Synergistic actions of apomorphine and m-chlorophenylpiperazine on ejaculation, but not penile erection in rats

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ABSTRACT

It has been suggested that dopamine (DA) and serotonin (5-HT) and their receptors, particularly D₂-like and 5-HT_{2C} receptors, may play a significant role in the control of male sexual function. The purpose of this study was to investigate whether the combination of a dopamine receptor agonist apomorphine and a 5-HT₂ receptor agonist m-CPP would potentiate penile erection and ejaculation in male rats. Systemic administration of either apomorphine (0.01–0.1 mg/kg, s.c.) or m-CPP (0.01–0.3 mg/kg, i.p.) dose-dependently elicited penile erections, but did not induce ejaculation. When combined, there was a drastic increase in both the incidence of ejaculation and the amount of ejaculated seminal materials, while the proerectile effect induced by each drug was not potentiated. The proejaculatory effect induced by the combination of apomorphine (0.1 mg/kg, s.c.) and m-CPP (0.3 mg/kg, i.p.) was completely blocked by pretreatment with the D₂-like receptor antagonists haloperidol and sulpiride, but not by the D₁-like receptor antagonist SCH-23390. The synergistic action for ejaculation was also blocked by domperidone, the D₂-like receptor antagonist that does not cross the blood-brain barrier. The rats pretreated with the 5-HT_{2C} receptor antagonist SB242084 did not show the synergistic action by the combination of apomorphine and m-CPP, whereas the rats pretreated with the 5-HT_{2A} receptor antagonist ketanserin and the 5-HT_{2B} receptor antagonist SB204741 showed the combination-induced synergistic action. These results suggest that the combination of a small dose of apomorphine and m-CPP potently and selectively facilitates the ejaculatory response through the activation of D₂-like and 5-HT_{2C} receptors, respectively. The D₂-like receptors involved in the synergistic action may be, at least in part, located in the peripheral sites.

Ejaculation is defined as a complex physiological process that results in the expulsion of the seminal fluid from the urethral meatus (19, 22). This process consists 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 occur reflexively, and require coordination of autonomic and somatic nervous systems (7, 9, 16) in order to achieve effective delivery of semen. A variety of neurotransmitters distributed in supraspinal and spinal sites are important for the controlling ejaculation. Among the several neurotransmitters, it has been suggested that the dopaminergic and serotonergic systems have both excitatory and inhibitory influences on penile erection and ejaculation at the supraspinal level (10,

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14). Previous studies in our laboratory have revealed that systemic administration of the serotonin (5-HT)-releasing agents such as *p*-chloroamphetamine (PCA) and fenfluramine can induce ejaculation in conscious and anesthetized rats (31, 32). Pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine or 5-HT_{1/2} and 5-HT₂ receptor antagonists (methysergide, ritanserin and ketanserin) significantly abolished the ejaculatory response induced by PCA or fenfluramine (26, 32, 33). In addition, it has been shown that 5-HT₂ receptor agonist 5-methoxydimethyltryptamine facilitates ex copula seminal emission and ejaculation (20, 27, 28). 5-HT₂ receptors can be divided into three subtypes: 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors (11). It has been shown that systemic administration of *m*-chlorophenylpiperazine (*m*-CPP), a 5-HT₂ receptor agonist, produces ex copula responses such as penile erection in rats (1, 3). Furthermore, it has been recently reported that *m*-CPP possesses the ability to induce ejaculation as well as penile erection in rats (34), and that these responses are primarily associated with 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 (30), which is associated with the seminal emission and ejaculation phases of the ejaculatory process.

On the other hand, microinjection of apomorphine, a nonselective dopamine (DA) receptor agonist, into the medial preoptic area (MPOA) and paraventricular nucleus (PVN) facilitated seminal emission, and this effect was completely blocked by the D₂ receptor antagonist raclopride (13, 25). Two separate DA receptor populations have been identified: D₁-like receptors (D₁ and D₅) and D₂-like receptors (D₂, D₃ and D₄). The selective D₂ receptor agonist quinolorane administered both systemically and microinjected into the MPOA facilitated seminal emission (4). Recent studies have demonstrated that i.c.v. injection of quinolorane induced rhythmic bursts of bulbospongiosus muscle contractions that are responsible for the propulsion of semen from the prostatic urethra to the urethral meatus as well as its forceful expulsion (6). These results indicate the possibility that the proejaculatory effect of apomorphine is likely mediated at supraspinal levels, and the effect may be related to the activation of D₂ receptors. Recently, Stafford *et al.* (30) reported that the combination of Ro600175 and the 5-HT_{1A} receptor agonist 8-OH-DPAT synergistically elicits contraction of the vas deferens through the activation of

sympathetic preganglionic neurons in the spinal cord. However, 8-OH-DPAT has been reported to increase dopaminergic transmission into the MPOA by activation of D₂-like receptors (6, 18, 21). Thus, DA or 5-HT may promote ejaculation via the interaction of the DA and 5-HT receptor subtypes.

The purpose of the present study was to investigate whether the combination of a DA receptor agonist apomorphine and a 5-HT₂ receptor agonist *m*-CPP would enhance the ejaculatory response in rats. Additionally, we have examined to clarify the role of DA and 5-HT₂ receptor subtypes in the combination-induced response.

MATERIALS AND METHODS

Animals. Adult male Wistar-ST strain rats (Japan SLC, Hamamatsu, Japan), weighing 300–400 g (10–14 weeks old), were used for the present study. They were housed in a two rat per wire-bottomed stainless-steel cage (35 × 42 × 20 cm). 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 : 00–17 : 00 h. Each rat was used only once except for the dose-response experiment (Fig. 2). All animal procedures were approved by the Ethics 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.

Drugs and treatments. The following drugs were used: apomorphine hydrochloride hemihydrate, R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH-23390), haloperidol, (–)-sulpiride, domperidone, N-(1-methyl-1H-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea (SB204741), 6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride (SB242084) (all were obtained from Sigma-RBI, St. Louis, MO), 1-(3-chlorophenyl)piperazine hydrochloride (*m*-CPP; Tocris Cookson, Ellisville, MO), and ketanserin tartrate (Research Biochemicals International, Natick, MA). Apomorphine was dissolved in 0.1% L-ascorbic acid solution and diluted with sterilized saline. *m*-CPP, SCH-23390, ketanserin and SB242084 were dissolved in sterilized saline. Haloperidol, (–)-sulpiride and SB204741 were suspended in 0.5% Tween 80/saline solution. Apomorphine was injected subcutaneous (s.c.) and other drugs were injected intraperi-

toneally (i.p.) in a volume of 1 mL/kg body weight. m-CPP was injected i.p. immediately after s.c. apomorphine. Dopamine receptor antagonists such as SCH-23390, haloperidol, (-)-sulpiride and domperidone were injected 30 min before the combination of apomorphine and m-CPP. 5-HT₂ receptor antagonists such as ketanserin, SB204741 and SB242084 were injected 20, 30 and 20 min before the combination of apomorphine and m-CPP, respectively.

Observation of penile erections, ejaculation and other behavior. 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 drug administration, 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 apomorphine and m-CPP administration, or combination of both, 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 placed on filter paper for 30 min, and then weighed. A penile erection was defined as previously described (3, 17, 34); repeated pelvic thrusts immediately followed by an upright position on the hind limbs, an emerging, engorged penis and licking of the penis. The number of penile erections and yawns, or the duration of self-grooming behavior was simultaneously observed following the combination of apomorphine and m-CPP using an event-recorder (MATYS, model CIF-E12; Toyo Sangyo, Toyama).

Statistics. Data were expressed as the means ± S.E.M. The statistical significance of differences between means was analyzed with a one-way analysis of variance (ANOVA) followed by either Dunnett's test or Newman-Keuls test. The difference between the incidence of penile erection and ejaculation was analyzed by the Fisher's exact probability test.

RESULTS

Effects of apomorphine and m-CPP alone, or combination of both, on penile erection and ejaculation in rats
Systemic administration of either apomorphine

(0.01–0.1 mg/kg, s.c.) or m-CPP (0.01–0.3 mg/kg, i.p.) dose-dependently elicited penile erections in rats, but did not induce ejaculation (Table 1). There was a slight incidence of the ejaculatory response at 0.3 mg/kg dose of m-CPP, but no incidence at lower doses (0.01–0.1 mg/kg). However, the combination of apomorphine (0.1 mg/kg) and m-CPP (0.3 mg/kg) drastically induced ejaculation (Fig. 1A); all animals receiving both drugs had ejaculations with a large amount of seminal materials (53.8 ± 16.8 mg/kg, $n = 8$). By contrast, the synergistic action on penile erections was not observed by the combination (Fig. 1B). None of the vehicle-injected rats had the ejaculatory response during the observation period.

Dose-dependent effects

As shown in Table 2, the combination of a fixed dose (0.1 mg/kg) of apomorphine and the small doses (0.01–0.3 mg/kg) of m-CPP dose-dependently increased both the incidence of ejaculation and the weight of seminal materials in comparison with apomorphine alone. The proerectile effect of apomorphine was not potentiated by the lower doses of m-CPP (data not shown). Likewise, the combination of a fixed dose (0.3 mg/kg) of m-CPP and the small doses (0.01–0.1 mg/kg, i.p.) of apomorphine dose-dependently induced the ejaculatory response in rats. The proerectile effect of m-CPP was not potentiated by the lower doses of apomorphine (data not shown). Since a marked proejaculatory effect was seen in rats injected with the combination of apomorphine (0.1 mg/kg) and m-CPP (0.3 mg/kg), the dose of both drugs was chosen for the subsequent studies.

Effect on other behaviors

Systemic administration of apomorphine (0.1 mg/kg, s.c.) and m-CPP (0.3 mg/kg, i.p.) induced yawning and self-grooming in rats, respectively. As shown in Fig. 2, in contrast to ejaculation, the combined treatment did not potentiate the apomorphine-induced yawning and the m-CPP-induced self-grooming, respectively.

Effect of DA receptor antagonists on the ejaculatory response induced by the combination of apomorphine and m-CPP

To determine the involvement of the DA receptor subtype(s) in the synergistic action for ejaculation, several DA receptor antagonists were used for this purpose. As shown in Fig. 3A, pretreatment with the D₂-like receptor antagonists haloperidol (0.1 mg/kg, i.p.) and (-)-sulpiride (25 mg/kg, i.p.) completely

Table 1 Effects of apomorphine and m-CPP on ejaculation and penile erection in rats

Dose (mg/kg)	Ejaculation	Penile erection
	Incidence (%)	Incidence (%)
VEH	0	33.3
Apomorphine (0.01)	0	50
Apomorphine (0.03)	0	50
Apomorphine (0.1)	0	100*
VEH	0	25
m-CPP (0.01)	0	25
m-CPP (0.1)	0	62.5
m-CPP (0.3)	12.5	100**

* $P < 0.05$, ** $P < 0.01$, compared with vehicle (VEH)

blocked the ejaculatory response induced by the combination of apomorphine (0.1 mg/kg, s.c.) and m-CPP (0.3 mg/kg, i.p.), whereas the response induced by the combination was not inhibited by the D_1 -like receptor antagonist SCH-23390 (0.1 and 0.3 mg/kg, i.p.). Furthermore, the synergistic action to the combination of apomorphine and m-CPP was also dose-dependently inhibited by domperidone, a D_2 -like receptor antagonist that does not cross the blood-brain barrier (Fig. 3A). In contrast, penile erection induced by the combination of apomorphine and m-CPP was not blocked by pretreatment with SCH-23390, haloperidol, (–)-sulpiride (25 mg/kg, i.p.) or domperidone (Fig. 3B). Penile erection induced by apomorphine (0.1 mg/kg, s.c.) alone was significantly inhibited by haloperidol (0.1 mg/kg, i.p.), but not by domperidone (3.0 mg/kg, i.p.) (data not shown).

Effect of 5-HT₂ receptor antagonists on the ejaculatory response induced by the combination of apomorphine and m-CPP

To determine the involvement of the 5-HT₂ receptor subtype(s) in the synergistic action on ejaculation, several 5-HT₂ receptor antagonists were used for this purpose. As shown in Fig. 4A, pretreatment with the 5-HT_{2C} receptor antagonist SB242084 (0.03 and 0.1 mg/kg, i.p.) dose-dependently blocked the ejaculatory response induced by the combination of apomorphine (0.1 mg/kg, s.c.) and m-CPP (0.3 mg/kg, i.p.), whereas the response induced by the combination was not inhibited by the 5-HT_{2A} receptor antagonist ketanserin (0.03 and 0.1 mg/kg, i.p.) and the 5-HT_{2B} receptor antagonist SB204741 (0.1 and 0.3 mg/kg, i.p.). In contrast, penile erection induced by the combination of apomorphine and m-CPP was not blocked by pretreatment with ketanserin, SB204741, and SB242084 (Fig. 4B). Penile erection induced by m-CPP (0.3 mg/kg, i.p.) alone was com-

Fig. 1 Effect of apomorphine (0.1 mg/kg, s.c.), m-CPP (0.3 mg/kg, i.p.) or combination of apomorphine (0.1 mg/kg) and m-CPP (0.3 mg/kg) on ejaculation (A) and penile erection (B) in rats. (A) and (B) denote the weight of seminal materials and the number of penile erections during the observation period (for 30 min), respectively. Each column represents the means $\pm$ S.E.M. for 8 animals. The symbol indicates a significant difference (* $P < 0.05$, ** $P < 0.01$) from vehicle (VEH)-treated animals. The values expressed are the number of rats per group showing ejaculatory response.

pletely inhibited by SB242084 (0.1 mg/kg, i.p.), but not by ketanserin and SB204741 (data not shown).

DISCUSSION

The present study clearly demonstrated that the combination of a dopamine receptor agonist apomorphine and a 5-HT₂ receptor agonist m-CPP potently facilitates the ejaculatory response in rats. In fact, when systemically co-administered with the sub-threshold doses, there was the drastic increase in both the incidence of ejaculation and the amount of ejaculated seminal materials (Fig. 1 and Table 2). We have recently reported that systemic administra-

Table 2 Effects of combination of apomorphine and m-CPP on ejaculation in rats

Dose (mg/kg)	Ejaculation	
	Proportion of ejaculating animals	Weight (mg)
Apomorphine (0.1) alone	0/8	0
+ m-CPP (0.01)	3/8	9.5 ± 6.8
+ m-CPP (0.1)	5/8*	25.2 ± 16.7
+ m-CPP (0.3)	8/8**	51.8 ± 16.8**
m-CPP (0.3) alone	1/8	0.7 ± 0.4
+ apomorphine (0.01)	3/8	3.3 ± 2.3
+ apomorphine (0.03)	5/8	26.7 ± 14.1
+ apomorphine (0.1)	8/8**	53.1 ± 17.5**

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

tion of a relatively high dose of m-CPP produced ejaculation in rats; the ED_{50} value of m-CPP for inducing ejaculation was 0.6 mg/kg (0.597–0.602 mg/kg, 95% confidence intervals), while, in this study, the ED_{50} value of m-CPP obtained by the combination with the fixed dose (0.1 mg/kg) of apomorphine was 0.023 mg/kg (0.017–0.032 mg/kg, 95% confidence intervals), indicating a drastic reduction in the threshold for ejaculation. Thus, the relative potency of the proejaculatory effect of m-CPP was 26-fold increase by the combination of apomorphine.

The combination of apomorphine and m-CPP may selectively potentiate the ejaculatory response in rats. In the present study, the proerectile effect of each compound was not enhanced by the combination (Fig. 1 and Table 1). We previously demonstrated that the dose-response curves for penile erection induced by m-CPP are inverted U-shaped curves with the maximum effect at 0.6 mg/kg (number of penile erections/30 min; 4.3 ± 0.8) (34). Furthermore, neither the m-CPP-induced self-grooming nor the apomorphine-induced yawning was also enhanced by the combination of apomorphine and m-CPP (Fig. 2). These results indicate that the combination with a small dose of apomorphine and m-CPP may act synergistically to induce ejaculation without affecting other characterized behavioral response induced by each compound.

It has been shown that apomorphine exhibits a relatively high affinity for D_1 -like (D_1 and D_5) and D_2 -like (D_2 , D_3 and D_4) dopamine receptor subtypes (K_i values are 100, 10, 15, 2.5 and 50 nM for human cloned D_1 , D_2 , D_3 , D_4 and D_5 receptors, respectively) (12), and elicits almost full agonistic activity at D_2 -like receptors. Also, m-CPP has been shown to exhibit a relatively high affinity for $5-HT_{2A}$, $5-HT_{2B}$ and $5-HT_{2C}$ receptor subtypes (K_i values are 80, 40 and 16 nM for human cloned $5-HT_{2A}$, $5-HT_{2B}$

Fig. 2 Effect of apomorphine (0.1 mg/kg, s.c.), m-CPP (0.3 mg/kg, i.p.) or combination of apomorphine (0.1 mg/kg) and m-CPP (0.3 mg/kg) on self-grooming and yawning in rats. Results of the duration of self-grooming and the number of yawning are shown as means $\pm$ S.E.M. for 8 animals.

and $5-HT_{2C}$ receptors, respectively) (17). The proerectile effects of apomorphine and m-CPP have shown to be mediated by the activation of D_2 -like and $5-HT_{2C}$ receptors, respectively (12, 17, 23). The results obtained in the present study also indicate that activation of both D_2 -like receptor by apomorphine and $5-HT_{2C}$ receptor by m-CPP may involve the synergistic action for inducing ejaculation induced by the combination of both compounds. This is evidenced by the findings that the synergistic action for the co-administered apomorphine and m-CPP was completely abolished by pretreatment with the D_2 -like receptor antagonists (haloperidol and sulpiride), but not by the D_1 -like receptor antagonist (SCH-23390), while pretreatment with the selective $5-HT_{2C}$ receptor antagonist (SB242084), but not the $5-HT_{2A}$ and $5-HT_{2B}$ receptor antagonists (ketanserin and SB204741), dose-dependently blocked the ejaculatory response induced by the combination. The lack of effects of these antagonists on the proerectile effect induced by the combination (Fig. 1), confirms the importance of the simultaneous activa-

Fig. 3 Effect of DA receptor antagonists on the combination of apomorphine and m-CPP induced ejaculation (A) and penile erection (B) in rats. (A) and (B) denote the weight of seminal materials and the number of penile erections, which were pretreated with the D_1 -like and D_2 -like receptor antagonists, respectively. Each column represents the means $\pm$ S.E.M. for 8 animals. The symbol indicates a significant difference (** $P < 0.01$) from vehicle (VEH)-treated animals. SCH = SCH-23390, HAL = haloperidol, SUL = sulpiride, DOM = domperidone.

tion of D_2 -like receptor by apomorphine and 5-HT_{2C} receptor by m-CPP, respectively.

Several studies have shown that systemic and intracerebral (*e.g.*, MPOA and PVN) administration of apomorphine can facilitate seminal emissions in rats, and this effect was blocked by the D_2 -like receptor antagonist (4, 13). Furthermore, systemic administration of apomorphine in anaesthetized rats has been shown to evoke the supraspinal-mediated bursting pattern response in the sympathetic vas deferens nerve that corresponds to ejaculation (29). These results suggest the possibility that the proejaculatory effect of apomorphine results in the activation of D_2 -like receptor at supraspinal level as with its proerectile effect (4). However, the result obtained in the present study demonstrates that D_2 -like receptor which mediated the synergistic action induced by the combination of apomorphine and

Fig. 4 Effect of 5-HT₂ receptor antagonists on the combination of apomorphine and m-CPP induced ejaculation (A) and penile erection (B) in rats. (A) and (B) denote the weight of seminal materials and the number of penile erections, which were pretreated with the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor antagonists, respectively. Each column represents the means $\pm$ S.E.M. for 8 animals. The symbol indicates a significant difference (* $P < 0.05$) from vehicle (VEH)-treated animals.

m-CPP may be located in the peripheral sites rather than the supraspinal level. Thus, the proejaculatory effect of the co-administered apomorphine and m-CPP was dose-dependently blocked by pretreatment with domperidone (0.3–3.0 mg/kg), a D_2 -like receptor antagonist that does not cross the blood-brain barrier (2). As shown here and in previous studies (8, 24), the doses of domperidone used did not affect the erectile activity induced by apomorphine alone (*i.e.*, central D_2 -like receptors-mediated response) (2) or the combination of apomorphine and m-CPP, suggesting both the selective antagonism and the importance of peripheral D_2 -like receptors. Recently, identification of peripheral D_2 receptors in the seminal vesicle of rats and humans has been established (15) and dopamine itself contracts the seminal vesicles (5), suggesting the possibility that dopamine D_2 receptor localized in the seminal vesicles may modulate seminal emission in

rats. Further investigations will be needed to elucidate this consideration.

The results of this study may provide useful information as to the treatment of patients with ejaculatory dysfunction. It is noteworthy that a prominent synergistic action was induced by the combination of a small dose of apomorphine and m-CPP without affecting the proerectile activity. Although apomorphine has been used for the treatment of sexual dysfunction, its side-effects may limit the use of higher doses and the narrowing therapeutic modality. The addition of m-CPP or the selective 5-HT_{2C} receptor agonist may lower the effective dose of apomorphine and provide better treatment with fewer side-effects.

In conclusion, the results of the present study demonstrate that the combination of a small dose of apomorphine and m-CPP results in a potentiation for inducing ejaculation and this synergistic action is likely mediated by the simultaneous activation of D₂-like and 5-HT_{2C} receptors, respectively.

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