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Intracavernosal Pressure Monitoring in Mice: Responses to Electrical Stimulation of the Cavernous Nerve and to Intracavernosal Drug Administration

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Abstract: With the development of transgenic mice to evaluate mechanisms of erectile function, it appears particularly advantageous to develop a standardized mouse model of penile erection. The purpose of the study reported here was to evaluate the novel application of intracavernosal pressure (ICP) monitoring in the mouse during electrophysiologic and pharmacologic induction of penile erection. In anesthetized adult male mice, the cavernous nerves (CN) were isolated unilaterally, and the corpora cavernosa were exposed. A 24-gauge angiocath (intravenous catheter) was inserted into the right corpus cavernosum to monitor the ICP, and a 30.5-gauge needle was inserted into the left corpus cavernosum for intracavernosal drug administration. ICP was recorded during CN-stimulated or pharmacostimulated erections. Electrical stimulation of the CN significantly increased the ICP (from 10.09 ± 2.01 to 34.62 ± 2.71 mm Hg, $P < .05$), which then returned to baseline pressure after termination of the electrical stimulation. Pretreatment with intracavernosal administration of the nitric oxide synthase inhibitor, nitro-L-arginine methyl ester (0.1 mg), inhibited the electrical stimulation–induced changes in ICP (7.17 ± 1.46 vs 10.38 ± 2.17 mm Hg, not significant [NS]). Also, intracavernosal administration of papaverine (0.4 mg) produced a significant increase in ICP (from 8.51 ± 0.69 to 26.37 ± 5.7 mm Hg, $P < .05$). We concluded that this technique might be applied to perform quantitative erection physiologic experiments with the mouse as an economical and experimentally advantageous animal model, particularly with the development of transgenic mice to evaluate mechanisms of erectile function.

Key words: Nitric oxide, penile erection, papaverine, transgenic mice.

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The scientific understanding of penile erection has improved greatly in recent years as a result of efforts to investigate the pathophysiologic elements of erectile dysfunction and to define the physiologic, pharmacologic, and molecular biologic aspects of the erectile process (Lue, 1992; Andersson and Wagner, 1995; Burnett, 1997, 1999). The use of various animal models has contributed significantly to this understanding. In 1863, Eckhardt demonstrated that electrical stimulation of the sacral nerve roots in dogs resulted in erection, which established the role of pelvic innervation in this response (Eckhardt, 1863). Over a century later, a number of animal species, including the cat, dog, monkey, rabbit, and rat have been commonly used to study the physiologic and the pharmacologic elements of penile erections (Lue et al, 1983; Lin et al, 1985; Andersson et al, 1987; Carati et al, 1987; Stackl et al, 1988; Azadzoi et al, 1990; Holmquist et al, 1991; Wang et al, 1994; Giuliano et al, 1995).

The application of various techniques to record penile erections in animal models in vivo has figured prominently in the research progress. In addition to the assessment of copulatory behavior within natural contexts of erectile function, neurostimulated or pharmacostimulated erectile responses have been monitored by such methods as visual assessment, scoring various erectile parameters, video recording the extent of the erectile response (Quinlan et al, 1989; Wang et al, 1994), penile plethysmography (Sjostrand and Klinge, 1979), and direct vascular pressure recordings within the cavernous and spongious corporal bodies of the penis (Lue et al, 1983; Wang et al, 1994; Lin and Lin, 1996; Bernabe et al, 1999). The latter technique basically involves insertion of a needle or a catheter into the corporal body of the penis, whereby recordings representing pressure changes within the penile tissue can be obtained. This technique has been thought to be a significant advance because it provides an objective and accurate quantitative index to evaluate penile erections. Its application has been extended to species of increasingly small scale, such as the rat (Burnett et al, 1992; Chen et al, 1992; Mills et al, 1992; Martinez-Pineiro et al, 1994; Rehman et al, 1998).

Recently, the mouse species has been used as a model to investigate physiologic erectile function. In a study involving genetically engineered mice that lack the neuronal nitric oxide synthase-α (NOS-α) isoform, copulatory

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behavior and the absolute presence or absence of an erectile response to cavernous nerve (CN) electrical stimulation were used as the indices of penile erection (Burnett et al, 1996). To our knowledge, this was the first study in mice that was an in vivo investigation of CN stimulation–induced responses, although erectile measurements were only qualitative. The aim of this study was to describe a model to monitor intracavernosal pressure (ICP) in mice as a quantitative method for assessing the erectile response. In this in vivo model, penile erections were induced via direct CN electrical stimulation and via intracavernosal administration of papaverine hydrochloride.

Materials and Methods

Animals

Adult male C57BL6 mice (body weight, 25 to 32 grams) were used in this study. Animals were housed in a temperature-controlled environment under a 14-hour light/10-hour dark cycle until the day of the experiments. Mice had access to food and water ad libitum. All experiments were conducted in accordance with the Johns Hopkins University School of Medicine guidelines for the care and use of animals.

Surgical Procedures

Mice were anesthetized intraperitoneally, with 40 mg/kg pentobarbital sodium (Nembutal, Abbot Laboratories, North Chicago, Ill). The bladder and prostate were exposed via a midline suprapubic incision, and testes and epididymides were repositioned into the abdomen after they were divided from their scrotal attachments. Bilateral CN, located lateral to the urethra, were identified and isolated. The penis was denuded of skin, and the scrotum was incised at the midline to expose the base of the penis. Small portions of the ischiocavernous muscles were dissected bilaterally to expose each penile crus. To monitor the ICP, a heparinized (100 U/mL), 24-gauge angiocath (Insyte-N, Becton Dickinson Vascular Access, Sandy, Utah) attached to polyethylene (PE)-50 tubing was inserted into the right corpus cavernosum. For intracavernosal drug administration, a separate cannula (30.5-gauge needle, attached to PE-10 tubing and 25 μL Hamilton syringe) was inserted into the left corpus cavernosum. Because the corporal bodies communicate in the mouse penis (Burnett et al, 1996) similar to that of the rat penis (Rehman et al, 1998), physiologic effects resulting from the administration of a drug into one corporal body can be successfully monitored via a separate apparatus applied to the other one.

ICP Monitoring

The cannula inserted into the right corpus cavernosum was connected to a pressure transducer and an amplifier unit (Harvard Apparatus, Holliston, Mass). The amplifier was connected to a data acquisition module (DI-190, Dataq Instruments, Akron, Ohio), and the data were recorded on a computer with Windaq/Lite recording software (Dataq).

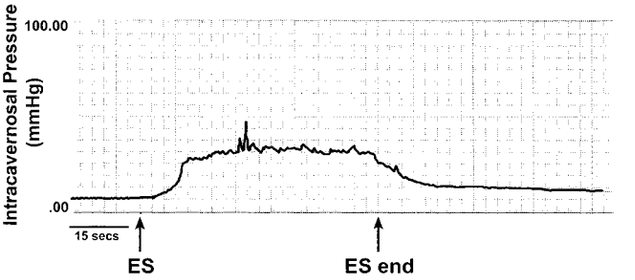

Figure 1. Actual tracing showing the changes in the intracavernosal pressure following cavernous nerve electrical stimulation. ES indicates electrical stimulation started; ES end, electrical stimulation terminated.

CN-Electrical Stimulation

A stainless-steel bipolar electrode with parallel hooks (0.7- to 1-mm apart) was placed around the nerve and positioned by a micromanipulator. The electrode cable was attached to a Grass S48 stimulator (Quincy, Mass), and the following stimulation parameters were used: 16 Hz, 5 milliseconds duration, 0.5 to 4 volts.

Intracavernosal Drug Administration

Nitro-L-arginine methyl ester (L-NAME, 0.1 mg total) and papaverine hydrochoride (0.4 mg total) were prepared in a volume of 10 to 15 μL and injected via a cannula inserted into the left corpus cavernosum. Drugs were purchased from Sigma (St Louis, Mo), and solutions were prepared in 0.9% NaCl on the day of the experiment.

Statistical Analysis

Data were determined as the maximum ICP after CN stimulation or drug administration and were presented as mean ± SEM. One-way analysis of variance (ANOVA) test was used (Sigma Stat statistical software, Version 2.0, Jandel Scientific, Chicago, Ill) to compare responses with the baseline pressure, followed by post hoc analysis with Dunnett test. Statistical significance was defined as $P < .05$.

Results

In anesthetized mice, the baseline ICP was 10.1 ± 2.0 mm Hg (n = 6, Figures 1 and 2). CN electrical stimulation significantly increased the ICP (34.6 ± 2.7 mm Hg; Figure 2), which then returned to baseline pressures after the stimulation ended (Figure 1). Direct electrical stimulation of the CN was performed for approximately 60 seconds. Tumescence began between 6 to 9 seconds after the start of electrical stimulation, and detumescence occurred within 5 seconds of stopping electrical stimulation. The consecutive stimulation of the CN, 10 to 15 minutes after termination of the initial electrical stimulation, produced similar increases in ICP (data not shown).

To establish quantitatively that the CN-stimulated erectile responses were mediated by release of nitric oxide

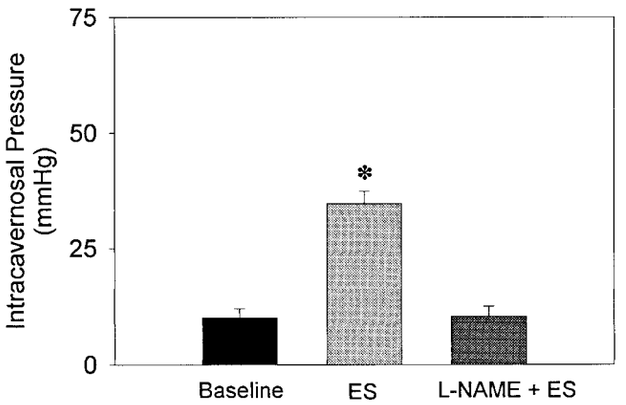

Figure 2. Intracavernosal pressure responses induced by cavernous nerve electrical stimulation. Values are mean ± SEM and illustrate the resting intracavernosal pressures (baseline) and electrical stimulation–induced peak pressures (ES) before and after intracavernosal administration of 0.1 mg nitro-L-arginine methyl ester ([LNAME] L-NAME + ES, n = 6). Statistical difference defined as $P < .05$.

from the nerve terminals, CN stimulation was repeated 10 minutes after intracavernosal administration of L-NAME (0.1 mg), a nonselective inhibitor of the enzyme NOS that produces nitric oxide. As shown in Figure 2, in mice pretreated with L-NAME, electrical stimulation of the CN did not cause a significant increase in ICP (7.17 ± 1.46 vs 10.38 ± 2.17 mm Hg).

The ICP increased significantly (26.4 ± 5.7 mm Hg, n = 4) within 8 to 10 minutes after administration of papaverine hydrochloride (0.4 mg; Figures 3 and 4) and was sustained about 5 minutes prior to returning to baseline pressures in 20 to 25 minutes. Injection volumes containing only 0.9% NaCl did not appreciably change ICP recordings from baseline levels.

Discussion

Animal models of penile erection, whether occurring in natural contexts or following experimental induction, have played a crucial role in furthering the study of penile erection. In this study, we evaluated the application of ICP monitoring of penile erection in the mouse. This monitoring was performed with electrical stimulation of the CN and following the intracavernosal administration of the erectogenic agent papaverine hydrochloride under pentobarbital anesthesia. To our knowledge, there have not been any prior published reports of ICP monitoring in the mouse following any particular erectile stimulus. Similar to investigations in other experimental animal models, our work in the mouse demonstrated that the mouse may feasibly serve as an animal species by which to carry out investigative work of penile erection in vivo.

With ICP monitoring in the mouse, we have been able

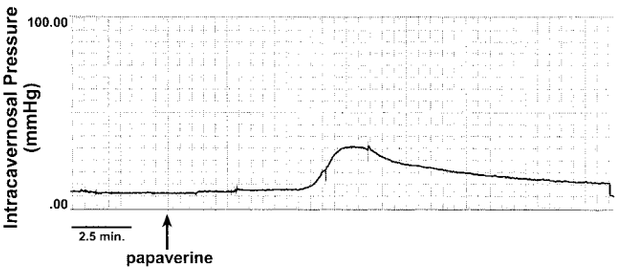

Figure 3. Actual tracing showing the changes in the intracavernosal pressure following intracavernosal administration of 0.4 mg papaverine.

to characterize the features of the erectile response in the anesthetized mouse following direct CN electrical stimulation and intracavernosal pharmacostimulation. The baseline ICP recording in anesthetized mice ranged between 4 and 14 mm Hg, similar to that recorded in rats (Chen et al, 1992). Following electrical stimulation of the CN, we showed ICP measurements of approximately 35 mm Hg. A graded increase in ICP was noted with stepwise increases in voltage during stimulation, as shown by Mills et al (1992). Our determination of maximal ICP response in the mouse following CN electrical stimulation provides a reasonable indication of the autonomic regulatory basis of erection in this model. Additional electrical stimulation of somatic nerve structures supplying the pelvic region, such as the pudendal nerve, would be necessary to achieve truly maximal ''suprasystolic'' pressure elevations in the penis (Giuliano et al, 1995). Following pharmacostimulation with papaverine hydrochloride, ICP measurements rose to approximately 27 mm Hg within 8 to 10 minutes, similar to effects measured in rats (Chen et al, 1992; Rehman et al, 1998). The slightly lower pressure response with pharmacostimulation compared with

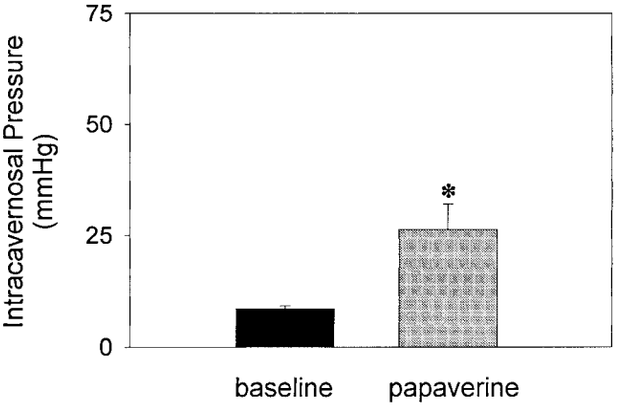

Figure 4. Intracavernosal pressure responses induced by intracavernosal administration of 0.4 mg papaverine. Values are mean ± SEM and illustrate the resting intracavernosal pressures (baseline) and papaverine-induced peak pressures (n = 4). Statistical difference defined as $P < .05$.

electrical stimulation in these studies appears consistent with the effects of a pharmacologic stimulus that operates through only 1 proerectile mechanism.

We carried out the additional experiment involving pharmacologic blockade of NOS-signal transduction of penile erection to provide further support for the erection-monitoring technique we have developed in mice. Significant investigative work in recent years has established that nitric oxide is the principal mediator of penile erections (Kim et al, 1991; Burnett et al, 1992; Rajfer et al, 1992). Pretreatment with L-NAME, an inhibitor of the enzyme NOS, measurably inhibited the response associated with CN stimulation, which is consistent with nitric oxide–mediation of erectile responses in mice (Burnett et al, 1996).

Several technical caveats are worth emphasizing from our development of this experimental mouse model of penile erection. Proper insertion and securement of a cannula into the designated corporal body is necessary for precise and reproducible recordings of erections. After multiple trials, we identified that a heparinized, 24-gauge angiocath provided a reliable device for ICP monitoring. The pinpoint tip of the cannula adequately fits into the penile corpus cavernosum without leakage, and with anticoagulation, remains patent throughout the experiment. During proper cannula insertion, an instant surge of a small amount of blood into the cannula is identified with a concomitant rise in pressure recording. Further confirmation of placement is achieved with the injection of small amount of saline solution, after which there should be straightening of the penis without leakage of saline solution into surrounding tissue. These experiments were facilitated by a secure understanding of pelvic anatomy and autonomic innervation of the penis in mice. We have confirmed that this anatomy resembles that previously described in the rat (Langworthy, 1965; Quinlan et al, 1989), and we have supported its functional importance in the mouse species. Obviously, the anatomical findings in the mouse are much smaller than that in the rat and other larger animal species. However, the CN emanating discretely as a fiber from each major pelvic ganglion, situated bilaterally dorsolateral to the prostate, can be identified even with the naked eye and hooked acceptably with an electrical probe.

The major importance of this investigation beyond establishing that ICP monitoring can be accomplished in the mouse is that high-quality, quantitative experimental studies of penile erection can seemingly be accomplished at the mouse species level. We perceive that ICP monitoring in mice offers a useful scientific tool for experimental erection studies. An increasing interest has been given to genetically engineered mice that may lend insight to particular mechanisms involved in the male sexual response (Burnett et al, 1996, 1998). Additional transgenic mouse models have been developed and are currently under study that may further clarify erectile mechanisms. An additional purpose behind developing an experimental mouse model of penile erection is that such a model either in genetically intact or disrupted mice has economical advantages associated with reduced purchase and maintenance costs of animals.

In summary, we have significantly advanced the use of the mouse animal model for the evaluation of penile erection, particularly adding ICP monitoring as a means to record experimentally induced erectile responses. The technique has been applied in this investigation to the peripheral neuromediation of penile erections in the anesthetized mouse. Further investigation is necessary to confirm our findings and to possibly extend the work to other erectile contexts.

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