Evidence that m-chlorophenylpiperazine-induced hyperthermia in rats is mediated by stimulation of 5-HT$_{2C}$ receptors

Prospective m-CPP-induced hyperthermia in rats is mediated by stimulation of 5-HT$_{2C}$ receptors. Pretreatment with low doses of metergoline (5-HT$_1$/5-HT$_2$ antagonist), mesulergine and mianserin (5-HT$_{2C}$/5-HT$_{2A}$ antagonists) blocked m-CPP-induced hyperthermia. Pretreatment with propranolol (β-adrenergic receptor antagonist that also has binding affinity for 5-HT$_1$A, 5-HT$_1$B and 5-HT$_3$ sites), yohimbine (α$_2$-noradrenergic antagonist that also has binding affinity for 5-HT$_2$B sites), MDL-72222 or ondansetron (5-HT$_3$ antagonists) did not attenuate m-CPP-induced hyperthermia. Only high doses of ketanserin, LY-53857 and ritanserin (5-HT$_{2A}$/5-HT$_{2C}$ antagonists) as well as spiperone (5-HT$_1$A/5-HT$_{2A}$/D$_2$ antagonist) attenuated m-CPP-induced hyperthermia. Daily administration of m-CPP produced complete tolerance to its hyperthermic effect by day 5. However, there was no cross-tolerance to 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, a 5-HT$_{2A}$ agonist that also has high affinity for 5-HT$_{2C}$ receptors)-induced hyperthermia. m-CPP-induced increases in temperature were found to be significantly less in the Fawn-Hooded (FH) rat strain as compared to the Wistar rat strain; in prior studies, FH rats have been found to be subsensitive to other 5-HT$_{2C}$-mediated pharmacologic responses. Altogether, these findings suggest that m-CPP-induced hyperthermia in rats is mediated by selective stimulation of 5-HT$_{2C}$ receptors.

Keywords: Ketanserin, Metergoline, Mesulergine, Ondansetron, Propranolol, Ritanserin, Spiperone, Tolerance

Introduction

Radioligand binding studies have demonstrated the existence of multiple receptors for serotonin (5-HT). Recently, similarities between 5-HT$_{1C}$ and 5-HT$_{2C}$ receptors led to the nomenclature change in which the former 5-HT$_{1C}$ receptor was renamed 5-HT$_{2C}$, and the former 5-HT$_{2A}$ receptor designated 5-HT$_{2A}$ (Humphrey et al. 1993).

m-Chlorophenylpiperazine (m-CPP) is an active metabolite of the antidepressant drug, trazodone (Caccia et al. 1981). In radioligand binding studies, m-CPP possesses an approximately 10-fold higher affinity for 5-HT$_{2C}$ versus 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{2A}$ sites (Hoyer 1988). In functional studies, administration of m-CPP produces effects opposite to those of the highly selective 5-HT$_{1A}$ agonist, 8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT), on food intake, temperature and locomotor activity. In drug discrimination studies, the stimulus effects of m-CPP have been suggested to be mediated predominantly by a combination of agonist activity at 5-HT$_{2C}$ receptors and antagonist activity at 5-HT$_{2A}$ receptors (Fiorella et al. 1995). Various investigators have demonstrated that stimulation of 5-HT$_{2C}$ receptors is involved in mediating m-CPP-induced anxiety (Kennett 1993), penile erection (Berendsen et al. 1990), decreases in food intake (Kennett and Curzon 1991), decreases in locomotor activity (Kennett and Curzon 1988) and discriminative effects of m-CPP (Callahan and Cunningham 1994). Recently, we have demonstrated that m-CPP-induced increases in plasma prolactin are also mediated by stimulation of 5-HT$_{2C}$ receptors (Aulakh et al. 1992b).

In rats, systemic administration of arylpiperazine 5-HT agonists such as m-CPP and MK-212 induces hyperthermia (Gudelsky et al. 1986; Wozniak et al. 1989), whereas systemic administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT produces hypothermia (Wozniak et al. 1988). MK-212-induced hyperthermia has been suggested to be mediated by central 5-HT$_{2A}$ receptors (Gudelsky et al. 1986; Nash et al. 1989). Recently, m-CPP or TFMPP-induced hyperthermia in heat-adapted rats (28°C) has been reported to be mediated by stimulation
of postsynaptic 5-HT$_{2A}$ and/or 5-HT$_{2C}$ receptors (Klodzinska and Chojnacka-Wojcik 1992).

The purpose of the present study was to investigate the role of various 5-HT receptor subtypes in mediating m-CPP-induced hyperthermia at an ambient temperature of 21°C. Therefore, we studied the effects of 5-HT receptor subtype-selective antagonists on m-CPP-induced hyperthermia in Wistar rats. Since most of the available antagonists of 5-HT$_{2C}$ receptors are also potent antagonists at 5-HT$_{2A}$ sites, we explored two other possible measures of 5-HT$_{2C}$ versus 5-HT$_{2A}$ selectivity in thermoregulation: (a) as 1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane (DOI) administration also produces hyperthermia in rats (Pranzatelli 1990), and DOI-induced hyperthermia has recently been reported to be mediated by stimulation of 5-HT$_{2A}$ receptors (Mazzola-Pomietto et al. 1995), we investigated the possible time-dependent development of tolerance to m-CPP and also checked for cross-tolerance to DOI-induced hyperthermia in m-CPP-tolerant rats; (b) in addition, we compared the effects of various doses of m-CPP on rectal temperature in Wistar and Fawn-Hooded (FH) rat strains since other 5-HT$_{2C}$-mediated effects are reported to be attenuated (Aulakh et al. 1988, 1989, 1992a) and 5-HT$_{2A}$-mediated effects to be accentuated (Gudelsky et al. 1985) in the FH rat strain relative to the Wistar and Sprague-Dawley rat strains.

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**Materials and methods**

Male rats of Wistar (Charles River, Kingston, New York) and Fawn-Hooded (FH, Frederick Cancer Research and Development Center, Frederick, Md.) strains weighing approximately 225 g at the beginning of the study were used. Animals were housed four per cage in a temperature-controlled (21±1°C) room with a 12-h light/dark cycle (lights on at 6:00 a.m.). Animals had free access to Purina rat chow and water at all times. Separate groups of animals were used for each dose of the antagonist studied in the antagonist studies as well as for the time-course and tolerance studies.

Animals were brought into the test environment (ambient temperature 21±1°C) at least 1 h prior to any recording. Animals were housed five or six per cage in Plexiglas cages with bedding during temperature studies. Rectal temperature was measured with a rectal and digital thermometer (Sensortek, Clifton, N.J.), all recordings being made between 10 a.m. and 1 p.m. Each animal received several habituating exposures to the rectal probe, which was inserted 2.5 cm into the colon, while each rat was held lightly by the tail.

**Time-course study**

In this study, 12 animals were used. The animals were divided into two groups of six each. The first group received an acute challenge of saline while the other group received an acute challenge of m-CPP (2.5 mg/kg) intraperitoneally. Rectal temperature was recorded at baseline and 30, 60 and 90 min after saline or m-CPP injection.

**Antagonist studies**

Vehicle (DMSO or saline) or each antagonist was injected intraperitoneally (IP) 30 min before administrating saline or m-CPP (2.5 mg/kg). This dose of m-CPP was chosen based on our previous work (Fozard 1984; Tricklebank et al. 1985; Costall et al. 1987; Aulakh et al. 1992b). m-CPP or saline was injected IP on the side opposite the antagonist injection. The rectal temperature was recorded at baseline (before vehicle or antagonist injection) and 30 min after m-CPP injection.

**Tolerance study**

In this study, 12 animals were used. The animals were divided into two groups of six each. The two groups received daily injections of either saline or m-CPP (2.5 mg/kg per day) for 5 days. On day 6, both saline and m-CPP-treated (2.5 mg/kg per day×5) animals were challenged with DOI (2.5 mg/kg) to check for cross-tolerance. Rectal temperature was recorded daily 30 min after administration of saline or m-CPP. In the case of DOI, rectal temperature was recorded at 60 min, since DOI produces its peak effect on temperature at 60 min (Murphy et al. 1993).

**Strain comparison study**

In this study, 24 animals (12 Wistar and 12 FH) were used. The animals were injected IP with two doses (1.25 mg/kg and 2.5 mg/kg) of m-CPP and their rectal temperatures were recorded at baseline (before m-CPP injection) and 30, 60 and 90 min after m-CPP injection.

**Drugs**

The following drugs were used: metergoline (Farmitalia, Milan, Italy), m-CPP hydrochloride, MDL-72222 hydrochloride, l-propanol hydrochloride, mesulergine hydrochloride, mianserin hydrochloride, ritanserin, ketanserin tartrate, LY-53857 maleate, spiperone hydrochloride, (+) DOI hydrochloride (Research Biochemicals, Natick, Mass.), yohimbine hydrochloride (Sigma, St Louis, Mo.) and ondansetron (Glaxo Pharmaceuticals, Ware, UK). m-CPP, DOI, mesulergine, mianserin, propranolol, ketanserin, LY-53857, yohimbine and ondansetron were dissolved in 0.9% saline. All other drugs were dissolved in 100% DMSO. The volume injected was 0.1 ml/100 g body weight. All drug doses given in the text refer to the salt.

**Data analysis**

For strain comparison and time course studies, repeated measures analysis of variance was used to test for main and interaction effects. Significant effects were further analyzed using a priori contrasts. For the antagonist studies, the data used were changes in rectal temperature from baseline at 30 min after m-CPP administration. The data were analyzed using one-way analysis of variance accompanied by two tailed Dunnett's t-tests. For the tolerance study, the data used were changes in rectal temperature from baseline at 30 min after m-CPP administration, respectively. The data were analyzed using a two tailed Student's t-test by comparing saline-treated animals versus m-CPP-treated animals.

**Results**

Administration of a 2.5 mg/kg dose of m-CPP produced hyperthermia in Wistar rats (Fig. 1). Analysis of variance showed a significant [F(1, 10)=25.57, P<0.001] m-CPP drug effect. Further analysis revealed that m-CPP-induced increases in temperature were significantly higher