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Differential contributions of nitric oxide synthase isoforms at hippocampal formation to negative feedback regulation of penile erection in the rat

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1 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.

2 Adult, male Sprague-Dawley rats that were anaesthetized 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.

3 Microinjection bilaterally of a NO donor, S-nitroso-N-acetylpenicillamine (0.25 or 1 nmoles), or the NO precursor, L-arginine (1 or 5 nmoles), into the hippocampal CA1 or CA3 subfield or dentate gyrus elicited a significant reduction in baseline ICP.

4 Bilateral hippocampal application of a NO trapping agent, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (10 nmoles), significantly potentiated the elevation in ICP induced by intracavernous administration of papaverine (400 μg).

5 Microinjection bilaterally into the HF of equimolar doses (0.5 or 2.5 pmoles) of two selective neuronal NOS inhibitors, 7-nitroindazole or N^{ω}-propyl-L-arginine; or equimolar doses (50 or 250 pmoles) 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 a potent endothelial NOS inhibitor, N^5-(1-iminoethyl)-L-ornithine (18 or 92 nmoles), was ineffective. Neither of these inhibitors, furthermore, affected baseline ICP.

6 These results suggest that NO generated *via* both neuronal and inducible NOS at the HF may participate in negative feedback regulation of penile erection.
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Keywords: CA1 or CA3 subfield; dentate gyrus; hippocampal formation; intracavernous pressure; neuronal, inducible and endothelial nitric oxide synthase; nitric oxide; penile erection; negative feedback

Abbreviations: aCSF, artificial cerebrospinal fluid; AG, aminoguanidine; carboxy-PTIO, carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; eNOS, endothelial nitric oxide synthase; HF, hippocampal formation; HR, heart rate; ICP, intracavernous pressure; iNOS, inducible nitric oxide synthase; L-Arg, L-arginine; L-NIO, N^5-(1-iminoethyl)-L-ornithine; MSAP, mean systemic arterial pressure; 7-NI, 7-nitroindazole; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NPLA, N^{ω}-propyl-L-arginine; SAP, systemic arterial pressure; SMT, S-methylisothiourea; SNAP, S-nitroso-N-acetylpenicillamine

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 & Wagner, 1995; Giuliano *et al*., 1995; Meston & Frolich, 2000). The role of NO in central regulation of penile erection, on the other hand, is much less documented.

One potential site of action 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 & Ploog (1962) and Dua & MacLean (1964), the HF has long been assigned a role in the regulation of erectile functions (Steers, 1990; Andersson & Wagner, 1995; Giuliano *et al*., 1995). Our laboratory (Chen *et al*., 1992b; 1997) 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.

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Nitric oxide synthase (NOS) is an enzyme involved in the generation of NO from L-arginine (Moncada & 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 conspicuously present in the pyramidal layer of Ammon's horn and molecular layer of dentate gyrus (Valtschanoff *et al*., 1993). That these may represent nNOS-immunoreactive HF neurons has been demonstrated (Doyle & Slater, 1997; Lopez-Figueroa *et al*., 1998; Jinno *et al*., 1999; Reagan *et al*., 1999). Hippocampal eNOS immunoreactivity is present in endothelial cells of blood vessels (Doyle & Slater, 1997; Gajkowska & Mossakowski, 1997), or in pyramidal cells (Dinerman *et al*., 1994). However, exhibition of iNOS immunoreactivity in HF is reportedly sparse (Lee *et al*., 1999a).

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, but not by eNOS, in the HF may play an active role in negative feedback inhibition of penile erection.

Methods

Animals and general preparations

The experimental procedures used in this study conformed to the guidelines of our 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 Experimenal Animal Center, National Science Council, Taiwan, Republic of China. Animals were anaesthetized initially with chloral hydrate (400 mg kg^{-1}, 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 anaesthetic level by intravenous infusion of chloral hydrate (40 mg kg h^{-1}). Previous studies (Chang *et al*., 1998a, b; 2000; 2001) indicated that this management scheme provided satisfactory anaesthetic level while preserving the capacity of central cardiovascular regulation. SAP was recorded through a pressure transducer (Gould P23XL, Valley View, OH, U.S.A.) 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, U.S.A.), 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 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 23ID) 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, U.S.A.), stored on magneto-optical disk (Kyocera FRE-3651W-5P, Kyoto, Japan), and displayed continuously on a computer monitor. Intracavernous injection of papaverine was used to elicit an increase in ICP (Chen *et al*., 1992a; Chang *et al*., 1998a; 2001). This vascular smooth muscle relaxant induces penile erection by promoting increase in inflow of arterial blood, distension of sinusoids and possible restriction of venous outflow (Lue & Tanagho, 1987). Local application of papaverine to the corpus cavernosus has been used in therapeutic management of impotent patients (Soli *et al*., 1998).

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, U.S.A.). The stereotaxic co-ordinates 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 injection 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 a non-nitrate NO donor (Harrison & Bates, 1993), S-nitroso-N-acetylpenicillamine (SNAP; RBI, Natick, MA, U.S.A.); the NO precursor (Szabo, 1996), L-arginine (L-Arg; RBI); a NO trapping agent (Yoshida *et al*., 1994; Rand & Li, 1995), 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO; RBI); the selective nNOS inhibitors, 7-nitroindazole (7-NI; RBI) (Moore *et al*., 1993; Moore & Handy, 1997; Maren, 1998; Osborne & Coderre, 1999) and N^{ω}-propyl-L-arginine (NPLA; Tocris Cookson, Bristol, U.K.) (Zhang *et al*., 1997; Lee *et al*., 1999b; Cooper *et al*., 2000); the selective iNOS inhibitors, aminoguanidine (AG; RBI) (Griffiths *et al*., 1993; Corbett & McDaniel, 1996; Moore & Handy, 1997; Mattson *et al*., 1998; Osborne & Coderre, 1999) and S-methylisothiourea (SMT; Tocris Cookson) (Szabo *et al*., 1994; Southan *et al*., 1995; Wildhirt *et al*., 1996; Mitaka *et al*., 2000); a potent eNOS inhibitor (Rees *et al*., 1990; Wilderman & Armstead, 1998; McDuffie *et al*., 1999), N^5-(1-iminoethyl)-L-ornithine (L-NIO; Tocris Cookson) and a vasodilator, 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 SNAP and papaverine, which used respectively 0.2% DMSO and saline as the solvent, all test agents were dissolved in artificial cerebrospinal fluid (aCSF). The composition of

aCSF was (mM): NaCl 117, $NaHCO_3$ 25, Glucose 11, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2 and NaH_2PO_4 1.2.

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 sections stained with Neutral red, aided by the addition of 1% Evans blue into the injection medium.

Statistical analysis

All values are expressed as mean $\pm$ s.e.mean. 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 a non-nitrate NO donor, SNAP (0.25 or 1 nmoles) evoked a dose-dependent reduction in baseline ICP (Figure 1) without significant changes in SAP or HR (Table 1). Comparable observations (Figure 1 and Table 1) were made by augmenting the endogenous production of NO *via* administration bilaterally into the HF of the NO precursor, L-Arg (1 or 5 nmoles). Local application of their respective vehicle, DMSO or aCSF, on the other hand, did not induce appreciable changes in baseline ICP, SAP or HR (Figure 1, Table 2).

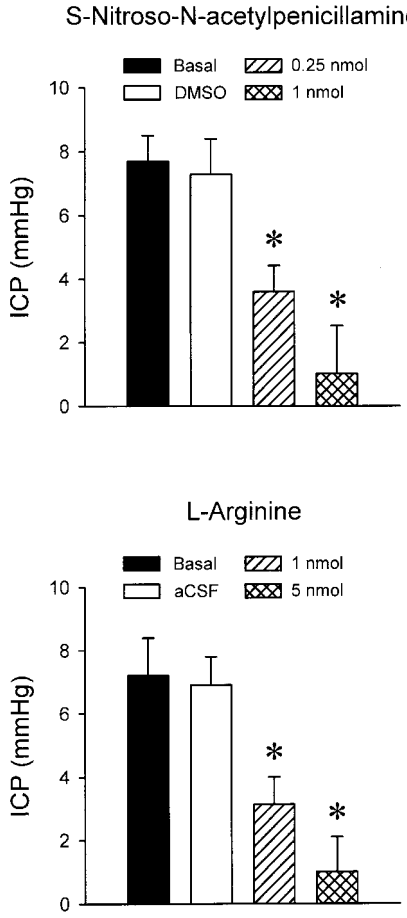

Figure 1 Maximal effects on intracavernous pressure (ICP) of microinjection bilaterally into the HF of SNAP (0.25 or 1 nmoles), 0.2% DMSO, L-Arg (1 or 5 nmoles) or aCSF. The maximal values measured during 10 min of baseline recording and within 30 min after administration of SNAP, L-Arg or either vehicle are presented as mean $\pm$ s.e.mean, $n=5-7$ animals per group. $^*P<0.05$ *vs* pretreatment control group (Basal) in the Dunnett analysis or DMSO or aCSF group in the Scheffé analysis.

We further differentiated the contribution of NOS isoforms to the endogenous NO generated by L-Arg at the HF in eliciting the descending inhibition on penile erection. Co-microinjection into the HF (Figure 2) of L-Arg (5 nmoles), together with either the selective nNOS inhibitor, 7-NI (2.5 pmoles) or the selective iNOS inhibitor, AG (250 pmoles), blunted approximately 40–60% of the decrease in ICP induced by L-Arg (5 nmoles) and aCSF. Co-administration of L-Arg (5 nmoles) and the potent eNOS inhibitor, L-NIO (92 nmoles), on the other hand, exerted minimal antagonizing effect (Figure 2).

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 trapping agent, carboxy-PTIO (10 nmoles), 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 or HR (Table 1). The pre-treatment by itself also did not elicit discernible alterations in baseline ICP, SAP or HR (Table 2).

Differential involvement of nNOS, iNOS or eNOS at hippocampal formation in negative feedback inhibition on penile erection

Our third series of experiments was designed to further decipher the relative contributions of NOS isoforms at HF to negative feedback inhibition of penile erection. Pre-treatment by hippocampal application of equimolar doses (0.5 or 2.5 pmoles) of two selective nNOS inhibitors, 7-NI or NPLA,

Table 1 Changes in systemic arterial pressure or heart rate during evaluation of treatment effects of test agents on intracavernous pressure

Test agent	*MSAP* (mm Hg)	*HR* (b.p.m.)
DMSO	77.5±4.2	363.4±12.4
aCSF	74.6±3.9	359.8±12.8
SNAP	77.4±6.0	365.8±13.8
L-Arg	76.3±3.6	357.2±13.7
L-Arg + aCSF	77.2±2.9	364.1±9.8
L-Arg + 7-NI	78.6±2.9	370.3±11.7
L-Arg + AG	78.9±5.4	378.1±14.5
Carboxyl-PTIO	74.7±4.5	367.1±14.6
7-NI	78.6±4.8	365.2±12.6
NPLA	78.0±4.1	362.5±11.9
AG	77.4±4.2	354.2±13.7
SMT	78.4±4.0	361.1±13.0
L-NIO	78.2±4.2	361.2±11.8

Maximal changes in mean system arterial pressure (MSAP) or heart rate (HR) during the evaluation of effects on baseline or papaverine-evoked increase in intracavernous pressure 30 min after microinjection bilaterally into the HF of 0.2% DMSO, aCSF, SNAP (5 nmoles), L-Arg (5 nmoles), L-Arg (5 nmoles)+aCSF, L-Arg (5 nmoles)+7-NI (2.5 pmoles), L-Arg (5 nmoles)+AG (250 pmoles), carboxy-PTIO (10 nmoles), 7-NI (2.5 nmoles), NPLA (2.5 pmoles), AG (250 pmoles), SMT (250 pmoles) or L-NIO (92 nmoles). For clarity, only the effects of the higher dose of each test agent were given. Values presented are mean±s.e.mean, $n=5-6$ animals per group. No significant difference ($P>0.05$) exists among the treatment groups in one-way analysis of variance.

Table 2 Effects of test agents on baseline intracavernous pressure, mean systemic arterial pressure or heart rate

Test agent	*ICP* (mm Hg)	*MSAP* (mm Hg)	*HR* (b.p.m.)
aCSF	7.7±1.3	78.6±4.5	368.1±10.1
Carboxyl-PTIO	7.9±1.5	79.4±3.8	368.5±12.5
7-NI	7.8±1.2	77.9±3.2	361.1±10.5
NPLA	7.7±1.3	78.1±3.7	460.6±12.8
AG	7.6±1.1	78.1±3.2	362.8±10.4
SMT	7.8±1.3	78.6±4.1	359.7±13.1
L-NIO	7.7±1.2	78.4±3.2	359.2±13.2

Maximal effects on baseline intracavernous pressure (ICP), mean system arterial pressure (MSAP) or heart rate (HR), 30 min after microinjection bilaterally into the hippocampal formation of aCSF, carboxyl-PTIO (10 nmoles), 7-NI (2.5 pmoles), NPLA (2.5 pmoles), AG (250 pmoles), SMT (250 pmoles) or L-NIO (92 nmoles). For clarity, only the effects of the higher dose of each test agent were given. Values presented are mean±s.e.mean, $n=5-6$ animals per group. No significant difference ($P>0.05$) exists among the treatment groups in one-way analysis of variance.

significantly and dose-dependently augmented the papaverine-evoked elevation in ICP (Figure 3) without changing MSAP or HR (Table 1). Similar observations (Figure 4 and Table 1) were made with microinjection bilaterally into the HF of equimolar doses (50 or 250 pmoles) of two selective iNOS inhibitors, AG or SMT. On the other hand, the papaverine-evoked increase in ICP remained essentially unchanged after local administration of a potent eNOs inhibitor, L-NIO (18 or 92 nmoles), into the HF (Figure 5). Hippocampal administration of aCSF or all these NOS inhibitors also elicited minimal alterations in baseline ICP, SAP or HR (Table 2).

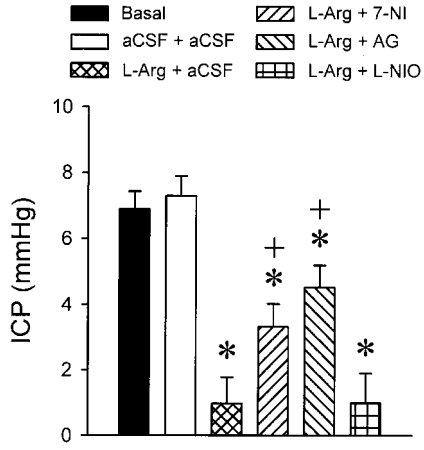

Figure 2 Maximal effects on ICP of co-microinjection bilaterally into the HF of aCSF+aCSF, or L-Arg (5 nmoles) together with aCSF, 7-NI (2.5 pmoles), AG (250 pmoles) or L-NIO (92 nmoles). The maximal values measured during 10 min of baseline recording and within 30 min after administration of the test agents are presented as mean±s.e.mean, $n=5-7$ animals per group. *$P<0.05$ *vs* pretreatment control group (Basal) in the Dunnett analysis or aCSF+aCSF group in the Scheffé analysis, and *$P<0.05$ *vs* L-Arg+aCSF group in the Scheffé analysis.

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 (Figure 6). Further quantitative analysis, however, revealed subtle topographic differences in the efficacy of the test agents. Microinjection sites on which SNAP or L-Arg elicited >80% reduction in baseline ICP were located in the CA1 subfield or dentate gyrus of the HF. Similarly, microinjection of carboxy-PTIO, 7-NI, 7-NPLA, AG or SMT 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 an efficacy ⩽5%, microinjection of L-NIL into the CA1 or CA3 subfield or DG, or the other test agents into the hilus, stratum radiatum or subiculum, was essentially ineffective.

Discussion

The present study provided the first demonstration that NO generated by nNOS and iNOS, but not by eNOS, in the HF plays an active role in negative feedback regulation of penile erection. Correlations between hippocampal EEG activity during sexual behaviour revealed that male rats exhibit slow, high-amplitude waves after intromission and ejaculation (Kurtz & 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

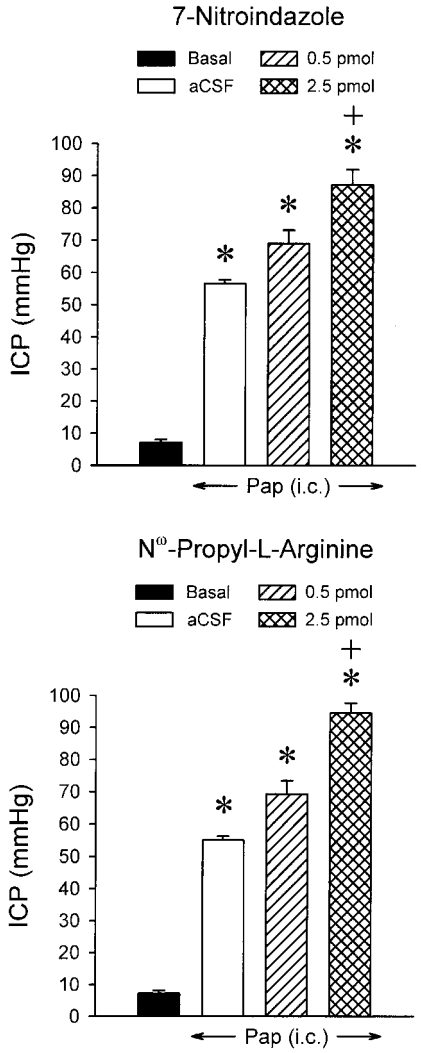

Figure 3 Maximal effect of intracavernous (i.c.) administration of papaverine (400 μg; Pap) on ICP, evaluated 30 min after animals were pretreated with microinjection bilaterally into the HF of equimolar doses (0.5 or 2.5 pmoles) of 7-NI, NPLA or aCSF. Values are presented as mean$\pm$s.e.mean, $n=5-7$ animals per group. *$P<0.05$ *vs* pretreatment control group (Basal) in the Dunnett analysis, and $+P<0.05$ *vs* aCSF group in the Scheffé analysis.

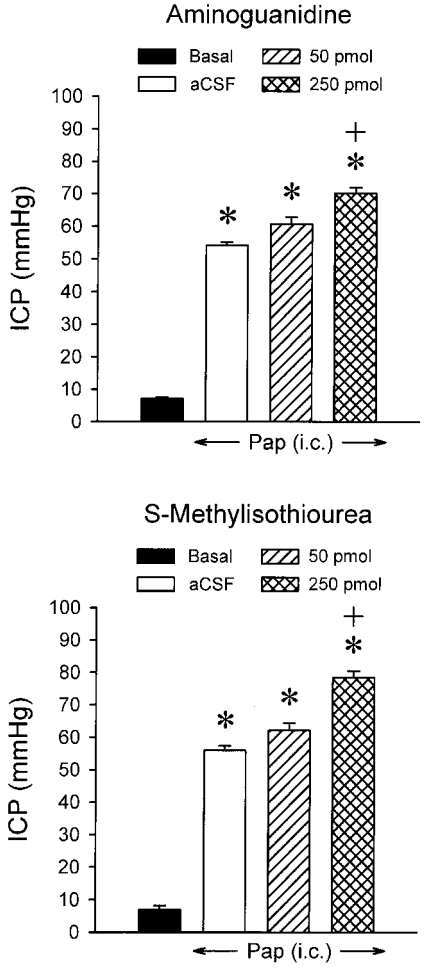

Figure 4 Maximal effect of i.c. administration of papaverine (400 μg; Pap) on ICP, evaluated 30 min after animals were pretreated with microinjection bilaterally into the HF of equimolar doses (50 or 250 pmoles) of AG, SMT or aCSF. Values are presented as mean$\pm$s.e.mean, $n=5-7$ animals per group. *$P<0.05$ *vs* pretreatment control group (Basal) in the Dunnett analysis, and $+P<0.05$ *vs* aCSF group in the Scheffé analysis.

an increase exogenously or endogenously in the amount of NO at the HF elicited a descending inhibition on penile erection. Furthermore, inhibitors of both nNOS and iNOS blunted the engagement of endogenous NO in this inhibitory process. 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. Our results further suggested that the contribution of eNOS to this process is minimal.

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

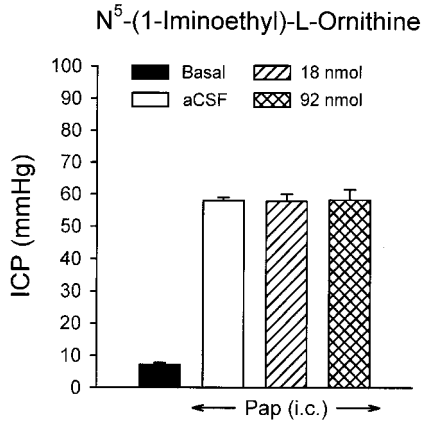

Figure 5 Maximal effect of i.c. administration of papaverine (400 μg; Pap) on ICP, evaluated 30 min after animals were pretreated with microinjection bilaterally into the HF of L-NIO (18 or 92 nmoles) or aCSF. Values are presented as mean$\pm$s.e.mean, $n=5-7$ animals per group. No significant difference ($P>0.05$) exists among the treatment groups in one-way analysis of variance.

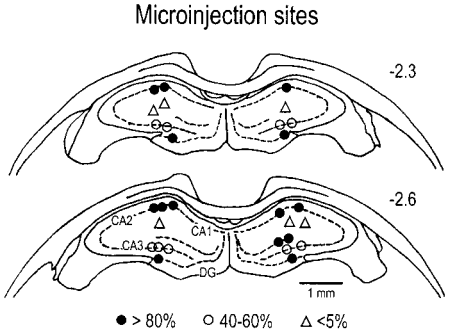

Figure 6 Diagrammatic representation of the HF at two rostral–caudal levels showing the location of sites in the CA1 or CA3 subfield or dentate gyrus (DG) where microinjection of test agents or vehicle was delivered. Shown are sites on which L-Arg or SNAP elicited >80, 40–60 or <5% reduction in baseline ICP; and where carboxy-PTIO or 7-NI, NPLA, AG, SMT or L-NIO reversed the papaverine-evoked increase in ICP by >80, 40–60 or <5%. For clarity, only 15% of the total microinjection sites are included. Numbers on the right side of each diagram represent the distance from the bregma.

physiologic conditions seemingly contradicts the general contention (Murphy *et al*., 1993; Szabo, 1996; Wong *et al*., 1996) that iNOS is induced only by pro-inflammatory 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. Whereas iNOS and nNOS at the ventrolateral medulla are responsible respectively for sympatho-inhibition and sympatho-excitation, the present study indicated that these two NOS isoforms at the HF function synergistically in negative feedback of penile erection. 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. Whether our unveiled function of iNOS may take origin from these glial cells in the HF remains to be clarified.

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. Hippocampal pyramidal neurons of CA1 or CA3 subfield and granule cells of dentate gyrus express nNOS mRNA (Lopez-Figueroa *et al*., 1998; Reagan *et al*., 1999). Immunohistochemical studies also revealed that nNOS-containing HF neurons are present in mouse (Jinno *et al*., 1999) or human (Doyle & Slater, 1997). Of particular relevance to the present study is that Jinno *et al*. (1999) reported that cells immunoreactive to nNOS are more conspicuously present in the CA1 subfield and dentate gyrus than in the CA3 area. Manifestation of iNOS immunoreactivity in HF is reportedly sparse in human (Lee *et al*., 1999a). However, preliminary results from our laboratory (Chang, Chan & Chan, unpublished data) revealed that iNOS-immunoreactive neurons that exhibited a topographic distribution pattern comparable to nNOS were also present in the HF. Hippocampal eNOS immunoreactivity in rat (Gajkowska & Mossakowski, 1997) or human (Doyle & Slater, 1997) is found in endothelial cells of blood vessels, or CA1 pyramidal cells in rat (Dinerman *et al*., 1994). Our pharmacologic results suggested that this NOS isoform may not play an active role in negative feedback mechanism on penile erection.

An important premise for the interpretation of our results is the selectivity of our test agents. Handy & Moore (1998) commented that, on the balance of evidence presently available and until even more selective antagonists are available, 7-NI is a useful experimental tool to study the functional roles of nNOS. The low K_i value indicates that NPLA 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 doses, 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. AG has been reported to be 26 times more potent in inhibiting iNOS than nNOS activity (Moore & Handy, 1997). In addition, calcium-dependent NOS activity is not significantly altered by AG (Mattson *et al*., 1998). That comparable results were obtained from treatments with SMT, another selective inhibitor of iNOS (Szabo *et al*., 1994; Wildhirt *et al*., 1996; Mitaka *et al*., 2000), again validated a functional role for iNOS at the HF in negative feedback inhibition on penile erection. That L-NIO is a potent eNOS inhibitor has been amply documented (Rees *et al*., 1990; Wilderman & Armstead, 1998; McDuffie *et al*., 1999).

Four 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-NI, NPLA, AG, SMT or L-NIO 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 haemodynamic 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. Fourth, i.c. injection of papaverine may activate nociceptive afferents in the erectile tissues. As such, it is possible that the ascending sensory inputs initiated by tumescence of the penis, which trigger the negative feedback regulatory mechanism on penile erection in the HF, may be contaminated by nociceptive information. This possibility was deemed unlikely because our animals were appropriately anaesthetized. Furthermore, the increase in ICP elicited by i.c. administration of papaverine was not accompanied by discernible changes in SAP or HR, an indicator of nociception.

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 &

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 at 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-cyclic GMP pathway (Agullo *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 α_1- or α_2-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.

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