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NORADRENERGIC MODULATION OF GLUTAMATE-INDUCED EXCITATORY RESPONSES IN SINGLE NEURONS OF THE RED NUCLEUS: AN ELECTROPHYSIOLOGICAL STUDY

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Abstract—The effect induced by noradrenaline (NA) on the spiking activity evoked by glutamate (Glu) on single neurons of the mesencephalic red nucleus (RN) of the rat was studied extracellularly. Long-lasting microiontophoretic applications of the amine induce a significant and reversible depression of the responsiveness of RN neurons to Glu. This effect was mediated by noradrenergic alpha$_2$ receptors since it was mimicked by application of clonidine, an alpha$_2$ adrenoceptor agonist, and blocked or at least reduced by application of yohimbine, an antagonist of NA for the same receptors. The effect appears homogeneously throughout the nucleus and is independent of the effect of NA on baseline firing rate. Application of isoproterenol, a beta adrenoceptor agonist, either enhanced or depressed neuronal responses to Glu in a high percentage (86%) of the tested neurons. Moreover, application of timolol, a beta adrenoceptor antagonist, was able to strengthen the depressive effects induced by NA application on neuronal responsiveness to Glu. Although these data suggest some involvement of beta adrenergic receptors in the modulation of neuronal responsiveness to Glu, the overall results indicate a short-term depressive action of NA, mediated by alpha$_2$ receptors, on the responsiveness of RN neurons and suggest that stress initially leads to an attenuation of the relay function of the RN.

Key words: noradrenaline, glutamate, red nucleus, electrophysiology, microiontophoresis.

INTRODUCTION

The red nucleus (RN) is a mesencephalic structure involved in various aspects of motor control including, according to recent theories, even phonation (Martin and Ghez, 1988; Schmied et al., 1988; Hicks and Onodera, 2012). The classical opinion about the RN is that it is closely related in evolutionary terms to the development of the limbs (ten Donkelaar, 1988). The RN in mammals is arranged in two parts: the ventrolateral one, characterized by cells of big size and therefore named magnocellular (RMC), directly controls the distal motor periphery through the rubrospinal tract. The dorsomedial part, characterized by cells of smaller size and thus named parvicellular (RPC), forms an integral part of a more elaborate neural network, including the motor cortex and the cerebellum, and contributes to the processing and improvement of motor programs (Reid et al., 1975; Kennedy et al., 1986; Padel, 1993). Several studies, however, call into question the existence of a strict anatomical and functional segregation between the two parts already in the cat (Horn et al., 2002; Pong et al., 2002) and even more in rodents (Shieh et al., 1983; Kennedy and Humphrey, 1987), probably in parallel with the development of skilled motor actions. Anyway, the RMC part and the direct path to the periphery, the rubrospinal tract, appear less significant, almost vestigial, in humans, at least in adults (Patt et al., 1994) while the rubrocerebellar path retains its importance (Onodera and Hicks, 2009).

However, despite this apparent functional regression, a reorganization of the rubrospinal path seems to be essential in the compensatory mechanisms activated following strokes that impair the function of the corticospinal path (Takenobu et al., 2014). Moreover, a malfunction of the RN is related to the genesis of cerebellar tremor (Lefranc et al., 2014) and RN neuronal activity appears modified in many disorders from multiple sclerosis (Klaas et al., 2013) to Parkinson's (Lewis et al., 2013) and even Alzheimer's disease (Langkammer et al., 2014).

Various neurotransmitters contribute to define the function of RN neurons. One of them is glutamate (Glu), released by corticorubral fibers and by a good number of cerebellorubral fibers, which convey the cerebellar output (Bernays et al., 1988; Nieoullon et al., 1988). Many types of ionotropic and metabotropic Glu receptors are located throughout the RN (Billard et al., 1991; Shigemoto et al., 1992), suggesting that the role of this amino acid in the nucleus is widespread and very articulate.

The function of neuroamines within the RN is also complex. A dense plexus of serotonergic nerve terminals reach the RN (Bosler et al., 1983) originating in the nucleus raphe dorsalis (Pierce et al., 1976; Bernays et al., 1988) and, as suggested by some functional results (Satoh et al., 2014), also in the nucleus raphe magnus.

Noradrenergic fibers, although scattered, are present throughout the RN (Swanson and Hartman, 1975) and

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Abbreviations: BFR, background firing rate; BFR, baseline firing rate; Glu, glutamate; ISO, isoproterenol; NA, noradrenaline; RMC, magnocellular; RN, red nucleus; RPC, parvicellular; SD, standard deviation; YO, yohimbine hydrochloride.

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originate from the ventral adrenergic cell groups in the pons and medulla, in part corresponding to the middle tegmental catecholamine radiation (Lindvall and Bjorklund, 1974).

Both amines modulate the baseline firing rate (BFR) of rubral neurons (Licata et al., 1995; Ciranna et al., 1996). Furthermore, in the RN, noradrenaline (NA) and 5-hydroxytryptamine (5-HT) exert a selective control on neuronal responses to GABA (Ciranna et al., 2000; Licata et al., 2001), released by a dense population of small-sized interneurons and by fibers arising from the mesencephalic reticular formation and from the *substantia nigra* (Fu et al., 1996). Since high levels of NA are found in the RN (Versteeg et al., 1976), we questioned whether NA is also able to influence the responsiveness of RN neurons to Glu and therefore to modify the effects evoked by cortical and cerebellar input.

The noradrenergic system modulates a variety of behavioral functions, involving attention and memory circuits, aimed to ''the facilitation of processing of relevant, or salient, information'' (Berridge and Waterhouse, 2003). In particular, this system is active during stress.

A possible control by NA on the neuronal responsiveness to Glu in the RN would imply that the functions of this nucleus may be affected by any behavioral state implicating an activation of the noradrenergic system, e.g., stress. This research was aimed to verify if and how the responsiveness of RN neurons to Glu can be modified in the presence of NA and which noradrenergic receptors might be involved.

EXPERIMENTAL PROCEDURES

The experimental procedures used were already described in various papers previously published (Licata et al., 1995, 2001; Ciranna et al., 2000).

Experiments were performed on 32 adult male Wistar rats (200–250 g, Morini) that were deeply anesthetised with urethane (intraperitoneal injection 1.5 g/kg). Acquisition and care of laboratory animals conformed with 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 Ethics Committee of the University of Catania.

Heart rate was monitored continuously by a ratemeter equipped with two cutaneous microprobes. Supplementary doses of urethane (0.6 g/kg) were administered by intramuscular injection during the experiment if heart rate increased more than 370–380 beats per min showing that the animal was emerging from anesthesia. Loss of the toe-pinch reflex was used as an indicator of surgical anesthesia. A gel of agar–agar (2%) was used to cover the exposed tissue and prevent desiccation. Body temperature, recorded by a thermocouple, was maintained between 38 and 39 °C by a heating pad.

The head was held in a stereotaxic frame, small holes were drilled in the skull unilaterally and a multi-barrel glass microelectrode was positioned with a micromanipulator driven by hand at coordinates (Paxinos and Watson, 2007) corresponding to the RN (A: 3.70–2.70, L: 0.60–1.40, H: 2.80–2.00).

The final point of each penetration in the RN was stained by a negative current ejection of Pontamine Sky Blue (Sigma) (20 μA–5/10 min) delivered through the recording electrode. Electrode tracks and recording sites were identified in serial coronal sections of the RN (60-μm-thick), cut using frozen tissue and stained with Neutral Red (Fig. 1A, left). The sites that were not positively identifiable as belonging to RMC or RPC, were excluded from successive statistical analysis.

Recording and drug microiontophoresis

Five-barrel microelectrodes were used to record the spiking activity of single RN neurons and to apply drugs by microiontophoresis.

The spiking activity of single RN neurons was recorded extracellularly with one barrel (impedance: 7–12 M) of the microelectrode filled with a 4% solution of Pontamine Sky Blue in 3 M NaCl.

Three barrels of the microelectrode were used for iontophoresis, containing monosodium glutamate (Glu, Sigma 100 mM, pH 8.0) and two of the following: noradrenaline hydrogen tartrate (NA, Sigma, 200 mM, pH 4.0), clonidine hydrochloride (CLO, Tocris, 50 mM, pH 5.0), L-isoproterenol hydrochloride (ISO, Sigma, 50 mM, pH 5.0), yohimbine hydrochloride (YO, Sigma, 20 mM, pH 4.5–5.0), timolol maleate (TIM, Tocris, 20 mM, pH 4.5–5.0). Before barrel filling and penetration, pH values of the solutions were routinely controlled and adjusted if necessary.

The microiontophoretic system (Neurophore BH-2, Medical System Corp) balanced currents automatically through a barrel filled with 3 M NaCl to neutralize any voltage shift caused by the applied currents.

Retaining currents of 2–10 nA (positive for Glu, negative for the remaining drugs) were applied to the barrels to reduce drug leakage during electrode penetrations.

Glu was applied with brief (30 s) negative current pulses (intensity up to 60 nA), while NA agonists and antagonists were applied with longer lasting positive currents (up to 20 min, 2–20 nA).

Spikes were rated as unitary and then processed only if they had a signal-to-noise ratio of at least 3:1 (Fig. 1A, right) and could be discriminated on the basis of spike shape and amplitude, which remained unmodified during the tests.

Pulses amplified (300 Hz to 10 kHz band-pass) were recorded and processed by a PC provided with a peripheral device for acquiring signals (interface: Cambridge Electronic Design 1401, software: SPIKE2) that analyzed spike sequences on and off line.

After recognizing the spiking activity related to a single unit, three applications (30 s pulses) of Glu were routinely followed (if possible) by three applications performed during continuous ejection of NA or one of its agonists. In some cases the sequence of applications was repeated during simultaneous application of an NA antagonist specific for a noradrenergic receptor. Glu

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