Central modulation of the NO/cGMP pathway affects the MPOA-induced intracavernous pressure response

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Sato, Yoshikazu, Weixin Zhao, and George J. Christ. Central modulation of the NO/cGMP pathway affects the MPOA-induced intracavernous pressure response. Am J Physiol Regulatory Integrative Comp Physiol 281: R269–R278, 2001.—Alterations in the nitric oxide (NO)/cGMP levels in hypothalamic nuclei, including the medial preoptic area (MPOA), regulate critical aspects of sexual behavior and penile reflexes. However, the effects of altered central nervous system (CNS) NO/cGMP levels at the end organ level, that is, on the magnitude/quality of the erection so achieved [intracavernous pressure (ICP) response], has yet to be evaluated. The goal of this report was to evaluate the effects of intrathecal administration of modulators of NO and cGMP levels on ICP responses to stimulation of the MPOA and cavernous nerve in rats in vivo. In all cases, intrathecal administration of compounds that increase and decrease cGMP and NO levels, respectively, was associated with corresponding increases and decreases in the MPOA-stimulated ICP response. Specifically, sodium nitroprusside (SNP), 8-bromo-cGMP, and sildenafil increased the MPOA-stimulated ICP response, whereas Nω-nitro-L-arginine methyl ester reduced it. None of the intrathecal treatments had detectable effects on blood pressure or the cavernous nerve-stimulated ICP response, although intravenous sildenafil increased the latter. These data clearly indicate that intrathecal administration of modulators of NO and cGMP levels on ICP responses to stimulation of the MPOA and cavernous nerve in rats in vivo. In all cases, intrathecal administration of compounds that increase and decrease cGMP and NO levels, respectively, was associated with corresponding increases and decreases in the MPOA-stimulated ICP response. Specifically, sodium nitroprusside (SNP), 8-bromo-cGMP, and sildenafil increased the MPOA-stimulated ICP response, whereas Nω-nitro-L-arginine methyl ester reduced it. None of the intrathecal treatments had detectable effects on blood pressure or the cavernous nerve-stimulated ICP response, although intravenous sildenafil increased the latter. These data clearly indicate that intrathecal drug administration affects central and not peripheral neural mechanisms and, moreover, documents that CNS NO/cGMP levels can affect erectile capacity per se (i.e., ICP) in the rat model.

nitric oxide; guanosine 3′,5′-cyclic monophosphate; rat; erection

Penile erection is a complex neurovascular event in which alterations in the tone and compliance of arterial and corporal smooth muscle cells play major regulatory roles (1, 15, 16). Specifically, visual or tactile sexual stimulation elicits activation of central nervous system (CNS) pathways responsible for relaxation of the vascular beds (i.e., helicine arterioles) that supply the penis, as well as relaxation of the corporal smooth muscle cells that comprise the bulk of the erectile tissue. Rapid and syncytial relaxation of corporal and arterial smooth muscle ensures transmission of systemic blood pressure (BP) into the penile vascular spaces, producing the increased blood flow and pressure required for rigidity and sustained erection.

Numerous studies have documented that nitric oxide (NO) released from peripheral nitrergic nerves (14) plays a prominent role in ensuring corporal smooth muscle relaxation under a wide range of physiological conditions. Neuronal NO diffuses from neuronal processes to subjacent myocytes and activates soluble guanylate cyclase to increase intracellular cGMP levels (2, 13, 15, 16, 18). Increases in intracellular cGMP levels, in turn, result in activation of protein kinase G, which presumably mediates, at least in part, the cellular hyperpolarization, decreased intracellular calcium levels, and smooth muscle relaxation that are a prerequisite to erection. The documented clinical success of sildenafil (i.e., Viagra), a phosphodiesterase (PDE) type 5 inhibitor, has further solidified the importance of the peripheral NO/cGMP pathway in the modulation of erection and the etiology of erectile dysfunction (25).

More recently, modulation of a variety of CNS functions has been ascribed to the NO/cGMP pathway (22, 33), including various aspects of sexual physiology and penile erection (29, 40, 44, 47–49). More specifically, alterations in hypothalamic NO levels (e.g., in the medial preoptic area (MPOA) and paraventricular nucleus (PVN)) have been shown to modulate both copulatory performance and reflexive penile erections (29, 40, 47–49). Not surprisingly, NO synthase (NOS)-containing cells have been localized to CNS areas that modulate penile erection (e.g., hypothalamus, spinal cord at T12-L3, L5-S1 levels) (12, 58), and furthermore, PDEs, including PDE5, are also located in the CNS (5, 36, 55, 56). Overall, these data indicate a potentially important regulatory role of a CNS NO/cGMP pathway on the behavioral and reflexive components of erectile function but provide no information concerning the quality/magnitude of the erection per se.

The goal of the current investigation, therefore, was to quantify the effects of altered CNS NO/cGMP levels on the ICP responses to electrical stimulation of the MPOA, in a well-documented rat model in vivo. The rationale for this approach is related to the critical
role played by the MPOA in integrating sexual physiology and penile erection and, furthermore, to the absolute importance of ICP to penile rigidity and sexual intercourse. To this end, we examined the effects of intrathecal administration of modulators of NO and cGMP levels on the ICP response elicited by central (MPOA) and peripheral (cavernous nerve) electrical stimulation, respectively, in the same animal (48, 50).

MATERIALS AND METHODS

Experimental animals and design. A total of 44 male Sprague-Dawley rats (Taconic Farms, Germantown, NY), weight 350–425 g, was used in this study. The animals were housed under a 12:12-h light-dark cycle. Food and water were supplied ad libitum. Rats were divided into five experimental groups as follows: 1) control group (saline; n = 5), 2) sodium nitroprusside (SNP) group (100 μg SNP; n = 6), 3) N^ω-nitro-L-arginine methyl ester (l-NAME) group (1 mg l-NAME; n = 6), 4) 8-Bromo-cGMP (8-Br-cGMP) group (100 μg 8-Br-cGMP; n = 6), and 5) PDE5 inhibition/sildenafil group (Viagra, 1 mg; n = 6 and 100 μg; n = 6). For intrathecal administration, drugs were dissolved in 50 μl saline and injected into the cisterna magna at the level of atlantooccipital membrane with a 28-gauge insulin needle. Control animals received an injection of vehicle alone.

For intravenous injections, sildenafil was dissolved in 200 μl saline and injected into the cisterna magna at the level of atlantooccipital membrane with a 28-gauge insulin needle. Control animals received an injection of vehicle alone. The concentration was calculated as follows. The volume of CSF of rats has been calculated to be ~1.5–2 ml according to Ref. 4. Using this estimate, and, furthermore, assuming that sildenafil was homogenously distributed in the CSF, we estimated that the calculated concentration (~750 nM) would be roughly equivalent to the maximum serum concentration of sildenafil reported in impotent men. A second and much higher concentration of sildenafil was also selected to determine if any further increase in the measured ICP response was possible. Note that given the rate of CSF formation in rats (~2.5 μl/min; Refs. 3, 31) and the estimated volume of the rat CSF (1.5–2.0 ml; Ref. 4), the concentration of intrathecally administered compounds would be expected to change <10% during the time course of these studies.

Surgical procedures. All surgical procedures were identical to those previously described (17, 46, 50). Briefly, anesthesia was induced by an intraperitoneal injection (35 mg/kg) of pentobarbital sodium (Abbott Laboratories, North Chicago, IL). Anesthesia was maintained during the course of the experimental protocol (2–3 h) by a subsequent injection of pentobarbital sodium (5–10 mg/kg). Rats were placed in the supine position, and systemic mean arterial BP was monitored via a 20-gauge cannula placed in the left carotid artery. The bilateral cavernous nerves were isolated, and unilateral crural of corpus cavernosum were exposed. Rats were fixed to a stereotaxic headholder (Kopf 900, David Kopf Instruments, Tujunga, CA) in flat skull position, and the electrode was placed in the MPOA. The stereotaxic coordinates for the tip of the electrode were 0.1–0.4 mm posterior, 0.4–0.6 mm lateral, and 8.8 mm ventral according to the atlas of Paxinos and Watson (41). The lower part of body was then rotated, and a 22-gauge needle was inserted unilaterally into the crus of the corpus cavernosum. Systemic BP and ICP lines were connected

![Fig. 1. Experimental protocol. As illustrated, 2 distinct, albeit similar, experimental protocols were used for the experiments assessing the effects of nitric oxide (NO) modulators and cGMP modulators, respectively, on the intracavernous pressure (ICP) response. In short, the difference was that protocol 2 (B) used 1 additional medial preoptic area (MPOA) and cavernous nerve (CN) stimulation in the presence of N^ω-nitro-L-arginine methyl ester (l-NAME) to further evaluate the mechanistic basis for the observed effects. As illustrated for protocol 1 (A), control MPOA and CN stimulations were obtained 5 min apart, while continuously monitoring ICP and systemic blood pressure (BP). After the control CN stimulation, saline, l-NAME, or sodium nitroprusside (SNP) were intrathecally administered. At 30 and 35 min after drug administration, respectively, MPOA and CN stimulations were again performed. As shown for protocol 2, control responses were obtained in identical fashion to protocol 1, and then the MPOA and CN stimulations were performed at 30 and 35 min after 8-Bromo-cGMP (8-Br-cGMP), saline, or sildenafil administration. Finally, tissues were exposed to l-NAME, and the MPOA and CN were stimulated a third time. ▼ And ▼, electrical stimulation of the MPOA and CN, respectively. Arrows indicate an intrathecal injection.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00248.2017)
to a pressure transducer, which was connected via a transducer amplifier to a data-acquisition board (MacLab8e7, ADI Instrument, Milford, MA). Real-time display and recording of pressure measurements were performed on a Macintosh computer (MacLab software V3.4, ADI Instruments). Cannulation of the external jugular vein provided the source of the intravenous sildenafil injections. Details of all other surgical procedures have been previously described (17, 46, 50).

Experimental protocol. As described elsewhere (50), central (MPOA) and peripheral (cavernous nerve) electrical stimulations were performed in the same animal before and after (i.e., 30 min postinjection; see Fig. 1) intrathecal drug or saline (i.e., vehicle) administration in a total of 44 rats. In another four rats, a stable response to 1 mA of cavernous nerve stimulation (i.e., 2 consecutive ICP responses that differ by <10%) was established, and the rats were then injected with an intravascular bolus of sildenafil (1 mg/kg, dissolved in 200 μl saline). Subsequently, the cavernous nerve only was stimulated 30 and 60 min postaddition of sildenafil.

Neural stimulation protocol. Briefly, the cavernous nerve was stimulated with a custom-made delicate stainless steel bipolar hook electrode (17, 46). Stimulation parameters were current at 1 mA, frequency of 20 Hz, pulse width of 0.22 ms, and duration of 1 min. Electrical stimulation of the MPOA was performed with a stainless-steel bipolar concentric electrode (SNE-100, Rhodes Medical Instruments, Woodlands Hills, CA). MPOA stimulation was applied by square-wave pulses of 2-ms duration, 75 Hz, 30 Hz for 1 min using a Grass S88 stimulator coupled with a constant-current isolation unit (PSIU/6, Grass, West Warwick, RI; Ref. 50). The rationale for the selected level of both central (75 μA; Ref. 50) and peripheral (1 mA; Refs. 17, 46) current stimulation was based on our previous observations in rats of similar age and weight, which indicated they produced a stable, reproducible, and submaximal ICP response.

Histological examination. After completion of the in vivo experiments, electrical coagulation of the stimulation site was performed for subsequent histological confirmation of the anatomical locus. Rat brains were then removed and fixed by 10% formaldehyde-saline for 2–3 wk. Thirty-micrometer-thick frozen sections were stained with Toluidine-Blue O (Sigma, St. Louis, MO) to confirm the location of electrical stimulation. Only animals in which electrodes were verified to be in the anterior MPOA, as previously reported (50), were used in these studies.

Methylene blue injections. To approximate the potential CNS distribution of the intrathecally injected compounds, we injected methylene blue into the cisterna magna. This seems a reasonable research strategy in light of the accepted use of this compound as a general measure of CSF flow through the brain and spinal column (27, 30). For the assessment of the diffusion/spread of intrathecally injected methylene blue, brain and spinal cord were harvested 35 (n = 2) and 65 (n = 2) min after injection. The tissues were then placed in 4% paraformaldehyde for 3 h and then switched to a 30% sucrose PBS solution. Thirty-micrometer frozen sections were obtained the following day on a Zeiss cryostat, and tissue was placed on histology slides for light microscopy. All photographs were taken at ×200 magnification.

Drugs and solutions. SNP (as NO donor; Sigma), L-NAME (as NOS inhibitor; Sigma), 8-Br-cGMP (as nonhydrolyzable cGMP agonist; Sigma), and sildenafil citrate (a PDE5 inhibitor; Pfizer, New York, NY; Refs. 10, 11) were used in this study.

Data reduction and analysis. All statistical analyses were performed using StatView 4.5 software (Abacus Concepts, Berkeley, CA). Changes in ICP after the cavernous nerve and the MPOA stimulation were expressed as a fraction of the mean arterial pressure during the stimulus period; i.e., an ICP-to-BP ratio was calculated and presented as the arithmetic mean ± SE. A repeated-measures ANOVA with post hoc test (Fisher’s protected least-significant difference method) was used for comparison of group means for parameters of interest between drug-treated and control animals. All differences were considered significant at P < 0.05.

RESULTS

Electrode placement. As highlighted in Fig. 2, for all animals used in these studies, the stimulation sites

<table>
<thead>
<tr>
<th>Table 1. Effects of NO modulation on MPOA-induced</th>
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<tr>
<td>Preintrathecal Injection</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>SNP</td>
</tr>
<tr>
<td>L-NAME</td>
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</tbody>
</table>

Values are means ± SE (n = 6 for each group). NO, nitric oxide; ICP, intracavernous pressure; BP, blood pressure; SNP, sodium nitroprusside; L-NAME, N^\circ-\text{nitro-L-arginine methyl ester}; MPOA, median preoptic area.
(coagulation marks) were located within the area defined by the MPOA (bregma 2.0 ± 0.1 mm; Ref. 41) and previously shown to elicit a reproducible and sustained increase in ICP (50). There was no apparent variation in the distribution of the coagulation marks within or among the experimental groups.

Central effects of NO modulators. As shown in the representative examples of the MPOA-stimulated ICP response in a control animal in Fig. 3, there was no obvious difference in the MPOA-stimulated ICP responses before and after intrathecal injection of saline. However, after intrathecal injection of l-NAME (1 mg) the ICP response was almost completely eliminated (Table 1). This latter observation clearly supports a role for NO in the MPOA-stimulated ICP response (50). Consistent with the data shown in Fig. 2, the representative examples displayed in Fig. 4 clearly indicate that the MPOA-stimulated, NO-mediated, ICP response can be differentially modulated by manipulation of CSF NO levels. That is, the MPOA-stimulated ICP response can be increased or decreased by the intrathecal administration of SNP (Fig. 4A) and L-NAME (Fig. 4B), respectively (Table 1). The data for all such experiments are graphically depicted in Fig. 5A and summarized in Table 1.

As illustrated in Fig. 5, repeated-measures ANOVA revealed that intrathecal administration of SNP and L-NAME was associated with a statistically significant increase and decrease, respectively, in the mean MPOA-stimulated ICP-to-BP ratio. Importantly, as shown for the mean data depicted in Fig. 5B, in contrast to observations with MPOA stimulation, there was no detectable effect of intrathecal injection of NO modulators on the cavernous nerve-stimulated ICP-to-BP ratio (Table 2). In addition, there was no effect of intrathecal injection of NO modulators on the resting ICP or BP levels.

Central effects of cGMP modulators. As documented by the representative examples shown in Fig. 6, intra-

![Fig. 4. Representative examples of the effects of NO modulators on the MPOA-stimulated ICP response. A: SNP group; B: l-NAME group. Note that increasing cerebrospinal fluid (CSF) NO levels via the NO donor SNP increases the ICP response to the same level of current stimulation in the same animal, whereas decreasing CSF NO levels has the opposite effect. Solid bar, corresponding stimulation period (i.e., 1 min).](image)

![Fig. 5. Graphical summary of the average (expressed as the arithmetic mean ± SE) effects of intrathecal administration of NO modulators on the ICP response to stimulation of the MPOA and CN. A: effects of NO modulation on the ICP-to-BP ratio during MPOA stimulation in all animals. A repeated-measures ANOVA revealed that there was significant difference among the 3 groups through 2 stimulations [F(2,16) = 12.8, P < 0.001]. **Significantly different from corresponding value in control animals; P < 0.01 [post hoc Fisher’s protected least-significant difference (PLSD)]. B: effects of intrathecal administration of NO modulators on ICP-to-BP ratio during CN stimulation. A repeated-measures ANOVA revealed that there was no significant difference among the 3 treatment groups through 2 stimulations [F(2,15) = 1.85, P = 0.191].](image)

<table>
<thead>
<tr>
<th>No.</th>
<th>Control</th>
<th>SNP</th>
<th>L-NAME</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.65 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.64 ± 0.02</td>
<td>0.56 ± 0.03</td>
<td>0.62 ± 0.05</td>
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Table 2. Effects of NO modulation on CN-induced ICP-to-BP ratio response

Values are means ± SE (n = 6 for each group). CN, cavernous nerve.
thetic administration of either 8-Br-cGMP (Fig. 6A) or sildenafil (Fig. 6, B and C) was associated with a significant increase in the MPOA-stimulated ICP response (see Table 3). Moreover, increasing the sildenafil concentration from nominally maximal therapeutic levels (by 2 log orders of magnitude; see MATERIALS AND METHODS) had no additional effect on the magnitude of the MPOA-stimulated ICP response. The mean of many such experiments are graphically depicted in Fig. 7A and summarized in Table 3.

Repeated-measures ANOVA revealed that intrathecal administration of 8-Br-cGMP and both the 1 µg and 100 µg sildenafil doses were all associated with statistically significant increases, relative to control values on the same animal, in the mean value of the ICP-to-BP ratio during stimulation of the MPOA (Table 3). For all treatment groups, the subsequent intrathecal administration of L-NAME produced a significant diminution in the MPOA-stimulated ICP-to-BP ratio (Table 3). Note that once again, there was no detectable effect of any of the treatments on the cavernous nerve-stimulated ICP-to-BP ratio in the same animal (Fig. 7B; Table 4). In addition, there was no effect of intrathecal injection of cGMP modulators on the resting ICP or BP levels.

**Intravenous administration of sildenafil.** To document that sildenafil could indeed increase the cavernous nerve-stimulated ICP-to-BP ratio if present at sufficient levels in the blood, another series of experiments was conducted on four distinct rats (see MATERIALS AND METHODS). In these experiments, a control cavernous nerve-stimulated ICP response (1 mA) was obtained on each rat before the intravenous injection of sildenafil (1 mg/kg). The cavernous nerve was then stimulated on two successive occasions in each rat, at 30 and 60 min postsildenafil injection. The corresponding ICP-to-BP ratio for the control, 30, and 60 min responses were 0.62 ± 0.07, 0.80 ± 0.05, and 0.83 ± 0.06, respectively. One-way repeated-measures ANOVA with a post hoc Fisher’s PLSD test (see MATERIALS AND METHODS) revealed that the 30 and 60 min postsildenafil responses were both significantly greater than the control response (P < 0.04), but not different from each other.

**Evaluating the physiological relevance of MPOA- and cavernous nerve-induced changes in ICP.** To highlight the potential physiological relevance of the observed effects of the NO/cGMP modulators on the MPOA- and cavernous nerve-induced ICP responses, we compared the frequency of erections before and after intrathecal or intravenous drug administration. The data are summarized in Table 5. As shown, there were no visible erections associated with the control MPOA-induced ICP responses (see Tables 1 and 3). However, after intrathecal administration of each of the NO/cGMP modulators, visible erections were observed in at least one-half of the animals in all treatment groups. In contrast, there was no change in the frequency of erections observed before and after intrathecal administration of NO/cGMP modulators after cavernous nerve stimulation in these same animals.

**Table 3. Effects of cGMP modulation on MPOA-induced ICP-to-BP ratio response**

<table>
<thead>
<tr>
<th></th>
<th>Preintrathecal Injection</th>
<th>35 min Postinjection</th>
<th>L-NAME (65 min Postinjection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.46 ± 0.04</td>
<td>0.40 ± 0.04</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>cGMP</td>
<td>0.49 ± 0.03</td>
<td>0.65 ± 0.04</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Sildenafil (1 µg)</td>
<td>0.48 ± 0.04</td>
<td>0.61 ± 0.05</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>Sildenafil (100 µg)</td>
<td>0.48 ± 0.04</td>
<td>0.66 ± 0.04</td>
<td>0.19 ± 0.04</td>
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</tbody>
</table>

Values are means ± SE (n = 6 for each group).
After intravenous injection of sildenafil, visible erections were noted in all rats. These last data reflect the submaximal nature of the control cavernous nerve-stimulated ICP response used in these studies and, moreover, document that sildenafil can further increase this response in a physiologically relevant manner.

Assessment of potential CNS distribution of injected compounds. In an attempt to preliminarily assess the potential CNS distribution of the injected compounds, intrathecal injections of methylene blue (0.1 M in 50 μl; see MATERIALS AND METHODS) were performed on an additional four rats. In these experiments, anesthetized rats received an intrathecal injection of methylene blue and then were killed 35 (n = 2) or 65 (n = 2) min later. The brain and spinal cord were harvested, fixed, and sectioned for light microscopy. As shown in Fig. 8, significant blue staining was routinely observed in the third ventricle, as well as in spinal vertebra C1-C5. In one animal at the 65 min time point, staining down to the level of T1 was observed (data not shown). These data provide evidence consistent with a potentially wide distribution and supraspinal locus of action for the injected compounds.

DISCUSSION

Given the critical role played by CNS pathways in integrative erectile physiology/function, identification of relevant molecular targets that are critical regulators of erectile function seems a cogent therapeutic

<table>
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<tr>
<th>Drug</th>
<th>MPOA Stimulation</th>
<th>Cavernous Nerve Stimulation</th>
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<tr>
<td></td>
<td>Control</td>
<td>Posttreatment</td>
</tr>
<tr>
<td>Control</td>
<td>0/6</td>
<td>5/6</td>
</tr>
<tr>
<td>cGMP</td>
<td>0/6</td>
<td>3/6</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>0/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>ND</td>
<td>ND</td>
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ND, not done; ic, intercavernous.
strategy (6, 7, 26, 38, 51). However, a vastly improved understanding of the central neural control of erectile function is still an absolute prerequisite to the identification of truly selective molecular targets in the CNS. As described in detail elsewhere (23, 37, 39, 40), several groups have studied central neural modulation of the behavioral (i.e., copulatory) and reflexive aspects of sexual function in the rat. A few investigations have also specifically implicated a role for CNS NO levels in the modulation of sexual behavior and penile erection (35, 40, 47–49). Such studies mainly focused on monitoring mounting, intromission, and the frequency of the observed erections, rather than on characterization of the physiology of the end organ. Although monitoring the aforementioned parameters provides undeniably important information concerning the role and context of the central NO/cGMP pathway in certain aspects of sexual behavior and reflexive penile erections, it does not precisely, nor directly, address the impact of this pathway on the ability of the penis to generate and/or sustain the relatively high-pressure, rigid erections required for intercourse.

Therefore, in light of the critical role of penile rigidity in both erectile physiology and dysfunction, we focused the current investigation on the role played by the NO/cGMP pathway in the MPOA-induced increases in ICP. The rationale for the selection of the MPOA as the site of CNS stimulation is related to its importance as an integrator of sexual behavior and penile erection (2, 23, 24, 28, 34, 37, 45, 50, 54). On the other hand, the rationale for using ICP as a measure of erectile capacity is related to the critical role ICP plays in both erectile physiology and dysfunction. In fact, ICP is an important predictor of penile rigidity and erectile function in both rats (17, 46) and men (19) and, as such, is a major determinant of erectile capacity and function per se. The primary goal of these initial studies then was to gauge the impact of the central NO/cGMP pathways on this important aspect of sexual physiology.

In that regard, the main finding of the current investigation was that modulation of the NO/cGMP pathway in the CNS (i.e., CSF) via the intrathecal drug injection had a dramatic impact on the central (i.e., MPOA; Figs. 3, 4, 5A, 6A, 7A) but not peripheral (i.e., cavernous nerve; Figs. 5B and 7B) neural activation of pressure responses in the specialized vascular spaces in the penis (i.e., ICP). This was true despite the fact that sildenafil was clearly capable of further increasing the cavernous nerve-stimulated ICP response after an intravenous injection (1 mg/kg, see RESULTS). Perhaps the best indication of the physiological relevance of the changes in the MPOA-induced ICP response mediated by the NO/cGMP modulators is the fact that before intrathecal administration, none of the rats showed any evidence of penile erection, whereas afterward, visible erections were noted in most, but not all, rats in each of the treatment groups (see Table 5). Although there is no absolute cutoff value for the ICP-to-BP ratio that guarantees an erection, it appears from these preliminary studies that the physiological scenario in

Fig. 8. Representative methylene blue staining in the brain and spinal cord of rats after intrathecal injection (see MATERIALS AND METHODS). A: cross section of spinal cord at C2; dorsal column at top. B: cross section of spinal cord at C5; dorsal column at top. C: coronal section through brain, with the 3rd ventricle on the right. Magnification was ×200 in all panels.
rats is consistent with previous observations in men, where human clinical studies have documented that the ICP-to-BP ratio measured in patients during a rigid papaverine-induced erection is ≈0.6 (1). Furthermore, despite the well-known diagnostic limitations of the penile brachial index (PBI), there is indeed general agreement that the vast majority of patients with a PBI <0.6 suffers from erectile dysfunction (32, 52).

More specifically, with respect to the current report, when the ICP surpassed ≈60% of the systemic pressure (i.e., ICP-to-BP ratios ≳0.6), the probability of visible erections apparently begins to increase quite dramatically. The positive correlation between ICP-to-BP ratio and penile erection is apparent even in the case of cavernous nerve stimulation, where despite the higher initial baseline response (≈0.6 ICP-to-BP to start, with 2 of 4 animals displaying visible erections), intravenous sildenafil administration produced visible erections in all four animals, while simultaneously increasing the ICP-to-BP ratio from 0.6 to ≳0.8. As such, this report provides strong support for the concept that the modulation of the NO/cGMP pathway in the MPOA is capable of producing physiologically relevant changes in erectile capacity per se.

Our current observations are also consistent with the localization of NO neurons in hypothalamic nuclei such as the MPOA (8, 58). Furthermore, NOS-containing cells are also found at spinal levels in areas known to be involved in ensuring penile erection (L3–S2 levels; Ref. 12). Not surprisingly, NO-stimulated guanylate cyclase activity is widely distributed in the CNS (58) and usually juxtaposed at very short distances from NOS neurons (20). In vivo studies have further demonstrated that NO donors increased cGMP levels in the hypothalamus (9, 44). In summary, the neuroanatomical literature is consistent with our in vivo observation that NO-cGMP activity in the MPOA modulates proerectile pathways to increase ICP in the penis and thus affects erection.

To gain preliminary insight into the putative CNS site(s) of action of the intrathecally injected compounds, a series of studies were conducted with methylene blue (Fig. 8). Although the expected diffusional paths of the NO/cGMP modulators are not likely to be identical to that of methylene blue, blue staining was observed in the third ventricle, as well as in the cervical vertebrae (C1–C5; Fig. 8), and, in one instance, as low as the T1 level of the spinal column. Such observations are consistent with a potentially widespread CNS locus of action, although further work is required to more precisely identify those sites.

From a mechanistic standpoint, prior in vivo studies have indicated that NO is capable of modifying neuronal activity. With respect to erectile physiology, NO is thought to enhance catecholamine (i.e., dopamine) release and inhibit reuptake, resulting in increased extracellular dopamine levels (26, 29). Of particular note in the current experiments is the fact that intrathecal injection of NO modulators had no detectable effect on basal ICP or BP. This stands in stark contrast to other studies documenting a significant effect of NO modulators when they are directly injected into hypothalamic nuclei on copulatory behavior and penile reflexes. In this regard, the obvious differences between intrathecal injection and direct intranuclear deposition of NO (i.e., much greater local NO/cGMP concentrations would be achieved with the latter method) probably account for this discrepancy. Nonetheless, it is clear that intrathecal injection of sildenafil produces a physiological profile that is qualitatively similar to that observed clinically after systemic administration of Viagra, where there is no detectable effect of sildenafil in the absence of sexual stimulation.

In addition, subsequent to 1-NNAME administration, the MPOA-stimulated ICP responses in the 8-Br-cGMP and sildenafil groups were significantly reduced (Figs. 6 and 7A; Tables 1 and 3), although not completely ablated, as observed in control or SNP-treated rats (Figs. 3, 4, and 5A). However, it is important to note that only the intrathecal administration of 8-Br-cGMP was associated with a statistically significant 1-NNAME-resistant portion of the MPOA-induced ICP increase, relative to the control response after 1-NNAME (Fig. 7A). This latter observation is consistent with a role for the cGMP pathway in the proerectile MPOA-induced ICP response and, moreover, reflects the fact that the effects of this nonhydrolyzable cGMP analog should nominally be unaltered by 1-NNAME.

As such, the action of sildenafil in the CNS appears similar to its presumed role in the peripheral nervous system. The CNS effect though is not surprising in light of the fact that PDE5 (36) and most of the other known PDEs are present in the CNS (5, 55–57). Furthermore, even if sildenafil at the concentrations used in these studies exerts some of its effects via actions on, for example, the PDE1 isoform, such an effect would not change the main conclusion of this report concerning the involvement of the NO/cGMP pathway in this process. Moreover, nonspecific actions of sildenafil on neuronal human ether-a-go-go-related gene channels (21) also cannot be excluded, although the robust inhibitory actions of 1-NNAME (Figs. 6 and 7) argue against such a possibility as a major contributing factor. Thus PDE inhibitors that cross the blood-brain barrier might provide a strategy for the improved therapy of erectile dysfunction.

**Perspectives**

The vast majority of treatments for erectile dysfunction has had, as their sole goal/effect, increased penile rigidity and erectile capacity. Certainly, such a therapeutic approach highlights the glaring disparity between the treatment of erectile dysfunction and the reality of sexual physiology/intimacy. With this background in mind, the current findings not only provide further evidence in support of the hypothesis that the activation of a NO/cGMP pathway is involved in MPOA-induced ICP response in the rat model, but also document that such changes are physiologically relevant. As the precise locus of the observed effect is not clear, one cannot determine whether it reflects direct
actions of the NO/cGMP pathway on the MPOA or more indirect effects on the MPOA via actions on other CNS regions/nuclei. Nonetheless, modulation of the NO/cGMP pathways has an unequivocally significant effect on the MPOA-induced ICP response, in the absence of a peripheral effect. That is, the increased ICP response was always confined to activation of the MPOA (but not the cavernous nerve) at the same level of neural stimulation in the same animal. Taken together, these observations indicate that it should be possible to manipulate CNS NO/cGMP targets to not only condition or modify sexual behavior and physiology overall, but, moreover, to increase the efficacy and rigidity of the erection per se, that is, to increase erectile capacity. Such therapies may provide the requisite strategy for truly treating the “whole” person rather than just the end organ.

REFERENCES


