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Ejaculatory response induced by a 5-HT_2 receptor agonist *m*-CPP in rats: Differential roles of 5-HT_2 receptor subtypes

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Abstract

It has been reported that systemic administration of *m*-CPP (1-[3-chlorophenyl] piperazine hydrochloride), a 5-HT_2 receptor agonist, produces a 5-HT_{2C} receptor-mediated *penile erections* and self-grooming in rats. In the present study, we examined the ability of *m*-CPP to induce ejaculation in rats and determined which 5-HT_2 receptor subtypes may be involved in the *m*-CPP-induced ejaculation. The ejaculatory response was assessed by weighing the seminal materials accumulated over 30 min. In Experiment 1, systemic administration of *m*-CPP (0.1–3.0 mg/kg, i.p.) produced a dose-dependent increase in both the incidence of ejaculation and the weight of the seminal materials. The inverted U-shaped dose-response effects of *m*-CPP on penile erection and genital grooming were also observed, with maximum effects at 0.6 mg/kg. Pretreatment with SB242084 (0.1 and 0.3 mg/kg, i.p.), a selective 5-HT_{2C} receptor antagonist, dose-dependently attenuated the ejaculatory response induced by *m*-CPP (3.0 mg/kg). The proejaculatory effect of *m*-CPP was also attenuated by ketanserin (0.3 and 1.0 mg/kg, i.p.), a 5-HT_{2A} receptor antagonist, whereas SB204741 (0.1 and 0.3 mg/kg, i.p.), a selective 5-HT_{2B} receptor antagonist, significantly potentiated the *m*-CPP-induced ejaculatory response. Penile erection and genital grooming induced by *m*-CPP (0.3 mg/kg, i.p.) was only blocked by SB242084. In Experiment 2 (termed as corset test), in rats fitted with a corset at the thoracic level to prevent the loss of seminal materials by genital grooming, the proejaculatory effect of *m*-CPP was more efficiently detected than in the non-fitted animals: the ED_{50} value for inducing ejaculation was reduced to less than 50% of the ED_{50} in non-fitted animals. In this test, the proejaculatory effect of *m*-CPP (0.6 mg/kg, i.p.) was completely blocked by SB242084 (0.3 mg/kg, i.p.), whereas ketanserin (0.3 mg/kg, i.p.) or SB204741 (0.3 mg/kg, i.p.) did not affect the *m*-CPP -induced ejaculation.

From these observations, it is suggested that the 5-HT_2 receptor agonist *m*-CPP at *low* doses (0.3–1.0 mg/kg) *possesses* the proejaculatory as well as proerectile effects in rats that are primarily associated with the activation of 5-HT_{2C} receptors, and that the activation of 5-HT2B receptors may produce an inhibitory effect on ejaculation induced by a high dose (3.0 mg/kg) of *m*-CPP. Furthermore, the results of the present study also indicate that the corset test employed in this study may be useful for detecting the proejaculatory effect of the compounds.

Keywords: *m*-CPP; Ejaculation; 5-HT_2 receptor subtypes; Penile erection; Rat

1. Introduction

Although significant advances in the understanding of the neurophysiology and pharmacology of penile erection have been made in the past decade, many problems remain to be elucidated as related to other sexual functions, particularly ejaculation. Ejaculation is defined as a complex process that is composed of three distinct phenomena: seminal emission (secretion of the mixed fluids composing semen into the posterior urethra), ejaculation (expulsion of semen from the posterior urethra to the outside), and bladder neck closure. These events are *reflexive* in nature and require coordination between autonomic and somatic nervous systems following appropriate central and peripheral stimuli (Kimura 1972; Coolen et al., 2004; Giuliano and Clement 2005a) in order to achieve effective delivery of semen. Numerous studies have indicated that the thoracolumbar and lumbosacral levels of the spinal cord are the primary sites for achieving the seminal emission, ejaculation and bladder neck closure phases of the

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ejaculatory process. Recently, Truitt and Coolen (2002) reported that the spinothalamic cells located in laminae VII and X in lumbar segments 3 and 4 appear to play an important role in the induction of ejaculation in rats.

Pharmacological studies have suggested that several neurotransmitters and their receptors contribute to the ejaculatory process. Recently, attention has been drawn to the role of serotonin (5-HT) and its receptors, particularly 5-HT_2, 5-HT_{1A} or 5-HT_{1B} receptors, in the control of ejaculation (Giuliano and Clement 2005b; de Jong et al., 2006). For example, administration of 5-methoxydimethyltryptamine, a 5-HT_2 receptor agonist, facilitates *ex copula* seminal emission and ejaculation in both neurally intact and spinal transected rats (Mas et al., 1985; Renyi 1985). On the other hand, 8-OH-DPAT, a 5-HT_{1A} agonist, increases the copulatory rate and facilitates ejaculatory behavior but inhibited *ex copula* ejaculation in rats and dogs (Schnur et al., 1989; Lee et al., 1990; Yonezawa et al., 2004a). Previous studies in our laboratory have revealed that systemic administration of the 5-HT-releasing agents such as *p*-chloroamphetamine (PCA) and fenfluramine can induce ejaculation in conscious and anesthetized rats (Yonezawa et al., 2000, 2004b). Pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine or 5-$HT_{1/2}$ and 5-HT_2 receptor antagonists (methysergide, ritanserin or ketanserin) significantly abolishes the ejaculatory response induced by PCA or fenfluramine, suggesting that 5-HT and its receptors, particularly the 5-HT2 receptor subtype, may play an important role in the mechanism of ejaculation induced by these agents (Yonezawa et al., 2004b, 2005).

It has been shown that 5-HT_2 receptors can be divided into three subtypes: 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (Hoyer et al., 1994). This classification is based on their close structural homology, pharmacology and signal transduction pathways (Hoyer et al., 1994). Stafford et al. (2006a) have recently shown that systemic administration of PCA elicits a rhythmic burst of a branch of the hypogastric nerve activity, coherent with rhythmic pressure increases in the *vas deferens* and contraction of bulbospongiousus muscles, which together comprise ejaculation in anesthetized rats. They subsequently found that administration of Ro600175, a selective 5-HT_{2C} receptor agonist, induces ejaculation-related responses which closely resemble the PCA-induced responses (2006b). These results suggest that the activation of 5-HT_{2C} receptor may be involved in the proejaculatory action of PCA or other 5-HT-related compounds. It has been shown that systemic administration of *m*-CPP, a 5-HT_2 receptor agonist, produces *ex copula* responses such as penile erection in rats (Berendsen et al., 1990; Bagdy and Makara 1995). The proerectile effect of *m*-CPP in rats is attenuated by pretreatment with a selective 5-HT_{2C} receptor antagonist, whereas a selective 5-HT_{2A} or 5-HT_{2B} receptor antagonists does not affect the erectile response (Millan et al., 1997), suggesting that the activation of 5-HT_{2C} receptors may be mainly involved in *ex copula* penile erection induced by *m*-CPP. These results also suggest the possibility that administration of *m*-CPP may induce ejaculation in rats in a similar manner as penile erection. However, to our knowledge, the proejaculatory effect of *m*-CPP in rats has not been reported previously.

The purpose of the present study was thus to investigate the ability of *m*-CPP to induce ejaculation in rats and to clarify the role of 5-HT_2 receptor subtypes in the *m*-CPP-induced response, by using a new evaluating method for ejaculation. Penile erection and genital grooming induced by *m*-CPP were also observed to accurately evaluate the effect of the drug on male sexual functions.

2. Materials and methods

2.1. Animals

Adult male Wistar–ST strain rats (Japan SLC, Hamamatsu, Japan), weighing 300–350 g, were housed in a two rat per wire-bottomed stainless-steel cage (35 × 42 × 20 cm), except during the corset test, when they were housed individually. Lighting was controlled on a 12:12 h light–dark cycle (lights on at 09:00). Constant temperature (22–24 °C) and humidity (50–60%) were maintained and food and water were available *ad libitum*. The experiments were carried out between 11:00 and 18:00 h. All animal procedures were approved by the Committee of Animal Experiments, Tohoku Pharmaceutical University and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

The following drugs were used: *m*-CPP (1-[3-chlorophenyl] piperazine, Research Biochemicals International, Natick, MA); ketanserin hydrochloride, *N*-(1-methyl-5-indolyl)-*N*′-(3-methyl-5-isothiazoyl) urea (SB204741), 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)pyrid-5-yl-carbamoyl]indoline (SB242084) (all were obtained from Sigma Chemical Co., St. Louis, MO). Ketanserin, SB242084 and *m*-CPP were dissolved in sterilized saline and SB204741 was suspended in 0.5% tween 80/saline solution. All drugs were given intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. Each antagonist was injected 30 min before the *m*-CPP injection.

2.3. Observation of ejaculation, penile erection and genital grooming

Prior to the testing, rats were placed in individual transparent plastic cages (35 × 40 × 18 cm) for at least 30 min to adapt to the novel environment. A paper towel was laid in the observation cage to confirm the occurrence of ejaculation. Before the administration of *m*-CPP, the presence of seminal materials on the paper towel as well as that adhering to the shaft of the penis was checked in order to alleviate any effect of spontaneous seminal emission. If seminal materials were detected, the test was discontinued and restarted the next day. In the present study the incidence of ejaculation was determined 30 min after *m*-CPP administration by the presence of seminal materials on the paper towel as well as that adhering to the shaft of the penis. If the animal ejaculated, the coagulated seminal materials were retrieved from the paper towel and from the shaft of the penis and were placed on filter paper for 30 min, and then weighed. A penile erection was defined as previously described

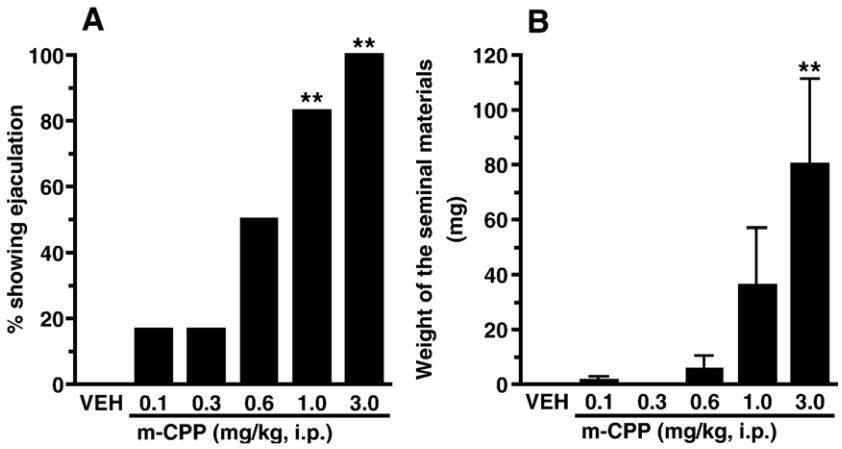

Fig. 1. Ejaculatory response induced by the 5-HT_2 receptor agonist *m*-CPP in rats. (A) and (B) denote the incidence of ejaculation and the weight of seminal materials accumulated over 30 min, respectively. Each column in (B) represents the mean±S.E.M. of six animals. The symbol indicates a significant difference (**$P<0.01$) from vehicle (VEH)-treated animals.

(Berendsen et al., 1990; Kimura et al., 2004); repeated pelvic thrusts immediately followed by an upright position on the hind limbs, an emerging, engorged penis and licking of the penis (i.e., genital grooming). The number of penile erections and the duration of genital grooming were simultaneously observed for 30 min following *m*-CPP injection by an event-recorder (MATYS, model CIF-E12, Toyo Sangyo, Toyama).

2.4. Corset test

A previous study in rats showed that the ejaculated seminal materials induced by some 5-HT-related drugs are lost by licking of the penis (Berendsen et al., 1990). To confirm the ability of *m*-CPP to induce ejaculation more certainly, we examined the effect of *m*-CPP on ejaculation while preventing the occurrence of genital grooming. Briefly, immediately after the *m*-CPP injection, each animal was fitted with a corset, made from thick cotton cloth, at the thoracic level to prevent the animal from bending over to groom its penis. Thirty minutes after this treatment, the ejaculated seminal materials were retrieved from the paper placed under each cage and from the shaft of the penis, and then weighed.

2.5. Statistical analysis

Data were expressed as the means±S.E.M. The statistical significance of differences between treatments were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni test. The differences between the incidences of ejaculation were analyzed by the Fisher's exact probability test. $P<0.05$ was considered as statistically significant.

3. Results

3.1. Experiment 1

3.1.1. Effects of m-CPP on ejaculation, penile erection and genital grooming in rats

As shown in Fig. 1A, systemic administration of *m*-CPP (0.1–3.0 mg/kg, i.p.) dose-dependently induced ejaculation in

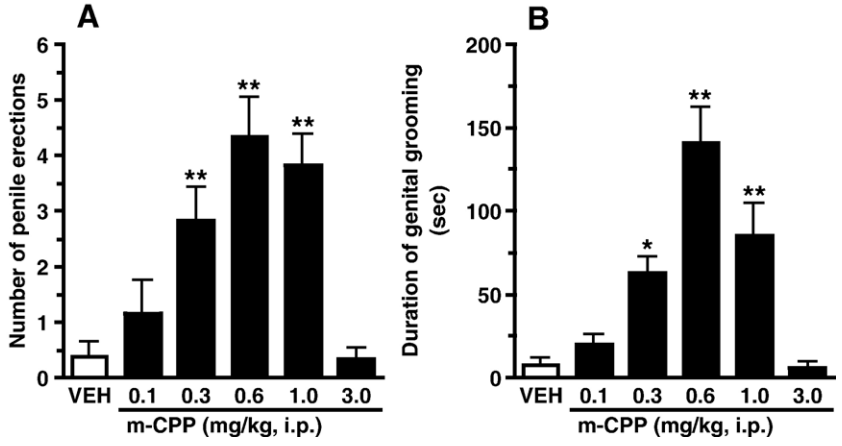

Fig. 2. Penile erection and genital grooming induced by the 5-HT_2 receptor agonist *m*-CPP in rats. (A) and (B) denote the number of penile erections and the duration of genital grooming during the observation period (for 30 min), respectively. Each column represents the mean±S.E.M. of six animals. The symbol indicates a significant difference (*$P<0.05$, **$P<0.01$) from vehicle (VEH)-treated animals.

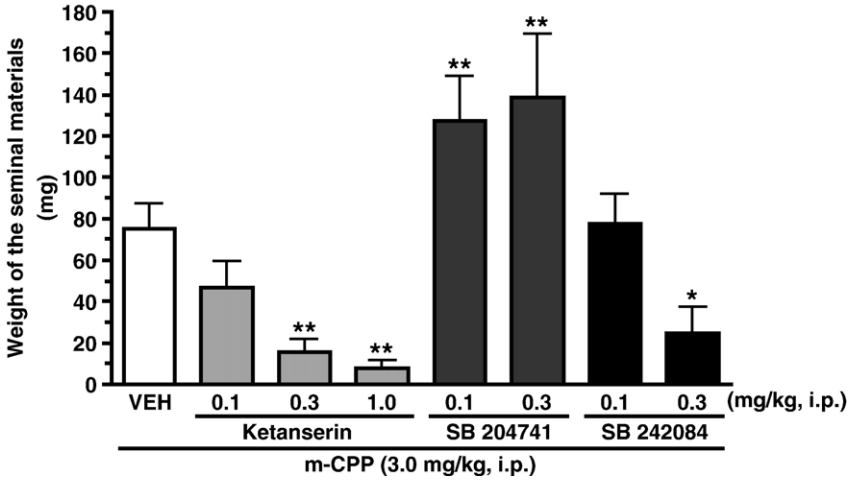

Fig. 3. Effects of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor antagonists on *m*-CPP-induced ejaculation in rats. Each antagonist was administered i.p. 30 min before *m*-CPP (3.0 mg/kg, i.p.) injection. Results of the weight of seminal materials are shown as mean±S.E.M. of eight animals. The symbol indicates a significant difference (*$P<0.05$, **$P<0.01$) from vehicle (VEH)-treated animals.

rats. A marked proejaculatory effect was seen in rats injected with *m*-CPP at doses of 1.0 and 3.0 mg/kg. Although one of the six rats had an ejaculation (the presence of seminal material in the shaft of the penis) at 0.1 and 0.3 mg/kg doses of *m*-CPP, the amount of each ejaculate was very little (Fig. 1B). *m*-CPP also dose-dependently increased the amount of ejaculated seminal materials during the observation period (Fig. 1B: ANOVA; $F(5,30)=4.54$, $P<0.01$). Post-hoc analysis revealed that compared to vehicle-treated animals, the increase in the amount of ejaculated seminal materials by *m*-CPP was statistically reliable at 3.0 mg/kg dose of *m*-CPP (Bonferroni test, $P=0.008$; 1.0 mg/kg, $P=0.096$). In contrast, *m*-CPP showed bell-shaped dose-response effects related to the number of penile erections (Fig. 2A: ANOVA; $F(5,30)=10.70$, $P<0.001$) and the duration of genital grooming (Fig. 2B: ANOVA; $F(5,30)=9.07$, $P<0.001$); the maximum effects of both responses were seen in rats injected with 0.6 mg/kg dose of *m*-CPP, whereas 0.1 and 3.0 mg/kg doses were ineffective. Post-hoc analysis revealed that compared to vehicle-treated animals, the increase in both the number of penile erections and the duration of genital grooming by m-CPP were statistically reliable at 0.3, 0.6 and 1.0 mg/kg doses of *m*-CPP (Bonferroni test).

3.1.2. Effect of 5-HT_2 receptor antagonists on m-CPP-induced ejaculation in rats

Several selective antagonists were used to determine which 5-HT_2 receptor subtypes may be involved in the *m*-CPP-induced ejaculation in rats. The 3.0 mg/kg dose of *m*-CPP was selected for the experiments because it was the most effective and reliable dose for producing ejaculation (Fig. 1). As shown in Fig. 3, pretreatment with the selective 5-HT_{2C} receptor antagonist SB242084 (0.1 and 0.3 mg/kg, i.p.) and the 5-HT_{2A} receptor antagonist ketanserin (0.1–1.0 mg/kg, i.p.) dose-dependently *reduced* the *m*-CPP-induced ejaculation. In contrast, pretreatment with SB204741 (0.1 and 0.3 mg/kg, i.p.), a selective 5-HT_{2B} receptor antagonist, *significantly* potentiated the *m*-CPP-induced ejaculation (Bonferroni test, $P<0.01$); the weight of seminal materials accumulated over 30 min was 1.5- to 2-fold higher than that of *m*-CPP alone.

3.1.3. Effect of 5-HT_2 receptor antagonists on m-CPP-induced penile erection and genital grooming in rats

Experiments were also carried out using the selective antagonists to determine the involvement of 5-HT_2 receptor subtypes in the *m*-CPP-induced penile erection and genital grooming in rats. The 0.3 mg/kg dose of *m*-CPP was selected for the experiments because it was the minimum dose to effectively and reliably produce penile erection and genital grooming in rats (Fig. 2). As shown in Table 1, pretreatment with SB242084 (0.3 mg/kg) significantly inhibited the *m*-CPP -induced penile erection (Bonferroni test, $P<0.01$) and genital grooming (Bonferroni test, $P<0.01$), whereas pretreatment with ketanserin (0.3 mg/kg) and SB204741 (0.3 mg/kg) did not affect these responses.

3.2. Experiment 2

3.2.1. m-CPP-induced ejaculation in the corset test

To prevent the loss of ejaculates by genital grooming, we examined the effect of *m*-CPP on ejaculation in rats that were fitted with a corset at the thoracic level. As shown in Fig. 4 (A and B), both the incidence of ejaculation and the weight of the seminal materials induced by *m*-CPP were increased in

Table 1
Effect of 5-HT_2 receptor antagonists on *m*-CPP-induced penile erection and genital grooming in rats

Treatment (mg/kg, i.p.)	*n*	Number of penile erection	Duration of genital grooming (s)
m-CPP (0.3) alone	10	2.4±0.3	70.9±8.2
Ketanserin (0.3)+*m*-CPP (0.3)	6	2.8±0.6	68.0±13.4
SB204741 (0.3)+*m*-CPP (0.3)	6	2.5±0.6	55.9±17.0
SB242084 (0.1)+*m*-CPP (0.3)	6	0.8±0.3*	49.7±13.7
(0.3)+*m*-CPP (0.3)	6	0.5±0.3**	7.5±4.9**

*$P<0.05$, **$P<0.01$, compared with *m*-CPP alone.

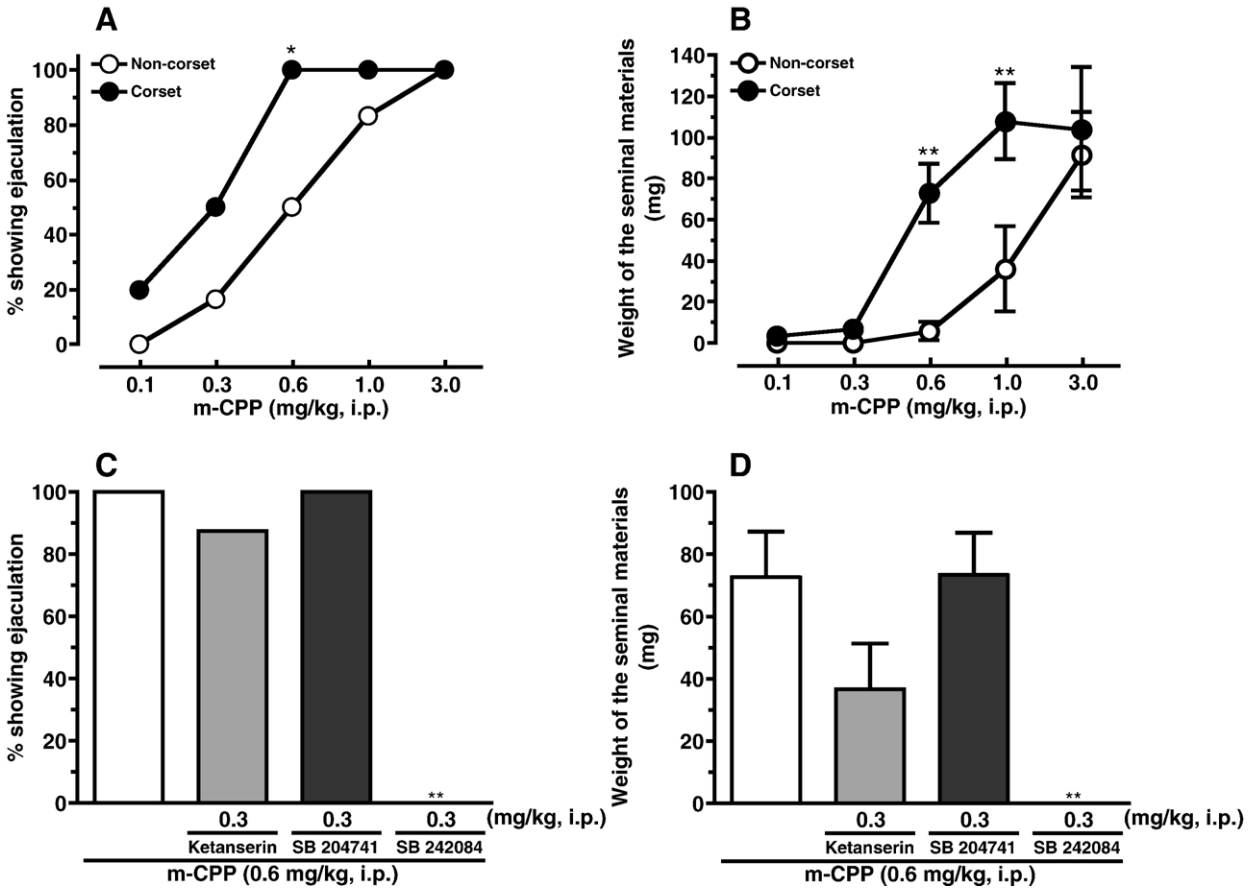

Fig. 4. Changes in the ability of *m*-CPP to induce ejaculation (upper graph) and the effects of the 5-HT_2 receptor antagonists on the *m*-CPP -induced ejaculation (lower graph) in corset test. Immediately after the *m*-CPP injection, each animal was fitted with a corset at the thoracic level to prevent the animal from bending to groom its penis. (A) and (B) denote the incidence of ejaculation and the weight of seminal materials in both corset and non-corset tests, respectively. Each point in (B) represents the mean ± S.E.M. of six to eight animals. The symbol indicates a significant difference (*$P<0.05$, **$P<0.01$) from non-corseted animals. (C) and (D) denote the incidence of ejaculation and the weight of seminal materials in corset fitted animals, which were pretreated with the 5-$HT2_A$, 5-$HT2_B$ and 5-$HT2_C$ receptor antagonists, respectively. Each column in (D) represents the mean ± S.E.M. of six animals. The symbol indicates a significant difference (**$P<0.01$) from vehicle (VEH)-treated animals.

fitted animals compared to non-fitted animals. In particular, a prominent proejaculatory effect was seen in rats injected with 0.6 (Bonferroni test, $P<0.01$) and 1.0 mg/kg (Bonferroni test, $P<0.01$) doses of *m*-CPP. Although three of the six rats fitted with corset had an ejaculation (the presence of seminal material in the shaft of the penis) at 0.3 mg/kg dose of *m*-CPP (Fig. 4A), the amount of each ejaculate was very little (Fig. 4B). The ED_{50} values for inducing ejaculation in fitted and non-fitted animals were 0.28 and 0.6 mg/kg, respectively. The effects of the selective antagonists on the *m*-CPP-induced ejaculation in the corset test were subsequently investigated. As shown in Fig. 4 (C and D), the proejaculatory effect of *m*-CPP (0.6 mg/kg) in rats fitted with a corset was completely inhibited by pretreatment with SB242084 (0.3 mg/kg) (Bonferroni test, $P<0.01$), whereas pretreatment with ketanserin (0.3 mg/kg) and SB204741 (0.3 mg/kg) did not affect the response.

4. Discussion

The major finding of the present study was that systemic administration of *m*-CPP, a 5-HT_2 receptor agonist, dose-dependently induced ejaculation in rats. In particular, a prominent proejaculatory effect (a significant increase in both the proportion of ejaculating animals and the amount of seminal materials) was seen in rats injected with 1.0 and 3.0 mg/kg doses of *m*-CPP (Fig. 1). To our knowledge, this is the first report to clearly show the ability of *m*-CPP to induce ejaculation in rats. The present study also demonstrated that the dose-response curves for both penile erection and genital grooming induced by *m*-CPP are inverted U-shaped curves with the maximum effect at 0.6 mg/kg, which is in agreement with the findings of previous reports (Berendsen et al., 1990; Stancampiano et al., 1994; Bagdy and Makara 1995; Graf et al., 2003).

The reason why the proejaculatory effect of *m*-CPP in rats was not reported previously is obscure; however, it is probably related to the difference in experimental conditions (e.g., in the present study a paper towel was laid on the observation chamber in order to confirm the occurrence of ejaculation) or the fact that the previous studies were not focused on the aspect of sexual functions, particularly ejaculation. Furthermore, the results of the present study indicate that the excessive genital grooming induced by *m*-CPP (Bagdy et al., 1992), which typically occurs

concomitant with the expression of penile erection in rats (Fig. 2), may interfere with the proejaculatory effect of *m*-CPP. In fact, in the rats fitted with a corset at the thoracic level to prevent genital grooming (corset test; Fig. 4 A and B), the proejaculatory effect of *m*-CPP was more efficiently detected than that of the non-fitted animals; the ED_{50} value for inducing ejaculation was reduced to less than 50% of the ED_{50} value in non-fitted animals. These results suggest that the 5-HT_2 receptor agonist *m*-CPP possesses the ability to induce ejaculation as well as penile erection in rats within a similar dose range (0.3–1.0 mg/kg). In addition, it is also suggested that when the proejaculatory action of the compounds that induce excessive genital grooming are assessed accurately in rats, such a study should be conducted using a device to prevent the expression of this behavior (e.g., the use of a corset at the thoracic level).

It has been shown that the affinity of *m*-CPP for human 5-HT_{2C} receptors (Ki: 16 nM) was 2.5 and 5.3 times higher than those for human cloned 5-HT2B (Ki: 40 nM) and 5-HT_{2A} receptors (Ki: 85 nM) (Kimura et al., 2004). Behavioral studies in rats have shown that the decrease in locomotor activity, hypophagia, anxiety, penile erection and self-grooming induced by *m*-CPP are primarily mediated by activation of 5-HT_{2C} receptors (Kennett and Curzon 1988; Gleason et al., 2001; Millan et al., 1997; Graf et al., 2003). The results obtained in the present study also indicate that the induction of ejaculation induced by *m*-CPP might involve activation of 5-HT_{2C} receptors. This is confirmed by the findings indicating that the ejaculatory response induced by *m*-CPP was abolished by pretreatment with the selective 5-HT_{2C} receptor antagonist SB242084 (Kennett et al., 1997a), as assayed by both non-corset (*m*-CPP 3.0 mg/kg; Fig. 3) and corset (*m*-CPP 0.6 mg/kg; Fig. 4C and D) tests. In the later test, neither the 5-HT_{2A} receptor antagonist ketanserin nor the 5-HT_{2B} receptor antagonist SB204741 (Forbes et al., 1995) affected the *m*-CPP -induced ejaculation (Fig. 4 C and D). Furthermore, it is noteworthy that the dose of SB242084 (0.3 mg/kg) that completely blocked the *m*-CPP-induced ejaculation also attenuated both penile erections and genital grooming induced by *m*-CPP, which has been previously shown in the 5-HT_{2C} receptor-mediated responses (Millan et al., 1997; Graf et al., 2003). These results, taken together, suggest that not only ejaculation but also other sexual responses induced by *m*-CPP may be primarily mediated by the activation of 5-HT_{2C} receptors. Support for this view comes from the recent finding that the selective 5-HT_{2C} receptor agonist Ro600175 can elicit a specific patterned burst response in the *vas deferens* nerve in rats (Stafford et al., 2006b), which is associated with the seminal emission and ejaculation phases of the ejaculatory process. In addition, a recent study in our laboratory showed that, when assayed by corset test, systemic administration of MK212 (0.3–1.0 mg/kg, i.p.), a preferential 5-HT_{2C} receptor agonist (Walker et al., 2005), dose-dependently induced ejaculation in rats, which was completely blocked by SB242084 (unpublished observation). However, the potential role for 5-HT_{2A} receptors cannot be ruled out, because ejaculation induced by the highest dose (3.0 mg/kg) of *m*-CPP was dose-dependently blocked by the 5-HT_{2A} receptor antagonist ketanserin.

The stimulatory effect of *m*-CPP on *ex copula* ejaculation is in contrast with previous findings (Mendelson and Gorzalka 1990; Pomerantz et al., 1993) that treatment with *m*-CPP to male rats and monkeys declined the percentage of animals achieving *in copula* ejaculation. Contradictory results on *ex copula* and *in copula* ejaculation have also been shown in rats treated with the 5-HT-releasers such as *p*-chloroamphetamine (Foreman et al., 1992; Yonezawa et al., 2005). Although the mechanism(s) for these contradictory results could not be clearly defined, the genito-sensory systems may be partially related to these differences. This is supported by the fact that when the occurrence of genital grooming was prevented by a fitted with corset, the percentage of rats showing ejaculation and the amount of ejaculate were much higher compared to the non-corset rats (Fig. 4A and B). This result indicates that the genito-sensory stimulation (e.g., genital grooming or insertion of penis into vagina) in the process of copulation may suppress the *m*-CPP-induced ejaculation. Further investigations will be needed to elucidate this consideration.

It is well established that ejaculation is a complex physiological process that consists of three distinct successive phases; seminal emission, ejaculation and bladder neck closure (Kimura 1972; Kimura et al., 1975). Numerous studies have indicated that the central command of ejaculation is located at the thoracolumbar and the lumbosacral levels of the spinal cord (Giuliano and Clement 2005a,b; Truitt and Coolen, 2002). Recent immunohistochemical and *in situ* hybridization studies in rats indicated the presence of *a* high density of 5-HT_{2C} receptors in the lumbosacral spinal cord (Bancila et al., 1999; Helton et al., 1994), which contributes to the generation of penile erection. In the present study, the finding that the doses of *m*-CPP that induced ejaculation were very similar to that of penile erection is noteworthy. These results, taken together, suggest that the activation of 5-HT_{2C} receptors located in the spinal cord may involve the *m*-CPP-induced sexual responses. This is supported by a recent report which indicated that the ejaculation-related responses in rats evoked by the selective 5-HT_{2C} receptor agonist Ro600175 are mediated by the activation of sympathetic preganglionic neurons in the lumbosacral spinal cord, and that these responses are blocked by a 5-$HT_{2B/2C}$ receptor antagonist (Stafford et al., 2006b). Electrophysiological studies have confirmed the spinal facilitatory action of *m*-CPP on genital reflex pathways in rats (Steers and de Groat, 1989). However, a peripheral site of action of *m*-CPP cannot be ruled out, because peripheral 5-HT may also be related to PCA-induced ejaculation (Yonezawa et al., 2005) and the presence of 5-TH_{2C} receptors has been identified in male reproductive organs, such as the seminal vesicle and *vas deferens* (Kim and Paick 2004).

One particularly interesting finding in the present study is that pretreatment with SB204741, a selective 5-HT_{2B} receptor antagonist, *significantly* potentiated the proejaculatory effect of a high dose (3.0 mg/kg) *m*-CPP. In fact, the weight of seminal materials accumulated over 30 min was 1.5- to 2-fold higher than with *m*-CPP alone. These results suggest that the activation of 5-HT_{2B} receptor may interfere inhibitory with the expression of ejaculation induced by *m*-CPP. This is probably due to the

fact that the high dose of *m*-CPP, but not the lower doses used in the present study, activated both 5-HT_{2C} and 5-HT_{2B} receptors, so that the 5-HT_{2C} receptor-mediated ejaculatory response might have been masked by the 5-HT_{2B} receptor activation. Based on these observations, we speculate that the activation of 5-HT_{2C} and 5-HT_{2B} receptors may play opposite roles in the control of the ejaculatory process in rats. Such opposite effects mediated by these 5-HT receptors have been previously observed in self-grooming, anxiety and feeding behavior in rats (Kennett et al., 1997b; Graf et al., 2003).

In conclusion, the results of the present study suggested that *the* 5-HT_2 receptor agonist *m*-CPP at *low* doses (0.3-1.0 mg/kg) *possesses* the proejaculatory as well as proerectile effects in rats that are primarily associated with the activation of 5-HT_{2C} receptors. The activation of 5-HT2B receptors may produce an inhibitory effect on ejaculation induced by a high dose (3.0 mg/kg) of *m*-CPP. Furthermore, the present study also indicated that the corset test employed may be useful for detecting the proejaculatory effect of the compounds.

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