Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 82 (2005) 744 – 750
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Evidence for an involvement of peripheral serotonin in *p*-chloroamphetamine-induced ejaculation of rats

Akihiko Yonezawa [a,*], Masaru Yoshizumi [a], Manabu Ebiko [a], Toshihiko Iwanaga [b], Yukio Kimura [c], Shinobu Sakurada [a]

[a] *Department of Physiology and Anatomy, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan*
[b] *Department of Functional Morphology, Laboratory of Histology and Cytology, Hokkaido University Graduate School of Medicine, Sapporo 060-0818, Japan*
[c] *Towada Urology Hospital, Towada City 034-0001, Japan*

Received 27 August 2005; received in revised form 24 November 2005; accepted 1 December 2005

Abstract

The purpose of the present study was to determine the mechanism of the ejaculatory response induced by the 5-HT-releasing compound *p*-chloroamphetamine (PCA) in rats. The ejaculatory response was assessed by weighing the coagulated seminal materials accumulated over 1 h. Intraperitoneal injection of PCA (0.5–5.0 mg/kg) produced a dose-related increase in both the incidence of ejaculation and the weight of the accumulated seminal materials. The ejaculatory response induced by PCA (5.0 mg/kg) was abolished by pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine, the 5-HT receptor antagonists methysergide and MDL72222, or by the selective 5-HT reuptake inhibitor citalopram, suggesting that the 5-HT-releasing property of PCA mainly involved the expression of ejaculation. Neither the section of the spinal cord at thoracic (T8–9) level nor the lumbosacral spinal 5-HT denervation by intrathecal (i.t.) injection of 5,7-dihydroxytryptamine affected the ejaculatory response induced by PCA. The i.t. injection of PCA (20–160 μg/rat) at lumbosacral spinal level did not exert the systemic PCA-like prominent effect on ejaculation, whereas i.t. injection of lidocain at the same site completely abolished the response to systemic PCA. Additionally, the peripherally administered 5-HT (0.1 and 0.25 mg/kg, i.p.) enhanced the proejaculatory effect at a threshold dose (1.0 mg/kg, i.p.) of PCA. From these observations, it is concluded that the ejaculatory response induced by PCA is mainly a spinally mediated reflex response that is triggered by the release of 5-HT in the peripheral sites.

Keywords: PCA; Ejaculation; Peripheral serotonin; Rat

1. Introduction

The amphetamine derivative *p*-chloroamphetamine (PCA) has been known as an indirect serotonergic agonist by its serotonin (5-HT)-releasing action from nerve terminals or other 5-HT containing cells (Fuller, 1992; Holzwarth et al., 1984). It has been shown that behavioral and neuroendocrine responses induced by PCA are mainly due to its 5-HT-releasing action, as indicated by their prevention by inhibition of 5-HT synthesis or by blockade of 5-HT receptor with the selective antagonists (Trulson and Jacobs, 1976; Van de Kar and Bethea, 1982; Ogren, 1986). The selective 5-HT reuptake inhibitors can prevent most of the behavioral and neuroendocrine effects of PCA, suggesting that PCA must be taken up through the 5-HT transporter into 5-HT nerve terminals or other 5-HT containing cells to induce 5-HT release (Fuller, 1992; Rudnick and Wall, 1992). In vivo dialysis study has also demonstrated that the 5-HT behavioral syndrome induced by PCA correlates well with increasing 5-HT in the central nervous system (CNS) (Hutson and Curzon, 1989).

Pharmacological experiments have indicated that 5-HT-releasing compounds, such as PCA and fenfluramine, exert a complex action for the male sexual behavior and functions. Systemic administration of PCA (1.0–2.5 mg/kg) inhibits male rat copulatory behavior, and this inhibition can be blocked by 5-HT receptor antagonist (Foreman et al., 1992). By contrast, the similar doses of PCA and fenfluramine induce ex copula penile responses (e.g. glans erection and penile cup; Maeda et

* Corresponding author. Tel.: +81 22 234 4181; fax: +81 22 275 2013.
E-mail address: yonezawa@tohoku-pharm.ac.jp (A. Yonezawa).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.12.001

al., 1994; Yonezawa et al., 2000) and increase the sex-related behavior, e.g. genital grooming (Yonezawa, unpublished observation) in rats, suggesting that the compounds possess the contradictory effects on male sexual behavior and functions.

With regard to ejaculation, PCA exerts a drastic stimulatory effect on the response. It has been shown that systemic administration of PCA (the dose over 2.5 mg/kg) induces a marked ejaculatory response in both conscious and anesthetized rats (Humphries et al., 1981; Renyi, 1985; Yonezawa et al., 2000). We have recently examined the effects of PCA and fenfluramine on the ejaculatory response in anesthetized rats, and indicated that intermittent ejaculations were caused by these compounds, and a large amount of ejaculate consisting of solid and/or liquid materials were ejected during the test period (Yonezawa et al., 2000, 2004). Although the behavioral or neuroendocrine responses induced by PCA appears to be involved in the 5-HT-releasing action of the compound in the CNS, an enhancement of synaptic 5-HT level in the CNS results in an inhibition of ejaculation, as reported in several clinical and laboratory studies (Mos et al., 1999; Ahlenius and Larsson, 1999). Therefore, the exact mechanisms, especially the site of PCA action in mediating ejaculation, remain unknown.

The purpose of our present study was to determine the mechanisms of the ejaculatory response induced by PCA, especially the site of action. Additionally, we have also examined whether the combination with the peripherally administered 5-HT would enhance the ejaculatory response induced by PCA.

2. Materials and methods

2.1. Animals

Adult male Wistar-ST strain rats (Japan SLC, Hamamatsu, Japan), weighing 350–400 g, were used. The animals were housed in standard stainless-steel cage (35 × 42 × 20 cm; 2–3 rats per cage) at a constant temperature (22–24 °C) and a relative humidity (50–60%) under controlled 12 h light–dark cycle (lights on at 09:00). They have free access to standard food pellets and water. The experiments were carried out between 11:00 and 18:00 h. Each rat was used only once. All animal procedures were approved by the Committee of Animal Experiments, Tohoku Pharmaceutical University and were carried out in accordance with NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

The following drugs were used: PCA, *p*-chlorophenylalanine methylester hydrochloride (*p*-CPA), 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT), methysergide maleate, MDL 72222, 5-hydroxytryptamine creatinine sulfate (5-HT), citalopram hydrobromide, nomifensine maleate (all were obtained from Sigma Chemical Co., USA), and lidocaine hydrochloride (Fujisawa-Astora, Japan). All drugs, except for MDL 72222 or intrathecal (i.t.) injection of PCA and 5,7-DHT, were dissolved in sterilized saline and given intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. MDL 72222 was dissolved in 25% DMSO. For i.t. injection, PCA and 5,7-DHT were dissolved in sterilized artificial cerebrospinal fluid (A-CSF; 126.7 mM NaCl, 2.5 mM KCl, 2.0 mM $MgCl_2$, 1.3 mM $CaCl_2$). 5,7-DHT (50 μg/10 μl) was injected i.t. following desipramine (25 mg/kg, i.p.) and the animals were tested 10–12 days after the treatment. *p*-CPA (150 mg/kg, i.p.) was injected 24, 48 and 72 h before the administration of PCA.

2.3. Observation of ejaculation

Prior to the testing, rats were placed individually in a transparent plastic cage (35 × 40 × 18 cm) for a period of at least 30 min. A paper towel was laid in the observation cage to facilitate the confirmation of the occurrence of ejaculation. Before PCA administration, the existence of seminal materials on the paper towel and those that adhered on the shaft of the penis were checked in order to remove the effect by spontaneous ejaculation. If it was detected, treatment with PCA was carried out in the next day. The ejaculatory response was observed for 60 min after PCA administration, and then the coagulated seminal materials were retrieved from the paper and from the shaft of the penis. The seminal materials were placed and dried on filter paper for 30 min, and then the weight was measured.

2.4. Intrathecal (i.t.) catheterization

The i.t. injection of drugs was performed with a polyethylene catheter inserted into the spinal subarachnoid space, according to the method of Ando et al. (2001). Briefly, rats were implanted with a polyethylene catheter (tapered to an appropriate size by heating and filled with A-CSF) through a laminectomy between L1 and L2 under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). The tip of the inserted catheter was positioned at L4–L5 and the free end of the catheter was allowed to protrude from the skin of the back. Rats showing any neurologic dysfunction, such as paralysis or urine incontinence, after catheterization were eliminated. Rats were housed individually and allowed to recover from surgery for at least 1 week.

2.5. Spinal cord transection

Rats were anesthetized with sodium pentobarbital (55 mg/kg, i.p.) and maintained under light to conserve body temperature. The skin and muscles over the thoracic vertebrae were incised and laminectomy was performed. The spinal cord was transected at thoracic (T8–T9) level and then the skin and muscles were sutured. Spinalization was performed 5–6 days prior to testing. The bladder of the spinal rat was compressed manually 3–4 times a day following surgery. In sham operated rats, laminectomy was done but the spinal cord was not transected.

2.6. Measurement of spinal 5-HT

Immediately after the PCA testing, 5,7-DHT ($n=6$)- and vehicle ($n=6$)-treated rats were decapitated and the lumbosacral spinal cord were removed. The tissue was homogenized in 0.1 N perchloric acid (500 μl) containing 1 mM EDTA·2Na, centrifuged at 12,000 rpm (2 °C, 20 min), and the supernatant removed and stored at −80 °C until assayed. The 5-HT determination was performed by the method of Murai et al. (1988), using a high performance liquid chromatography (HPLC; ECD-300, EICOM, Japan).

2.7. Statistics

Data were expressed as the means±S.E.M. The statistical significance of differences between means was analyzed with Mann–Whitney *U*-test or analysis of variance (ANOVA) followed by the Fisher's PLSD post hoc test. The difference between the incidence of the ejaculatory responses was analyzed by the Fisher's exact probability test.

3. Results

3.1. Ejaculatory response induced by i.p. and i.t. administration of PCA

As shown in Fig. 1A, i.p. administration of PCA (0.5–5.0 mg/kg) dose-dependently induced the ejaculatory response in rats. A marked proejaculatory effect (all animals ejaculated a large amount of seminal materials) was seen in rats injected with PCA at doses over 2.5 mg/kg. The highest dose (5.0 mg/kg) produced the seminal materials with a mean of 125.9±20.1 mg during the observation period. Although there was only a small amount of ejaculate, PCA at a dose of 1.0 mg/kg induced the ejaculatory response in about half of animals (Fig. 1A), suggesting an approximate threshold dose that produce the ejaculatory response. In contrast, the direct application of PCA (20–160 μg) at lumbosacral spinal level did not exert a systemic PCA-like prominent effect on the ejaculatory response (Fig 1B). There was a slight incidence of the ejaculatory response even at a high dose (160 μg), but no incidence at a lower dose (20 μg). None of the vehicle (saline or A-CSF)-injected rats had the ejaculatory response during the observation period (saline: $n=8$, A-CSF: $n=6$).

Table 1
Effects of *p*-CPA, citalopram, nomifensin, methysergide and MDL 72222 on ejaculation induced by PCA (5 mg/kg)

Treatment	Dose (mg/kg)	Ejaculation	
		Weight (mg)	Incidence (%)
PCA alone	5	157.6±25.7	100
PCA+ p-CPA	150×3	17.8±9.1**	25
PCA+citalopram	3	6.3±6.1**	20
PCA+citalopram	10	0**	0
PCA+nomifensin	3	99.8±37.5	100
PCA+nomifensin	10	71.0±37.4	100
PCA+methysergide	10	45.1±16.4*	70
PCA+MDL 72222	4	44.5±26.7*	75

*$p<0.05$, **$p<0.01$, compared with PCA control (n=8-10).

3.2. Involvement of 5-HT-releasing action in PCA-induced ejaculatory response

Several pharmacological experiments were carried out to examine the involvement of 5-HT system in the PCA-induced ejaculatory response in rats. The 5.0 mg/kg dose of PCA was selected for the experiments because it was the most effective and reliable dose for producing the ejaculatory response. The data obtained are summarized in Table 1. Pretreatment with the 5-HT synthesis inhibitor *p*-CPA (150 mg/kg, i.p.×3) and the 5-HT receptor antagonists

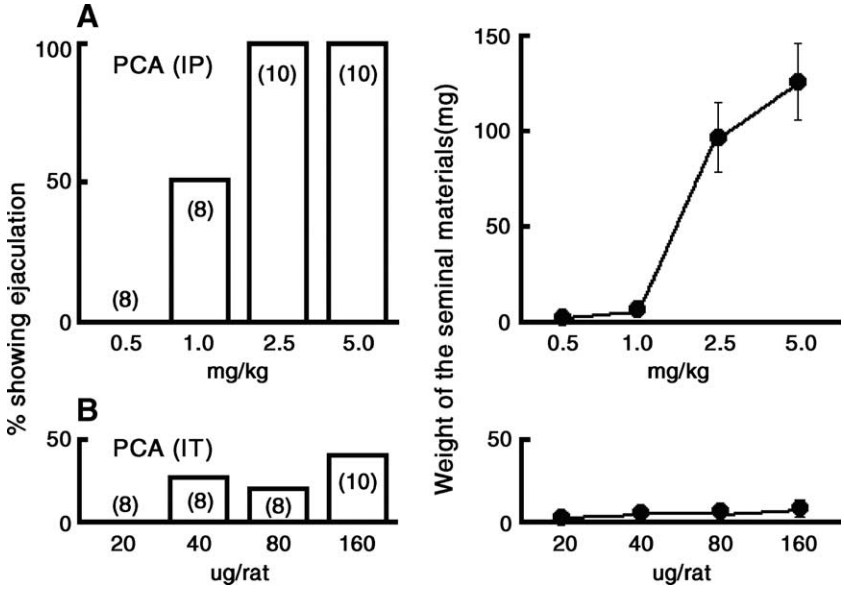

Fig. 1. Effects of systemic (A) and intrathecal (B) administration of *p*-chloroamphetamine (PCA) on the incidence of ejaculation and the weight of seminal materials in rats. Results of the weight of seminal materials are shown as mean±S.E.M. for 8–10 animals. The values expressed are the number of rats used in each treatment.

methysergide (10 mg/kg, i.p.) or MDL 72222 (4 mg/kg, i.p.) significantly inhibited the PCA-induced ejaculatory response. The ejaculatory response induced by PCA was completely inhibited by the selective 5-HT reuptake inhibitor citalopram (3 and 10 mg/kg i.p.) but not by the selective dopamine reuptake inhibitor nomifensine (3 and 10 mg/kg i.p.). These data suggest that the ejaculatory response induced by PCA is mainly due to its 5-HT releasing action, and then released 5-HT stimulates the post-synaptic 5-HT receptors.

3.3. Sites of action

To determine whether the proejaculatory effects of PCA are mediated by either the central (supraspinal and/or spinal) or peripheral sites, or a combination of both, several experiments were carried out for this purpose. As shown in Fig. 2A, the ejaculatory response induced by PCA (5.0 mg/kg, i.p.) was not affected by the spinal cord transection at thoracic level; there was no statistically significant difference in both the number of ejaculated rats and the amount of seminal materials produced by PCA between sham operated and transected rats. On the other hand, i.t. pretreatment with a local anesthesia lidocaine (1 mg) at the lumbosacral spinal level completely abolished PCA-induced ejaculatory response (Fig. 2B). This inhibition was shown to have a tendency to recover until 60 min after i.t. lidocaine. Pretreatment with i.t. 5,7-DHT produced a marked depletion of the lumbosacral spinal 5-HT content (>90%; Fig. 2D-right), while the ejaculatory response induced by PCA (2.5 and 5 mg/kg, i.p.; Fig 2C-left) was not affected by 5,7-DHT. These results suggest that the ejaculatory response induced by PCA is mainly a spinally mediated response that did not involve the descending 5-HT pathway from the supraspinal sites.

3.4. Effect of peripherally administered 5-HT on PCA-induced ejaculatory response

Because systemic administration of PCA can produce the 5-HT-releasing action in the peripheral sites, we examined a possible involvement of peripheral 5-HT on PCA-induced ejaculatory response. As shown in Fig. 3, the ejaculatory response induced by 1.0 mg/kg dose of PCA (the 50% effective dose in producing ejaculation; Fig. 1A) was potentiated markedly by peripherally administered 5-HT (0.1 and 0.25 mg/kg, i.p.). The treatment with 5-HT,

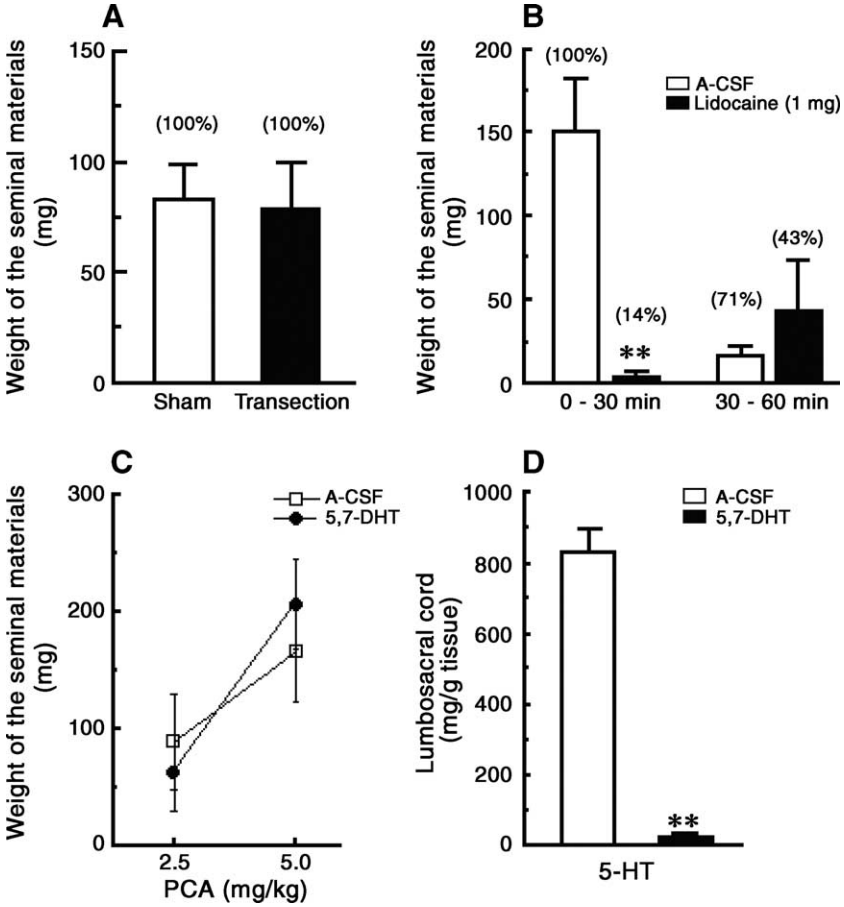

Fig. 2. Effects of spinal cord transection (A) and intrathecal (i.t.) pretreatment with lidocaine (B) or 5,7-DHT (C) on the ejaculatory response induced by systemic PCA in rats. (A) Transection of the spinal cord at thoracic (T8–T9) level was performed 5–6 days prior to administration of PCA (5.0 mg/kg, i.p.). (B) Lidocaine was administered i.t. 5 min prior to administration of PCA (5.0 mg/kg, i.p.). (C) 5,7-DHT (50 μg/10 μl) was administered i.t. following desipramine (25 mg/kg, i.p.) and the animals were tested 10–12 days after the treatment. 5-HT contents in the lumbosacral spinal cord for VEH- and 5,7-DHT-treated animals are shown in C (right). Results are shown as mean±S.E.M. for 6–9 animals. Percentage values in (A) and (B) represent the number of rats per group showing ejaculatory response. The symbol (**) indicates a significant difference ($p<0.01$) from vehicle (VEH)-treated animals.

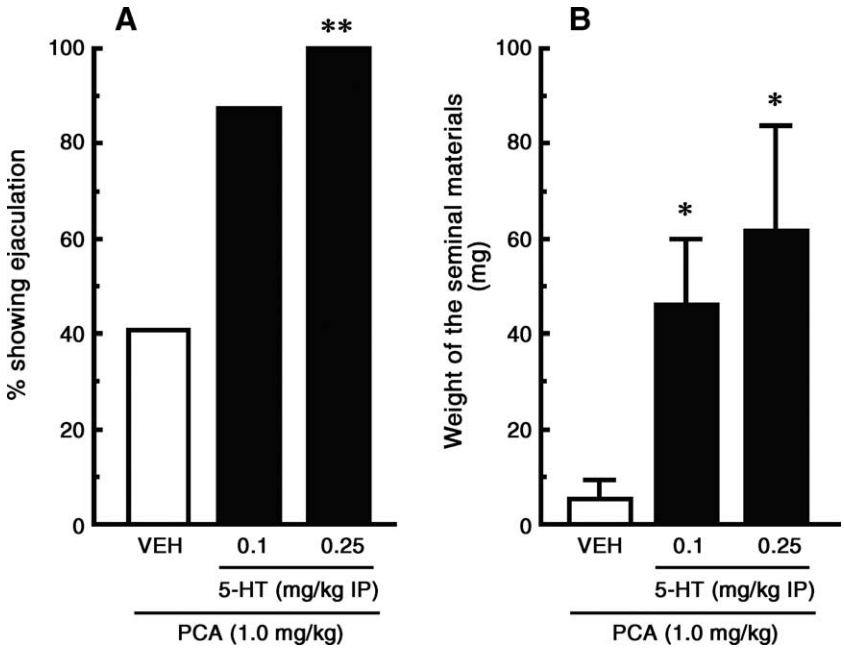

Fig. 3. Effect of peripherally administered 5-HT on the incidence of ejaculation (A) and the weight of seminal material (B) induced by a threshold dose of PCA in rats. 5-HT was administered i.p. 10 min after PCA (1.0 mg/kg, i.p.). Results of the weight of seminal material are shown as mean ± S.E.M. for 8–10 animals. The symbol indicates a significant difference (*$p<0.05$, **$p<0.01$) from vehicle (VEH)-treated animals.

10 min after PCA, dose-dependently increased both the number of ejaculated rats (Fig. 3A) and the amount of ejaculate (Fig. 3B) in comparison with PCA alone. No potentiation was observed in combination of the higher dose (5.0 mg/kg, i.p.) of PCA and 5-HT (0.25 mg/kg, i.p.) (data not shown). 5-HT (0.1 and 0.25 mg/kg, i.p.) alone did not induce the ejaculatory response in rats.

4. Discussion

Here, we present for the first time an involvement of peripheral 5-HT in PCA-induced ejaculatory response of rats. A potent proejaculatory effect (all animals ejaculated a large amount of seminal material) was observed at doses over 2.5 mg/kg of PCA (Fig. 1A), in accordance with previous reports (Humphries et al., 1981; Renyi 1985). Our previous study in anesthetized rats showed that treatment with the same doses of PCA can induce a marked ejaculatory response (e.g., intermittent ejaculations) and the response lasted for a relatively long period (Yonezawa et al., 2000). A prominent proejaculatory effect of PCA under either conscious or anesthetized state may be useful for studying the physiological mechanisms underlying sexual function in male rats.

It is well documented that systemic administration of PCA causes an acute increase in the activity of serotonergic system through a mechanism involving the release of 5-HT from nerve terminals and/or other 5-HT-containing cells (Fuller, 1992; Holzwarth et al., 1984). The results obtained in the present study also indicate that the 5-HT-releasing property of PCA mainly involved the expression of ejaculation, as well as inducing behavioral and neuroendocrine response (Hutson and Curzon, 1989; Van de Kar and Bethea, 1982). This is evidenced by the findings that the ejaculatory response induced by PCA is abolished by pretreatment with the 5-HT synthesis inhibitor *p*-CPA, or by pretreatment with the 5-HT receptor antagonists methysergide ($5\text{-HT}_{1/2}$ receptor antagonist) and MDL-72222 (5-HT_3 receptor antagonist). Furthermore, the selective 5-HT reuptake inhibitor citalopram completely blocked the proejaculatory effect of PCA. These results suggest that PCA may be taken up through the 5-HT transporter into 5-HT nerve terminals and/or other 5-HT-containing cells, which in turn produces the release of 5-HT and results in expression of ejaculation. This hypothesis is supported by our recent finding that administration of fenfluramine, the 5-HT-releasing compound, can also induce the ejaculatory response in rats, which is abolished by the above pharmacological manipulation (Yonezawa et al., 2004). The lack of effect of nomifensin, a catecholamine reuptake inhibitor, also confirms the importance of the 5-HT system in the PCA-induced ejaculatory response.

It is well established that ejaculation is a reflex that consists of two successive motor phases, emission (secretion of the mixed fluids composing semen into the posterior urethra) and ejection (expulsion of semen from the urethra) (Kimura, 1972; Bohlen et al., 2000). The lumbosacral spinal cord has been shown to be the important site for achieving emission and ejection phases in rats (Coolen et al., 2004). Truitt and Coolen (2002) have recently demonstrated that the spinothalamic cells located in laminae VII and X in lumber segments 3 and 4 may play an important role in generation of ejaculatory response in rats. In the present study, the transection of the spinal cord above the lumbosacral level did not affect the ejaculatory response induced by PCA, in accordance with the previous result of acute transection in anesthetized rats (Yonezawa et al., 2000). On the other hand, i.t. injection of lidocaine at the lumbosacral level completely inhibited the proejaculatory effect of PCA, and this inhibition was partially restored until

60 min after i.t. lidocaine. These results, taken together, suggest that the major site of action of PCA-induced ejaculatory response may be localized to the lumbosacral spinal cord level in rats.

Pharmacological studies have indicated that the spinal ejaculatory neural system is under descending inhibitory and excitatory control from the supraspinal sites. The descending serotonergic system in the lumbosacral spinal cord can mediate both facilitatory and inhibitory effects on the expression of ejaculation (Marson and McKenna, 1992; Mas et al., 1985). In the present study, the lumbosacral spinal serotonergic denervation by i.t. injection of a neurotoxin 5,7-DHT did not affect the ejaculatory response induced by PCA. Indeed, in spite of a marked decrease of 5-HT content (>90%) in the lumbosacral spinal cord by 5,7-DHT pretreatment, both the incidence of ejaculation and the amount of seminal material were not changed as compared to those in vehicle-treated rats. This result suggests that the PCA-induced ejaculatory response is not affected by the supraspinal descending serotonergic projection. Furthermore, i.t. injected PCA did not produce systemic PCA-like prominent effects on ejaculation; there was a slight incidence of the ejaculatory response even at a high dose (160 μg), but no incidence at a lower dose (20 μg). Previous experiments in anesthetized rats showed that i.t. injection of PCA induces a release of spinal 5-HT (Hammond et al., 1983) and that iontophoretically administered PCA may release 5-HT from spinal terminals (Neuman and White, 1982). These results, taken together, suggest that when PCA is injected systemically, the release of 5-HT involving the expression of ejaculation may occur mainly at the peripheral sites rather than the spinal level.

This proposal is supported by the present findings that the combination of a small dose (0.1 and 0.25 mg/kg, i.p.) of 5-HT and a threshold dose of PCA (1.0 mg/kg) obviously potentiated the ejaculatory response in rats. It is well known that systemically administered 5-HT cannot cross the blood–brain barrier, suggesting the mechanism of potentiating effect is limited to the peripheral sites. It has been shown that PCA induces a significant increase in plasma 5-HT level (Martin and Artigas, 1992), which is derived from peripheral organs, and that male reproductive organs contain relatively high concentration of 5-HT (Di Sant'Agnese et al., 1987; Iwanaga et al., 1987; Hanyu et al., 1987). McKenna et al. (1991a,b) have demonstrated that intraurethral application of 5-HT produces a significant decrease in the threshold for urethro-genital reflex in female rats, which closely resembled the reflex in male rats (Chung et al., 1988; McKenna et al., 1991a,b). Thus, it is speculated that, in certain conditions, peripherally released 5-HT may play a role in the control of ejaculatory function. A number of reports have documented that 5-HT produces a rapid depolarization in many peripheral afferent fibers, especially visceral afferent fibers, and these effects are mediated by 5-HT_2 and/or 5-HT_3 receptors (Cohen, 1992; Tokunaga et al., 1998; Fu and Longhurst, 1998). These results indicate the possibility that the release of 5-HT, which involves the ejaculatory response induced by PCA, may be generated in the peripheral sites, including reproductive organs. However, the direct action of PCA for the spinal ejaculatory neural system is also necessary, because none of the animals was induced the ejaculatory response by 5-HT itself.

The result of this study may provide useful information as to the effects of selective 5-HT reuptake inhibitors (SSRIs) for the ejaculatory function in men. SSRIs have been used for the treatment of premature ejaculation. It is considered that the increase in synaptic 5-HT levels in the CNS may be related to ejaculation-delaying effect of SSRIs, but the ability in this effect of SSRIs has been shown to be greatly different from each other (Waldinger et al., 1998). Although this reason has not been clarified, it can be speculated from our present study that peripherally increased 5-HT may interfere with ejaculation-delaying effect of SSRIs. Further study is necessary for this consideration.

In conclusion, the results of the present study demonstrate that the major sites of action of PCA-induced ejaculatory response are localized to both the lumbosacral spinal cord and peripheral regions. The systemically administered PCA may induce the 5-HT release in peripheral sites, which subsequently triggers to activate the lumbosacral ejaculation center, and may produce the expulsion of seminal materials. The direct activation of spinal ejaculatory center induced by PCA may also involve the expression of the response.

References

Ahlenius S, Larsson K. Synergistic actions of the 5-HT1A receptor antagonist WAY-100635 and citalopram on male rat ejaculatory behavior. Eur J Pharmacol 1999;379;1–6.

Ando R, Watanabe C, Kawamura S, Yonezawa A, Sakurada T. Involvement of spinal NK2 and NMDA receptors in aversive behavior induced by intra-arterial injection of capsaicin. Pharmacol Biochem Behav 2001;68;105–13.

Bohlen D, Hugonnet CL, Mills RD, Weise ES, Schmid HP. Five meters of H_2O: the pressure at the urinary bladder neck during human ejaculation. Prostate 2000;44;339–41.

Chung SK, McVary KT, McKenna KE. Sexual reflexes in male and female rats. Neurosci Lett 1988;94;343–8.

Cohen ML. 5-HT_3 receptors in the periphery. In: Hamon M, editor. Central and peripheral 5-HT3 receptors. London: Academic Press; 1992. p. 19–32.

Coolen LM, Allard J, Truitt WA, McKenna KE. Central regulation of ejaculation. Physiol Behav 2004;83;203–15.

Di Sant'Agnese PA, Davis NS, Chen M, de Mesy Jensen KL. Age-related changes in the neuroendocrine (endocrine-paracrine) cell population and the serotonin content of the guinea pig prostate. Lab Invest 1987;57;729–36.

Foreman MM, Hall JL, Love RL. Effects of fenfluramine and *para*-chloroamphetamine on sexual behavior of male rats. Psychopharmacology 1992;107;327–30.

Fu LW, Longhurst JC. Role of 5-HT3 receptors in activation of abdominal sympathetic C fibre afferents during ischaemia in cats. J Physiol 1998;509;729–40.

Fuller RW. Effects of *p*-chloroamphetamine on brain serotonin neurons. Neurochem Res 1992;17;449–56.

Hammond DL, Tyce GM, Yaksh TL. Drug-induced alterations in the efflux of 5-hydroxytryptamine and of 5-hydroxyindoleacetic acid into superfusates of the rat spinal cord. Eur J Pharmacol 1983;87;441–8.

Hanyu S, Iwanaga T, Kano K, Fujita T. Distribution of serotonin-immunoreactive paraneurons in the lower urinary tract of dogs. Am J Anat 1987;180;349–56.

Holzwarth MA, Sawetawan C, Brownfield MS. Serotonin-immunoreactivity in the adrenal medulla: distribution and response to pharmacological manipulation. Brain Res Bull 1984;13;299–308.

Humphries CR, Paxinos G, O'Brien M. Mechanisms of PCA-induced hypothermia, ejaculation, salivation and irritability in rats. Pharmacol Biochem Behav 1981;15;197–200.

Hutson PH, Curzon G. Concurrent determination of effects of *p*-chloroamphetamine on central extracellular 5-hydroxytryptamine concentration and behavior. Br J Pharmacol 1989;96;801–6.

Iwanaga T, Hanyu S, Fujita T. Serotonin-immunoreactive cells of peculiar shape in the urethral epithelium of the human penis. Cell Tissue Res 1987;249;51–6.

Kimura Y. Posterior urethrogram as a method to study ejaculation. Tohoku J Exp Med 1972;106(1);89–91.

Maeda N, Matsuoka N, Yamaguchi I. Possible involvement of the septo-hippocampal cholinergic and raphe-hippocampal serotonergic activations in the penile erection induced by fenfluramine in rats. Brain Res 1994;652; 181–9.

Marson L, McKenna KE. A role for 5-hydroxytryptamine in descending inhibition of spinal sexual reflexes. Exp Brain Res 1992;88;313–20.

Martin F, Artigas F. Simultaneous effects of *p*-chloroamphetamine, d-fenfluramine, and reserpine on free and stored 5-hydroxytryptamine in brain and blood. J Neurochem 1992;59;1138–44.

Mas M, Zahradnik MA, Martino V, Davidson JM. Stimulation of spinal serotonergic receptors facilitates seminal emission and suppresses penile erectile reflexes. Brain Res 1985;342;128–34.

McKenna KE, Knight KC, Mayers R. Modulation by peripheral serotonin of the threshold for sexual reflexes in female rats. Pharmacol Biochem Behav 1991;40;151–6.

McKenna KE, Chung SK, McVary KT. A model for the study of sexual function in anesthetized male and female rats. Am J Physiol 1991;261; R1276–8.

Mos J, Mollet I, Tolboom JT, Waldinger MD, Olivier B. A comparison of the effects of different serotonin reuptake blockers on sexual behavior of the male rat. Eur Neuropsychopharmacol 1999;9;123–35.

Murai S, Saito H, Masuda Y, Itoh T. Rapid determination of norepinephrine, dopamine, serotonin, their precursor amino acids, and related metabolites in discrete brain areas of mice within ten minutes by HPLC with electrochemical detection. J Neurochem 1988;50;473–9.

Neuman RS, White SR. Serotonin-like actions of quipazine and CPP on spinal motoneurones. Eur J Pharmacol 1982;81;49–56.

Ogren SO. Serotonin receptor involvement in the avoidance learning deficit caused by *p*-chloroamphetamine-induced serotonin release. Acta Physiol Scand 1986;126;449–62.

Renyi L. Ejaculations induced by *p*-chloroamphetamine in the rat. Neuropharmacology 1985;24;697–704.

Rudnick G, Wall SC. *p*-Chloroamphetamine induces serotonin release through serotonin transporters. Biochemistry 1992;31;6710–8.

Tokunaga A, Saika M, Senba E. 5-HT2A receptor subtype is involved in the thermal hyperalgesic mechanism of serotonin in the periphery. Pain 1998;76;349–55.

Truitt WA, Coolen LM. Identification of a potential ejaculation generator in the spinal cord. Science 2002;297;1566–9.

Trulson ME, Jacobs BL. Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. Eur J Pharmacol 1976;36;149–54.

Van de Kar LD, Bethea CL. Pharmacological evidence that serotonergic stimulation of prolactin secretion is mediated via the dorsal raphe nucleus. Neuroendocrinology 1982;35;225–30.

Waldinger MD, Hengeveld MW, Zwinderman AH, Olivier B. Effect of SSRI antidepressants on ejaculation: a double-blind, randomized, placebo-controlled study with fluoxetine, fluvoxamine, paroxetine, and sertraline. J Clin Psychopharmacol 1998;18;274–82.

Yonezawa A, Watanabe C, Ando R, Furuta S, Sakurada S, Yoshimura H, et al. Characterization of *p*-chloroamphetamine-induced penile erection and ejaculation in anesthetized rats. Life Sci 2000;67;3031–9.

Yonezawa A, Yoshizumi M, Ebiko M, Kimura Y, Sakurada S. Penile erection and ejaculation induced by the serotonin releasers in male rats. J Tohoku Pharmaceutical University 2004;51;133–41.