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Short communication

NMDA receptor antagonists block development of tolerance to m-CPP-induced increases in ACTH concentrations in rats

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Abstract

We have recently demonstrated that a single administration of *m*-chlorophenylpiperazine (m-CPP, a preferential 5-HT_{2C} receptor agonist) produces tolerance to its stimulatory effect on adrenocorticotropic hormone (ACTH) concentrations when challenged 24 h later with the same dose of m-CPP. In the present study, we studied the effects of pretreatment with various *N*-methyl-D-aspartate (NMDA) receptor antagonists on development of tolerance to m-CPP's stimulatory effect on ACTH concentrations. Pretreatment with various NMDA receptor antagonists such as 5,7-dichlorokynurenic acid (1.0 mg/kg), 3-amino-1-hydroxy 2-pyrrolidone (1.0 mg/kg), dizocilpine (0.1 mg/kg) and ifenprodil (1.0 mg/kg) injected 30 min before the first injection of m-CPP (2.5 mg/kg) blocked development of tolerance to m-CPP's stimulatory effect on ACTH concentrations in rats injected 24 h later with the same dose (2.5 mg/kg) of m-CPP. These findings suggest that tolerance to postsynaptic 5-HT_{2C} receptor-mediated response is initiated though stimulation of NMDA receptor complex and, furthermore, demonstrate a functional interaction between the 5-HT and glutamate systems.

Keywords: 5,7-Dichlorokynurenic acid; HA-966 (3-amino-1-hydroxy-2-pyrrolidone); Dizocilpine; Phencyclidine; Ifenprodil; CPP ((±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid); 5-HT_{2C} receptor

1. Introduction

m-Chlorophenylpiperazine (m-CPP), a metabolite of the antidepressant drug trazodone, has mixed agonist and antagonist action at several serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes. In radioligand binding studies, m-CPP possesses an approximately 10-fold higher affinity for 5-HT_{2C} vs. 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_3 sites (Hoyer, 1988). In functional studies, various investigations have led to the conclusion that most of m-CPP's effects are predominantly mediated by its 5-HT_{2C} agonist effects (Aulakh et al., 1992, 1995; Berendsen et al., 1990; Kennett and Curzon, 1991; Murphy et al., 1991). Acute administration of m-CPP enhances plasma concentrations of adrenocorticotropic hormone (ACTH) by stimulation of 5-HT_{2C} receptors (Bagdy et al., 1989). We have recently demonstrated that a single administration of m-CPP produces tolerance to its stimulatory effect on ACTH concentrations on subsequent rechallenge (24 h later) with the same dose of m-CPP (Mazzola-Pomietto et al., 1994b).

There are few reports in the literature that suggest a functional interaction between the 5-HT and glutamate systems. Pretreatment with various NMDA receptor antagonists was reported to counteract the long lasting attenuation of postsynaptic 5-HT_{1A} receptor-mediated responses such as increased corticosterone secretion and inhibition of the cage leaving response produced by single injections of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in rats (Ross et al., 1992). In another study, pretreatment with the NMDA receptor antagonist (+)-MK-801 was shown to protect against 5-HT depletions induced by methamphetamine, 3,4-methylene-dioxymethamphetamine and *p*-chloroamphetamine in rats (Farfel et al., 1992).

The purpose of the present study was to further explore this functional interaction between the 5-HT and glutamate systems. Therefore, we studied the effects of pretreatment with various NMDA receptor antagonists on development of tolerance to 5-HT_{2C} receptor-mediated m-CPP-induced ACTH secretion in rats.

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2. Materials and methods

Male Wistar rats obtained from Charles River (Kingston, NY, USA) weighing approximately 250 g were used. The animals were housed three per cage in a temperature-controlled (21 ± 1°C) room with a 12-h light-dark cycle (lights on at 06:00 a.m.). Animals had free access to Purina rat chow and water at all times. Separate groups of animals were used for each NMDA antagonist studied.

In the antagonist studies, vehicle or various NMDA antagonists were injected intraperitoneally (i.p.) 30 min before the first injection of m-CPP (2.5 mg/kg) or saline. The selection of the 2.5 mg/kg dose of m-CPP was based on our previous work (Aulakh et al., 1992). The selection of NMDA antagonist doses was based on the published literature and our previous work (Farfel et al., 1992; Mazzola-Pomietto et al., 1995; Ross et al., 1992). Vehicle or NMDA antagonist plus m-CPP-treated animals were subsequently challenged 24 h later with the same dose (2.5 mg/kg) of m-CPP or saline. The animals were killed 30 min after saline or m-CPP injection between 11:00 and 11:30 a.m.

The rats were killed by decapitation and trunk blood was collected in centrifuge tubes containing 0.5 ml of ethylenediamine-tetraacetic acid (EDTA). After centrifugation, plasma samples were collected and stored at −70°C. The plasma concentrations of ACTH were measured by radioimmunoassay as described elsewhere (Nicholson et al., 1984).

2.1. Drugs

The following drugs, m-CPP hydrochloride, 5,7-dichlorokynurenic acid, ifenprodil tartrate, dizocilpine [(+)-MK-801] maleate, phencyclidine (PCP) hydrochloride, (+)-3-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and (+)-3-amino-1-hydroxy-2-pyrrolidone (HA-966) (Research Biochemicals, Natick, MA, USA) were used in the study. 5,7-Dichlorokynurenic acid was dissolved in 100% dimethylsulfoxide (DMSO). All other drugs were dissolved in 0.9% saline. The volume injected was 0.1 ml/100 g of body weight. The doses given in the text refer to the salt.

2.2. Data analysis

For statistical analysis, various NMDA receptor antagonists were grouped according to their site of action. The data were analyzed using one-way analysis of variance accompanied by contrasts specified a priori comparing each NMDA receptor antagonist plus m-CPP or saline plus m-CPP or saline group vs. vehicle plus m-CPP or saline plus m-CPP or saline group. All data are presented as means ± S.E.M.

3. Results

The effects of 24 h prior pretreatment with vehicle or various NMDA receptor antagonists plus m-CPP on baseline plasma concentrations of ACTH in rats are shown in Table 1. Analysis of variance showed an overall nonsignificant [$F(6,35) = 0.97$, $P > 0.05$] treatment effect on baseline plasma ACTH concentrations.

The effects of pretreatment with vehicle or various NMDA receptor antagonists on development of tolerance to m-CPP-induced ACTH secretion are shown in Fig. 1. For ifenprodil (polyamine site NMDA receptor antagonist) and CPP (NMDA site antagonist), analysis of variance showed a trend for significant [$F(4,24) = 2.43$, $P = 0.07$] treatment effect. Further analysis revealed that pretreatment with ifenprodril (1.0 mg/kg) but not CPP (10.0 mg/kg) blocked development of tolerance since m-CPP administration produced significant increases in ACTH levels only in the ifenprodil-treated group relative to the vehicle-treated group (Fig. 1).

For (+)-MK-801 and PCP (channel blocking NMDA receptor antagonists), analysis of variance showed an overall significant [$F(4,24) = 4.17$, $P < 0.05$] treatment effect. Further analysis revealed that pretreatment with (+)-MK-

Table 1
Effects of 24 h prior pretreatment with NMDA antagonists + m-CPP on plasma concentrations of adrenocorticotropic hormone (ACTH, pg/ml) levels in rats

24 h pretreatment		Acute challenge	ACTH (pg/ml)
NMDA antagonists (mg/kg)	m-CPP (mg/kg)		
(DMSO)	Saline	Saline	64.0 ± 8.2
CPP (10.0)	m-CPP (2.5)	Saline	53.7 ± 6.0
Ifenprodil (1.0)	m-CPP (2.5)	Saline	69.6 ± 7.3
MK-801 (0.1)	m-CPP (2.5)	Saline	77.0 ± 12.9
PCP (1.0)	m-CPP (2.5)	Saline	51.5 ± 1.8
5,7-DCKA (1.0)	m-CPP (2.5)	Saline	63.4 ± 6.5
HA-966 (1.0)	m-CPP (2.5)	Saline	73.3 ± 16.8

Values are expressed as means ± S.E.M. from 6 animals. There was no significant difference between NMDA antagonist + m-CPP-treated animals and DMSO + saline-treated animals.

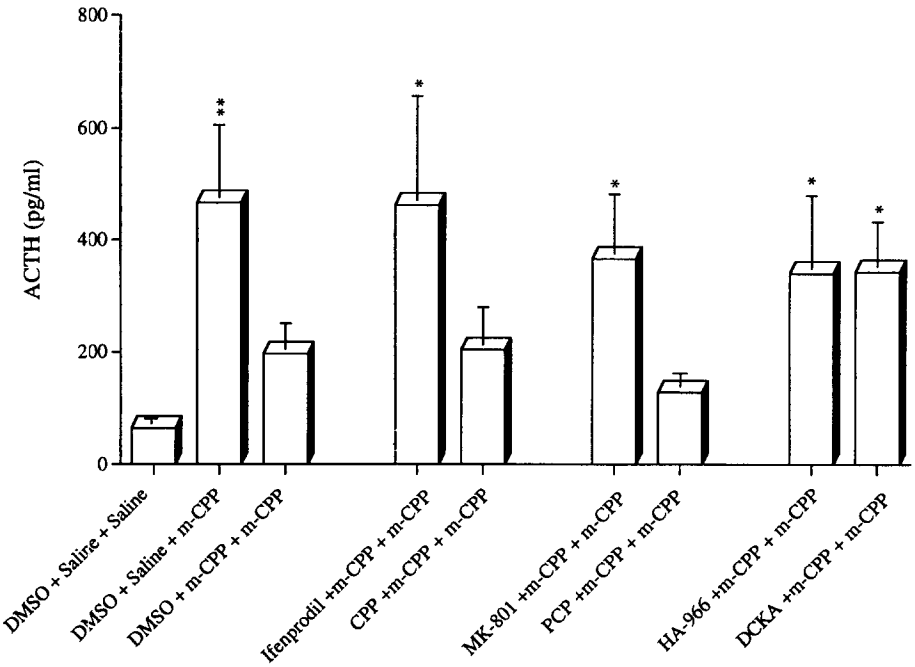

Fig. 1. The effects of 24 h prior pretreatment with vehicle or various NMDA receptor antagonists on development of tolerance to m-CPP-induced ACTH secretion in rats. Values are expressed as means ± S.E.M. from 5–6 animals. $^{*}P < 0.05$; $^{**}P < 0.01$, significantly different from vehicle-treated (DMSO + saline + saline) group.

801 (0.1 mg/kg) but not PCP (1.0 mg/kg) blocked development of tolerance since m-CPP administration produced significant increases in ACTH levels only in the (+)-MK-801-treated group relative to the vehicle-treated group (Fig. 1).

For 5,7-dichlorokynurenic acid and HA-966 (glycine site NMDA receptor antagonists), analysis of variance showed an overall significant [$F(4,24) = 3.27$, $P < 0.05$] treatment effect. Further analysis revealed that pretreatment with both 5,7-dichlorokynurenic acid (1.0 mg/kg) and HA-966 (1.0 mg/kg) blocked development of tolerance, since m-CPP administration produced significant increases in ACTH levels in these two groups relative to the vehicle-treated group (Fig. 1).

4. Discussion

The demonstration of rapid development of tolerance to m-CPP-induced increases in ACTH secretion following a single injection of m-CPP in the present study is consistent with a previous report from this laboratory (Mazzola-Pomietto et al., 1994b). m-CPP-induced ACTH secretion in rats has been suggested to be mediated by stimulation of postsynaptic 5-HT_{2C} receptors (Bagdy et al., 1989; Mazzola-Pomietto et al., 1994a,b). In a previous report from this laboratory, we have demonstrated rapid development of tolerance to m-CPP-induced hypophagia and hyperthermia following daily administration of m-CPP for 1–3 days (Aulakh et al., 1994). m-CPP-induced hypophagia (Aulakh et al., 1995; Kennett and Curzon, 1991) and hyperthermia (Mazzola-Pomietto et al., 1996) are also mediated by stimulation of postsynaptic 5-HT_{2C} receptors. Other investigators have also demonstrated development of tolerance to m-CPP-induced hypoactivity following its repeated administration (Sills et al., 1985). It is of note that m-CPP-induced hypoactivity is also mediated by stimulation of 5-HT_{2C} receptors (Kennett and Curzon, 1988). It is possible that the observed tolerance to 5-HT_{2C} receptor-mediated responses following daily administration of m-CPP may be related to pharmacokinetic factors. However, this possibility seems unlikely in view of the fact that brain m-CPP levels were essentially identical in acute m-CPP-treated (2.5 mg/kg) and daily m-CPP-treated (2.5 mg/kg × 2 or 7 days) animals killed 30 min after the last injection of m-CPP (our unpublished observations).

The rapid development of tolerance to m-CPP-induced ACTH secretion following a single injection of m-CPP seems to be specific to pre-exposure to m-CPP since we did not observe similar rapid tolerance to DOI (a 5-HT_{2A}/5-HT_{2C} receptor agonist)-induced ACTH secretion following a single injection of DOI (Mazzola-Pomietto et al., 1994b). Furthermore, a pre-exposure to acute administration of DOI (2.5 mg/kg) also did not induce tolerance to m-CPP-induced ACTH secretion when challenged 24 h later with a 2.5 mg/kg dose of m-CPP (Mazzola-Pomietto et al., 1994b). These findings also suggest that glucocorticoids themselves do not play any significant role in development of rapid tolerance to m-CPP-induced ACTH secretion. It is also of note that daily administration of m-CPP (2.5 mg/kg/day) for 13 days failed to produce tolerance to its stimulatory effect on corticosterone secretion (Maz-

zola-Pomietto et al., 1994b). Other investigators have also reported failure of chronic m-CPP (5 mg/kg b.i.d. for 15 days) to produce tolerance to its stimulatory effect on corticosterone secretion (Ulrichsen et al., 1992). There are several studies in the literature demonstrating differential effects of serotonin agonists including m-CPP on ACTH and corticosterone secretion (Bagdy et al., 1989; Mazzola-Pomietto et al., 1994b; Van de Kar et al., 1992).

The present study further demonstrates that pretreatment with 5,7-dichlorokynurenic acid and HA-966 (glycine site NMDA receptor antagonists), (+)-MK-801 (channel blocking NMDA receptor antagonist) and ifenprodil (polyamine site NMDA receptor antagonist) 30 min before the first injection of m-CPP blocked development of tolerance to m-CPP-induced ACTH secretion. Our results are consistent with another study in which pretreatment with various NMDA receptor antagonists was shown to counteract the long-lasting attenuation of postsynaptic 5-HT_{1A} receptor-mediated responses in rats (increased corticosterone secretion and inhibition of the cage leaving response) produced by a single injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Ross et al., 1992). In the present study, pretreatment with PCP (1.0 mg/kg) and CPP (10.0 mg/kg) did not block development of tolerance to m-CPP-induced ACTH secretion whereas both PCP (1.0 mg/kg) and CPP (50.0 mg/kg) counteracted attenuation of 5-HT_{1A} receptor-mediated responses (Ross et al., 1992). In the case of CPP (NMDA site antagonist), it is possible that a higher dose (50.0 mg/kg) as used by Ross et al. (1992) may have blocked development of tolerance to m-CPP-induced ACTH secretion in the present study also. Only further experimentation will clarify this. On the other hand, unlike (+)-MK-801, pretreatment with PCP (another channel blocking NMDA receptor antagonist) did not block development of tolerance to m-CPP-induced ACTH secretion in the present study. However, it is well known that PCP can interact with two binding sites: the NMDA receptor PCP site and another PCP site on σ receptors (Quirion et al., 1987), while (+)-MK-801 has a high degree of selectivity for PCP over σ receptor sites (Wong et al., 1986). While the PCP binding site on NMDA receptors has been well characterized (Fagg, 1987; Lodge and Anis, 1982; Snell and Johnson, 1986), little is currently known about PCP's interaction with σ receptors. Thus, it is possible that an interaction of PCP at σ receptors may be responsible for failure of PCP to block development of tolerance to m-CPP-induced ACTH secretion in the present study.

The mechanism by which various classes of NMDA receptor antagonists block development of tolerance to m-CPP-induced ACTH secretion is not easily explainable. In previous reports from this laboratory, we have demonstrated that acute administration of the same doses of these NMDA receptor antagonists as used in the present study do not affect baseline ACTH levels and, furthermore, do not affect m-CPP-induced increases in plasma ACTH concentrations – except for PCP and 5,7-dichlorokynurenic acid which significantly potentiated m-CPP's effect (Mazzola-Pomietto et al., 1994a). Therefore, the possibility of a direct interaction between 5-HT_{2C} receptors and NMDA receptor antagonists seems unlikely. Rahman and Neuman (1993) investigated the mechanisms of desensitization (tolerance) of 5-HT_2 receptor-mediated enhancement of NMDA depolarization in rat cortical neurons. These investigators demonstrated that activation of protein kinase C contributes to desensitization of the 5-HT_2 receptor-mediated enhancement. Thus, it is possible that an interaction of NMDA receptor antagonists with protein kinase C may be responsible for the present findings. However, only further experimentation will clarify this.

In summary, the present study demonstrates that NMDA receptor antagonists block development of tolerance to m-CPP-induced 5-HT_{2C} receptor-mediated ACTH secretion in rats. It will be interesting to explore in the future studies whether these NMDA receptor antagonists would also block development of tolerance to other 5-HT_{2C} receptor-mediated functions such as m-CPP-induced hypophagia (Aulakh et al., 1995; Kennett and Curzon, 1991), hypoactivity (Sills et al., 1985) and hyperthermia (Mazzola-Pomietto et al., 1996) in rats. Furthermore, it will also be interesting to explore if pretreatment with protein kinase C inhibitors would also block development of tolerance to 5-HT_{2C} receptor-mediated functions.

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