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Short Communication

Nucleus of the solitary tract chemical stimulation induces extracellular norepinephrine release in the lateral and basolateral amygdala

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ABSTRACT

The NTS catecholaminergic neurons, activated by a variety of afferent stimuli, are ideally situated to coordinate afferent signaling to multiple brain regions. In particular, there is evidence that systemic epinephrine injections induce a significant increase of norepinephrine (NE) in the amygdala during enhanced memory, which can be disrupted by NTS chemical blockade or interruption of vagal afferents. The present experiment was conducted to obtain information about the levels of NE release induced by activation of the whole NTS, which projects to the lateral and basolateral amygdala. Therefore, we compared NE levels before and after general stimulation of the NTS and the amygdala in anesthetized rats, without any behavioral or vagal stimulation, to find out the degree of noradrenergic activation modulated by the NTS through all its projections to the lateral and basolateral amygdala, as well as the degree of noradrenergic activation which may occur locally in the amygdala through rapid and general activation of this structure.

Introduction

The nucleus of the solitary tract (NTS) is the first relay and the major interface between sensory visceral afferents and the central nervous system; accordingly, the majority of vagal afferents project to the NTS, and these projections influence many homeostatic functions, including the cardiovascular reflex, food intake, stress, and cognitive processes [1–4]. The NTS catecholaminergic neurons, activated by a variety of afferent stimuli, are ideally situated to coordinate afferent signaling to multiple brain regions [5–7], including the hippocampus, hypothalamus, amygdala, nucleus accumbens, and dorsal motor nucleus of the vagus. The NTS and the basolateral amygdala (BLA) are highly and functionally connected; electrophysiological studies demonstrated that the firing interval in the amygdala neurons increases significantly after electrical stimulation of NTS neurons [8,9]. In particular, there is evidence that systemic epinephrine injections induce a significant increase of norepinephrine (NE) in the amygdala during enhanced memory, which can be disrupted by NTS chemical blockade [10]. Furthermore, vagal nerve stimulation improves cognitive processing and induces increases of NE release in the BLA [11]. Interestingly, some findings indicate that NTS neurons also project directly to the locus coeruleus (LC) [12,13], a structure that provides the major source of NE innervations to the BLA as well as to several other structures [14,15]. Thus, vagal activation or direct stimulation of the NTS may initiate NE release in the amygdala, either by direct NTS action on the BLA [16] or via polysynaptic pathway involving the LC [14].

The present experiment was conducted to obtain information about the levels of NE release induced by activation of the NTS, which projects to the lateral and basolateral amygdala [16]. Therefore, we compared NE levels before and after general stimulation of the whole NTS and the amygdala to find out the degree of noradrenergic activation modulated by the NTS through all its projections to the lateral and basolateral amygdala, as well as the degree of noradrenergic activation which may occur locally in the amygdala through rapid and general activation of this structure.

Twelve male Sprague–Dawley rats (Instituto de Neurobiología breeding colony, weighing 250–300 g) were individually housed with access to food and water and maintained at 23 °C in an inverted 12-h/12-h dark–light cycle. All experimental procedures were performed during the dark phase and were in compliance with the NIH Guide for Care and Use of Laboratory Animals and Rules in Health Matters (Ministry of Health, México). During the microdialysis experiment, rats were deeply anaesthetized with urethane (1.0 ml/kg i.p) and, using standard stereotaxic procedures, a stainless steel injector was placed unilaterally, in left NTS (AP = −13.3 mm, L = +1.0 mm, V = −7.6 mm from bregma); also,

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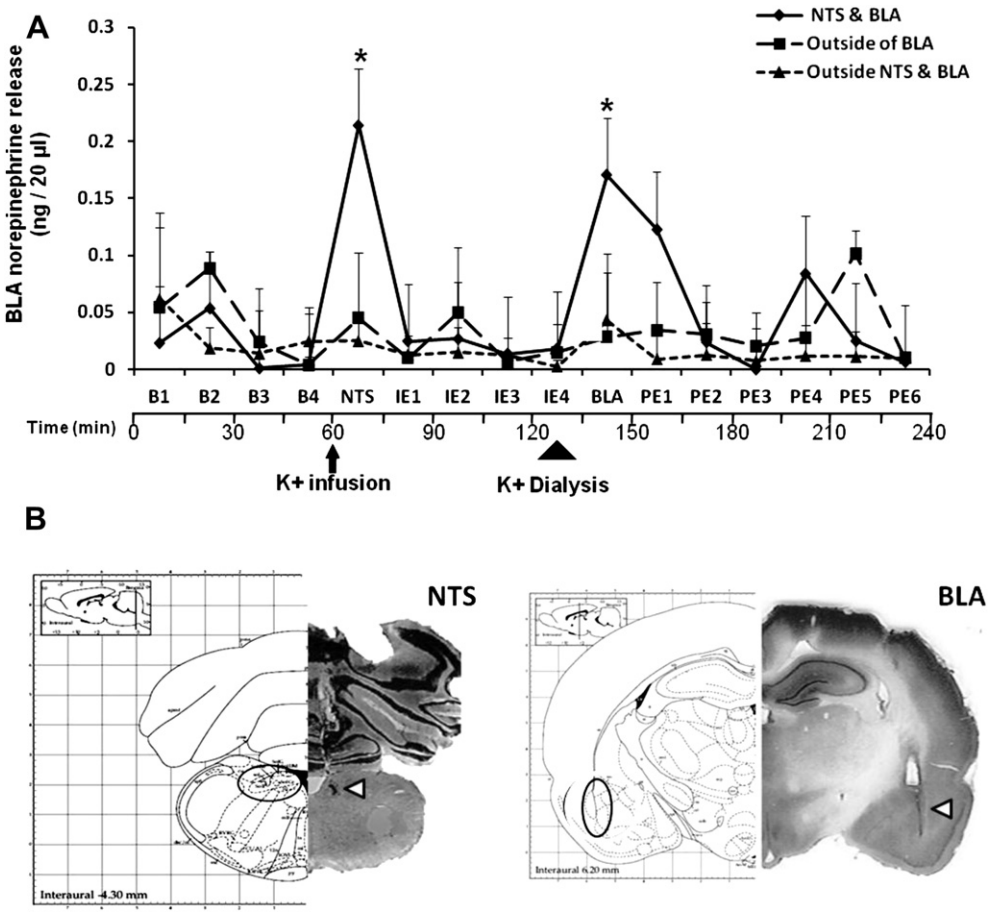

Figure 1. (A) Changes in the release of NE in the BLA after stimulation of the NTS (thin arrow) or direct chemical stimulation of the BLA (wide arrow) with high potassium ($n = 6$; $^*P \leq 0.05$). Release observed in animals with misplaced location of lateral or basolateral microdialysis probe (Square-dotted line) ($n = 4$, $P > 0.05$); release observed in animals with misplaced location of NTS injector and lateral and basolateral amygdala probe (triangle-dotted line) ($n = 6$, $P > 0.05$). (B) Diagrams and representative photomicrographs illustrating the placement (arrowheads) of the injector and microdialysis probe in the NTS and BLA. The circles on the diagrams represent approximately the areas considered to be correct for injector and microdialysis probe localization.

a microdialysis probe (BAS, West Lafayette, IN) was placed ipsilateral in the left BLA (AP = −2.8, $L = \pm 5.0$ mm, $V = -8.5$ mm from bregma) according to the atlas of Paxinos and Watson [17]. The microdialysis was started after the injector and microdialysis probe had been inserted. The probe in the BLA was continuously perfused with Ringer solution (4.7 mM KCl, 2 µl/min); the first 60-min sample was discarded, and then 16 samples were collected at 15-min intervals in 300-µl Eppendorf tubes containing 5 µl 0.1 M $HClO_4$, to prevent NE oxidation; 3,4-Dihydroxybenzylamine (DHBA) dissolved in the $HClO_4$, was used as internal standard. The initial four samples were considered baseline release (B1-B4); during sample five collection, high potassium Ringer solution was infused (110 mM KCl, 1.5 µl/min) by the micro-injectors in the NTS. Four additional inter-stimulus samples (IE1–IE4) were collected over 60 min. During sample 10 collection, high potassium Ringer solution (110 mM KCl, depolarizing compound), instead of regular Ringer (4.7 mM KCl), was infused via the microdialysis probe into the BLA at the same rate (2.0 µl/min) for 15 min. Six additional post-stimulus samples (PE1–PE6) were collected with normal Ringer to evaluate the effects on NE release. Samples were immediately frozen at −80 °C for further analysis. The NE levels in the dialysis samples (30 µl) were analyzed by reverse-phase liquid chromatography with electrochemical detection (BAS CC-5E) using an HPLC system (BAS PM-80) with a 3-µm phase II ODS column (BAS) and a mobile phase consisting of 15 ml of HPLC grade acetonitrile containing 190 mg disodium EDTA, 230 mg octyl sodium sulfate, and 7.08 g chloroacetic acid adjusted to pH 3.0. The data obtained (expressed as ng/20 µl sample) were analyzed using the general linear models procedure of SAS (2008), and LSMEANS multiple comparison tests were used to obtain and analyze treatment methods. Results with $P \leq 0.05$ were considered significant. At the end of microdialysis experiments, rats were overdosed with sodium pentobarbital and intracardially perfused with 0.9% saline. Brains were removed from skulls and stored in 4% paraformaldehyde solution for 24 h at 4 °C. The brains were then immersed in a 30% sucrose solution at 4 °C, frozen at −40 °C, and cut in a microtome (Leica) to obtain coronal slices (50 µm thick). After staining with cresyl violet, the slices were examined under a light microscope to determine the needle tip injection and probe tip locations in the NTS or BLA, respectively, according to the atlas of Paxinos and Watson [17]. Only six animals out of a total of sixteen, in which the probe tip was inside the BLA boundaries, and which had very similar anterior and posterior brain coordinates as well as similar and precise bilateral injector localization in the NTS, were considered as correct data in the present results (see representative pictures in Fig. 1B). As can be seen in Fig. 1B, the correct target area for the NTS injectors includes all NTS sub-regions (gelatinous, lateral and medial). However, tests made with pontamine blue stain (data not shown) indicated a minimal diffusion into nearby areas such as the hypoglossal nucleus, of the nucleus parasolitary and of the “Probst’s bundle”. The target area of the amygdala that was included in the data was limited to the anterior, posterior, and