Psychopharmacology (2007) 190:221–231
DOI 10.1007/s00213-006-0619-5

ORIGINAL INVESTIGATION

Role of atypical opiates in OCD. Experimental approach through the study of 5-HT$_{2A/C}$ receptor-mediated behavior

M. Olga Rojas-Corrales · Juan Gibert-Rahola · Juan A. Mico

Received: 29 June 2006 / Accepted: 16 October 2006 / Published online: 11 November 2006

Abstract
Rationale The selective serotonin (5-HT) reuptake inhibitors (SSRIs) represent the first-line pharmacotherapy for obsessive–compulsive disorder (OCD), and atypical antipsychotic drugs, which block 5-HT2A receptors, are used in augmentation strategies. Opiate drugs are also effective in treatment-refractory OCD and Tourette syndrome. The 5-HT2A-related behavior (i.e., head twitch) has been related with tics, stereotypes, and compulsive symptoms observed in Tourette syndrome and OCD.
Objectives The aim of this study was to explore whether 5-HT2A-related behavior is affected by atypical opiate drugs.
Materials and methods Head-twitch response was induced in mice by administration of either 5-hydroxytryptophan (5-HTP) or the 5-HT2A/C agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Dose–effect curves of atypical opiate drugs [(±)-tramadol, (−)-methadone and levorphanol], morphine, and other psychoactive drugs (fluvoxamine, desipramine, nefazodone, and clozapine) were performed. Opioid mechanisms were investigated by administration of naloxone.
Results All the opiates tested reduced both 5-HTP and DOI-induced behavior in a naloxone-reversible fashion, atypical opiates being more effective. The effects of the other drugs depended on the protocol, clozapine being the most effective.
Conclusions Combined 5-HT and opioid properties result in a greater efficacy in antagonizing 5-HT2A-related behavior. These results provide behavioral evidence to support convergent effects of the 5-HT and opioid systems in discrete brain areas, offering the potential for therapeutic advances in the management of refractory stereotypes and compulsive behaviors.

Keywords Atypical opiates · Head twitch · Opioid · 5-HT2A · Compulsive behavior · Stereotypes

M. O. Rojas-Corrales · J. Gibert-Rahola · J. A. Mico (✉)
Group of Research and Development in Neuropsychopharmacology, Department of Neuroscience, Faculty of Medicine, University of Cádiz,
Plz Falla 9,
11003 Cádiz, Spain
e-mail: juanantonio.mico@uca.es

Introduction

Obsessive–compulsive disorder (OCD) is a severe, highly prevalent (2–3% of the population worldwide) and chronically disabling disorder that usually emerges during childhood or adolescence (Karno et al. 1988; Weissman et al. 1994). The illness is characterized by intrusive thoughts (obsessions) and repetitive behaviors (compulsions). Sometimes, OCD is associated with tics, particularly related to Tourette syndrome (Pauls 1992), a disorder within the OCD spectrum. Although its pathophysiology is not yet fully elucidated, clinical evidences strongly implicate an abnormal regulation of brain serotoninergic function. Currently, the selective serotonin (5-HT) reuptake inhibitors (SSRIs) represent the first-line pharmacotherapy for OCD. Nevertheless, they have a slow onset of action and 30–50% of patients do not respond at all to these agents.

The effects of 5-HT are mediated by a large number of receptor subtypes (Barnes and Sharp 1999); among them, 5-HT2A/C subtype has been suggested to be involved in both the pathogenesis and the treatment strategies of various neuropsychiatric disorders. It has been proposed that the reduction in 5-HT2C receptor responsivity induced by chronic SSRIs may be of particular relevance to their unique efficacy in OCD (Kennett et al. 1994a) and may

explain the long latency period and high doses necessary for anti-OCD effects (Dougherty et al. 2004). Moreover, atypical antipsychotic medications, which block 5-HT2A/C receptors, are used in combination with SSRIs in augmentation strategies for OCD (Dougherty et al. 2004). In this sense, it has been suggested that the simultaneous specific blockade of 5-HT2A receptors and activation of an unknown constellation of other 5-HT receptors indirectly, as a result of 5-HT uptake inhibition, might have greater therapeutic efficacy than either action alone in treatment-refractory OCD (Marek et al. 2003).

The beneficial effects of opioids in mental illness have been known for a hundred years (Kraepelin 1921), but their introduction in clinical practice has been limited by their addictive properties and the introduction of effective nonaddictive treatments (i.e., drugs affecting monoaminergic systems). Nevertheless, opioid system has been implicated in OCD (Insel and Pickar 1983; Keuler et al. 1996; Urraca et al. 2004), and opiate drugs have been proved to be effective in treatment-refractory OCD (Goldsmith et al. 1999; Koran et al. 2005; Shapira et al. 1997a; Shapira et al. 1997b; Warneke 1997). However, the mechanisms of opiate relief of OCD symptoms are unknown, but an interaction of opioid and monoaminergic systems is not ruled out. In fact, agonists at μ-opioid receptors have proved to suppress 5-HT2A-mediated electrophysiological responses in the medial prefrontal cortex (Marek and Aghajanian 1998a,b) which provides a potential mechanism for convergent effects of serotoninergic and opioid systems in the brain.

It is known that some opiates, called here atypical opiates, are able to inhibit monoamine reuptake. These opiates are structurally identifiable: phenanthrene opiates without an oxygen bridge between C4 and C5 and the C6-OH moiety, such as levorphanol, and nonphenanthrene opiates, such as tramadol and methadone (Codd et al. 1995). Taking into account the aforementioned relationship of the opioid and the 5-HT systems, these drugs which involve both μ-opioid and serotoninergic properties would be good candidates for the development of new strategies in psychiatric research. In this regard, it is not surprising that atypical opiates induce antidepressant effects in rodents (Rojas-Corrales et al. 1998, 2002, 2004) and humans (De Montis et al. 1982; Fanelli and Montgomery 1998; Shapira et al. 2001; Spencer 2000). This is interesting, taking into account that due to the lack of OCD models useful for drug discovery, all compounds currently used for OCD were developed first as antidepressants. In fact, these atypical opiates have been proved to be effective in OCD (Goldsmith et al. 1999; Shapira et al. 1997a,b). In this sense, we aimed to study if 5-HT2A/C-related mechanism may be involved in these beneficial effects of atypical opiates and may offer the potential for therapeutic advances in stereotypes and compulsive behaviors related with OCD.

We used an experimental approach through the study of 5-HT2A receptor-mediated behavior: the head-twitch response induced by different experimental designs. This response has been often used to study the function of 5-HT2A receptors in the brain in vivo (Schreiber et al. 1995; Vickers et al. 2001) and has been related with impulsivity (Evenden and Ryan 1999) and the generation of obsessive–compulsive symptoms. Moreover, it has been proposed that shakes or twitches of the head in mice can serve as an animal model of tics seen in Tourette's disorder (Dursun and Handley 1996; Gaynor and Handley 2001). The aim of this study was to explore whether these atypical opiates (opiates which inhibit serotonin reuptake) could modify the 5-HT2A-related behavior in vivo. We have compared these effects with those induced by the typical opiate morphine and several psychoactive drugs affecting differently the serotoninergic neurotransmission.

Materials and methods

Animals

Albino male mice of the CD1 strain (25–30 g) provided by the "Servicio de Experimentación y Producción Animal" (SEPA) of the University of Cádiz were used. Animals were housed in groups of five animals per cage and were maintained under standard conditions: 12-h light–dark schedule (light on at 8:00 A.M.) with ad libitum food and water and constant temperature (21±1°C). The experiments were carried out between 09:00 and 15:00 hours. The experimental protocols were reviewed and approved by the Local Committee for Animal Experimentation of the Faculty of Medicine at the University of Cádiz (License number 079604). Animal care and use procedures have been carried out in accordance with the Declaration of Helsinki and conformed to the European Directive regarding the protection of animals used for experimental and other scientific purposes (86/609-EEC) and Spanish Law (RD 1201/2005). Naive mice were used only once and ten subjects were used per group. Drugs were administered (s.c. or i.p.) in an injection volume of 0.1 ml/10 g of body weight (Iwarson et al. 1994)

Method and experimental design

The head-twitch response is a very distinctive behavior related to serotoninergic mechanisms that has been well defined as a rapid movement of the head and neck of the rodent. This behavior may be induced either nonspecifically by administration of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT (Corne et al. 1963), or specifically by administration of 5-HT2A/C agonists such as DOI (Gessner

and Page 1962), and it is considered as a specific behavioral model for the activation of serotoninergic neurons (Schreiber et al. 1995). This behavior has been often used as an experimental model to study the function of 5-HT2 receptors, or more specifically of 5-HT2A receptors in the brain in vivo (Schreiber et al. 1995; Vickers et al. 2001). Although DOI has similar affinity for both 5-HT2A and 5-HT2C receptors, the head-twitch behavior appears to be mediated primarily by 5-HT2A receptors as this effect is blocked by selective 5-HT2A but not 5-HT2C antagonists (Dursun and Handley 1996; Kennett et al. 1994b; Kleven et al. 1997; Schreiber et al. 1995). We have used different experimental designs to induce this behavior in mice and to explore how atypical opiates are able to modify it. The rodents were placed individually in transparent polycarbonate observation boxes equipped with holed steel lids (10×24×8 cm, IFFA CREDO®) containing no bedding material to facilitate the evaluation of the head-twitch responses.

5-HTP-induced head-twitch response

Previous data showed us that single i.p. administration of 100 mg/kg of 5-HTP-induced one to two head twitches per minute, 30 min after, without side effects. In these conditions, we studied the possible potentiation of this behavior performing the dose–effect curves of the drugs. Psychoactive drugs underlying different mechanisms were: a selective inhibitor of noradrenaline reuptake, desipramine; a selective inhibitor of 5-HT reuptake, fluvoxamine; nefazodone which inhibits serotonine and noradrenaline reuptake and blocks 5-HT2-receptors; and clozapine, a psychoactive drug which preferentially blocks 5-HT2 receptors. Morphine was used as a typical opiate, and (±)-tramadol, R-(−)-methadone and levorphanol were used as atypical opiates which enhance serotonin levels. The number of head twitches was scored 30 min after i.p. administration of 100 mg/kg of 5-HTP in 2 min; drugs were injected 60 min before the test.

5-HTP plus carbidopa-induced head-twitch response

Carbidopa, an inhibitor of aromatic amino acid decarboxylation, was associated to 5-HTP to reduce its peripheral decarboxylation and enhance CNS effects; this design provides a larger number of head twitches which allow a wide range to study drug effects. Carbidopa (25 mg/kg i.p.) was administered 15 min before 5-HTP (100 mg/kg i.p.). The number of head twitches was scored 10 min after 5-HTP administration in 1 h (in periods of 10 min, during the first minute of each period, total observation time: 6 min). (±)-Tramadol was injected 15 min before the test as the standard atypical opiate that binds to μ-opioid receptor and enhances both noradrenline and serotonine levels.

DOI-induced head-twitch response

The dose of the 5-HT2A/C receptor agonist DOI (Ichikawa and Meltzer 1995) was selected on the basis of a previous dose–effect curve performed (1.25–10 mg/kg, data not shown) (Darmani and Gerdes 1995). Animals were placed immediately after DOI administration (2.5 mg/kg i.p.) in the observation boxes. The number of head twitches was scored 10 min after DOI administration in 1 h (in periods of 10 min, during the first minute of each period; total observation time, 6 min). Psychoactive drugs as desipramine, fluvoxamine, nefazodone, and clozapine and opiates as morphine, (±)-tramadol, (+)-tramadol, (±)-tramadol, (−)-methadone, and levorphanol were administered 30 min before the test. In a subsequent series of experiments, naloxone was s.c. administered 15 min before the test to study the opioid involvement in the effect of the drugs.

Drugs

All drug doses are given in terms of the salt, in mg per kg of body weight. Drugs were dissolved in water. If necessary, a few drops of acetic acid were added. 5-Hydroxytryptophan, carbidopa, (±)-1-(2,5-dimetoxi-4-iodofenil)-2-aminopropano HCl (DOI), and naloxone HCl were obtained from Sigma-Aldrich-Química (Madrid, Spain); (±)-tramadol HCl, (+)-tramadol HCl, and (-)-tramadol HCl were gifts given generously by Grünenthal-Andrómaco (Madrid, Spain); levorphanol tartrate and (−)-methadone HCl were purchased from Research Biochemicals Inc. (Natick, MA); nefazodone HCl and clozapine base were gifts generously given by Bristol-Myers-Squib (Madrid, Spain) and Sandoz (Madrid, Spain), respectively, morphine HCl was obtained from the Ministry of Health and Consume of Spain.

Statistics

Data are expressed as mean ± standard error of the total number of head twitches in 5-HTP-induced responses. Normal distribution of data was analyzed by Kolmogorov–Smirnov test, and nonparametric tests were used when appropriate; i.e., Mann–Whitney *U*-test after significant Kruskal–Wallis *H*-test. Normally distributed data were analyzed using one-way analysis of variance. Data from DOI and 5-HTP plus carbidopa-induced responses were expressed as the mean ± standard error of the number of head twitches per minute and were analyzed by repeated measures analysis of variance. Areas under curves (AUC, 0–1 h) were also calculated by the trapezoidal rule and one-way analysis

Table 1 Effect of different opiate and psychoactive drugs on the number of head twitches induced 30 min after administration of 100 mg/kg of 5-HTP in 2 min

Drug	mg/kg	Mean	S.E.	Drug	mg/kg	Mean	S.E.
DMI	–	0.90	±0.28	MOR	–	1.00	±0.33
	5	1.30	±0.90		5	0.40	±0.31
	10	2.30	±0.80		10	0.00	±0.10*
	20	0.60	±0.31		20	0.00	±0.10*
FVX	–	2.00	±0.68	(±)-TRM	–	1.00	±0.39
	5	7.90	±0.88*		5	0.10	±0.10*
	10	9.80	±1.31*		10	0.10	±0.10*
	20	10.56	±2.92*		20	0.20	±0.13*
NFZ	–	1.00	±0.45	(−)-MET	–	0.70	±0.30
	5	2.20	±0.63		5	0.00	±0.00*
	10	2.67	±0.99		10	0.00	±0.00*
	20	2.10	±0.55		20	0.00	±0.00*
CLZ	–	1.00	±0.30	LEV	–	0.70	±0.21
	1,25	0.80	±0.42		1.25	0.50	±0.22
	2,5	0.00	±0.10*		2.5	0.40	±0.22
	5	0.00	±0.10*		5	0.10	±0.10

DMI Desipramine, *FVX* fluvoxamine, *NFZ* nefazodone, *CLZ* clozapine, *MOR* morphine, *(±)-TRM* (±)-tramadol, *(−)-MET* (−)-methadone, *LEV* levorphanol

*$p<0.05$ vs control (saline treated)

was subsequently performed. Significant analyses of variance were followed by Student–Newman–Keuls post hoc test. A p value of <0.05 was considered to be significant.

Results

5-HTP-induced head-twitch response

Table 1 resumes the effect of the different CNS active drugs underlying different mechanisms of action on the head-twitch response induced by 100 mg/kg of 5-HTP. The selective noradrenaline reuptake inhibitor, desipramine, did not induce any significant effect in this paradigm at the doses tested (5–20 mg/kg). Fluvoxamine, a selective inhibitor of 5-HT reuptake induced a significant ($p<0.05$) enhancement of head-twitch response at 5, 10, and 20 mg/kg. Nefazodone (5–20 mg/kg) did not modify significantly this behavior, and clozapine significantly reduced the number of head twitches at 2.5 and 5 mg/kg.

On the other hand, morphine (4–8 mg/kg), (±)-tramadol (10–40 mg/kg), and (−)-methadone (2–8 mg/kg) reduced strongly the number of head twitches while levorphanol (0.5–2 mg/kg) slightly reduced this behavior, although it does not reach statistical significance.

5-HTP plus carbidopa-induced head-twitch response

The inhibitor of aromatic amino acid decarboxylation, carbidopa, enhanced the number of spontaneous 5-HTP-

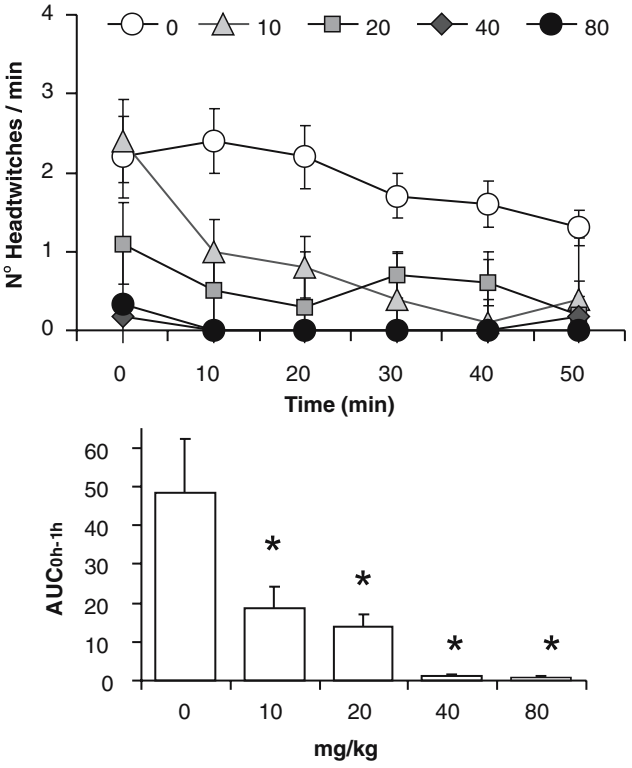

Fig. 1 Effect of (±)-tramadol (i.p.) on the head-twitch response induced by 5-HTP plus carbidopa. *Upper graph*: time–course evolution of the number of head twitches (HT) per minute, in periods of 10 min, in 1 h after drug administration. *Open circles*: saline, *triangles*: 10 mg/kg of (±)-tramadol, *squares*: 20 mg/kg of (±)-tramadol, *diamonds*: 40 mg/kg of (±)-tramadol, and *closed circles*: 80 mg/kg of (±)-tramadol. *Lower graph*: areas under curves from the time–course evolution curves. *Asterisks*: $p<0.05$ vs saline group

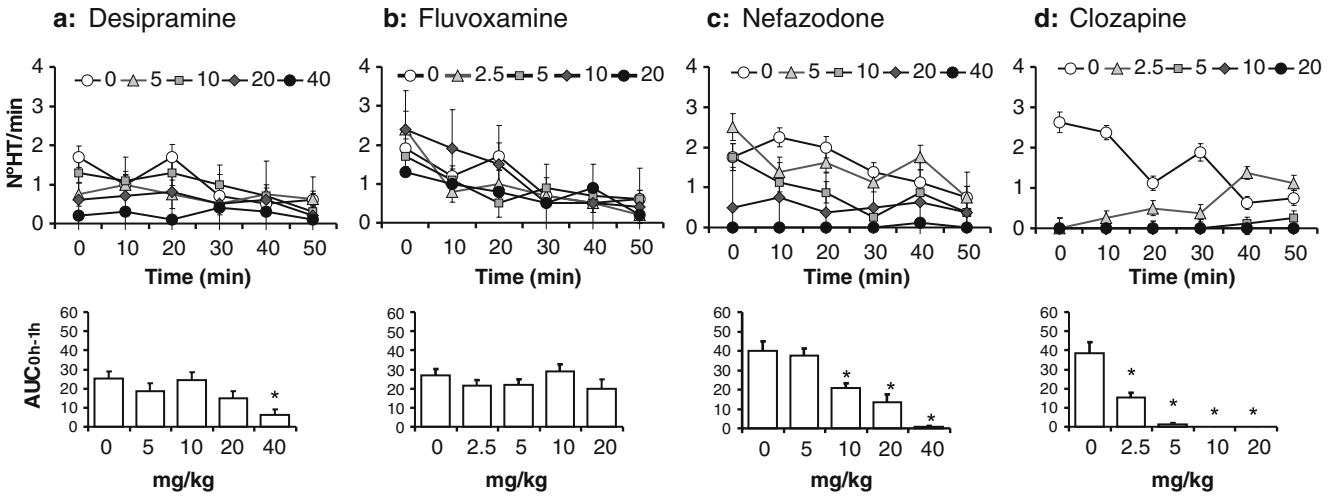

Fig. 2 Effect of several psychoactive drugs (i.p.), affecting differentially serotoninergic neurotransmission, on the head-twitch response induced by the selective 5-HT2A/C agonist DOI. *Upper graphs*: time–course evolution of the number of head twitches (HT) per minute, in periods of 10 min, in 1 h after drug administration. *Open circles*: saline, *triangles*: 5 mg/kg of desipramine and nefazodone and 2.5 mg/kg of fluvoxamine and clozapine, *squares*: 10 mg/kg of desipramine and nefazodone and 5 mg/kg of fluvoxamine and clozapine, *diamonds*: 20 mg/kg of desipramine and nefazodone and 10 mg/kg of fluvoxamine and clozapine, *closed circles*: 40 mg/kg of desipramine and nefazodone and 20 mg/kg of fluvoxamine and clozapine. *Lower graphs*: areas under curves from the time–course evolution curves. *Asterisks*: $p<0.05$ vs saline group

induced head twitches in the animals. Upper graph in Fig. 1 shows the time evolution of the number of head twitches. Repeated analysis of variance shows an effect of time in each experimental group (intra-subject effect, $p<0.05$) and an effect of treatment (inter-subject effect $p<0.001$). Subsequent one-way analysis of variance of the AUC values obtained for each time–effect curve (Fig. 1, lower graph), shows that (±)-tramadol reduced dose-dependently and significantly ($p<0.05$) this behavior at all the doses tested (10–80 mg/kg).

DOI-induced head-twitch response

Monoaminergic drugs: Figure 2 shows the effect of monoaminergic drugs in DOI-induced head-twitch response. Figure 2a shows the effect of desipramine in the DOI-induced response. Repeated analysis of variance shows an intra-subject ($p<0.05$) and inter-subject ($p<0.001$) effects. Analysis of AUC 0–1 h values shows that only 40 mg/kg of desipramine was able to significantly reduce this behavior. On the other hand (Fig. 2b), administration of fluvoxamine (2.5–20 mg/kg) did not induce any signifi-

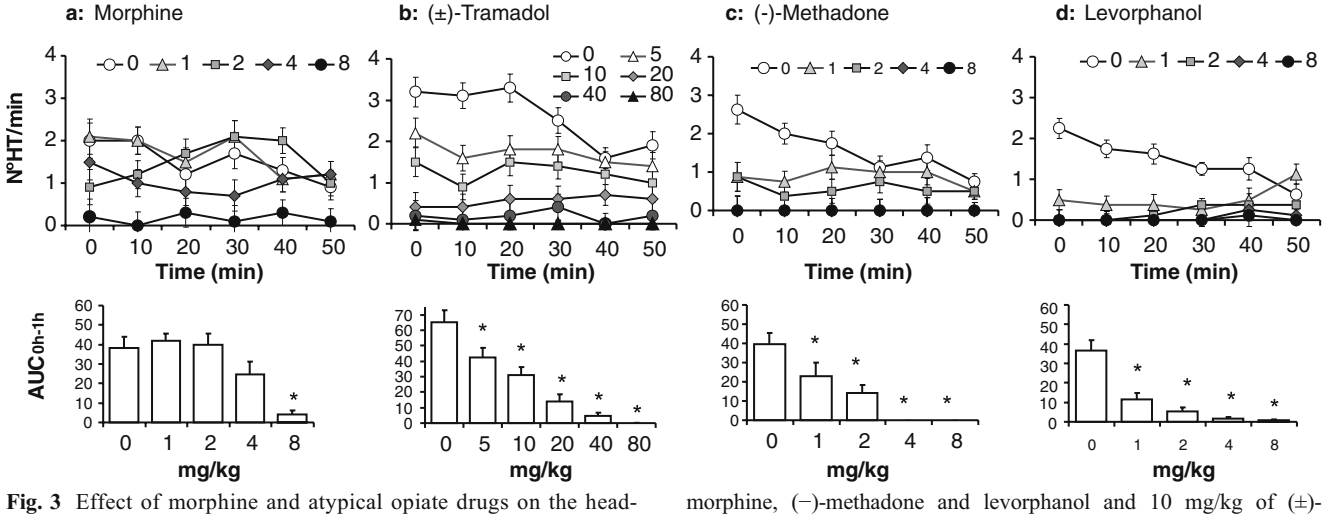

Fig. 3 Effect of morphine and atypical opiate drugs on the head-twitch response induced by the selective 5-HT2A/C agonist DOI. *Upper graphs*: time–course evolution of the number of head twitches (HT) per minute, in periods of 10 min, in 1 h after drug administration. *Open circles*: saline, *triangles*: 1 mg/kg of morphine, (−)-methadone and levorphanol and 5 mg/kg of (±)-tramadol, *squares*: 2 mg/kg of morphine, (−)-methadone and levorphanol and 10 mg/kg of (±)-tramadol, *diamonds*: 4 mg/kg of morphine, (−)-methadone and levorphanol and 20 mg/kg of (±)-tramadol, *closed circles*: 8 mg/kg of morphine, (−)-methadone and levorphanol and 40 mg/kg of (±)-tramadol. *Lower graphs*: areas under curves from the time–course evolution curves. *Asterisks*: $p<0.05$ vs saline group

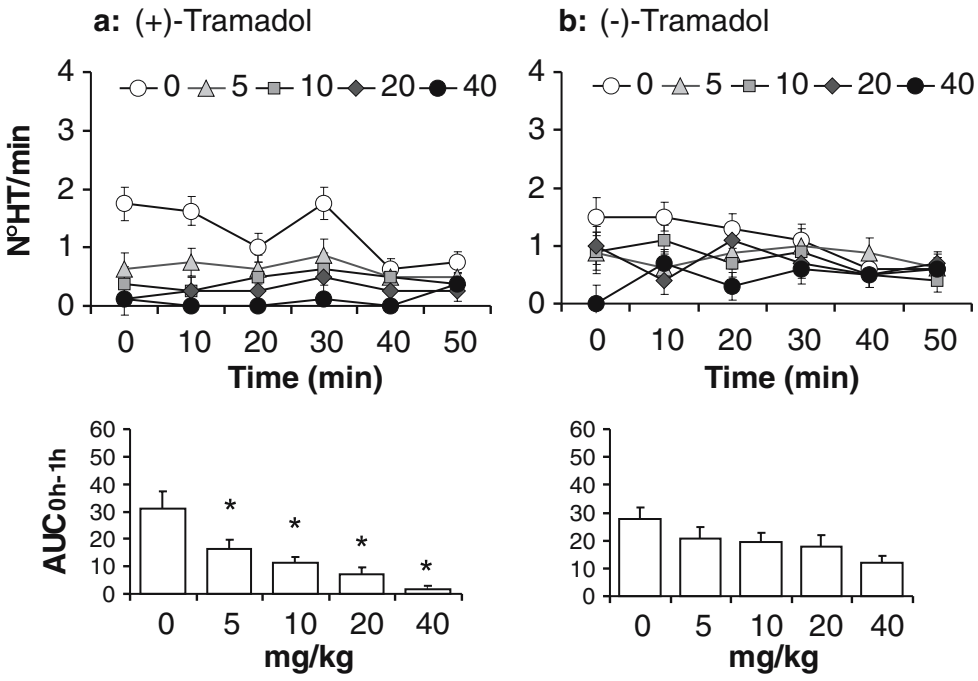

Fig. 4 Effect of the isomers of tramadol on the head-twitch response induced by the selective 5-HT2A/C agonist DOI. **a** Effect of (+)-tramadol and **b** effect of (−)-tramadol. *Upper graphs*: time–course evolution of the number of head twitches (HT) per minute, in periods of 10 min, in 1 h after drug administration. *Open circles*: saline, *triangles*: 5 mg/kg, *squares*: 10 mg/kg, *diamonds*: 20 mg/kg, *closed circles*: 40 mg/kg. *Lower graphs*: areas under curves from the time–course evolution curves. *Asterisks*: $p<0.05$ vs saline group

cant effect (inter-subject effect, $p=0.330$). AUC 0–1 h values show more clearly the slight modifications, not significant, induced by fluvoxamine vs saline-treated mice. Figure 2c shows a decrease in the number of head twitches per minute along the time (intra-subject effect, $p<0.001$) and in nefazodone-treated mice (inter-subject effect, $p<0.001$). This effect was dose-dependent; nefazodone-induced AUC 0–1 h values at 10 (20.94±02.50), 20 (13.44±4.28), and 40 (0.63±0.63) mg/kg were significantly different ($p<0.05$) from saline (40.00±4.77).

The antipsychotic drug, clozapine, blocked the DOI-induced behavior. Moreover, the block of head twitches was maintained throughout the test period by clozapine at 10 and 20 mg/kg, as it is shown in Figure 2d. AUC 0–1 h values show that clozapine at 2.5 (15.31±2.47) and 5 (1.25±0.94) mg/kg also induced a significant effect vs the saline-treated group (38.44±5.96), albeit short-lived compared to higher doses. Opiate drugs: morphine was able to reverse the DOI-induced head twitches (inter-subject effect, $p<0.001$) (Fig. 3a). Doses of 1 and 2 mg/kg of morphine scarcely modified ($p>0.05$) AUC 0–1 h values (head twitches per time) (41.75±3.86 and 39.75±6.03, respectively) and 4 mg/kg slightly reduced, although not significantly, this parameter vs the saline control group (38.25±5.53). Conversely, 8 mg/kg of morphine was able to reduce significantly this parameter (4.25±1.83, $p<0.05$ vs saline).

The inhibition of the number of DOI-induced head twitches by (±)-tramadol is shown in Fig. 3b (inter-subject effect, $p<0.001$). AUC 0–1 h values show that this effect was dose-dependent at 5 (42.50±6.10), 10 (31.25±4.86), 20 (14.00±4.57), 40 (4.50±2.44), and 80 (0.25±0.25) mg/kg and the significant vs the saline-treated group (65.25±7.91). Figure 3c shows the effect of (−)-methadone (1–8 mg/kg). Administered at 4 and 8 mg/kg, (−)-methadone totally abolished this behavior during the whole observation period. Post hoc analysis of AUC 0–1 h values also shows a significant effect of 1 (22.81±7.14) and 2 (14.06±4.43) mg/kg, with respect to control group (39.69±5.91). The other opiate drug studied, levorphanol, also abolished the head twitches at 2–8 mg/kg at the beginning of the observation period. However, this abolishment was only conserved along the time when 4–8 mg/kg was administered. Significant AUC 0–1 h values vs saline (36.56±5.55) were obtained for 1 (11.56±3.27), 2 (5.31±2.24), 4 (1.56±1.05), and 8 (0.63±0.63) mg/kg of levorphanol.

Figure 4 shows the data obtained with the two isomers of (±)-tramadol. Administration of (5–40 mg/kg) of (+)-tramadol dose-dependently reduced (±)-DOI-induced syndrome (Fig. 4a). Conversion of the time–course data to AUC 0–1 h values shows significant effects of 5 (16.56±3.31), 10 (11.25±2.22), 20 (7.19±2.34), and 40 (1.88±0.91) mg/kg compared with the control saline group (31.25±5.96). Conversely, (−)-tramadol (5–40 mg/kg) reduced slightly, but not significantly, the number of head twitches per time (inter-subject effect, $p=0.063$), Fig. 4b.

Effect of naloxone

As opiates induced a strong effect in reducing the number of DOI-induced head twitches, we studied the involvement of opioid receptors in this behavior by coadministration of 0.8 mg/kg of naloxone. Therefore, naloxone was associated to either the minimum effective dose of either nefazodone

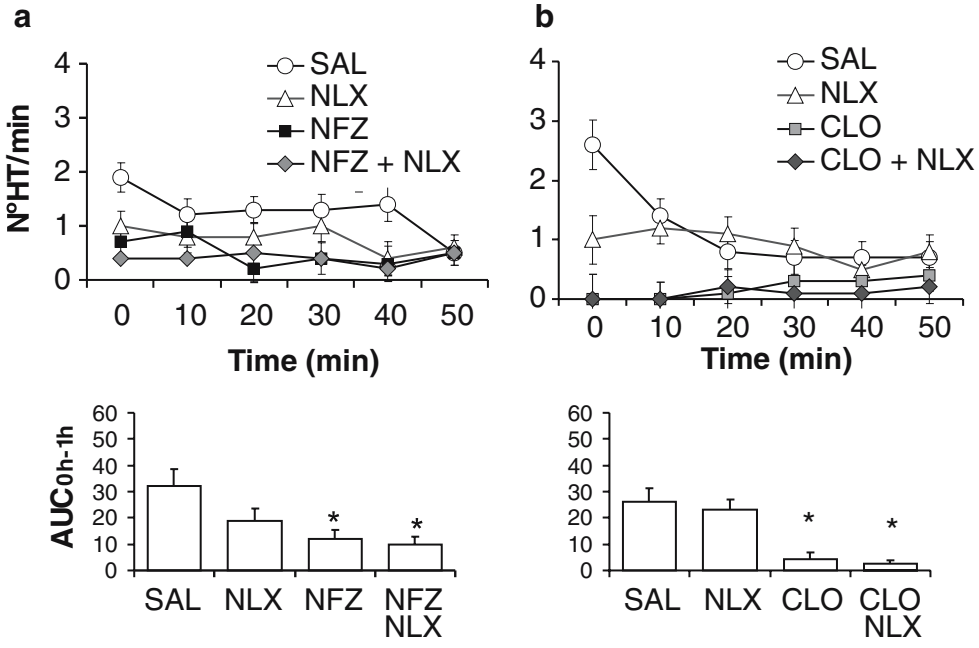

Fig. 5 Effect of naloxone in the reduction of the number of head twitches per minute induced by 5-HT2 blocking agents. **a** Nefazodone and **b** clozapine. *Upper graphs*: time–course evolution of the number of head twitches (HT) per minute, in periods of 10 min, in 1 h after drug administration. *Open circles*: saline (*SAL*), *triangles*: 0.8 mg/kg of naloxone (*NLX*), *squares*: 10 mg/kg of nefazodone (*NFZ*) or 2.5 mg/kg of clozapine (*CLO*), *diamonds*: 0.8 mg/kg of *NLX* plus 10 mg/kg of *NFZ* or 2.5 mg/kg of *CLO*. *Lower graphs*: areas under curves from the time–course evolution curves. *Asterisks*: $p<0.05$ vs saline group

(see Fig. 2c), clozapine (see Fig. 2d), morphine (see Fig. 3a), (±)-tramadol (see Fig. 3b), or methadone (see Fig. 3c). As it is shown in Fig. 5, naloxone neither modified the effect of nefazodone (Fig. 5a) nor the effect of clozapine (Fig. 5b). Conversely, naloxone was able to reduce significantly the effect of morphine, (±)-tramadol, and (−)-methadone (Fig. 6)

Discussion

This study explores and compares the effect of opiates with serotoninergic properties with those of morphine and other psychoactive drugs in the head-twitch behavior. Head-twitch behavior in mice has been related at the behavioral level as an experimental model for the activation of the CNS 5-HT2A receptors. DOI-induced head-twitch response has been claimed to serve as an animal model of tics seen in Tourette's disorder (Dursun and Handley 1996; Gaynor and

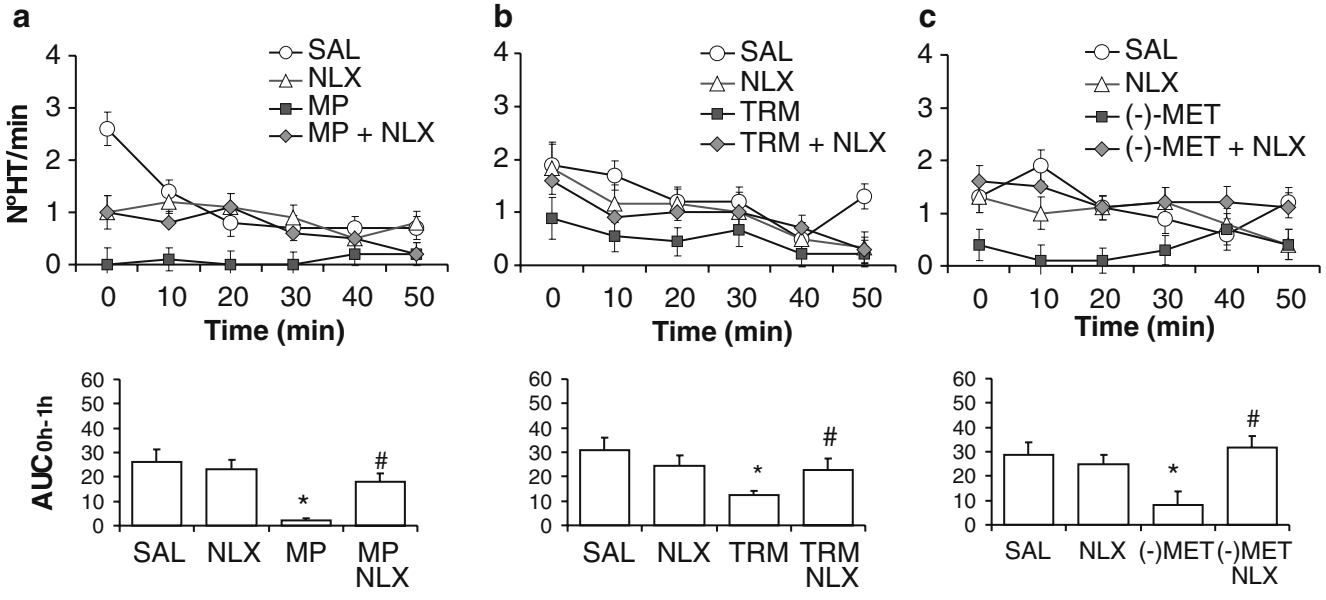

Fig. 6 Effect of naloxone in the reduction of the number of head twitches per minute induced by opiate drugs **a** morphine, **b** (±)-tramadol and **c** (−)-methadone. *Upper graphs*: time–course evolution of the number of head twitches (*HT*) per minute, in periods of 10 min, in 1 h after drug administration. *Open circles*: saline (*SAL*), *triangles*: 0.8 mg/kg of naloxone (*NLX*), *squares*: 8 mg/kg of morphine (*MP*) or 5 mg/kg of (±)-tramadol (*TRM*) or 1 mg/kg of (−)-methadone (*MET*), *diamonds*: 0.8 mg/kg of *NLX* plus *MP* or *TRM* or *MET*. *Lower graphs*: areas under curves from the time–course evolution curves. *Asterisks*: $p<0.05$ vs saline group, *number signs*: $p<0.05$ vs *MP* or *TRM* or *MET*

Handley 2001; Hayslett and Tizabi 2005), and the relationship between tics and OCD compulsions have been previously suggested (Leckman et al. 1994).

Our results show that all the opiates tested reduced both 5-HTP and DOI-induced head twitches. Studies from our laboratory show no sedative effect induced by these atypical opiate drugs at doses used; conversely, they induced antidepressant-like effects implying, many times, motor responses (Rojas-Corrales et al. 1998, 2002, 2004). Moreover, it has been specifically demonstrated that the μ-opioid receptor agonists suppress DOI-induced head shakes without simultaneously having effects on locomotor activity (Marek 2003). Association of carbidopa to 5-HTP enhances the frequency of head twitches and confirms the dose-dependent blocking effect of (±)-tramadol. Tramadol is a racemic mixture of two enantiomers, each one displaying different mechanisms of action (Codd et al. 1995). (+)-Tramadol, which inhibits preferentially 5-HT reuptake and binds to μ-opioid receptors, reduced DOI-induced head twitches, as the racemic form did. On the other hand, (−)-tramadol, which preferentially inhibit noradrenaline reuptake, induced a less potent, nonsignificant, antagonism of the number of head twitches. From these results, we suggest that μ-opioid receptor was involved in the effect of opiates, especially if we bear in mind that, in agreement with previous results (Sun et al. 2003; Marek 2003), the effects of all opiates were blocked by naloxone.

These results agree with the positive effects found with opiates in OCD in clinical practice (Warneke 1997). Tramadol has shown positive effects in the treatment of Tourette's syndrome (Shapira et al. 1997a,b), and morphine has shown beneficial effects in the treatment of resistant obsessive–compulsive disorder in a placebo-controlled double blind study (Koran et al. 2005). Recently, it has been reported that there was a case of worsening of OCD during methadone tapering (Khazaal et al. 2006). Furthermore, several studies have demonstrated that the symptoms of Tourette's syndrome can be attenuated by modulation of the opioid system (Kurlan et al. 1991; McConville et al. 1994; Meuldijk and Colon 1992; Sandyk 1986a,b), and some authors suggest an involvement of μ-opioid receptor gene in OCD with tics (Urraca et al. 2004). Nevertheless, other mechanisms than opioid may underlie the reduction of DOI-induced head twitches, as morphine was only effective at the highest doses tested, while tramadol, which binds with modest affinity to the μ-opioid receptor, reduced the head twitches from the lowest dose tested. Similarly, methadone and levorphanol, both inhibiting the 5-HT reuptake, reduced this behavior at all the doses tested.

Acute inhibition of 5-HT reuptake by fluvoxamine administration increased the number of head twitches induced nonspecifically by 5-HTP, probably by an indirect action at 5-HT2A receptors. Along these lines, acute inhibition of serotonin reuptake did not inhibit the specific (DOI-induced) 5-HT2A-related behavior. These results are consistent with reports showing that acute fluvoxamine potentiated 5-HTP-induced head-twitch response (Pawlowski and Melzacka 1986) and failed to modify the number of DOI-induced shakes (Kawakami et al. 2005). Nevertheless, SSRIs have been proved to be effective after chronic treatment, where a reduction in 5-HT2 receptor responsivity may have occurred in animal models related to human OCD (Nurnberg et al. 1997). Regarding clinical studies in OCD, SSRIs also display clinical efficacy only after chronic treatment, and it has been recently published that relapses in OCD symptoms may not depend solely on short-term changes in serotonin availability (Berney et al. 2006). On the other hand, our results and other's show that inhibition of noradrenaline reuptake by desipramine failed to modify the head-twitch response induced by 5-HTP (Xu et al. 2006), but decreased that induced by DOI (Kawakami et al. 2005). An interaction between the serotoninergic (5-HT2) and noradrenergic (alpha-adrenergic) systems in the modulation of compulsory responding (head shakes) has been previously suggested (Koskinen et al. 2003), possibly involving an enhancement of noradrenergic inhibitory transmission from the locus coeruleus nucleus to the dorsal raphe nucleus.

The atypical antipsychotic clozapine (approved for suicide prevention in USA), have a suppressant effect on both 5-HTP and DOI-induced head shakes, probably due to its 5-HT2A receptor-blocking properties; consistently, naloxone did not affect this effect. Besides, nefazodone (discontinued for inducing hepatic failure), which inhibits 5-HT uptake both in vivo and in vitro while antagonizing the 5-HT2A receptor (Kent 2000; Stahl 1998), slightly (no significant) increased 5-HTP-induced head-twitch response and reduced, dose dependently, DOI-induced head-twitch response. The discrepancies may underlie the ability of nefazodone to inhibit 5-HT reuptake while blocking 5-HT2A receptors.

From a neurobiological perspective, specific parts of the frontostriatal system have been reported to be altered in patients with OCD, and changes in anatomically connected distant regions have been reported (Lopez-Ibor Alcocer et al. 2000; Pujol et al. 2004). In fact, cortico–striato–thalamo-cortical circuitry seems to be dysfunctional in OCD (Friedlander and Desrocher 2006). Similarly, tics are believed to be a result of dysregulation of cortical–subcortical circuitry (Graybiel and Canales 2001; Mink 2001). More specifically, tics are presumably due to failed inhibition within cortico–striato–thalamo–cortical circuits. Dysregulation of this circuitry, which is also involved in opiate reward (Mangold et al. 2000), may cause a relative lack of serotonin in the feedback loop between the thalamus and the orbito-frontal cortex, the caudate nucleus, and globus pallidus and produce a compensatory increase in 5-HT2A receptor. This mechanism probably underlies the

increase in 5-HT2A receptor binding that has been found in the caudate nuclei of untreated patients with OCD (Adams et al. 2005). Therefore, 5-HT2A receptors, widespread in the central nervous system, and enriched in these regions, are likely to be involved in the regulation of this circuitry by clinically effective drugs. In fact, 5-HT2A/C receptor antagonism appears to be an important feature of antipsychotic and antidepressant drugs with broad pharmacological profiles (Leysen 2004), and down-regulation of these receptors have been implicated in the SSRI-induced relief of OCD symptoms.

On the other hand, it has been suggested that enhanced 5-HT2 receptor activation might underlie the beneficial action of SSRIs. Case studies have reported that the use of hallucinogens with 5-HT2 receptor agonistic properties, such as lysergic acid diethylamide (LSD) and psilocybin, results in relief of OCD symptoms (Delgado and Moreno 1998), and 5-HT2A/C receptor antagonism has been postulated to play a role in the generation of obsessive–compulsive symptoms in patients with a psychotic disorder. Besides, specific agonists as mCPP have been shown to exacerbate symptoms in some OCD patients (Zohar et al. 1987), although not in all (Khanna et al. 2001), and many hallucinogens, including LSD and phencyclidine, caused the head-twitch response in mice (Corne et al. 1963). Although atypical antipsychotic drugs may induce or exacerbate OC symptoms in patients with schizophrenia, they may also be effective as adjunctive treatment for treatment-refractory OCD (Atmaca et al. 2002). In fact, blockade of 5-HT2A receptors has been suggested to be an important component of the therapeutic effects the new generation of so-called atypical antipsychotic (Leysen 2004) effective in augmenting SSRIs effects in OCD. On the other hand, it remains unclear whether the atypical antipsychotics may induce obsessive–compulsive symptoms in some patients and over what time course (Ongur and Goff 2005).

The differential capacity of atypical antipsychotic drugs to block 5-HT2A receptors in different regions may also underlie the clinical discrepancies found with such drugs (Atmaca et al. 2002; Shapira et al. 2004). In fact, atypical antipsychotic drugs which preferentially blocked 5-HT2A receptors have got in Europe the indication for OCD (risperidone) and Tourette's syndrome (risperidone, ziprasidone and olanzapine) (Leysen 2004). In this sense, low doses of risperidone have clearly shown a therapeutic effect in resistant OCD (Hollander et al. 2003). Risperidone is an effective 5-HT2A antagonist in the medial prefrontal cortex, but not in the orbitofrontal cortex (Bergqvist et al. 1999). Such comparative data are lacking for the other atypical antipsychotics.

The effect of atypical opiate tramadol may also involve 5-HT2 mechanisms, as it decreases the number of 5-HT2 receptors in frontal cortex (Hopwood et al. 2001) and inhibits 5-HT2C receptor function and the specific binding in a competitive manner (Ogata et al. 2004). Our results, and some obtained by others (Sun et al. 2003), demonstrate that typical (morphine) and atypical (tramadol, methadone, and levorphanol) opioids damp down the number of 5-HTP-induced head twitches after acute administration in a naloxone-reversible fashion, being atypical ones more effective. Therefore, both opioid and 5-HT2 receptors, which have a close correspondence distribution in both the rodent and primate neocortex, may be involved in those effects.

Head-twitches (in mice) and wet-dog shakes (in rats) induced by drugs such as the selective 5-HT2A/2C receptor agonist DOI and its structural analogues, were suggested to be mediated via central 5-HT2A receptors in the medial prefrontal cortex (Willins and Meltzer 1997), and these models were used as an in vivo test of 5-HT2A receptor pharmacology (Barnes and Sharp 1999). Indeed, DOI-induced head-twitch response is completely antagonized by ketanserin, a selective 5-HT2A receptor antagonist (Darmani et al. 1990). Recently, it has been published that signaling responses induced by DOI are blocked by lithium (Basselin et al. 2005). Lithium appears to act against obsessions and compulsions in several conditions and has been effective in acute OCD (Swartz and Shen 1999)

The key findings of this study are: (1) atypical opiates which enhance serotonin levels (by inhibiting its reuptake), and also morphine, counteract both specific and nonspecific 5-HT2A-mediated behavior. (2) Atypical opiates inhibit the DOI-induced selective responses through an opioid mediated mechanism. (3) A combination of 5-HT and opioid properties results in a greater efficacy in antagonizing behaviors related with stereotypes and compulsory responding. These results may link with the role of opiates in mental illness and provide behavioral support to a mechanism for convergent effects of 5-HT and opioid systems in the brain. Further pharmacologic studies in ethologic animal models would be of interest to dissect the complex interactive biological systems involved in these psychiatric disorders and further explore the beneficial effects of these opiates. Overall, atypical opiates seem to offer the potential for therapeutic advances in stereotypes and compulsive symptoms.

Acknowledgements The authors thank the financial support provided by "Fondo de Investigación Sanitaria" from the Ministry of Health of Spain (PI031430).

References

Adams KH, Hansen ES, Pinborg LH, Hasselbalch SG, Svarer C, Holm S, Bolwig TG, Knudsen GM (2005) Patients with obsessive–compulsive disorder have increased 5-HT2A receptor binding in the caudate nuclei. Int J Neuropsychopharmacol 8:391–401

Atmaca M, Kuloglu M, Tezcan E, Gecici O (2002) Quetiapine augmentation in patients with treatment resistant obsessive–compulsive disorder: a single-blind, placebo-controlled study. Int Clin Psychopharmacol 17:115–119

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152

Basselin M, Chang L, Seemann R, Bell JM, Rapoport SI (2005) Chronic lithium administration to rats selectively modifies 5-HT2A/2C receptor-mediated brain signaling via arachidonic acid. Neuropsychopharmacology 30:461–472

Bergqvist PB, Dong J, Blier P (1999) Effect of atypical antipsychotic drugs on 5-HT2 receptors in the rat orbito-frontal cortex: an in vivo electrophysiological study. Psychopharmacology (Berl) 143:89–96

Berney A, Sookman D, Leyton M, Young SN, Benkelfat C (2006) Lack of effects on core obsessive–compulsive symptoms of tryptophan depletion during symptom provocation in remitted obsessive–compulsive disorder patients. Biol Psychiatry 59:853–857

Codd EE, Shank RP, Schupsky JJ, Raffa RB (1995) Serotonin and norepinephrine uptake inhibiting activity of centrally acting analgesics: structural determinants and role in antinociception. J Pharmacol Exp Ther 274:1263–1270

Corne SJ, Pickering R, Warner BJ (1963) A method for assessing the effect of drugs on the central actions of drugs on the central action of 5-hydroxytriptamine. Br J Pharmacol 20:106–120

Darmani NA, Gerdes CF (1995) Temporal differential adaptation of head-twitch and ear-scratch responses following administration of challenge doses of DOI. Pharmacol Biochem Behav 50:545–550

Darmani NA, Martin BR, Pandey U, Glennon RA (1990) Pharmacological characterization of ear-scratch response in mice as a behavioral model for selective 5-HT2-receptor agonists and evidence for 5-HT1B- and 5-HT2-receptor interactions. Pharmacol Biochem Behav 37:95–99

De Montis GM, Devoto P, Tagliamonte A (1982) Possible antidepressant activity of methadone. Eur J Pharmacol 79:145–146

Delgado PL, Moreno FA (1998) Hallucinogens, serotonin and obsessive–compulsive disorder. J Psychoact Drugs 30:359–366

Dougherty DD, Rauch SL, Jenike MA (2004) Pharmacotherapy for obsessive–compulsive disorder. J Clin Psychol 60:1195–1202

Dursun SM, Handley SL (1996) Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest 5HT2A receptors are active under physiological conditions. Psychopharmacology (Berl) 128:198–205

Evenden JL, Ryan CN (1999) The pharmacology of impulsive behaviour in rats VI: the effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. Psychopharmacology (Berl) 146:413–421

Fanelli J, Montgomery C (1998) Use of the analgesic tramadol in antidepressant potentiation. Psychopharmacol Bull 32:442

Friedlander L, Desrocher M (2006) Neuroimaging studies of obsessive–compulsive disorder in adults and children. Clin Psychol Rev 26:32–49

Gaynor CM, Handley SL (2001) Effects of nicotine on head-shakes and tryptophan metabolites. Psychopharmacology (Berl) 153:327–333

Gessner PK, Page IH (1962) Behavioral effects of 5-methoxy-*N*,*N* dimethyltryptamine, other tryptamines and L.S.D. Am J Physiol 203:167

Goldsmith TB, Shapira NA, Keck PE, Jr. (1999) Rapid remission of OCD with tramadol hydrochloride. Am J Psychiatry 156:660–661

Graybiel AM, Canales JJ (2001) The neurobiology of repetitive behaviors: clues to the neurobiology of Tourette syndrome. Adv Neurol 85:123–131

Hayslett RL, Tizabi Y (2005) Effects of donepezil, nicotine and haloperidol on the central serotonergic system in mice: implications for Tourette's syndrome. Pharmacol Biochem Behav 81:879–886

Hollander E, Baldini Rossi N, Sood E, Pallanti S (2003) Risperidone augmentation in treatment-resistant obsessive–compulsive disorder: a double-blind, placebo-controlled study. Int J Neuropsychopharmacol 6:397–401

Hopwood SE, Owesson CA, Callado LF, McLaughlin DP, Stamford JA (2001) Effects of chronic tramadol on pre- and post-synaptic measures of monoamine function. J Psychopharmacol 15:147–153

Ichikawa J, Meltzer HY (1995) DOI, a 5-HT2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. Brain Res 698:204–208

Insel TR, Pickar D (1983) Naloxone administration in obsessive–compulsive disorder: report of two cases. Am J Psychiatry 140:1219–1220

Iwarson K, Lindberg L, Waller T (1994) Common non-surgical techniques and procedures. In: Svendsen P, Hau J (eds) Handbook of laboratory animal science. Selection and handling of animals in biomedical research. CRC, Boca Raton (Florida), pp 229–272

Karno M, Golding JM, Sorenson SB, Burnam MA (1988) The epidemiology of obsessive–compulsive disorder in five US communities. Arch Gen Psychiatry 45:1094–1099

Kawakami Y, Kitamura Y, Araki H, Kitagawa K, Suemaru K, Shibata K, Gomita Y (2005) Effects of monoamine reuptake inhibitors on wet-dog shakes mediated by 5-HT2A receptors in ACTH-treated rats. Pharmacol Biochem Behav 81:65–70

Kennett GA, Lightowler S, de Biasi V, Stevens NC, Wood MD, Tulloch IF, Blackburn TP (1994a) Effect of chronic administration of selective 5-hydroxytryptamine and noradrenaline uptake inhibitors on a putative index of 5-HT2C/2B receptor function. Neuropharmacology 33:1581–1588

Kennett GA, Wood MD, Glen A, Grewal S, Forbes I, Gadre A, Blackburn TP (1994b) In vivo properties of SB 200646A, a 5-HT2C/2B receptor antagonist. Br J Pharmacol 111:797–802

Kent JM (2000) SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. Lancet 355:911–918

Keuler DJ, Altemus M, Michelson D, Greenberg B, Murphy DL (1996) Behavioral effects of naloxone infusion in obsessive–compulsive disorder. Biol Psychiatry 40:154–156

Khanna S, John JP, Reddy LP (2001) Neuroendocrine and behavioral responses to mCPP in obsessive–compulsive disorder. Psychoneuroendocrinology 26:209–223

Khazaal Y, Krenz S, Benmebarek M, Zullino DF (2006) Worsening of obsessive-compulsive symptoms under methadone tapering. Prog Neuropsychopharmacol Biol Psychiatry 30:30(7):1350–1352 (originally published online 21 April 2006 at http://www.sciencedirect.com)

Kleven MS, Assie MB, Koek W (1997) Pharmacological characterization of in vivo properties of putative mixed 5-HT1A agonist/5-HT(2A/2C) antagonist anxiolytics. II. Drug discrimination and behavioral observation studies in rats. J Pharmacol Exp Ther 282:747–759

Koran LM, Aboujaoude E, Bullock KD, Franz B, Gamel N, Elliott M (2005) Double-blind treatment with oral morphine in treatment-resistant obsessive–compulsive disorder. J Clin Psychiatry 66:353–359

Koskinen T, Haapalinna A, Sirvio J (2003) Alpha-adrenoceptor-mediated modulation of 5-HT2 receptor agonist induced impulsive responding in a 5-choice serial reaction time task. Pharmacol Toxicol 92:214–225

Kraepelin E (1921) Manic-depressive insanity and Paranoia. E&S Livingstone, Edinburgh.

Kurlan R, Majumdar L, Deeley C, Mudholkar GS, Plumb S, Como PG (1991) A controlled trial of propoxyphene and naltrexone in patients with Tourette's syndrome. Ann Neurol 30:19–23

Leckman JF, Walker DE, Goodman WK, Pauls DL, Cohen DJ (1994) "Just right" perceptions associated with compulsive behavior in Tourette's syndrome. Am J Psychiatry 151:675–680

Leysen JE (2004) 5-HT2 receptors. Curr Drug Targets CNS Neurol Disord 3:11–26

Lopez-Ibor Alcocer MI, Ortiz Alonso T, Encinas Mejias M, Fernandez A, Maestu F, Lopez-Ibor Alino JJ (2000) New advances in neuroimaging in the diagnosis of obsessive–compulsive disorder. Actas Esp Psiquiatr 28:304–310

Mangold DL, Peyrot M, Giggey P, Wand GS (2000) Endogenous opioid activity is associated with obsessive–compulsive symptomology in individuals with a family history of alcoholism. Neuropsychopharmacology 22:595–607

Marek GJ (2003) Behavioral evidence for mu-opioid and 5-HT2A receptor interactions. Eur J Pharmacol 474:77–83

Marek GJ, Aghajanian GK (1998a) 5-Hydroxytryptamine-induced excitatory postsynaptic currents in neocortical layer V pyramidal cells: suppression by mu-opiate receptor activation. Neuroscience 86:485–497

Marek GJ, Aghajanian GK (1998b) The electrophysiology of prefrontal serotonin systems: therapeutic implications for mood and psychosis. Biol Psychiatry 44:1118–1127

Marek GJ, Carpenter LL, McDougle CJ, Price LH (2003) Synergistic action of 5-HT2A antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. Neuropsychopharmacology 28:402–412

McConville BJ, Norman AB, Fogelson MH, Erenberg G (1994) Sequential use of opioid antagonists and agonists in Tourette's syndrome. Lancet 343:601

Meuldijk R, Colon EJ (1992) Methadone treatment of Tourette's disorder. Am J Psychiatry 149:139–140

Mink JW (2001) Neurobiology of basal ganglia circuits in Tourette syndrome: faulty inhibition of unwanted motor patterns? Adv Neurol 85:113–122

Nurnberg HG, Keith SJ, Paxton DM (1997) Consideration of the relevance of ethological animal models for human repetitive behavioral spectrum disorders. Biol Psychiatry 41:226–229

Ogata J, Minami K, Uezono Y, Okamoto T, Shiraishi M, Shigematsu A, Ueta Y (2004) The inhibitory effects of tramadol on 5-hydroxytryptamine type 2C receptors expressed in *Xenopus* oocytes. Anesth Analg 98:1401–1406

Ongur D, Goff DC (2005) Obsessive–compulsive symptoms in schizophrenia: associated clinical features, cognitive function and medication status. Schizophr Res 75:349–362

Pauls DL (1992) The genetics of obsessive compulsive disorder and Gilles de la Tourette's syndrome. Psychiatr Clin North Am 15:759–766

Pawlowski L, Melzacka M (1986) Inhibition of head twitch response to quipazine in rats by chronic amitriptyline but not fluvoxamine or citalopram. Psychopharmacology (Berl) 88:279–284

Pujol J, Soriano-Mas C, Alonso P, Cardoner N, Menchon JM, Deus J, Vallejo J (2004) Mapping structural brain alterations in obsessive–compulsive disorder. Arch Gen Psychiatry 61:720–730

Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Mico JA (2002) Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats. Life Sci 72:143–152

Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Mico JA (2004) Antidepressant-like effect of tramadol and its enantiomers in reserpinized mice: comparative study with desipramine, fluvoxamine, venlafaxine and opiates. J Psychopharmacol 18:404–411

Rojas-Corrales MO, Gibert-Rahola J, Mico JA (1998) Tramadol induces antidepressant-type effects in mice. Life Sci 63:PL175–180

Sandyk R (1986a) Naloxone withdrawal exacerbates Tourette syndrome. J Clin Psychopharmacol 6:58–59

Sandyk R (1986b) Tourette syndrome: successful treatment with clonidine and oxycodone. J Neurol 233:178–179

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. J Pharmacol Exp Ther 273:101–112

Shapira NA, Keck PE, Jr., Goldsmith TD, McConville BJ, Eis M, McElroy SL (1997a) Open-label pilot study of tramadol hydrochloride in treatment-refractory obsessive–compulsive disorder. Depress Anxiety 6:170–173

Shapira NA, McConville BJ, Pagnucco ML, Norman AB, Keck PE, Jr. (1997b) Novel use of tramadol hydrochloride in the treatment of Tourette's syndrome. J Clin Psychiatry 58:174–175

Shapira NA, Verduin ML, DeGraw JD (2001) Treatment of refractory major depression with tramadol monotherapy. J Clin Psychiatry 62:205–206

Shapira NA, Ward HE, Mandoki M, Murphy TK, Yang MC, Blier P, Goodman WK (2004) A double-blind, placebo-controlled trial of olanzapine addition in fluoxetine-refractory obsessive–compulsive disorder. Biol Psychiatry 55:553–555

Spencer C (2000) The efficacy of intramuscular tramadol as a rapid-onset antidepressant. Aust N Z J Psychiatry 34:1032–1033

Stahl SM (1998) Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action. J Clin Psychiatry 59 Suppl 4:5–14

Sun HL, Zheng JW, Wang K, Liu RK, Liang JH (2003) Tramadol reduces the 5-HTP-induced head-twitch response in mice via the activation of mu and kappa opioid receptors. Life Sci 72:1221–1230

Swartz CM, Shen WW (1999) Is episodic obsessive compulsive disorder bipolar? A report of four cases. J Affect Disord 56:61–66

Urraca N, Camarena B, Gomez-Caudillo L, Esmer MC, Nicolini H (2004) Mu opioid receptor gene as a candidate for the study of obsessive compulsive disorder with and without tics. Am J Med Genet B Neuropsychiatr Genet 127:94–96

Vickers SP, Easton N, Malcolm CS, Allen NH, Porter RH, Bickerdike MJ, Kennett GA (2001) Modulation of 5-HT(2A) receptor-mediated head-twitch behaviour in the rat by 5-HT(2C) receptor agonists. Pharmacol Biochem Behav 69:643–652

Warneke L (1997) A possible new treatment approach to obsessive–compulsive disorder. Can J Psychiatry 42:667–668

Weissman MM, Bland RC, Canino GJ, Greenwald S, Hwu HG, Lee CK, Newman SC, Oakley-Browne MA, Rubio-Stipec M, Wickramaratne PJ et al (1994) The cross national epidemiology of obsessive compulsive disorder. The Cross National Collaborative Group. J Clin Psychiatry 55 Suppl:5–10

Willins DL, Meltzer HY (1997) Direct injection of 5-HT2A receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. J Pharmacol Exp Ther 282:699–706

Xu LF, Chu WJ, Qing XY, Li S, Wang XS, Qing GW, Fei J, Guo LH (2006) Protopine inhibits serotonin transporter and noradrenaline transporter and has the antidepressant-like effect in mice models. Neuropharmacology 50:934–940

Zohar J, Mueller EA, Insel TR, Zohar-Kadouch RC, Murphy DL (1987) Serotonergic responsivity in obsessive–compulsive disorder. Comparison of patients and healthy controls. Arch Gen Psychiatry 44:946–951