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CARDIOVASCULAR RESPONSES TO ELECTRICAL STIMULATION OF SYMPATHETIC NERVES IN THE PITHED MOUSE

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Abstract

The pithed rat model has been used extensively to study peripheral cardiovascular responses to electrical stimulation of the sympathetic nervous system, as pithing eliminates central and reflex effects. However, since the transgenic mouse has become a standard and economical model organism, an electrically stimulated pithed mouse would facilitate a variety of studies. We have developed surgical techniques, drug doses and stimulation parameters for an electrically stimulated pithed mouse to study peripheral sympathetic nerve effects on blood pressure. Similar to the pithed rat, the pithed mouse showed voltage and frequency-dependent blood pressure responses to a pulsed train of electrical stimuli. In addition, α -adrenergic stimulation with phenylephrine gave a marked systolic pressor response, while the β_2 agonist salbutamol lowered diastolic blood pressure. Furthermore, pithed transgenic mice unable to synthesize catecholamines in adrenergic cells displayed smaller pressor responses than pithed control mice. In summary, the electrically stimulated pithed mouse can be used to study peripheral effects of the sympathetic system on cardiovascular dynamics unencumbered by central responses.

Keywords

pithed mouse; catecholamine; electrical stimulation; blood pressure; epinephrine; phenylethanolamine N-methyltransferase

Introduction

The pithed rat has been extensively used to study peripheral cardiovascular responses to electrical stimulation of the sympathetic nervous system since pithing eliminates central and reflex effects by inserting a steel rod through the brainstem and spinal cord, rendering the

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animal brain dead. Application of an electrical current through the steel rod activates peripheral sympathetic nerves, while destruction of the brainstem prevents central reflex responses to the pressor effects of peripheral nerve stimulation (Balt et al., 2001; Streefkerk et al., 2002). Despite the wide use of the pithed rat, a literature search revealed very limited use of the pithed mouse (Lopez-Guerrero et al., 2005; McCafferty et al., 1999) and no accounts of electrical stimulation of the sympathetic nervous system in the mouse. However, an electrically stimulated pithed mouse could facilitate studies of gene function. Here we present a technique to measure electrically stimulated cardiovascular responses in the pithed mouse that provides the same type of information that was available from the pithed rat. Many genes are expressed both in the brain and peripheral nervous system. The brain mediates reflex responses to cardiovascular changes. A reliable model for studying the peripheral effects of gene manipulation, without central or reflex effects, could clarify the locus of gene actions. The pithed mouse reflected peripheral effects of the sympathetic system on cardiovascular dynamics unencumbered by central responses.

Methods

Experiments were performed on mice (C57Bl/6) weighing 20–30 g and were approved by the University of California San Diego Animal Subjects Committee. The Animal was anesthetized with an intraperitoneal injection comprising 100 mg/kg of ketamine and 5 mg/kg of xylazine. An incision was made in the neck of the mouse and the trachea was exposed. A polyethylene tracheal cannula (PE-50) was inserted through the mouth to the trachea. A low power microscope was used to visualize the throat, so the tip of the cannula could be seen and guided into the trachea. The cannula was then secured by tying a thread around the trachea to prevent the cannula from slipping out during the pithing process. Mice were pithed by insertion of a steel rod, 0.5 mm thick and 10 cm long, into an eye socket and through the entire length of the spinal column. Pithing renders the animal brain dead, so animals were mechanically ventilated at a rate of 105 breaths/min (SAR 830-p; CWE, Ardmore, PA, USA). A grounding rod was inserted under the skin. Bilateral vagotomy was performed and both carotid arteries were cannulated with heat stretched polyethylene catheters (PE-10). The blood pressure was monitored and recorded using a heparinized saline-filled catheter placed in the right common carotid artery. The catheter in the right common carotid artery was connected to a blood pressure transducer (Statham P23XL, Gould-Statham Instruments Inc.) the one in the left common carotid artery was used for injection of drugs. The mouse temperature was regularly checked with a thermometer and kept around 36.5–37°C with a heat lamp throughout the whole 2-hour duration of the experiments.

We used freshly dissolved gallamine triethiodide (Sigma Chemical, St Louis, MO, USA) from a bottle stored at 4°C. A dose of 0.02 mmol/kg (20 mg/kg) was used to prevent muscular contractions during electrical stimulation. Heparin 150 IU/kg was also injected (Fisher Scientific, Fair Lawn, NJ, USA) and heparinized saline was used in all cannulae to prevent blood clotting.

Mice were allowed to stabilize for 20 min after pithing. In order to determine the sympathetic nervous response to different patterns of electrical stimulation, 7 mice were stimulated with 8 different voltages (3, 4, 5, 6, 7, 8, 9 and 10V) with a 1-minute rest between two stimulation steps. At each of these eight steps, a one millisecond (ms) long stimulus of given intensity was applied continuously for 30 seconds at 5Hz, 10Hz and 15Hz with 30 seconds rest between these three steps (S44 stimulator, Grass Medical Instruments, Quincy, Mass.).

In order to select the optimal frequency of electrical stimulation, another group of 6 wild type mice and six PNMT/TH knockout mice were stimulated with 3V at six different frequencies (2, 4, 6, 8, 10 and 15 Hz) with a 1-minute rest between each stimulation period. A 3V stimulus

of 1ms duration was applied continuously for 30s at each given frequency. Five minutes after the last stimulation, 0.04 $\mu\text{mol/kg}$ (8 $\mu\text{g/kg}$) of phenylephrine HCl (American Regent laboratory, NY 11967) was injected intra-arterially and the blood pressure was recorded until return to baseline, usually 10 to 15 minutes. Finally, mice were injected with 0.05 $\mu\text{mol/kg}$ (12 $\mu\text{g/kg}$) of the beta-2 adrenergic agonist salbutamol (Sigma Chemical, St Louis, MO, USA) and the blood pressure was recorded for an additional 15 minutes. An equal 100- μl volume was used for each injection.

Data Acquisition And Analysis

The pressure transducer signal was amplified (Gould Brush amplifier, Clevite Corp., Brush Instruments Division; Cleveland, OH) and then sampled at 250 Hz using a WinDaq acquisition system (version 3.16 DATAQ Instruments; Akron, OH) for real time monitoring and data storage. Systolic blood pressure, diastolic blood pressure and pulse rate values were extracted from the stored data with software we created with LabVIEW (version 6.0, National Instruments; Austin, TX). Statistical significance was determined with *t*-test by comparing SBP response between WT and KO mice to different stimulation frequencies. Data are presented as mean $\pm$ SEM.

PNMT/TH Knockout Mice

We generated the PNMT/TH knockout mice by crossing the PNMT knockout mice (Bao et al., 2007) with C57BL mice that had loxP sequence inserted around the tyrosine hydroxylase gene, the kind gift of Richard Palmiter. Phenotype was checked with catecholamine assays (Durrett and Ziegler, 1980), which detected no epinephrine and decreased levels of norepinephrine in the blood of the PNMT/TH knockout mice. In this experiment, 5 PNMT/TH knockout mice and 7 wild type mice were pithed and the right common carotid artery was cannulated.

Results

Seven pithed wild type mice were electrically stimulated at 3V with 1ms spikes for 30 seconds at six different frequencies (2, 4, 6, 8, 10 and 15Hz), with a 60s rest between two consecutive frequency steps. Blood pressure increased with increasing frequency of electrical stimulation (Fig. 1B). In addition, α -adrenergic stimulation with phenylephrine gave a pressor response and the β_2 agonist salbutamol lowered diastolic blood pressure after the pulsed train of electrical stimuli.

Six pithed wild type mice were stimulated with eight different voltages (3, 4, 5, 6, 7, 8, 9 and 10V). At each stimulus voltage, a 30s train of stimulation was applied at given frequencies (5, 10 and 15 Hz for 30s, respectively, with a 60s rest between two consecutive steps). There was a voltage-dependent increase in arterial blood pressure from 3V to 5V at all three frequencies. Higher voltages did not yield a linear blood pressure response and were associated with deleterious effects, such as occasional decreases of baseline blood pressure and cardiac arrhythmias (Fig. 2A). Only 2 mice remained stable through the highest voltage. As shown in Fig. 2B, alterations in voltage and frequency of electrical stimulation did not significantly change the pulse rate ($p > 0.2$).

We studied six transgenic mice in which tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) were selectively knocked out in PNMT producing cells so that epinephrine producing cells in the adrenal did not synthesize catecholamines. As shown in Fig. 3, a smaller pressor response was induced by electrical stimulation at 3V in pithed PNMT/TH knockout mice than in six pithed wild type mice ($p < 0.05$).

Discussion

The electrically stimulated pithed rat has been useful for study of the peripheral sympathetic nervous system. However, the mouse is more economical to breed and more amenable to genetic manipulation. The mouse is about one tenth of the size of the rat, so microsurgical technique is necessary. Beside the obvious differences from the rat due to size, the pithed mouse has different drug sensitivity, does not tolerate high voltage electrical stimulation, and has a good pressor response to low voltage stimulation.

Adequate neuromuscular blockade must be provided to prevent muscle contraction during electrical stimulation (Elayan et al., 2002a, b, c). Unlike other neuromuscular blockers, gallamine triethiodide has been used extensively in the pithed rat because it lacks ganglion-blocking and histamine-releasing effects (Clark and Mitchelson, 1976; Dunlap and Brown, 1983; McCafferty et al., 1999; Narayanan and Aronstam, 1986). In our study, we found that 20 mg/kg of gallamine provided adequate neuromuscular blockade at 3, 4, 5 and 6V, using frequencies up to 15Hz (Fig. 2A). At 5V, blood pressure responses approached the maximum. Further increases in voltage were associated with deleterious effects, such as decreases of baseline blood pressure and cardiac arrhythmias. Thus, we do not recommend stimulation at greater than 5 volts in the pithed mouse, even though higher voltages are useful in the pithed rat. Long experiments can be carried out with low levels of stimulation that avoid fatigue of the mouse nervous system. For example, at 3V the pithed mouse gave increasing pressor responses to 2, 4, 8, 10 and 15 Hz stimuli and remained responsive to the α -adrenoceptor agonist phenylephrine (Lopez-Guerrero et al., 2005) and the β_2 agonist salbutamol (Fig. 1). Hence, it is possible to glean much information from a single animal in a properly designed experiment.

Targeted knockout of catecholamine synthesis in epinephrine producing cells decreased the blood pressure response to electrical stimulation of pithed mice (Fig. 3), suggesting that the pithed mouse model suffices to detect functional changes of the adrenergic system. The selective catecholamine-synthesis defective mice exhibited fairly substantial pressor responses to electrical stimulation, since catecholamine synthesis was blocked only in adrenergic cells and because of the release of neuropeptide Y and/or ATP under intensive electrical stimulation (Kennedy et al., 1997). In summary, the electrically stimulated pithed mouse gives quantitative peripheral hemodynamics unencumbered by central responses, and thus may be used to probe the role of peripheral sympathetic nervous regulation of the cardiovascular system.

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Fig. 1.

A. Original arterial blood pressure response to 30s long pulsed trains of a 3V/1ms electrical stimulation at 2, 4, 6, 8, 10 and 15 Hz in the wild type pithed mouse. Each stimulation step lasted 30s and was followed by a 60s rest. Gallamine triethiodide (0.02 mmol/kg) was injected 10 min prior to electrical stimulation to prevent muscular contractions. Note blood pressure responses to vehicle (saline), 0.04 μ mol/kg of phenylephrine (Phe.) and 0.05 μ mol/kg of salbutamol (Salb.), with an equal 100- μ l volume for each injection. B. Blood pressure percent changes from baseline summarized for procedures performed in Fig. 1A on seven wild type mice. The data is presented as mean $\pm$ SEM. SBP = systolic blood pressure.

Fig. 2.

Blood pressure change (A) and pulse rate change (B) during spinal cord electrical stimulation in pithed mice (number of mice tested is given in brackets). Pulsed train stimuli of different intensities (from 3V to 10V) were applied for 30s at three different rates (5, 10 and 15 Hz). Each stimulation step was followed by 60s rest. Data is presented as mean $\pm$ SEM.

Fig. 3.

Systolic blood pressure (SBP) change in response to electrical stimulation in pithed wild type and PNMT/TH knockout mice (number of mice tested is given in brackets). At each stimulation step, a 3V/1ms electrical stimulus was delivered as a 30s long pulsed train at a given frequency. The stimulus frequency varied from 2Hz to 15Hz with a 60s rest between two consecutive steps (* $p < 0.05$). Data is presented as mean $\pm$ SEM.