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CHEMICAL LESIONING AND GLUTAMATE ADMINISTRATION REVEAL A MAJOR ROLE FOR THE NUCLEUS TRACTUS SOLITARIUS IN THE CARDIAC-SOMATIC REFLEX IN RATS

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Abstract—Many patients suffer from secondary muscle hyperalgesia after experiencing angina pectoris. In this study, we examined the role of the nucleus tractus solitarius (NTS) and glutamate receptors in modulating cardiac-evoked muscle hyperalgesia induced by pericardial capsaicin, which was monitored by recording electromyogram (EMG) activity from the spinotrapezius muscle in the anesthetized rat. Unilateral chemical lesioning of the commissural NTS with the neurotoxin ibotenic acid significantly depressed the cardiac-somatic reflex; the EMG responses decreased to 56.4±6.9% of that of the controls (5 of 5). Microinjection of the excitatory amino acid glutamate, at 10, 20, and 50 nmol, into the commissural NTS increased the EMG response, in a dose-dependent manner, to 116.9±4.9%, 143.9±10.2%, and 214.2±15.8% (n=8), respectively, of that of the controls. In contrast, microinjection of the *N*-methyl-D-aspartate (NMDA) receptor antagonist (+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d]-cyclohepten-5,10-imine maleate (MK-801) at 4 and 6 nmol, decreased the EMG response to 45.2±10.6% and 36.8±14.3%, respectively, of that of the controls (n=8 for each dose). Similarly, the metabotropic glutamate receptor (mGluR) antagonist (RS)-a-methyl-4-carboxyphenylglycine (MCPG), at 2.5 and 5 nmol, decreased the EMG response to 65.2±16.3% and 57.0±4.2%, respectively, of that of the controls. When a combination of MK-801 and MCPG was administrated, the EMG response further decreased to 22.5±13.2% (n=6) of that of the controls. However, administration of a non-NMDA receptor antagonist 6, 7-dinitroquinoxaline-2, 3-dione (DNQX), at 2 and 5 nmol, had no effect on the EMG response. These results suggest that the NTS is involved in the facilitation of the cardiac-somatic reflex, and that the NMDA receptor and mGluRs play an important role in mediating this effect.

Key words: cardiac-somatic reflex, nucleus tractus solitarius, chemical lesion, glutamate stimulation, glutamate receptors, rat.

Cardiac patients often experience cardiac-related secondary muscle hyperalgesia, including deep muscle-like incapacitating pain, persistent postoperative musculoskeletal pain, and chronic myofascial pain (Levine and Mascette, 1989; Romano, 1999). Jou et al. (2001a) developed an animal model, using rats, for studying cardiac-evoked muscle hyperalgesia. In this model, electromyogram (EMG) activity in paraspinal muscles was evoked by intrapericardial infusion of bradykinin or an algogenic chemical mixture. The authors suggested that this reflex was produced by cardiac sympathetic afferents converging on neurons in the upper thoracic spinal dorsal horn, evoking hyperalgesia of paraspinal muscles (Jou et al., 2000). The contribution of supraspinal neurons to cardiac-related muscle hyperalgesia remains unknown. The nucleus tractus solitarius (NTS), located in the medulla oblongata, plays an important role in the processing of cardiac nociception. Pericardial application of pain-inducing compounds (e.g. capsaicin, bradykinin, or an inflammatory exudate solution) significantly increases c-Fos expression in the NTS (Albutaihi et al., 2004; Fang et al., 2004). There is evidence that stimulation of the NTS can elicit antinociception in the rat tail-flick test (Aicher and Randich, 1990; Morgan et al., 1989). In contrast, in pathological states, the NTS is likely to facilitate nociceptive responses, because unilateral lesions of the NTS can abolish hyperalgesia induced by intraperitoneal (i.p.) administration of lipopolysaccharide (LPS) and have a mild analgesic effect as well (Wiertelak et al., 1997). However, it is unknown whether the NTS exerts a descending influence on cardiac nociception, whether inhibitory or facilitatory.

Capsaicin is a specific activator of nociceptive sensory neurons having C and Aδ fibers (Holzer, 1991), and it has become a valuable tool for studying the function of afferent fibers. It has been widely used in models of hyperalgesia in both animals (Alexandra et al., 2005) and humans (Asbjorn et al., 2003). It has been reported that application of capsaicin to the surface of the rat heart activates both vagal and sympathetic afferent fibers (Schultz and Ustinova, 1998). Our previous studies indicate that pericardial capsaicin can produce a repeatable cardiac-somatic reflex that was a suitable index for investigating angina pectoris-evoked hyperalgesia and its modulation (Sun et al., 2011; Liu et al., 2011).

Glutamate is the principal excitatory neurotransmitter in the NTS, and in addition to the well-recognized fast synaptic transmission at ionotropic glutamate receptors (NMDAR and non-NMDAR), glutamate also mediates slow synaptic neurotransmission within the NTS at metabotropic glutamate receptors (mGluR) (Glaum and Miller, 1992; Foley et al., 1998). Both NMDAR and mGluR are reported to be involved in hyperalgesia (Coutinho et al.,

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Abbreviations: BP, blood pressure; DNQX, 6, 7-dinitroquinoxaline-2, 3-dione; EMG, electromyogram; i.p., intraperitoneal; LPS, lipopolysaccharide; MCPG, (RS)-a-methyl-4-carboxyphenylglycine; mGluRs, metabotropic glutamate receptors; MK-801, (+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d]-cyclohepten-5, 10-imine maleate; NMDA, *N*-methyl-D-aspartate; NTS, nucleus tractus solitarius.

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1998; Volker et al., 1994). In the present study, we used ibotenic acid and glutamate, to specially eliminate and activate neuronal cell body (but not passing fibers), respectively, in the NTS; and we examined the effect of these treatments on the cardiac-somatic reflex induced by pericardial capsaicin in rats anesthetized with pentobarbital sodium. If glutamate was effective in modulating the cardiac-somatic reflex, we would further examine the effects of the *N*-methyl-D-aspartate (NMDA) receptor antagonist (+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d]-cyclohepten-5,10-imine maleate (MK-801), the non-NMDA receptor antagonist 6, 7-dinitroquinoxaline-2, 3-dione (DNQX), and the mGluR antagonist (RS)-a-methyl-4-carboxyphenylglycine (MCPG) on this reflex to determine which receptors mediate the effects of glutamate.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on adult male Sprague–Dawley rats (280–310 g), provided by the Experimental Animal Center of Shaanxi Province, Xi'an, China. All animals were housed singly and maintained on a 12-h light/dark cycle with free access to food and water. The experimental protocol was approved by the Institutional Animal Center of Xi'an Jiaotong University and was in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, eighth edition, revised 2011). All efforts were made to minimize the number of animals used and any distress to the animals.

Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p., SCRC, Shanghai, China). The right carotid artery was catheterized to monitor blood pressure (BP), and the left jugular vein was catheterized to permit continuous infusion of sodium pentobarbital (10–15 mg/kg/h) using a syringe pump (KD100; KD Scientific, Inc., Holliston, TX, USA). A tracheal cannula was inserted for artificial ventilation (55–60 breaths/min, 5.0–6.0 ml tidal volume). Body temperature was monitored with a rectal thermometer and was maintained at 37 °C with a heating pad. Arterial BP and pupil diameter were monitored to confirm an adequate level of anesthesia.

To activate cardiac nociceptors, rats underwent midsternal incision from the first rib to the third rib to expose the thymus, and a silicone catheter (0.020 ID, 0.037 OD, 14–16 cm long) with four small holes in the distal 2 cm was inserted into the pericardial sac between the lobes of the thymus. The lobes of the thymus and every layer of tissue were sutured, and the animals were placed in the prone position during recovery.

EMG recordings

To record EMG activity, a concentric electrode was inserted in the left spinotrapezius muscle at an angle of approximately 30°. EMG signals were amplified and bandpass-filtered (1.0 kHz) with a Dual-Trace Amplifier (AVM-11; Nihon Kohden, Tokyo, Japan) and were monitored with an oscilloscope (VC-10; Nihon Kohden, Tokyo, Japan) and an audio monitor. Signals were collected and analyzed using the Biological Experimental System (BL-420; TaiMeng, Chengdu, Sichuan, China). Experiments were performed within a Faraday cage to decrease electrical noise.

Cardiac stimulation

The nociceptive cardiac-somatic reflex was evoked by pericardial administration of capsaicin as described previously (Jou et al., 2001a). Briefly, 2 h after surgery, capsaicin (0.655 nmol/0.2 ml in 2–4 s) was infused into the pericardial sac to evoke an EMG response and was withdrawn after 60 s. After the capsaicin infusion, warm saline was infused and then withdrawn to remove any remaining capsaicin. There was a minimum 40-min interval between each injection.

Microinjections into the NTS

After surgery, the animal in a prone position was placed in the stereotaxic apparatus. A partial occipital craniotomy was made. The dura and arachnoid were incised to expose the dorsal surface of the brainstem at the level of the obex. The calamus scriptorius was identified and served as the anterior/posterior and lateral stereotaxic zero coordinate—this location was situated 14.30 mm caudal to the bregma (Paxinos and Watson, 1986). Because the commissural NTS plays a relatively major role in cardiac nociception (Albutaihi et al., 2004; Fang et al., 2004) and the effects of the drugs showed no significant difference between the right and left sides of the NTS in the preliminary experiments, microinjections were performed uniformly in the left commissural NTS at predetermined coordinates that were 0.5 mm rostral and 0.5 mm lateral to the calamus scriptorius, and 0.5 mm ventral to the dorsal surface of the medulla. Unilateral NTS lesion was chosen on the basis of the previous reports, in which it was suggested that bilateral NTS lesions result in unstable physiological responses and poor survivability of animals (Buller et al., 2001; Wiertelak et al., 1997).

Microinjections of saline, glutamate, and its antagonists were performed 15–20 min before capsaicin infusion into the pericardial sac to evoke an EMG response. Drug tests were performed every 40 min in the same animal. Microinjection of ibotenic acid was performed 1 or 2 h before the pericardial capsaicin according to previous study, at which time frame, ibotenic acid could effectively disrupt the cell bodies but leave passing axons intact (Randich and Gebhart, 1990). Recovery responses were tested 90 min after microinjecting the drugs. The microinjection volume was 100 nl.

Relationship between hemodynamic changes and the cardiac-somatic reflex

In the preliminary experiment, microinjection of drugs into NTS could induce changes in BP. It was unknown whether the effect of drugs on the cardiac-somatic reflex was induced by the BP changes. To clarify this issue, the effect of hemodynamic changes on this reflex was observed. A bolus injection (0.5–0.6 ml) of the ganglionic blocker, hexamethonium bromide (10 mg/kg), or noradrenaline (0.01 mg/kg) was administered via the jugular vein over a period of 60 s. Capsaicin injection was performed 15–20 min after drug injection to determine the effects on evoked EMG activity. Heart rate and BP were monitored to ascertain the efficacy of each drug.

Drugs

All the drugs were obtained from Sigma (St. Louis, MO, USA). Capsaicin stock solution was dissolved in saline containing 10% ethanol and 10% Tween 80 to a concentration of 3.28 μmol/ml and was diluted to the final concentration of 3.28 nmol/ml. Glutamate (10, 20, and 50 nmol/100 nl), MK-801 (4 and 6 nmol/100 nl), DNQX (2 and 5 nmol/100 nl), and ibotenic acid (13 nmol/100 nl) were dissolved in saline. MCPG was first solubilized in NaOH (1 M) before dilution with saline to the working concentrations (2.5 and 5 nmol/100 nl). All drugs were adjusted to pH 7.2–7.6. Drug doses were selected based on previous studies (Randich et al., 1988; Andrea et al., 1999) and preliminary experiments.

Histology

To identify the location of the injection sites within the NTS, 2% Pontamine Sky Blue dye (100 nl) was microinjected into the same