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Short communication

Paraventricular nucleus controls 5-HT_{2C} receptor-mediated corticosterone and prolactin but not oxytocin and penile erection responses

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Abstract

There is evidence that administration of the 5-HT_{2C} receptor agonist, *m*-chlorophenylpiperazine (m-CPP), increases plasma concentrations of oxytocin, prolactin, corticosterone, induces penile erections and excessive grooming in male rats. To test the hypothesis that the hypothalamic paraventricular nucleus mediates these neuroendocrine and behavioural responses, we measured the effects of m-CPP (0.6 mg/kg i.v.) on these parameters in chronically cannulated, freely moving male rats after surgical lesion of the paraventricular nucleus or sham operation. Paraventricular nucleus lesion markedly attenuated prolactin, corticosterone and excessive grooming, but not oxytocin and penile erection responses to m-CPP. In contrast, both oxytocin and corticosterone responses to the dopamine receptor agonist, apomorphine (0.05 mg/kg i.v.), were attenuated in lesioned rats. The present studies suggest that the paraventricular nucleus mediates m-CPP-induced prolactin, corticosterone and probably also excessive grooming responses, and the mechanisms of apomorphine and m-CPP action on oxytocin secretion and penile erection responses differ.

Keywords: Apomorphine; 5-HT (5-hydroxytryptamine, serotonin); Grooming, excessive; Penile erection; Oxytocin; Prolactin; Corticosterone; 5-HT_{2C} receptor; m-CPP (1-3-chlorophenylpiperazine); Hypothalamic paraventricular nucleus

1. Introduction

m-Chlorophenylpiperazine (m-CPP), a metabolite of the antidepressant, trazodone, has been one of the most extensively studied serotonin (5-hydroxytryptamine, 5-HT) receptor agonists in humans (Kahn and Wetzler, 1991). Its primary use has been as a probe of the status of brain 5-HT function (Kahn and Wetzler, 1991; Murphy et al., 1991). Behavioural effects and hormonal responses are among the most frequently used parameters of the so-called challenge strategy (Murphy et al., 1991). Administration of m-CPP has several behavioural and neuroendocrine effects in rats also, e.g., increases plasma concentrations of corticosterone, prolactin, oxytocin, induces penile erections and causes excessive grooming (Bagdy et al., 1989a,b, 1992; Berendsen et al., 1990; Aulakh et al., 1992). However, very little is known about mechanisms, especially anatomical sites involved in these responses. We now studied whether the hypothalamic paraventricular nucleus mediates m-CPP-induced increases in plasma concentrations of corticosterone, prolactin, oxytocin, induction of penile erections and excessive grooming.

2. Materials and methods

Male Wistar rats (LATI, Godollo, Hungary), weighing 280–340 g on the day of surgery were used. The animals had free access to food and water, and were housed 5 per cage under constant conditions with a

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12:12 h light-dark cycle (lights on at 06.00 a.m.) at least one week before further experimentation.

2.1. Paraventricular lesion

Animals were randomly selected for lesion or sham operation. Paraventricular lesions and sham operations were performed under pentobarbital anesthesia (40 mg/kg i.p.) with a rotating knife destroying an inverted cone of tissue containing the paraventricular nucleus. In sham-operated rats, the knife was lowered 5 mm into the brain without turning (Makara et al., 1981; Bagdy and Makara, 1994). Only data from rats with complete destruction of the paraventricular nucleus based on histological examination were included (Bagdy and Makara, 1994).

2.2. Experimental protocol and drug administration

At least 2 days before drug challenge, polyethylene cannulae (PE-50) were implanted in the left femoral artery and vein under halothane anesthesia (Bagdy et al., 1992). The animals were housed in separate cages after cannulation. All experiments were performed 3–5 days after hypothalamic surgery. Baseline samples of blood were obtained 20–30 min before drug injection. m-CPP (1-(3-chlorophenyl)piperazine) hydrochloride (0.6 mg/kg) or apomorphine hydrochloride (0.05 mg/kg; both Research Biochemicals, Wayland, MA, USA) were administered into the femoral vein. Samples of blood (950 μl) were collected by free flow through the intra-arterial cannula in polyethylene tubes containing EDTA. Blood loss was compensated by saline replacement. Blood samples were cooled and centrifuged at 4°C. Aliquots of plasma were removed and stored at −70°C until the assay.

2.3. Behavioural observations

Penile erections and grooming behaviour were observed and quantified for 30 min as described previously (Bagdy et al., 1992). Animals were scored in their home (single) cages by three observers, all of whom were blind to the treatment. Drug administration and blood sampling were carried out by a fourth person. The characteristic behavior of penile erection consisted of pelvic thrusts immediately followed by an upright position presenting an emerging, engorged penis which the rat proceeds to lick, eating the ejaculate. Grooming was also continuously observed for 30 min, and grooming bouts which exceeded 1 min were counted. Grooming behaviour was also quantified by scoring each animal every 15 s (data not shown). Vibration, face and head washing, body grooming, scratching, paw licking, head shake and genital grooming were included as components of grooming behaviour.

2.4. Hormone measurements

Plasma hormone concentrations were measured directly by radioimmunoassay (RIA) without prior extraction. Plasma samples of lesioned animals and sham operated controls were run in the same assays. Corticosterone RIA developed in the Institute of Experimental Medicine, prolactin RIA using materials kindly supplied by the NIDDK Rat Pituitary Hormone Distribution Program and a commercial oxytocin RIA kit (RI 081 OT RIA KIT, Institute for Research, Production

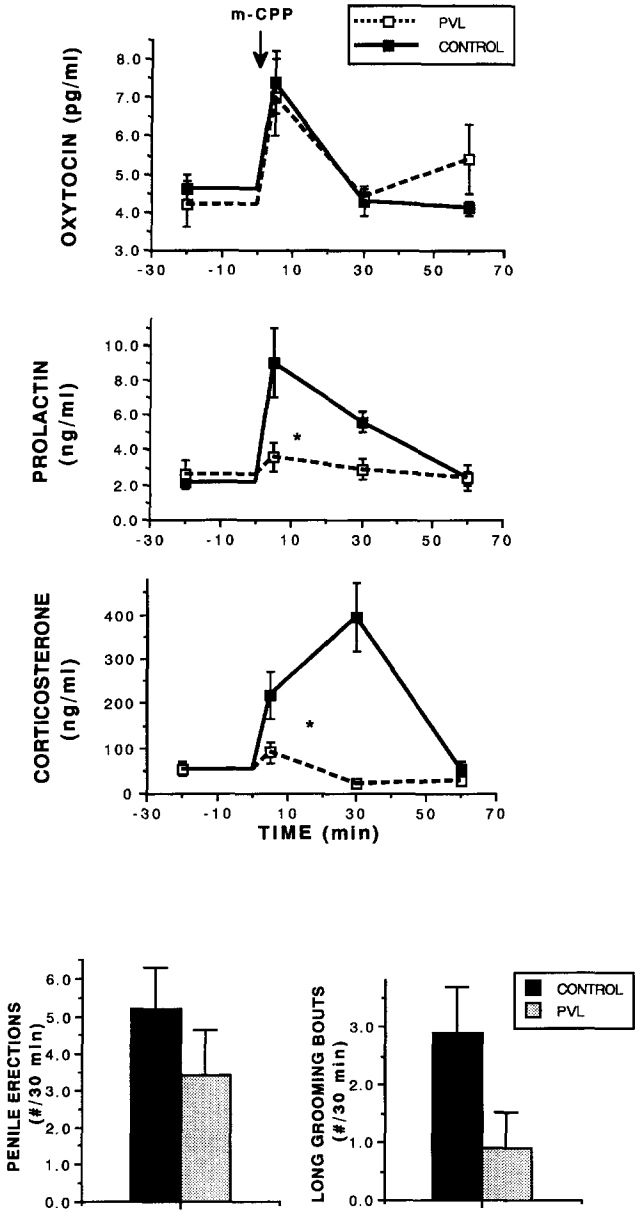

Fig. 1. Effects of m-CPP (0.6 mg/kg i.v.) on plasma oxytocin, prolactin and corticosterone concentrations, penile erections and grooming behaviour in sham operated control (black squares, $n = 10$) and paraventricular nucleus lesioned (PVL, open squares, $n = 8$) rats. * Significant effect of the lesion ($P < 0.05$).

and Application of Radioisotopes, Prague, Czechoslovakia) were used for hormone measurements (Bagdy and Makara, 1994). Detection limits were 1.2 ng/ml, 0.8 ng/ml and 2.6 pg/ml, intraassay coefficients of variation were 4.4, 7.8 and 6.6% for corticosterone, prolactin and oxytocin, respectively.

2.5. Statistical methods

One-way analysis of variance (ANOVA) for repeated measures was used for the time curves, and Duncan's multiple range test was used for post-hoc comparisons using Super Anova (Brain Power, Calabasas, CA, USA). The Mann-Whitney *U*-test was used for the statistical evaluation of behavioural measures (Stat View SE + Graphics, Brain Power, Calabasas, CA, USA). Mean (±S.E.M.) values of 8–10 (m-CPP) and 4–5 (apomorphine) animals are reported.

3. Results

m-CPP (0.6 mg/kg i.v.) caused elevations in plasma oxytocin, prolactin and corticosterone concentrations and increased the number of penile erections and long grooming bouts. Lesion of the hypothalamic paraventricular nucleus prevented prolactin and corticosterone responses. The difference in the number of long grooming bouts between lesioned and sham operated rats showed a strong trend ($P = 0.06$). In contrast, oxytocin and penile erection responses were not different in the two groups (Fig. 1).

Apomorphine-induced (0.05 mg/kg i.v.) oxytocin and corticosterone responses were signifcantly lower in the lesioned rats. The effect of the surgery on prolactin concentrations was not significant (Fig. 2). Behavioural observations were not made after apomorphine treatment.

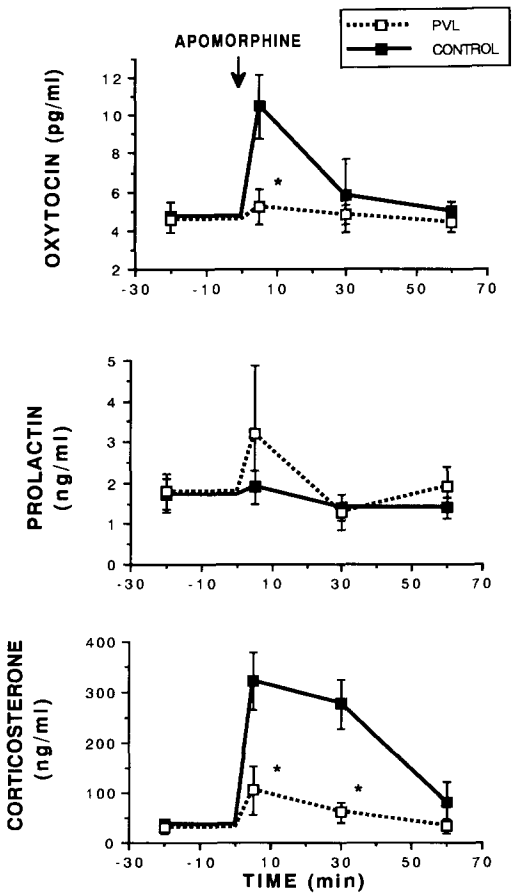

Fig. 2. Effects of apomorphine (0.05 mg/kg i.v.) on plasma oxytocin, prolactin and corticosterone concentrations in sham operated control (black squares) and paraventricular nucleus lesioned (PVL, open squares) rats. * Significant effect of the lesion ($P < 0.05$).

4. Discussion

m-CPP has about 10-fold higher affinity for 5-HT_{2C} (formerly 5-HT_{1C}) sites than to any other 5-HT_1 or 5-HT_2 receptors (Murphy et al., 1991; Humphrey et al., 1993). Prolactin, oxytocin, penile erection and excessive grooming responses all have been shown to be blocked by antagonists that have high affinity for 5-HT_{2C} receptors (e.g., metergoline, mianserin), but not by ketanserin, a 5-HT_{2A} receptor antagonist, that has 20–50 times lower affinity for 5-HT_{2C} receptors (Berendsen et al., 1990; Bagdy et al., 1989b, Aulakh et al., 1992). A partial antagonistic effect of the 5-HT_{2A}/5-HT_{2C} receptor antagonist, ritanserin, but not ketanserin on m-CPP-induced adrenocorticotropin (ACTH) responses has also been described (Bagdy et al., 1989b). These results suggest that all these effects are mediated mainly by 5-HT_{2C} receptors. In this study we used a relatively low dose of the drug (0.6 mg/kg i.v.). Plasma oxytocin, penile erection and excessive grooming responses had been pharmacologically characterized at this dose (Bagdy et al., 1992). Our purpose with the low dose was to avoid unwanted other receptor stimulation. In addition, the conclusions of our study are more comparable to those of clinical challenge studies that usually used low (0.1 mg/kg i.v.) doses.

The hypothalamic paraventricular nucleus plays a very important role in neuroendocrine regulation. It is the main source of corticotropin releasing hormone (CRH), the most important regulator of pituitary adrenocortical function in humans and in rats (Makara et al., 1984). In addition, several peptides endowed with prolactin releasing activity originate from the paraventricular nucleus. One of those is oxytocin. Plasma oxytocin derives either from the paraventricular or the supraoptic nucleus of the hypothalamus. In this study corticosterone and prolactin, but not oxytocin responses to m-CPP were blocked after paraventricular nucleus lesion. Thus, we may conclude that

m-CPP-induced corticosterone responses are mediated by hypothalamic factors, most likely CRH and/or vasopressin. Indeed, m-CPP is capable of releasing CRH from explanted hypothalami in vitro, and pretreatment with anti-CRH serum prevents ACTH responses to m-CPP in vivo (Calogero et al., 1989, 1990). Our data suggest that prolactin responses after 5-HT_{2C} receptor stimulation with m-CPP are mediated by peptides of paraventricular nucleus origin which are capable of releasing this hormone (e.g. thyrotropin-releasing hormone, vasoactive intestinal peptide, polypeptide histidine isoleucine-27, arginine vasopressin). The origin of oxytocin responses is, however, not the paraventricular nucleus, but most likely the supraoptic nuclei of the hypothalamus.

Interestingly, in earlier studies, paraventricular nucleus lesions had different effects on hormone responses to the 5-HT_{2A}/5-HT_{2C} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). DOI (1.0 mg/kg i.v.) induced corticosterone and prolactin responses were partially attenuated, oxytocin responses were completely prevented by lesion of the paraventricular nucleus (Bagdy and Makara, 1994). Interestingly, 5-HT releaser-induced prolactin responses are also attenuated after paraventricular nucleus lesions (Rittenhouse et al., 1993). This means that the mechanisms of m-CPP- and DOI-induced corticosterone and prolactin responses differ at least partially, while oxytocin responses to the two drugs are elicited by completely different mechanisms. Namely, our data suggest that the main origin of the m-CPP-induced oxytocin response is the supraoptic, but those of DOI are the paraventricular nuclei of the hypothalamus. These data suggest that challenge studies with the two drugs examine different serotonergic functions.

The effects of paraventricular nucleus lesion on the hormonal responses induced by the 5-HT_{1A} receptor partial agonist, ipsapirone, also contrast with those to m-CPP. Prolactin responses after ipsapirone (1.0 mg/kg i.v.) were not affected by lesion of the paraventricular nucleus (Bagdy and Makara, 1994). In addition, ipsapirone (2.0 mg/kg i.v.)-induced oxytocin responses were blocked after the lesion (Bagdy, 1992). Both effects oppose those of m-CPP. Corticosterone responses were absent in paraventricular nucleus-lesioned animals after ipsapirone as well as after m-CPP. These results suggest that the mechanisms of ipsapirone-induced oxytocin and prolactin responses differ from those of m-CPP-induced ones.

There is evidence that administration of the dopamine receptor agonist, apomorphine, causes oxytocin release and induces penile erections in male rats, and lesion of the hypothalamic paraventricular nucleus prevents oxytocin and apomorphine-induced penile erections (Argiolas et al., 1987; Melis et al., 1989). From these and other results the authors concluded that oxytocin of hypothalamic paraventricular nucleus origin mediates penile erection after dopaminergic stimuli (Argiolas et al., 1987). Our finding that paraventricular nucleus lesion prevented apomorphine-induced oxytocin responses also supports this hypothesis. In addition, it was shown by the same group in a recent paper that m-CPP-induced penile erections are not mediated by oxytocin of paraventricular nucleus origin (Stancampiano et al., 1994). In our study, neither the number of penile erections nor plasma oxytocin responses to m-CPP administration were significantly altered by paraventricular nucleus lesions. Thus, our data suggest that the mechanism of 5-HT_{2C} receptor agonist-induced penile erection is different from that induced by apomorphine, namely, the hypothalamic paraventricular nucleus does not play a significant role in m-CPP-induced penile erection. These data and conclusion are in agreement with those of Stancampiano et al. (1994). Interestingly, the number of excessive grooming bouts showed a strong, almost significant difference between lesioned and control animals. These data suggest that the mechanisms of 5-HT_{2C} receptor agonist-induced penile erections and excessive grooming differ. This is surprising, because we have found similar dose-response curves and pharmacological profiles of these m-CPP-induced behaviours previously. Both behaviours were mediated by 5-HT_{2C} receptors, and they showed bimodal correlation with the dose of the 5-HT receptor agonist (Bagdy et al., 1992).

In conclusion, the present studies suggest that the hypothalamic paraventricular nucleus or neural pathways close to it mediate m-CPP-induced corticosterone, prolactin but not oxytocin and penile erection responses. Excessive grooming responses also depend on an intact paraventricular nucleus. On the basis of our paraventricular nucleus lesion studies we conclude that 5-HT_{1A} receptor stimulation induced prolactin, and 5-HT_{2A} receptor stimulation induced oxytocin prolactin, corticosterone, and dopamine receptor stimulation-induced penile erection responses are mediated by mechanisms different from those after 5-HT_{2C} receptor stimulation.

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