Involvement of Raphe-Hippocampal Serotonergic and Septo-Hippocampal Cholinergic Mechanisms in the Penile Erection Induced by FR121196, a Putative Cognitive Enhancer

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ABSTRACT—FR121196 (*N*-[4-acetyl-1-piperazinyl]-4-fluorobenzenesulfonamide), a putative cognitive enhancer, induced penile erection in naive rats; the dose-response curve was bell-shaped with the maximum response obtained at the dose of 3.2 mg/kg. The response to FR121196 was abolished in rats treated with intra-raphe injections of 5,7-dihydroxytryptamine or systemic injections of *p*-chlorphenylalanine (150 mg/kg, i.p. for three consecutive days) as well as in rats with electrolytic medial-septum lesion or surgical fimbria-fornix lesion. In addition, the penile erection induced by FR121196 (3.2 mg/kg) was dose-dependently attenuated by pindolol (0.1-3.2 mg/kg), a serotonin $(5\text{-HT})_1$ antagonist with β-antagonistic activity, but not by metoprolol, a selective β-antagonist. The inhibitory activity was shared by ICS205-930, a 5-HT_3 antagonist, but not by ketanserin, a 5-HT_2 antagonist, or sulpiride, a dopamine D_2 antagonist. Scopolamine (0.032-1 mg/kg), but not methyl-scopolamine (0.032-1 mg/kg), also attenuated the penile erection induced by FR121196. Neurochemical analysis revealed that intraperitoneal injection of FR121196 significantly elevated the levels of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus and that raphe-lesion significantly reduced both 5-HT and 5-HIAA levels without affecting choline-acetyltransferase activity in all cortical and subcortical regions examined. It is thus postulated that FR121196 facilitates the raphe-hippocampal serotonergic pathway resulting in an activation of the septo-hippocampal cholinergic pathway and finally induces the penile erectile response.

Keywords: FR121196, Penile erection, Septo-hippocampal pathway, Median- and dorsal-raphe nuclei

FR121196, a newly introduced putative cognitive enhancer, ameliorated memory deficit in various rat models of dementia, possibly through its action on the hippocampus (1) that plays a crucial role in memory formation (2-4). Also, the drug facilitated long term potentiation (LTP) of the mössy fiber-CA3 system of the guinea pig hippocampal slice preparation in a manner dependent on intact cholinergic as well as somatostatinergic neuronal activities (5). Apart from the relevancy between the LTP phenomena and memory (6), which is still to be determined, FR121196 is undoubtedly a potential hippocampal activator.

FR121196 scarcely induced any behavioral changes in naive rats except for penile erection, and its effective dose as well as the dose-response curve was comparable to those for memory enhancement. Interestingly, a series of electrophysiological studies by MacLean (7) has shown that the hippocampus and its afferent are important for the expression of penile erection. The hippocampal formation is densely innervated by cholinergic nerve fibers derived from cells in the medial septum (8, 9) as well as serotonergic projections from the median and dorsal midbrain raphe nuclei (10, 11). On the other hand, cholinergic activation also induces apart from penile erections (12) yawning behavior, which is one of self grooming behaviors in rats (13, 14). Dopaminergic activation also induces yawning and penile erections (14). Activation of the serotonergic system, however, only induces penile erections (15). We thus propose that an activation of serotonergic afferents to the hippocampus may be involved in the penile erection induced by FR121196.

The present study was designed to assess the possible neuronal mechanisms underlying the penile erection induced by FR121196.

MATERIALS AND METHODS

Animals

Male Fischer 344 rats at the age of 9 weeks were purchased from Charles River Inc. (Atsugi) and housed six per cage (25.5 × 38 × 17 cm) in a temperature controlled room (22 ± 1℃) under a 12 : 12 light/dark cycle with lights on at 8:00. Food and water were provided ad libitum. One week later, the behavioral tests were carried out between 13:30 and 19:00 in the same room where the rats were housed. All animals were handled 3 min a day for 3 successive days before the behavioral tests. The rats were tested in groups of six, and various doses of drugs were given in semi-randomized order. Immediately after the drug injection, each rat was placed in a perspex box (17 × 27 × 13 cm) and its behavior was observed for 60 min, during which time the number of penile erections was counted. 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. Incidence of the response was counted and expressed as the number of penile erections per hour. A mirror was situated behind each box to facilitate observation of the animal.

Lesioned rats

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 the vehicle or doses of a drug in the first session. In the following sessions, each of them received a dose of the drug in ascending order, and the rats that 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. An interval of at least 3 days was provided between each session, and the series of tests was finished within 4 weeks at the longest.

Neurosurgeries

Medial-septum (MS) lesion: Handled rats were assigned to two groups: medial-septum-lesioned and sham-operated. The rats were anesthetized with pentobarbital (50 mg/kg, i.p.), placed in a stereotaxic apparatus and lesioned on the medial-septum by electrocoagulation with a radiofrequency generator (RGF-4A; Muromachi Kikai Co., Tokyo). The coordinates of the electrode placement were: 0.8 mm anterior to the bregma, on the midline, and 7.0 mm ventral from the skull surface according to the map of König and Klippel (16). A stainless-steel electrode insulated to 0.2 mm from the top was introduced, and a radiofrequency current was delivered for 60 sec with the temperature set at 60℃. The sham-operated rats were subjected to the same procedures except for electrical coagulation. The behavioral test was started 7 days after surgery.

Fimbria-fornix (FF) lesion: A micro-surgery knife (No. 715; Feather, Mino) 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 a microknife 8.0 mm laterally and retrieving it. For the sham lesions, only the sagittal sinus was transected. The behavioral test was started 7 days after surgery.

Median- and dorsal-raphe lesion: Specific lesions of serotonergic neurons were made by stereotaxically placed local injections of 5,7-dihydroxytryptamine (5,7-DHT, in 1 μl of 0.1% ascorbic acid − 0.9% saline solution, 0.2 μl/min) through a 26-gauge cannula connected by polyethylene tubing to a gear-driven Hamilton syringe. The sham animals were injected with the vehicle. Injections into the median raphe nucleus were made at the coordinates 8.3 mm posterior to the bregma, on the midline, and 8.0 mm ventral from the skull surface; injections into the dorsal raphe nucleus were made at the coordinates 8.7 mm posterior to the bregma, on the midline, and 6.3 mm ventral from the skull surface. Both nuclei were injected with 2 μg of 5,7-DHT in a volume of 1 μl. The rats were pretreated with desipramine (25 mg/kg, i.p.) 30−45 min prior to the local injections to protect noradrenergic neurons from the lesion (17). The behavioral test was started 14 days after the lesionings.

At the end of the experiments, the placement and size of the lesions were checked by histological examination on 10-μM-thick slices subjected to Nissl staining. All animals subjected to medial-septum and FF-lesions were used for the behavioral examination; however, 3 out of 15 of the raphe-lesioned animals died within 2 weeks after surgery.

Anatomical orientation of each lesion is presented in Fig. 1.

Biochemical analysis

Brain regional monoamine contents were measured in the raphe lesioned rats by a high performance liquid chromatography (HPLC) system. Two weeks after the surgery, each rat was sacrificed by decapitation, and the whole brain was dissected out and sectioned into the following regions on ice: hippocampus; striatum; anterior part of the cortex (C1) including the prefrontal cortex; and posterior part of the cortex (C2) including the parietal, occipital and temporal cortex. The effects of FR121196 on the brain neurochemistry were also determined 30 min after i.p. injection of the drug (0.1, 1, 10, 100 mg/kg) or vehicle for its action on the brain regional contents of monoamines.

Levels of serotonin (5-HT), 5-hydroxyindoleacetic acid

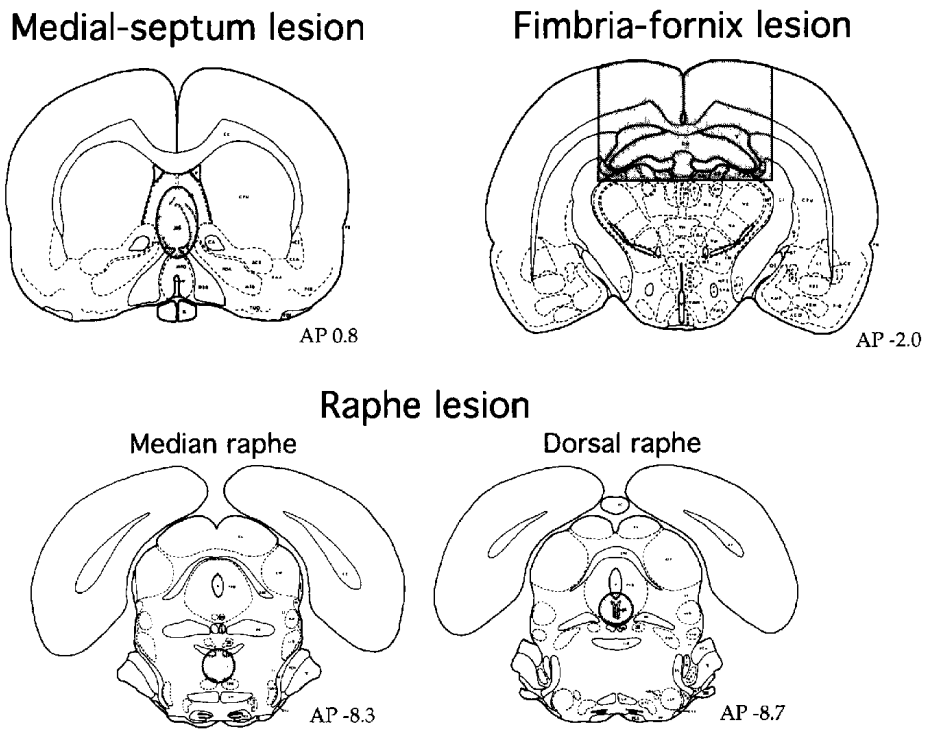


Fig. 1. Schematic drawings of anatomical locations of medial-septum, fimbria-fornix and median- and dorsal-raphe lesions in a coronal section. Surgical procedures and orientations of the lesions were described in Materials and Methods. The stippled areas indicate the representative extent and placement of each lesion.

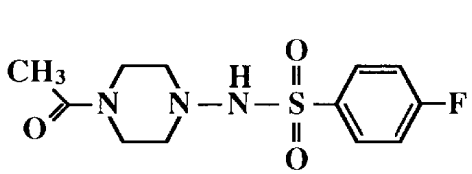
(5-HIAA), norepinephrine (NE), epinephrine (E) and dopamine (DA) were measured in stored tissues of each rat. A reverse phase HPLC method with electrochemical detection was used for quantification of monoamines. Briefly, brain tissues were extracted in 0.2 M perchloric acid (PCA) containing isoproterenol as an internal standard. The extracted solution was centrifuged at 20,000 × *g* for 15 min at 4℃, and the supernatant was adjusted to pH 3.0 with 1 M CH_3COONa and injected into a Eicompac MA50 DS liquid chromatography system equipped with an Eicom ECD100 electrochemical detector (Eicom Co., Kyoto); pump speed was 1 ml/min. The mobile phase consisted of a 0.1 M acetate-citrate buffer (pH 3.5) containing 100 μM sodium octane sulphonate as the ion pair reagent, 1 mM EDTA and 17% methylalcohol.

To characterize the changes in the integrity of brain cholinergic neurons, the choline acetyltransferase (ChAT) activity was determined in the brain of raphe-lesioned rats. The ChAT activity was measured by the method of Fonnum (18), using ^{14}C-Acetyl Coenzyme A (New England Nuclear, MA, Boston, USA), and expressed as nmol ACh/hr/mg protein.

Proteins were measured by the method of Lowry (19).

Drugs

FR121196 (*N*-[4-acetyl-1-piperazinyl]-4-fluorobenzenesulfonamide, Fig. 2), sulpiride hydrochloride and ICS205-930 ((3-tropanyl)-1*H*-indole-3-carboxylic acid-ester HCl) were synthesized in our research laboratories. 5,7-Dihydroxytryptamine creatinine sulfate (5,7-DHT), (*dl*)-*p*-chlorophenylalanine methyl ester (PCPA), (±)pindolol hydrochloride and (±)metoprolol tartrate were purchased from Sigma (St. Louis, MO, USA). Ketanserin tartrate was purchased from Research Biochemicals, Inc. (Natick, MA, USA). Scopolamine hydrobromide and methyl-scopolamine hydrobromide were purchased from Nacalai Tesque (Kyoto). Pindolol and sulpiride was dissolved in 1% tartrate-saline with pH adjusted to 6.0−7.0. All other drugs were dissolved in physiological saline and given intraperitoneally in a volume of 1 ml/kg. Antagonists and FR121196 were injected 15 min and just before the start of the behavioral experiments, respectively.



FR121196

Fig. 2. The chemical structure of FR121196.

Statistics

All data are expressed as the mean±S.E.M. In the behavioral experiments, statistical significance was estimated with the Mann-Whitney *U*-test. For comparison of brain neurochemistry between lesioned and sham or FR121196- and vehicle-treated rats, statistical analysis was made by Student's *t*-test, with a P value of 0.05. or less indicating a significant difference.

RESULTS

Effects of FR121196 on penile erection in rats

Figure 3 shows penile erections in rats after an i.p. injection of FR121196. FR121196 (0.1 – 10 mg/kg) increased the number of penile erections in a bell-shaped dose-response curve with statistically significant and maximum increases above the spontaneous value at 1 and 3.2 mg/kg of the compound. No sign of other abnormal behavior was observed in rats dosed with FR121196 up to 10 mg/kg.

Effects of 5-HT depletion by systemic PCPA administration or lesioning the median- and dorsal-raphe nuclei on FR121196-induced penile erections

Figure 4 shows the effects of 5-HT depletion by systemic treatment with PCPA or median- and dorsal-raphe lesions and sham operation on the penile erection induced by FR121196. The doses of 0.32 ($P<0.01$), 1 ($P<0.05$) and 3.2 mg/kg ($P<0.01$) of FR121196 produced statistically significant erections in the saline-pretreated rats. On the other hand, pretreatment with 150 mg/kg of PCPA 72, 48 and 24 hr before the behavioral test markedly reduced the penile erection induced by FR121196, and the

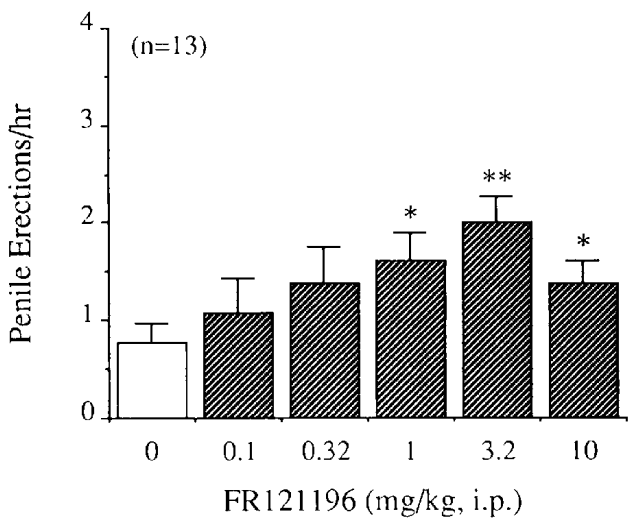


Fig. 3. Effects of FR121196 on penile erection in rats. The ordinate represents the mean number of penile erections in 1 hr following i.p. administration of FR121196. *P<0.05, **P<0.01: Statistically significant compared with the vehicle-treated group (by the Mann-Whitney *U*-test). Each column represents the mean±S.E.M. The number of rats tested is given in parentheses.

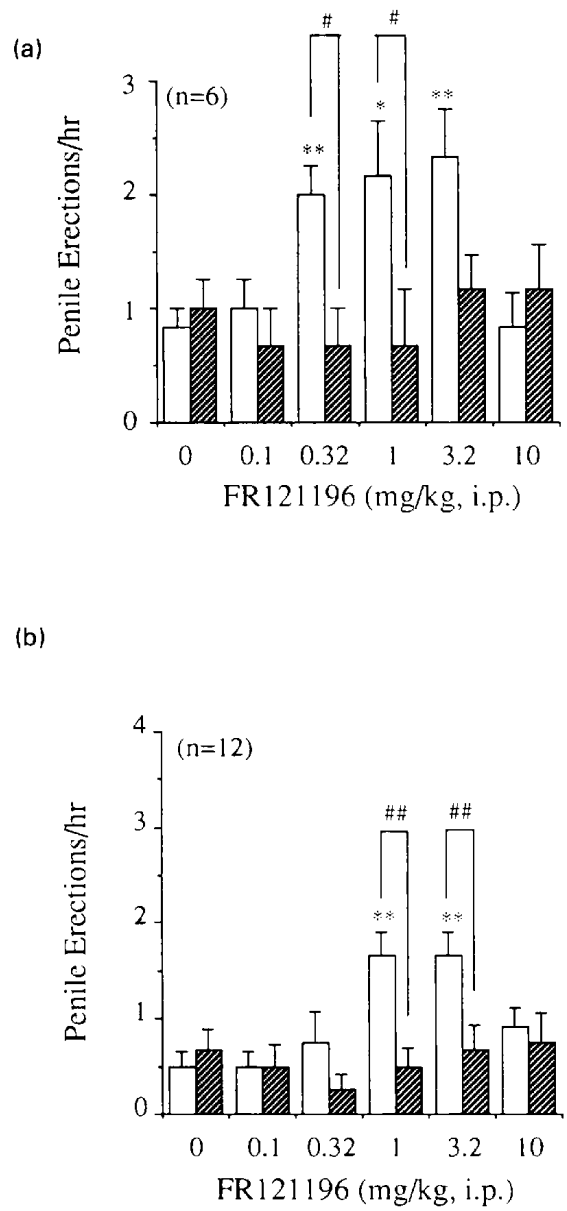


Fig. 4. Effects of PCPA pretreatment (a) and median- and dorsal-raphe lesion (b) on the penile erection induced by FR121196. PCPA (150 mg/kg) was administered 72, 48 and 24 hr before the behavioral test. Medial- and dorsal-raphe lesion by 5,7-DHT was performed as described in Materials and Methods. Two weeks after surgery, the behavioral tests were started and repeated as in the other lesion studies. The ordinate represents the mean number of penile erections in 1 hr following i.p. administration of FR121196. *P<0.05, **P<0.01: Statistically significant compared with the vehicle control in sham animals. #P<0.05, ##P<0.01: Statistically significant compared with the same dosed group of sham animals (by the Mann-Whitney *U*-test). Each column represents the mean±S.E.M. The number of rats tested is given in parentheses. a: □ Saline, ▨ PCPA. b: □ Sham, ▨ Raphe lesion.

changes were statistically significant ($P < 0.05$) at 0.32 and 1 mg/kg of FR121196 compared to those in the saline-pretreated controls (Fig. 4a).

In the raphe-lesioned rats, the compound failed to induce penile erection above the spontaneous level, whereas in the sham-operated rats, a statistically significant response occurred at the doses of 1 and 3.2 mg/kg (Fig. 4b).

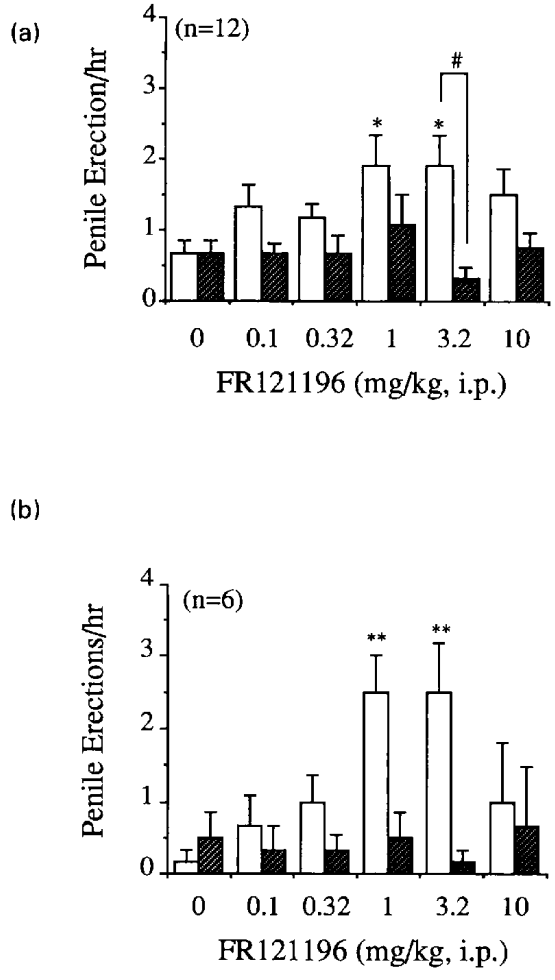


Fig. 5. Effects of medial-septum (a) and fimbria-fornix (b) lesions on the penile erection induced by FR121196. Electrical coagulation of the medial septum and sham operation and knife cut of the fimbria-fornix and sham operation were performed as described in Materials and Methods. Seven days after surgery, the behavioral tests were started and repeated as each rat experienced all dosages of the drug with at least 3-day intervals. Ordinate represents the mean number of penile erections that were observed in 1 hr following i.p. administration of FR121196. $^{*}P<0.05$, $^{**}P<0.01$: Statistically significant compared with the vehicle control group in sham animals. $^{\#}P<0.05$: Statistically significant compared with the same dosed group of sham animals (by the Mann-Whitney *U*-test). Each column represents the mean±S.E.M. The number of rats tested is given in parentheses. a: ☐ Sham, ▨ Medial-septum lesion. b: ☐ Sham, ▨ Fimbria-fornix lesion.

Effects of septo-hippocampal cholinergic denervation on the penile erection

The effects of MS lesion and sham operation on penile erection induced by FR121196 are shown in Fig. 5a. In

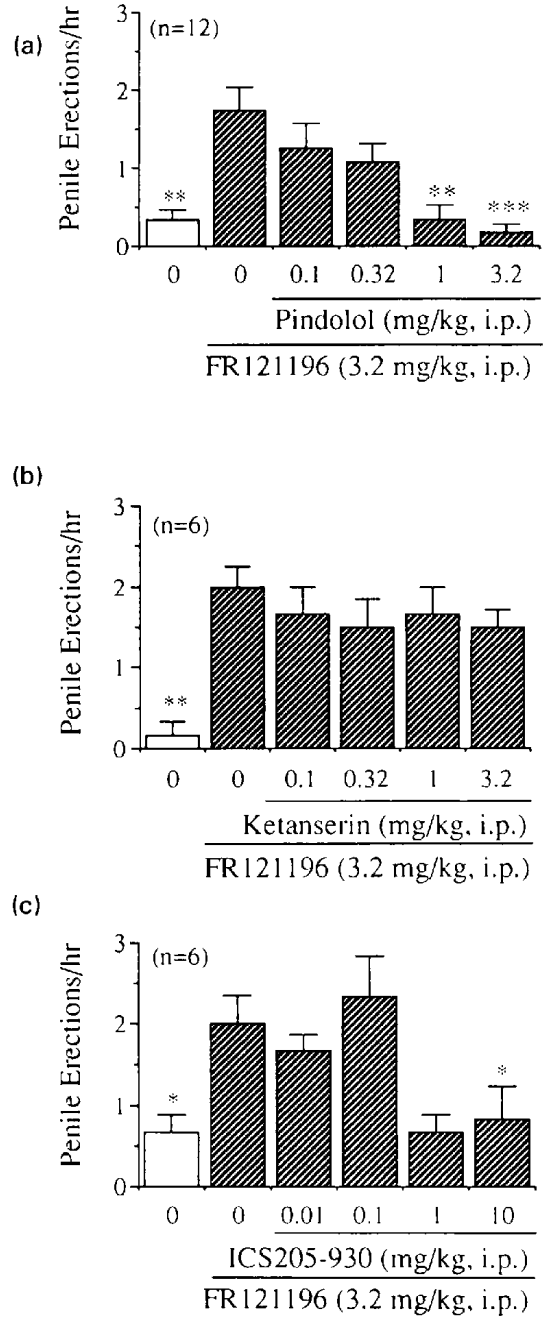


Fig. 6. Effects of pindolol (a), ketanserin (b) and ICS205-930 (c) on the penile erection induced by FR121196. Each antagonist was administered 15 min before FR121196 treatment. The ordinate represents the mean number of penile erections that were observed in 1 hr following i.p. administration of FR121196. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$: Statistically significant compared with the vehicle control group in the FR121196-treated animals (by the Mann-Whitney *U*-test). Each column represents the mean±S.E.M. The number of rats tested is given in parentheses.

Table 1. Effects of cholinergic and dopaminergic antagonists on penile erection induced by FR121196

Antagonist	Dose (mg/kg)	n	Incidence of penile erections/hr
	0	6	1.67±0.42
Scopolamine (i.p.)	0.032	6	1.00±0.26
+	0.1	6	1.33±0.71
FR121196 (3.2 mg/kg, i.p.)	0.32	6	0.33±0.33*
	1	6	0**
	0	6	1.83±0.54
Methyl-scopolamine (i.p.)	0.032	6	1.00±0.26
+	0.1	6	1.17±0.31
FR121196 (3.2 mg/kg, i.p.)	0.32	6	2.33±1.02
	1	6	1.00±0.52
	0	12	1.67±0.26
Sulpiride (i.p.)	1	12	1.42±0.29
+	3.2	12	1.42±0.38
FR121196 (3.2 mg/kg, i.p.)	10	12	1.25±0.28
	32	12	1.58±0.31
	0	12	1.75±0.28
Pindolol (i.p.)	0.1	12	1.25±0.33
+	0.32	12	1.08±0.23
FR121196 (3.2 mg/kg, i.p.)	1	12	0.33±0.19**
	3.2	12	0.17±0.11***

Antagonists were administered intraperitoneally 15 min before administration of FR121196. The ordinate represents the mean number of penile erections of 6–12 rats in 1 hr ±S.E.M. The mean number of penile erections of the vehicle-vehicle administered control rats was 0.3–0.5/hr, and this value was significantly lower than that of the vehicle-FR121196 administered groups. *$P<0.05$, **$P<0.05$, ***$P<0.001$: Statistically significant compared with the vehicle-treated group (by the Mann-Whitney *U*-test).

the MS lesioned rats, all doses of FR121196 failed to induce penile erection above the spontaneous level. In the sham-operated rats, however, statistically significant responses occurred at several doses with the maximum number of erections observed at 1 mg/kg.

Similar results were obtained in the FF-lesioned rats: FR121196 failed to induce penile erections above the spontaneous level (Fig. 5b).

Effects of serotonergic receptor antagonists on FR121196-induced penile erections

The effects of serotonergic receptor antagonists on the penile erection induced by FR121196 were investigated (Fig. 6 and Table 1). Doses of pindolol (0.1–3.2 mg/kg), a 5-HT_1 and β-adrenoceptor antagonist (20), dose-dependently and significantly abolished the penile erection induced by 3.2 mg/kg of FR121196 (Fig. 6a), whereas metoprolol (0.32–10 mg/kg), a β-adrenoceptor-selective antagonist without 5-HT_1-receptor-blocking activity, failed to affect the response (Table 1). Ketanserin (0.1–3.2 mg/kg), a 5-HT_2 antagonist had little effect on the response (Fig. 6b). On the contrary, ICS205-930 (0.01–10 mg/kg), a specific 5-HT_3 antagonist (21), significantly attenuated the response induced by FR121196 (Fig. 6c).

Effects of cholinergic and dopaminergic antagonists on the penile erection induced by FR121196

Table 1 summarizes the effects of scopolamine, methyl-scopolamine and sulpiride on the penile erections induced by FR121196. Scopolamine (0.032–1 mg/kg) dose-dependently reduced the number of penile erections induced

Table 2. Changes in monoamines and ChAT activities in the brain areas of raphe-lesioned rats

Brain region	Monoamine contents (ng/mg protein)					ChAT activity (nmol ACh/hr /mg protein)
	NE	DOPAC	DA	5-HIAA	5-HT	
Hippocampus						
Sham	11.65±0.23	0.413±0.040	1.580±0.508	11.85±0.81	13.19±0.67	48.46±0.97
Lesion	10.56±0.38*	0.390±0.061	1.690±0.629	3.74±1.10***	3.53±1.45***	48.34±0.85
Cortex anter.						
Sham	8.64±1.89	4.240±1.060	25.96±6.670	10.10±2.25	13.37±2.98	41.29±0.48
Lesion	10.73±0.38	4.370±0.412	22.43±4.630	4.74±1.27	5.26±1.76*	41.90±0.86
Cortex poster.						
Sham	7.570±1.26	0.957±0.204	6.450±1.060	8.640±1.48	11.12±2.28	43.86±0.48
Lesion	6.700±1.38	0.711±0.122	4.800±0.929	2.670±0.57**	3.17±0.78*	44.93±0.30
Striatum						
Sham	7.590±0.57	24.05±0.712	230.6± 9.36	20.50±1.36	14.47±0.84	106.52±4.55
Lesion	7.090±0.34	24.75±3.630	226.2±21.09	10.11±1.34***	6.61±1.10***	104.88±2.80

Each value represents the mean±S.E.M. Six animals were used in each group. Brain monoamines and ChAT activities were determined 2 weeks following the raphe lesion. *$P<0.05$, **$P<0.01$, ***$P<0.001$: Statistically significant compared to the corresponding region in the sham animals by Student's *t*-test.

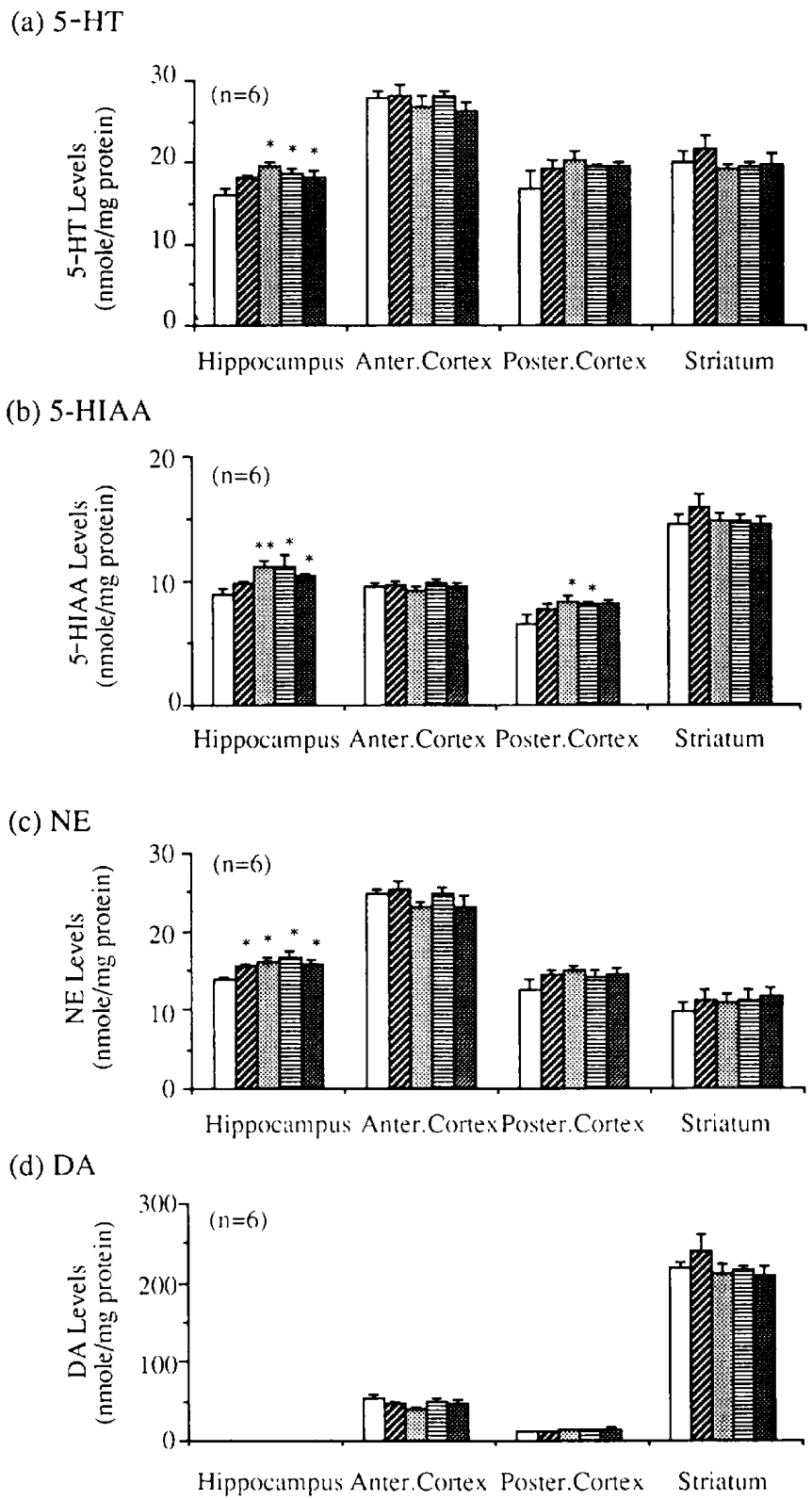


Fig. 7. Effects of FR121196 on brain regional monoamine contents. Doses of FR121196 (0.1, 1, 10, 100 mg/kg) or vehicle were administered i.p. 30 min before sacrifice of the animals. Brain regional monoamines were determined by HPLC as described in Materials and Methods. $^{*}P<0.05$, $^{**}P<0.01$: Statistically significant compared to the corresponding region of the vehicle-administered animals by Student's *t*-test. □ 0 mg/kg, ▨ 0.1 mg/kg, ▤ 1 mg/kg, ▤ 10 mg/kg, ■ 100 mg/kg.

by FR121196 (3.2 mg/kg). In contrast, neither a peripheral acting muscarinic antagonist, methyl-scopolamine (0.032 – 1 mg/kg), nor sulpiride (1 – 32 mg/kg), a dopamine D_2 blocker, affected the responses induced by FR121196.

Brain neurochemistry

Effects of median- and dorsal-raphe lesion by 5,7-DHT on brain regional monoamines and ChAT activities: The levels of brain regional monoamines after median- and dorsal-raphe lesion are presented in Table 2. The treatment significantly reduced 5-HT and 5-HIAA levels in almost all the cortical and subcortical brain areas. The most striking effect was observed in the hippocampus where 5-HT and 5-HIAA were decreased by 73 and 68% of the values in sham rats, respectively. NE, DA and their metabolites in the brain areas were hardly changed in the lesioned rats compared with the sham control. On the other hand, the lesion hardly affected ChAT activities in all brain regions examined.

Effects of intraperitoneal administration of FR121196 on brain regional monoamines: Figure 7 illustrates the effect of systemic administration of FR121196 on the brain regional monoamine levels. Intraperitoneal injection of FR121196 significantly elevated 5-HT and 5-HIAA levels in the hippocampus in a bell-shaped dose-response manner, and the maximum increase was obtained at doses of 1 and 10 mg/kg by 125 and 121% of the respective values in vehicle-treated rats (Fig. 7, a and b). A statistically significant increase in the 5-HIAA level was also observed in the posterior cortex (Fig. 7b). The drug hardly affected NE and DA levels in any other brain regions examined except for the hippocampus where the NE level was slightly but significantly increased (Fig. 7, c and d).

DISCUSSION

MacLean (7), after a series of electrophysiological studies, concluded that the hippocampus and its afferents are important for the expression of penile erection. There are two major afferents to the hippocampus: the cholinergic nerves derived from MS (8, 9) and the serotonergic nerves from the raphe nuclei (10, 11). It is also known that the septo-hippocampal cholinergic nerve activity is controlled by dopaminergic nerves derived from the ventral tegmental area (A10) (22, 23). These histochemical mappings of the hippocampal afferents coincide with the pharmacological evidence that dopaminergic (14, 24, 25), serotonergic (15, 26, 27) and cholinergic (12) stimulants induce penile erection.

The present study clearly demonstrated that FR121196 induced penile erection in naive rats that had been completely abolished by PCPA pretreatment or neurotoxic lesioning of the median- and dorsal-raphe using 5,7-DHT. The dose regime of PCPA-pretreatment reportedly depletes brain 5-HT levels by more than 90% (28), whereas the present neurochemical analysis showed that the raphe lesion specifically depleted 5-HT and its major metabolite 5-HIAA in the whole limbic area including the hippocampus (Table 2). The latter observation coincides with the previous findings that serotonergic nerve terminals derived from the median- and dorsal-raphe nuclei are widely distributed in brain areas such as the cerebral cortex, septum and hippocampus (10, 11). We thus theorize that the response induced by FR121196 is mediated by an activation of serotonergic neurons that originated in the raphe nucleus and reached the hippocampus, and this would be supported by the present finding that FR121196 increased hipocampal 5-HT and 5-HIAA. Because the ratio of 5-HT to 5-HIAA hardly changed following FR121196 treatment, both the synthesis and release of serotonin might be enhanced by the drug. In this regard, it is interesting to note that the penile erection induced by fenfluramine, a serotonin reuptake inhibitor and releaser, was also cancelled by lesioning of the median and dorsal raphe nuclei as well as by the septo-hippocampal deafferentations (29).

The present study further demonstrated that pindolol, a β-adrenoceptor antagonist with 5-HT_1-blocking activity (20), but not metoprolol, a β-adrenoceptor antagonist without 5-HT_1-blocking activity (30), dose-dependently attenuated the penile erection induced by FR121196. The finding is in line with the hypothesis that central 5-HT_{1B}- and/or 5-HT_{1C}-receptor activities play a crucial role in sexual behavior (15, 27). In addition, ICS205-930, a 5-HT_3 antagonist (21), but not ketanserin, a 5-HT_2 antagonist (31), attenuated the penile erection. These results in view of the above discussions suggest that a stimulation of 5-HT_1 and 5-HT_3 receptors through an activation of the raphe-hippocampal pathway is responsible for the penile erection induced by FR121196. In support of the assumption, a growing body of evidence indicates that not only 5-HT_1 but also 5-HT_3 receptors are densely distributed within the limbic areas including the hippocampus (32, 33). On the other hand, it has been shown that the serotonergic raphe-hippocampal pathway exerts a powerful influence on the electrical activity of the hippocampus (34 – 36) whereas a direct application of 5-HT into the hippocampus or a systemic administration of 5-HT releaser increased hippocampal acetylcholine release (37, 38). Furthermore, it is interesting to note that stimulation of the 5-HT_1 receptors enhances ACh release in the hippocampus (38). An activation of the intra-hippocampal 5-HT_3 receptors is also postulated to enhance ACh release via the enhancement of 5-HT transmission (39). It is thus

tempting to speculate that FR121196 stimulates 5-HT_1 and 5-HT_3 receptors through raphe-hippocampal serotonergic activation that in turn causes hippocampal ACh release and finally leads to penile erection.

The above-mentioned speculation is substantiated by the present finding that scopolamine but not methylscopolamine attenuated the penile erection induced by FR121196. In addition, the erectile effects of FR121196 were completely abolished by MS- or FF-lesion, which has been shown to significantly reduce hippocampal ChAT activities (1). Although we have previously reported that dopaminergic stimulants, apomorphine and amantadine, induced penile erection in a manner antagonizable by scopolamine, MS- or FF-lesion (12, 40), the present data on sulpiride exclude the possible involvement of a dopaminergic mechanism in the action of FR121196. FR121196 appears to indirectly activate the septo-hippocampal cholinergic pathway through a mechanism independent from dopaminergic nerve activity to induce penile erection. In fact, FR121196 revealed little affinity for the receptors to the known mediators including acetylcholine, dopamine and serotonin (unpublished observation of N. Maeda et al.).

In conclusion, the present study strongly suggest that an indirect activation of the septo-hippocampal cholinergic pathway through effects on the raphe-hippocampal serotonergic neurons is involved in the penile erection induced by FR121196. It is noteworthy that the memory enhancing effect of the compound is cancelled not only by septo-hippocampal cholinergic (1) but also raphe-serotonergic denervation (41). A common mechanism might be involved, at least in part, in the erection stimulating and memory enhancing activities of FR121196.

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