Differential ICP responses elicited by electrical stimulation of medial preoptic area

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Sato, Yoshikazu, and George J. Christ. Differential ICP responses elicited by electrical stimulation of medial preoptic area. Am. J. Physiol. Heart Circ. Physiol. 278:H964–H970, 2000.—Recent findings indicate a complex role for the medial preoptic area (MPOA) in modulating penile erection. To further investigate this important area we measured changes in intracavernous pressure (ICP) elicited by electrical stimulation of the MPOA and evaluated the contribution of the cavernous nerve to the ICP responses after bilateral transection of the cavernous nerve (CN). In all experiments electrical stimulation was performed unilaterally in anesthetized male rats. Two distinct patterns of ICP response were seen after electrical stimulation of the MPOA: 1) increases in ICP during electrical stimulation (pattern 1, n = 10 rats) and 2) increases in ICP after electrical stimulation was terminated (pattern 2, n = 10 rats). For pattern 1, increases in ICP during stimulation exhibited a stable plateau without contraction of striated penile muscles, and bilateral transection of the CN eliminated the ICP responses. For pattern 2, increases in ICP observed after stimulation were lower, more variable, and accompanied by significant amplitude variations ("peaks"), caused by contraction of striated penile muscles. Bilateral transection of the CN eliminated the pattern 2 ICP response but did not alter striated muscle contraction. Histological studies documented that pattern 1 and pattern 2 responses occurred via electrical stimulation of the anterior and posterior areas of the MPOA, respectively. Thus both responses appear to result from activation of the CN, but the pattern 2 response apparently involves contraction of the striated penile muscles as well.

intracavernous pressure; striated penile muscle

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MATERIALS AND METHODS

Animal Preparation and Pretreatment

A total of 26 sexually mature (22) male Sprague-Dawley rats (Taconic Farms, Germantown, NY), ranging in age from 16 to 20 wk old and in weight from 350 to 400 g, were used in this study. They were housed under a 12:12-h light-dark cycle. Food and water were supplied ad libitum.

Experimental Design

The animals were divided into two equal groups of 10 rats each, according to the observed pattern of the ICP response elicited by stimulation of the MPOA (see description below). Six additional rats showed no ICP response to central nervous system stimulation (nonresponders). A schematic depiction of the stimulation schedule is provided in Fig. 1, and a detailed description is given in the text below.
and a second group (n = 10 rats) conducted in the absence of any other manipulation (5). The rats were further subdivided into two additional groups of equal size: a control group (A) and for bilateral transections of CN group (B).

Response pattern 1. These animals (n = 10 rats) showed an increased in ICP during electrical stimulation of the MPOA. All animals were stimulated twice, with a 10-min interval between successive stimulations. The rats were further subdivided into two additional groups of equal size: a control group in which the two consecutive MPOA stimulations were conducted in the absence of any other manipulation (n = 5 rats), and a second group (n = 5 rats) that underwent a bilateral transection of the cavernous nerve between successive MPOA stimulations. It is important to note that when adjacent fine branches of the penile nerves were identified (24, 29), they were transected as well.

Response pattern 2. These animals (n = 10 rats) showed increases in ICP after termination of electrical stimulation of the MPOA. They were subdivided and studied using the identical experimental design and protocol described above.

Experimental Set Up and Preparation

Anesthesia was induced by an intraperitoneal injection (35 mg/kg) of pentobarbital sodium (Abbott, North Chicago, IL). Anesthesia was maintained during the experimental protocol (2–3 h) by a subsequent injection of pentobarbital sodium (5–10 mg/kg) every 45–60 min as required for maintenance of anesthesia. Rats were placed in the supine position. Systemic mean arterial blood pressure was monitored via a 20-gauge cannula placed in the left carotid artery. The striated penile muscles were exposed. The 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 8.8–9.0 mm ventral from the skull surface, according to the atlas of Paxinos and Watson (23). By convention, we stimulated the right side of the MPOA. These coordinates were modified from previously reported stereotaxic positions (13). The lower part of body was then rotated, and a 20-gauge needle was inserted unilaterally into the crus of the corpus cavernosum. Systemic blood pressure (BP) and ICP lines were connected to a pressure transducer, which was connected via a transducer amplifier to a data acquisition board (MacLab/8e7, ADInstruments, Milford, MA). Real-time display and recording of pressure measurements were performed on a MacIntosh computer (MacLab software version 3.4, ADInstruments). Details of all other surgical procedures have been previously described (6, 26, 30).

Neurostimulation Protocol

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, 150 μA, and 30 Hz for 2 min using a Grass 88 stimulator coupled with a constant-current isolation unit (PSIU6, Grass, West Warwick, RI). The interval between the first and second electrical stimulations was 10 min, and the entire experimental protocol is depicted and described in Fig. 1.

Analysis of ICP Response

In light of the differential response patterns we observed to stimulation of the MPOA (see Neurostimulation Protocol and Results), we analyzed the ICP response using two distinct methods. For response pattern 1, the ICP at the plateau of the response was measured and presented as the mean ± SE of the ratio of ICP to BP (10, 26). For response pattern 2, in addition to the ICP-to-BP ratio at the plateau of the response, maximum peak pressure (MPP) was measured to describe “peaks” in response pattern 2 (1) (see Fig. 2).

Analysis of Visible Penile and Muscle Response

To further evaluate the nature of differential MPOA responses, visible erectile responses were also quantitated. Specifically, penile erection (penile body and glans erection), contraction of striated penile muscles, and ejaculation during and after electrical stimulation were assessed visually.

Penile body erections. Tumescence comprised extension and reddening of the distal penile body during ICP response and was defined as a positive response (29). Dorsal movement of penile body was measured as elevation or dorsiflexion (any angle) of the penile body during ICP response and was defined as a positive response (16).

Penile glans erection. E1 was an engorgement of base of the glans. E2 was a dilation of the distal glans but with no more than the lateral margin of the glans being parallel to each other. E3 (cup) was an intense flaring of the tip of the glans so that tip diameter was greater than the base of the glans (16). Glans erections were expressed as the maximum intensity in the responses that we could observe during the pattern 1 or 2 ICP response.

![Fig. 2. Peaks and plateau in idealized example of response pattern 2. Maximum peak pressure was expressed as ICP of highest peak on a plateau in response pattern 2.](https://example.com/fig2.png)
Ejaculation. If semen was seen from urethral meatus, we recognized a positive ejaculation.

Contraction of striated penile muscles. Ischiocavernous and bulbocavernous muscle (distal and proximal) were exposed, and visual evidence of muscle contractions corresponding to response patterns 1 and 2 was evaluated.

Histological Examination

After completion of the experiments, we performed electrical coagulation of the stimulation site for subsequent histological confirmation of the anatomic locus. Rat brains were then removed and fixed by 10% formaldehyde-saline for 48 h. Frozen sections (30 µm) were stained by toluidine blue O (Sigma, St. Louis, MO) to confirm the location of electrical stimulation. Brains from all 26 animals (i.e., 20 responders and 6 nonresponders) were examined.

Data Analysis

All statistical analyses were performed using the StatView 4.5 software package (Abacus Concepts, Berkeley, CA). A repeated-measured ANOVA design (two groups × two stimulations) with post hoc multiple comparisons (Fisher’s least significant difference) was utilized as appropriate for comparison of the ICP response between the control group and bilateral CN transection group within each distinct response pattern. All differences were considered significant at $P < 0.05$. Unless otherwise noted, all data are expressed as means ± SE of the ICP-to-BP ratio (i.e., BP and MPP).

RESULTS

Two distinct patterns of increase in ICP were elicited by electrical stimulation of the MPOA. Each pattern is described separately below.

Characterization of Pattern 1 Response

The first pattern of response (pattern 1) was characterized by increases in ICP during stimulation of the MPOA (Fig. 3A). In these animals, ICP increased immediately after the stimulation was initiated and

Table 1. Summary of visible parameters in pattern 1 and pattern 2 response

<table>
<thead>
<tr>
<th>Pattern 1 Response</th>
<th>Pattern 2 Response</th>
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<tbody>
<tr>
<td><strong>Intact CN</strong> (first stimulation)</td>
<td><strong>After bilateral CN transection (second stimulation)</strong></td>
</tr>
<tr>
<td><strong>After bilateral CN transection (second stimulation)</strong></td>
<td><strong>Intact CN</strong> (first stimulation)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>n 10</td>
<td>5</td>
</tr>
<tr>
<td>Penile body erections</td>
<td></td>
</tr>
<tr>
<td>Tumescence</td>
<td>+</td>
</tr>
<tr>
<td>Dorsal movement</td>
<td>-</td>
</tr>
<tr>
<td>Glans erections</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>+</td>
</tr>
<tr>
<td>E2</td>
<td>-</td>
</tr>
<tr>
<td>E3</td>
<td>-</td>
</tr>
<tr>
<td>Ejaculation</td>
<td>-</td>
</tr>
<tr>
<td>Striated muscle contractions</td>
<td>-</td>
</tr>
</tbody>
</table>

n, No. of rats; +, parameter was observed in all rats; -, parameter was not observed in any rats except ++ and +++*. No. in parentheses indicates no. of rats that showed E1 and E2, respectively (see MATERIALS AND METHODS). In control group, findings during second ICP response (n = 5) were same as first one (not shown). CN, cavernous nerve.
was associated with penile tumescence (penile body and penile glans; E1; see MATERIALS AND METHODS) (Fig. 3A, Table 1). No detectable contraction of the striated penile muscles, dorsal movement of penile body, nor moderate (E2) or strong (E3) glans erections were observed during stimulation/generation of the ICP response (Table 1). To evaluate the contribution of the CN to the observed response, a bilateral transection of the CN was performed in five rats before the second stimulation. As illustrated, there was no detectable increase in ICP (Fig. 3B) and a significant decrease in ICP-to-BP ratio (Table 2) on stimulation of the MPOA, after a bilateral transection of the CN. In the control group, the first and second ICP responses were indistinguishable (Fig. 3A, Table 2). As such, we can rule out any time-dependent alteration in the responsivity of the MPOA to electrical stimulation over the course of these experiments.

Characterization of Pattern 2 Response

A second pattern of response (pattern 2) was characterized by an increase in ICP immediately after termination of stimulation of the MPOA. These responses were characterized by a more modest increase in the plateau of the ICP response, were less stable, and were accompanied by amplitude variations (i.e., peaks) superimposed on the ICP response plateau due to contractions of striated penile muscles (Fig. 4A). Penile body tumescence, glans erection (E1 and E2), and penile dorsal movement corresponding to contractions of striated penile muscles were also observed during the typical pattern 2 response (Table 1). As such, the observed responses appeared qualitatively similar to reflexive erections (22).

After bilateral transection of the CN, the second MPOA stimulation did not elicit any detectable sustained (i.e., plateau) increase in ICP (Fig. 4B, Table 3). Furthermore, neither penile elongation, glans erection, nor penile dorsal movement were observed after CN transection (Table 1). However, peaks corresponding to the contraction of striated penile muscle were still observed (Fig. 4B), although MPP after transection of CN was significantly smaller than that before transection (Table 4). Once again, in the control group (i.e., intact CN), the ICP responses observed after the first and second stimulation were statistically indistinguishable (Table 3).

Histological Examination

As shown in Fig. 5, the coagulation marks (stimulation site) in the pattern 1 responses were located in the anterior area of the MPOA (Fig. 5, A and B), whereas the coagulation marks in the pattern 2 responses were found in the posterior area of the MPOA (Fig. 5, C and D). The stimulation site of six nonresponders was located in areas lateral and dorsal to the effective MPOA stimulation sites (Fig. 5, A–D).

Table 2. Effect of bilateral CN transection on ICP-to-BP ratio in pattern 1 response

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>First stimulation</th>
<th>Second stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>0.82 ± 0.04</td>
<td>0.81 ± 0.03</td>
</tr>
<tr>
<td>Bil CN transection group</td>
<td>5</td>
<td>0.78 ± 0.04</td>
<td>0.08 ± 0.01*</td>
</tr>
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</table>

Values are means ± SE, n is no. of rats. Two-way ANOVA for repeated measures revealed significant differences among various groups (ANOVA F(1, 8) = 113.7, P < 0.0001; see MATERIALS AND METHODS). *Significantly different from corresponding value within same group [post hoc Fisher's protected least significant difference (PLSD)], P < 0.01 [after bilateral (Bil) CN transection].
Table 3. Effect of bilateral CN transection on ICP-to-BP ratio in pattern 2 response

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>First stimulation</th>
<th>Second stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.48 ± 0.05</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Bil CN transection</td>
<td>5</td>
<td>0.47 ± 0.03</td>
<td>0.05 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE, n is no. of rats/group. Two-way ANOVA for repeated measures revealed significant differences among various groups (ANOVA F(1, 8) = 7.6, P < 0.02; see MATERIALS AND METHODS).

DISCUSSION

The main finding in this study was the apparent anatomic specificity of the two distinct response patterns observed on electrical stimulation of the MPOA. Specifically, response patterns 1 and 2 were observed during stimulation of the anterior MPOA (Fig. 3A) and after termination of stimulation of the posterior MPOA (Fig. 4A), respectively. The specificity of the hypothalamic stimulation site is further evidenced by the fact that the anatomic sites of stimulation for the six animals that did not experience any increase in ICP during or after stimulation (see Fig. 5) were located lateral and dorsal to the stimulation sites consistently associated with the pattern 1 and 2 responses. Each response pattern is considered in detail below.

Characterization of Response Pattern 1

Response pattern 1 was associated with robust, reproducible, and sustainable increases in ICP (Fig. 3A) that were nearly completely eliminated by bilateral transection of the CN (Fig. 3B). Additionally, the magnitude and duration of the pattern 1 increases in ICP were similar to that reported by others during stimulation of the MPOA, and moreover, were very reminiscent of previously observed ICP responses to direct electrical stimulation of the CN (6, 26; see Fig. 3A). Therefore, the increased ICP response observed during stimulation of the anterior MPOA is presumed to involve relaxation of the arterial and trabecular smooth muscle of the penis via autonomic efferents traveling in the CN. Note that a previous investigation (13) reported a response similar to the pattern 1 response documented here but stated that it occurred by activation of the posterior MPOA, as opposed to the anterior MPOA stimulation we report here (see Fig. 3A and Fig. 5). Comparison of the stereotaxic coordinates reveals that the posterior region described by Guiliano and colleagues (13) is the same as the anterior region we describe here. Therefore, the “apparent” anatomic discrepancy is related to nomenclature and not to differential effects observed after stimulation of the same region.

Neural Efferents Mediating Response Pattern 1

Efferent fibers originating from the anterior hypothalamus (e.g., medial forebrain bundle pathway) are...
known to connect to the sacral parasympathetic nucleus through the periaqueductal gray (3, 15, 19, 32). Such anatomic findings further support the hypothesis that electrical stimulation of the anterior MPOA induces arterial and corporal smooth muscle relaxation in the penis through activation of parasympathetic nerves innervating the lower urogenital organs.

Characterization of Response Pattern 2

A distinct series of events, that is, response pattern 2, was observed after stimulation of the posterior MPOA (Figs. 4A and 5). Response pattern 2 was characterized by less stable, but equally reproducible, change in ICP that was further characterized by amplitude variations or by the superimposed presence of peaks on the plateau of the ICP (compare Fig. 4A, Tables 3 and 4). The more modest increase in ICP, but not the peaks, was nearly completely eliminated by bilateral transection of the CN (Fig. 4B, Tables 3 and 4). Additionally, the penile elongation, glans erection, and penile dorsal movement that were observed after stimulation of the posterior MPOA were also eliminated by bilateral transection of the CN. In this regard, response pattern 2 shares many of the physiological and behavioral characteristics reported for other erectile events and contexts. This includes characteristics such as penile reflexes (3, 14, 22), noncontact erections (3, 28), penile erections induced by apomorphine (1–3), and erectile events that occur during copulatory performance (mount, intromission, and ejaculation) (3, 11, 14). Furthermore, similar response patterns have been observed by other investigators after termination of electrical stimulation of posterior hypothalamic regions (5, 17).

The pattern 2 ICP response and penile tumescence are assumed to reflect at least some degree of relaxation of the arterial and trabecular smooth muscle of the penis after a lower level of activation of CN efferents. Such vascular filling may be responsible, at least in part, for the much greater amplitude of the peaks before sectioning of the CN (see Table 4 and Fig. 4B). However, it is also conceivable that the ischiocavernosus and bulbocavernosus muscles are differentially affected by CN transection. Regardless, a portion of the efferent neuronal pathways associated with the peaks appears not to be contained in the CN.

Neural Efferents Mediating Response Pattern 2

Many neural fibers from the posterior hypothalamic area (which includes our posterior MPOA) traverse the periventricular pathway and project to the lower spinal cord where pudendal motoneurons are located [the Onuf’s nucleus (cat, dog, monkey and human) and dorsomedial and dorsolateral nucleus (rat); Refs. 15, 18, 21, 32]. Consistent with these anatomic findings, another recent study has documented that transection of the pudendal nerve eliminated the peaks but not the plateau of the ICP response elicited by apomorphine (1). Although the precise physiological meaning of the ICP increase after termination of the stimulus is unclear, it would seem that it likely reflects the removal of descending inhibitory pathways rather than the activation of stimulatory pathways (as observed for the pattern 1 responses).

Physiological Significance of Two Distinct Response Patterns Within MPOA

The existence of these two distinct response patterns within the MPOA may have important implications for copulatory performance in rats, where rigid erections must be achieved very quickly (generally lasting 200–400 ms) (22). That is, the requisite rapid achievement of penile erection in the rat may require an integrative mechanism to coordinate vascular filling and penile striated contraction. For example, at least theoretically, it would seem necessary to fill the corporal space with blood before the peak pressures commensurate with copulatory performance can be achieved. However, although the current report provides further evidence that the MPOA is intimately involved in integrating copulatory performance in the rat, the current studies only begin to address the complexity of this important brain region (9, 19, 22).

In conclusion, these data confirm previous studies (2, 12) documenting the importance of the CN pathway to the control of penile erection elicited by activation of hypothalamic pathways. Furthermore, the current observations provide strong evidence for the hypothesis that the distinct patterns of ICP response observed are indeed related to a relatively specific activation of discrete areas within the hypothalamus by electrical stimulation. In addition, in conjunction with the extant literature, this report also supports the supposition that electrical stimulation of the posterior MPOA affects erectile function via neuronal pathways that are at least partially distinct from the neuronal pathways responsible for the much larger ICP increases seen on stimulation of the anterior MPOA. As such, the rat appears to provide a good model system for exploring further the complex integration of the activity of peripheral and central pathways and their subsequent effects on penile erection.

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