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Evidence for involvement of 5-HT_2 and 5-HT_{1C} receptors in the behavioral effects of the 5-HT agonist 1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI)

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DOI (1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2) has recently been suggested as a selective 5-HT_2 receptor agonist, but its behavioral effects have not been previously reported. In naive rats, DOI induced dose-dependent shaking behavior, the novel behavior 'skin jerks' (paraspinal muscle contractions), and forepaw tapping of the 'serotonin syndrome.' These behaviors had a similar dose-response and time course and were blocked by the 5-HT_2/5-HT_{1C} antagonists mianserin, ritanserin, and methysergide. Skin jerks, unlike other behaviors, were not blocked by l-propranolol or phenoxybenzamine, drugs with little activity at 5-HT_2/5-HT_{1C} sites. Differences in the pharmacology and neuroanatomy between skin jerks and shaking behavior suggest that the 5-HT_{1C} receptor may participate in skin jerks and the 5-HT_2 receptor in shaking behavior, but drug coaffinities for 5-HT_2 and 5-HT_{1C} receptors require further investigation.

Delineation of the serotonin (5-HT) receptors which mediate serotonergic behaviors has been the focus of considerable research. Behavioral correlates have been identified for only a few of the seven 5-HT binding sites described in the rat (5-HT_{1A-C}, 5-$HT_{2A(H),B(L)}$, 5-HT_3, 5-HT uptake). Shaking behaviors evoked by serotonin agonists, such as head shakes and wet-dog shakes, have been linked to 5-HT_2 receptor activation [1, 20, 27], but serotonergic behaviors such as myoclonus and other motor abnormalities of the 'serotonin syndrome' [14] have been ascribed both to 5-HT_{1A} [8, 15, 26] and 5-HT_2 [11, 19, 22] receptors. Selective 5-HT_2 agonists have only recently become available to help resolve these issues.

Glennon and co-workers have identified several phenylisopropylamine derivatives as potential 5-HT_2 agonists, including 1-(2,5-dimethoxy-4-substituted phenyl)-2-ami-

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nopropanes, such as DOB (bromo-substitution) and DOI (iodo-substitution) [5, 6]. The selectivity of DOB for the agonist-labelled 5-HT_2 site is 60-fold greater than for the antagonist-labelled 5-HT_2 site [6, 16, 25] and 8000-fold more than the 5-HT_{1A} site [25]. This improves on the selectivity of the earlier putative 5-HT_2 agonist, quipazine [3, 7], which is active as well at the 5-HT_{1B}-like 5-HT terminal autoreceptor [17, 24]. The curious co-affinity of 5-HT_2 agonists for 5-HT_{1C} sites also raises a new issue: do 5-HT_2 for 5-HT_{1C} sites have different behavioral correlates? DOB is 157-fold more selective for 5-HT_2 agonist-labelled sites than 5-HT_{1C} sites, compared to 25-fold for quipazine, 33-fold for ketanserin, and 1.7-fold for l-propranolol [25].

We previously reported the effects of DOI on locomotor activity [23]. This is the first report of the syndrome of behaviors induced by DOI. The capacity as blockers of putative 5-HT_{1A} (l-propranolol, spiroxatrine), 5-HT_2 (ritanserin, mianserin, methysergide) (8), and other receptor antagonists were also studied. Mianserin possesses the highest affinity for 5-HT_{1C} binding sites of the drugs studied [5].

Drugs were prepared before each experiment and dosages (1 ml/kg) were calculated according to body weights determined on the day of testing. DOI is commercially available only as a racemic mixture, but (−)-DOI is the more active isomer [5]. L-Propranolol (with warming) (Ayerst), (±)-DOI HCl (1-(2,5-dimethoxy-4-iodophenylaminoproprane)-2) (RBI), mianserin HCl (with HCl) (RBI), methysergide maleate (with HCl) (Sandoz), and sulpuride (with HCl) (Sigma) were sonicated in 0.9% saline. Drug vehicle was 50% each of ethanol and propylene glycol for ritanserin (Janssen), phenoxybenzamine (RBI), and spiroxatrine (RBI). Haloperidol (McNeil) was obtained in solution (5 mg/ml).

Male Sprague–Dawley, 175–200 g rats (Charles River) were housed 3 to a cage with free access to food and water under constant temperature (23°C) and a 12-h light–dark cycle. After acclimatizing to test room conditions in their home cages, rats were placed in a clear plastic 21 × 24 × 45 cm test cage between 10.00 and 18.00 h. Rats received only one dose of a single drug. Doses for dose–response experiments were selected on the basis of preliminary experiments. Antagonists were injected intraperitoneally 45 min prior to agonists, which were injected subcutaneously. Therefore, each rat received two injections at each testing session: either an agonist or saline, and either an antagonist or its vehicle.

An observer blind to drug treatment counted the number of head shakes and wet-dog shakes [12] in the 30 min following drug injection, and recorded them at 5-min intervals. At those same times, a score was awarded for forepaw tapping (reciprocal forepaw treading), rearing (both forepaws in air), vertical head movements/sniffing, hyper-reactivity to handling, hindlimb abduction, tail abnormalities (stiffening or snake-like movement), lateral head weaving (slow side-to-side head turning), body tremor (oscillations), skin jerks, backing, and pivoting (tight circling). Using a graded scoring method, behaviors were rates as absent (0), trace (1), mild or infrequent (2), moderate or intermediate (3), severe or continuous (4) [21]. Activity counts (Automex II, Columbus Instruments) were automatically recorded simultaneously. Body temperature was then taken rectally with a Becton-Dickinson digital thermometer at 20°C ambient temperature. Data were analyzed by analysis of variance (ANOVA),

and for significant main effects, multiple comparisons between groups were made using the Newman–Keuls test. ID_{50}s (the dose of blocker to halve response frequency or magnitude) [12] were computed by linear regression after log-logit transformation of the data.

DOI evoked dose-dependent shaking behavior at low doses, including head shakes and wet-dog shakes, lasting 60–90 min (Fig. 1). The maximum shaking response occurred at 3–6 mg/kg DOI. When head shakes and wet-dog shakes were counted

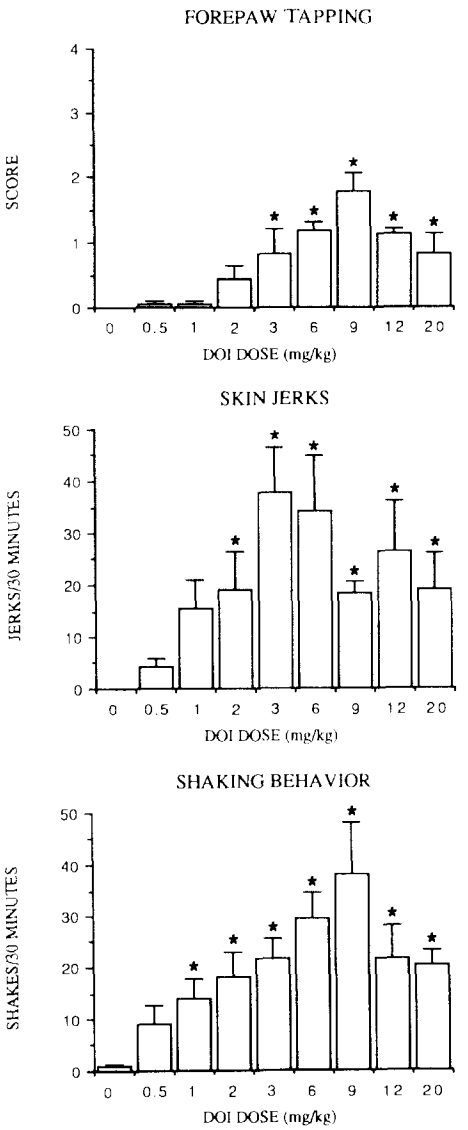

Fig. 1. DOI dose–response studies. Shakes of either head or trunk and skin jerks were counted immediately following drug injection. Forepaw tapping was scored at 5-minute intervals. Data are means ± S.E.M. of 5–7 rats for each dose of DOI. $P < 0.05$, Neuman–Keuls test, compared to dose 0 (saline).

TABLE I

ID_{50}s (mg/kg) FOR BLOCKERS OF DOI-EVOKED BEHAVIORS

ID_{50}s were calculated from 5 to 8 doses (mg/kg) of ritanserin (0.05–1), mianserin (0.01–5), l-propranolol (1–40), and phenoxybenzamine (1–20); $n = 4$–6 rats/dose. The dose of 3 mg/kg DOI was chosen from dose–response studies shown in Fig. 1. [a]No significant effect. [b]Biphasic response, reduction at low doses only. –, not measured. r correlation coefficient of linear regression.

	Ritanserin		Mianserin		l-Propranolol		Phenoxybenzamine	
	ID_{50}	(r)	ID_{50}	(r)	ID_{50}	(r)	ID_{50}	(r)
Shaking behavior	0.41	(0.91)	0.02	(0.89)	25.2	(0.97)	b	
Skin jerks	0.13	(0.91)	0.05	(0.75)	a		a	
Forepaw tapping	0.23	(0.99)	0.21	(0.87)	26.0	(0.76)	16.9	(0.78)
Hyperthermia	0.49	(0.79)	–		1.38	(0.91)	< 1	
Activity counts	0.30	(1.00)	–		32.3	(1.00)	0.84	(1.00)

separately at 3 mg/kg DOI, wet-dog shakes tended to occur earlier than head shakes but with overlap.

DOI also induced dose-related forepaw tapping of the serotonin syndrome. The dose threshold of forepaw tapping (3 mg) was similar to DOI-evoked shaking behavior (1 mg) and skin jerks (2 mg). These behaviors did not differ in their time course.

DOI induced easily quantitated paraspinal muscle contractions over the back which briefly drew up the skin. These were called 'skin jerks' for their characteristic appearance. The ratio of skin jerks and shaking behavior was approximately one at all DOI doses, yet the behaviors were not time-locked events. The frequency of skin jerks (like shaking behavior) was evenly distributed over each 5-min interval. We also noted that skin jerks typically occurred when the rat was in motion (action-associated). The DOI-evoked syndrome lacked coarse, palpable truncal myoclonus. However, skin jerks resemble myoclonus, and electrophysiological studies will be required for further characterization.

At 20°C ambient temperature, DOI 3 mg/kg raised body temperature 1.5 ± 0.3°C as compared to saline, but there was no significant dose effect. Ritanserin, l-propranolol, methysergide, haloperidol, and phenoxybenzamine prevented the increase (Tables I and II), but only L-propranolol (> 20 mg/kg) and phenoxybenzamine (> 10 mg/kg) significantly reduced temperature (-2.5 and -1.2°C) below values for saline-injected rats, respectively. The putative 5-HT_{1A} drug spiroxatrine, however, did not reduce temperature, and higher doses could not be tested due to poor drug solubility.

Several drug pretreatments significantly reduced DOI-induced shakes, skin jerks, and forepaw tapping. Each behavior was blocked by the 5-HT_2 antagonists ritanserin, mianserin, and methysergide ($P < 0.0001$), but only skin jerks were not blocked by the 5-HT_{1A} antagonist l-propranolol. Mianserin was the most potent blocker of DOI-induced skin jerks and the only drug to block shakes and skin jerks at a lower dose than forepaw tapping. The ID_{50} of shaking behavior of mianserin was 30-fold

TABLE II

EFFECTS OF DRUGS TESTED AT SINGLE DOSES AS BLOCKERS

Vehicle or blocker was injected 45 min prior to 3 mg/kg DOI, and behaviors were studied for the next 30 min. Temperature was taken at the end of the session. Data are means ± S.E.M. for 4–6 rats. –, not measured.

Drugs	Dose (mg/kg)	Temperature (°C)	Shakes	Skin jerks	Forepaw tapping	Activity counts
Saline + vehicle		37.1 ± 0.2	0	0	0	1124 ± 325
DOI + vehicle		38.5 ± 0.2	34 ± 4	42 ± 8	2.1 ± 0.2	1486 ± 216
DOI + blocker						
Spiroxatrine	10	37.9 ± 0.3	0*		0*	87 ± 40*
Methysergide	20	37.2 ± 0.4*	0*	0*	0*	1059 ± 315
Haloperidol	2.5	36.8 ± 0.2*	0*		0*	134 ± 47*
Sulpuride	25	37.7 ± 0.4	45 ± 9		2.4 ± 0.3	1706 ± 315

*$P < 0.05$ compared to vehicle.

less than ritanserin and 1200-fold less than l-propranolol. Phenoxybenzamine resembled l-propranolol but had biphasic effects. Except for methysergide, drugs which significantly reduced shaking behavior also reduced activity counts. The blocking effects of the neuroleptic haloperidol were not imitated by the non-neuroleptic sulpuride.

There was no dose-dependence to other behaviors scored as moderate to mild (vertical head movements/sniffing, hunching), mild to trace (head weaving, rearing, tail abnormalities, hyper-reactivity) or trace to absent (body tremor, backing, pivoting, hindlimb abduction). Blocker studies also showed no consistent effects across categories of blockers on these behaviors.

This is the first report that the selective 5-HT_2 agonist DOI induces shaking behavior, skin jerks, and forepaw tapping behavior of the serotonin syndrome. The finding that a 5-HT_2 agonist evokes shaking behavior was expected [1, 8, 15, 20, 27], whereas the induction of a principal, serotonin syndrome behavior had been proposed by some [8, 11, 19, 22] but not others [5, 26]. The low affinity of DOI at 5-HT_{1A} sites [25] suggests that DOI did not evoke forepaw tapping by direct action at 5-HT_{1A} sites. Since 5-HT_{1A} agonists also induce forepaw tapping [8], effectors for forepaw tapping may be influenced by drugs acting at either 5-HT_2 or 5-HT_{1A} receptors.

Skin jerks are an easily and reliably quantifiable serotonergic model. Since skin jerks are of spinal origin [2] and there are 5-HT_{1C} [13] but few spinal 5-HT_2 receptors [18], skin jerks may be 5-HT_{1C} receptor-mediated. Shaking behavior, in contrast, is mediated by hippocampus or midbrain, where 5-HT_2 receptors are also present [12, 16, 27, 28]. In further support of the pharmacologic differentiation of skin jerks and shaking behavior, shaking behavior but not skin jerks was blocked by l-propranolol, a drug with little activity at 5-HT_{1C} or 5-HT_2 receptors. However, DOI time course and dose-response did not allow putative 5-HT_{1C} and 5-HT_2 receptor-mediated behaviors to be differentiated. Although mianserin was the most potent blocker, like others drugs active at 5-HT_{1C} sites, it is also active at 5-HT_2 sites and was equally

effective in blocking shaking behavior and skin jerks. The pharmacologic relation of 5-HT_{1C} to 5-HT_2 receptors is itself yet undefined and remains an important area for further investigation. Selective 5-HT_{1C} agonists or 5-HT_2 agonists without 5-HT_{1C} properties will be necessary to resolve this issue.

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