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

Akihiko Yonezawa1, Masaru Yoshizumi1, Shin-nosuke Ise1, Chizuko Watanabe1, Hirokazu Mizoguchi1, Katsuo Furukawa2, Hiromichi Tsuru3, Yukio Kimura3, Masahito Kawatani4 and Shinobu Sakurada1

1Department of Physiology and Anatomy, Tohoku Pharmaceutical University, Sendai, Japan; 2Department of Pharmacology, Toho University School of Medicine, Tokyo, Japan; 3Towada Urology Hospital, Towada, Japan; and 4Department of Neurophysiology, Akita University School of Medicine, Akita, Japan

(Received 24 December 2008; and accepted 8 January 2009)

ABSTRACT

It has been suggested that dopamine (DA) and serotonin (5-HT) and their receptors, particularly D2-like and 5-HT2c 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 antagonist 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-HT2c receptor antagonist SB242084 did not show the synergistic action by the combination of apomorphine and m-CPP, whereas the rats pretreated with the 5-HT2a receptor antagonist ketanserin and the 5-HT2b 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-HT2c 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,
The following drugs were reported that the effect may be related to the activation of D$_2$ receptors. Additionally, we have examined to clarify the role of DA and 5-HT 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-yl)oxy]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-
Dopamine and serotonin receptors in ejaculation

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(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 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
The present study clearly demonstrated that the combination of a dopamine receptor agonist apomorphine and a 5-HT\textsubscript{2} 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 administration 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\textsubscript{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\textsubscript{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).

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

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Ejaculation</th>
<th>Penile erection</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEH</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Apomorphine (0.01)</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Apomorphine (0.03)</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Apomorphine (0.1)</td>
<td>0</td>
<td>100*</td>
</tr>
<tr>
<td>VEH</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>m-CPP (0.01)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>m-CPP (0.1)</td>
<td>0</td>
<td>62.5</td>
</tr>
<tr>
<td>m-CPP (0.3)</td>
<td>12.5</td>
<td>100**</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, compared with vehicle (VEH)

**Discussion**

The present study clearly demonstrated that the combination of a dopamine receptor agonist apomorphine and a 5-HT\textsubscript{2} 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 administration...
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$_3$) 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$_2B$ 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 sulphide), 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-

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

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

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

**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 ± 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.

**Fig. 4** Effect of 5-HT$_2$ 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 ± S.E.M. for 8 animals. The symbol indicates a significant difference (*$P < 0.05$) from vehicle (VEH)-treated animals.
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 D$_2$-like and 5-HT$_2C$ receptors, respectively.

Acknowledgments

This study was supported by Grant-in-Aid for Scientific Research (C) (KAKENHI 19591884) from Japan Society for the Promotion of Science.

REFERENCES