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Research Report

Autonomic and respiratory responses to microinjection of L-glutamate into the commissural subnucleus of the NTS in the working heart–brainstem preparation of the rat

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ABSTRACT

Changes in heart rate (HR), thoracic sympathetic nerve activity (tSNA) and frequency of phrenic nerve discharge (PND) in response to microinjection of L-glutamate before and after local microinjection of ionotropic or metabotropic glutamate receptors antagonists into the commissural subnucleus of the NTS (comNTS) were investigated. The experiments were performed in an in situ unanesthetized decerebrated working heart–brainstem preparation (WHBP), and the main findings were as follows: (a) microinjection of increasing concentrations of L-glutamate (5, 25, 50, 250 and 500 mM) into the comNTS produced bradycardia, increase in tSNA and reduction in the frequency of the PND in a concentration-dependent manner; (b) both bradycardia and increase in tSNA were almost abolished by kynurenic acid (KYN, 250 mM, a nonselective ionotropic glutamate receptor antagonist); (c) the reduction in the frequency of the PND was reversed to an increase in the frequency of the PND after KYN and this increase was blocked by the sequential microinjection of MCPG (100 mM, a nonselective metabotropic glutamate receptor antagonist); and (d) microinjection of increasing concentrations of *trans*-ACPD (0.5, 1.0, 2.5, 5.0 and 10 mM, a metabotropic glutamate receptor agonist), elicited bradycardia and increase in the frequency of the PND in a concentration-dependent manner, which were blocked by MCPG. Taken together, these data indicate that L-glutamate and its ionotropic receptors are involved in the sympathoexcitatory, bradycardic and reduction in the frequency of the PND responses whereas/although its metabotropic receptors are involved in the bradycardic and mainly in the increase in the frequency of the PND to microinjection of L-glutamate into the comNTS in the WHBP.

1. Introduction

The nucleus tractus solitarius (NTS) is the site in the brain where afferents mediating different cardiovascular and respiratory reflexes establish their primary synapse. The commissural subnucleus of the NTS (comNTS) encompasses a region that extends from the rostral edge of the area postrema to 1 mm caudal to the calamus scriptorius (CS; Drhuva et al., 1998). The chemoreceptor afferents have been reported to terminate predominantly in the region of the comNTS caudal

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to the CS (0–0.5 mm caudal, 0–0.5 mm lateral and 0.3–0.5 deep with respect to the calamus scriptorius; Sapru, 1996, 2004). On the other hand, there is evidence that the baroreceptor and cardiopulmonary afferents terminate in a region rostral and lateral to chemoreceptor projection site (Vardhan et al., 1993; Chitravanshi et al., 1994; Chitravanshi and Sapru, 1995, 1996; Marchenko and Sapru, 2000). Although the excitatory amino acid (EAA) L-glutamate is considered the most important neurotransmitter released in the NTS by the afferents of cardiovascular reflexes (Talman et al., 1980; Talman, 1989; Vardhan et al., 1993; Zhang and Mifflin, 1993), the involvement of this EAA in the neurotransmission of the chemoreflex, for example, is controversial due to different experimental approaches (Vardhan et al., 1993; Zhang and Mifflin, 1993; Haibara et al., 1995, 1999; Machado and Bonagamba, 2005). In addition, cardiovascular responses to microinjection of L-glutamate into the comNTS may also vary in accordance with the experimental approach used. Microinjection of L-glutamate into the comNTS rostral and lateral to the CS of anesthetized or unanesthetized rats produces baroreflex-like responses (Leone and Gordon, 1989; Talman et al., 1980; Talman, 1989; Canesin et al., 2000), whereas L-glutamate microinjected into the comNTS at the CS level of unanesthetized rats elicits increase in arterial pressure and bradycardia, a pattern of cardiovascular adjustments similar to that produced by the chemoreflex activation (Machado and Bonagamba, 1992; Colombari et al., 1994, 1996; Machado, 2001, 2004).

With respect to the respiratory effects of EAA receptor activation in the NTS, the data available are also controversial. Studies by Mizusawa et al. (1994) performed in unanesthetized rats showed that local microinjection of L-glutamate into the comNTS, caudal to the CS, increases ventilation by acting on NMDA receptors. In addition, studies by Bonham and McCrimmon (1990), performed in rats under urethane anesthesia, showed that the microinjection of DL-homocysteic acid into the NTS, from 0.8 mm rostral to 0.2 mm caudal to CS and from the midline to 2 mm lateral, inhibits phrenic nerve discharge by activation of receptors involved in the Hering–Breuer reflex. Besides, studies from Vitagliano et al. (1994), performed in rats under urethane anesthesia, showed that unilateral microinjection of L-glutamate or a metabotropic glutamate receptor agonist (*trans*-ACPD) into the NTS, 1.4 mm rostral and 0.5 lateral with respect to CS, produced apnea.

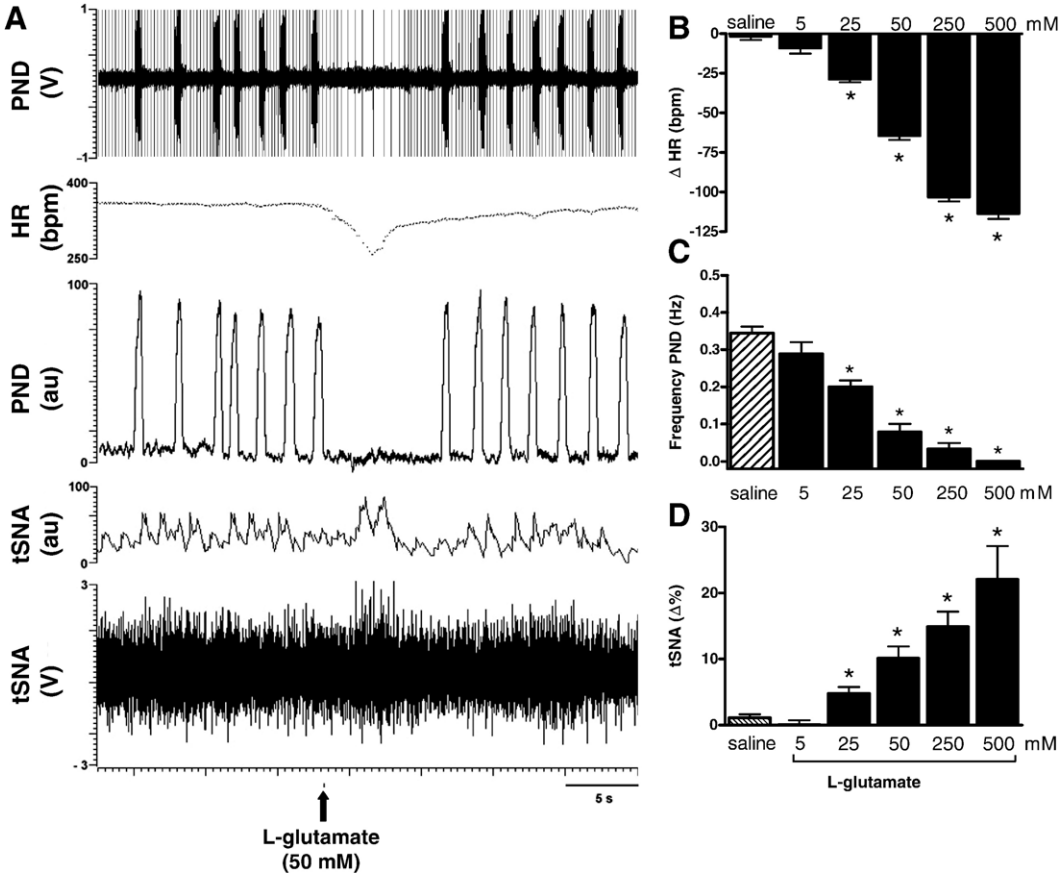

Fig. 1 – Effects of microinjection of L-glutamate into the comNTS on heart rate (HR), frequency of phrenic nerve discharge (PND) and thoracic sympathetic nerve activity (tSNA). Panel A illustrates a group of tracings from a representative WHBP showing the raw tracings of ECG and PND, the changes in HR, ∫PND, ∫tSNA and the raw tSNA in response to microinjection of L-glutamate (50 mM) into the comNTS. Panels B–D represent the changes in the heart rate (ΔHR, bpm), in the frequency of the PND (Hz) and in the tSNA (Δ%), respectively, in response to microinjection of increasing concentrations of L-glutamate into the comNTS (5, 25, 50, 250 and 500 mM in 20 nl; *n* = 9).