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NORADRENERGIC MODULATION OF NEURONAL RESPONSES TO *N*-METHYL-D-ASPARTATE IN THE VESTIBULAR NUCLEI: AN ELECTROPHYSIOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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Abstract—Excitatory responses evoked by *N*-methyl-D-aspartate (NMDA) in the vestibular nuclei (VN) of the rat were studied *in vivo* during microiontophoretic application of noradrenaline (NA) and/or its agonists and antagonists. Ejection of NA-modified excitatory responses mediated by NMDA receptors (NMDAR) in all neurons tested; the effect was enhancement in 59% of cases and depression in the remaining 41%. Enhancements prevailed in all VN with the exception of the lateral vestibular nucleus, where both effects were recorded in an equal number of cases. The enhancing action of NA on NMDAR-mediated responses was mimicked by the noradrenergic beta-receptor agonist isoproterenol, the beta$_1$ specific agonist denopamine and the alpha$_2$ agonist clonidine. These effects were blocked respectively by the generic beta-receptor antagonist timolol, the beta$_1$ antagonist atenolol and the alpha$_2$ antagonist yohimbine. In contrast, application of the alpha$_1$ receptor agonist cirazoline and the specific alpha$_1$ antagonist prazosin respectively mimicked and partially antagonized the depression of NMDAR-mediated excitations induced by NA. Double-labeling immunohistochemical techniques demonstrated broad colocalization of NMDAR (specifically NR1 and NR2 subunits) with noradrenergic receptors (alpha$_1$, alpha$_2$ and beta$_1$) in many VN neurons; only minor differences were found between nuclei. These results indicate that NA can produce generalized modulation of NMDAR-mediated excitatory neurotransmission in VN, which may in turn modify synaptic plasticity within the nuclei.

Key words: NMDA receptors, noradrenaline, vestibular complex, firing rate, microiontophoresis.

INTRODUCTION

The vestibular nuclei (VN) constitute a sensorimotor complex involved in the control of posture (Pompeiano, 1972; Sarkisian, 2000), eye movements (Pompeiano, 1972; Ito, 1991; Sarkisian, 2000) and motor learning (Broussard and Kassardjian, 2004).

Primary vestibular afferents to the VN are mostly glutamatergic (Zhang et al., 2011). The important role played by glutamate in these nuclei is confirmed by the high concentration of the neurotransmitter (Li et al., 1996) and its receptors (De Waele et al., 1994; Vidal et al., 1996), found throughout the whole complex. A significant and selective noradrenergic projection from the locus coeruleus is also delivered to VN (Schuerger and Balaban, 1993, 1999). Modulation of glutamatergic neurotransmission by noradrenaline (NA) has been described in various central structures such as the hippocampus (Stanton et al., 1989; Segal et al., 1991), prefrontal cortex (Ji et al., 2008a,b), amygdala (Ferry et al., 1997) and cerebellar cortex (Pompeiano, 2006). We found in a previous study of VN that GLU-evoked excitatory effects on secondary vestibular neurons are modulated by NA (Barresi et al., 2009).

Ionotropic AMPA and *N*-methyl-D-aspartate receptors (NMDAR) are widely colocalized in VN (Chen et al., 2000). The NMDAR participate in various learning mechanisms throughout the CNS (Malenka and Nicoll, 1993; Dineley et al., 2001; Antic et al., 2010). Specifically, in the VN, a structure characterized by intrinsic plasticity (Gittis and du Lac, 2006), NMDAR are related to long-term potentiation and depression (Scarduzio et al., 2012).

We set out to determine whether NA application could modulate NMDAR-mediated responses in VN, and which types of noradrenergic receptor may be involved. We also explored the extent to which NMDAR are co-localized with noradrenergic receptors in single neurons of the VN. Noradrenergic effects, mediated by various receptors, and implicated in learning have been described (Cahill and McGaugh, 1996; Gibbs et al., 2010; McIntyre et al., 2012). A possible involvement of NA in mechanisms of synaptic plasticity in VN by a modulation of NMDAR-mediated responses is discussed.

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Abbreviations: ANOVA, analysis of variance; ATE, atenolol; CIRA, cirazoline hydrochloride; CLO, clonidine hydrochloride; DENO, denopamine; ISO, L-isoproterenol hydrochloride; LVN, lateral vestibular nucleus; MBA, mean background activity; MVN, medial vestibular nucleus; NA, noradrenaline; NMDA, *N*-methyl-D-aspartate; NMDAR, NMDA receptors; PBS, phosphate-buffered saline; PRA, prazosin hydrochloride; SD, standard deviation; SIOC, semi-quantitative indicator of co-localization; SpVN, spinal vestibular nucleus; SVN, superior vestibular nucleus; VN, vestibular nuclei; YO, yohimbine hydrochloride.

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EXPERIMENTAL PROCEDURES

Electrophysiological and double-labeling immunohistochemical techniques were employed.

Electrophysiology and microiontophoresis

Experiments were performed on 18 Wistar rats deeply anesthetized with urethane (1.5 g/kg i.p.). Acquisition and care of laboratory animals conformed to the European Communities Council Directive (86/609/EEC), guidelines in the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and with Italian law. The experimental protocol was approved by the IACUC (International Animal Care and Use Committee) of the University of Catania. Toe pinch reflex, heart rate and respiratory rate were used to monitor anesthetic levels. Supplementary doses of anesthetic were administered when necessary. Body temperature was maintained between 37 and 38 °C with a heating pad, and a gel of agar-agar (2%) covered the exposed tissue to prevent desiccation. Small holes were drilled in the skull and a five-barrel glass microelectrode was introduced at coordinates corresponding to the VN (Paxinos & Watson, 1997). The final point of each penetration was marked by ejecting Pontamine Sky Blue (Sigma, Milano, Italy) from the recording electrode (negative current pulse of 20 μA for 20 min). At the end of the experiment, the brain was removed and fixed in 10% formalin. Electrode tracks and recording sites were identified in 60 μm coronal sections through the VN that were stained with Neutral Red.

Five-barrel glass microelectrodes were used to record single unitary activity of secondary vestibular neurons and to apply drugs with microiontophoresis. The recording barrel (resistance 6–10 MΩ) was filled with a 4% solution of Pontamine Sky Blue in 3 M NaCl. Action potentials acquired, analyzed and stored using a personal computer (interface: Cambridge Electronic Design 1401, software: Spike2) were checked for their unitary nature and processed if they remained unmodified in amplitude during the test and had a signal to noise ratio of at least 3:1. Three barrels of the micropipette were used for microiontophoresis and contained *N*-methyl-D-aspartate (NMDA, Sigma, Milano, Italy, 100 mM, pH 8.0) and two of the following: norepinephrine hydrogen tartrate (NA, Sigma, Milano, Italy, 100 mM, pH 4.0–5.0), cirazoline hydrochloride (CIRA, Tocris Bioscience, Milano, Italy, 50 mM, pH 4.5–5.0), prazosin hydrochloride (PRA, Tocris Bioscience, Milano, Italy, 5 mM, pH 4.5–5.0), clonidine hydrochloride (CLO, Tocris Bioscience, Milano, Italy, 50 mM, pH 4.5–5.0), yohimbine hydrochloride (YO, Sigma, Milano, Italy, 20 mM, pH 4.5–5.0), L-isoproterenol hydrochloride (ISO, Sigma, Milano, Italy, 20 mM, pH 4.5–5.0), timolol maleate (TIM, Tocris Bioscience, Milano, Italy, 20 mM, pH 4.5–5.0) denopamine (DENO, Sigma, Milano, Italy, 50 mM, pH 4.5–5.0) and atenolol (ATE, Sigma, Milano, Italy, 10 mM, pH 4.5–5.0). All drugs were dissolved in water with the exception of DENO, which was dissolved in dimethyl sulfoxide. The microiontophoretic system (Neurophore BH-2, Harvard Apparatus, Holliston, MA, USA) balanced currents automatically through a barrel filled with 3 M NaCl to neutralize any voltage shift due to the applied currents. Retaining currents (2–10 nA) of appropriate polarity were constantly applied to prevent drug leakage. NMDA was applied with brief (30 s) negative current pulses (intensity up to 80 nA), while NA agonists and antagonists were ejected with longer-lasting currents (up to 20 min, 1–20 nA). Whenever a single unit was isolated, applications of NMDA (30 s pulses) were routinely followed by three (or more) applications performed during continuous application of NA or one of its agonists at low doses that did not by themselves modify the firing rate of the tested neuron. NMDA was then pulsed for at least 5 min after cessation of NA ejection to ascertain recovery. In some cases the sequence of applications was repeated during simultaneous application of NA and an antagonist specific for a specific type of noradrenergic receptor. The retention–ejection cycle of NMDA applications was determined in each neuron by the duration of the response and the recovery after the NMDA response. A short-lasting, high-dose application of NA was finally tested to ascertain the effect of the amine on the background firing rate.

The firing rate of each recorded unit was calculated and integrated over 1 s bins for analysis and 5 s bins for display. The mean firing rate, calculated over a sequence of 180 values (3 min) before any drug application, was defined as the mean background activity (MBA). If the standard deviation (SD) of this parameter exceeded 50% of the MBA, the unit was excluded from analyses.

Following a drug ejection, a neuron was defined as responsive if its MBA was differed by at least 2 SD from the MBA for at least 20 s. The parameters used to quantify the response were the magnitude (*M*) and the contrast (*C*). *M* indicated the absolute intensity of the effect, and was defined as the difference between the number of spikes recorded during the response and the number recorded during a period with the same duration immediately preceding drug ejection. *C* (in %) was the ratio between these two values and indicated the signal-to-noise value. The effects of NA agonists on the responses to NMDA application were expressed as modifications of the *M* and *C* values induced by NMDA applied to the same neuron.

As a rule at least three single responses to microiontophoretic applications of NMDA alone (control) were compared with at least three responses to NMDA obtained during continuous application of an NA agonist. Modification of an NMDA response was regarded as significant if at least one of the mean values of the parameters used (*M*, *C*) differed significantly from the mean values recorded during the preliminary control (two-tailed Student's *t*-test and Mann–Whitney *U* test for non-parametric data). The trend of the two parameters *M* and *C* could only differ significantly if the NA agonist influenced the background firing rate. In fact, in a few cases, NA application modified the background activity even at very low doses of 1–2 nA or 0 (no retention current).