Characterization of the 5-HT receptor subtypes involved in the motor behaviours produced by intrathecal administration of 5-HT agonists in rats

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1 The motor behavioural effects of intrathecal injections of 5-hydroxytryptamine (5-HT) and a variety of 5-HT receptor agonists were examined in adult Wistar rats to establish; (a) which 5-HT receptor subtype/s elicit each behaviour and (b) whether these receptors are located within the spinal cord.

2 Intrathecal injection of 5-methoxy-*N,N'*-dimethyltryptamine (5-MeODMT), ($\pm$)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) or 2,5-dimethoxy- α ,4-dimethylbenzene ethamine hydrochloride (DOM) produced dose-related back muscle contractions (BMC) and wet dog shakes (WDS) which were both markedly attenuated by intraperitoneal pretreatment with either ritanserin (1 mg kg^{-1}), ketanserin (0.16 mg kg^{-1}) or mianserin (0.6 mg kg^{-1}) indicating the involvement of 5-HT₂ receptors in both these motor behaviours. Both fluoxetine ($1\text{--}20 \text{ mg kg}^{-1}$, i.p.) and high doses of 5-HT ($50 \mu\text{g}$) following fluoxetine (5 mg kg^{-1} , i.p.) also elicited BMC, further confirming the involvement of 5-HT in this behaviour.

3 Intrathecal 5-carboxamidotryptamine (5-CT) evoked a marked wet-dog shake response without producing any BMC. Intrathecal pretreatment with 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) enhanced, while in contrast 2-methyl-5-HT pretreatment attenuated, 5-HT agonist-induced BMC without affecting WDS. These data suggest that the spinal 5-HT₂ receptors mediating BMC are positively modulated by 5-HT_{1A} but negatively influenced by 5-HT₃ receptor activation and may be of a different subtype to the supra-spinal 5-HT₂ receptors which elicit WDS.

4 A contrast, reciprocal forepaw treading, lateral head weaving, flat body posture and Straub-tail were evoked by 5-MeODMT, 8-OH-DPAT or 5-CT but not by DOI or DOM indicating that these behaviours were not produced by 5-HT₂ receptor activation alone. Ritanserin (1 mg kg^{-1} , i.p.) or ketanserin (0.16 mg kg^{-1} , i.p.) pretreatment reduced the reciprocal forepaw treading induced by high intrathecal doses of either 5-MeODMT ($25 \mu\text{g}$) or 5-CT ($50 \mu\text{g}$) suggesting that this behaviour may be facilitated by 5-HT₂ receptor activation.

5 Intrathecal injection of 5-HT ($0.05\text{--}50 \mu\text{g}$, after systemic fluoxetine, 5 mg kg^{-1} , i.p.), or 1-(3-chlorophenyl)piperazine (mCPP) produced dose-related forepaw-licking and grooming, neither of which were attenuated by ketanserin (0.16 mg kg^{-1} , i.p.) pretreatment suggesting these behaviours may be mediated by 5-HT_{1C} receptors. In contrast, 2-methyl-5-HT (50 and $100 \mu\text{g}$) produced sideward tail-flicks, not evoked by any other 5-HT agonist and could therefore be mediated by spinal 5-HT₃ receptor activation.

6 These data provide behavioural evidence for the existence of spinal 5-HT₂ receptors which produce a novel motor behaviour, BMC. Ligand binding studies and dose-response studies with a range of selective 5-HT antagonists are required to establish whether BMC and WDS are mediated by different subtypes of 5-HT₂ receptors.

Keywords: Bulbospinal 5-hydroxytryptaminergic neurones; 5-HT₂ receptors; 5-HT behavioural syndrome

Introduction

Within the central nervous system ligand binding studies have identified 5-HT₁, 5-HT₂, 5-HT₃ and recently 5-HT₄ binding sites, furthermore subtypes of 5-HT_{1(A to E)} and possibly 5-HT_{2(A and B)} have also been proposed (for recent reviews see Peroutka, 1988; Hartig, 1989; Frazer *et al.*, 1990; Bobker & Williams, 1990). Three of the putative 5-HT receptors appear to be linked to different second messenger systems; all the 5-HT₁ subtypes (apart from 5-HT_{1C}) are coupled to adenylate cyclase (Barbaccia *et al.*, 1983), the 5-HT₂ (and 5-HT_{1C}) to phosphoinositide hydrolysis (Conn & Sanders-Bush, 1984; 1986) and the 5-HT₃ forms an ion gating channel (Bobker & Williams, 1990), while the 5-HT₄ receptor is positively coupled to adenylate cyclase (Dumuis *et al.*, 1989). Of all these different 5-HT receptors, however, only four (5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D} and 5-HT₂) have so far been cloned (Hartig, 1989; Branchek *et al.*, 1990) so much effort is currently being made to identify functional correlates for each 5-HT receptor subtype, a problem addressed in the current behavioural study.

It is well established that bulbospinal neurones from the nuclei raphe obscurus and pallidus, which contain 5-hydroxytryptamine (5-HT), project to the ventral horn of the spinal cord (Fuxe, 1965) where many of these neurones make synaptic contact with α -motoneurones (Ulfhake *et al.*, 1987). There is considerable evidence to suggest that 5-HT released from these descending raphe neurones facilitates spinal motoneurone excitability. For example the iontophoretic application of 5-HT onto spinal motoneurones augments the effect of excitatory amino acids (White & Neuman, 1980) and stimulation of bulbospinal raphe neurones produces excitatory postsynaptic potentials in motoneurones (Fung & Barnes, 1989). In addition, Barbeau & Bédard (1981) showed the precursor to 5-HT, 5-hydroxytryptophan, augments hindlimb EMG in spinalised rats, an effect which was enhanced by destruction of bulbospinal raphe neurones with the 5-hydroxytryptaminergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT). However, the physiological role of these bulbospinal raphe neurones in the control of normal spinal motor function is poorly understood but could be more readily investigated if a motor behavioural model was available to examine the effect of 5-HT on spinal motor function in conscious animals.

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The various components of the 5-HT behavioural syndrome appear to involve activation of the caudal raphe neurones which innervate cranial and spinal motor nuclei (Jacobs, 1976; McCall & Aghajanian, 1979). However, of all the components of the 5-HT syndrome, only Straub-tail and tremor are unaffected by transverse lesions of the CNS down to the spinal cord while hindlimb abduction is abolished by spinal transection (Jacobs & Klemfuss, 1975), implying that these may be the only behaviours resulting from an action of 5-HT at the spinal level. More recent studies have shown that intrathecal administration of relatively high doses of the non-selective 5-HT agonist 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT) induce dose-related contractions of the muscles of the back in rats (Fone *et al.*, 1989). These back muscle contractions (BMC) are potentiated by intrathecal pretreatment with 5,7-DHT, as would be expected if this behaviour results from the activation of 5-HT receptors located postsynaptic in relation to bulbospinal raphe neurones (Fone *et al.*, 1989). The present study attempts to characterize more fully the 5-HT receptor subtype/s involved in the production of BMC by examining the effect of, and interaction between, a variety of 5-HT agonists given intrathecally and 5-HT antagonists administered systemically to conscious rats.

Methods

Intrathecal cannulation and behavioural monitoring

Male Wistar rats (weighing between 260 and 320 g) were maintained on a 12 h light-dark cycle and given food and water *ad libitum*. Indwelling intrathecal cannulae (7.5 cm long polyethylene tubing, 0.63 mm o.d. Portex) were implanted along the spinal subarachnoid space of rats under sodium methohexitone anaesthesia (Brietal 60 mg kg⁻¹, i.p.) as described previously (Yaksh & Rudy, 1972; Fone *et al.*, 1987). Animals were habituated to their behavioural recording chamber during the post-operative week and again for 30 min before each of a maximum of ten intrathecal injections of the selected 5HT agonist/s, which were given at three or four day intervals.

Following each intrathecal injection (10 µl washed in with 15 µl saline) the number of back muscle contractions (BMC, brief caudo-rostral contractions of the superficial muscles of the back), wet dog shakes (WDS, including head shakes) and yawns were counted separately and the time spent forepaw-licking and face washing (collectively referred to as forepaw-licking) and the total time spent grooming (including forepaw-licking) were recorded for 30 min post-injection. In addition lateral head weaving, reciprocal forepaw treading, flat body posture and Straub-tail were each scored separately on a scale of 0–3 (0 absent, 1 present < 50%, 2 present > 50% and 3 present all of the time) for 20 s once every min for 20 min (maximum score being 60 for each behaviour).

Drug administration

Dose-response studies to examine the effect of each selected 5-HT agonist were performed in eight separate groups of rats which received either, 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT, 0.1 to 100 µg, *n* = 9), 1-(3-chlorophenyl)piperazine (mCPP, 10 to 100 µg, *n* = 5), 5-MeODMT (2 to 100 µg, *n* = 8), 5-carboxamidotryptamine (5-CT, 0.1 to 100 µg, *n* = 6), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 1 to 50 µg, *n* = 6), 2,5-dimethoxy-α,4-dimethylbenzene ethamine (DOM, 2 to 25 µg, *n* = 7), 2-methyl-5-HT (2-Me-5-HT, 1 to 100 µg, *n* = 7) or 5-HT (0.5 to 200 µg, *n* = 6 given 30 min after clorgyline, 1 mg kg⁻¹ i.p. or 0.05 to 50 µg, *n* = 5 given 30 min after fluoxetine, 5 mg kg⁻¹, i.p.). Subsequent to these dose-response studies the behavioural effect of a selected dose of some of the 5-HT agonists (groups above) was re-examined following pretreatment (–30 min) with either a 5-HT₂ antagonist or another 5-HT agonist. The behavioural effects of mCPP (50 µg) and 5-HT (50 µg) were re-examined after ketan-

serin (0.16 mg kg⁻¹, i.p.), 5-MeODMT (25 µg) and 2-Me-5-HT (50 µg) after ritanserin (1 mg kg⁻¹ and 0.16 mg kg⁻¹, i.p., respectively) and DOI (10 µg) after ketanserin (0.16 mg kg⁻¹, i.p.) and mianserin (0.6 mg kg⁻¹, i.p.) while the behavioural response to 8-OH-DPAT (0.1 to 100 µg) was re-examined after pretreatment with DOI (2 µg). In two additional groups of rats the behavioural response to DOI (2, 10 and 50 µg) was recorded following intrathecal pretreatment (–30 min) with saline and either 8-OH-DPAT (2 µg, *n* = 8) or 2-Me-5-HT (2 µg, *n* = 7). In a further group of rats, BMC were counted for 30 min following repeated intrathecal injection of DOI (10 µg, *n* = 4), the second injection being 24 h after the first, followed by further twice daily injections for four days. Five rats were decapitated 9 min after intrathecal injection of a mixture of cold and [¹²⁵I]-DOI (10 µg containing 6.85 kBq in 10 µl saline washed in with 15 µl saline) and the distribution of radioactivity was examined in the intrathecal cannula, brain and spinal cord which were removed, sonicated (10–15 s MSE sonicator) in 90% methanol and counted (200 s) on a gamma counter (Mini-Instruments Ltd, type 6-20). In another group of six rats, which did not have indwelling intrathecal cannula, the behavioural response to fluoxetine (1 to 20 mg kg⁻¹, i.p.) was examined for 30 min post-injection and compared with the effect of saline. A final group of rats was decapitated 35 min after a single intrathecal injection of either saline (*n* = 5) or 8-OH-DPAT (2 µg, *n* = 5), and the thoraco-lumbar spinal cord removed and stored at –60°C prior to determining levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by high performance liquid chromatography with electrochemical detection as described in detail by Fone *et al.* (1989).

Drugs

The following drugs were used: 8-hydroxy-2-(di-n-propylamino)tetraline hydrobromide (8-OH-DPAT), 1-(3-chlorophenyl)piperazine dihydrochloride (mCPP), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) and 3-(2-aminoethyl)-2-methyl-1-*H*-indol-5-ol maleate (2-Me-5-HT) (Research Biochemicals Inc.); [¹²⁵I]-(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride ([¹²⁵I]-DOI) (New England Nuclear), 5-methoxy-N,N'-dimethyltryptamine hydrochloride (5-MeODMT) 5-hydroxytryptamine hydrochloride (5-HT) and N-methyl-N-propargyl-3-(2,4-dichlorophenoxy) propylamine hydrochloride (clorgyline) (Sigma); 2,5-dimethoxy-α,4-dimethylbenzene ethamine hydrochloride (DOM provide by Janssen), 5-carboxamidotryptamine maleate (5-CT, Glaxo), fluoxetine hydrochloride (Eli Lilly & Co.) and mianserin hydrochloride (Research Biochemicals Inc.): these were all dissolved in saline (0.154 M sodium chloride). Ketanserin and ritanserin (Janssen) were dissolved in a minimum volume of 0.1 M tartaric acid or 0.04 M lactic acid in dextrose, respectively before being diluted to the required concentration in saline (0.154 M sodium chloride).

Analysis of results

Behavioural experiments were performed using a blind protocol as described previously (Fone *et al.*, 1987) and values given are mean (± s.e.mean for parametric data). Analysis of variance (ANOVA) was used for the statistical analysis of the behavioural studies followed by Wilcoxon rank test (behaviours which were scored on a rating scale) or Dunnett's test (behaviours counted or timed).

Results

Effect of systemic fluoxetine and clorgyline on intrathecal 5-hydroxytryptamine-induced behaviours

Systemic administration of the 5-HT uptake inhibitor fluoxetine (1, 5 and 20 mg kg⁻¹, i.p.) evoked readily quantifiable,

dose-related, brief contractions of the superficial muscles of the back (BMC) resulting in caudal-rostral movements of the skin without any movements of the vertebrae. These BMC began within 2–3 min of injection, lasted for 20–30 min (Figure 1), and were not accompanied by any WDS. Even the highest dose of fluoxetine failed to elicit any of the previously well characterized components of the 5-HT behavioural syndrome (scores for reciprocal forepaw treading 2, lateral head weaving 3, flat body posture 8 and Straub-tail 0 were not significantly different from those obtained with saline, being 1, 2, 4 and 5 respectively, from a possible maximum of 60 each). In addition, 30 min after a submaximally effective dose of fluoxetine (5 mg kg^{-1} , i.p.), intrathecal injection of 5-HT re-initiated

BMC in a dose-related manner (Figure 1), once again without eliciting WDS (even $50 \mu\text{g}$ 5-HT evoked only 2 ± 2 in 30 min) or any of the other components of the 5-HT behavioural syndrome. Following fluoxetine (5 mg kg^{-1} , i.p.), intrathecal 5-HT produced significantly more forepaw-licking ($P < 0.02$, ANOVA) and grooming ($P < 0.001$, ANOVA) than saline (which caused $63 \pm 14 \text{ s}$ and $114 \pm 32 \text{ s}$ in 30 min, respectively) both these behaviours being maximal ($117 \pm 25 \text{ s}$, $P < 0.001$ and $283 \pm 55 \text{ s}$, $P < 0.05$, respectively) with the highest dose of 5-HT.

In contrast, following pretreatment with the monoamine oxidase A inhibitor, clorgyline, intrathecal administration of 5-HT (0.05 – $200 \mu\text{g}$) elicited WDS, which exhibited a bell-shaped response to increasing dose (being maximal with $100 \mu\text{g}$ 5-HT), but did not produce any of the other behaviours monitored (Table 1).

Behavioural effects of intrathecal 5-hydroxytryptamine agonists

The most marked behavioural effect, which began within the first 2 min of the intrathecal injection of the non-selective 5-HT agonist 5-MeODMT, or DOI or DOM which are selective agonists for both 5-HT_{1C} and 5-HT_2 receptors (Glennon *et al.*, 1988; Hoyer, 1988) was BMC (Figure 2). The number of BMC elicited by 5-MeODMT ($r = 0.959$), DOI ($r = 0.958$) and DOM ($r = 0.997$) were linearly correlated to $\log_{10}$ dose according to the method of least squares ($P < 0.001$), the ED_{50} values being 10.6 , 8.5 and $11.0 \mu\text{g}$, respectively. With all three agonists BMC were accompanied by WDS, which typically began after, but within 2–3 min of, the first BMC and were approximately ten fold fewer in number (Figure 2). Irrespective of whether BMC or WDS were initiated by 5-MeODMT, DOI or DOM they were attenuated by pretreatment with low doses of one of the 5-HT_2 receptor antagonists, ketanserin, ritanserin or mianserin (Table 2). Although the higher doses of 5-MeODMT also evoked flat body posture, reciprocal forepaw treading and Straub-tail, as previously documented (Fone *et al.*, 1989), none of these behaviours were quantitatively different from values obtained with saline treatment with any dose of DOI or DOM (data not shown). Following twice daily intrathecal injection the number of BMC produced by DOI ($10 \mu\text{g}$, 93 ± 27 in 30 min) rapidly declined with the second (70 ± 23 at +24 h), fourth (26 ± 12 at +48 h, $P < 0.05$ Dunnett's test following a significant ANOVA $F = 3.55$, $P = 0.038$, compared with the response to the first injection) and sixth (13 ± 11 at +72 h, $P < 0.05$) intrathecal injections. Intrathecal injection of 8-OH-DPAT (0.1 to $100 \mu\text{g}$) a selective 5-HT_{1A} receptor agonist (Tricklebank *et al.*, 1985) failed to evoke any marked behavioural effects until the highest dose ($100 \mu\text{g}$), which produced significantly more flat body posture, reciprocal forepaw treading and lateral head weaving than saline (Table 3), but these

Figure 1 Total number (mean with s.e.mean shown by vertical bars) of back muscle contractions (BMC) observed in 30 min following: (a) intraperitoneal injection of saline ($0.1 \text{ ml } 100 \text{ g}^{-1}$) or fluoxetine (1 , 5 and 20 mg kg^{-1} as indicated, $n = 6$) or (b) intrathecal injection of saline ($10 \mu\text{l}$ washed in with $15 \mu\text{l}$ saline) or 5-hydroxytryptamine (0.05 to $50 \mu\text{g}$, $n = 5$) given 30 min after fluoxetine (5 mg kg^{-1} , i.p.). In (b) column 0 is the number of BMC elicited by intrathecal saline from 30–60 min after fluoxetine (5 mg kg^{-1} , i.p.). Note the re-initiation of BMC by 5-HT. * $P < 0.05$ and *** $P < 0.001$ Dunnett's test following a significant ANOVA, from the effect of the appropriate saline control.

Table 1 Behavioural effects of intrathecal 5-hydroxytryptamine (5-HT) following clorgyline

5-HT dose (μg)	0	0.05	5	50	100	200
Wet dog shakes	1 ± 1	1 ± 1	1 ± 1	8 ± 2	$40 \pm 6^{***}$	$24 \pm 7^{***}$
Back muscle contract.	9 ± 2	7 ± 3	11 ± 4	8 ± 3	6 ± 2	6 ± 1
Flat body posture	1.5 (0–6)	0.2 (0–1)	0.0 (0)	0.0 (0)	11.0 (0–36)	0.3 (0–2)
Reciprocal forepaw treading	0.0 (0)	0.0 (0)	0.8 (0–3)	0.7 (0–3)	0.3 (0–1)	0.5 (0–1)
Lateral head weaving	2.3 (0–5)	3.0 (0–6)	2.4 (1–5)	1.3 (0–4)	1.3 (1–2)	1.3 (1–4)
Straub tail	0 (0)	0.4 (0–1)	0.6 (0–2)	0.0 (0)	0.7 (0–3)	0.7 (0–3)

Comparison of the effect of intrathecal saline with that of 5-HT (0.05 – $200 \mu\text{g}$, $n = 6$) given 30 min after clorgyline (1 mg kg^{-1} , i.p.) on the total number of wet dog shakes and back muscle contractions (mean $\pm$ s.e.mean in 30 min) counted separately. All other behaviours were scored separately on a scale of 0–3, (0 = absent to 3 = present 100% of the time, for 20 s once every minute for the first 20 min post-injection, maximum score = 60), values given are mean (range) from six rats. *** $P < 0.001$ Dunnett's test from the effect of saline after a significant ANOVA ($F = 16.099$, $P < 0.001$).

Figure 2 Dose-response studies showing the total number (mean with s.e.mean shown by vertical bars) of back muscle contractions (BMS, a) and wet dog shakes (WDS, b) observed in 30 min following the intrathecal administration (10 μ l washed in with 15 μ l saline) of; 8-OH-DPAT (■, 0.1 to 100 μ g, $n = 9$), mCPP (○, 10 to 100 μ g, $n = 5$), 5-MeODMT (●, 2 to 100 μ g, $n = 8$), 5-CT (◇, 0.1 to 100 μ g, $n = 6$), DOI (□, 1 to 50 μ g, $n = 6$), DOM (▲, 2 to 25 μ g, $n = 7$) or 2-Me-5-HT (△, 1 to 100 μ g, $n = 7$). mCPP (10 to 100 μ g, $P < 0.01$), 5-MeODMT (25 to 100 μ g, $P < 0.001$), 2-Me-5-HT (50 μ g, $P < 0.01$), DOI (10 to 50 μ g, $P < 0.001$) and DOM (2 to 25 μ g, $P < 0.001$) elicited significantly more BMC and mCPP (50 and 100 μ g, $P < 0.05$), 5-MeODMT (25 to 100 μ g, $P < 0.001$), 5-CT (50 μ g, $P < 0.05$; 100 μ g, $P < 0.01$), DOI (50 μ g, $P < 0.001$) and DOM (10 and 25 μ g, $P < 0.001$) produced significantly more WDS than saline, Dunnett's test following a significant ANOVA. The $\log_{10}$ dose-response curves for BMC and WDS were linearly correlated for 5-MeODMT, DOM and DOI according to the method of least squares. See text for abbreviations.

behaviours were typically only observed from 3 to 4 min after the injection. No WDS or BMC were evoked by any dose of 8-OH-DPAT used in the present study (Figure 2). Intrathecal pretreatment with DOI (2 μ g, -30 min) did not significantly

affect any of the behaviours produced by any dose of 8-OH-DPAT, as illustrated for the highest dose of DOI in Table 3. In contrast, intrathecal pretreatment of rats with 8-OH-DPAT (2 μ g, -30 min) approximately doubled the total number of BMC produced by the lowest and highest doses of DOI ($P < 0.05$ at both 2 and 50 μ g), although it did not significantly enhance the effect of intermediate doses (Figure 3). In contrast, 8-OH-DPAT pretreatment did not affect the WDS concomitantly evoked by intrathecal injection of DOI (Figure 3). In a separate acute biochemical study, a single intrathecal injection of 8-OH-DPAT (2 μ g given 35 min prior to decapitation) significantly increased ($P < 0.05$ Student's t test) the levels of both 5-HT (from 40.7 ± 3.4 to 57.1 ± 6.1 pmol mg^{-1} protein) and 5-HIAA (from 24.0 ± 2.0 to 32.3 ± 3.0 pmol mg^{-1} protein) in the ventral portion of the

Figure 3 Comparison of the effect of pretreatment (-30 min intrathecal) with either saline 25 μ l (□) or 8-hydroxy-2-(di- n -propylamine)tetraline (8-OH-DPAT, 2 μ g, ■) on the total number (mean with s.e.mean shown by vertical bars) of back muscle contraction (a) and wet dog shakes (b) recorded in 30 min following the intrathecal injection of ($\pm$)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI, 2 to 50 μ g, $n = 8$). * $P < 0.05$ from saline pretreated controls, Dunnett's test following a significant ANOVA.

Table 2 Effect of selected 5-hydroxytryptamine (5-HT) antagonists on 5-HT agonist-induced back muscle contractions and wet dog shakes

Pretreatment	5-HT agonist					
	DOI (10 μ g)	DOM (10 μ g)	5-MeODMT (25 μ g)	mCPP (50 μ g)	2-Me-5-HT (50 μ g)	5-CT (50 μ g)
Control	108 $\pm$ 16 (8 $\pm$ 3)	101 $\pm$ 11 (3 $\pm$ 5)	186 $\pm$ 18 (10 $\pm$ 2)	18 $\pm$ 3 (5 $\pm$ 1)	20 $\pm$ 2 (1 $\pm$ 1)	0 $\pm$ 0 (46 $\pm$ 11)
Ketanserin	38 $\pm$ 14* (0 $\pm$ 0)	—	—	5 $\pm$ 2** (1 $\pm$ 1)	—	0 $\pm$ 0 (7 $\pm$ 2)*
Ritanserin	—	1 $\pm$ 1** (1 $\pm$ 1)	17 $\pm$ 10** (0 $\pm$ 0)	—	†1 $\pm$ 1* (0 $\pm$ 0)	—
Mianserin	4 $\pm$ 1** (0 $\pm$ 0)	—	—	—	—	—

Effect of intraperitoneal pretreatment with either ketanserin (0.16 mg kg^{-1}), ritanserin (1 mg kg^{-1} and †0.16 mg kg^{-1}) or mianserin (0.6 mg kg^{-1}) on the total number (mean $\pm$ s.e.mean) of back muscle contractions (upper value) and wet dog shakes (lower value in parentheses) elicited in 30 min following the intrathecal administration of the selected 5-HT agonist ($n = 5$ to 8) at the dose shown in parentheses. * $P < 0.05$ and ** $P < 0.01$ from agonist alone.

DOI, ($\pm$)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DOM, 2,5-dimethoxy- α ,4-dimethylbenzene ethamine hydrochloride; 5-MeODMT, 5-methoxy-N,N'-dimethyltryptamine; mCPP, 1-(3-chlorophenyl)piperazine; 2-Me-5-HT, 2-methyl-5-HT; 5-CT, 5-carboxamidotryptamine.

Table 3 Behavioural effects of intrathecal 8-hydroxy-2-(di-n-propylamino)tetraline (8-OH-DPAT)

8-OH-DPAT dose (μg)	0	0.1	1	10	100	100 plus DOI
Flat body posture	1.7 (0–4)	2.8 (0–7)	3.4* (1–6)	2.9 (1–5)	35.0** (32–37)	35.8** (31–40)
Reciprocal forepaw treading	1.4 (0–3)	1.2 (0–3)	1.9 (0–5)	2.6 (0–7)	10.1** (7–14)	9.1* (4–13)
Lateral head weaving	2.0 (0–4)	3.0 (0–5)	3.4 (0–8)	2.9 (0–7)	14.0** (11–17)	13.5** (10–17)

Behavioural effects of 8-OH-DPAT (0.1–100 μg intrathecally, in 10 μl washed in with 15 μl saline) compared with the effect of saline (25 μl). Each behaviour was scored on a scale of 0–3 (0 = absent to 3 = present 100% of the time) for 20 s once every minute for the first 20 min post-injection (maximum score = 60), values given are mean (range) from nine rats. * $P < 0.05$ and ** $P < 0.01$ Wilcoxon rank test from the effect of saline after a significant Kruskal-Wallis test ($P < 0.001$ for each behaviour). 100 plus DOI = 8-OH-DPAT (100 μg) given 30 min after intrathecal injection of ($\pm$)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 2 μg), all other 8-OH-DPAT injections were given 30 min after saline (25 μl , intrathecally).

thoraco-lumbar spinal cord, without affecting either indoleamine level in the dorsal spinal cord or brainstem (data not shown).

Intrathecal administration of mCPP which shows some selectivity for 5-HT_{1C} over the other 5-HT₁ ligand binding sites (Hoyer, 1988) produced a limited number of WDS and BMC, which were significantly greater ($P < 0.05$, 50 and 100 μg and $P < 0.01$, 10 to 100 μg , respectively) than observed with saline but neither of these behaviours was linearly correlated to $\log_{10}$ dose according to the method of least squares (Figure 2). The few WDS and BMC evoked by mCPP (50 μg) were, however, significantly reduced ($P < 0.05$, Table 2) by pretreatment with the selective 5-HT₂ receptor antagonist ketanserin (0.16 mg kg⁻¹, i.p.) at a dose which shows some selectivity for 5-HT₂ rather than 5-HT_{1C} receptors (Hoyer, 1988; Kennett & Curzon, 1988). Figure 4 shows that mCPP also induced grooming of the body and, in particular, licking of the forepaws and face-washing behaviour (collectively referred to as forepaw-licking) which both occurred predominantly between 2 and 10 min from injection and increased with dose. Neither of these grooming behaviours was significantly altered by ketanserin pretreatment (Figure 4). Intrathecal mCPP injection also elicited significantly more yawns than saline (Figure 4). However, yawning usually commenced after the grooming behaviours, typically being maximal 10 to 20 min after the injection and was significantly reduced ($P < 0.05$) by ketanserin pretreatment (Figure 4).

5-CT which has high affinity for 5-HT₁-like receptor subtypes (Connor *et al.*, 1986; Hoyer, 1988) was the most potent of all the 5-HT agonists in producing WDS, which were significantly greater in incidence than observed with saline, increased with dose and were significantly reduced by ketanserin (Figure 2 and Table 2). 5-CT also evoked marked flat body posture (without accompanying hindlimb abduction) and some lateral head weaving, neither of which was altered by ketanserin (Figure 5). Reciprocal forepaw treading demonstrated a bell-shaped relationship to the intrathecal dose of 5-CT, the maximum effect (with 50 μg 5-CT) being attenuated by ketanserin pretreatment (Figure 5). No BMC were observed with any dose of 5-CT (Figure 2).

Intrathecal injection of even the highest dose of the 5-HT₃ receptor agonist, 2-Me-5-HT (100 μg), did not elicit significantly more reciprocal forepaw treading (mean score of 2 from a maximum of 60), lateral head weaving (3), flat body posture (0), Straub-tail (0) or yawns (11 ± 2 mean $\pm$ s.e.mean in 30 min) than saline (being 2, 3, 2, 0 and 17 ± 3 , respectively). The limited number of BMC evoked by 2-Me-5-HT were significantly greater than observed with saline at all doses used but failed to increase with dose, being maximal with 50 μg (Figure 2), and were significantly attenuated by ritanserin (0.16 mg kg⁻¹, i.p.) pretreatment (Table 2). The highest two doses of 2-Me-5-HT (50 and 100 μg) produced sideward tail-flicks, (each flick lasting for less than a second) which appeared with almost clockwork regularity with the highest dose (reaching 442 ± 90 jerks in 30 min following 100 μg) and yet were not evident with any of the other 5-HT agonists used.

Intrathecal pretreatment with 2-Me-5-HT (2 μg , which elicited little behavioural effect alone) produced a small but significant attenuation of the number of the BMC evoked by the highest dose (50 μg) of the selective 5-HT₂ receptor agonist DOI (from 179 ± 14 to 127 ± 14 in 30 min, $P < 0.05$ Dunnett's test following ANOVA), without affecting the response to 2 or 10 μg DOI (data not shown).

Distribution of [¹²⁵I]-DOI given intrathecally

Following intrathecal injection, only $20 \pm 7\%$ of the total radioactive DOI administered was recovered from the brain and spinal cord while $19 \pm 4\%$ remained in the cannula, with

Figure 4 Effect of intrathecal injection of 1-(3-chlorophenyl)piperazine (mCPP, 10 to 100 μg , $n = 5$) alone or following pretreatment with ketanserin (0.16 mg kg⁻¹, i.p., -30 min) on the total number of yawns (a) and time (s) spent forepaw-licking and face washing (b) and grooming (c) i 30 min post-injection, values given represent mean (s.e.mean shown by vertical bars) in nine rats; 50 + ketanserin = mCPP (50 μg) given 30 min after ketanserin (0.16 mg kg⁻¹, i.p.). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ from saline-treated controls and † $P < 0.05$ from mCPP (50 μg) alone, Dunnett's test following ANOVA.

Figure 5 Effect of ketanserin (Ket) pretreatment (0.16 mg kg^{-1} , i.p., –30 min) on (a) the reciprocal forepaw treading, (b) lateral head weaving, (c) flat body posture and (d) Straub-tail (given as a mean score with a maximum of 60, rated on a scale of 0–3 once every min for 20 min post-injection, see methods for further details) evoked by the intrathecal injection of 5-carboxamidotryptamine (5-CT, 1 to $100 \mu\text{g}$, $n = 6$). * $P < 0.05$ Wilcoxon rank test following a significant Kruskal-Wallis from the effect of saline and † $P < 0.05$ from $50 \mu\text{g}$ 5-carboxamidotryptamine without ketanserin.

the rest presumably being in the cerebrospinal fluid which was lost during the dissection procedure. Nine minutes after intrathecal injection of [^{125}I]-DOI, $97 \pm 2\%$ of the total radioactivity recovered from the entire CNS tissue was within the spinal cord, highest levels ($68 \pm 8\%$) being in the thoracic segments.

Discussion

The present study examined the behavioural effects of intrathecal injection of a variety of 5-HT agonists which show some selectivity for different 5-HT receptor subtypes (Bradley *et al.*, 1986; Hoyer, 1988; Bobker & Williams, 1990). Although relatively high doses (1 to $10 \mu\text{g}$) of the various 5-HT agonists were required to elicit many of the behaviours reported, the strikingly different effects suggest that some pharmacological selectivity was maintained. Furthermore the present study shows that [^{125}I]-DOI is principally restricted to the spinal

cord for at least 9 min post-intrathecal injection, in agreement with a previous study (Johnson *et al.*, 1989) using intrathecal [^{14}C]-RX 77368 (a thyrotrophin-releasing hormone analogue), and also suggested that a large proportion of the total dose of DOI administered remains either in the cerebrospinal fluid or the cannula at this time.

Considerable evidence suggests that head twitches and WDS in rats are mediated by 5-HT₂ receptors (Bédard & Pycock, 1977; Yap & Taylor, 1983; Lucki *et al.*, 1984; Yocca *et al.*, 1990) and in the present study both WDS and BMC were attenuated by a low dose of one of the 5-HT₂ antagonists, ketanserin, ritanserin or mianserin, irrespective of the initiating agonist. Furthermore, the rank order of potency of the 5-HT agonists at producing BMC (DOI $\geq$ DOM = 5-MeODMT $\geq$ 5-HT = mCPP = 2-Me-5-HT > 8-OH-DPAT = 5-CT) was similar to that for WDS (5-CT > DOI = DOM > 5-MeODMT > mCPP > 5-HT = 2-Me-5-HT = 8-OH-DPAT), consistent with both behaviours being 5-HT₂ receptor-mediated. However, 5-CT potentially evoked WDS without eliciting any BMC and intrathecal 5-HT only evoked WDS when given following clorgyline and BMC after fluoxetine treatment, suggesting these two behaviours are produced by different mechanisms. Although McKenna & Peroutka (1989) proposed the existence of two 5-HT₂ receptor subtypes (DOI binding 5-HT_{2A} while ketanserin labels both 5-HT_{2A} and 5-HT_{2B} sites) such differences in ligand binding are probably due to separate G-protein-dependent agonist affinity states of one common receptor (Lyon *et al.*, 1987; Leonhardt & Titeler, 1989). Without determining dose-response curves for WDS and BMC using a range of antagonists it is impossible to determine whether these behaviours are mediated by different 5-HT₂ receptor subtypes or one receptor at two anatomically distinct locations.

The present results, together with the previously observed potentiation of BMC following intrathecal 5,7-DHT (Fone *et al.*, 1989) suggests that this motor behaviour is elicited by the activation of 5-HT₂ receptors located postsynaptic to bulbo-spinal raphe nerve terminals, either on spinal motoneurons or interneurons. Iontophoretic studies show that 5-HT depolarizes spinal motoneurons (Connell & Wallis, 1988; Roberts *et al.*, 1988), an effect not mimicked by 2-Me-5-HT, 8-OH-DPAT or mCPP, and 5-HT₂ agonists excite both facial (Rasmussen & Aghajanian, 1990) and spinal motoneurons (Dixon & Fone, 1991) all of which correlate with the current pharmacological profile reported for 5-HT agonist-induced BMC. However, 5-CT is a full excitatory agonist on motoneurons, an effect poorly antagonized by ketanserin (Roberts *et al.*, 1988; Connell & Wallis, 1989); which fails to agree with the current findings on BMC. Following intrathecal administration, 5-HT agonists presumably affect dorsal horn neurones that are responsive to 5-HT (El-Yassir *et al.*, 1988) which in turn may indirectly influence the activity of motoneurons. It would therefore be surprising if all 5-HT agonists and antagonists had an identical pharmacological profile of activity on a single cell (α -motoneurons) as on a motor behaviour (BMC), even if motoneurone discharge produces this behaviour.

One of the criteria empirical to classifying a functional response as 5-HT₁-like, is that it is mimicked by 5-CT (Bradley *et al.*, 1986; Frazer *et al.*, 1990). Although 5-CT has greater binding affinity for 5-HT₁ than 5-HT₂ receptors (Hoyer, 1988), the present study suggests that this selectivity may be lost *in vivo*, since 5-CT was the most potent agonist in eliciting WDS. A similar conclusion was made by Toda & Okamura (1990) who showed that 5-CT-induced contractions of primate arteries are ketanserin-sensitive. In anaesthetized rats, intrathecal injection of 5-CT ($25\text{--}200 \mu\text{g}$) and low doses of 5-HT ($20\text{--}100 \mu\text{g}$) enhance while α -methyl-5-HT and high doses of 5-HT ($100\text{--}600 \mu\text{g}$) attenuate sympathetic neuronal discharge, but ketanserin pretreatment only prevents the inhibitory effects (Yusof & Coote, 1988). Thus at comparable doses to those used in the present study, 5-CT mimicked the effect of 5-HT on sympathetic discharge yet it failed to evoke BMC. It is therefore unlikely that BMC directly result from

these cardiovascular effects of 5-HT agonists. Alteration in local spinal cord blood flow could, however, contribute to some of the observed behaviours, but intrathecal pretreatment with 5,7-DHT, which enhanced 5-HT agonist-induced BMC (Fone *et al.*, 1989), would not be expected to potentiate the vascular effects of 5-HT agonists. Flat body posture and reciprocal forepaw treading are the only behaviours produced by the selective 5-HT_{1A} agonist 8-OH-DPAT (Middlemiss & Fozard, 1983), thought to be mediated by 5-HT_{1A} receptor activation (Tricklebank *et al.*, 1985). In the present intrathecal study, only the highest dose of 8-OH-DPAT (100 µg) produced flat body posture and reciprocal forepaw treading which with our previous finding that these behaviours are unaffected by intrathecal 5,7-DHT (Fone *et al.*, 1989) implies that they are mediated by supraspinal receptors. Intrathecal administration of DOI or DOM, which have high affinity for 5-HT₂ receptors, evoked only WDS and BMC in agreement with previous studies showing that the complete 5-HT behavioural syndrome is not elicited by 5-HT₂ receptor activation alone (Lucki *et al.*, 1984; Goodwin & Green, 1985). Although simultaneous administration of DOI has been shown to potentiate the reciprocal forepaw treading induced by systemic 8-OH-DPAT (Arnt & Hyttel, 1989; Berendsen *et al.*, 1989), this was not observed in the present intrathecal study. In agreement with previous studies (Goodwin & Green, 1985; Jackson & Kitchen, 1989) however, systemic pretreatment with 5-HT₂ antagonists reduced the reciprocal forepaw treading evoked by intrathecal 5-MeODMT or 5-CT as expected if the positive interaction between 5-HT₂ and the 5-HT_{1A} receptors which cause reciprocal forepaw treading occurs at the supraspinal level. Conversely the 5-HT_{1A} agonists 8-OH-DPAT and buspirone inhibit the ketanserin-sensitive head twitches evoked by DOI (Arnt & Hyttel, 1989), quipazine (Yocca *et al.*, 1989) or 5-HTP following carbidopa (Goodwin & Green, 1985) suggesting that 5-HT_{1A} receptor activation attenuates 5-HT₂ receptor function. In contrast, in the present study intrathecal pretreatment with 8-OH-DPAT (which alone did not elicit BMC) significantly enhanced DOI-induced BMC. One explanation for this discrepancy is that positive coupling exists between the 5-HT_{1A} and 5-HT₂ receptors in the ventral spinal cord which elicit BMC, but not at supraspinal sites involved in mediating other 5-HT-induced behaviours. The present biochemical study showed ventral horn 5-HT levels were elevated 35 min after intrathecal 8-OH-DPAT (an equivalent time to the potentiation of DOI-induced BMC caused by 8-OH-DPAT), as expected if 5-HT release from bulbospinal raphe neurones had decreased as previously documented following 8-OH-DPAT (Hjorth & Magnusson, 1988). The potentiation of BMC produced by 8-OH-DPAT pretreatment could thus result from postsynaptic supersensitivity to the 5-HT₂ agonist precipitated by an acute reduction in 5-HT release from bulbospinal raphe neurones. In support of this view, tolerance rapidly developed to repeated 5-HT₂ agonist injection in the present study, as previously noted for DOM-induced WDS (Leysen *et al.*, 1989) and supersensitivity occurs following depletion of ventral horn 5-HT by intrathecal 5,7-DHT (Fone *et al.*, 1989).

Although forepaw-licking and whole body grooming were apparent with 5-HT following fluoxetine, of all the other 5-HT agonists used only mCPP, which has slightly higher affinity for 5-HT_{1C} than other 5-HT receptor subtypes (Hoyer, 1988), elicited dose-related grooming as formerly noted in 5 day old rats (Jackson & Kitchen, 1989). Pretreatment with ketanserin (0.16 mg kg⁻¹, i.p.), a dose that selectively antagonizes 5-HT₂ and not 5-HT_{1C} receptors (Kennett & Curzon, 1988), abolished the few BMC produced by mCPP without affecting grooming, implicating 5-HT_{1C} receptors in grooming. Hylden & Wilcox (1983) demonstrated similar biting and scratching in mice given intrathecal 5-HT, which was potentiated by fluoxetine (Larson & Wilcox, 1984). Although Berendsen *et al.*, (1990) recently suggested hindlimb scratching may involve 5-HT_{1D} receptors, the existence of this receptor subtype in the rat is controversial (Hoyer & Middlemiss, 1989).

Intrathecal injection of the selective 5-HT₃ receptor agonist 2-Me-5-HT (Kilpatrick *et al.*, 1987; Barnes *et al.*, 1989) produced sideward tail-flicks, not observed with any other 5-HT agonist used. Systemic administration of 8-OH-DPAT to rats with tails hanging freely from restraining cylinders also produces tail-flicks (Millan *et al.*, 1989) but none was observed with the current intrathecal injection in unrestrained rats, suggesting that spinal 5-HT_{1A} receptors are not involved in this behaviour. Even large intrathecal doses of 2-Me-5-HT failed to evoke the classical 5-HT behavioural syndrome and the few BMC were attenuated by ritanserin, however, 2-Me-5-HT reduced DOI-induced BMC in a non-competitive manner. Although rodent spinal motoneurons are unresponsive to iontophoresis of 2-Me-5-HT (Connell & Wallis, 1989), ligand binding studies show 5-HT₃ receptors concentrated throughout the grey matter of the spinal cord (Waeber *et al.*, 1989), providing a mechanism for the observed behavioural interaction.

Figure 6 summarizes the mechanisms involved in the induction of BMC. BMC are evoked by activation of 5-HT₂ receptors located postsynaptic to bulbospinal raphe neurones, possibly on thoracic α-motoneurons. Activation of 5-HT_{1A} receptors located on raphe soma in the brainstem facilitates 5-HT₂ receptor-induced BMC, while activation of 5-HT₃ receptors possibly located on spinal interneurons or dorsal horn neurones attenuate BMC.

Figure 6 Diagrammatic representation of the location of the 5-HT receptors involved in the production of back-muscle contractions (BMC). From the current data and Fone *et al.* (1989), BMC are elicited by the activation of 5-HT₂ receptors located postsynaptic to bulbospinal raphe neurones in the ventral horn of the spinal cord, possibly on thoracic α-motoneurons. Activation of 5-HT_{1A} receptors, probably located on the brainstem raphe soma, potentiates this motor behaviour. In contrast, the apparently non-competitive inhibition of BMC by a 5-HT₃ agonist, suggests that the receptors involved in this effect are not located on motoneurons but possible on spinal interneurons. The effect of activation of terminal autoreceptors remains to be determined.

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