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Involvement of 5-HT_2 receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats

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1 The behavioural effects of the intrathecal injection of a thyrotrophin-releasing hormone (TRH) analogue L-orotyl-L-histidyl-prolineamide (CG 3509, 0.5 μg), the non-selective 5-HT_1 and 5-HT_2 receptor agonist 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT, 2–100 μg) and the selective 5-HT_2 receptor agonist 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM, 2-25 μg) were compared with the response of systemically administered 5-MeODMT (2 mg kg^{-1}, i.p.) in rats, to establish whether the agonist-induced behaviours were mediated by bulbospinal 5-HT_1 or 5-HT_2 receptors.

2 Intrathecal injection of 5-MeODMT or DOM produced dose-related back muscle contractions (a previously undocumented behaviour) and wet-dog shakes which were both markedly attenuated by ritanserin pretreatment (1 mg kg^{-1}, i.p.) indicating the involvement of 5-HT_2 receptors. In contrast, reciprocal forepaw treading, flat body posture and Straub-tail were evoked by 5-MeODMT but not by DOM indicating that these behaviours were not produced by 5-HT_2 receptor activation alone. However, as ritanserin pretreatment reduced the reciprocal forepaw treading induced by intrathecal 5-MeODMT, this behaviour may be facilitated by 5-HT_2 receptor activation.

3 Intrathecal 5,7-dihydroxytryptamine (5,7-DHT, 2 × 150 μg) treatment decreased thoraco-lumbar spinal cord 5-HT (−95%) and potentiated the back muscle contractions produced by intrathecal DOM injection without altering the wet-dog shake behaviour. None of the components of the 5-HT syndrome produced by 5-MeODMT (2 mg kg^{-1}, i.p.), with the exception of a small increase in wet-dog shakes, was significantly altered by intrathecal 5,7-DHT (which reduced thoraco-lumbar spinal cord 5-HT by 84%). Taken together these data suggest that the only 5-HT agonist-induced behaviour mediated by the activation of 5-HT_2 receptors located postsynaptic to bulbospinal 5-hydroxytryptaminergic (5-HTergic) neurones was back muscle contractions.

4 The wet-dog shake and forepaw licking behaviors produced by intrathecal CG 3509 (0.5 μg) were attenuated when ritanserin was administered intrathecally 30 min before, but not when it was given at the same time as CG 3509 and neither behaviour was altered by intrathecal 5,7-DHT. This suggests that bulbospinal 5-HTergic neurones are not involved in the production of these TRH analogue-induced behaviours and that the 5-HT_2 receptors which mediate these behaviours are not located in the spinal cord.

Introduction

The systemic administration of drugs which increase synaptic levels of 5-hydroxytryptamine (5-HT) or agonists which act on postsynaptic 5-HT receptors produces a well-defined behavioural syndrome including reciprocal forepaw treading, hindlimb abduction, lateral head weaving, Straub tail, wet-dog shakes and hyperactivity in the rodent (Grahame-Smith, 1971; Jacobs, 1976; Deakin & Green, 1978) which all have clear motor components and can be

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used to assess 5-hydroxytryptaminergic (5-HTergic) neuronal function. The neuronal substrates which mediate these behaviours (except for hindlimb abduction) are thought to be the pontine and medullary raphe nuclei and their synaptic contacts in the lower brain stem and spinal cord (Jacobs & Klemfuss, 1975; Jacobs 1976; Bédard & Pycock, 1977; McCall & Aghajanian, 1979) though the localisation of the 5-HT receptor sub-types involved has not been established.

The tripeptide thyrotrophin-releasing hormone (TRH) is also present in high concentrations in the ventral horn of the spinal cord of a number of experimental animals (Hökfelt *et al.*, 1975; Kardon *et al.*, 1977; Lechan *et al.*, 1984; Fone *et al.*, 1987a) and man (Bennett *et al.*, 1986), where it co-exists with 5-HT and the undecapeptide substance P in descending medullary raphe nerve terminals (Johansson *et al.*, 1981; Pelletier *et al.*, 1981; Gilbert *et al.*, 1982). All three neurotransmitters have been localised, by electronmicroscopy, within boutons making close synaptic contact with ventral horn motoneurones (Atsumi *et al.*, 1985; Ulfhake *et al.*, 1987) consistent with the proposal that these neurones regulate spinal motor output. When applied iontophoretically to spinal motoneurones TRH has similar electrophysiological effects to substance P and 5-HT, facilitating the activation of motoneurones produced by the excitatory amino acids glutamate or aspartate (White, 1985). In addition, the intrathecal administration of TRH or TRH analogues produces clearly defined motor behaviours, including a dose-related wet-dog shake and marked forepaw-licking (Fone *et al.*, 1987b; 1988; Johnson *et al.*, 1988), which resemble components of the 5-HT-syndrome. Furthermore, Barbeau & Bédard (1981) reported that TRH, like 5-HT, increases spontaneous EMG activity in the hindlimb of chronic spinalised rats and as these effects were blocked by the 5-HT antagonist cyproheptadine, they suggested that TRH may act on 5-HT receptors situated on motoneurones or interneurones in the lumbar spinal cord of the rat. An alternative explanation for these findings, however, is that TRH releases 5-HT from bulbospinal nerve terminals.

The present study therefore, firstly determined whether any of the behaviours evoked by intrathecal administration of the non-selective 5-HT_1 and 5-HT_2 receptor agonist 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT) or the selective 5-HT_2 receptor agonist 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM), involved activation of spinal 5-HT receptors. Secondly we investigated whether the behaviours produced by the intrathecal injection of the TRH analogue CG 3509 (L-orotyl-L-histidyl-prolineamide) were also mediated via the same spinal 5-HT receptors.

Methods

Intrathecal cannulation and behavioural recording techniques

An intrathecal cannula was implanted in male Wistar rats under sodium methohexitone anaesthesia (60 mg kg^{-1}, i.p.) so that the caudal tip of the cannula was at the thoraco-lumbar junction of the spinal cord (Yaksh & Rudy, 1976; Fone *et al.*, 1987b). Following a seven day recovery period, rats were placed in a sound-proof chamber, to which they had previously been habituated, and the number of back muscle contractions, the number of wet-dog shakes and the time spent forepaw-licking following the intrathecal injection of saline (10 μl + 20 μl wash-in), 5-MeODMT, DOM or CG 3509 were recorded for 30 min using a camera and a video-recorder, as described previously (Fone *et al.*, 1987b). In addition, in those rats receiving i.p. or intrathecal injections of 5-MeODMT or DOM, lateral head weaving, reciprocal forepaw treading, flat body posture and Straub-tail were scored on a scale of 0–3 (0 absent, 1 present < 50% of the time, 2 present > 50% of the time and 3 present all of the time), each behaviour being monitored for 20 s once every 2 min with i.p. injections or once every min with intrathecal injections. In addition, the number of turns through 90° and wet-dog shakes were counted for 20 min following the i.p. injection of 5-MeODMT.

Drug administration

In one group of rats (A in Table 1) CG 3509 (0.5 μg, $n = 7$) was administered intrathecally at four day intervals 30 min after the injection of (a) vehicle i.p. (0.04 M lactic acid in 5% dextrose on day 7), (b) ritanserin (1 mg kg^{-1}, i.p., on day 11), (c) ritanserin 5 μg intrathecally (on day 19) and (d) vehicle (0.04 M lactic acid in 5% dextrose) given intrathecally (on day 23). Ritanserin (5 μg) was also given intrathecally at the same time as CG 3509 (0.5 μg, on day 15). A second group of rats (B in Table 1, $n = 8$) received intrathecal injections, at four day intervals, of saline or 5-MeODMT (2, 10, 25, 50 and 100 μg) and four days later 5-MeODMT (25 μg) was re-administered 30 min after ritanserin (1 mg kg^{-1}, i.p.). A third group of rats (C in Table 1, $n = 7$) were given saline and DOM (2, 10 and 25 μg) intrathecally at four day intervals and the response to DOM (10 μg) was re-examined 30 min after ritanserin (1 mg kg^{-1}, i.p.).

In two further groups of rats (D in Table 1), the behavioural response to the intrathecal injection of CG 3509 (0.5 μg) was compared two days before and ten days after vehicle (10 μl, 5.68 mM ascorbate in 0.154 M saline, $n = 5$) or 5,7-dihydroxytryptamine (5,7-DHT, 2 × 150 μg, $n = 6$) which were both given

Table 1 Protocol for drug administration

Days	*7*	*9*	*11*	*13*	*15*	*17*	*19*	*21*	*23*	*25*	*27*	*31*
Group A	CG 3509 vehicle (i.p.)		CG 3509 ritanserin (i.p.)		CG 3509 ritanserin (i.t.)		CG 3509 ritanserin (i.t.)		CG 3509 vehicle (i.t.)			
Group B	Saline		5-MeODMT		5-MeODMT		5-MeODMT		5-MeODMT		5-MeODMT	5-MeODMT ritanserin (i.p.)
Group C	Saline		DOM		DOM*		DOM		DOM ritanserin (i.p.)			
Group D	5-MeODMT	CG 3509	5,7-DHT*	5,7-DHT*			5-MeODMT	CG 3509				
Group E	DOM	5,7-DHT	5,7-DHT				DOM					

For each group of rats (A to E, $n = 7$–11), the behavioural response to the test drug (on the upper line) was examined with the pretreatments listed (on the lower line). In groups B and C the different doses of 5-MeODMT and DOM respectively, were given in a randomised order. For further details of the doses of each drug used see the methods. * Five rats in group D received vehicle instead of 5,7-DHT.
5,MeODMT = 5-methoxy-N,N'-dimethyltryptamine; DOM = 2,5-dimethoxy-α,4-dimethyl-benzene ethamine HCl; 5,7-DHT = 5,7-dihydroxytryptamine; CG 3509 = L-orotyl-L-histidyl-prolineamide.

intrathecally 11 and 13 days after surgery and 1 h after desipramine (25 mg kg^{-1}, i.p.). Furthermore, in the same rats, the behavioural response in the first 20 min following i.p. injection of the non-selective 5-HT$_1$ and 5-HT$_2$ agonist 5-MeODMT (2 mg kg^{-1}, i.p.) was assessed four days before and eight days after treatment with vehicle or 5,7-DHT. In a separate group of rats (E in Table 1, $n = 7$), the behavioural response to DOM (10 μg intrathecally) was also compared two days before and ten days after 5,7-DHT treatment (2 × 150 μg administered as detailed above).

Biochemical assays

In animals given 5,7-DHT (see above) the thoracolumbar spinal cord, brain stem and hypothalamus were dissected (on the same day as the last behavioural test), extracted and maintained at −80°C prior to assay. Levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined by high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.d.) and TRH and substance P levels were measured by well characterized radioimmunoassays (RIAs) as previously described in detail (Fone *et al.*, 1987a).

Drugs

L-Orotyl-L-histidyl-prolineamide (CG 3509, Grunenthal GMBH), 5-methoxy-N,N'-dimethyltryptamine hydrochloride (5-MeODMT), 5,7-dihydroxytryptamine creatinine sulphate (Sigma) and 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM provided by Janssen) were dissolved in saline (0.154 M sodium chloride). Ritanserin (Janssen) was dissolved in 0.04 M lactic acid in dextrose.

Analysis of results

All behavioural experiments were performed using a blind protocol as previously described (Fone *et al.*, 1987b). Values given are mean ± s.e.mean and Student's t test was used for statistical analysis unless otherwise indicated in the text.

Results

Behaviours produced by intrathecal or intraperitoneal 5-MeODMT administration

Within 2 min of intrathecal injection, 5-MeODMT (2–100 μg) produced wet-dog shakes and readily quantifiable, brief, contractions of the muscles of the

back without accompanying hindlimb movements; subsequently referred to as 'back muscle contractions'. As shown in Figure 1, both the number of wet-dog shakes and the number of back muscle contractions recorded in the 30 min post-injection period were linearly correlated to $\log_{10}$ dose 5-MeODMT (within the range of 1–50 μg, but did not increase further with 100 μg 5-MeODMT). With all but the lowest dose (back muscle contractions) or the 2 and 10 μg doses (wet-dog shakes) of 5-MeODMT the incidence of both behaviours was significantly greater than observed with saline. Intrathecal 5-MeODMT also caused components of the 5-HT-syndrome (including flat body posture, reciprocal forepaw treading, and Straub tail) which although not pronounced, increased in a dose-related manner, however, lateral head weaving was not evoked by this route of administration (Table 2). Ritanserin (1 mg kg^{-1}, i.p.) pretreatment significantly attenuated the number of wet-dog shakes and back muscle contractions and the amount of reciprocal forepaw treading produced by intrathecal 5-MeODMT, although none of the other components of the 5-HT-syndrome were affected by this treatment (Figure 2 and Table 2). Intrathecal 5-MeODMT also caused scratching of the torso and caudally directed bites (as reported by Hylden & Wilcox, 1983; Larson & Wilcox, 1984), but these were not quantified in the present study.

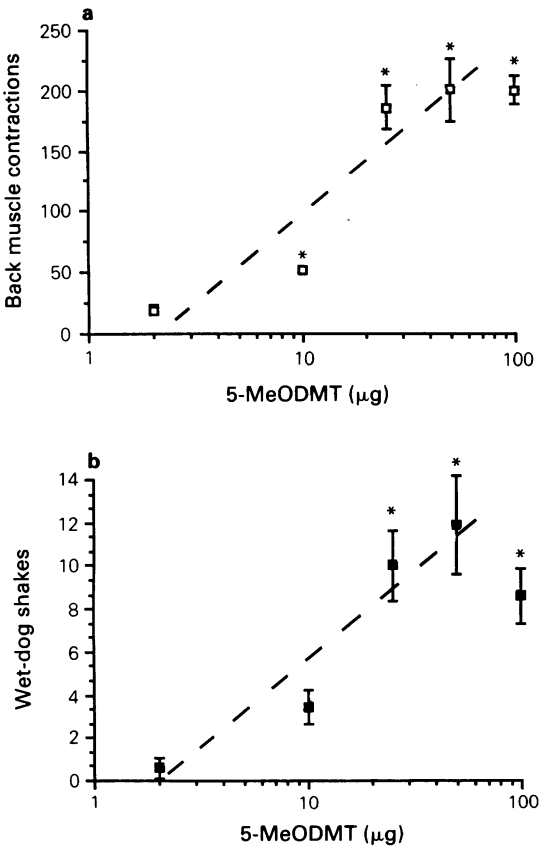

Figure 1 The total number (mean ± s.e.mean, $n = 8$) of (a) back muscle contractions and (b) wet-dog shakes produced in 30 min following the intrathecal administration of 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT, 2-100 μg) was significantly different ($P < 0.05$, ANOVAR) from the effect of saline (7 ± 3 and 1 ± 1 in 30 min, respectively) with all but the lowest dose (back muscle contractions) and the 2 and 10 μg dose (wet-dog shakes). The $\log_{10}$ dose-responses were both linearly correlated ($r = 0.959$ (a) and 0.929 (b), $P < 0.01$) from 1 to 50 μg, according to the method of least squares.

The systemic administration of 5-MeODMT (2 mg kg^{-1}, i.p.), which had previously been shown to evoke a submaximal behavioural response (Nisbet & Marsden, 1984), produced lateral head weaving, flat body posture, hindlimb abduction, reciprocal forepaw treading, wet-dog shakes and hyperlocomotion (assessed by counting the number of turns) but not back muscle contractions as observed after intrathecal injection of 5-MeODMT. With the exception of a small, but significant increase in the number of wet-dog shakes, none of these behaviours produced by i.p. 5-MeODMT were significantly altered by intrathecal 5,7-DHT pretreatment (Table 3), although the apparent reduction in flat body posture following 5,7-DHT treatment only just failed to reach significance.

Behaviours produced by intrathecal administration of DOM

Figure 3 shows that the intrathecal injection of DOM (2–25 μg) caused back muscle contractions and wet-dog shakes, both of which were significantly

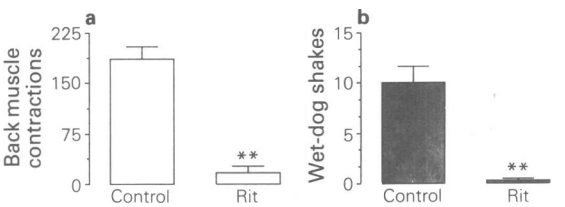

Figure 2 Effect of ritanserin (Rit, 1 mg kg^{-1} i.p., −30 min) pretreatment on the total number (mean with s.e.mean shown by vertical bars, $n = 8$) of back muscle contractions (a) and wet-dog shakes (b) produced in 30 min following the intrathecal injection of 5-methoxy-N,N'-dimethyltryptamine (25 μg, $n = 8$). ** $P < 0.001$, Student's t test.

Table 2 Behavioural effect of intrathecal 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT)

5-MeODMT dose (μg)	*0*	*2*	*10*	*25*	*50*	*100*	*25 plus Rit*
Flat body posture	1.0 (0–4)	0.0 (0–0)	4.6 (0–36)	14.7* (1–34)	21.8* (0–32)	16.4** (4–44)	12.6 (2–31)
Reciprocal forepaw treading	0.3 (0–1)	0.5 (0–2)	0.5 (0–3)	3.3* (2–6)	7.4* (5–9)	6.0* (3–8)	1.0† (0–3)
Straub-tail	1.0 (0–3)	1.3 (0–6)	2.9* (0–6)	2.9* (0–6)	3.1* (1–6)	9.1* (1–17)	1.1 (0–3)
Lateral head weaving	3.8 (1–6)	3.9 (2–6)	2.6 (1–6)	2.4 (0–6)	3.8 (2–6)	2.8 (0–8)	4.9 (2–8)

Scoring procedures are described in the Methods. The maximum score per behaviour = 60, values given are mean (range) from eight animals. * $P < 0.05$, ** $P < 0.01$ Wilcoxon rank test from the effect of saline following significant Kruskal-Wallis test and † $P < 0.01$ from the effect of 5-MeODMT (25 μg). 25 plus Rit = 5-MeODMT (25 μg) given 30 min after ritanserin (1 mg kg^{-1}, i.p.).

greater than the effect in saline. Both behaviours appeared to increase in a dose-related manner and were virtually abolished by ritanserin pretreatment (1 mg kg^{-1}, i.p., Figure 3). All doses of DOM also produced an immediate and marked extension of the hindlegs together with chewing and licking of the hindlegs and abdomen. In addition some yawning was observed with the highest dose of DOM. However, no tail elevation, flat body posture, lateral head weaving or reciprocal forepaw treading were evident with any dose of DOM (average behavioural scores for 25 μg DOM being 0, 1, 6 and 1 respectively, from a possible maximum of 60). Neither was there any evidence of hyperlocomotion when compared with saline.

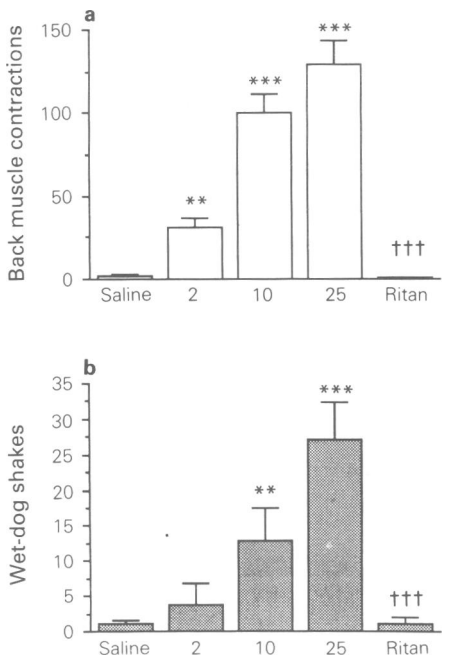

Figure 3 A comparison of the total number (mean ± s.e.mean, $n = 7$) of back muscle contractions (a) and wet-dog shakes (b) produced by the intrathecal injection of saline or 2,5-dimethoxy-α,4-dimethylbenzene ethamine HCl (DOM) (2,10 and 25 μg) given alone with the response to DOM (25 μg) given 30 min after ritanserin (Ritan, 1 mg kg^{-1}, i.p.). ** $P < 0.001$ and *** $P < 0.0001$, ANOVAR from rats given saline and ††† $P < 0.0001$ from rats given DOM (25 μg) alone.

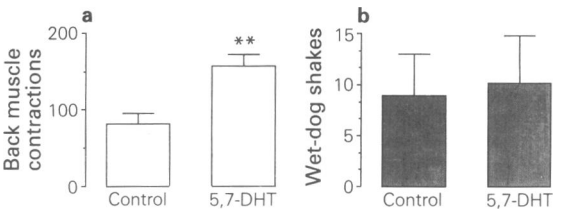

Figure 4 Comparison of the number of back muscle contractions (a) and wet-dog shakes (b) produced in 30 min by intrathecal 2,5-dimethoxy-α,4-dimethylbenzene ethamine HCl (DOM, 10 μg) administration two days before (Control) with that evoked ten days after intrathecal injection of 5,7-dihydroxytryptamine 5,7-DHT (2 × 150 μg, $n = 6$). ** $P < 0.01$, Student's t test from the control response.

Intrathecal pretreatment with 5,7-DHT, which significantly reduced thoraco-lumbar 5-HT levels (95% below that in vehicle-treated controls), significantly enhanced the number of back muscle contractions evoked by intrathecal DOM administration (from 82 ± 14 to 157 ± 16 in 30 min with 10 μg DOM, $P < 0.01$) without altering the wet-dog shake response (Figure 4).

Behaviours produced by intrathecal CG 3509 administration

The intrathecal injection of CG 3509 (0.5 μg) produced marked wet-dog shake and forepaw-licking

Table 3 Effect of intrathecal 5,7-dihydroxytryptamine (5,7-DHT) on the behavioural response to 5-methoxy-N,N'-dimethyltryptamine (5-MeODMT, 2 mg kg^{-1}, i.p.)

	Vehicle ($n = 5$)		*5,7-DHT* ($n = 6$)	
Treatment	*before*	*after*	*before*	*after*
Wet-dog shakes	2 ± 2	1 ± 1	1 ± 1	6 ± 2*
Number of turns	78 ± 15	89 ± 17	60 ± 8	59 ± 20
Flat body posture	11.6 (10–14)	10.4 (0–17)	9.5 (9–16)	7.5 (5–10)
Forepaw treading	5.6 (3–10)	3.2 (0–6)	3.3 (2–5)	2.2 (1–5)
Head weaving	7.8 (6–10)	9.4 (7–12)	9.5 (9–10)	7.5 (5–10)
Hindlimb abduction	12.4 (10–16)	15.6 (13–21)	13.2 (9–18)	14.7 (15–20)

The number of wet-dog shakes and turns through 90° were counted (mean ± s.e.mean) and the other behaviours were scored mean (range) as described in the Methods, the maximum score being 30 for each behaviour. * $P < 0.05$ Student's t test from before 5,7-DHT treatment.

There was no significant difference in the behavioural score within the groups (before and after either treatment, Wilcoxan Rank test) nor between vehicle and 5,7-DHT groups after treatment (Mann Whitney U test).

behaviours, with the peak effect at 6–9 min as reported previously (Fone *et al.*, 1987b; Johnson *et al.*, 1988) but unlike intrathecal 5-HT agonist administration it did not produce any back muscle contractions. The wet-dog shakes and forepaw-licking behaviours induced by CG 3509 were attenuated by a 30 min intrathecal pretreatment with ritanserin and wet-dog shakes were also reduced by i.p. ritanserin pretreatment (Figure 5), but neither behaviour was significantly reduced when ritanserin was given intrathecally at the same time as CG 3509 (Figure 5).

Both the wet-dog shakes and forepaw-licking behaviours induced by CG 3509 were unaltered by intrathecal injections of vehicle or 5,7-DHT (Figure 6a and b).

Effect of 5,7-DHT on brain and spinal cord 5-HT, TRH and substance P levels

Intrathecal 5,7-DHT administration markedly depleted 5-HT, TRH and substance P levels in the thoraco-lumbar ventral horn (reduced by 84%, 96% and 59%, respectively, from levels in vehicle-treated controls; Figure 7), but levels were unchanged in the hypothalamus, and only TRH levels were significantly increased in the brainstem (Figure 7). 5,7-DHT treatment also caused a small decrease in the 5-HT and TRH levels in the dorsal horn of the spinal cord and although substance P levels appeared to be reduced in this region, compared with vehicle-treated controls, this did not reach significance (Figure 7).

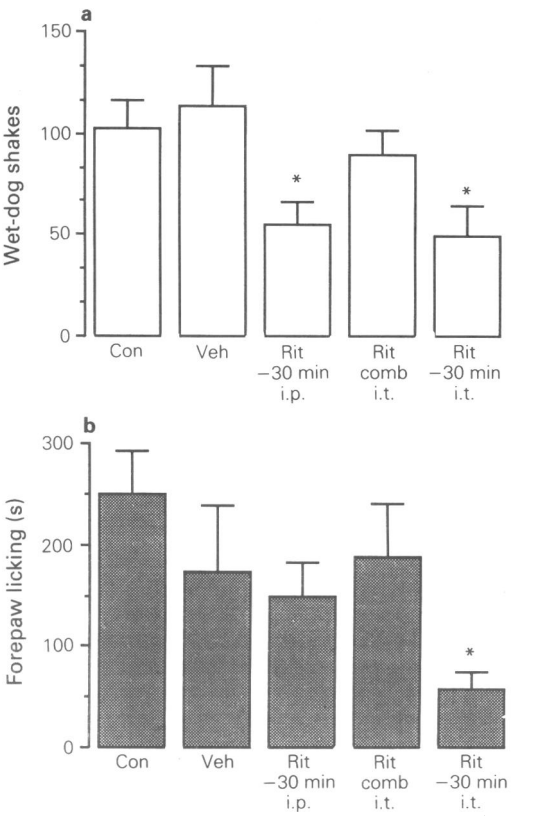

Figure 5 Effect of the pretreatment with either vehicle (Veh) intrathecally, ritanserin 1 mg kg^{-1} i.p. (Rit −30 min, i.p.) or ritanserin 5 μg intrathecally given at the same time (Rit comb i.t.) or 30 min before (Rit −30 min i.t.) CG 3509, on the wet-dog shake (a) and the forepaw-licking (b) behaviours (mean with s.e.mean shown by vertical lines over 30 min, $n = 8$) produced by the intrathecal administration of 0.5 μg CG 3509 alone (Con). * $P < 0.02$, Student's t test from the appropriate control.

Discussion

Intrathecal administration of the 5-HT agonist, 5-MeODMT produced the characteristic 5-HT behavioural syndrome previously observed following systemic administration of 5-HT agonists or drugs

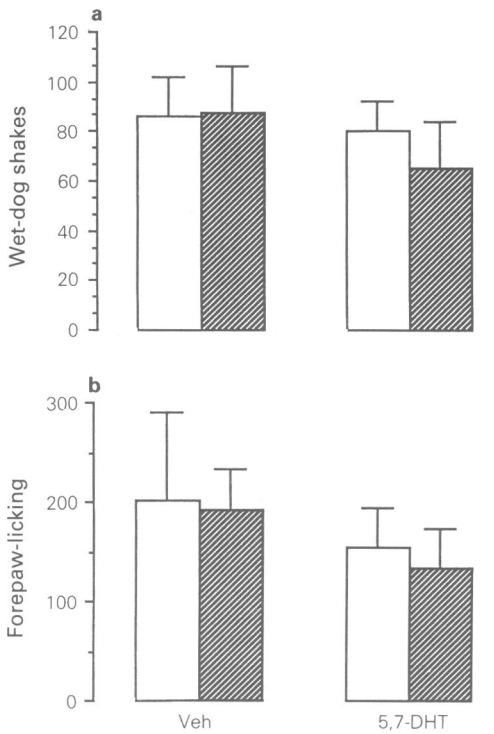

Figure 6 A comparison of the wet-dog shake (a) and the forepaw-licking (b) behaviours evoked in 30 min following intrathecal CG 3509 (0.5 μg) administration given two days before (open columns) and ten days after (filled columns) treatment with vehicle (10 μl, 5.68 mM ascorbate in 0.154 M saline, $n = 5$) or 5,7-dihydroxytryptamine (5,7-DHT, 2 × 150 μg intrathecally, $n = 6$).

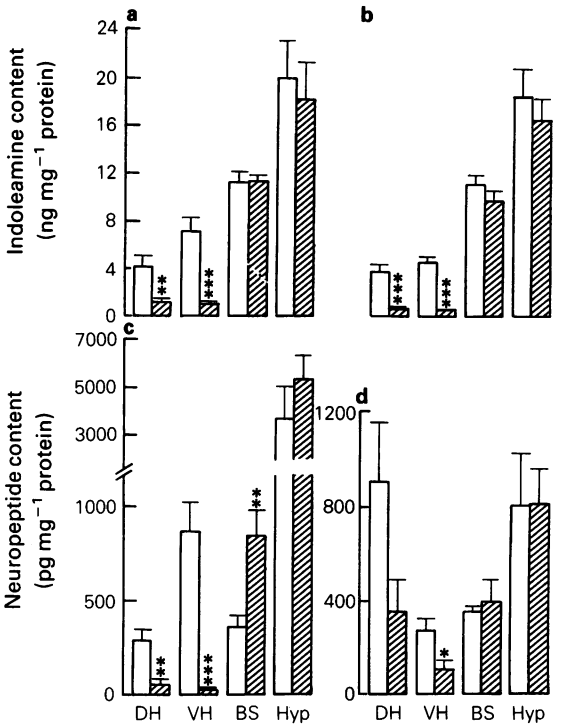

Figure 7 The effect of vehicle (open columns, $n = 5$) and 5,7-dihydroxytryptamine (hatched columns, 2 × 150 μg intrathecally, $n = 6$) treatment on (a) 5-hydroxytryptamine, (b) 5-hydroxyindoleacetic acid, (c) thyrotrophin-releasing hormone and (d) substance P levels (mean with s.e.mean shown by vertical bars) in the thoraco-lumbar dorsal (DH) and ventral (VH) horns, brain stem (BS) and hypothalamus (Hyp). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$; Student's t test from vehicle-treated controls.

which increase synaptic 5-HT levels (Grahame-Smith, 1971; Jacobs, 1976), with the exception that lateral head weaving was not discernable. In addition, intrathecal 5-MeODMT caused marked, dose-related back muscle contractions, a characteristic and readily quantifiable motor response which does not appear to have been described previously. The close similarity of the dose-response curves for the wet-dog shakes and back muscle contractions produced by intrathecal 5-MeODMT is consistent with the idea that these behaviours are mediated by a common 5-HT receptor subtype. Since both these behaviours and the reciprocal forepaw treading evoked by intrathecal 5-MeODMT were reduced by pretreatment with the selective 5-HT$_2$ receptor antagonist ritanserin, 5-HT$_2$ receptors are implicated in these three behaviours. Furthermore, intrathecal injection of the selective 5-HT$_2$ receptor agonist DOM (Glennon, 1987) also produced wet-dog shakes and back muscle contractions which were both attenuated by ritanserin, while none of the other 5-MeODMT-induced behaviours (including reciprocal forepaw treading) were observed. Taken together these results suggest that 5-HT$_2$ receptor activation may facilitate reciprocal forepaw treading but activation of this receptor alone is not sufficient to elicit this behaviour. In addition, as back muscle contractions were only evoked when 5-HT agonists were given intrathecally, and were not seen following the systemic administration of 5-MeODMT, this behaviour is likely to be mediated by spinal 5-HT$_2$ receptors.

Previous studies suggest that while 5-HT-induced wet-dog shakes are inhibited in a dose-related manner by 5-HT$_2$ receptor antagonists such as ketanserin and pirenpirone (Yap & Taylor, 1983; Koshikawa *et al.*, 1985) and are produced by the systemic administration of the 5-HT$_2$ receptor agonist

1 - (2,5 - dimethyl - 4 - iodophenyl) - 2 - aminopropane (Heaton *et al.*, 1988), the other components of the 5-HT-syndrome are largely mediated by 5-HT_1-like receptors (Lucki *et al.*, 1984; Goodwin & Green, 1985; Tricklebank *et al.*, 1985; Kennett & Curzon, 1988). For this reason the presence of another 5-HT_2 receptor-mediated behaviour, namely the back muscle contractions described in the present study, provides a very useful tool to investigate further the neuropharmacology of spinal 5-HT.

In the present study, intrathecal 5,7-DHT administration depleted 5-HT (−84%), TRH (−96%) and substance P (−59%) from the ventral horn of the spinal cord where the indoleamine and neuropeptides co-exist (Johansson *et al.*, 1981; Pelletier *et al.*, 1981; Gilbert *et al.*, 1982). With the exception of an elevation in brainstem TRH, previously reported following i.c.v. 5,7-DHT (Manaker *et al.*, 1985), intrathecal 5,7-DHT injection had no effect on indoleamine or peptide levels in the hypothalamus or brainstem. The increase in brainstem TRH probably results from 5,7-DHT causing degeneration of 5-HT-peptidergic terminals in the ventral horn of the spinal cord without affecting the brainstem perikarya (Towle *et al.*, 1984), so preventing nerve terminal synthesis of 5-HT without reducing the neuronal cell body synthesis of TRH which consequently accumulates in the brainstem soma. The lack of effect of 5,7-DHT on substance P levels in the brainstem can not at present be explained. Within the ventral horn postsynaptic supersensitivity to 5-HT agonists would be expected following such a marked depletion of 5-HT, since similar lesions of the 5-HTergic system produce a parallel increase in the 5-MeODMT-induced head twitch response and the number of cortical 5-HT_2 receptors in mice (Heal *et al.*, 1985). As 5,7-DHT treatment, in the present study, markedly enhanced the DOM-induced back muscle contractions, this behaviour would appear to be mediated by activation of spinally-located 5-HT_2 receptors. In contrast, neither the wet-dog shake response to DOM nor any of the components of the 5-HT-syndrome induced by 5-MeODMT (apart from a small increase in the number of wet-dog shakes) were altered by 5,7-DHT treatment. The present results therefore suggest that bulbospinal 5-HT-ergic neurones are not involved in the lateral head weaving, flat body posture, hindlimb abduction, reciprocal forepaw treading, hyperactivity (number of turns) or wet-dog shakes produced by 5-HT agonists. In comparison, Deakin & Green (1978) showed that microinjection of 5,7-DHT into the rostral cervical spinal cord (C_1 and C_2) decreased spinal 5-HT levels by 70% and significantly enhanced the head weaving, Straub-tail and hindlimb abduction produced by 5-MeODMT (2 mg kg^{-1}, i.p.). However, the neurotoxin may have spread rostrally from the latter injection site so destroying 5-HT neurones not affected by the more caudal intrathecal injection procedure used in the current study. In agreement with this proposal, i.c.v. 5,7-DHT has been shown to enhance 5-MeODMT-induced hindlimb abduction and reciprocal forepaw treading (Nisbet & Marsden, 1984) and injections of 5,7-DHT into the most caudal thoracic segment (T_{13}) of the rat, only increased the Straub-tail and not the hindlimb abduction, head weaving or reciprocal forepaw treading produced by i.p. 5-MeODMT (Dickinson *et al.*, 1984).

Intrathecal administration of the TRH analogue CG 3509 produced wet-dog shake and forepaw-licking behaviours as previously described (Fone *et al.*, 1987b; Johnson *et al.*, 1988), but did not produce back muscle contractions as would be expected if TRH directly activates spinal 5-HT_2 receptors. Both the wet-dog shake and forepaw-licking behaviours were attenuated by a 30 min intrathecal pretreatment with ritanserin although neither response was altered when ritanserin was given intrathecally at the same time as CG 3509. We have previously shown that in the first 9 min following intrathecal administration of radiolabelled TRH or TRH analogues the radioactivity remains principally confined to the spinal cord (Fone *et al.*, 1987b; Johnson *et al.*, 1988). Since 30 min pretreatment with ritanserin was required to obtain antagonism of the TRH analogue-induced behaviours, the 5-HT_2 receptors involved in these effects may be located rostral to the spinal cord.

The degeneration of bulbospinal 5-HT neurones (which contain TRH) produced by 5,7-DHT increases spinal TRH receptor number according to binding studies (Sharif *et al.*, 1983; Ogawa *et al.*, 1985) and augments the facilitation of spinal reflexes normally evoked by TRH (Barbeau & Bédard, 1981; Clarke & Stirk, 1983). Thus several lines of evidence suggest that the intrathecal 5,7-DHT treatment, used in the present study, would induce up-regulation of both 5-HT and TRH receptors in the spinal cord. Despite this, intrathecal 5,7-DHT did not affect the wet-dog shakes or the forepaw-licking produced by intrathecal TRH analogue administration, supporting the proposal that these behaviours are not mediated by bulbospinal 5-HTergic neurones. In accordance with this proposal previous work suggests that the brain area involved in mediating 5-HT- and TRH-induced wet-dog shake behaviour is in the medial brainstem, between the colliculi and the anterior commissure, as lesions in this region reduce the shaking produced by 5-hydroxytryptophan (5-HTP) and water immersion (Wei *et al.*, 1973; Bédard & Pycock, 1977; Wei, 1981), while micro-injection of TRH into the medial brainstem (Wei, 1981) or the area of the locus coeruleus (Kalivas & Horita, 1981; Webster *et al.*, 1982)

caused vigorous wet-dog shakes. Since intrathecal 5,7-DHT significantly enhanced the 5-MeODMT-but had no effect on the TRH analogue-induced shaking, the 5-HT- and TRH-induced wet-dog shakes may be mediated by separate 5-HTergic pathways. In agreement with this idea, Drust & Connor (1983) found that while 5-HT-induced wet dog shakes were blocked by the 5-HT antagonist methysergide and enhanced by 5,7-DHT, neither treatment effected the TRH-induced shaking.

In summary, both i.p. and intrathecal injection of the non-selective 5-HT agonist 5-MeODMT produced components of the 5-HT-syndrome, but back muscle contractions were only observed with intrathecal injections and so may be dependent upon marked stimulation of spinal 5-HT receptors, while lateral head weaving appears to be mediated by more rostral neurones as it was not evoked by this route of administration. 5-HT$_2$ receptors may be involved in the wet-dog shakes and back muscle contractions evoked by the 5-HT agonists 5-MeODMT or DOM, as these behaviours were attenuated by ritanserin. Intrathecal 5,7-DHT treatment, which would be expected to increase 5-HT and TRH receptors in the spinal cord, was without effect on the wet-dog shakes and forepaw-licking behaviours produced by intrathecal CG 3509, or the lateral head weaving, flat body posture, hindlimb abduction, reciprocal forepaw treading or hyperlocomotion produced by i.p. injection of 5-MeODMT, indicating that spinal 5-HT and TRH receptors are not involved in mediating these behaviours. Spinal cord 5-HT$_2$ receptors located postsynaptically in relation to bulbospinal 5-HTergic nerve terminals are implicated in 5-HT-agonist induced back muscle contractions, since 5,7-DHT pretreatment significantly enhanced this behaviour. However, the wet-dog shake behaviour produced by TRH and 5-HT agonists may not be mediated by the same spinal pathways as they were differentially affected by 5,7-DHT.

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