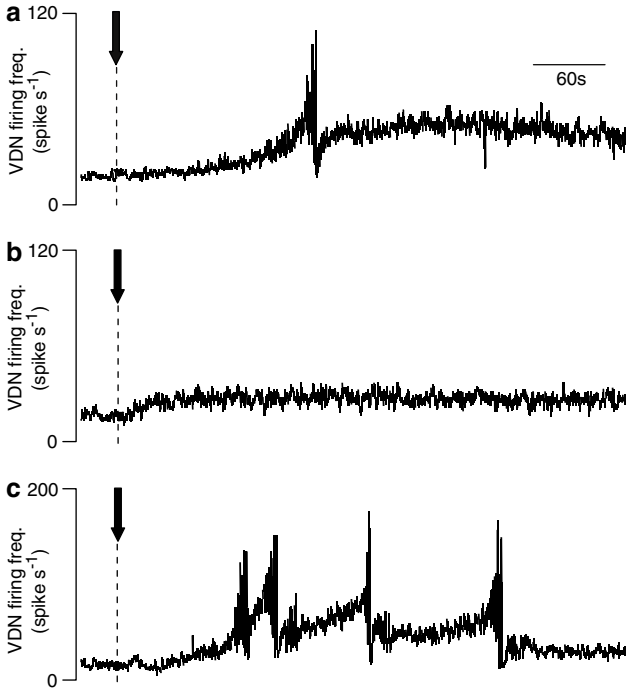

Figure 1 Effects of 8-OH-DPAT and Ro600175 on VDN activity in CNS-intact rats. Traces show VDN firing frequency following administration of: (a) 1.0 mg kg^{-1} Ro600175 alone, (b) 1.0 mg kg^{-1} 8-OH-DPAT alone and (c) 1.0 8-OH-DPAT and 1.0 mg kg^{-1} Ro600175 in combination. Within each response, one shown in (a) and four in (c), there are repetitive intense bursts of increased activity. Traces show recordings from separate rats. Compounds administered at time indicated by arrows.

cocktail of 1.0 mg kg^{-1} of each agonist was 5.0±0.9, which was significantly increased ($P<0.001$) compared to the effect of each agonist alone. Rhythmic contractions of the striated pelvic muscles (bulbospongiosus) could be observed simultaneously with responses in the VDN. Forceful emission of fluid from the penis was also seen.

The number of burst pattern responses was dose-dependent, significantly increasing from 1.5±0.9 to 5.0±0.7 and 5.0±0.9, respectively ($P=0.0233$; Figure 3a). Furthermore, the latency from administration to the responses decreased from 357±22 s at the lowest dose to 64±22 and 50±4 s, respectively at the higher doses ($P<0.0001$; Figure 3b). Also, postdrug VDN firing frequency increased above baseline from 5.1±0.6 to 21.2±8.5 and 50.8±20.8 spikes s^{-1} following each dose of cocktail however these increases did not reach statistical significance ($P=0.0901$). In addition to this, the average maximum burst frequency during ejaculatory-like responses also increased from 46.5±7.6 to 92.9±25.1 and 125.1±35.8 spikes s^{-1}, but despite the clear trend they were not significant ($P=0.3398$; Figure 3c). Other measured variables of the responses remained unchanged between doses as follows: number of bursts of high-frequency activity within responses (3.0±0.1 bursts response^{-1}; $P=0.7454$), frequency of bursts within responses (0.44±0.01 bursts s^{-1}; $P=0.7530$) and total duration of the responses ($P=0.2184$; Figure 3d). A second administration of the agonist cocktail (at the same dose) evoked further responses, which remained unchanged from the first administration.

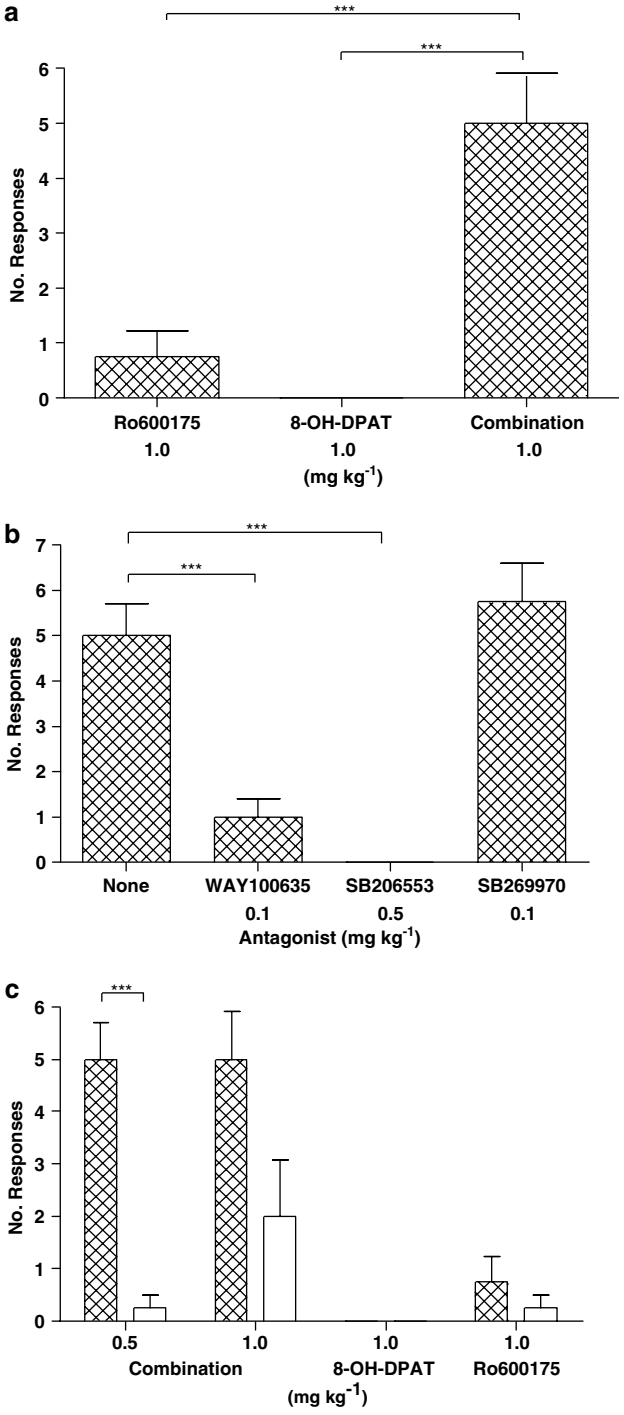

Figure 2 Number of burst pattern responses elicited following administration of 5-HT receptor agonists and antagonists. (a) Effect of 1.0 mg kg^{-1} Ro600175, 1.0 mg kg^{-1} 8-OH-DPAT or combination of both at 1.0 mg kg^{-1} each in the CNS-intact animal. (b) Effect of combination of agonists (0.5 mg kg^{-1} of each agonist) in the absence of antagonists (none) compared to the effect of agonist combination following preadministration of 0.1 mg kg^{-1} WAY100635, 0.5 SB206553 or 0.1 mg kg^{-1} SB269970. (c) Effect of spinal transection at T8/9 (open columns) on number of responses evoked by the combination at two doses (0.5 mg kg^{-1} or 1.0 mg kg^{-1}) or single dose of either agonist at 1.0 mg kg^{-1} compared to the CNS-intact animal (filled columns). ***$P<0.001$ using the unpaired Student's t-test. $n=4$ for all groups.

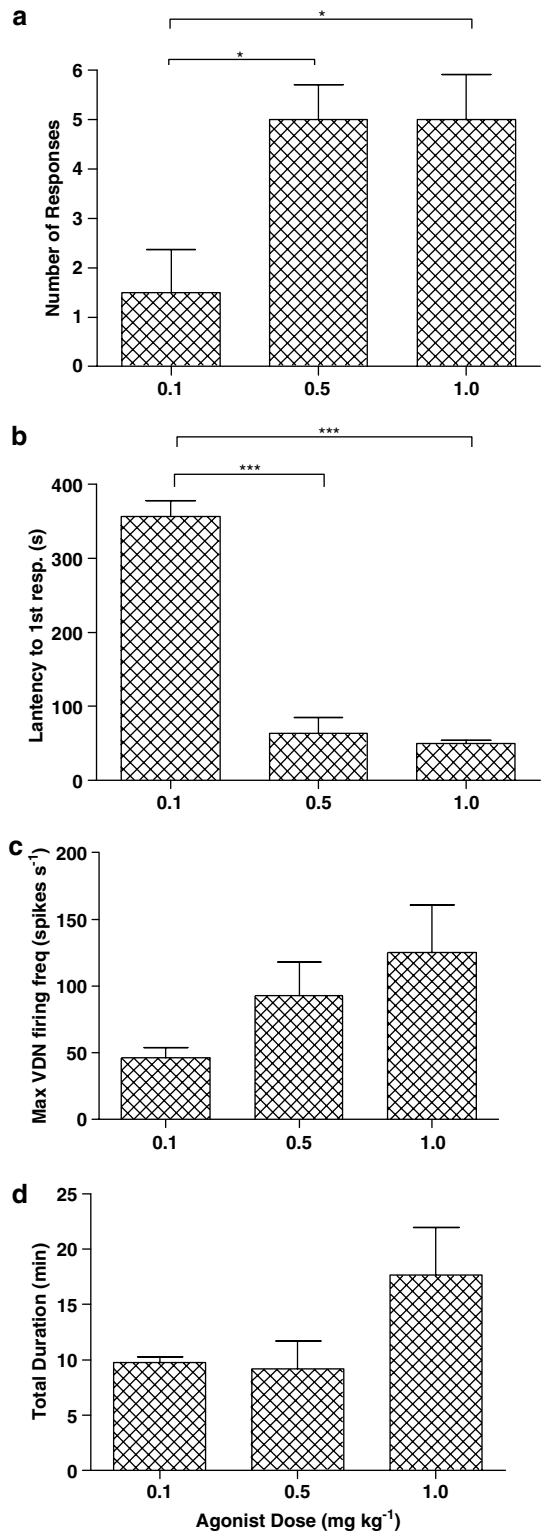

Figure 3 Effect of agonist combination on characteristics of burst pattern responses. Effect of increasing doses of agonists on: (a) number of burst pattern responses after a single administration, (b) latency to initiation of burst pattern responses, measured as the time from administration to the first burst of the initial response, (c) mean maximum VDN firing frequency during synchronous bursts and (d) total duration of responses, measured as the time from administration to the end of the final PCA response. $*P<0.05$, $**P<0.01$, $***P<0.001$. $n=4$ for all groups.

Blood pressure fluctuated (rises and falls) by approximately 10 mmHg over a period of up to 60 s immediately following the administration of the cocktail. Thereafter, it returned to baseline and remained stable during the period when bursts of activity appeared in the VDN.

Group 2: Preadministration of antagonists

5-HT$_{2B/C}$ antagonist The combination of 8-OH-DPAT and Ro600175 (both at 0.5 mg kg^{-1}) failed to elicit any burst pattern responses in the four animals that were pretreated with 0.5 mg kg^{-1} SB206553 (5-HT$_{2B/C}$ antagonist; Figure 2c; $P=0.0058$ compared to agonist combination alone). Responses returned following a subsequent administration of the agonist combination given 2 h later.

5-HT$_{1A}$ antagonist Burst pattern responses were observed following the combination of agonists (0.5 mg kg^{-1} 8-OH-DPAT and 0.5 mg kg^{-1} Ro600175) in three out of four animals pretreated with WAY100635 (0.1 mg kg^{-1}; 5-HT$_{1A}$ antagonist). This reduced the mean number of burst pattern responses to 1.0 ± 0.4 (Figure 2c; $P=0.0027$), compared to the animals given the cocktail alone.

5-HT$_7$ antagonist Several burst pattern responses were observed following the combination of agonists (0.5 mg kg^{-1} 8-OH-DPAT and 0.5 mg kg^{-1} Ro600175) in all four animals pretreated with SB269970 (0.1 mg kg^{-1}; 5-HT$_7$ antagonist). A subsequent higher dose of SB269970 (1.0 mg kg^{-1}) also failed to prevent the elicitation of burst pattern responses following a second dose of the cocktail. The mean number of responses was 5.75 ± 0.85 after the low dose and 4.75 ± 1.11 after the high dose of SB269970, which were not significantly different compared to the first or second administration of combination alone ($P=0.5239$ and $P=0.0683$ respectively).

Group 3: Spinal animals

In four rats given Ro600175 (1.0 mg kg^{-1} i.v.), VDN tonic activity showed an increase of 31 ± 17 spikes s^{-1}, but only in one rat was there a single burst pattern response, which occurred after the first administration (Figure 2b).

8-OH-DPAT (1.0 mg kg^{-1} i.v.) failed to elicit any increase in VDN activity or bursting pattern responses in any of the four spinal rats (Figure 2b).

In the group of four spinal rats given the middle dose of combination of the two agonists (0.5 mg kg^{-1} i.v. for both), there was an increase in baseline tonic activity. This was measured at 37 ± 3 spikes s^{-1} following the first dose and 31 ± 9 spikes s^{-1} after the second dose. However, burst pattern responses were only elicited by the combination of agonists in one of these rats. In this animal, one burst pattern response occurred after the first dose and two such responses after a second dose. Thus, significantly fewer responses were observed in the spinal rat than the CNS-intact rat at this dose ($P=0.0007$; Figure 2b).

In a further four spinal rats, the effect of a higher dose of Ro600175 and 8-OH-DPAT (both at 1.0 mg kg^{-1} i.v.) was studied. Burst pattern responses were observed after both doses of agonists in three out of four rats. This gave a mean of 2.0 ± 1.1 responses following the first dose and 2.8 ± 1.4 following the second (Figure 2b). This was not significantly

different to the number obtained from the CNS-intact rats ($P=0.0781$).

The characteristics of the responses (i.e. number of synchronous bursts, frequency of synchronous bursts, maximum VDN firing frequency, latency to and duration of responses) elicited in the spinal rats were not significantly different to those observed in the CNS-intact rats at the same dose.

Discussion

These experiments on anaesthetised rats have shown that the 5-HT$_{2C}$ receptor agonist Ro600175 given intravenously can elicit burst pattern responses in the sympathetic nerve to the VDN. These intense bursts of nerve activity have all the characteristics of responses in the VDN that are elicited by PCA (i.v.), which we have previously shown are owing to an action in the central nervous system and strongly associated with contractions of the vas deferens, ejection of a seminal plug and rhythmic contraction of the bulbospongiosus muscles (Stafford *et al*., 2003; 2006a). It therefore seems reasonable to conclude that a 5-HT pathway in the CNS has an excitatory effect on sympathetic circuits in the CNS that are concerned with emission mediated by 5-HT$_{2C}$-like receptors. As this effect is still present in the spinal rat, although less robust, there is a strong likelihood that the 5-HT$_{2C}$ agonist was mimicking that action of supraspinal 5-HT terminals synapsing directly on sympathetic preganglionic neurones projecting to the vas deferens and/or the spinal neuronal network controlling their excitability. A direct effect on the sympathetic neurones is supported as there is abundant evidence from extracellular and intracellular recordings that sympathetic preganglionic neurones are directly excited by 5-HT and this is *via* a 5-HT$_2$-like receptor (McCall *et al*., 1987; Coote 1988; Ramage, 1988; Clement & McCall, 1990). However, the lowered probability of evoking the response in the spinal animal suggests this alone may not be sufficient to cause a relatively synchronised intense activation of the VDN neuronal population.

In the rat with intact CNS, the burst pattern response in the VDN became more robust, being more predictable and more numerous, when Ro600175 was combined with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. This is not too unlike the effect on ejaculation of 8-OH-DPAT observed *in-copula*, which shows that activation of 5-HT$_{1A}$ receptors enhances the triggering of ejaculation induced by afferent stimulation (Ahlenius *et al*., 1981). Thus, together these studies support the idea that activation of 5-HT$_{1A}$ receptors can increase the excitability of neuronal networks involved in emission and ejaculation. The clear increase in predictability of evoking a burst pattern response in the spinal animal when 8-OH-DPAT was combined with Ro600175 suggests that part of this facilitatory action is occurring on intraspinal networks.

The effects of 5-HT$_2$ receptor activation upon ejaculation have previously been disputed, with reports of inhibition (Foreman *et al*., 1989; Watson & Gorzalka, 1991) and facilitation of ejaculation (Mas *et al*., 1985). Ro600175 is a centrally active 5-HT$_{2C}$ agonist with over 150-fold selectivity over 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors ($pK_i=8.8$, 6.0 and 5.8, respectively) and over a thousand-fold selectivity over other 5-HT receptor subtypes (Martin *et al*., 1998). The robustness of the responses to Ro600175 at a dose of 1.0 mg kg^{-1} strongly indicates that 5-HT$_{2C}$ receptors were activated rather than any other 5-HT receptor subtype. This was confirmed by using SB206553, a centrally active 5-HT$_{2B/C}$ antagonist (Kennett *et al*., 1996), which completely abolishes the PCA-like responses. This present report is the first detailing the use of selective 5-HT$_{2C}$ receptor activation to investigate the control of emission. The results clearly demonstrate the participation of 5-HT$_{2C}$ receptors in an *ex-copula* model. Furthermore, it is evident that Ro600175 has an action upon lumbosacral 5-HT$_{2C}$ receptors as the response still occurs in the spinal animal.

Bancila *et al*. (1999) conducted immunocytochemical studies into the location of 5-HT$_{2C}$ receptors within the spinal cord, with particular reference to neurones involved in the control of penile erection. A high level of 5-HT$_{2C}$-labelled neurones were identified in the dorsal horn neurones of L5-S1, in particular in areas that receive primary afferents from dorsal penile nerve. This suggests that activation of these receptors could modulate the transmission of sensory information from these primary afferents. It is possible that a proportion of the receptors identified by Bancila *et al*. (1999) also influence the processes involved in ejaculation in addition to erection, although in our study we did not test the ejaculatory response to sensory afferent stimulation.

8-OH-DPAT is a 5-HT$_{1A}$ receptor agonist with moderate affinity for 5-HT$_7$ receptors (Middlemiss & Fozard, 1983; Plassat *et al*., 1993). In the experiments presented here antagonism of the responses was achieved using a moderate dose of WAY100635, a selective 5-HT$_{1A}$ antagonist, at a similar dose to that which has previously been demonstrated to antagonise 8-OH-DPAT-induced facilitation of ejaculation (Ahlenius & Larsson, 1997). The antagonism by WAY 100635 would appear to diminish the possibility that the effect of 8-OH-DPAT was mediated by supraspinal dopamine D$_2$-like receptors, which we have recently shown can initiate the responses in the VDN (Stafford & Coote, 2006b). However, we cannot rule out the possibility for some of the enhancement by 8-OH-DPAT being mediated *via* supraspinal D$_2$ receptors, as when it is given intracerebroventricularly, it elicits rhythmic contractions of the bulbospongiosus muscles, a marker of the expulsion phase of ejaculation (Clement *et al*., 2006).

It is possible that activation of 5-HT$_7$ receptors contributes to the excitatory action of both 8-OH-DPAT and Ro600175, as both compounds do have affinity for 5-HT$_7$ receptors ($pK_i=6.6$ and 5.6, respectively). However, the selectivity of the agonists and antagonists used in these studies suggests that the effects are primarily mediated through 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors. Nonetheless, the involvement of 5-HT$_7$ receptors was directly investigated by using a preadministration of SB269970, a 5-HT$_7$ antagonist. This compound failed to prevent the elicitation, or reduce the number, of PCA-like responses at the two doses tested, suggesting that 5-HT$_7$ receptors are not involved in the generation of these responses. Therefore, we conclude that the effects of 8-OH-DPAT are primarily mediated *via* 5-HT$_{1A}$ receptors.

Complete antagonism of the generation of VDN burst responses is not achieved using WAY100635. This result is expected as administration of Ro600175 alone, in the absence of any antagonist, induces several responses. This suggests that the few VDN responses evoked by Ro600175 observed following pretreatment with WAY100635 are primarily a result of activation of 5-HT$_{2C}$ receptors. Furthermore, the results presented here demonstrate that activation of 5-HT$_{1A}$ recep-

tors by administration of 8-OH-DPAT does not normally induce emission responses in VDN, but rather causes a synergistic facilitation of the excitatory effects of 5-HT$_{2C}$ activation by Ro600175 as described above. Indeed, antagonism of 5-HT$_{2C}$ receptors completely prevented the elicitation of any VDN burst responses. These data indicate the existence of an excitatory descending pathway acting on postsynaptic 5-HT$_{2C}$ receptors. Activation of such lumbosacral receptors causes an excitement of the neuronal network and if the trigger threshold is met, activation of a pattern generator for emission and possibly ejaculation.

Fewer responses were observed in the spinal animal than in the CNS-intact animal using the same dose of the combination of agonists. This may be owing to actions of one or both agonists at supraspinal sites, which are lost following spinal transection, in addition to the proposed lumbosacral actions. An alternative explanation is that a supraspinal excitatory reflex loop is lost following spinal transection, as previously suggested by Hubscher & Johnson (1999; 2000), and is well established to occur in the micturition reflex (de Groat *et al*., 1981).

We suggest that in the CNS-intact rat 8-OH-DPAT primarily suppresses the previously documented tonic serotonergic inhibitory pathway (Marson & McKenna, 1992; 1994) by activation of somatodendritic 5-HT$_{1A}$ autoreceptors. There is substantial evidence from previous investigations to support this. In the spinal animal, this tonic descending inhibition is no longer present as the pathways have been disrupted, so it would be expected that 8-OH-DPAT would no longer exert its facilitatory effects to the same degree. However, 8-OH-DPAT did have a facilitatory action in combination with Ro600175 in the spinal rat. This surprising result may be difficult to explain with current understanding. One possible explanation to consider is a 5-HT$_{1A}$-mediated inhibition of glycine interneurones, which, at least in spinal cord slices *in vitro*, keep up a constant barrage of inhibitory post-synaptic potentials (IPSPs) on the membrane of sympathetic preganglionic neurones (Miyazaki *et al*., 1989; Lewis & Coote, 1990). An alternative explanation may be that, at the concentrations used, 8-OH-DPAT activates postsynaptic D$_2$-like receptors in the spinal cord, although the effect of spinal dopamine receptor activation on sexual reflexes is currently unclear.

Summary

The anaesthetised rat model established previously by us to measure ejaculatory-like neural responses in a nerve to the vas deferens has been used to examine the activation of specific 5-HT receptors. The main findings of these studies are that activation of lumbosacral 5-HT$_{2C}$ receptors in both intact and spinal rats can evoke emission associated with ejaculatory-like responses indicative of sexual responses in the male rat. This suggests a descending excitatory role for 5-HT systems in addition to the well-documented inhibitory pathways. This 5-HT$_{2C}$-mediated response is synergistically potentiated by coactivation of 5-HT$_{1A}$ receptors in CNS-intact rats. The new findings also suggest that part of this enhancement is at the level of a lumbosacral pattern generator.

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