Role of the Dopaminergic, Serotonergic and Cholinergic Link in the Expression of Penile Erection in Rats

Noriaki Maeda, Nobuya Matsuoka* and Isamu Yamaguchi
Basic Research Group, Tsukuba Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5–2–3, Tokodai, Tsukuba, Ibaraki 300-26, Japan

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ABSTRACT—The neural mechanisms underlying the penile erection induced by serotonergic, cholinergic and dopaminergic stimulants were comparatively investigated. Fenfluramine (0.1–10 mg/kg, i.p.), pilocarpine (0.032–3.2 mg/kg) and apomorphine (0.01–1 mg/kg) induced penile erection in rats with bell-shaped dose-response curves. The penile erection induced by fenfluramine (1 mg/kg) was dose-dependently antagonized by pindolol (0.1–3.2 mg/kg), a 5-HT₁ antagonist, or scopolamine (0.032–1 mg/kg), a muscarinic antagonist, but not by sulpiride (1–32 mg/kg), a dopaminergic antagonist. The penile erection induced by pilocarpine (0.32 mg/kg) was countered by pindolol or scopolamine but not by sulpiride, while that induced by apomorphine (0.032 mg/kg) was countered by all three antagonists. Septo-hippocampal cholinergic deafferentations by medial septum lesioning or fimbria-fornix transection also significantly attenuated the penile erection induced by fenfluramine or apomorphine, but scarcely affected that induced by pilocarpine. Raphe lesion by injections of 5,7-dihydroxytryptamine, a serotonergic neurotoxin, into the median- and dorsal-raphe nuclei significantly attenuated the penile erections induced by fenfluramine and apomorphine but not that by pilocarpine. These results suggest that a neuronal link between the dopaminergic, serotonergic and cholinergic systems plays a crucial role in the expression of penile erection; dopaminergic stimulation causes an activation of the raphe serotonergic neurons which in turn enhances the septo-hippocampal cholinergic pathway and results in expression of penile erection.

Keywords: Penile erection, Cholinergic, Serotonergic, Dopaminergic, Septo-hippocampal pathway

Electrical stimulation of various brain areas has demonstrated that the hippocampus and its afferents play a crucial role in expressing penile erection (1). The brain area is densely innervated by cholinergic nerve fibers derived from cells in the medial septum (MS) (2, 3), suggesting the view that the cholinergic nerve activity in the hippocampus functions as an important signal in the expression of penile erection.

On the other hand, the hippocampal formation receives dense serotonergic projections from the median and dorsal midbrain raphe nuclei (4–6). Previous pharmacological studies have shown that serotonergic stimulants such as 5-HT reuptake inhibitors or 5-HT₁B receptor agonists induce penile erection in rats (7, 8) and in primates (9), an effect that is attenuated by drugs with 5-HT₁ antagonist activity such as propranolol or pindolol (8). It is thus speculated that an activation of central specific 5-HT₁ receptor subtype(s) is involved in the penile erections (10).

Both direct and indirect dopaminergic stimulants also induce penile erection in male rats (11, 12), an effect which is attenuated by dopaminergic as well as cholinergic antagonists (11–13). It appears that dopaminergic nerves in the upstream of the septo-hippocampal cholinergic nerves regulate the expression of penile erection.

These findings prompted us to investigate the role of interactions between the serotonergic, cholinergic and dopaminergic systems that might underlie the mechanism of penile erection. In the present paper, we examined the effects of neuronal blockade by drugs or by surgical deafferentations of the hippocampal serotonergic and cholinergic systems on penile erection in rats. Possible interplay between the limbic serotonergic, cholinergic and dopaminergic pathways in relation to penile erection are discussed.

* To whom correspondence should be addressed.
MATERIALS AND METHODS

Animals

Male Fischer-344 rats at the age of 9 weeks were purchased from Charles River, Inc. (Atsugi) at least one week before the experiments. All animals were housed 6 to a stainless mesh cage (28 x 38 x 17 cm) in a temperature controlled room (22 ± 1°C) under a 12:12 light/dark cycle with lights on at 8:00, and given food and water ad libitum. Experiments were carried out between 13:30 and 19:00 in the room where animals were housed.

Behavioral procedures

All animals were handled 3 min a day for three successive days before the behavioral tests. The rats were tested in groups of six, and various doses of fenfluramine, apomorphine and pilocarpine were given in semi-randomized order. Immediately after the drug injection, each rat was placed in a Perspex box (25 x 25 x 35 cm), and its behavior was observed for 60 min, during which time the number of penile erections was counted. A mirror was situated behind each box to facilitate observation of the animal. A penile erection was defined as repeated pelvic thrusts immediately followed by an upright position presenting an emerging, engorged penis which the rat proceeds to lick. Antagonists were injected 15 min before the behavioral observation.

Lesioned and sham-operated rats were assigned to receive all doses throughout a series of test sessions. Briefly, each group of six rats was administered with vehicle or doses of a drug in the first session. In the next session, each rat received a dose of the drug in ascending order, and those which had received the largest dose were given the vehicle. In this way, the test sessions were repeated the same number of times as the total number of vehicle and drug doses. At least 3 days intervened between each session, and a series of tests was finished within 4 weeks at the longest.

Surgical procedures

The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed on a stereotaxic apparatus with the incisor bar set 2.3 mm below the intra-aural line. In all lesions, stereotaxic coordinates were positioned according to the atlas of König and Klippel (14). In each lesion, the body temperature of the rats was maintained at 37°C until they recovered from anesthesia after the operations. At the end of the experiments, the placement and

Fig. 1. Schematic drawings of the position of medial-septum (MS), fimbria-fornix (FF), median-raphe and dorsal-raphe lesions in a coronal section approximately at the level of 0.8 mm anterior, 2.0 mm posterior, 8.3 mm posterior and 8.7 mm posterior from the bregma, respectively, according to the atlas of König and Klippel (ref. 14). The stippled areas indicate the representative extent of the lesion.
Brain Mechanisms of Penile Erection

Effects of pindolol on drug-induced penile erection

The effects of pindolol on the penile erections produced by fenfluramine, pilocarpine and apomorphine were investigated (Fig. 2). Fenfluramine, pilocarpine and apomorphine at the dosages of 1 mg/kg, 0.32 mg/kg and 0.032 mg/kg were used as the respective optimal dosages of each drug to induce the maximal response. Doses of pindolol (0.1–3.2 mg/kg), a 5-HT1 antagonist, attenuated the penile erections induced by fenfluramine (1 mg/kg), pilocarpine (0.32 mg/kg) and apomorphine (0.032 mg/kg) in a dose-dependent manner, and its activity was almost the same irrespective of the stimulants used. ED50 values were around 0.2–0.4 mg/kg.

Effects of scopolamine on drug-induced penile erection in rats

The effects of scopolamine on the penile erections produced by fenfluramine, pilocarpine and apomorphine were investigated (Fig. 3). Cholinergic blockade by scopolamine (0.032–1 mg/kg) also produced a dose-dependent inhibition of the response not only by pilocarpine but also those by fenfluramine and apomorphine, as shown in Fig. 3. In each experiment, the rats receiving larger dosages than 0.32 mg/kg of scopolamine showed statistically significant changes (P < 0.05 by the Mann Whitney U-test) when compared with the control (stimulant alone-treated) group. The effectiveness of scopolamine to block the responses was comparable with that of pindolol.

Effects of sulpiride on drug-induced penile erection in rats

The effects of sulpiride on the penile erections produced by fenfluramine, pilocarpine and apomorphine

size of the lesions were checked by histological examination on 10-µm-thick slices subjected to Nissl staining. The location and size of each lesion are shown schematically in Fig. 1.

Medial-septum (MS) or fimbria-forrnix (FF) lesion: The procedures for lesioning of the septo-hippocampal pathway used in the present study were similar to those used in the previous study (15). In brief, for MS lesion, an electrode (0.7 mm in diameter and insulated except for the top 0.2 mm) was placed at 0.8 mm anterior from the bregma on the midline and 7.0 mm ventral from the surface of the skull. A radiofrequency current was delivered from a lesion generator (Radionics, Burlington, MA, USA; model RFG-4A) for 60 sec through the electrode. The temperature was set at 60°C. Sham operation was done in the same way except for delivery of the radiofrequency current. For FF lesion, a microsurgery knife (Feather, No. 715) mounted on a stereotaxic apparatus was positioned at 4.0 mm lateral to the midline, 2.0 mm posterior to the bregma and lowered 5.0 mm below the surface of the skull. The FF was transected bilaterally by driving the microknife 8.0 mm laterally and retrieved at this position. For the sham lesions, only the sagittal sinus was transected. The behavioral test was started 7 days after each operation.

Dorsal- and median-raphe lesion: Specific lesions of serotonergic neurons were made by stereotactically placed local injections of 5,7-dihydroxytryptamine (5,7-DHT, in 1 μl of 0.2% ascorbic acid-saline solution, 0.2 μl/min) through a 26-gauge cannula connected by polyethylene tubing to a gear-driven Hamilton syringe. Sham animals were injected with the vehicle. Injections into the median raphe nucleus were made at the coordinates of 8.3 mm posterior to the bregma, on the midline, 8.0 mm ventral from the skull surface; injections into the dorsal raphe nucleus were made at the coordinates of 8.7 mm posterior to the bregma, on the midline, 6.3 mm ventral from the skull surface. Both nuclei were injected with 2 μl of 5,7-DHT in a volume of 1 μl, and the rats were pretreated with desipramine (25 mg/kg, i.p.) 30–45 min prior to the local injections in order to protect noradrenergic neurons from the lesion (16). The behavioral test was started 14 days after the operation.

Drugs

The following drugs were used: (±)-fenfluramine hydrochloride and (±)-sulpiride hydrochloride (synthesised in our research laboratories); pilocarpine hydrochloride, apomorphine hydrochloride, (±)-pindolol hydrochloride and 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT) (Sigma Chemical Co., St. Louis, MO, USA); scopolamine hydrobromide and methyl-scopolamine hydrobromide (Nacalai Tesque, Kyoto). Pindolol and sulpiride were dissolved in 1% tartrate-saline with the pH adjusted to 6.0–7.0. 5,7-DHT was dissolved in sterile saline containing 0.2% ascorbic acid immediately before use and injected into the raphe nuclei. All other drugs were dissolved in physiological saline and given intraperitoneally in a volume of 1 ml/kg. Control animals were injected with an equivalent volume of vehicle. Antagonists and agonists were injected at 15 min before and just before the start of the behavioral experiments, respectively. The dosages of antagonists were selected based on our previous observations (15).

Statistics

All results are expressed as means±S.E.M. In the behavioral experiments, the statistical significance was estimated with the Mann-Whitney U-test by considering a P-value less than 0.05 as statistically significant.

RESULTS

Effects of sulpiride on drug-induced penile erection in rats

The effects of sulpiride on the penile erections produced by fenfluramine, pilocarpine and apomorphine were investigated. Pindolol and scopolamine to block the responses was comparable with that of pindolol.
were investigated (Fig. 4). Sulpiride (1–32 mg/kg), a dopamine D₂ blocker, failed to affect the penile erection induced by fenfluramine or pilocarpine, but moderately reduced the number of responses induced by apomorphine.

Effects of septo-hippocampal cholinergic denervation on drug-induced penile erections

The effects of the three stimulants on penile erections in MS-lesioned rats are shown in Fig. 5. Fenfluramine (0.1–10 mg/kg) increased the number of penile erections with a bell-shaped dose-response curve, with statistically significant (P<0.01) responses occurring at the doses of 0.32 and 1 mg/kg, and the maximum response was obtained at 1 mg/kg. The penile erections induced by the drug were fewer in the MS-lesioned rats compared with the sham rats, and the difference was statistically significant (P<0.05) at the dose of 1 mg/kg, as shown in Fig. 5A. On the other hand, pilocarpine (0.032–3.2 mg/kg) facilitated the penile erection with a bell-shaped dose response in both groups with or without the MS lesion. Apomorphine (0.01–1 mg/kg) increased the number of penile erections with a bell-shaped dose-response curve with statistically significance (P<0.01) at 0.032–0.32 mg/kg of the drug in sham rats, while the drug hardly affected the response in MS-lesioned rats (Fig. 5C).

Also in the FF-lesioned rats, the maximum response at 1 mg/kg of fenfluramine was significantly (P<0.01) reduced compared with that in sham rats, and the change was more pronounced than in the MS-lesioned rats (Fig.
6A). On the other hand, pilocarpine (0.032 – 3.2 mg/kg) caused comparable penile erections to the sham and FF-lesioned rats with statistically significant (P < 0.05) effects at 0.32 and 1 mg/kg (Fig. 6B), whereas apomorphine (0.01 – 1 mg/kg) significantly induced penile erection in the saline-pretreated rats but not in the FF-lesioned rats (Fig. 6C).

Effects of lesioning of the raphe nuclei on drug-induced penile erection

Figure 7 shows the effect of raphe lesion on penile erection induced by fenfluramine, pilocarpine or apomorphine. The doses of 0.32 (P < 0.05) and 1 mg/kg (P < 0.01) of fenfluramine gave statistically significant responses in the sham rats. In the raphe lesioned rats, significant erections were still observed at the dose of 1 mg/kg of the drug; however, the maximum response was significantly (P < 0.01) attenuated compared to that in the sham-operated rats. The lesion hardly affected the responses induced by pilocarpine, and the dose-response relationships were comparable between the lesioned and sham-operated rats (Fig. 7B). On the other hand, penile erections induced by apomorphine were completely abolished by the lesion (Fig. 7C). The difference between the lesioned and sham rats was statistically significant (P < 0.01) at 0.032 mg/kg of apomorphine.
DISCUSSION

The present study confirmed the earlier observations (7, 11–13) that fenfluramine, pilocarpine and apomorphine induced penile erection in naive rats. In addition, we demonstrated here that the penile erection induced by the drugs was dose-dependently attenuated by scopolamine. These results suggest that acetylcholine is the common mediator of penile erection induced by serotonergic and dopaminergic activations. We further provided evidence for the central locus of the cholinergic mechanism regulating the expression of penile erection. Septo-hippocampal deafferentations not only by MS but also by FF-lesions significantly attenuated the penile erection induced by fenfluramine and apomorphine with minimal effect on that induced by pilocarpine. It has been widely accepted that the cells in the MS and the diagonal band of Broca (DB) send cholinergic nerve fibers to the hippocampus through the FF (2, 3), and their activities play an important role in the regulation of penile erection (1). Indeed, we have previously shown that the MS lesion produced 50% reduction in the hippocampal choline...
acetyltransferase (ChAT) activity, whereas FF lesion almost completely depleted the activity (17). These results favor the view that fenfluramine and apomorphine indirectly activate the septo-hippocampal cholinergic neurons to induce penile erection. Because the penile erection by pilocarpine was hardly affected by the septo-hippocampal lesions, it is considered that pilocarpine produced the response as a consequence of the direct stimulation of post-synaptic muscarinic receptors existing in the hippocampus. These results taken together suggest that the septo-hippocampal cholinergic system exerts a powerful modulatory role in the expression of penile erection and that the cholinergic mechanism is downstream to the serotonergic or dopaminergic mechanisms.

The fenfluramine-induced penile erection was also attenuated by pindolol, a β-receptor antagonist with 5-HT₁ blocking activity (18) but not by metoprolol, a β-receptor antagonist without 5-HT₁ blocking activity (19, 20). It has been postulated that central 5-HT₁B and/or 5-HT₂C (5-HT₁C in previous nomenclature)-receptor activities play a crucial role in the expression of penile erection (8, 10), and that fenfluramine enhances 5-HT release and reduces 5-HT re-uptake (21, 22). These observations taken together suggest that an indirect activation of 5-HT₁B and/or 5-HT₂C receptors is involved in the penile erection induced by fenfluramine. The view was strengthened by the present observation that the raphe lesion made by injection of 5,7-DHT into the raphe nuclei, which specifically depleted 5-HT and its major metabolite 5-HIAA in the brain (20), attenuated the penile erection induced by fenfluramine. Nerve terminals derived from the median- and dorsal-raphe nuclei are widely distributed in the whole brain areas such as the cerebral cortex, septum and hippocampus (4, 5), and the serotonergic raphe-hippocampal pathway is postulated to exert a powerful influence on the neuronal activity of the hippocampus (23–25). In fact, direct application of 5-HT to the hippocampus increased hippocampal ACh release determined by microdialysis (26), and systemic administration of p-chloroamphetamine, a 5-HT releaser, increased hippocampal ACh release (27). In addition, recent electrophysiological studies have demonstrated that the septo-hippocampal pathway is negatively regulated by hippocampal GABAergic interneurons (28), and that the serotonergic raphe-hippocampal pathway modulates the septo-hippocampal excitability by disinhibiting these inhibitory interneurons (29, 30). We thus speculate that the raphe-serotonergic mechanism is upstream to the septo-hippocampal cholinergic mechanism for the expression of penile erection. Although the speculation is in line with the present result that raphe-lesioning hardly affected the pilocarpine-induced penile erection, it is contradicted by the result that the response to pilocarpine was inhibited by pindolol. One of the possible explanations could be that a serotonergic system located downstream to the hippocampus also takes a part in the regulation of penile erection. In fact, it has been suggested that the subspinal descending serotonergic system could also be involved in sexual behavior (31). In this respect, it is interesting to note that the present raphe-lesion failed to affect the 5-HT content in the spinal cord (data not shown). There is also a possibility that serotonergic control on the cavernous tone of the penis might participate in the drug-induced penile erection (32).

Another important finding in the present study was that lesions of the raphe nuclei as well as pindolol treatment totally canceled the penile erection induced by apomorphine. Furthermore, the apomorphine-induced penile erection was significantly attenuated by a dose (32 mg/kg) of sulpiride which hardly affected the fenfluramine- and pilocarpine-induced penile erection. From these results, it is suggested that dopaminergic neurons located upstream to the raphe-hippocampal serotonergic neurons regulate the penile erection. This view may parallel recent neurochemical evidence by Ferre and Artigas who found that apomorphine increased 5-HT release from the rat raphe nuclei (33). In addition, there is evidence that dopaminergic cell bodies in the ventral tegmental area (A10) and substantia nigra (A9) send projections to the raphe nuclei which is believed to control serotonergic neural activity in the nuclei (34). This evidence combined with the above discussions strongly suggest that dopaminergic stimulation by apomorphine causes raphe-hippocampal serotonergic activation which in turn activates the septo-hippocampal cholinergic pathway to cause penile erection. An alternative explanation of apomorphine’s action is that the drug could directly activate the septo-hippocampal cholinergic pathway as well as indirectly activate the paths via the raphe stimulation. In vivo microdialysis studies of Nilsson et al. (27) revealed that systemic administration of apomorphine increases hippocampal ACh release, which is specifically blocked by a 6-hydroxydopamine lesion of the A10. The septo-hippocampal cholinergic pathway is known to be under the control of the dopaminergic nerve fibers derived from the cell bodies in the A10 (3, 35). Therefore, A10 dopaminergic neurons might play a positive modulatory role in the septo-hippocampal cholinergic pathway in the drug induced penile erection in rats. Taken together, it is assumed that dopaminergic stimulation comes down to hippocampal serotonergic and cholinergic activation finally to cause penile erection.

In conclusion, the present results provided novel findings regarding the limbic ACh-5-HT-dopamine interplay that exerts an important role in drug-induced penile erection in rats.
REFERENCES


