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Parabrachial complex glutamate receptors modulate the cardiorespiratory response evoked from hypothalamic defense area

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ABSTRACT

To characterize the possible role of glutamate in the interaction between Hypothalamic Defense Area (HDA) and Parabrachial complex (PBc) nuclei, cardiorespiratory changes were analyzed in response to electrical stimulation of the HDA (1 ms pulses, 30–50 μA given at 100 Hz for 5 s) before and after the microinjection of the nonspecific glutamate receptor antagonist kynurenic acid (50 nl, 5 nmol), NMDA receptor antagonist MK-801 (50 nl, 50 nmol), non-NMDA receptor antagonist CNQX (50 nl, 50 nmol) or metabotropic glutamate receptor antagonist MCPG (50 nl, 5 nmol) within the PBc. HDA stimulation evoked an inspiratory facilitatory response, consisting of an increase in respiratory rate ($p<0.001$) due to a decrease in expiratory time ($p<0.01$). The respiratory response was accompanied by a pressor ($p<0.001$) and a tachycardic response ($p<0.001$).

Kynurenic acid within the lateral parabrachial region (lPB) abolished the tachycardia ($p<0.001$) and decreased the magnitude of blood pressure response ($p<0.001$) to HDA stimulation. Similarly, the magnitude of the tachycardia and the pressor response was decreased after the microinjection of MK-801 ($p<0.01$ and $p<0.001$, respectively) and CNQX ($p<0.05$ in both cases) into the lPB. Kynurenic acid microinjection in this region produced an inhibition of the tachypnea ($p<0.001$) to HDA stimulation but the respiratory response persisted unchanged after MK-801 or CNQX microinjection into the lPB.

Kynurenic acid within the medial parabrachial region (mPB) abolished the tachycardia ($p<0.01$) and decreased the magnitude of the pressor response ($p<0.001$) to HDA stimulation. MK-801 and CNQX microinjection in this region decreased the magnitude of the tachycardia ($p<0.05$, in both cases) and pressor response ($p<0.05$, in both cases). The respiratory response evoked by HDA stimulation was not changed after the microinjection of kynurenic acid, MK-801 or CNQX within the mPB.

No changes were observed in the cardiorespiratory response evoked to HDA stimulation after MCPG microinjection within lPB and mPB.

These results indicate that glutamate PBc receptors are involved in the cardiorespiratory response evoked from the HDA. The possible mechanisms involved in these interactions are discussed.

1. Introduction

The parabrachial complex (PBc), located within the dorsolateral pons, is an important brainstem site within the context of central autonomic regulatory responses. PBc participates in a variety of visceral regulatory functions that range from blood pressure and respiratory control to taste and feeding (Biondolillo et al., 2009; De Gobbi et al., 2009; Hayward, 2007; Lara et al., 1994, 2002; Norgren and Pfaffmann, 1975). The functional heterogeneity of the PBc is reflected in its anatomy; at least 11 parabrachial subnuclei have been identified on the basis of their unique cytoarchitecture, chemical phenotype, and circuitry (Chamberlin and Saper, 1992; Fulwiler and Saper, 1984; Herbert et al., 1990).

In rat, the PBc modulates respiration in two different ways. Glutamate microstimulation of neurons located within the medial region of the parabrachial complex (mPB) evoked a prolongation of expiration, while stimulation of neurons located within the lateral parabrachial region (lPB) evoked a decrease in the duration of expiration together with a facilitation of inspiratory activity (Chamberlin and Saper, 1994, 1998; Lara et al., 1994). Parabrachial neurons are also involved in a topographic organized control of bulbar laryngeal motoneurons (Lara et al., 2002). In addition, there is evidence which suggests that the PBc plays an important role in central cardiovascular control (Coote et al., 1973; Lara et al., 1994). These studies demonstrated that in all locations where respiratory responses were elicited by electrical stimulation of the PBc a cardiovascular response was also observed (Lara et al.,

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1994). A similar effect was observed with glutamate at most of these sites. This response comprised an increase in blood pressure with a small increase in heart rate (Lara et al., 1994). Recent studies demonstrate that the lPB is one of several brainstem regions involved in the descending modulation of the cardiac baroreflex during defensive behavior (Hayward, 2007; Nosaka et al., 1993).

The stimulation of the hypothalamic defense area (HDA) produces a cardiorespiratory response similar to that evoked by the stimulation of cell bodies located within the PBc (Lara et al., 1994). This response includes tachypnea, tachycardia and a marked pressor response (Hilton and Redfern, 1986). It is known that the PBc is a major secondary relay within the pons for transmission of cardiovascular and respiratory information from the nucleus tractus solitarii (NTS) (Spyer, 1990). In fact the PBc, and mainly the lPB has been shown, using neuroanatomical techniques, to be reciprocally connected with forebrain structures involved in cardiorespiratory regulation (Fulwiler and Saper, 1984; Moga et al., 1990a,1990b). Specific stimulation with glutamate of cell bodies located within lPB evokes a pressor response associated with tachycardia and tachypnea, while stimulation of cell bodies of the mPB evokes the same cardiovascular response, hypertension and tachycardia, associated with bradypnea (Lara et al., 1994).

The similarity of the responses to HDA and PBc stimulation suggested a possible interaction between these cardiorespiratory regions. Recently, we have demonstrated that the microinjection of muscimol, a GABA agonist that inhibits cell somata by sustained hyperpolarization, within the PBc modifies the cardiorespiratory response evoked by HDA stimulation (Diaz-Casares et al., 2009). Muscimol microinjection within the lPB abolished the respiratory response to HDA stimulation and decreased the pressor response. Muscimol microinjected within the mPB decreased the magnitude of the pressor and tachycardic responses to HDA stimulation without modifying the respiratory response.

Glutamate activates metabotropic and ionotropic (NMDA and non-NMDA) receptors (van den Pol et al., 1990). By employing immunocytochemical and in situ hybridization techniques, previous studies have demonstrated the presence of both metabotropic and ionotropic receptors in different nuclei of the PBc and Kölliker-Fuse (Chamberlin and Saper, 1995; Guthmann and Herbert, 1999a,1999b). Activation of vagal afferent fibers releases glutamate within the PBc (Saleh et al., 1997). An ascending excitatory pathway involving glutamate from the NTS to the PBc has been described (Jhamandas and Harris, 1992). In vitro studies also show that glutamate agonists depolarize neurons in the PBc (Zidichouski and Jhamandas, 1993), and lPB stimulation causes local glutamate release, which depolarizes lPB neurons by NMDA and non-NMDA receptors (Zidichouski et al., 1996).

Moreover, the blockade of glutamate receptors and the microinjections of glutamate into the PBc and Kölliker-Fuse, elicit a variety of cardiovascular and respiratory responses indicating that this amino acid is an important neurotransmitter for mediating autonomic functions in these regions (Bazil and Gordon, 1990; Boon and Milsom, 2008; Chamberlin and Saper, 1992, 1994; Jhamandas and Harris, 1992; Lara et al., 1994; Miura and Takayama, 1991; Zidichouski and Jhamandas, 1993; Zidichouski et al., 1996).

Therefore, the purpose of this work was to demonstrate the possible implications of glutamate receptors within the different PBc subnuclei in the cardiorespiratory response to HDA stimulation. To confirm this hypothesis, microinjection of glutamate unspecific receptor antagonist, kynurenic acid, was delivered within the PBc. The study was also carried out with the specific NMDA receptor antagonist, MK-801 and with non NMDA receptor antagonist, CNQX. To characterize the role of metabotropic receptors, MCPG (antagonist of metabotropic glutamate receptors) was microinjected within the same regions. The cardiorespiratory response evoked during electrical stimulation of the HDA was analyzed before and after the microinjections of the different glutamate receptor antagonists.

2. Materials and methods

2.1. Animals and housing

Studies were performed on 92 male SPF Sprague–Dawley rats of 250–350 g (Charles River, Barcelona, Spain). Animals were housed six per cage in a temperature-controlled room (22–24 °C) and maintained on a 12:12 h light/dark cycle (light at 7:00 am) in the Animal House of the University of Malaga. Food and water were available ad libitum. All experimental protocols were performed in accordance with the recommendations of the European Union directive (86/609/EU) for animal care and experimental procedure and the experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía. Every attempt was made to reduce animal suffering, discomfort and to decrease the number of animals needed to obtain reliable results.

2.2. General procedures

Anesthesia was induced with sodium pentobarbitone (60 mg kg^{-1} i.p, initial dose, supplemented as necessary with 2 mg kg^{-1} i.v.) Catheters were inserted into a femoral artery for the measurement of arterial blood pressure and a femoral vein for the administration of drugs. The trachea was cannulated below the larynx for the measurement of airflow through a Fleish pneumotachograph. An air filled catheter was introduced into the esophagus for the indirect measurement of pleural pressure. The animals breathed spontaneously a mixture of humidified O_2 enriched room air. End tidal CO_2 was monitored during the experiment with a fast response CO_2 analyser (ADC FM1), values ranged from 3 to 5%. Rectal temperature was maintained at 37–38 °C by a servo controlled heating pad.

The depth of anesthesia was assessed by observing the presence or absence of a significant withdrawal reflex to pinching a paw and the absence of alterations in arterial blood pressure and heart rate. Throughout the experiment, a stable level of these variables, was used as an indication of the anesthetic level and any changes under resting conditions were countered by supplemental anesthetic doses.

The animals were positioned in a stereotaxic frame with the upper incisor bar 3.3 mm below interaural line (Paxinos and Watson, 2005), and fixed by clamps on the spinous processes of C7 and L2.

2.3. HDA stimulation and parabrachial complex interactions

Two burr holes were drilled into the skull to allow access to the right HDA and the right pons through the cerebellum. A concentric bipolar electrode (Rhodes Medical electrodes, NE-100) was positioned in the right HDA according to the coordinates of the atlas of Paxinos and Watson (2005). The HDA was stimulated with 1 ms pulses, 30–50 μA given at 100 Hz for 5 s. The HDA was located on the basis of the characteristic cardiorespiratory response evoked upon electrical stimulation in the rat (Yardley and Hilton, 1986).

A glass microelectrode was positioned stereotaxically into different regions of the pons ipsilateral to the stimulated HDA. The microelectrode was filled with kynurenic acid 100 mM, MK-801 1 M, CNQX 1 M or MCPG 100 mM. All drugs were dissolved in a solution of sodium–phosphate buffer saline (PBS, pH 7.4 ± 0.1) with 0.05% Evans blue which served to mark microinjection sites. Microinjections of PBS-Evans blue alone were used for control purposes.

Microinjection volumes of 50 nl were programmed with a pump controller (Ultra Micro Pump II, Micro 4 WPI) driving a 0.5 μl microsyringe attached to the microelectrode. The volume injected was measured by observing the displacement of the microsyringe plunger wire. Only one microinjection was delivered in each animal and only those in which 50 nl was microinjected were considered for further analysis.