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Short communication

Meta-chlorophenylpiperazine induced changes in locomotor activity are mediated by 5-HT_1 as well as 5-HT_{2C} receptors in mice

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Abstract

1-(*meta*-chloro)phenylpiperazine (*m*-CPP) is a 5-HT receptor agonist which has been purported to be relatively selective for the 5-HT_{2C} receptor. In particular, the hypolocomotion produced by *m*-CPP has been suggested to be mediated by 5-HT_{2C} receptors. *m*-CPP binds with high affinity to 5-HT_1 as well as 5-HT_2 receptors, thus effects of *m*-CPP on locomotor activity may be due to the physiologic summation of the actions of *m*-CPP at 5-HT_1 as well as 5-HT_2 receptors. The present study investigated the effects of *m*-CPP alone and in the presence of the 5-HT_2 receptor antagonist 6-methyl-1-(1-methyethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate (LY53857), the 5-HT_{1A} receptor antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]- ethyl}-*N*-(2pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100,635), and the 5-$HT_{1B/1D}$ receptor antagonist 2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-corboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide (GR 127935) on locomotor activity. Administration of *m*-CPP alone (0.3–10 mg/kg) produced a dose-related decrease in locomotor activity. The 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (3.0 mg/kg) in combination with *m*-CPP produced a slight leftward shift of the dose–response curve of *m*-CPP. The 5-HT_{1A} receptor antagonist WAY 100,635 (1.0 mg/kg) in combination with *m*-CPP did not alter the *m*-CPP dose–response curve. The non-selective 5-HT_2 receptor antagonist LY53857 (1.0 mg/kg) in combination with *m*-CPP unmasked a hyperlocomotion produced by *m*-CPP. Furthermore, the hyperlocomotion produced by *m*-CPP in the presence of LY53857 (1.0 mg/kg) was blocked by both the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (3.0 mg/kg) and the 5-HT_{1A} receptor antagonist WAY 100,635 (1.0 mg/kg). The present results demonstrate that the hyperlocomotion seen with the combination of *m*-CPP and LY53857 is mediated by 5-HT_1 receptors. Taken together the data indicate that *m*-CPP affects locomotor activity by the physiologic summation of agonist activity at the 5-HT_{2C} receptor as well as the 5-HT_1 receptor family. © 1998 Published by Elsevier Science B.V.

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1. Introduction

Meta-chlorophenylpiperazine (*m*-CPP) has been previously demonstrated to decrease locomotor activity in rats (Kennett and Curzon, 1988; Lucki et al., 1989). The decrease in spontaneous locomotion has been ascribed to activation of 5-HT_{2C} receptors (Lucki et al., 1989; Kennett et al., 1994). However, *m*-CPP has affinity for the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer, 1988; Hamik and Peroutka, 1989), suggesting that *m*-CPP may be capable of altering locomotor activity by actions at more than one serotonin receptor subtype. Moreover, in drug discrimination paradigms the predominate action(s) of *m*-CPP have been ascribed to agonist activity at 5HT_{2C} receptors, antagonist activity at 5-HT_{2A} receptors (Fiorella et al., 1995) and to a lesser extent activation of 5-HT_{1B} receptors (Callahan and Cunningham, 1994). In addition, *m*-CPP has been shown to partially substitute for the 5-HT_1 agonist RU 24969 in rats trained to discriminate RU 24969 from saline (Gardner, 1989). Taken together, these data indicate that *m*-CPP is not a selective agonist at the 5-HT_{2C} receptor.

Previous work has focused on the antagonism of a fixed dose of *m*-CPP (Kennett and Curzon, 1988; Lucki et al., 1989) by various serotonin antagonists in rats. In the present study, we investigated the effects of the non-selective 5-HT_2 receptor antagonist 6-methyl-1-(1-methyethyl)-ergoline-88-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate (LY53857) on the effects of

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m-CPP administered over a broad range of doses in mice. LY53857 blocked the decreases in locomotor activity produced by *m*-CPP, but also unmasked a hyperlocomotion. Based on previous work demonstrating 5-HT_1 agonists are capable of increasing locomotor activity (Green et al., 1984; Tricklebank et al., 1986; O'Neill et al., 1996), we investigated the effects of *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100,635) and 2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-corboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide (GR 127935) on the hyperlocomotion produced by *m*-CPP in the presence of LY53857. Interestingly, both 5-HT_1 receptor antagonists were capable of fully reversing the hyperlocomotion seen with *m*-CPP and LY53857.

2. Methods

2.1. Subjects

Male Crl:CF1® BR (Harlan Sprague Dawley, Indianapolis, IN) mice weighing 20–35 g at the time of testing were housed in groups of 17 in a large colony room with food and water available continuously. The lights were on between 6.00 a.m. and 6.00 p.m. Studies were conducted between 8.00 a.m. and 12 noon in a quiet room.

2.2. Procedure

Locomotor activity was measured with a 20 station Photobeam Activity System (San Diego Instruments, San Diego, CA) with seven photocells per station. Animals were weighed, injected and immediately placed individually in a polypropylene cage ($40.6 \times 20.3 \times 15.2$ cm). Data were collected for 15 min and expressed as total ambulations. Eight mice were used per group. Each animal was used only once.

2.3. Data analysis

Locomotor activity was recorded as the number of ambulations, where ambulation was defined as the sequential breaking of adjacent photobeams. P values comparing the drug-treated groups to control groups were based on an analysis of variance with Dunnett's method of multiple comparisons (Kirk, 1982). A *P*-value of < 0.05 was considered as significant. All computations were done using JMP v3.1 (SAS Institute, Cary, NC).

2.4. Drugs

The following drugs were used in this study: 6-methyl-1-(1-methyethyl)ergoline-813-carboxylic acid 2-hydroxy-1-methylpropyl ester maleate (LY53857), *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY 100,635 MCI), and 2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-corboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide (GR 127935 MCI), (Lilly Research Labs, Indianapolis, IN and Lilly Research Centre, Erl Wood, Windlesham, Surrey, UK), and 1-(*m*-chlorophenyl)piperazine (*m*-CPP MCI), Sigma Chemical Co., St. Louis, MO. LY53857, WAY 100,635 and *m*-CPP were dissolved in delonized water. GR 127935 was dissolved in a minimal amount of 0.1 N hydrochloric acid and deionized water. All drugs and drug vehicles were injected s.c. in a volume of 10 ml/kg. Doses refer to the indicated salt form of each drug. Antagonists were administered concurrently with *m*-CPP.

3. Results

Administration of *m*-CPP alone (0.3–10 mg/kg) produced a dose-related decrease in spontaneous activity (Fig. 1, closed squares). In the presence of 3.0 mg/kg GR 127935, the *m*-CPP dose–response curve was shifted slightly to the left of the *m*-CPP alone curve (Fig. 1, closed circles. In the presence of 1.0 mg/kg WAY 100,635, the *m*-CPP dose response curve was not appreciably altered (Fig. 1, closed triangles). In the presence of LY53857 (1.0 mg/kg), lower doses (0.1 and 0.3 mg/kg) of *m*-CPP produced a dose-related increase in activity which was significant at the 3.0 mg/kg dose of *m*-CPP (Fig. 1, open

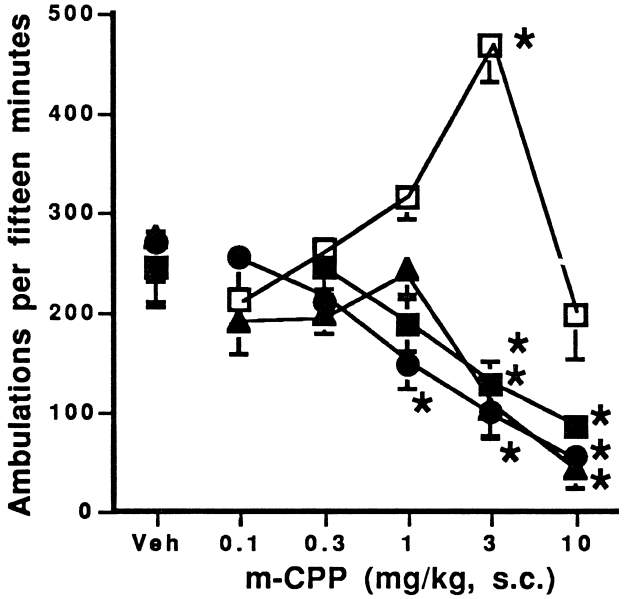

Fig. 1. Dose-related effects of *m*-CPP administered alone (closed squares) and in the presence of 3.0 mg/kg GR 127935 (closed circles), 1.0 mg/kg WAY 100,635 (closed triangles), or 1.0 mg/kg LY53857 (open squares) on locomotor activity in mice. Each point represents the mean of one observation in each of eight animals. The vertical lines represent ±S.E.M. $^{*}P < 0.05$ versus respective control group (Dunnett's). Points above Veh represent the effects of vehicle alone.

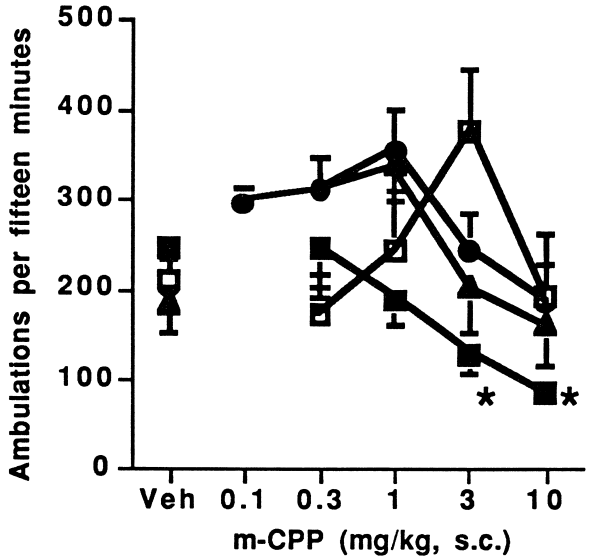

Fig. 2. Dose-related effects of *m*-CPP administered alone (closed squares), or in the presence of 1.0 mg/kg LY53857 (open squares), 1.0 mg/kg LY53857 and 3.0 mg/kg GR 127935 (closed circles), or 1.0 mg/kg LY53857 and 1.0 mg/kg WAY 100,635 (closed triangles). Each point represents the mean of one observation in each of eight animals. The vertical lines represent ±S.E.M. $^{*}P < 0.05$ versus respective control group (Dunnett's). Points above Veh represent vehicle + vehicle + vehicle (closed squares), vehicle + vehicle + vehicle (open squares), vehicle + 1.0 mg/kg LY53857 + 3.0 mg/kg GR 127935 (closed circles) and vehicle + 1.0 mg/kg LY53857 + 1.0 mg/kg WAY 100,635 (closed triangles).

squares). A higher dose of *m*-CPP (10 mg/kg) did not increase activity in the presence of LY53857 (Fig. 1, open squares). When 3.0 mg/kg GR 127935, 1.0 mg/kg WAY 100,635 or 1.0 mg/kg LY53857 were administered alone they had no effect on spontaneous activity (data not shown).

The increase in activity produced by *m*-CPP in the presence of LY53857 (Fig. 2, open squares) was antagonized by administration of 3.0 mg/kg GR 127935 (Fig. 2, closed circles); in the presence of GR 127935, no dose of *m*-CPP significantly altered locomotor activity relative to the respective vehicle control condition. The increase in activity was also antagonized by 1.0 mg/kg WAY 100,635 (Fig. 2, closed triangles). The combination of 1.0 mg/kg LY53857 with 3.0 mg/kg GR 127935 or 1.0 mg/kg WAY 100,635 was without effect on spontaneous activity.

4. Discussion

m-CPP produced a dose-related decrease in locomotor activity in mice as has been previously reported in rats (Kennett and Curzon, 1988; Lucki et al., 1989). In the presence of GR 127935 (3.0 mg/kg), the *m*-CPP dose–response curve was shifted slightly to the left. The 5-HT_2 receptor antagonist LY53857 (1.0 mg/kg) blocked the hypolocomotion produced by *m*-CPP. Moreover, in the presence of LY53857, *m*-CPP produced a dose-related increase in locomotor activity. The present findings are consistent with the previous finding that *m*-CPP produces hyperlocomotion in 5-HT_{2C} knockout mice but hypolocomotion in wild type mice (Das and Tecott, 1996). In addition, the hyperlocomotion produced by *m*-CPP in the presence of LY53857 was blocked by both the 5HT_{1A} receptor antagonist, WAY 100,635 (1.0 mg/kg) and the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (3.0 mg/kg). The dose of 1.0 mg/kg WAY 100,635 was chosen based on antagonism of 8-OH-DPAT induced hyperthermia in the rat (Fletcher et al., 1996). A dose of 3.0 mg/kg of GR 127935 was chosen based on previous work in mice showing this dose was capable of antagonizing the effects of RU 24969 on locomotor activity (O'Neill et al., 1996). The effectiveness of both a 5-HT_{1A} receptor antagonist and a 5-$HT_{1B/1D}$ receptor antagonist in reversing the hyperlocomotion seen with *m*-CPP and LY53857 is somewhat surprising. One possible interpretation is that activation of both 5HT_{1A} and 5-$HT_{1B/1D}$ receptors are necessary under these conditions to produce hyperlocomotion and therefore blockade of either the 5-HT_{1A} or 5-$HT_{1B/1D}$ receptor is capable of reversing the hyperlocomotion caused by *m*-CPP and LY53857. Overall, the present data demonstrate that the effects of *m*-CPP on locomotor activity are due to the physiologic summation of at least effects on 5HT_{2C} and 5-HT_1 receptors.

Although previous investigators have concluded that the effects of *m*-CPP are due solely to the activation of 5-HT_{2C} receptors, several lines of evidence suggest that the situation is more complicated. Previous investigators have shown that the nonselective 5-HT receptor antagonists metergoline (Kennett and Curzon, 1988; Lucki et al., 1989), mianserin and cyproheptadine (Kennett and Curzon, 1988) reverse *m*-CPP hypolocomotion. These same investigators have also reported that the preferential 5-$HT_{2C/2A}$ antagonist ritanserin fails to reverse the effects of *m*-CPP on locomotor activity (Kennett and Curzon, 1988; Lucki et al., 1989). In addition, the 5-HT_{2C} receptor selective antagonist RS102221 also failed to antagonize the effects of *m*-CPP yet was effective in increasing food consumption (Bonhaus et al., 1997), an effect believed to be mediated by antagonism of 5-HT_{2C} receptors. However, the selective 5-$HT_{2B/2C}$ receptor antagonist SB-200646A was capable of reversing *m*-CPP hypolocomotion (Kennett et al., 1994). In addition, SB 242084, a selective and CNS active 5-HT_{2C} receptor antagonist was effective in inhibiting *m*-CPP hypolocomotion (Kennett et al., 1997). On the other hand, the increase, rather than the decrease in locomotion seen with administration of *m*-CPP in 5-HT_{2C} knockout mice (Das and Tecott, 1996) strongly implicate the involvement of the 5-HT_{2C} receptor in mediating *m*-CPP induced hypolocomotion. These results indicate that the pharmacology surrounding the effects of *m*-CPP on locomotor activity is complex. One possible factor is the unknown role that a splice variant of the 5-HT_{2C} receptor (Canton et al., 1996) might play in mediating *m*-CPP induced behaviors.

Results from drug discrimination studies further support the conclusion that *m*-CPP has complex pharmacological actions. The preferential 5-$HT_{2C/2A}$ receptor antagonist ritanserin has been shown to only partially antagonize the discriminative stimulus cue of *m*-CPP, and this occurred only at relatively high doses (10 and 20 mg/kg) of ritanserin (Fiorella et al., 1995). Other reports have demonstrated that the discriminative stimulus effects of *m*-CPP were mediated by both the 5-HT_{2C} receptor and 5-HT_{1B} receptor (Callahan and Cunningham, 1994). In addition, the finding that *m*-CPP partially substituted for RU24969 in rats trained to discriminate RU24969 from saline (Gardner, 1989) further supports a broad action of *m*-CPP at multiple serotonergic receptors. These previous results in drug discrimination studies are consistent with the present findings in implicating the involvement of 5-HT, receptors as well as 5-HT_2 receptors in mediating the effects of *m*-CPP on locomotor activity. Taken together the evidence demonstrates that the locomotor activity effects of *m*-CPP are not due solely to agonist activity at the 5-HT_{2C} receptor.

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