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行政院國家科學委員會補助專題研究計畫成果報告
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Involvement of Nitric Oxide Generated by nNOS and iNOS at Hippocampal Formation in Negative Feedback Regulation of Penile Erection in the Rat

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ABSTRACT: We established previously that a novel negative feedback mechanism for the regulation of penile erection, which is triggered by ascending sensory inputs initiated by tumescence of the penis, exists in the hippocampal formation (HF). This study further evaluated the participation of nitric oxide (NO) and the contribution of nitric oxide synthase (NOS) isoforms at the HF in this process. Adult, male Sprague-Dawley rats that were anesthetized and maintained with chloral hydrate were used, and intracavernous pressure (ICP) recorded from the corpus cavernosum of the penis was employed as our experimental index for penile erection. Microinjection bilaterally of a NO donor, S-nitro-N-acetylpenicillamine (0.25 or 1 nmol), or the NO precursor, L-arginine (1 or 5 nmol), into the hippocampal CA1 or CA3 subfield or dentate gyrus elicited a significant reduction in baseline ICP. Bilateral hippocampal application of a NO tamping agent, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (10 nmol), significantly potentiated the elevation in ICP induced by intracavernous administration of papaverine (400 μg). Microinjection bilaterally into the HF of equimolar doses (2.5 pmol) of two selective neuronal NOS inhibitors, 7-nitroindazole or Nω-propyl-L-arginine; or equimolar doses (250 pmol) of two selective inducible NOS inhibitors, aminoguanidine or S-methylisothiourea, significantly enhanced the magnitude and/or duration of the papaverine-induced elevation in ICP. In contrast, hippocampal application of an endothelial NOS inhibitor, N\(^5\)-(1-iminoethyl)-L-ornithine (92 nmol), was ineffective. Neither of these inhibitors, furthermore, affected baseline ICP. These results suggest that NO generated via both neuronal and inducible NOS at the HF may participate in negative feedback regulation of penile erection.

KEY WORDS: intracavernous pressure; nitric oxide synthase; CA1 or CA3 subfield; dentate gyrus
INTRODUCTION

In addition to its well-known function as an endothelial-derived relaxing factor that promotes relaxation of the blood vessels (Rees et al., 1989), nitric oxide (NO) is now established to be critically involved in erectile functions via an action on penile tissues (Andersson and Wagner, 1995; Giuliano et al., 1995; Meston and Frohlich, 2000). The role of NO in central regulation of penile erection, on the other hand, is much less documented; and one often mentioned central site of action is the paraventricular nucleus of hypothalamus (Argiolas and Melis, 1995).

Another potential site in the central nervous system for NO to exert its modulatory action on penile erection is the hippocampal formation (HF). Based on the classical electrophysiologic observations of MacLean and Ploong (1962) and Dua and MacLean (1964), the HF has long been assigned a role in the regulation of erectile functions (Steers, 1990; Andersson and Wagner, 1995; Giuliano et al., 1995). Our laboratory (Chen et al., 1992b, 1997) subsequently reported that electrical activation of the HF in rats elicits an elevation in intracavernous pressure (ICP), along with visible penile erection and ejaculation. We further identified a novel negative feedback regulatory mechanism on penile erection in the HF (Chang et al., 1998a), which is triggered by ascending sensory inputs initiated by tumescence of the penis during normal erectile processes.

Nitric oxide synthase (NOS) is an enzyme involved in the generation of NO from L-arginine (Moncada and Higgs, 1993). Of the three known NOS isoforms, it is generally contended that neuronal NOS (nNOS) and endothelial NOS (eNOS) are expressed constitutively (Moncada et al., 1992) and are normally present in subsets of neurons (Bredt et al., 1991) or endothelial cell in the brain (Dinerman et al., 1994). On the other hand, the inducible NOS (iNOS) exists in macrophage (Baek et al., 1993) or glial cells (Weldon et al., 1998), and
its activity is principally induced by inflammatory stimuli (Murphy et al., 1993; Szabo, 1996; Wong et al., 1996). NOS-containing neurons are conspicuous in the pyramidal layer of Ammon's horn and molecular layer of dentate gyrus (Vatschanoff et al., 1993). That these may represent nNOS-immunoreactive HF neurons was demonstrated in mouse (Jinno et al., 1999) or human (Doyle and Slater, 1997). Hippocampal pyramidal neurons of CA1 or CA3 subfield and granule cells of dentate gyrus also express nNOS mRNA (Lopez-Figueroa et al., 1998; Reagan et al., 1999). However, exhibition of iNOS immunoreactivity in HF is reportedly sparse in human (Lee et al., 1999a). Hippocampal eNOS immunoreactivity in rat (Gajkowska and Mossakowski, 1997) or human (Doyle and Slater, 1997) is found in endothelial cells of blood vessels.

The present study was carried out against the above background to address two important issues on the negative feedback regulatory machinery on penile erection in the HF. First, is NO at the HF involved in this regulatory process? Second, are all three NOS isoforms engaged? Our results suggest that NO generated via both nNOS and iNOS in the HF may play an active role in negative feedback inhibition of penile erection.

MATERIALS AND METHODS

Animals and general preparations

The experimental procedures used in this study conformed with the guidelines of the institutional committee on experimental animals. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

Adult, male Sprague-Dawley rats (230-275 g) were purchased from the Experimental Animal Center, National Science Council, Taiwan, Republic of
China. Animals were anesthetized initially with chloral hydrate (400 mg/kg, i.p.) to perform preparative surgery. This included cannulation of the left femoral artery and vein for the measurement of systemic arterial pressure (SAP) and maintenance of anesthetic level by intravenous infusion of chloral hydrate (40 mg/kg/h). SAP was recorded through a pressure transducer (Gould P23XL, Valley View, OH) and a universal amplifier (Gould 20-4615-58). HR was derived from the SAP signals (Yang et al., 1996). The trachea was intubated to maintain patency of the airway. Animals were therefore fixed to a stereotaxic headholder (Kopf 1404, Tujunga, CA), and the rest of the body was placed on a heating pad to maintain body temperature at 37°C throughout the experiment.

**Recording of intracavernous pressure**

The increase in intracavernous pressure (ICP) was used as our experimental index for penile erection (Chen et al., 1992a,b, 1997; Chang et al., 1998a,b, 2000, 2001). In brief, a 26-gauge needle filled with saline and connected to a pressure transducer (Gould 231D, Valley View, OH) was inserted into the corpus cavernosum on one side. Intracavernous (i.c.) administration of saline (250 μl) was routinely given at the beginning of the experiment to ensure the lack of leakage. During the experiment, ICP, SAP and HR signals were digitized (Adaptec AHA-1520A, Milpitas, CA), stored on magneto-optical disk (Kyocera FRE-3651W-5P, Kyoto, Japan), and displayed continuously on a computer monitor.

**Microinjection of test agents into the hippocampal formation**

Microinjection bilaterally of test agents into the HF was carried out with a stereotaxically positioned 27-gauge stainless steel needle connected to a 0.5-μl Hamilton microsyringe (Reno, NV). The stereotaxic coordinates were 2.3-3.2 mm posterior to the bregma, 3.6-4.4 mm from the cortical surface, and 1.5-2.4 mm lateral to the midline (Chang et al., 1998a,b, 2000, 2001). A total of 50 nl
was delivered over 1-2 min to allow for full diffusion of the injected solution. In all cases, microinjection of the vehicle served as the volume and solvent control.

Test agents

Test agents used in the present study were freshly prepared during the experiment. These included L-arginine (RBI, Natick, MA), S-nitro-N-acetylpenicillamine (RBI), 7-nitroindazole (RBI), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (RBI), N\(^6\)-propyl-L-arginine (Tocris Cookson, Bristol, UK), N\(^5\)-(1-iminoethyl)-L-ornithine (Tocris Cookson), aminoguanidine (RBI), S-methylisothiourea (Tocris Cookson), and papaverine (U-Liang Pharmaceuticals, Taiwan, Republic of China). The doses used were the same as in a recent study (Chan et al., 2001) when these test agents were used for the same purpose as in the present study. With the exception of S-nitro-N-acetylpenicillamine and papaverine, which used respectively 0.2% DMSO or saline as the solvent, all test agents were dissolved in artificial cerebrospinal fluid (aCSF).

Histology

At the conclusion of the experiment, the brain of the animal was removed and fixed in 30% sucrose in 10% formaldehyde-saline for at least 72 h. Histologic verifications of the microinjection site in the HF were carried out on frozen 25-μm frozen sections stained with Neutral red.

Immunofluorescence staining for NOS isoforms and neurons

Animals were processed for double immunofluorescence staining for NOS isoforms and neurons by modifying the procedures we reported previously (Shih et al., 1996). In brief, free-floating 25-μm sections of the forebrain were incubated for 60-72 h at 4°C in 0.1 M phosphate buffered saline containing two
primary antisera. These included a polyclonal antiserum (Santa Cruz Biotechnology, Santa Cruz, CA; 1:1000) raised in rabbit against nNOS (sc-648) or eNOS (sc-654), or in goat against iNOS (sc-650G), together with a mouse monoclonal antiserum (Chemicon International, Temecula, CA, MAB377; 1:1000) against neuron-specific nuclear protein (NeuN), a marker for neurons (Mullen et al., 1992; Kempermann et al., 1998; Liu et al., 1998; Newcomb et al., 1999). The same sections were subsequently incubated concurrently with two appropriate secondary antisera (Molecular Probes, Eugene, OR; 1:500). These included a goat anti-rabbit IgG conjugated with Alexa Fluor 488 (A11034) for nNOS or eNOS, or rabbit anti-goat IgG conjugated with Alexa Fluor 488 (A11078) for iNOS; together with a goat anti-mouse IgG conjugated with Alexa Fluor 568 (A11004) for NeuN. Under a Fluorview 500 laser scanning confocal microscope (Olympus, Tokyo, Japan), immunoreactivity for NOS isoforms and NeuN exhibited respectively green and red fluorescence when viewed individually, and yellow fluorescence indicated co-localization of nNOS, iNOS or eNOS with NeuN. Control sections processed without both primary antisera or substituting both antisera with normal rabbit, goat or mouse serum showed negative immunoreactivity.

**Statistical analysis**

All values are expressed as mean ± SEM. Differences between treatment groups were statistically assessed using one-way analysis of variance, followed by the Dunnett or Scheffé multiple-range test for a posteriori comparison of means. $P < 0.05$ was considered to be statistically significant.
RESULTS

Hippocampal application of nitric oxide donors activated descending inhibition on penile erection

To qualify for a role in the negative feedback machinery on erectile functions, NO at the HF must be able to elicit a descending inhibition on penile erection. Our first series of experiments explored this possibility. Increasing NO exogenously by microinjection bilaterally into the HF of either a non-nitrate NO donor, S-nitro-N-acetylpenicillamine (Harrison and Bates, 1993) (0.25 or 1 nmol; Fig. 1), or the NO precursor, L-arginine (Szabo, 1996) (1 or 5 nmol; Fig. 2), evoked a dose-dependent reduction in baseline ICP without discernible changes in SAP or HR. Local application of their respective vehicle, DMSO (Fig. 1) or aCSF (Fig. 2), on the other hand, did not induce appreciable changes in baseline ICP, SAP or HR.

Involvement of endogenous nitric oxide at hippocampal formation in negative feedback inhibition on penile erection

Our second series of experiments was designed to establish that the endogenous NO at HF is involved in negative feedback regulation of penile erection. By definition, a negative feedback mechanism must be triggered and should not be tonically active. Similar to our previous observations (Chang et al., 2000, 2001), i.c. administration of papaverine (400 µg) evoked a discernible elevation in ICP (7.8 ± 1.2 to 56.7 ± 1.8 mmHg) in animals that received hippocampal application of aCSF. Pretreatment with microinjection bilaterally into the HF of a NO tapping agent (Yoshida et al., 1994; Rand and Li, 1995), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (10 nmol), significantly potentiated the papaverine-evoked elevation in ICP (56.7 ± 1.8 to 84.8 ± 2.4 mmHg). This effect was not accompanied by appreciable changes in MSAP (74.7 ± 4.5 vs 73.8 ± 5.3 mmHg) or HR (367.1 ± 14.6 vs 356.2 ± 17.2
bpm). The pretreatment by itself also did not elicit discernible alterations in baseline ICP, SAP or HR (Table 1).

**Involvement of iNOS and nNOS at hippocampal formation in negative feedback inhibition on penile erection**

Our third series of experiments was designed to further decipher the relative contribution of NOS isoforms at HF in negative feedback inhibition of penile erection. Pretreatment by hippocampal application of an equimolar dose (2.5 pmol) of two selective nNOS inhibitors (Fig. 3), 7-nitroindazole (Moore et al., 1993; Moore and Handy, 1997; Maren, 1998; Osborne and Coderre, 1999) or N\(^\text{e}\)-propyl-L-arginine (Zhang et al., 1997; Lee et al., 1999b; Cooper et al., 2000), appreciably augmented the papaverine-evoked elevation in ICP without changing MSAP or HR. Similar observations were made with microinjection bilaterally into the HF of an equimolar dose (250 pmol) of two selective iNOS inhibitors (Fig. 4), aminoguanidine (Griffiths et al., 1993; Corbett and McDaniel, 1996; Moore and Handy, 1997; Mattson et al., 1998; Osborne and Coderre, 1999) or S-methylisothiourea (Szabo et al., 1994; Southan et al., 1995; Wildhirt et al., 1996; Mitaka et al., 2000). On the other hand, the papaverine-evoked increase in ICP remained essentially unchanged (58.1 ± 1.1 to 58.2 ± 3.2 mmHg) after local administration of an eNOS inhibitor (Rees et al., 1990; Wilderman and Armstead, 1998; McDuffie et al., 1999), N\(^5\)-(1-iminoethyl)-L-ornithine (92 nmol), into the HF. Hippocampal administration of aCSF or all these NOS inhibitors elicited minimal alterations in baseline ICP, MSAP or HR (Table 1).

**Topographic differences in efficacy of test agents in the hippocampal formation**

Histologic verifications confirmed that all effective microinjection sites in the HF were distributed randomly within the CA1 or CA3 subfield or dentate gyrus (Fig. 5). Further quantitative analysis, however, revealed subtle topographic differences in the efficacy of the test agents. Microinjection sites on which NO
donors elicited >80% reduction in baseline ICP were located in the CA1 subfield or dentate gyrus of the HF. Similarly, microinjection of NO trapping agent or nNOS or iNOS inhibitors into these two regions of the HF potentiated the papaverine-evoked elevation in ICP by >80%. On application locally to the CA3 subfield, NO donors elicited 40-60% reduction in baseline ICP; and NO trapping agent or nNOS or iNOS inhibitors also exhibited only 40-60% augmentation of the papaverine-evoked ICP. With a potency of ≤5%, microinjection of test agents into the hilus, stratum radiatum or subiculum was essentially ineffective.

**Differential distribution of NOS isoforms in the hippocampal formation**

Our double immunofluorescent staining experiments provided further credence to the identified topographic differences in efficacy of our test agents. As illustrated in Fig. 6A, co-localization of immunoreactivity for nNOS and the neuronal marker, NeuN, was present in the pyramidal cell layers of CA1 subfield and granule cell layers of dentate gyrus, but was appreciably less among the pyramidal cells in the CA3 area. A comparable distribution pattern was observed for the prevalent presence of iNOS immunoreactive neurons (Fig. 6B). Closer observation revealed an intriguing finding. Whereas iNOS-immunoreactivity co-localized overwhelmingly with NeuN-positive cells (Fig. 6E), hippocampal neurons that also manifested nNOS-immunoreactivity were less ubiquitous (Fig. 6D). Immunofluorescence indicative of eNOS was found primarily in blood vessels (Fig. 6C). Interestingly, many of these eNOS-positive vessels intermingled with the pyramidal or granule cells in Ammon's horn or dentate gyrus (Fig. 6F).
DISCUSSION

The present study provided the first demonstration that NO generated by nNOS and iNOS in the HF plays an active role in negative feedback regulation of penile erection. Correlations between hippocampal EEG activity during sexual behavior revealed that male rats exhibit slow, high-amplitude waves after intromission and ejaculation (Kurtz and Adler, 1973). This characteristic EEG manifestation is interpreted to indicate an inhibitory process or sexual satiety. We further proposed (Chang et al., 1998a) that the increase in magnitude of hippocampal EEG signals that invariably accompanies an elevation in ICP may represent the trigger for negative feedback inhibition on penile erection. It is therefore interesting to note that our results revealed that an increase in the amount of NO at the HF elicited a descending inhibition on penile erection. On the other hand, removal of endogenous NO by a NO trapping agent or blockade of nNOS or iNOS activity in hippocampal neurons potentiated the papaverine-evoked elevation in ICP. Taken together, it is likely that ascending sensory inputs initiated by tumescence of the penis may trigger the negative feedback inhibitory mechanism in the HF via NO generated by nNOS and iNOS.

We also identified topographic differences in the contribution of nNOS or iNOS from major divisions of the HF. Thus, microinjection of NO donors into the CA1 subfield or dentate gyrus was more efficacious than into the CA3 area in eliciting a reduction in baseline ICP. Likewise, compared to the CA3 subfield, nNOS or iNOS inhibitors were more effective when locally applied to the CA1 area or dentate gyrus in potentiating the papaverine-evoked elevation in ICP. Intriguingly, we also found that neurons that exhibited immunoreactivity against nNOS or iNOS were more conspicuously present in the CA1 subfield and dentate gyrus than in the CA3 area. Similar topographic distribution of nNOS immunoreactive neurons was reported by Jinno et al. (1999). Whereas eNOS-positive blood vessels intermingled with pyramidal cells in the Ammon's
horn and granule cells in the dentate gyrus, our pharmacologic results suggested that this NOS isoform may not play an active role in negative feedback mechanism on penile erection.

Another important contribution of the present study is to unveil a physiologic role for iNOS at the HF in NO-promoted negative feedback regulation of penile erection. This novel notion that iNOS is functionally active under physiologic conditions seemingly contradicts the general contention (Murphy et al., 1993; Szabo, 1996; Wong et al., 1996) that iNOS is induced only by proinflammatory stimuli. We noted, however, that a physiologic role for iNOS has been reported in the regulation of arterial pressure via an action on renal tubules (Mattson et al., 1998). Our laboratory (Chan et al., 2001) also demonstrated recently that iNOS in the rostral ventrolateral medulla, the medullary origin of sympathetic vasomotor tone, is tonically active under physiologic conditions at the levels of functional expression and molecular synthesis. Several studies (Murphy et al., 1993; Wong et al., 1996; Kitamura et al., 1998) indicate that NO may be generated in the central nervous system by iNOS present in microglia or astrocytes. Results from our double immunofluorescent staining experiment indicate that iNOS is also prevalently present in the pyramidal cells of the Ammon's horn and granule cells of the dentate gyrus.

An important premise for the interpretation of our results is the selectivity of our test agents, in particular the nNOS and iNOS inhibitors. Handy and Moore (1998) commented that, on the balance of evidence presently available and until even more selective antagonists are available, 7-nitroindazole is a useful experimental tool to study the roles of nNOS. The low Ki value indicates that N\textsuperscript{6}-propyl-L-arginine is a highly selective competitive inhibitor of nNOS (Zhang et al., 1997; Lee et al., 1999b; Cooper et al., 2000). That microinjection bilaterally into the HF of these two test agents, at equimolar dose, elicited comparable augmentation of the papaverine-evoked elevation of ICP therefore attested to the engagement of nNOS at the HF in negative feedback regulation
of penile erection. Aminoguanidine has been reported to be 26 times more potent in inhibiting iNOS than nNOS activity (Moore and Handy, 1997). In addition, calcium-dependent NOS activity is not significantly altered by aminoguanidine (Mattson et al., 1998). That comparable results were obtained from treatments with S-methylisothiourea, another potent inhibitor of iNOS (Szabo et al., 1994; Wildhirt et al., 1996; Milaka et al., 2000), again validated a functional role for iNOS at the HF in negative feedback inhibition on penile erection.

Three additional observations confirmed the specificity of our experimental observations. First, by definition, a negative feedback mechanism must be triggered and should not be tonically active. This prerequisite was satisfied when microinjection bilaterally of 7-nitroindazole, N⁶-propyl-L-arginine, aminoguanidine, S-methylisothiourea or N⁵-(1-iminoethyl)-L-ornithine) into the HF did not significantly alter baseline ICP. Second, all our results were obtained under minimal alterations in SAP and HR. Thus, the observed changes in ICP may not be secondary to hemodynamic perturbations. Third, the lack of significant effects by the vehicles on both baseline and papaverine-evoked increase in ICP ascertained that the physical action of microinjection and the chemical properties of both solvents were not a confounding factor.

In conclusion, the present study demonstrated that NO generated by nNOS or iNOS at the HF participates actively in the negative feedback regulation of penile erection. Melis and Argiolas (1997) suggest that NO is the common mediator for several neurotransmitters that control erectile functions at the central nervous system. It follows that NO may participate in negative feedback control of erectile functions by modulating the action of another neurotransmitter that is known to be engaged in this regulatory process in the HF. A likely candidate, in this regard, is norepinephrine. Presynaptic modulation of norepinephrine release by NO in the HF has been reported
(Lonart et al., 1992; Lauth et al., 1995; Satoh et al., 1996); and stimulation of α-adrenoceptors resulted in activation of the NO-cGMP pathway (Aguillo et al., 1995). Our laboratory demonstrated recently (Chang et al., 2001) that noradrenergic innervation of the HF that originates from the locus coeruleus plays an active role in negative feedback regulation of penile erection, possibly via at least α₁- or α₂-adrenoceptors in the HF. Speculatively, ascending sensory inputs initiated by tumescence of the penis may activate the α-adrenoceptors on pyramidal or granule cells via the locus coeruleus-HF noradrenergic projection. This in turn activates both nNOS and iNOS, and the NO thus generated may act presynaptically to promote further release of norepinephrine, resulting in the perpetuation of negative feedback inhibitory mechanism. This speculation, however, awaits further elucidation.

Acknowledgments

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FIGURE 1. Maximal effects on intracavernous pressure (ICP) of microinjection bilaterally into the hippocampal formation of S-nitro-N-acetylpenicillamine (SNAP; 0.25 or 1 nmol) or 0.2% DMSO. The maximal values measured during 10 min of baseline recording and within 30 min after administration of SNAP or DMSO are presented as mean ± SEM, n = 5-7 animals per group. *P < 0.05 vs baseline group (Basal) in the Dunnett analysis or DMSO group in the Scheffé analysis. Note lack of response in mean systemic arterial pressure (MSAP) or heart rate (HR).

FIGURE 2. Maximal effects on intracavernous pressure (ICP) of microinjection bilaterally into the hippocampal formation of L-arginine (1 or 5 nmol) or aCSF. The maximal values measured during 10 min of baseline recording and within 30 min after administration of L-arginine or aCSF are presented as mean ± SEM, n = 5-7 animals per group. *P < 0.05 vs baseline group (Basal) in the Dunnett analysis or aCSF group in the Scheffé analysis. Note lack of response in mean systemic arterial pressure (MSAP) or heart rate (HR).

FIGURE 3. Maximal effect of intracavernous (i.c.) administration of papaverine (400 μg) Pap) on intracavernous pressure (ICP), evaluated 30 min after animals were pretreated with microinjection bilaterally into the hippocampal formation of equimolar doses (2.5 pmol) of 7-nitroindazole (7-Ni), Nω-propyl-L-arginine (NPLA) or aCSF. Note lack of response in mean systemic arterial pressure (MSAP) or heart rate (HR). Values are presented as mean ± SEM, n = 5-7 animals per group. *P < 0.05 vs baseline group (Basal) in the Dunnett analysis, and *P < 0.05 vs aCSF group in the Scheffé analysis.
FIGURE 4. Maximal effect of intracavernous (i.c.) administration of papaverine (400 µg; Pap) on intracavernous pressure (ICP), evaluated 30 min after animals were pretreated with microinjection bilaterally into the hippocampal formation of equimolar doses (250 pmol) of aminoguanidine (AG), S-methylisothiourea (SMT) or aCSF. Note lack of response in mean systemic arterial pressure (MSAP) or heart rate (HR). Values are presented as mean ± SEM, n = 5-7 animals per group. *P < 0.05 vs baseline group (Basal) in the Dunnett analysis, and *P < 0.05 vs aCSF group in the Scheffé analysis.

FIGURE 5. Diagrammatic representation of the hippocampal formation at two rostral-caudal levels showing the location of sites in the CA1 or CA3 subfield or dentate gyrus (DG) where microinjection of test agents or vehicle was delivered. Shown are sites on which NO donors elicited >80% (●), 40-60% (○) or <5% (△) reduction in baseline ICP; and where NO trapping agent or NOS inhibitors potentiated the papaverine-evoked increase in ICP by >80% (●), 40-60% (○) or <5% (△). For clarity, only 30% of the total microinjection sites are included. Numbers on the right side of each diagram represent the distance from the bregma.

FIGURE 6. Laser scanning confocal microscope images of the hippocampal formation showing double immunofluorescence staining for nNOS (A,D), iNOS (B,E) or eNOS (C,F) and NeuN (A-F). D,E,F were obtained respectively from dentate gyrus, or CA1 or CA3 subfield. Note that immunoreactivity for individual NOS isoform exhibits green, NeuN manifests red, and double label displays yellow fluorescence. Scale bars, 150 µm in A,B,C; and 15 µm in D,E,F.
TABLE 1. Effects of test agents on baseline intracavernous pressure, mean systemic arterial pressure or heart rate

<table>
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<tr>
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<th>ICP (mmHg)</th>
<th>MSAP (mmHg)</th>
<th>HR (bpm)</th>
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<tr>
<td>L-NIO</td>
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<td>359.2 ± 13.2</td>
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Maximal effects on baseline intracavernous pressure (ICP), mean systemic arterial pressure (MSAP) or heart rate (HR), 30 min after microinjection bilaterally into the hippocampal formation of aCSF, DMSO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO; 10 nmol), 7-nitroindazole (7-NI; 2.5 pmol), N\textsuperscript{\text{\textdegree}}-propyl-L-arginine (NPLA; 2.5 pmol), aminoguanidine (AG; 250 pmol), S-methylisothiouria (SMT; 250 pmol), or N\textsuperscript{\text{\textdegree}}-trimethyl-L-ornithine (L-NIO; 92 nmol). Values presented are mean ± SEM, n = 5-7 animals per group. No significant difference (P > 0.05) exists among the treatment groups in one-way analysis of variance.
MSAP (mmHg)

HR (bpm)

ICP (mmHg)

SigmaPlot for Windows
Filename: Fig4(Pap-iNOSi)