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5-HT_4 receptors do not mediate the antidepressant-like behavioral effects of fluoxetine in a modified forced swim test

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Abstract

The receptors responsible for mediating the antidepressant effects of selective serotonin reuptake inhibitors are largely unknown. The role of the 5-HT_4 receptor in mediating the antidepressant-like effects of fluoxetine in a modified rat forced swim test was examined. Fluoxetine (20 mg/kg) decreased immobility and increased swimming, a pattern shown to represent its actions on the serotonergic system. The selective 5-HT_4 receptor antagonist, SB 204070A [8-amino-7-chloro-(*N*-butyl-4-piperidyl)methylbenzo-1,4-dioxan-5-carboxylatehydrochloride] (0.1–3 mg/kg), failed to change any of the active behaviors in the test compared with saline-treated animals. Upon combination, SB 204070A (3 mg/kg) failed to alter the effects of fluoxetine effects in the test. These data therefore suggest that activation of postsynaptic 5-HT_4 receptors, subsequent to reuptake inhibition by fluoxetine, is not necessary for its antidepressant-like behavioral effects in this test.

Keywords: Forced swim test; 5-HT_4 receptor; SB 204070A; Fluoxetine; Antidepressant

1. Introduction

Selective serotonin reuptake inhibitors, such as fluoxetine, are believed to exert their clinical antidepressant effects by blocking the reuptake of serotonin at the synapse, resulting in an elevation of extracellular 5-hydroxytryptamine (5-HT) concentrations in limbic regions of the brain which can act upon various postsynaptic 5-HT receptors (Goodnick and Goldstein, 1998). At least 14 different subtypes of 5-HT receptors have been identified (Barnes and Sharp, 1999), and it is still unclear as to which of them are most relevant to the pathogenesis of depression and the mechanism of action of antidepressants (see Cryan and Leonard, 2000). Human and animal studies have strongly implicated receptors of the 5-HT_1 and 5-HT_2 receptor families to play a critical role in the antidepressant response (Lucki et al., 1994; Barnes and Sharp, 1999; Cryan and Leonard, 2000; Cryan and Lucki, 2000); the role of other 5-HT receptor subtypes have been largely unexplored.

The 5-HT_4 receptor is a postsynaptically located seven-transmembrane spanning receptor present at highest densities in the brain, in limbic areas such as the olfactory tubercules, septum, hippocampus and amygdala as well as the basal ganglia (Bockaert et al., 1997). Such localization suggests that this receptor might play a role in mediating the psychotherapeutic effects of serotonergic compounds. Indeed, recent studies have shown that the 5-HT_4 receptor antagonists, GR 113808 ([1-[2-methylsulphonyl)amino]-ethyl]-4-piperidinyl]methyl 1-methyl-1 H-indole-3-carboxylate) (Silvestre et al., 1996), SB 207266A (*N*-(1-butyl-4-piperidinylmethyl)-3, 4-dihydro-2H-[1,3]-oxazino-[3,2-a]-indole-10-carboxamide hydrochloride) and SB 204070A [8-amino-7-chloro-(*N*-butyl-4-piperidyl)methylbenzo-1,4-dioxan-5-carboxylatehydrochloride] (Kennett et al., 1997), exhibit anxiolytic-like behaviors in various animal models of anxiety.

To our knowledge, there have been no published studies examining the role of 5-HT_4 receptors in animal behavior tests sensitive to antidepressants. The forced swim test (FST), as originally described by Porsolt et al. (1977), is

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the most widely used pharmacological model for assessing antidepressant activity (Weiss and Kilts, 1998). Rats develop immobility when they are placed in an inescapable cylinder of water. The immobile behavior is thought to reflect either a failure to persist in escape-directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli (Lucki, 1997). A broad spectrum of antidepressant drugs selectively reduces the development of behavioral immobility in the forced swim test (Borsini and Meli, 1988). However, the paradigm in its traditional form is unreliable in the detection of selective serotonin reuptake inhibitors (for review, see Borsini, 1995). In an effort to overcome this, we have made procedural modifications to the test which have enabled us to demonstrate that specific behavioral components of active behaviors in the forced swim test (namely, swimming and climbing) distinguished neurochemically distinct antidepressant drugs (see Lucki, 1997). The modified forced swim test differentiated swimming behavior, which was sensitive to selective serotonin reuptake inhibitors and 5-HT receptor agonists, and climbing behavior which was sensitive to tricyclic antidepressants and drugs with selective effects on catecholamine transmission (Detke et al., 1995; Lucki, 1997). The distinctive active behaviors produced by pharmacologically selective antidepressants persisted upon chronic treatment (Detke et al., 1997), and they were superimposable upon the combination of serotonergic and catacholaminergic compounds (Reneric and Lucki, 1998). The swimming behavior produced by fluoxetine is mediated by 5-HT because it was prevented by prior depletion of 5-HT with the tryptophan hydroxylase inhibitor parachlorophenylalanine, but not the climbing behavior produced by the norepinephrine reuptake inhibitor desipramine (Page et al., 1999). Although the modified rat forced swim test reliably detects the behavioral effects of serotonergic antidepressants, it is unclear which 5-HT receptors are responsible for the mediation of these antidepressant-sensitive behaviors. The following studies, therefore, were aimed at investigating the role of the 5-HT_4 receptor in mediating selective antidepressant-like effects in the modified rat forced swim test.

2. Materials and methods

2.1. Animals

A total of 73 male Sprague–Dawley rats (Charles River, Wilmington, MA, USA), weighing 300–400 g, were used in these studies. The animals were housed in pairs in polycarbonate cages and maintained on a 12-h light/dark cycle (lights on at 07:00 h) in a temperature-controlled (22°C) colony. The animals had free access to food and water. Animals were handled daily for at least 4 days prior to initiation of behavioral testing. Behavioral studies were carried out in the afternoon (12:00–18:00) during the month of May. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2. Rat forced swim test

The modified rat forced swim test was conducted essentially as described by Detke et al. (1995). Briefly, rats were placed individually in Pyrex cylinders (21 × 46 cm; Fisher Scientific) which were filled with water to a 30-cm depth. They were removed 15 min later, dried and placed in their home cage. Twenty-four hours after their first exposure, the animals were replaced in the swim apparatus for 5 min and behaviors were monitored from above by videocamera for subsequent analysis. Animals were randomly assigned to groups that received various drug treatments or control (0.9% saline). Injections were administered subcutaneously three times, 1, 5 and 23.5 h prior to the test session. The rater of the behavioral patterns was blind with respect to the experimental conditions being scored. A time sampling technique was employed, whereby, the predominate behavior in each 5-s period of the 300-s test was recorded. Climbing behavior consisted of upward directed movements of the forepaws along the side of the swim chamber. Swimming behavior was defined as movement (usually horizontal) throughout the swim chamber which also included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keep the rat's head above the water.

The first study examined in a dose–response fashion the effects of the highly potent and selective 5-HT_4 receptor antagonist SB 204070A (Wardle et al., 1994) on active behaviors in the modified rat forced swim test. SB 204070A has been shown to have over 5000 times more affinity for 5-HT_4 over other receptors (Wardle et al., 1994). Doses were selected from previous studies showing activity at these or lower doses in behavioral tasks (e.g. Kennett et al., 1997; Silvestre et al., 1996). The next set of studies examined the effects of SB 204070A (3 mg/kg), given either alone or in combination with the selective serotonin reuptake inhibitor fluoxetine (20 mg/kg) on active behaviors in the test. The dose of fluoxetine was selected from previous dose–response studies which showed maximal effects on both immobility and swimming behavior at this dose (Cryan and Lucki, 2000).

2.3. Drugs

All drugs were made up freshly prior to use and injected subcutaneously in a volume of 2 ml/kg. Both SB

204070A (SmithKline Beecham, Harlow, UK) and fluoxetine (Eli Lilly and Co., Indianapolis, IN) were dissolved in deionized water and sonicated mildly. For combination experiments, both drugs were made up in the same solution to eliminate any confounding stress effects of multiple injections. All drug doses were calculated as the base weight.

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was carried out in all studies. Overall statistical differences were analyzed further using Fisher's post-hoc tests.

3. Results

The 5-HT_4 receptor antagonist SB 204070A given alone did not alter immobility [$F(4,33) = 1.218$, $P = 0.32$], swimming [$F(4,33) = 0.97$, $P = 0.44$] or climbing [$F(4,33) = 1.52$, $P = 0.22$] behaviors in the rat forced swim test at all doses tested (see Fig. 1).

When SB 204070A (3 mg/kg) was administered in combination with fluoxetine (20 mg/kg), there was a significant overall effect of drug treatment on immobility [$F(3,31) = 12.89$, $P < 0.001$], on swimming [$F(3,31) = 21.336$, $P < 0.001$], but not on climbing [$F(3,31) = 1.67$, $P = 0.19$]. Post-hoc analysis demonstrated fluoxetine decreased immobility and increased swimming behavior. These effects were unaltered by combined treatment with the 5-HT_4 receptor antagonist SB 204070A, which had no effect on any behavioral parameter when administered on its own (see Fig. 2).

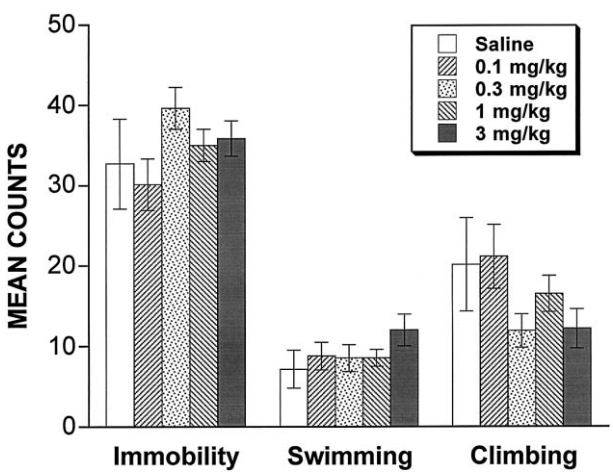

Fig. 1. The effects of the selective 5-HT_4 receptor antagonist, SB 204070A, on active behaviors in a modified rat forced swim test. SB 204070A [0.1 mg/kg, $n = 8$; 0.3 mg/kg, $n = 8$; 1 mg/kg, $n = 8$; 3 mg/kg, $n = 7$] failed to alter any of the behaviors in the forced swim test when compared with saline-treated animals [$n = 7$]. All bars represent mean values with vertical lines indicating 1 S.E.M.

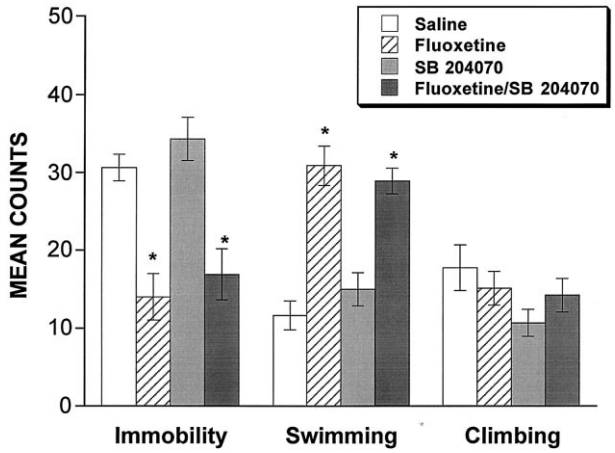

Fig. 2. The effects of the 5-HT_4 receptor antagonist SB 204070A alone or in combination with fluoxetine on active behaviors in a modified rat forced swim test. Fluoxetine alone [20 mg/kg, $n = 9$] and in combination with SB 204070A [3 mg/kg, $n = 9$] decreased immobility and increased swimming behavior compared with saline-treated animals [$n = 8$]. SB 204060A given alone [3 mg/kg, $n = 9$] failed to alter any behavioral parameter. All bars represent mean values with vertical lines indicating 1 S.E.M. Asterisks indicate groups that differed significantly from saline-treated animals: $^{*}P < 0.05$.

4. Discussion

The present studies demonstrate that the selective antidepressant-like effects of fluoxetine are not dependent on or modulated by 5-HT_4 receptors. Fluoxetine, as in previous studies, exhibited the characteristic behavioral effects of a selective serotonin reuptake inhibitor in the modified forced swim test, i.e. a decrease in immobility coupled with an increase in swimming behavior. The highly selective and potent 5-HT_4 receptor antagonist SB 204070A failed to alter either of these parameters or have an effect on climbing behavior at all doses tested. When combined with fluoxetine, SB 204070A failed to influence fluoxetine's effects on active behaviors in the test. This indicates that the increased synaptic 5-HT available, following subchronic fluoxetine administration, probably does not utilize 5-HT_4 receptors to manifest the behavioral antidepressant-like effects in the modified forced swim test.

The centrally mediated behavioral and neurochemical effects of 5-HT_4 receptor activation are still not well defined, although a putative role in cognitive performance and anxiety-related behaviors has been proposed (for review see Barnes and Sharp 1999; Meneses, 1999). 5-HT_4 receptor activation has also been associated with alterations in monoamine neurotransmission. Increases in 5-HT release in the hippocampus were demonstrated following direct administration of 5-HT_4 receptor agonists and 5-HT_4

receptor antagonists reduced the release of 5-HT, indicating the presence of an endogenous tone on the 5-HT_4 receptor (Ge and Barnes, 1996). 5-HT_4 receptor-mediated modulation of dopamine release has been demonstrated in various brain regions (Thorre et al., 1998; De Deurwaerdere et al., 1997; Steward et al., 1996). These alterations on dopaminergic neurotransmission may account for the ability of the 5-HT_4 receptor antagonist SDZ 205,557 [2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester] to antagonize cocaine-induced hyperactivity (McMahon and Cunningham, 1999).

The effects of 5-HT_4 receptor activation on cognitive processes is largely due to its proposed ability to facilitate cholinergic function, as assessed using in vivo microdialysis (e.g. Consolo et al., 1994). 5-HT_4 receptor agonists, such as BIMU1 (endo-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxamide, HCl), have been shown to enhance the performance of rats and mice in different behavioral models assessing both short-term and long-term memory. These actions of BIMU1 are likely to be 5-HT_4 receptor-mediated since they were prevented by the selective 5-HT_4 receptor antagonist, GR125487(1-[2-[(methylsulfonyl)-amino]ethyl]-4-piperidinyl-methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate) (Letty et al., 1997; Marchetti-Gauthier et al., 1997). In addition, the 5-HT_4 receptor partial agonist, RS 67333 (1(4-amino-5-chloro-2-methoxyphenyl)-3-(1-*n*-butyl-4-piperidinyl)-1-propanone), reversed the atropine-induced impaired performance of rats in the Morris water maze (Fontana et al., 1997), an effect blocked by the 5-HT_4 receptor antagonist RS 67532 (1-(4-amino-5-chloro-2-(3,5-dimethoxybenzyloxyphenyl)-5-(1-piperidinyl)-1-pentanone). 5-HT_4 receptor antagonists have been shown to be without effect on the performance of both naive and atropine-treated rats in the Morris water maze (Fontana et al., 1997), but the 5-HT_4 receptor antagonists SDZ 205,557 and GR 125487 elicited an amnesic effect in the mouse passive avoidance test (Galeotti et al., 1998).

The 5-HT_4 receptor antagonists SB 204070A, GR 113808 and SB 204070, at doses lower or comparable to used in our studies have marked anxiolytic properties in two animal models of anxiety, the rat social interaction test and the elevated plus-maze (Kennett et al., 1997; Silvestre et al., 1996). In contrast, the non-selective 5-$HT_{3/4}$ receptor antagonists, tropisetron and SDZ 205,557, reduced the anxiolytic-like action of diazepam in the mouse light/dark test and the rat social interaction test but did not produce changes when administered alone (Cheng et al., 1994). These results were replicated more recently in the mouse light/dark test using the highly selective 5-HT_4 receptor antagonists GR 113808 and SB 204070 (Costall and Naylor, 1997).

Because of the complex effects of 5-HT_4 receptor activation on serotonergic, cholinergic and dopaminergic neurotransmission, and the behavioral manifestations of this on animal models of memory, reward and anxiety, there was a likelihood that 5-HT_4 receptor antagonists could impact animal paradigms sensitive to antidepressant action. Here, we demonstrate that 5-HT_4 receptors are probably not involved in the behavioral antidepressant-like effects of fluoxetine in the forced swim test. Other 5-HT receptor subtypes such as the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptors, have been shown to be implicated in mediating the behavioral effects of selective serotonin reuptake inhibitors in the rat forced swim test (Lucki et al., 1994; De Vry, 1995; Borsini, 1995; Cryan and Lucki, 2000). Here, we conclude that the 5-HT_4 receptor does not appear to be a candidate receptor subtype for mediating fluoxetine's effects postsynaptically in the modified rat forced swim test.

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