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Cervical sympathectomy reduces neurogenic vasodilation in dura mater of rats

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ABSTRACT

Migraine may affect the autonomic nervous system, but the mechanisms remain unclear. The sympathetic and parasympathetic nervous systems may play different roles in the attack. To explore the effect of blocking the cervical sympathetic nerve on vasodilation of the meningeal vessels, jugular vein calcitonin gene-related peptide (CGRP) and meningeal blood flow changes were measured before and after transection of the cervical sympathetic nerve by electrically stimulating the trigeminal ganglion in Sprague–Dawley (SD) rats. We found that CGRP level and meningeal blood flow increased in both the sham-operated and sympathectomized groups ($p<0.05$). Compared with the sham-operated group, dural blood flow decreased significantly in the cervical sympathectomy group, but CGRP level was not significantly different between these two groups. The cervical sympathetic nerve may play an important role in the process of neurogenic dural vasodilation in rats; this effect is not entirely dependent on CGRP level.

1. Introduction

Migraine is a recurrent, unilateral pulsating headache of moderate-to-severe intensity that may last for 4–72 h. Considerable evidence based on clinical signs and diagnostic tests indicates that dysfunction of the sympathetic nervous system is involved (Yerdelen et al., 2008; Benjelloun et al., 2005; Thomsen and Olesen, 1995). This is supported by observations of autonomic symptoms such as nausea, vomiting, diarrhea, cutaneous vasoconstriction, vasodilation, piloerection, diaphoresis, photophobia, and abnormal pupillary reaction during migraine attacks (Peroutka, 2004).

At present, the precise pathophysiological mechanisms of the sympathetic nervous system in migraine remain unclear. Tore et al. (2010) found that cervical sympathectomy induced a significant increase in dura mater nitric oxide (NO) levels. In a clinical trial, salivary alpha amylase levels were significantly lower during the attack period and higher during the post-attack period in the migraine group when compared with the control group (Bugdayci et al., 2010). Patients with migraine had higher rest systolic blood pressure and lower heart rate induced by mental stress than controls. There were no significant differences between migraineurs and controls on the cold pressure test (Domingues et al., 2010). Hence, there are differing views that migraine is associated with sympathetic hypofunction (Mylius et al., 2003), sympathetic hyperfunction (Cortelli et al., 1991), sympathetic and parasympathetic hyperfunction (Yakinci et al., 1999) or sympathetic instability (Appel et al., 1992).

It is well known that blood vessels will contract after activation of the sympathetic nervous system, and that neurogenic dural vasodilation is substantially due to the release of calcitonin gene-related peptide (CGRP) from prejunctional trigeminal nerve fibers innervating the dural blood vessels (Akerman et al., 2003). Because CGRP levels in the cranial circulation have been found to be elevated during spontaneous or provoked migraine (Zagami and Goadsby, 1990; Juhasz et al., 2003), we hypothesized that sympathetic blockade would inhibit neurogenic dural vasodilation and be associated with CGRP. The aim of this study was to evaluate the effect of cervical sympathectomy on cerebral blood flow monitored by laser Doppler flowmetry and CGRP level detected by radioimmunoassay in migrainous rats.

2. Materials and methods

2.1. Preparation of experimental animals

Female adult Sprague–Dawley (SD) rats (200–250 g; $n=32$) were housed in a special room at constant temperature (22 °C) and relative humidity (50%), and maintained under a 12/12-h light–dark cycle (with light from 07:00 to 19:00 h) with food and water freely available. Rats were separated randomly into two groups: cervical sympathectomy group and sham operation group. Each group consisting of 16 rats was divided into two groups for dural blood flow monitoring and CGRP determination, respectively. All animal procedures and the protocols of the present investigation were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the study was approved by the local Institutional Animal Care and Use Committee (IACUC).

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2.2. Surgical removal of the superior cervical ganglion

The rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (at 0.4 ml/100 g body weight). Surgical sympathectomy was performed by right superior cervical ganglionectomy. A ventral midline incision 2.5 cm in length was made aseptically in the neck, the right sternocleidomastoid muscle was exposed and deflected, and the right carotid artery was displaced laterally to expose the superior cervical ganglion. The right superior cervical ganglion was excised by transecting the cervical preganglionic sympathetic nerve and the internal and external carotid nerves (Steinle et al., 2002). The wound was closed with monofilament silk. In the sham operation, the rats underwent the same procedures except for transection of the right superior cervical ganglion. All animals were kept for 12 days until the next experimental procedures were carried out. Right eye ptosis was used to confirm denervation, and only rats displaying good ptosis were used for the next experiments.

2.3. Trigeminal ganglion stimulation

Electrical stimulation of the trigeminal ganglia activating the trigeminovascular system is considered to be an effective and well-established method. This technique represents a standard method to evaluate meningeal inflammation. Twelve days after surgery, the anesthetized rats were placed in a stereotaxic frame and a longitudinal incision was made in the scalp to expose the skull. Two burr holes were drilled in the skull and a bipolar electrode (Rhodes NE-200) was lowered, using a micromanipulator, into each trigeminal ganglion (0.32–0.34 cm dorsal to bregma, 0.28–0.32 cm lateral from the midline, and 0.93 cm below the dural surface). Correct placement of the electrodes in the trigeminal ganglion was confirmed prior to paralysis by brief (1 s) electrical stimulation of the trigeminal ganglion which results in jaw movements due to the activation of the motor nerves of the ganglion. Only one trigeminal ganglion was electrically stimulated at 10 V, 5 Hz, and 5 ms for 10 min. These parameters are similar to those used by our group and others to stimulate the trigeminal ganglion.

2.4. Measurement of dural blood flow and CGRP

The dural blood flow was monitored by laser Doppler flowmetry. At the point 3 mm anterior and 3 mm right from the bregma, a round bone window of about 5 mm in diameter was drilled to expose the dura mater as a monitoring location. With the needle-type probes from moorLAB multi-channel laser Doppler flowmetry laboratory (UK Moor Company) positioned over the dural surface to avoid the large blood vessels, the exposed dura mater was covered with pieces of cotton soaked with isotonic saline to avoid drying and keep fluid contact between the dura and the needle probes throughout the experimental protocol. Monitoring of the sampling frequency was 2 Hz, flow unit (PU, perfusion unit). MoorVMS-PC V1.0 software was used to store and calculate the average blood flow value before and after stimulation.

CGRP levels were determined by radioimmunoassay. Every 1 ml of jugular venous blood was mixed with 15 μl of 7.5% EDTA·Na_2 and 20 μl of aprotinin (all reagents were from Beijing Puerweiye Bio Technology Co., Ltd.) in the Epoxy epoxide (EP) tube and centrifuged (4 °C, 3000 rpm, 20 min). An Sn-695B type immune counter (Shanghai Hesuo Rihuan Photoelectric Instrument Co. Ltd.) was used for radioimmunoassay by the Science and Technology Development Center, Chinese PLA General Hospital.

2.5. Statistical analysis

Each group's dural blood flow values and CGRP levels were expressed as mean ± standard deviation ($x \pm s$). The increased ratio of blood flow and CGRP levels after electrical stimulation was calculated as: [=(post-stimuli value/pre-stimuli value)×100%]. Paired Student's t-tests were used as appropriate to evaluate the statistical significance of differences between the two groups. Statistical significance was set at the $p<0.05$ level.

3. Results

3.1. Effects of trigeminal ganglion stimulation

After electrical stimulation, the dural blood flow significantly increased immediately and then remained at a stable level for 20 min (Figs. 1 and 2, $p<0.01$); the release of CGRP was significantly higher than at pre-stimulation. In the sham-operated group and sympathectomized group before stimulation the CGRP level was 44 ± 23 pg/m and 48 ± 21 pg/ml, respectively. After stimulation, the release of CGRP was shifted to 77 ± 42 pg/ml and 70 ± 19 pg/ml ($n=8$; $P<0.05$), respectively. CGRP is supposed to be an important marker of a successful rat model (Bergerot et al., 2006).

3.2. Effects of cervical sympathectomy on dural blood flow and the release of CGRP before stimulation

Compared to the basic dural blood flow before electrical stimulation, there was no significant difference in basic dural blood flow value and CGRP release between the two groups with cervical sympathectomy ($p>0.05$, Tables 1 and 2). The results indicated that amputation of the cervical sympathetic nerve has no significant effects on dural blood flow and CGRP levels in the normal rats.

3.3. Effects of cervical sympathectomy on dural blood flow in rats

After electrical stimulation of the trigeminal ganglion for 10 min the dural blood flow was measured. The dural blood flow of the sympathectomized group increased from 92 ± 20 to 119 ± 17 PU, while the dural blood flow changed from 118 ± 31 to 211 ± 65 PU in the sham-operated group. The net increase in dural blood flow was 93 ± 53 and 28 ± 13 PU, respectively, in the two groups ($p<0.05$). There was a significant difference in percentage increase between the sham-operated group (194 ± 46%) and sympathectomized group (136 ± 14)% ($p<0.01$) (Table 1).

3.4. Effects of cervical sympathectomy on the release of CGRP in rats

The release of CGRP was detected under the same conditions that the dural blood flow was measured. Although the release of CGRP increased in both the sham-operated and sympathectomized groups immediately after stimulation, the differences from before stimulation were not statistically significant. The net increase of CGRP was 33 ± 33 pg/ml and 22 ± 15 pg/ml, respectively, in the two groups, which was not statistically significant between the groups ($p>0.05$). There was also no significant difference in percentage increase between the sham-operated group (183 ± 77%) and sympathectomized group (169 ± 83%) ($p>0.05$) (Table 2).

4. Discussion

The experiments in this study demonstrated that cervical sympathectomy decreased the dural blood flow and had no effects on the CGRP release in rats with electrical stimulation of the trigeminal ganglion.

In the sham-operated rats, stimulation of the trigeminal ganglion caused long-lasting vasodilatation which was consistent with migraine pathophysiology. It is well accepted that migraine neurovascular syndrome is mainly due to cranial vasodilation with activation of the trigeminal system. Our study showed that electrical stimulation