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A novel behavioral model that discriminates between 5-HT_{2A} and 5-HT_{2C} receptor activation

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Abstract

2,5-Dimethoxy-4-iodoamphetamine (DOI), a serotonin (5-HT)$_{2A/2C}$ receptor agonist, elicits shaking behaviors in rodents, which have been reliably quantified as behavioral correlates of 5-HT_{2A} receptor activation. Such studies are lacking in the rabbit. As part of our research examining the role of the 5-HT_2 receptor in rabbits, we analyzed the behavioral effects of systemically administered DOI in rabbits. DOI (0.01–3 μmol/kg) or vehicle was injected, and two distinct behaviors, head bobs (vertical head movements) and body shakes (wet dog shakes), were counted for 90 min following the injection. DOI dose-dependently increased the number of head bobs and body shakes. The selective 5-HT_{2A} receptor antagonist ketanserin (1–3 μmol/kg), 1 h before DOI (0.3 μmol/kg) challenge, significantly attenuated head bobs, but not body shakes. In contrast, the selective 5-HT_{2C} receptor antagonists SDZ SER 082 (1–3 μmol/kg) and SB 206553 (1 μmol/kg) 30 min before challenge, significantly reduced body shakes but not head bobs produced by the same dose of DOI. This study establishes that, in rabbits, DOI mediates head bobs via 5-HT_{2A} receptors and body shakes via 5-HT_{2C} receptors. Thus, the rabbit provides a novel behavioral assay that discriminates between 5-HT_{2A} and 5-HT_{2C} receptor activation.

Keywords: DOI; Rabbit; 5-HT_{2A} receptor; 5-HT_{2C} receptor; Head bobs; Body shakes; Behavioral model

1. Introduction

Administration of compounds that increase synaptic serotonin (5-HT) (e.g., monoamine oxidase inhibitors, 5-HT precursors and 5-HT-releasing agents) or directly stimulate postsynaptic 5-HT receptors have been shown to produce a stereotypical behavioral syndrome in rodents (Jacobs, 1976). This hyperexcitability syndrome consists of two behavioral features, ''the 5-HT syndrome'' (forepaw treading, hindlimb abduction, lateral head weaving, straub tail, elongation of the trunk and resting tremor) and ''shaking behaviors'' (head shakes, head twitches and wet dog shakes). The 5-HT syndrome and shaking behaviors have been studied extensively as behavioral models for the activation of CNS 5-HT receptors in rodents (Bedard and Pycock, 1977; Grahame-Smith, 1971), and pharmacological analyses have been used to relate subsets of these behaviors with activation of different 5-HT receptors in the CNS. Administration of 5-HT_{1A} agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) elicits forepaw treading, hindlimb abduction, lateral head weaving, straub tail and elongation of the trunk (Hjorth et al., 1982; Tricklebank et al., 1984) but does not elicit head or body shakes, indicating that the 5-HT syndrome is mediated by 5-HT_{1A} receptors. In contrast, administration of partial 5-$HT_{2A/2C}$ receptor agonists such as 2,5-dimethoxy-4-iodoamphetamine (DOI), D-lysergic acid diethylamide (LSD) and quipazine induce dose-dependent shaking behaviors including head shakes, wet-dog shakes and head twitches in rodents (Pranzatelli, 1990; Vetulani et al., 1980; Schreiber et al., 1995). Head shakes, wet dog shakes and head twitches induced by DOI were blocked by mixed 5-$HT_{2A/2C}$ receptor antagonists such as ritanserin and mianserin, selective 5-HT_{2A} antagonists ketanserin and *R*-(+)-(2,3-dimethoxyphenyl)-1-[2-(4-flourophenylethyl)]-4-piperidine-methnol (MDL 100,907), but not with the selective 5-HT_{2C} receptor antagonists 1-(1-methylindol-5-yl)-3-(3-pyridyl) urea (SB 200646A) and SDZ SER 082, implicating 5-HT_{2A} receptors in mediating the shaking response (Dursun and Handley 1996; Pranzatelli, 1990; Schreiber et al., 1995; Wettstein et al., 1999; Willins and Meltzer, 1997). Indeed, there was a high degree of correlation between the potency of various antagonists to block DOI-induced shaking behaviors

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and their affinity at 5-HT_{2A} receptors. Thus, shaking behavior in rodents provide a consistent measure of 5-HT_{2A} receptor activation but not 5-HT_{2C} receptor activation.

Preliminary observations in the rabbit have indicated that DOI induced two distinct and easily identifiable behaviors consisting of a vertical head bob and a whole body shake. In the present study, we analyzed the behavioral effects of systemically administered DOI in rabbits to develop a behavioral model of 5-$HT_{2A/2C}$ receptor activation. Pharmacological analyses were performed to determine which DOI-induced behaviors were mediated by 5-HT_{2A} or 5-HT_{2C} receptors by pretreating with the selective 5-HT_{2A} receptor antagonist ketanserin and the selective 5-HT_{2C} receptor antagonists SB 206553 and SDZ SER 082. Additionally, radioligand binding assays were performed to determine the relative affinities of the antagonists used in the behavioral experiments.

2. Materials and methods

2.1. Animals

New Zealand white rabbits (Covance, Denver, PA, USA) weighing 1.6–1.8 kg were housed individually upon arrival. Male rabbits were utilized for the behavioral experiments, whereas rabbits of either gender were used in the binding experiments. They were kept under a 12/12-h light/dark cycle with access to 125 g of chow per day and free access to water in an AAALAC-approved animal facility maintained at 22 °C. Rabbits were adapted to their home cages for 3 days and were handled by a lab personnel for at least 2 days before the initiation of experiments. All animal experiments were carried out in accordance with the National Institute of Health guide ''Principles of Laboratory Animal Care'' (NIH publication No. 85-23, revised 1985) and were approved by our Institutional Animal Care and Use Committee.

2.2. Drugs

(±)-DOI hydrochloride, ketanserin hydrochloride, 5 methyl-1-(3-pyridil-carbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB 206553 hydrochloride), spiperone hydrochloride and methysergide maleate were purchased from RBI (Natick, MA, USA). (+)-*cis*-4,5,7*a*,8,9,10,11,11*a*-Octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]-naphthyridine (SDZ SER 082 fumarate), prazosin hydrochloride and 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)-phenyl-5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione (RS 102221 hydrochloride) were purchased from Tocris Cookson (Ballwin, MO, USA). DOI and ketanserin were dissolved in saline. SB 206553 and SDZ SER 082 were dissolved in deionized water. All drugs were prepared fresh on the day of the experiment. Drugs or their vehicle were injected subcutaneously (between the scapula) in a volume of 1 ml/kg.

2.3. Behavioral observations

The following behaviors were counted cumulatively at 5-min intervals and the mean ± S.E.M. scores were calculated for a 90-min observation period (or 30 min in some cases) after saline or DOI administrations: Head bobs (rapid sequential ''down-up'' movement of the head without any intervening behaviors), body shakes (a paroxystic shudder of the head, neck and trunk combined, similar to wet dog shakes in rodents) and grooming. With respect to the grooming behavior, the duration of time engaged in this activity over the 90-min period was also recorded. In addition, we also recorded head twitches (rapid side-to-side head movements) that are 5-HT_{2A} receptor-mediated behaviors normally elicited by DOI in the rodent. Finally, we also looked for the 5-HT_{1A} receptor-mediated 5-HT syndrome behaviors (forepaw treading, hindlimb abduction, lateral head weaving, straub tail and elongation of the trunk). All observations took place in the animal's home cage between 09:00 and 15:00 h.

2.4. Experimental procedures

Food hoppers and water bottles were removed immediately before DOI or saline administration. One group ($n = 6–16$) of rabbits was given saline (1 ml/kg) or DOI (0.01–3 μmol/kg) injections and was observed for 90 min for the indicated behaviors. Separate groups of 4–15 animals were pretreated with ketanserin (1 and 3 μmol/kg), SDZ SER 082 (1 and 3 μmol/kg) and SB 206553 (1 μmol/kg), or vehicle before testing with DOI (0.3 μmol/kg). The pretreatment interval for ketanserin was 60 min, whereas the pretreatment interval for SDZ SER 082 and SB 206553 was 30 min. Food and water were available during the antagonist or vehicle pretreatment interval but were removed immediately before DOI administration.

2.5. Radioligand binding assays

Immediately following sacrifice of the rabbit by decapitation, the brain was rapidly removed and the frontal cortex was dissected free and frozen on dry ice. Cortices were stored at −70 °C until used in receptor binding assays. On the day of the binding assay, the still frozen cortex was placed in 10 vol (by weight) of ice-cold homogenization buffer (50 mM Tris–HCl pH 7.4 at 0 °C) and immediately homogenized using a Brinkman Polytron (10 s at half power). All subsequent steps were performed at 0–4 °C. Tissue homogenate was centrifuged at 40,000 × *g* for 20 min. The resulting pellet was resuspended in 50 vol of homogenization buffer using a Brinkman Polytron (10 s at half power) followed by centrifugation as described above. The washed membrane fraction was dispersed in room temperature assay buffer (20 mM Tris–HCl pH 7.4 at 20 °C) using a Polytron.

2.5.1. 5-HT_{2A} receptor analysis

5-HT_{2A} receptors were analyzed using the selective antagonist [^{3}H]ketanserin (specific activity 63.3 Ci/mmol; New England Nuclear, Boston, MA, USA) in the presence of RS 102221 and prazosin (each at 30 nM, final concentration) to prevent binding to 5-HT_{2C} receptors and α_1-adrenoceptors, respectively. Rabbit cortical membranes were incubated at 25 °C with 0.4 nM [^{3}H]ketanserin and eight concentrations of test drug. The assay was initiated by the addition of washed membranes (4 mg) in a total volume of 1 ml. Nonspecific binding was defined by the addition of 100 nM spiperone. The mixture was incubated for 120 min at 25 °C before being terminated by rapid filtration through Whatman GF/B filters (presoaked in 0.5% polyethylenimine) followed by three washes each consisting of 5 ml of wash buffer (20 mM Tris–HCl, pH 7.4 at 4 °C). The amount of radioactivity retained on the filter was determined by liquid scintillation counting.

2.5.2. 5-HT_{2C} receptor analysis

5-HT_{2C} receptors were analyzed using [^{3}H]mesulergine (specific activity 74 Ci/mmol; Amersham Life Sciences, Arlington Heights, IL, USA) in the presence of spiperone (30 nM, final concentration) to prevent mesulergine binding to 5-HT_{2A} and dopamine D_2 receptors as previously described (Aloyo and Harvey, 2000). Rabbit cortical membranes were incubated at 25 °C with 0.4 nM [^{3}H]mesulergine and eight concentrations of test drug in a 1-ml final assay volume. The binding was initiated by the addition of washed membranes (4 mg). Nonspecific binding was defined by 1 μM methysergide. The assay was performed in glass tubes, and all pipettes were siliconized to prevent loss of the [^{3}H]mesulergine. The mixture was incubated for 120 min at 25 °C before being terminated by rapid filtration followed by three washes as described above. The amount of radioactivity retained on the filter was determined by liquid scintillation counting.

2.6. Data analysis

Behavioral data were subjected to an analysis of variance, and post hoc analyses were done by Dunnett's *t* test with the limit of significance set at $P < .05$. The ED_{50} values were calculated via linear regression. The K_i was calculated from the binding data using the nonlinear, curve-fitting program LIGAND (Munson and Rodbard, 1980) as modified for Macintosh computers.

3. Results

3.1. Baseline behaviors

The baseline (saline) frequency of head bobs and body shakes during the 90-min observation period was 0.6 ± 0.2 and 2.2 ± 0.5, respectively. Head twitches and the 5-HT syndrome behaviors (forepaw treading, hindlimb abduction, lateral head weaving, straub tail and elongation of the trunk) were absent in saline-treated animals. The frequency and duration for baseline grooming behavior was 51 ± 20 and 760 ± 553 s, respectively.

3.2. DOI dose–response curves

Systemically administered DOI (0.01–3 μmol/kg) elicited only two very distinct behaviors: head bobs and body shakes (Fig. 1). DOI at any of the doses tested did not induce other head or body movements that could be confused with a head bob or a body shake. Head twitches (induced by DOI in rodents) and the 5-HT syndrome behaviors were absent at all the doses of DOI tested. The frequency and duration of grooming behavior was only measured at the DOI dose of 0.3 μmol/kg. The frequency and duration of grooming at this dose of DOI was 46 ± 3 and 547 ± 325 s, respectively, which was not significantly different compared to baseline.

The dose-dependent activation of head bobs was significantly greater than that observed in the vehicle treated animals at all the doses tested except for 0.01 μmol/kg [$F(6,66) = 19.17$, $P < .05$]. DOI also dose-dependently elicited body shakes, with the highest three doses (0.3–3.0 μmol/kg) being significantly greater than that observed in vehicle treated animals [$F(6,71) = 13.84$, $P < .05$]. The ED_{50} values (95% confidence limits) for head bobs and body shakes were approximately 0.040 (0.01–0.12) and 0.160 (0.06–0.42) μmol/kg, respectively. This fourfold difference in the ED_{50} values was not significant due to the overlap of the 95% confidence intervals. The maximum number of head bobs and body shakes occurred at the 1.0 μmol/kg (approximately 0.35 mg/kg) dose of DOI with values of 38.7 ± 9.1 for head bobs and 17.7 ± 3.7 for body

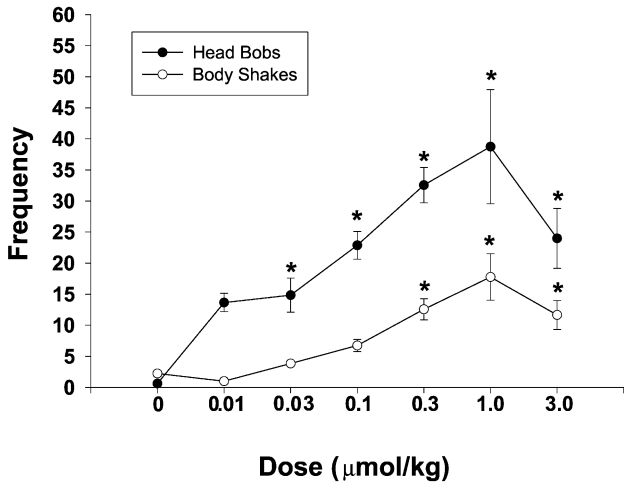

Fig. 1. DOI dose–response curves. Head bobs and body shakes were counted immediately following DOI injection for 90 min. Data are means ± S.E.M. of 6–16 rabbits for each dose of DOI. * $P < .05$, Dunnett's test, compared to Dose 0 (veh).

shakes. Immobility was seen in some animals at the 3.0 μmol/kg (approximately 1 mg/kg) dose of DOI, and this appeared to account for the drop in frequency of bobs and shakes at that dose. Based on the dose–effect curve (Fig. 1), we selected the 0.3 μmol/kg dose of DOI for the antagonist studies.

3.3. Effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonists on DOI-induced behaviors

Ketanserin (1 and 3 μmol/kg) pretreatment significantly attenuated head bobs induced by the 0.3-μmol/kg dose of DOI in the 90-min observation period (Fig. 2) [$F(2,13) = 129.04$, $P < .05$] but had no significant effect on the body shakes induced by DOI [$F(2,13) = 2.00$]. In contrast, pretreatment with the selective 5-HT_{2C} antagonist SB 206553 (1 μmol/kg) significantly attenuated body shakes but not head bobs produced by the same dose of DOI in the 90-min observation period (Fig. 3) [$F(2,16) = 25.49$, $P < .05$]. Similarly, pretreatment with the 5-HT_{2C} antagonist SDZ SER 082 (1 and 3 μmol/kg) significantly attenuated body shakes induced by the same dose of DOI (Fig. 3) [$F(2,17) = 27.38$, $P < .05$]. The low dose of SDZ SER 082 (1 μmol/kg) did not significantly attenuate head bobs. However, the high dose of SDZ SER 082 (3 μmol/kg) significantly attenuated head bobs induced by DOI [$F(2,17) = 6.23$, $P < .05$], which is in keeping with the binding data that demonstrated that SDZ SER 082 is far less selective for 5-HT_{2C} compared to 5-HT_{2A} receptors relative to SB 206553 (Table 1). The time course for the increase in head bobs and body shakes produced by DOI is presented in Fig. 4. The time course for ketanserin (1 μmol/kg) antagonism (Fig. 4, upper panel) illustrated that ketanserin significantly attenuated head bobs

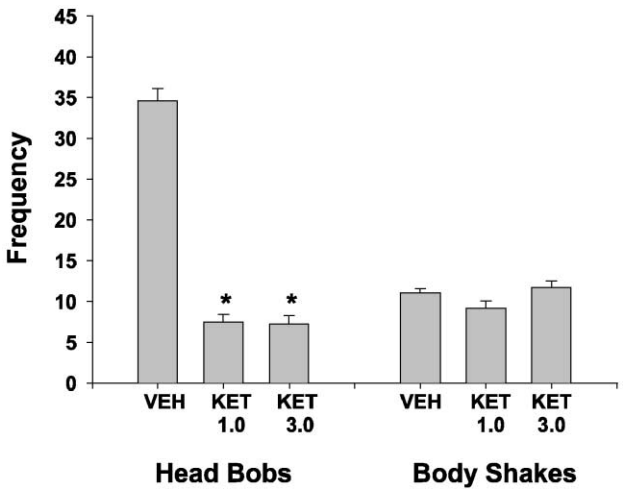

Fig. 2. Effect of the selective 5-HT_{2A} receptor antagonist pretreatment on DOI-induced behaviors. Animals were pretreated either with the selective 5-HT_{2A} receptor antagonist ketanserin (1 and 3 μmol/kg, $n = 4$/dose) or vehicle ($n = 8$) an hour before DOI challenge (0.3 μmol/kg). Behaviors were counted immediately after DOI injection for 90 min. Results are shown as means ± S.E.M. * Significantly different from the vehicle-pretreated group; Dunnett's test, $P < .05$. KET = ketanserin; VEH = vehicle.

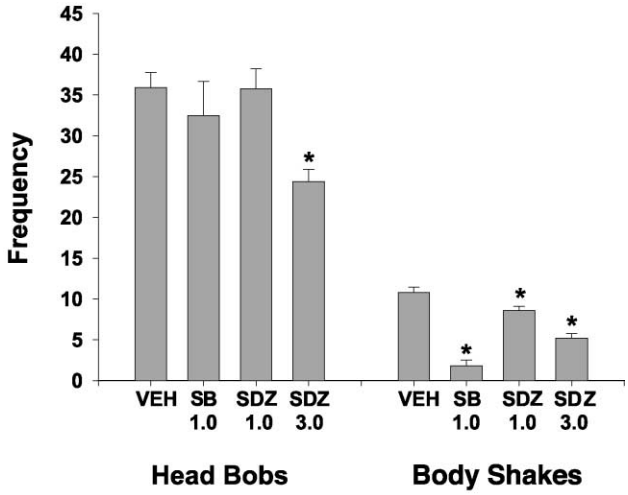

Fig. 3. Effects of the 5-HT_{2C} receptor antagonists pretreatment on DOI-induced behaviors. Animals were pretreated with either SDZ SER 082 (1 and 3 μmol/kg, $n = 5$/dose), SB 206553 (1 μmol/kg, $n = 5$) or vehicle ($n = 15$) half an hour before DOI challenge (0.3 μmol/kg). Behaviors were counted immediately after DOI injection for 90 min. Results are shown as means ± S.E.M. * Significantly different from the vehicle pretreated group; Dunnett's test, $P < .05$. SB = SB 206553; SDZ = SDZ SER 082; VEH = vehicle.

but not body shakes compared to the vehicle pretreated group at each of the three 30-min intervals [$F(2,20) = 8.88$, $P < .05$; $F(2,20) = 4.07$, $P < .05$; $F(2,20) = 7.86$, $P < .05$]. The time course for SB 206553 (1 μmol/kg) antagonism (Fig. 4, lower panel) illustrated that SB 206553 significantly attenuated body shakes but not head bobs compared to vehicle pretreated group in each of the 30-min intervals [$F(2,20) = 6.08$, $P < .05$; $F(2,20) = 6.37$, $P < .05$; $F(2,20) = 10.28$, $P < .05$].

3.4. Radioligand binding assays

Binding assays were performed to verify the selectivity of the antagonists employed in this study. The K_i for SB 206553 inhibition of mesulergine binding to the 5-HT_{2C} receptor was approximately 1 nM, whereas the K_i for SDZ SER 082 was approximately 10 nM (Table 1). Both SB 206553 and SDZ SER 082 exhibited a higher affinity for

Table 1
Binding affinities at the rabbit 5-HT_{2A} and 5-HT_{2C} receptors

	K_i	
Drug	5-HT_{2A}	5-HT_{2C}
SB 206553	1036 ± 177[a]	1.38 ± 0.1[b]
SDZ SER 082	469 ± 88[a]	10.0 ± 0.47[b]
Ketanserin	0.54 ± 0.10[c,d]	15.8 ± 0.67[b,d]

Each value is the mean ± S.E.M. of three experiments.
[a] Values are the K_i (nM) for displacement of [^{3}H]ketanserin.
[b] Values are the K_i (nM) for displacement of [^{3}H]mesulergine.
[c] Values are the K_i (nM) for displacement of [^{3}H]MDL 100,907.
[d] Values were taken from Aloyo and Harvey 2000.

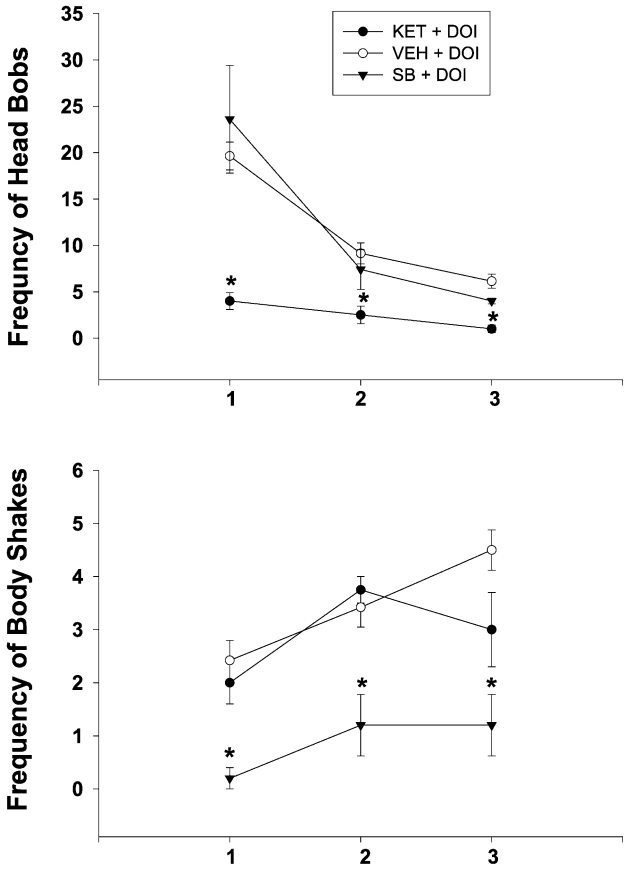

Fig. 4. Time course of DOI behaviors in the rabbit and effects of 5-$HT_{2A/2C}$ receptor antagonists. Data presented in Figs. 2 and 3 for total number of head bobs and body shakes were divided into three 30-min time intervals after DOI administration to illustrate the distribution of behavior over 90 min (1 = *0–29 min*, 2 = *30–59 min*, 3 = *60–89 min*). Animals were pretreated either with ketanserin (1 μmol/kg, $n = 4$), SB 206553 (1 μmol/kg, $n = 5$) or vehicle ($n = 15$) before DOI challenge (0.3 μmol/kg). Results are shown as means ± S.E.M. * Significantly different from the vehicle-pretreated group in the 30-min time interval; Dunnett's test, $P < .05$.

rabbit 5-HT_{2C} receptors compared to 5-HT_{2A} receptors; SDZ SER 082 was approximately 45-fold selective, whereas SB 206553 was approximately 750-fold selective. In contrast, ketanserin exhibited high affinity for 5-HT_{2A} receptors (0.5 nM) and was approximately 30-fold selective for the 5-HT_{2A} receptors compared to the 5-HT_{2C} receptors (Table 1).

4. Discussion

This study demonstrated that the selective 5-$HT_{2A/2C}$ receptor agonist DOI induced two easily quantifiable behaviors in the rabbit, head bobs and body shakes. These two behaviors were distinct and dose-dependent and were mediated by separate receptors in that head bobs were 5-HT_{2A} receptor-mediated and body shakes were 5-HT_{2C} receptor-mediated. DOI's relative selectivity for the 5-HT_{2A} receptor versus the 5-HT_{2C} receptor has been variously reported to be similar or up to 40-fold different (Barnes and Sharp, 1999; Fitzgerald et al., 1999; Nelson et al., 1999; Titeler et al., 1988; Wainscott et al., 1996). In our study, the ED_{50} value for the DOI-induced body shakes (5-HT_{2C} receptor mediated) was fourfold higher than that for the production of head bobs (5-HT_{2A} receptor mediated). Although the difference was not significant, the observed difference in the ED_{50} value may be due to the differential affinity of DOI for the 5-HT_{2A} over the 5-HT_{2C} receptor in the rabbit.

4.1. DOI-mediated head movements in rodents versus rabbits

Head shakes and head twitches have been used as terms to describe head movements induced by DOI in rodents (Darmani et al., 1992; Lucki et al., 1984; Pranzatelli, 1990; Schreiber et al., 1995; Simansky and Schechter, 1988; Wettstein et al., 1999). It is not clear whether these are indeed different behaviors or are simply different names for the same response. For example, Matthews and Smith (1980) defined head shake as a rapid lateral twitch of the head, whereas Darmani et al., (1992) described head shake as lateral movements of the head from side-to-side that they differentiate from the head twitch response. In the rabbit, DOI dose-dependently induces a vertical head movement and does not induce any other head jerking, head shaking or head twitching movements, which could be mistaken for a head bob or any other head movement. It has been clearly established in earlier reports on the behavioral effects of DOI that 5-HT_{2A} receptors mediate head shakes and head twitches in rodents (Schreiber et al., 1995; Wettstein et al., 1999). Indeed, in rodents, there was a high degree of correlation between the efficacy of various antagonists to block DOI-induced head movements and their affinity at the 5-HT_{2A} receptor but not at the 5-HT_{2C} receptor. Similarly, in the rabbit, head bobs induced by DOI were attenuated by the selective 5-HT_{2A} receptor antagonist ketanserin. Doses of ketanserin that significantly attenuated head bobs in rabbits were similar to the doses used to block DOI-induced head shakes and head twitches in rodents (Schreiber et al., 1995; Willins and Meltzer, 1997). Head bobs peaked in the first 30 min after DOI administration and then rapidly declined. This is similar to the rodent where the head twitch behavior has a rapid onset with a peak response at 30 min (Green and Backus, 1990). Hence, head movements whether they are head twitches or head shakes in rodents or head bobs in rabbits are mediated by 5-HT_{2A} receptors.

4.2. DOI-mediated wet dog shakes in rodents versus rabbits

DOI has been shown to induce wet dog shakes in rodents (Pranzatelli, 1990; Wettstein et al., 1999). A wet dog shake is described as a shudder of the head, neck and trunk musculature (Bedard and Pycock, 1977). Similar to the head movements induced by DOI, wet dog shakes in

rodents have also been correlated with the activation of the 5-HT_{2A} receptors (Handley and Dursun, 1992; Handley and Singh, 1986). For example, 5-$HT_{2A/2C}$ antagonists such as ritanserin and mianserin and the 5-HT_{2A} selective antagonists such as ketanserin and MDL 100,907 attenuated or blocked DOI-induced body shakes (Pranzatelli, 1990; Wettstein et al., 1999). The present study demonstrates that DOI also induces whole body shakes in rabbits that are similar in appearance to the wet dog shakes observed in rodents. In fact, body shakes continued to increase in frequency over the 90-min observation period similar to wet dog shakes, which have a slower onset and last for hours (Green and Backus, 1990). However, unlike the rodent, body shakes in the rabbit are 5-HT_{2C} receptor mediated. This is in contrast with studies conducted in rodents that have failed to detect any role of the 5-HT_{2C} receptor in the production of shaking behaviors although other body movements such as ear scratch responses in mice (Darmani, 1992) and back muscle contractions in rats (Pranzatelli, 1990) may be 5-HT_{2C} receptor mediated. In the rodent, there was no effect on DOI-induced shaking behaviors (head twitches, head shakes and wet dog shakes) after pretreatments with selective 5-HT_{2C} receptor antagonists such as SDZ SER 082 and SB 200646A (Willins and Meltzer, 1997; Schreiber et al., 1995; Wettstein et al., 1999). Hence, separate subtypes of 5-HT receptors in rodents and in rabbits mediate shaking behaviors pointing to species differences in 5-HT receptor-mediated behaviors. Indeed, there is substantial data showing species differences in the pharmacology of the 5-HT_2 receptors (Aloyo and Harvey, 2000; Johnson et al., 1993; Nelson et al., 1993). There is a high degree of correlation between the binding affinities of various 5-$HT_{2A/2C}$ receptor antagonists for the rabbit and human 5-HT_{2A} and 5-HT_{2C} receptors (Aloyo and Harvey, 2000), indicating similar pharmacological profiles for corresponding receptors. Therefore, the current results suggest that the rabbit provides an appropriate animal model for investigating 5-HT_2 receptor-mediated effects in humans.

4.3. Binding affinities of the antagonists

As discussed before, species differences in the pharmacology of 5-HT_2 receptors have been established for various 5-HT_{2A} and 5-HT_{2C} receptor antagonists (Aloyo and Harvey, 2000). There is a possibility then that the contrasting results observed in the rabbit and the rodent with respect to 5-HT_{2C} receptor's role in mediating DOI-induced shaking behaviors could be due to differences in the pharmacology of the antagonists used. The pharmacology (affinity and selectivity) of ketanserin at the 5-HT_2 receptors is similar in the rat and the rabbit. For example, in rabbit cortical membranes, ketanserin is approximately 30-fold selective for 5-HT_{2A} receptors compared to 5-HT_{2C} receptors, which is similar to the 40-fold selectivity observed in rats (Aloyo and Harvey, 2000). Yet, pretreatments with similar doses of ketanserin failed to attenuate DOI-induced body shakes in rabbits while attenuating wet dog shakes in rodents (Fone et al., 1991). Since DOI has been reported to be a selective 5-HT_2 receptor agonist with similar affinity and efficacy at both 5-HT_{2A} and 5-HT_{2C} receptors (Glennon et al., 1992), the selective 5-HT_{2C} receptor antagonists SDZ SER 082 and SB 206553 were employed to determine if the 5-HT_{2C} receptors played a role in mediating body shakes induced by DOI in rabbits. In rabbit cortex, both SB 206553 and SDZ SER 082 are 5-HT_{2C} receptor selective ligands (Table 1). SDZ SER 082 has about 47-fold selectivity for 5-HT_{2C} compared to 5-HT_{2A} receptors, which is similar to the selectivity (40-fold) reported earlier by Nozulak et al. (1995). SDZ SER 082 at the 1.0 μmol/kg (0.3 mg/kg) dose significantly attenuated body shakes in the rabbit but not DOI-induced shakes in the rat (Willins and Meltzer, 1997). Only the high dose of SDZ SER 082 (3.0 μmol/kg) significantly attenuated head bobs in the rabbit, suggesting that the effect on head bobs was probably due to the interaction at the 5-HT_{2A} receptors at that high dose. SB 206553 has about 750-fold selectivity for the 5-HT_{2C} receptors over the 5-HT_{2A} receptors in rabbit cortical tissue, which is substantially greater from the earlier reported values of the selectivity of this compound (140-fold, Kennett et al., 1996). The dose of SB 206553 (1 μmol/kg or 0.3 mg/kg) that potently abolished *m*-(chlorophenylpiperazine) (MCPP)-induced (5-HT_{2C} mediated) penile erections in the rat (Millan et al., 1997) also significantly attenuated body shakes but not head bobs in the rabbit. Taken together, the binding data support the conclusion that DOI-induced wet dog shakes are 5-HT_{2A} receptor mediated in rodent but DOI-induced body shakes are 5-HT_{2C} receptor mediated in the rabbit.

4.4. Other observed behaviors

In rodents, DOI has been shown to induce dose-related forepaw tapping (Pranzatelli, 1990), a component of the 5-HT syndrome (5-HT_{1A}-mediated behavior). However, the low affinity of DOI at the 5-HT_{1A} receptor (Teitler et al., 1987) calls into question whether DOI elicited forepaw tapping is 5-HT_{1A} or 5-HT_{2A} receptor mediated (Pranzatelli, 1990). In the rabbit, DOI did not induce any 5-HT syndrome behaviors allowing for a clear separation of 5-HT_{1A}- and 5-HT_{2A}-mediated behaviors. DOI also had no effect on the grooming behavior in the rabbit. This is again in agreement with the past literature demonstrating that grooming is a behavioral measure for dopamine D_1 receptor activation (Drago et al., 1999) and not 5-HT_{2A} or 5-HT_{2C} receptor activation. Immobility was observed (but not quantified) at the highest dose of DOI (3 μmol/kg) and the decrease in head bobs and body shakes correlated with the appearance of immobility. This immobility could be due to the direct effect of DOI since 5-HT_2 agonists have been shown to suppress locomotor activity in rats (Mittman and Geyer, 1991; Wing et al., 1990).

4.5. Rabbit as an appropriate model for studying 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor activation

Since a goal of a behavioral model is to enable investigation of the functional significance of biochemical or pharmacological changes, it is important to have a consistent and an unambiguous quantitative assessment of neurotransmitter/receptor activity. In the rabbit, DOI increases the number of head bobs and body shakes, movements that also occur spontaneously although at a much lower rate. Head bobs and body shakes are behavioral correlates of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor activation, respectively. To our knowledge, this is the first behavioral model to discriminate between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor activation. Thus, the rabbit provides a simple preparation to study 5-HT$_2$ receptor-induced behaviors and will be useful for investigating changes in 5-HT$_{2A}$ receptors that have been shown to play a role in clinical disorders such as depression and schizophrenia (Roth et al., 1998) and in 5-HT$_{2C}$ receptors that have been implicated in anxiety, temperature regulation and feeding (Barnes and Sharp, 1999).

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