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NORADRENALINE MODULATES NEURONAL RESPONSES TO GABA IN VESTIBULAR NUCLEI

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Abstract—The effects of noradrenaline (NA) on the inhibitory responses to GABA were studied *in vivo* in neurons of the vestibular nuclei of the rat using extracellular recordings of single unit electrical activity and a microiontophoretic technique of drug application in loco. NA application influenced GABA-evoked inhibitions in 82% of tested neurons, depressing them in 42% and enhancing them in 40% of cases. The more frequent action of NA on GABA responses was depressive in lateral and superior vestibular nuclei (50% of neurons) and enhancing in the remaining nuclei (56% of neurons). The most intense effect of NA application was the enhancement of GABA responses induced in a population of lateral vestibular nucleus neurons, characterized by a background firing rate significantly higher than that of other units. The alpha$_2$ noradrenergic receptor agonist clonidine mimicked the enhancing action of NA on GABA responses; this action was blocked by application of the specific alpha$_2$ antagonist yohimbine. The beta adrenergic agonist isoproterenol induced either depressive or enhancing effects on GABA responses; the former more than the latter were totally or partially blocked by application of the beta antagonist timolol. It is concluded that NA enhances GABA responses by acting on noradrenergic alpha$_2$ and to a lesser extent beta receptors, whereas depressive action involves beta receptors only.

These results confirm the hypothesis that the noradrenergic system participates in the regulation of the vestibulospinal and the vestibulo-ocular reflexes and suggest that conspicuous changes of NA content in brain due to aging or stress could lead to a deterioration in the mechanisms of normal vestibular function.

Key words: alpha$_2$-adrenoceptor, beta-adrenoceptor, microiontophoresis, firing rate, rat.

Vestibular nuclei process labyrinthine information contributing to postural control (Pompeiano, 1972; Sarkisian, 2000), regulation of ocular movements (Pompeiano, 1972; Ito, 1991; Sarkisian, 2000) and, according to more recent theories, to motor learning also (Broussard and Kassardjian, 2004). The integration of direct vestibular information with that filtered by the cerebellar network is the crucial task of vestibular nuclei (Gernandt and Gilman, 1959; McCrea et al., 2001) and specifically of the superior (SVN) and the lateral (LVN) nuclei, both involved in motor control, even if in different types of motor performance. Inputs to these nuclei are represented by the primary vestibular fibers, by a conspicuous projection from cerebellar cortex and cerebellar medial nucleus (Barmack, 2003) and by smaller groups of fibers such as trigeminal afferents (Diagne et al., 2006).

GABA exerts a primary role in the function of these nuclei because it is the neurotransmitter used by corticocerebellar fibers and also mediates the commissural inhibition existing between the two vestibular complexes. As such it is responsible for enhancing the sensitivity of vestibular neurons to head acceleration (Gliddon et al., 2005). Neuronal responses to GABA are inhibitory, short-lasting and intense in both LVN and SVN.

In addition to specific sensory information delivered to the vestibular complex by glutamatergic and, to a lesser extent, by cholinergic fibers (Barmack, 2003), a diffuse noradrenergic projection from locus coeruleus reaches all the vestibular nuclei (Schuerger and Balaban, 1993, 1999). Impairment of this pathway has severe consequences on vestibular function and is implicated in sensory mismatch during vertigo and motion sickness (Nishiike et al., 2001).

A noradrenergic control of GABA-evoked inhibition and, in more general terms, an interaction between GABAergic and noradrenergic systems is frequently observed in the CNS (Strahlendorf et al., 1979; Suzdak and Gianutsos, 1985; Solignac and Enero, 1993; Kawaguchi and Shindou, 1998; Beverly et al., 2000). As an example, in cerebellar Purkinje cells noradrenaline hydrogen tartrate (NA) evokes depression of the background firing rate involving various receptors (Parfitt et al., 1988) and, even at doses unable to induce any effect on the spontaneous firing rate, exerts a control on responsiveness to GABA selectively mediated by beta$_1$ receptors (Moises et al., 1979, 1980; Yeh and Woodward, 1983; Cheun and Yeh, 1992, 1996).

In the vestibular nuclei NA application induces a weak, almost homogeneous depression of the neuronal background firing rate, analogous to that observed in Purkinje cells and mediated by noradrenergic alpha$_2$ receptors in all the nuclei (Licata et al., 1993). The only exceptions are in the medial vestibular nucleus (MVN), where excitation involving noradrenergic alpha$_1$ and beta$_1$ receptors has been described (Podda et al., 2001).

The aim of this work was: a) to test the hypothesis that NA can either enhance and/or decrease the responsiveness to GABA in the neurons of the vestibular complex, b)

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Abbreviations: CLO, clonidine hydrochloride; ISO, L-isoproterenol hydrochloride; LVN, lateral vestibular nucleus; MVN, medial vestibular nucleus; NA, noradrenaline hydrogen tartrate; SpVN, spinal vestibular nucleus; SVN, superior vestibular nucleus; TIM, timolol maleate; YO, yohimbine hydrochloride.

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to try to identify the noradrenergic receptors involved. A secondary goal was to compare noradrenergic modulation of GABA-evoked inhibition in LVN and SVN; both belong to vestibular complex but control different motor tasks and have some important morphofunctional differences.

Modulation of inhibitory responses by NA would have interesting functional implications. For example, aging modifies the contents of NA and noradrenergic receptors in the brain (Scarpace and Abrass, 1988; Markowska et al., 1989; Cransac et al., 1996; Esler et al., 2002), and might impair vestibular function.

EXPERIMENTAL PROCEDURES

Animal preparation

Experiments were performed on Wistar rats deeply anesthetized with urethane (1.5 g/kg). Acquisition and care of laboratory animals conformed to the European Communities Council Directive (86/609/EEC), to the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and to Italian law. The experimental protocol was approved by the IACUC of the University of Catania. Every effort was made to minimize the number of animals used and their suffering.

Loss of toe-pinch reflex was used to indicate surgical anesthesia. Heart rate was monitored continuously during the experiments and supplementary doses of urethane were administered whenever the heart rate exceeded 370–380 beats per min. Body temperature was maintained with a heating pad, and a gel of agar-agar (2%) was used to cover the exposed tissue and to prevent desiccation.

The head was held in a stereotaxic frame, small holes were drilled in the skull and a multi-barrel glass microelectrode was positioned with a micromanipulator at coordinates corresponding to the nuclei of the vestibular complex (Paxinos and Watson, 1986).

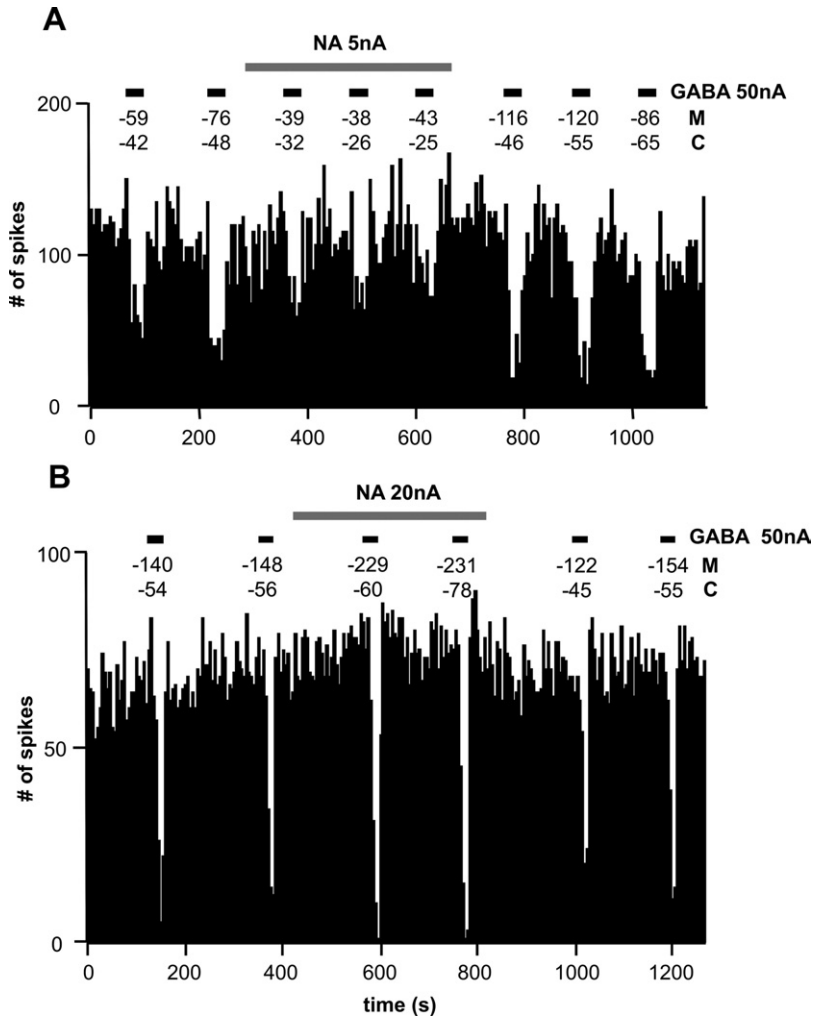

Fig. 1. Examples of modulation of responses to GABA by NA application. The histograms illustrate the number of spikes fired by two neurons of the LVN in successive 5-s bins. Each column indicates the number of spikes fired in a period of 5 s. The horizontal bars above the histograms indicate the duration of the ejection periods of the indicated drugs at the current given. The mean firing rates recorded in the absence of any drug application represent the background activity. The amount of each GABA-evoked inhibition is indicated by a depression of the firing and described by the values of magnitude M and contrast C, reported under the horizontal bars (a third parameter, the duration D is not shown). During NA application, inhibitory responses to GABA of the neuron A were depressed and those of neuron B were enhanced. Both effects were reversible and recovery was completed after less than 2–3 min from the end of NA application.