

Involvement of L-arginine/nitric oxide pathway at the paraventricular nucleus of hypothalamus in central neural regulation of penile erection in the rat

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The objective of this study is to investigate whether the L-arginine/nitric oxide pathway is involved in the neurotransmission of paraventricular nucleus of hypothalamus (PVN) activation-induced penile erection in the rat. Male adult Sprague-Dawley rats anesthetized with pentobarbital were used. The femoral artery was cannulated to measure systemic and mean arterial pressure (SAP and MAP), and heart rate (HR). A 26-gauge needle was inserted into corpus cavernosum to measure the intracavernous pressure (ICP) simultaneously with SAP, MAP and HR on a polygraph. Four groups of study were arranged: (1) stereotaxically delivery of L-arginine (500 nmol/500 nl) into PVN; (2) administration of a mixture (1 μ l) containing N^G-Nitro-L-arginine methyl ester (L-NAME) 500 nmol and L-arginine 500 nmol into PVN; (3) microinjection of saline 500 nl into PVN as a vehicle control; and (4) intracavernous injection of L-arginine (100 nmol/50 μ l). The ICP, SAP, MAP and HR were monitored for at least 2 h after each administration of the experimental agents. Upon administration of L-arginine into PVN, there was a significant increase of ICP from resting 9.6 ± 2.5 mmHg to peaked at 64.4 ± 9.8 mmHg after a latency of 3016.0 ± 1749.7 s and with a duration of 27.6 ± 15.8 min. There was no change of resting ICP after administration of the mixture of L-NAME and L-arginine into PVN. Application of saline to PVN and intracavernous injection of L-arginine failed to increase ICP. Based on elicitation of penile erection upon administration of L-arginine into PVN, and elimination of this L-arginine induced penile erection by co-administration of L-NAME with L-arginine, the results of this study suggest that L-arginine/nitric oxide pathway may be involved in the neurotransmission of PVN activation-induced penile erection in the rat.

International Journal of Impotence Research (2002) 14, 139–145. doi:10.1038/sj.ijir.3900825

Keywords: penile erection; L-arginine; nitric oxide; paraventricular nucleus of hypothalamus; intracavernous pressure; rat

Introduction

Penile erection is a process of initiation, filling and storage of blood in the corpora cavernosa. The 'initiation' of penile erection is induced chiefly through the activation of nervous system, either central or peripheral. A dysfunction of this system would then result in neurogenic impotence.¹

Although the peripheral nervous system responsible for penile erection has been investigated rather frequently,^{2–4} the mechanisms of central nervous system (CNS) participating in the penile erection is relatively less addressed. Previous studies have

demonstrated that penile erection was elicited after electrical and chemical (L-glutamate) stimulation of hippocampus,^{5,6} or of the paraventricular nucleus of hypothalamus (PVN)⁷ in the rat. The correlation of neural pathways between the hippocampus and the PVN in elicitation of penile erection may be in series in the rat.⁷ In addition, administration of apomorphine (dopamine receptor agonist) into PVN may elicit a penile erection mainly through activation of D2 receptor subtype in PVN of the rat. Dopaminergic neurotransmission may be involved in the PVN-activation induced penile erection.^{8,9}

Recent research has shown that both acetylcholine and neuronally mediated relaxation of the smooth muscles in corpora cavernosa in animals and humans involves production and release of nitric oxide (NO).^{10–12} Burnett *et al*, reported staining of nitric oxide synthase (NOS) in the pelvic plexus and the cavernous nerve.¹³

In addition to the demonstration of NOS in the urogenital tissues, previous immunohistochemical

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Received 21 August 2001; revised 16 October 2001; accepted 21 November 2001

localization studies have also demonstrated that positive staining of NOS in the neurons of PVN in the rat.^{14,15} This enzyme is also concentrated in the neural innervation of the posterior pituitary, in autonomic nerve fibers of the retina, in cell bodies and nerve fibers of the myenteric plexus in the intestine, in adrenal medulla, and in vascular endothelial cells. This enzyme catalyzes the production of NO from L-arginine which requires calmodulin.^{16,17} Isoforms of this enzyme was reported to be eNOS, iNOS and nNOS.¹⁸

As mentioned above, previous studies have suggested that PVN may participate in the central neural regulation of penile erection in the rat,^{7–9} and the neurons of PVN contain NOS.^{14,15} Taken together, therefore the aim of the present study is to investigate whether the L-arginine/NO pathway is involved in the neurotransmission to elicit penile erection through PVN in the rat. In this study, direct administration of the precursor (L-arginine) of the pathway into PVN was executed to investigate the physiological function of this pathway on penile erection.

Materials and methods

The rat model using the intracavernous pressure (ICP) as an experimental index for the evaluation of penile erection was used.¹⁹ Briefly, male adult Sprague-Dawley rats (200–300 g) anesthetized with pentobarbital sodium (50 mg/kg, i.p., with 20 mg/kg/h continuous i.v. infusion supplements) were used. An endotracheal intubation was set for a patent airway. The femoral artery was cannulated to measure systemic and mean arterial pressure (SAP and MAP) via a pressure transducer (Gould 23ID) and a pressure processor amplifier (Gould 13-4615-52). The heart rate (HR) was monitored utilizing a biotachometer amplifier (Gould 13-4615-65) which was triggered by arterial pulses. The femoral vein was also cannulated to infuse supplemental anesthetic agent continuously. The head of the rat was carefully mounted to a stereotaxic headholder (Kopf 900) with the lower body slightly rotated to expose the genital area. The penis was degloved and a 26-gauge needle connecting to a pressure transducer (Gould 23ID) via a PE-20 tube filled with saline was inserted into one side corpus cavernosum to measure the ICP simultaneously with SAP, MAP and HR on a polygraph (Gould ES 1000). The rat was placed on a heating pad to maintain its body temperature at 37°C during the whole experiment. Four groups of study were arranged.

L-arginine to PVN. A midline longitudinal incision was made on the scalp. A Hamilton microinjection needle was inserted into the PVN (coordinates: 1.5–2.0 mm caudal to the bregma, 5.5–6.5 mm below the dura and 0.5–1.0 mm lateral to the midline)²⁰ and L-

arginine (500 nmol/500 nl) was slowly administered into PVN over 1–2 min for an adequate diffusion of the chemical agent.

NG-Nitro-L-arginine methyl ester hydrochloride (L-NAME, NOS inhibitor) and L-arginine to PVN. A mixture (1 µl) containing L-NAME 500 nmol and L-arginine 500 nmol was slowly administered into PVN over 1–2 min.

Saline to PVN. Microinjection of saline 500 nl into PVN was done as a vehicle control.

L-arginine to corpus cavernosum. Intracavernous injection of L-arginine (100 nmol/50 µl) was executed to evaluate its local effect.

The ICP and hemodynamic parameters (SAP, MAP, HR) were monitored for at least 2 h after administration of studying chemical agent in each group.

Intracavernous injection of papaverine hydrochloride 0.4 mg/100 µl was executed at the completion of each experiment to assure that the penile cavernous tissues were still responding to external stimulation.

The rat brain was removed at completion of each experiment and was fixed in 30% sucrose in 10% formaldehyde for at least 3 days. The site of central administration of L-arginine, L-NAME and L-arginine, or saline is verified histologically with frozen section (25 µm in thickness) of the rat brain and stained with neutral red.

The difference of peak ICP before and after administration of chemical agents was statistically analyzed using Wilcoxon signed ranks test. A probability of $P < 0.05$ was considered statistically significant.

Results

Upon administration of L-arginine into the PVN, there were multiple episodes of increase in ICP (Figure 1). The characteristics of increase in ICP induced by L-arginine are shown in Table 1. There was no change of ICP (9.0 ± 1.8 mmHg vs 9.0 ± 1.8 mmHg, $n = 5$) upon concomitant administration of L-NAME and L-arginine into PVN (Figure 2). The sites of administration of chemical agents were histologically verified to be in the anatomical confine of the PVN (Figure 3). There was no change of ICP after administration of L-arginine outside or adjacent to the PVN (Figure 4). Administration of saline to PVN did not induce an increase of ICP (Figure 5). Intracavernous injection of L-arginine failed to increase ICP (9.0 ± 5.3 mmHg vs 9.0 ± 5.3 mmHg, $n = 6$; Figure 6).

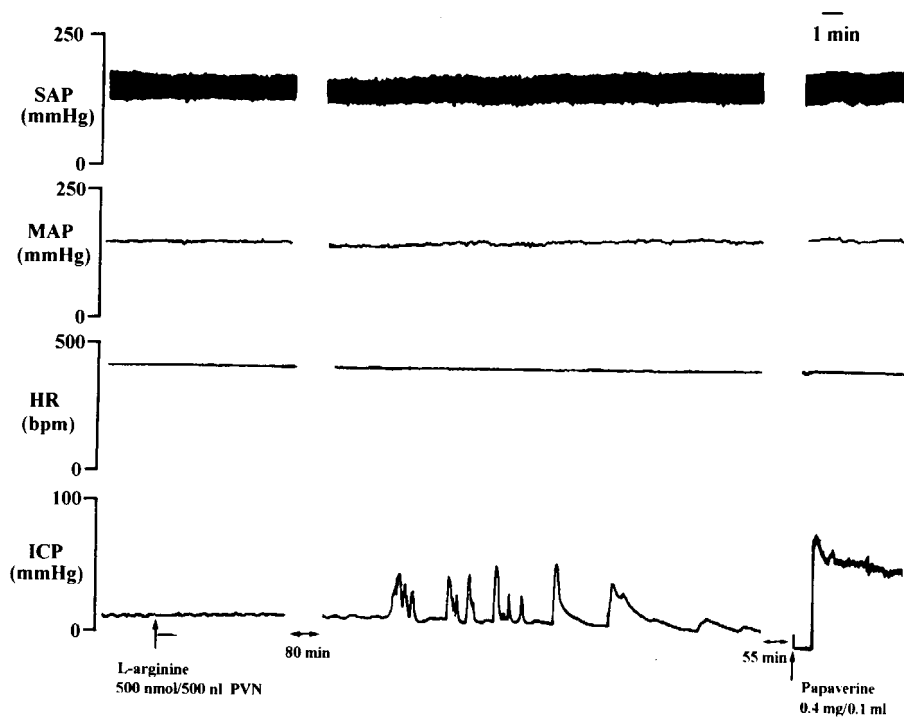


Figure 1 Time-course changes of systemic arterial pressure (SAP), mean arterial pressure (MAP), heart rate (HR) and intracavernous pressure (ICP) following microinjection of L-arginine (500 nmol/500 nl) into the paraventricular nucleus of hypothalamus. There was no simultaneous significant change of SAP, MAP and HR immediately before or during the period of elevation in ICP.

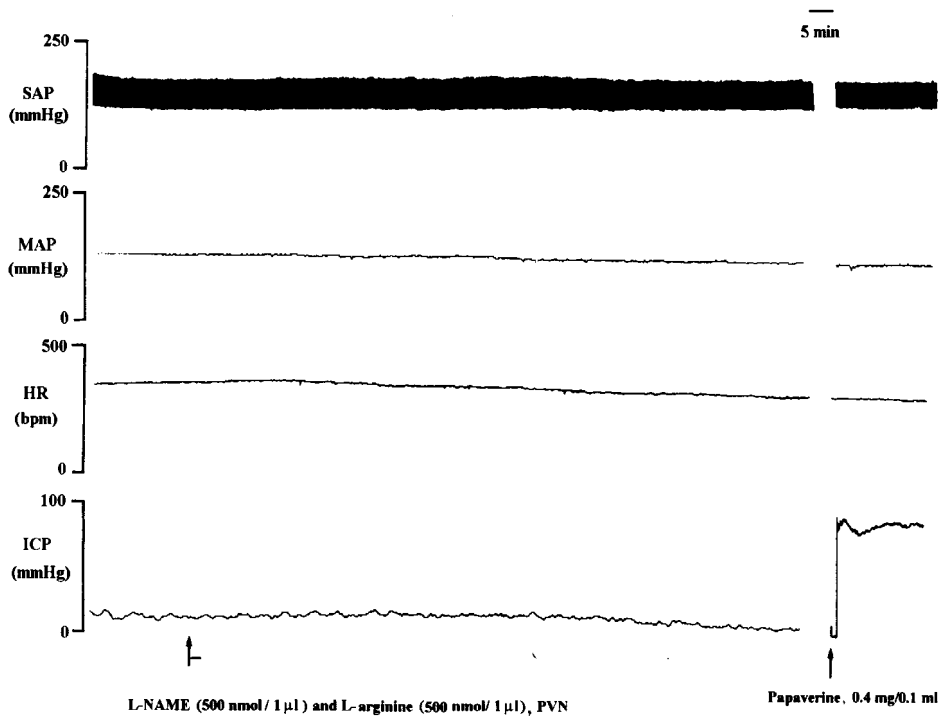


Figure 2 Time-course changes of systemic arterial pressure (SAP), mean arterial pressure (MAP), heart rate (HR) and intracavernous pressure (ICP) following microinjection of L-NAME (500 nmol/1 µl) and L-arginine (500 nmol/1 µl) into the paraventricular nucleus of hypothalamus. There was no increase of ICP upon concomitant administration of L-NAME and L-arginine into PVN. However, an increase of ICP was noted after intracavernous injection of papaverine (0.4 mg/0.1 ml).

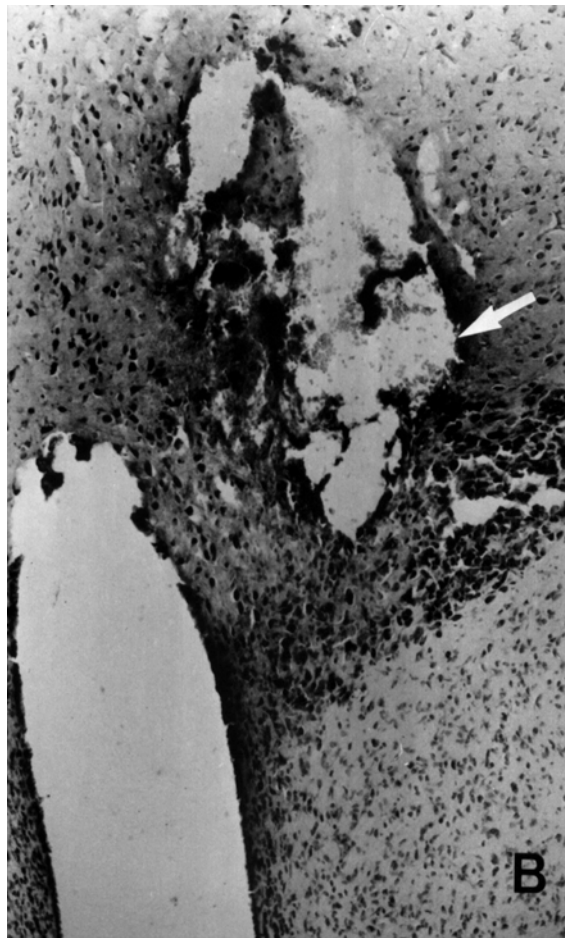
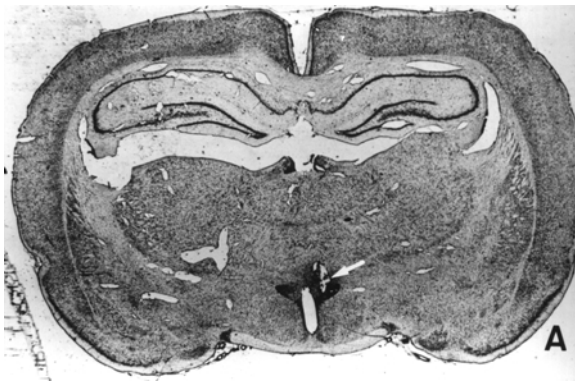


Figure 3 Photomicrograph of coronal section of rat forebrain shows the site (arrow) of microinjection of L-arginine (500 nmol/500 nl) into the paraventricular nucleus of hypothalamus. (A) Neutral red stain, $\times 10$; (B) neutral red stain, $\times 140$.

Discussion

In the current study, ICP was used as an experimental index for penile erection. This index allowed the experiment to have a sensitivity of detecting a change of 2 mmHg in ICP. This may provide a more objective and quantitative measurement of penile

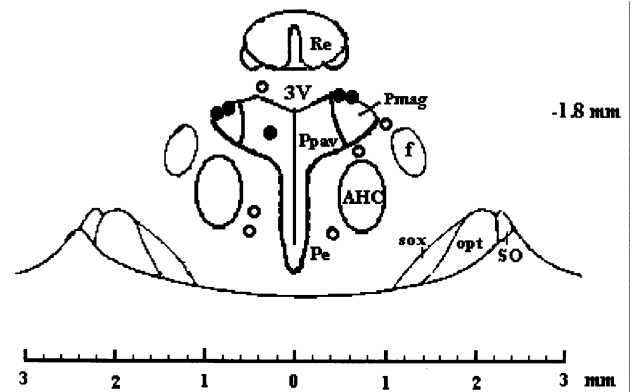


Figure 4 Diagrammatic illustration of hypothalamus relative to bregma demonstrating sites upon which administration of L-arginine induced an increase of intracavernous pressure (ICP). Sites upon administration of L-arginine failed to elicit elevation of ICP were indicated by an open circle. Abbreviations: AHC, anterior hypothalamus centralis; Pe, periventricular hypothalamic nucleus; Pmag, magnocellular subnucleus of paraventricular nucleus of hypothalamus; Ppav, parvocellular subnucleus of paraventricular nucleus of hypothalamus; Re, reuniens thalamic nucleus; SO, supraoptic nucleus; f, fornix; opt, optic tract; sox, supraoptic decussation; 3V, third

erectile status even at the ICP below 40 mmHg,⁵ which is the threshold pressure for visible penile erection, the basis for behavioral assessment.

NO in the penile tissues is considered a neurotransmitter to mediate penile erection.^{4,21} Through the action of NOS, NO and citrulline are produced from the precursor, ie L-arginine. In this study, intracavernous administration of L-arginine was ineffective to induce an increase of ICP. This finding suggested that the increase of ICP upon administration of L-arginine into PVN was a direct stimulatory effect of L-arginine to PVN neurons, not secondary to the local penile effect of L-arginine.

Conceptually, L-arginine injected intracavernously may induce penile erection. We estimated that after administration of L-arginine into PVN the amount of pharmacokinetic distribution of L-arginine in the local penile tissue would be much less than that in PVN. Therefore we injected only 100 nmol of L-arginine into the cavernous tissues in an attempt to evaluate the local penile effect of L-arginine. In this study, intracavernous administration of L-arginine (100 nmol) failed to induce an increase of ICP. Whether intracavernous application of a larger dose of L-arginine could induce penile erection awaits further clarification.

Previous investigation suggested that long-term oral administration of a large amount of L-arginine (928 mg/day) may significantly increase the maximal intracavernosal pressure upon electrical field stimulation of the cavernosal nerve in adult rats.²² Clinically, oral L-arginine 1500 mg/day for 17 days was ineffective (as compared with placebo) in the treatment of 30 patients with erectile dysfunction.²³ On the other hand, previous study has also shown

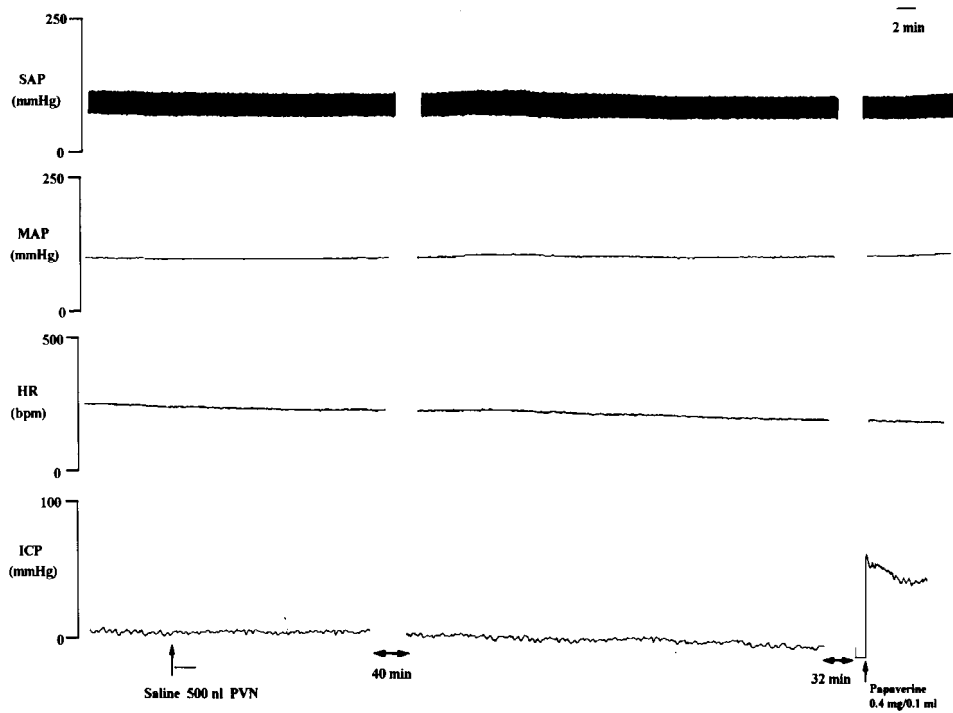


Figure 5 Time-course changes of systemic arterial pressure (SAP), mean arterial pressure (MAP), heart rate (HR) and intracavernous pressure (ICP) following administration of saline 500 nl into paraventricular nucleus of hypothalamus (PVN). There was no increase of ICP upon microinjection of saline into PVN. However, an increase of ICP was noted after intracavernous injection of papaverine (0.4 mg/0.1 ml).

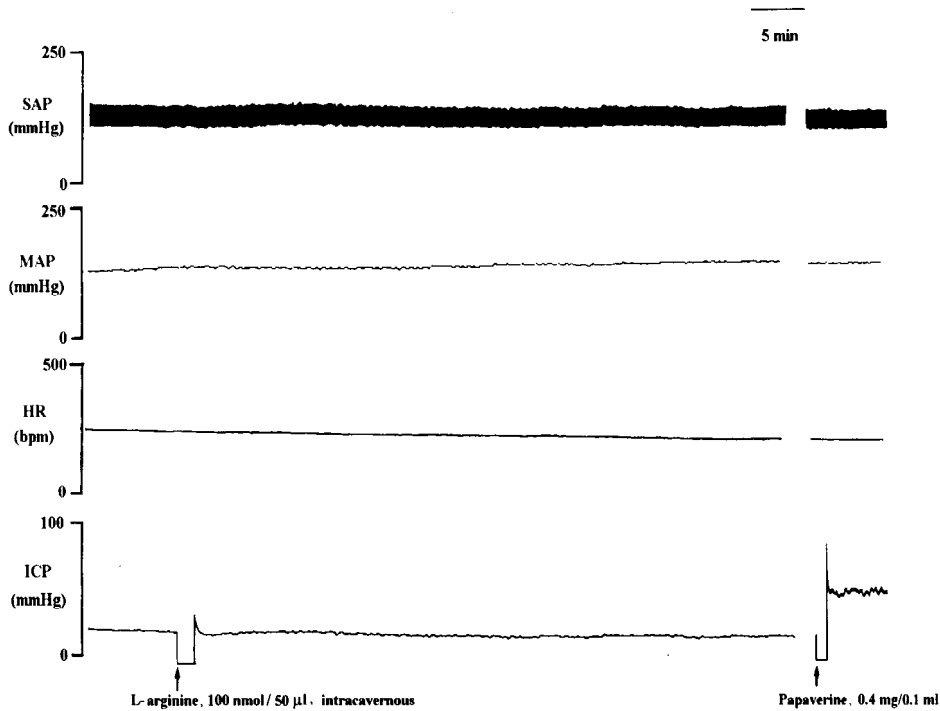


Figure 6 Time-course changes of systemic arterial pressure (SAP), mean arterial pressure (MAP), heart rate (HR) and intracavernous pressure (ICP) following intracavernous injection of L-arginine (100 nmol/50 µl). There was no increase of ICP upon intracavernous administration of L-arginine. However, an increase of ICP was noted after intracavernous injection of papaverine (0.4 mg/0.1 ml).

Table 1 Characteristics of change in intracavernous pressure (ICP) after administration of L-arginine (500 nmol/500 nl) into paraventricular nuclear of hypothalamus

	Mean \pm s.e.m.	Range
Resting ICP (mmHg)	9.6 \pm 2.5	1–16
Peak ICP (mmHg)	64.4 \pm 9.8*	44–100
Latency (s)	3016.0 \pm 1749.7	60–7440
Duration (min)	27.6 \pm 15.8	5–90

$n = 5$, * $P = 0.043$ vs resting ICP by Wilcoxon signed ranks test.

that six of 15 male patients with erectile dysfunction had improved erection after oral L-arginine 2800 mg/day for 2 weeks and no patient in the placebo group had a positive response.²⁴ Whether a large dose of L-arginine is necessary to improve the erectile function needs a large number of patients in a drug trial to elucidate this issue.

Nitroglycerin (NO donor) has been found to induce penile erection when injected intracerebroventricularly or into PVN. This penile erection-inducing effect was prevented by intracerebroventricular administration of methylene blue (guanylate cyclase inhibitor).²⁵ In the present study, administration of L-arginine into PVN induced penile erection in terms of an increase in ICP and this effect was eliminated by co-administration of L-arginine and L-NAME (NOS inhibitor). In addition, this penile erection-inducing effect is considered to be the result of a direct central effect of L-arginine because there was no increase of ICP after intracavernous injection of L-arginine. Extracellular NO metabolites (NO_2^- and NO_3^-) in the PVN have been reported to be increased with a corresponding increase of reflexive erections after administration of L-arginine into the PVN in the rats.²⁶ Taken together, it is suggested that the L-arginine-NO-guanosine 3',5'-cyclic monophosphate (cGMP) pathway may be involved in the nitrgergic neurotransmission of elicitation of penile erection after stimulation of PVN. Furthermore, penile erection induced by dopamine agonist, oxytocin and *N*-methyl-d-aspartic acid (NMDA) may be prevented by intracerebroventricular administration of L-NAME.^{25,27,28} All these results suggest that not only may NO precursor or donor induce penile erection, but also non-NO donors or precursors are also able to induce penile erection via the common mediator of NO.

In this study the peak increase of ICP elicited by administration of L-arginine to PVN is similar to those after administration of dopamine agonist into PVN or electrical stimulation of PVN.^{7,8} This also suggests that nitrgergic neurotransmission may be involved in the initial non-NO stimulation-induced penile erection. In this study, the onset latency period of the response to L-arginine was longer as compared with that after subcutaneous administration of dopamine agonist (apomorphine) or admin-

istration of apomorphine into PVN.⁸ We proposed that this longer latency may be due to slow diffusion of the experimental agent and uptake by the PVN neurons, and extra time necessary for metabolism of L-arginine to NO and citrulline. Although the latency period was relatively long in some animals, the elicitation of penile erection was associated with administration of L-arginine into PVN because there was no increase of ICP after administration of saline into PVN.

The results of the present study revealed that multiple episodes of increase in ICP were induced after administration of L-arginine into PVN. We speculated that these pressure spikes may be due to associated contractions of striated perineal muscles, the bulbospongiosus (BS) and the ischiocavernosus (IC) muscles, which are important for penile erection in rats.^{29,30} A direct descending projection from the PVN to the spinal nucleus of the bulbocavernosus (SNB) and the dorsolateral intermediolateral nucleus (CLN) in the lumbosacral area has been reported.^{31,32} Axons of motoneurons of the SNB and CLN supply the innervation to BS and IC muscles. Because we did not conduct recording of electromyography of the striated perineal muscles, this speculation awaits further investigation.

Oxytocin has been reported to elicit penile erection through activation of the NO synthase in the PVN, and the NO produced in the PVN subsequently stimulates PVN oxytocinergic neurons which project to extra-hypothalamic areas.²⁸ NO donors also induce penile erection by producing NO in the PVN, and then activates oxytocinergic neurotransmission to extra-hypothalamic areas, such as the hippocampus or the ventral medullaspinal cord.^{6,33,34} This NO donors induced penile erection is prevented by administration of oxytocin receptor antagonist [d(CH₂)⁵-Tyr(Me)²-Orn⁸]-vasotocin into lateral ventricle.³⁵ In this study, we considered that L-arginine produced NO in the PVN and then the latter induced penile erection by activating the PVN oxytocinergic neurons projecting to the extra-hypothalamic areas.

Previous study has shown that males with erectile dysfunction may have a positive response to oral L-arginine and no patient in the placebo group responded.²⁴ Heaton *et al* have proposed a therapeutic taxonomy and classified the treatments for erectile dysfunction into five classes: central initiator, peripheral initiator, central conditioner, peripheral conditioner, and other.³⁶ The results of this study showed that L-arginine may initiate penile erection through its action on a site (PVN) of CNS which may provide an academic basis to classify L-arginine as a central initiator. Accordingly, the implication of this experimental study was that L-arginine may be used clinically to induce penile erection through its action in the CNS.

Based on elicitation of penile erection upon administration of L-arginine into PVN, and elimina-

tion of this L-arginine induced penile erection by co-administration of L-NAME, the results of this study suggest that L-arginine/NO pathway may be involved in the neurotransmission of PVN activation-induced penile erection in the rat.

Acknowledgements

This study was supported by a research grant NSC 88-2314-B-075-043 from the National Science Council, Taiwan, Republic of China.

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