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Short communication

Selective serotonin reuptake inhibitors potentiate 8-OH-DPAT-induced stimulus control in the pigeon

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Abstract

The effects of two selective serotonin reuptake inhibitors, fluoxetine and citalopram, and a nonselective monoamine reuptake inhibitor, imipramine, were characterized in pigeons that had been trained to discriminate 0.64 mg/kg of 8-hydroxy-(2-di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), a 5-HT_{1A} receptor agonist, from saline. Neither fluoxetine, citalopram, nor imipramine generalized to the 8-OH-DPAT-induced stimulus cue. However, when administered in addition to 8-OH-DPAT, both fluoxetine (10 mg/kg) and citalopram (10 mg/kg) lowered the ED_{50} for generalization of 8-OH-DPAT from 0.16 mg/kg (8-OH-DPAT by itself) to 0.05 mg/kg (fluoxetine + 8-OH-DPAT) and 0.06 mg/kg (citalopram + 8-OH-DPAT). Under similar conditions, imipramine (1 mg/kg) had no effect on the generalization curve for 8-OH-DPAT. The data support the hypothesis that activation of the 5-HT_{1A} receptor may be relevant to the mechanism of action of serotonin reuptake inhibitors. © 1998 Elsevier Science B.V.

Keywords: Drug discrimination; 5-HT (5-hydroxy tryptamine, serotonin) reuptake inhibitor, selective; 5-HT_{1A} receptor; (Pigeon)

1. Introduction

The antidepressant activity of selective serotonin reuptake inhibitors is linked to their common ability to increase the concentration of serotonin (5-HT) in the synaptic cleft by inhibiting the activity of the 5-HT transporter. However, precisely which 5-HT receptors and pathways are involved in the antidepressant activity of selective serotonin reuptake inhibitors is not well understood (Fuller, 1992). The 5-HT_{1A} receptor subtype may be important since 5-HT_{1A} receptor selective compounds are effective in a number of animal models of depression (e.g., Schreiber and de Vry, 1993a). Similarly, two 5-HT_{1A} receptor agonists, buspirone and flesinoxan, have clinical antidepressant activity (Deakin, 1993).

The drug discrimination paradigm has been a useful tool for delineating the neuropharmacological properties of various drugs. However, there is very little information available on the discriminative stimulus effects of antidepressants (Zhang and Barrett, 1991), largely due to the difficulty of training animals to recognize such a stimulus at nontoxic doses. By virtue of its serotonin reuptake inhibiting property, fluoxetine can be considered an indirect agonist at all 5-HT receptor subtypes and thus might be expected to induce a complex discriminative stimulus. Although fluoxetine has not been used as a training drug, some information about its stimulus properties may be obtained from studies using other drugs as training cues. Selective serotonin reuptake inhibitors have not substituted for a number of other classes of compounds (e.g., cocaine: Cunningham and Callahan, 1991; 8-hydroxy-(2-di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), a 5-HT_{1A} receptor agonist: Arnt, 1989; *d*-lysergic acid diethylamide tartrate (LSD) or *N*-(3-Trifluoromethylphenyl)piperazine hydrochloride (TFMPP), agonists at several 5-HT receptors: Arnt, 1989; Kuhn et al., 1978). However, under some conditions, fluoxetine has substituted for ethanol (Maurel et al., 1997), a complex, compound stimulus and has partially substituted for fenfluramine (White and Appel, 1981), which has some 5-HT reuptake inhibiting properties in addition to increasing 5-HT release. In addition, selective serotonin reuptake inhibitors have been found to share stimulus properties with both a selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (de Beun et al., 1993) as well as a relatively selective 5-HT_{2C} receptor agonist, 6-chloro-2-(1-piperazinyl) pyrazine monohydrochloride (MK212) (Berendsen and Broekkamp, 1994) when tested in a cross-

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familiarization conditioned taste aversion procedure which gives results similar to those obtained in the more conventional drug discrimination paradigm (de Beun et al., 1993).

Further information about the stimulus properties of selective serotonin reuptake inhibitors has been obtained by combining a selective serotonin reuptake inhibitor with compounds which induce a known stimulus cue. For example, an acute injection of fluoxetine potentiated the stimulus effects of a low dose of fenfluramine (White and Appel, 1981) and cocaine (Cunningham and Callahan, 1991). Acute injections of fluoxetine augmented LSD-induced stimulus control in the rat (Fiorella et al., 1996), but, upon chronic administration, attenuated the LSD-induced stimulus in humans (Bonson et al., 1996). However, drugs such as LSD, cocaine, ethanol, and fenfluramine induce complex discriminative stimuli related to their broad receptor binding profiles or abilities to affect various neurotransmitter release and uptake activities. Consequently, these studies do not elucidate any one receptor subtype as being important for the stimulus effects of selective serotonin reuptake inhibitors like fluoxetine. Therefore, the present study was undertaken to investigate the interaction of the selective serotonin reuptake inhibitors fluoxetine and citalopram with the more specific 5-HT_{1A} cue induced by 8-OH-DPAT. This activity was compared to that of a nonselective monoamine reuptake inhibitor, imipramine.

2. Materials and methods

2.1. Subjects

Eight male White Carneaux pigeons (Palmetto Pigeon Plant, Sumter, SC) were housed in individual stainless steel cages with water and crushed oyster shells continuously available, except during experimental sessions. The pigeons were maintained at approximately 85% of their free feeding body weights by post-session supplemental feedings of Pigeon Chow Checkers (Purina Mills, St. Louis, MO). All testing was conducted during the illuminated phase of the light–dark cycle (0600–1800).

2.2. Procedure

2.2.1. Apparatus

The experiments were conduced in pigeon operant conditioning chambers (Med Associates, East Fairfield, VT) that were placed in light- and sound-attenuated enclosures equipped with ventilation fans and white noise generators. Mixed grain could be presented through an opening centered beneath the response keys which were transilluminated by white stimulus lights. During grain presentation, this opening was illuminated and the right and left key lights were extinguished.

2.2.2. Drug discrimination

Five pigeons were trained to peck on the right key after saline injection (i.m.) and on the left key after injection of 0.64 mg/kg of 8-OH-DPAT (i.m.). The injection appropriate keys were reversed for the other two pigeons. An additional 3 pigeons were trained in a similar manner but with a lower dose (0.16 mg/kg) of 8-OH-DPAT as the stimulus cue. During the training sessions, 30 consecutive responses on the injection-appropriate key resulted in 4 s access to grain. Responses on the inappropriate key reset the response requirement on the injection appropriate key. Substitution tests were conducted on Wednesday and Friday, if performance on the immediately preceding training days met the criterion of at least 90% correct responding. On test days, responding on either key resulted in grain presentation and the session lasted until 30 grain presentations occurred, or 30 min elapsed. The percentage of responses that occurred on the 8-OH-DPAT appropriate key and the total rate of responding on both response keys were recorded and averaged to determine dose response curves. All the pigeons had extensive exposure to this discrimination before the start of the present study. Dose response curves for fluoxetine ($n = 4$), 8-OH-DPAT ($n = 5$), imipramine ($n = 3$) and a single dose of citalopram

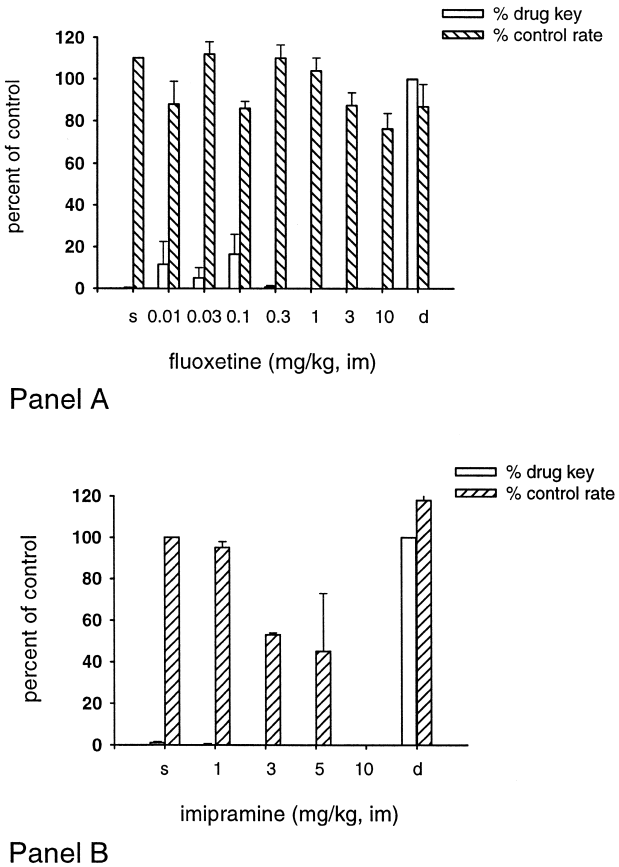

Fig. 1. Discriminative effects of various doses of fluoxetine (Panel A) or imipramine (Panel B) in pigeons trained to discriminate 0.64 mg/kg of 8-OH-DPAT from saline. s = saline control data; d = 8-OH-DPAT control data.

($n = 3$) were determined in the pigeons trained to discriminate 0.64 mg/kg of 8-OH-DPAT. In addition, fluoxetine (0.001–10 mg/kg) was also tested in pigeons trained to discriminate 0.16 mg/kg of 8-OH-DPAT. The highest doses of fluoxetine (10 mg/kg), citalopram (10 mg/kg) and imipramine (1 mg/kg) which were tested and found not to decrease response rates below approximately 80% of the saline control rates were then administered in addition to 0.64 mg/kg of 8-OH-DPAT (i.e., vehicle + 8-OH-DPAT, $n = 5$; fluoxetine + 8-OH-DPAT, $n = 3$; citalopram + 8-OH-DPAT, $n = 3$; imipramine + 8-OH-DPAT, $n = 3$).

2.3. Drugs

Imipramine and (±)8-hydroxy-(2-di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT HBr) were purchased from Research Biochemicals International, (Natick, MA). Fluoxetine and citalopram were synthesized at Eli Lilly (Indianapolis, IN).

All compounds were dissolved in normal saline and administered into the pectoral muscle (i.m.) in a volume of 1 ml/kg of body weight. 8-OH-DPAT was injected 20 min before the test session. When used in combination with 8-OH-DPAT, the fluoxetine, citalopram, imipramine, and saline were injected 40 min prior to 8-OH-DPAT; when used by themselves, these compounds were injected 60 min before the session began.

3. Results

Fluoxetine, tested at doses between 0.01 and 10 mg/kg, did not substitute for 8-OH-DPAT in the 0.64 mg/kg training dose group (Fig. 1, panel A). Similarly, in the 0.16 mg/kg training dose group, fluoxetine tested at doses between 0.001 and 10 mg/kg did not substitute for 8-OH-DPAT (data not shown). Tested at a single dose of 10 mg/kg, citalopram also did not substitute for 8-OH-DPAT (0.64 mg/kg trained group). Likewise, imipramine did not discriminate as 8-OH-DPAT, although doses above 1 mg/kg decreased rates of responding (Fig. 1, panel B).

When injected 40 min prior to 8-OH-DPAT, both fluoxetine (10 mg/kg) and citalopram (10 mg/kg) shifted the dose response curve for discrimination of 8-OH-DPAT to the left on the dose axis (Fig. 2, panel A). Similar injections of saline and imipramine (1 mg/kg) had no effect on the 8-OH-DPAT dose response curve (Fig. 2, panel B). The ED_{50} values for the recognition of the 8-OH-DPAT cue were, 0.05 mg/kg (fluoxetine + 8-OH-DPAT), 0.06 mg/kg (citalopram + 8-OH-DPAT), 0.19 mg/kg (imipramine + 8-OH-DPAT), 0.16 mg/kg (8-OH-DPAT

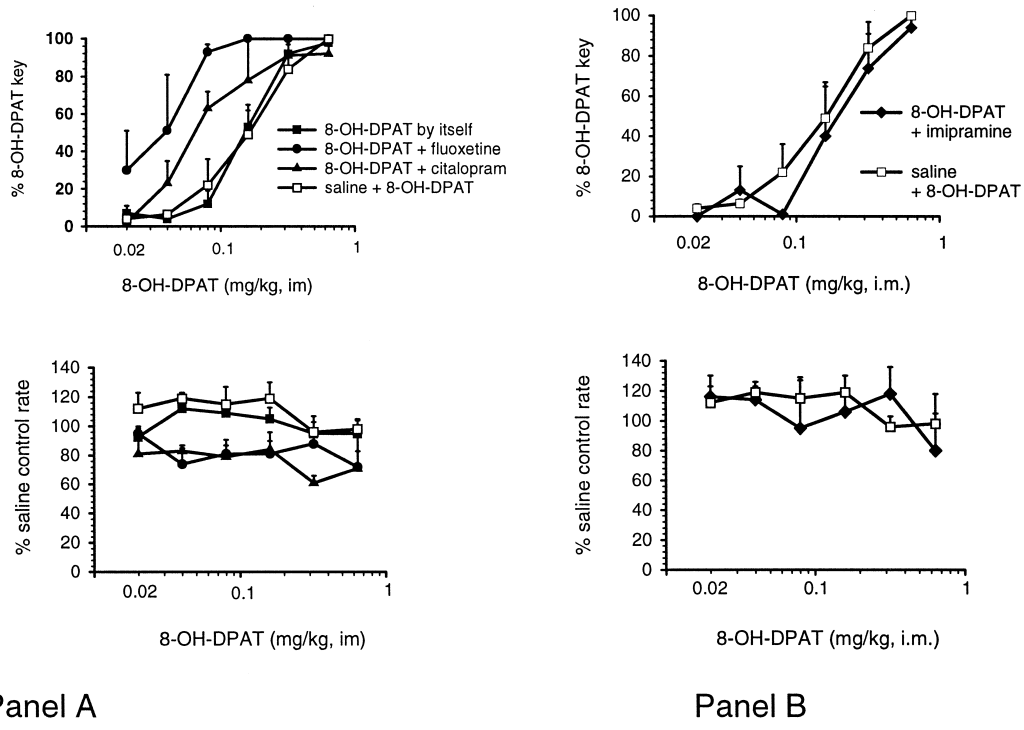

Fig. 2. Panel A (upper): The percent of the total responses on the 8-OH-DPAT key in response to various doses of 8-OH-DPAT injected by itself ($n = 5$) or in combination with 10 mg/kg of fluoxetine ($n = 3$), 10 mg/kg of citalopram ($n = 3$) or saline ($n = 5$). (Lower): The percent of the saline control rate in the same pigeons. Panel B (upper): The percent of the total responses on the 8-OH-DPAT key in response to various doses of 8-OH-DPAT in combination with 1 mg/kg of imipramine ($n = 3$). The data for saline +8-OH-DPAT has been replotted for convenience. (Lower): The percent of the saline control rate in the same pigeons.

alone), and 0.16 mg/kg (saline + 8-OH-DPAT). The combination of either fluoxetine or citalopram with 8-OH-DPAT resulted in a decreased rate of responding (Fig. 2, panel A), whereas no doses of 8-OH-DPAT alone decreased response rates.

4. Discussion

Fluoxetine, citalopram and imipramine were not discriminated as 8-OH-DPAT. This is in agreement with other studies that have found that reuptake inhibitors, such as citalopram (Arnt, 1989), are not discriminated as being similar to compounds that are specific for the 5-HT_{1A} receptor. Although selective serotonin reuptake inhibitors indirectly activate some of the 5-HT_{1A} receptors that may be responsible for the 8-OH-DPAT discrimination, such as the somatodendritic 5-HT_{1A} receptor (Schreiber and de Vry, 1993b), stimulus cues resulting from the indirect activation of other 5-HT receptor subtypes may interfere with recognition of the specific 5-HT_{1A} cue. In general, only compounds that have both high affinity and selectivity for the 5-HT_{1A} receptor have been found to substitute for training drugs, such as 8-OH-DPAT, that are also specific for this receptor. In the case of fluoxetine, substitution for 8-OH-DPAT was not obtained even when using a low training dose (0.16 mg/kg) of 8-OH-DPAT which greatly increases the sensitivity of the 5-HT_{1A} discrimination (Wolff and Leander, 1997).

Although not discriminated as being similar to 8-OH-DPAT, both fluoxetine and citalopram potentiated the stimulus effects of low doses of 8-OH-DPAT. Because neither fluoxetine nor citalopram exhibits high affinity for the 5-HT_{1A} receptor (Wong et al., 1991), this effect is most likely due to an indirect activation of 5-HT_{1A} receptors caused by increased 5-HT in the receptor vicinity. This effect may be specific for compounds with high selectivity for the 5-HT_{1A} receptor since selective serotonin reuptake inhibitors have not been found to potentiate all cues induced by compounds with selective activity at other 5-HT receptor subtypes. For instance, fluoxetine did not affect the *m*-chlorophenylpiperazine (*m*CPP)-induced stimulus cue (Callahan and Cunningham, 1994) which reflects activity at the 5-HT_{1B} and 5-HT_2 receptor sites or the cue in pigeons (Wolff and Leander, unpublished observations) induced by 0.32 mg/kg of (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), a compound with high affinity for the 5-$HT_{2A/C}$ receptor. That activation of the 5-HT_{1A} receptor is responsible for a component of the stimulus cue induced by selective serotonin reuptake inhibitors is supported by data obtained in a cross-familiarization conditioned taste aversion procedure in which preexposure to sertraline prevented the development of the conditioned taste aversion induced by 8-OH-DPAT (de Beun et al., 1993).

In contrast to the results obtained with a combination of fluoxetine or citalopram plus 8-OH-DPAT, the combination of imipramine plus 8-OH-DPAT did not affect the generalization curve for 8-OH-DPAT. This may be somewhat surprising since 5-HT_{1A} selective compounds are discriminated as being similar to imipramine (Zhang and Barrett, 1991). The dose of imipramine (1 mg/kg) which was administered in the present experiment may have been too low to reveal an effect on the generalization curve for 8-OH-DPAT. However, higher doses of imipramine were not studied because of their rate decreasing effects. It is also possible that other stimulus cues may have masked the effect of increased activation of the 5-HT_{1A} receptor. Imipramine induces a complex stimulus cue in which the effects of increased norepinephrine may be more important than 5-HT, since tomoxetine, but not fluoxetine, substitutes for the imipramine cue (Zhang and Barrett, 1991).

In summary, the discriminative stimulus effects of 8-OH-DPAT are increased by selective serotonin reuptake inhibitors such as fluoxetine and citalopram but not by a nonselective reuptake inhibitor, imipramine. The increased discrimination of low doses of 8-OH-DPAT caused by the combination of fluoxetine or citalopram with 8-OH-DPAT suggests that, in this situation, the selective serotonin reuptake inhibitors and a 5-HT_{1A} receptor agonist have additive effects and are functioning through a common neuronal system. Activation of the 5-HT_{1A} receptor may be important in the mechanism of action of selective serotonin reuptake inhibitors.

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