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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.

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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.

According to the atlas of Paxinos and Watson (2005), the stereotaxic coordinates to locate the HDA were from −2.0 to −2.2 caudal to bregma, 0.6 mm lateral to midline and 8 to 9 mm depth from the surface of the calota. To locate the different regions of the PBc the microelectrode was positioned according to the following parameters: 0 to −0.6 mm to interaural line, 1.5 to 3 mm lateral to midline and for depth 2–3 mm above interaural line.

The following protocol was used in each experiment:

a) Responses to HDA activation before PBS, kynurenic acid, MK-801, CNQX or MCPG were injected into the different pontine regions. Respiratory and cardiovascular changes were analyzed during HDA electrical stimulation.
b) Responses to HDA activation after PBS, kynurenic acid, MK-801, CNQX or MCPG were injected into the pontine regions. The cardiorespiratory responses to electrical HDA stimulations were characterized 4 min after the injection of PBS (50 nl, pH 7.4 ± 0.1, 5 s duration), kynurenic acid (50 nl, 5 nmol, pH 7.4 ± 0.1, 5 s duration), MK-801 (50 nl, 50 nmol, pH 7.4 ± 0.1, 5 s duration), CNQX (50 nl, 50 nmol, pH 7.4 ± 0.1, 5 s duration) or MCPG (50 nl, 5 nmol, pH 7.4 ± 0.1, 5 s duration). Only one microinjection was delivered in each animal and only two HDA stimulations, separated by 15 min, were given.

Electrical lesions (250 μA DC for 20 s) serve to locate HDA stimulation sites. Evans blue was used to locate the position of pontine microinjections. Brains were perfused with formal saline, serially sectioned (100 μm) at the level of the hypothalamus and the pons and counter stained with Neutral Red.

In summary HDA stimulation was elicited twice, separated by 15 min. The first one before microinjection and the second one 4 min following ipsilateral pontine microinjection.

Airflow, respiratory volume, pleural pressure (as an index of inspiratory activity) and arterial pressure were monitored and stored on digital tape for off-line analysis (Neuro-Corder DR-890). Measurements were made of inspiratory time, expiratory time, instantaneous respiratory frequency, mean blood arterial pressure and instantaneous heart rate. Recordings were taken for 3 min starting 30 s before the beginning of a stimulus. The 3 min window used for data analysis allowed a complete recovery of the evoked response. In all experiments baseline values for mean arterial blood pressure, heart rate and respiratory parameters were measured immediately prior to HDA stimulation. Changes in mean arterial blood pressure or heart rate were assessed by measuring the peak rise in blood pressure or heart rate observed during 5 s stimulation of the hypothalamus. Stimulus-evoked changes in respiratory parameters were measured as the average response observed during the 5 s stimulation of the defense area.

Only data from animals in which the histology showed that the microelectrodes were positioned within the HDA and the required pontine region were considered for statistical procedures. All data are expressed as mean ± SEM. For statistical comparisons, once the statistical normality (Kolmogorov–Smirnov test) and the homocedasticity (Bartlett's test) of the data were verified, a paired-sample test was applied to compare the control with the evoked response period for each animal. One-

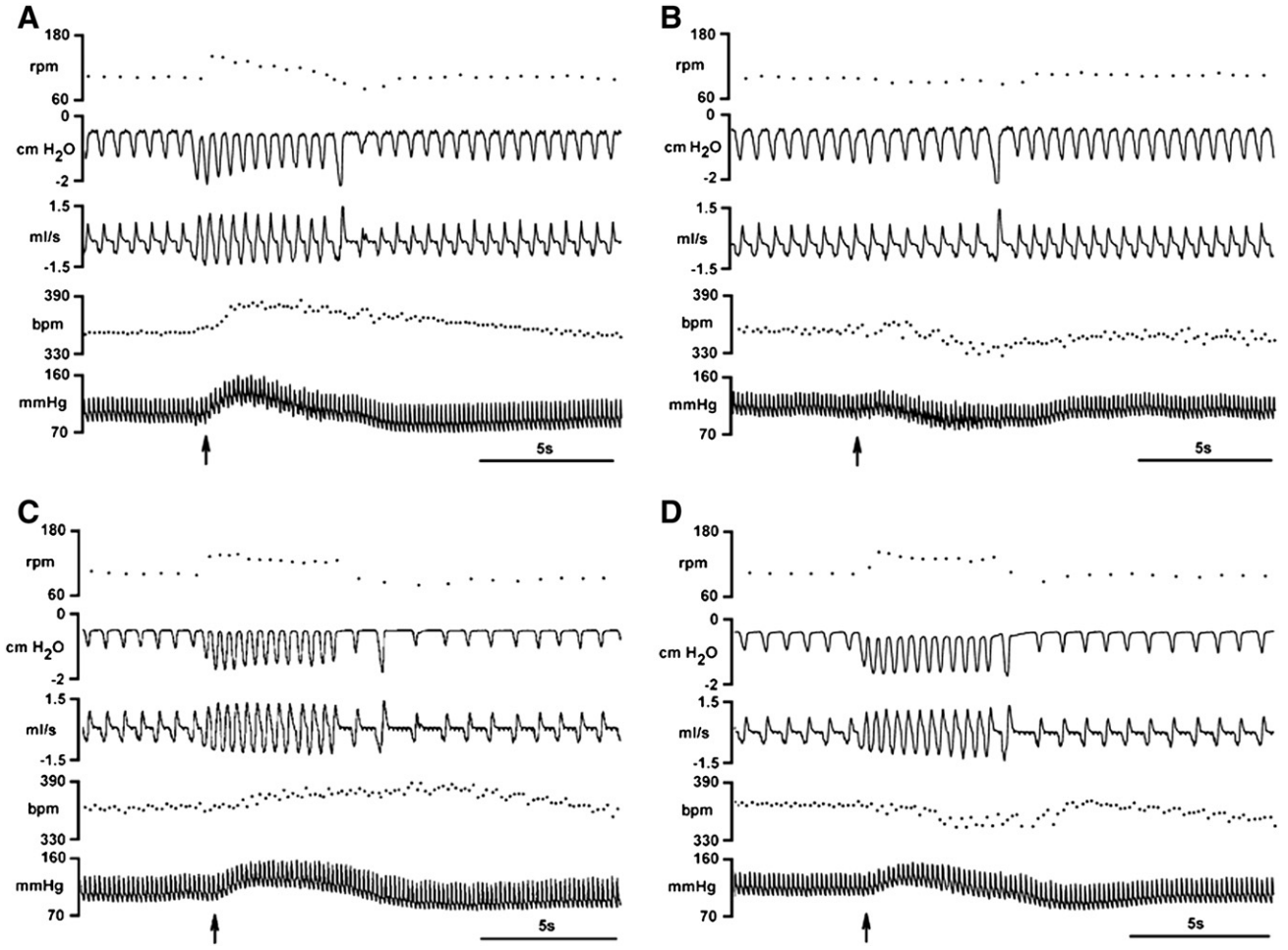

Fig. 1. Instantaneous respiratory rate (upper trace, respirations per minute — rpm), respiratory flow (ml/s), pleural pressure (cm H_2O), instantaneous heart rate (beats per minute — bpm) and blood pressure (mm Hg) showing the cardiorespiratory response evoked on HDA stimulation before (A, C) and after (B, D) the microinjection of kynurenic acid (50 nl) in the lPB (A, B) and in the mPB region (50 nl) (C, D). The arrows show the onset of the HDA electrical stimulation.

Table 1
Respiratory and cardiovascular changes before (Control) and during electrical stimulation of HDA (Stimulation), 4 min after the microinjection (kynurenic acid) and during electrical stimulation of HDA after kynurenic acid microinjection (Stimulation + kynurenic acid) within the lPB (n = 7) and the mPB (n = 8) regions. For statistical procedures we have only considered those histological verified sites of microinjection of kynurenic acid within the PB region in which cardiorespiratory changes were observed.

	Control	Stimulation	Kynurenic acid	Stimulation + kynurenic acid
lPB (n = 7)				
Ti (s)	0.195 ± 0.01	0.201 ± 0.01	0.202 ± 0.01	0.231 ± 0.01**
Te (s)	0.380 ± 0.03	0.250 ± 0.02*	0.476 ± 0.07†	0.422 ± 0.03
RR (rpm)	107.1 ± 6.4	138.1 ± 3.8**	93.5 ± 7.4†	93.9 ± 5.3
BP (mm Hg)	108.0 ± 1.0	141.7 ± 4.1***	120.3 ± 2.8††	127.1 ± 4.4
HR (bpm)	358.4 ± 10.5	386.1 ± 10.0***	368.2 ± 13.2	365.3 ± 13.5
mPB (n = 8)				
Ti (s)	0.206 ± 0.01	0.204 ± 0.01	0.204 ± 0.01	0.218 ± 0.02
Te (s)	0.424 ± 0.04	0.302 ± 0.04*	0.538 ± 0.06†	0.309 ± 0.03***
RR (rpm)	97.8 ± 5.9	124.5 ± 10.3*	84.5 ± 6.2†	117.9 ± 7.9**
BP (mm Hg)	106.6 ± 0.9	145.1 ± 3.0***	116.0 ± 1.8†	132.7 ± 3.6***
HR (bpm)	338.9 ± 8.9	366.4 ± 6.2**	343.7 ± 8.8	343.4 ± 10.1

Mean ± SEM. (Ti, inspiratory time; Te, expiratory time; RR, respiratory rate in respirations per minute; BP, blood pressure; HR, heart rate in beats per minute). Asterisks show differences between Control vs. Stimulation and kynurenic acid vs. Stimulation + kynurenic acid, *p<0.05, **p<0.01, and ***p<0.001. Crosses show differences between Control vs. kynurenic acid, †p<0.05 and ††p<0.01.

Table 3
Respiratory and cardiovascular changes before (Control) and during electrical stimulation of HDA (Stimulation), 4 min after the microinjection (CNQX) and during electrical stimulation of HDA after CNQX microinjection (Stimulation + CNQX) within the lPB (n = 8) and mPB (n = 7) regions. For statistical procedures we have only considered those histological verified sites of microinjection CNQX within the PB region in which cardiorespiratory changes were observed.

	Control	Stimulation	CNQX	Stimulation + CNQX
lPB (n = 8)				
Ti (s)	0.218 ± 0.01	0.208 ± 0.005	0.239 ± 0.01††	0.217 ± 0.004**
Te (s)	0.461 ± 0.04	0.295 ± 0.01**	0.488 ± 0.04	0.294 ± 0.01**
RR (rpm)	90.8 ± 5.3	119.4 ± 2.3***	84.5 ± 4.5	118.1 ± 3.3***
BP (mm Hg)	109.0 ± 0.5	149.3 ± 3.5***	118.5 ± 1.6††	146.7 ± 3.0***
HR (bpm)	365.0 ± 9.9	401.4 ± 11.2*	400.4 ± 12.8†	399.3 ± 17.4
mPB (n = 7)				
Ti (s)	0.220 ± 0.01	0.209 ± 0.002	0.244 ± 0.01	0.245 ± 0.01
Te (s)	0.492 ± 0.03	0.340 ± 0.02*	0.403 ± 0.01	0.301 ± 0.01
RR (rpm)	85.1 ± 3.4	110.3 ± 4.0***	92.8 ± 1.3	110.2 ± 1.8**
BP (mm Hg)	108.4 ± 1.1	147.7 ± 3.0***	121.4 ± 2.8†††	146.8 ± 5.7***
HR (bpm)	388.9 ± 16.5	438.2 ± 19.1***	388.0 ± 9.2	419.1 ± 8.6*

Mean ± SEM. (Ti, inspiratory time; Te, expiratory time; RR, respiratory rate in respirations per minute; BP, blood pressure; HR, heart rate in beats per minute). Asterisks show differences between Control vs. Stimulation and CNQX vs. Stimulation + CNQX, *p<0.05, **p<0.01, and ***p<0.001. Crosses show differences between Control vs. CNQX, †p<0.05, ††p<0.01, and †††p<0.001.

way analysis of variance with Student–Newman–Keuls post tests was used to compare different groups of animals. Significance was taken at a probability of <0.05.

3. Results

3.1. HDA stimulations

In all control groups electrical stimulation within HDA elicited a cardiovascular response consisting of an increase in mean arterial blood pressure accompanied with an increase in heart rate (Fig. 1A, C and Tables 1–4). The respiratory response consisted of an increase in respiratory rate, due to a decrease in expiratory time. No significant changes in inspiratory time were observed. Inspiratory activity, measured as pleural pressure, was also increased (p<0.05) (Fig. 1A, C and Tables 1–4).

3.2. HDA stimulations before and after lPB microinjections

PBS microinjected into the lPB (n = 4) did not produce, 4 min after its administration, changes in resting blood pressure (from 109.4 ± 0.7 to 107.8 ± 0.8 mm Hg), heart rate (from 365.8 ± 10 to 370.4 ± 9.8 bpm) or respiratory rate (from 97.4 ± 5.7 to 95.2 ± 5.4 rpm).

The microinjection of PBS (n = 4) within the lPB failed to produce changes in the amplitude of the HDA evoked cardiorespiratory response: respiratory rate, from 36.3 ± 15.1 rpm to 37.2 ± 12.8 rpm; blood pressure, from 31.3 ± 9.1 mm Hg to 30.5 ± 6.6 mm Hg and heart rate, from 40.2 ± 7.6 bpm to 38.3 ± 9.5 bpm (Fig. 3A, B, C respectively).

Table 2
Respiratory and cardiovascular changes before (Control) and during electrical stimulation of HDA (Stimulation), 4 min after the microinjection (MK-801) and during electrical stimulation of HDA after MK-801 microinjection (Stimulation + MK-801) within the lPB (n = 8) and mPB (n = 7) regions. For statistical procedures we have only considered those histological verified sites of microinjection of MK-801 within the PB region in which cardiorespiratory changes were observed.

	Control	Stimulation	MK-801	Stimulation + MK-801
lPB (n = 8)				
Ti (s)	0.224 ± 0.01	0.199 ± 0.02	0.233 ± 0.01	0.207 ± 0.01*
Te (s)	0.406 ± 0.05	0.275 ± 0.03**	0.360 ± 0.02	0.251 0.02*
RR (rpm)	100.9 ± 7.8	135.6 ± 11.2***	103.5 ± 6.3	135.4 ± 8.9***
BP (mm Hg)	108.9 ± 0.9	149.9 ± 1.9***	125.7 ± 2.4†††	152.1 ± 3.2***
HR (bpm)	345.4 ± 10.9	382.3 ± 12.8**	371.9 ± 9.8††	380.0 ± 13.8
mPB (n = 7)				
Ti (s)	0.226 ± 0.01	0.210 ± 0.01	0.245 ± 0.01†	0.230 ± 0.01*
Te (s)	0.402 ± 0.03	0.270 ± 0.01***	0.387 ± 0.02	0.268 ± 0.01***
RR (rpm)	97.3 ± 4.9	126.5 ± 5.4***	96.4 ± 4.9	122.6 ± 6.4***
BP (mm Hg)	107.6 ± 0.8	145.1 ± 5.0***	115.8 ± 1.4	142.3 ± 4.8***
HR (bpm)	336.4 ± 13.9	382.4 ± 20.4***	342.7 ± 13.4	374.0 ± 23.5**

Mean ± SEM. (Ti, inspiratory time; Te, expiratory time; RR, respiratory rate in respirations per minute; BP, blood pressure; HR, heart rate in beats per minute). Asterisks show differences between Control vs. Stimulation and MK-801 vs. Stimulation + MK-801, *p<0.05, **p<0.01, and ***p<0.001. Crosses show differences between Control vs. MK-801, †p<0.05, ††p<0.01, and †††p<0.001.

Table 4
Respiratory and cardiovascular changes before (Control) and during electrical stimulation of HDA (Stimulation), 4 min after the microinjection (MCPG) and during electrical stimulation of HDA after MCPG microinjection (Stimulation + MCPG) within the lPB (n = 7) and mPB (n = 7) regions. For statistical procedures we have only considered those histological verified sites of microinjection of MCPG within the PB region in which cardiorespiratory changes were observed.

	Control	Stimulation	MCPG	Stimulation + MCPG
lPB (n = 7)				
Ti (s)	0.196 ± 0.02	0.210 ± 0.003	0.267 ± 0.01††	0.211 ± 0.01**
Te (s)	0.477 ± 0.05	0.245 ± 0.01**	0.445 ± 0.04	0.249 ± 0.04**
RR (rpm)	91.2 ± 3.1	133.1 ± 9.5***	88.0 ± 5.8	136.3 ± 7.8***
BP (mm Hg)	110.8 ± 8.1	145.7 ± 7.1**	122.6 ± 7.5	159.0 ± 7.8**
HR (bpm)	390.8 ± 8.5	420.9 ± 7.3*	390.9 ± 8.3	418.1 ± 6.5*
mPB (n = 7)				
Ti (s)	0.248 ± 0.02	0.209 ± 0.01	0.304 ± 0.01†	0.220 ± 0.02**
Te (s)	0.366 ± 0.02	0.246 ± 0.03*	0.334 ± 0.03	0.251 ± 0.03
RR (rpm)	103.0 ± 6.0	134.6 ± 7.2**	98.8 ± 4.3	130.3 ± 7.5**
BP (mm Hg)	106.1 ± 2.7	144.2 ± 6.1***	120.4 ± 5.5†	146.9 ± 2.9**
HR (bpm)	429.1 ± 5.4	456.3 ± 5.4**	446.5 ± 6.5†	466.2 ± 3.8*

Mean ± SEM. (Ti, inspiratory time; Te, expiratory time; RR, respiratory rate in respirations per minute; BP, blood pressure; HR, heart rate in beats per minute). Asterisks show differences between Control vs. Stimulation and MCPG vs. Stimulation + MCPG, *p<0.05, **p<0.01, and ***p<0.001. Crosses show differences between Control vs. MCPG, †p<0.05 and ††p<0.01.

3.2.1. Kynurenic acid microinjection

Kynurenic acid microinjected the lPB (n = 7) (Fig. 2A) produced, 4 min after its administration, a significant increase in resting blood pressure ($p<0.01$) without changes in heart rate. Respiratory rate was decreased ($p<0.05$) due to an increase of expiratory time ($p<0.05$) (Fig. 1A, B and Table 1).

The microinjection of kynurenic acid into lPB abolished the tachypnea (from 31.0 ± 3.9 rpm to 0.4 ± 4.0 rpm, $p<0.001$, Figs. 1A, B, 3A and Table 1) and the tachycardia (from 27.7 ± 4.2 bpm to −2.9 ± 5.6 bpm, $p<0.001$, Figs. 1A, B, 3C and Table 1) to HDA electrical stimulation. Moreover, the amplitude of the increase in blood pressure was significantly smaller (from 33.7 ± 4.5 mm Hg to 6.8 ± 4.1 mm Hg, $p<0.001$, Figs. 1A, B, 3B and Table 1) after the kynurenic acid microinjection.

In some experiments (4 rats) the microinjection of kynurenic acid within the lPB did not produce changes neither in resting cardiorespiratory parameters (from 93.6 ± 4.7 rpm to 91.4 ± 4.8 rpm; blood pressure, from 105.8 ± 0.8 mm Hg to 107.4 ± 0.8 mm Hg and heart rate, from 384.4 ± 9.8 bpm to 380.4 ± 10 bpm) nor in the intensity of the cardiorespiratory HDA evoked response (from 30.3 ± 14.4 rpm to 32.2 ± 13.8 rpm; blood pressure, from 34.4 ± 8.2 mm Hg to 35.5 ± 9.4 mm Hg and heart rate, from 35.2 ± 10.8 bpm to 34.4 ± 12.4 bpm). These microinjections were located in the internal part (n = 3) and dorsal part (at level of interaural 0 mm, n = 1) of the lPB (Fig. 2A), and their effects were not statistically different to those of PBS.

No changes were observed during HDA stimulation when kynurenic acid microinjections were delivered to sites outside the lPB region, within the ventral spinocerebellar tract (n = 2) (Fig. 2A).

3.2.2. MK-801 microinjection

In 3 animals 500 nl of MK-801 were delivered within the lPB, external (n = 2) and ventral (n = 1) part. In all cases a respiratory depression was developed and were not used for experimental procedures and were not included in Fig. 2B as failures.

MK-801 microinjected into the central, ventral, external and crescent subnuclei of the lPB (n = 8) (Fig. 2B) produced, 4 min after its administration, a significant increase in blood pressure ($p<0.001$) and heart rate ($p<0.01$). No changes were observed on resting respiratory parameters (Table 2).

The microinjection of MK-801 into lPB did not produce changes on the respiratory response to HDA stimulation. The amplitude of the

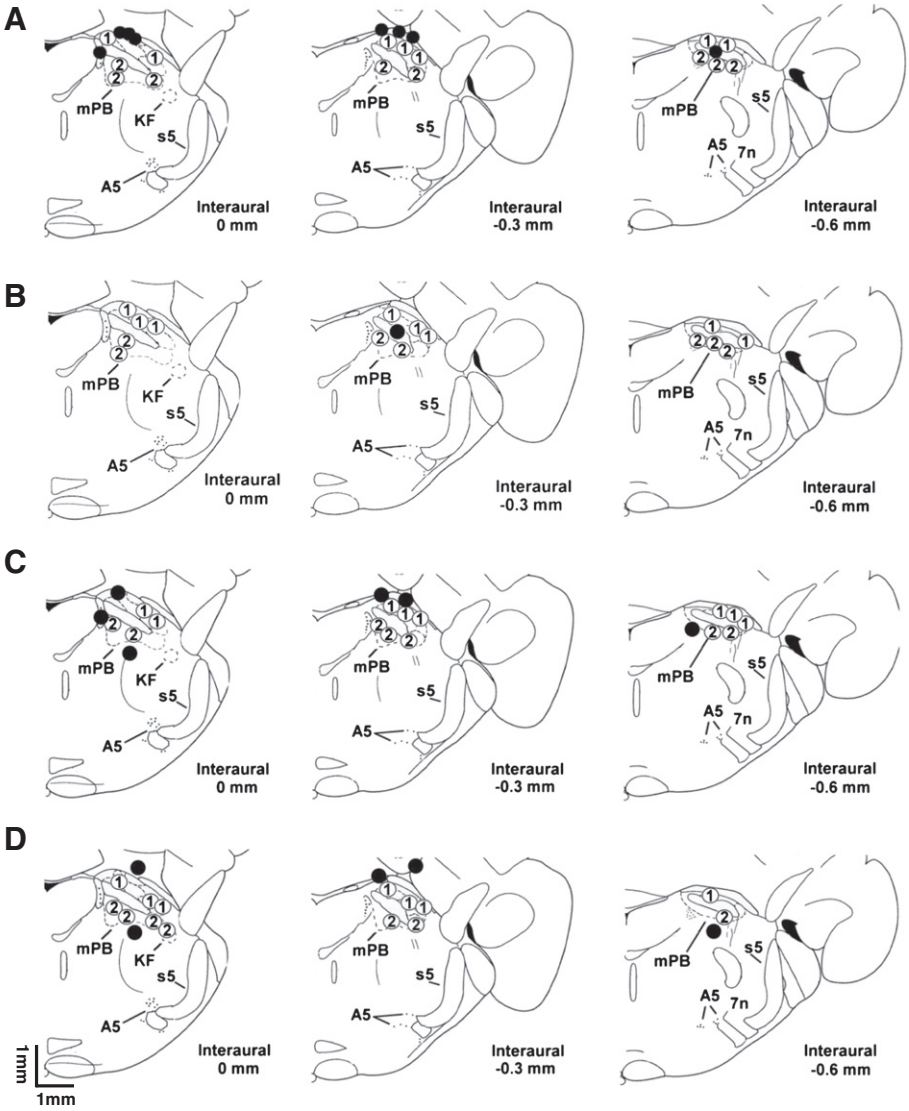

Fig. 2. Semi-schematic line drawings of coronal sections through the PB complex from rostral (left) to caudal (right), showing the sites where stimuli and microinjections were applied. The numbered circles show the location on the microinjections of kynurenic acid (A), MK-801 (B), CNQX (C) and MCPG (D) within lPB (numbered circles 1) and mPB (numbered circles 2). Filled black circles show the locations in which kynurenic acid (A), MK-801 (B), CNQX (C) and MCPG (D) failed to produce changes in the HDA evoked responses. (A5) A5 region. (s5) sensory root trigeminal nerve. (7n) facial nerve or its root.

respiratory response remained unchanged compared with control stimulations (from 34.6 ± 7.4 rpm to 31.8 ± 8.9 rpm) (Fig. 3A and Table 2).

On the contrary, MK-801 microinjection produced a decrease of the tachycardia (from 36.9 ± 7.3 bpm to 8.1 ± 7.7 bpm, $p<0.01$, Fig. 3C and Table 2) and the amplitude of the increase in blood pressure was significantly smaller (from 41.0 ± 1.3 mm Hg to 26.3 ± 2.0 mm Hg, $p<0.001$, Fig. 3B and Table 2) during HDA stimulation.

3.2.3. CNQX microinjection

Only locations from histological verified sites of microinjection of CNQX within the lPB region with cardiorespiratory changes were considered for statistical procedures.

No changes in resting cardiorespiratory parameters nor in the intensity of the cardiorespiratory HDA evoked response were observed after the microinjection of CNQX within the lPB, internal part (n = 2) and when the CNQX microinjection was delivered outside the lPB region, into the ventral spinocerebellar tract (n = 1) (Fig. 2C). These experiments were not included in the statistical study.

CNQX microinjected into the lPB (n = 8) (Fig. 2C) produced, 4 min after its administration, a significant increase in inspiratory time ($p<0.01$) without significant changes in expiratory time and respiratory rate. An increase in blood pressure ($p<0.01$) and heart rate ($p<0.05$) was also observed (Table 3).

On the other hand, CNQX microinjection within the lPB abolished the increase of heart rate (from 36.4 ± 6.6 bpm to −1.1 ± 12.8 bpm, $p<0.05$, Fig. 3C and Table 3) and reduced the amplitude of the increase in blood pressure (from 40.3 ± 3.6 mm Hg to 28.2 ± 3.9 mm Hg, $p<0.05$, Fig. 3B and Table 3) evoked by the stimulation of HDA. However, the microinjection of CNQX into lPB did not produce changes of the respiratory response to HDA stimulation (from 28.6 ± 4.6 rpm to 33.6 ± 4.7 rpm, Fig. 3A and Table 3).

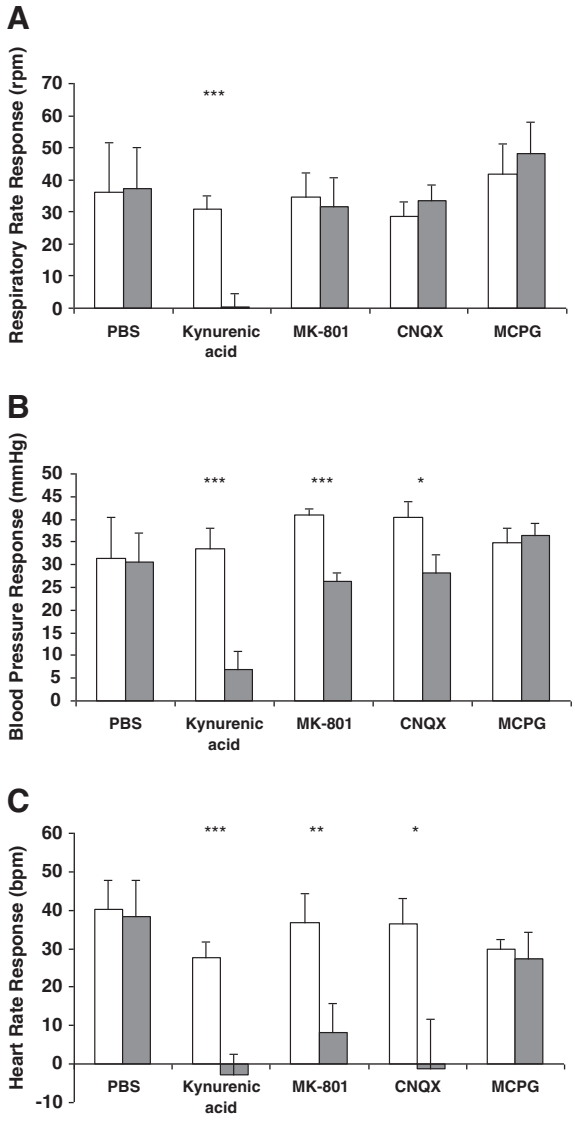

Fig. 3. Changes in the intensity of the cardiorespiratory response evoked on HDA stimulation (respiratory rate response in respirations per minute — rpm, blood pressure response — mm Hg and heart rate response in beats per minute — bpm) before (empty bar) and after (filled bar) microinjection of PBS, kynurenic acid, MK-801, CNQX and MCPG within the lPB. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

3.2.4. MCPG microinjection

Only locations from histological verified sites of microinjection of MCPG within the lPB region in which respiratory changes were observed have been considered for statistical procedures. In 3 rats the microinjection of MCPG did not produce changes neither in resting cardiorespiratory parameters nor in the intensity of the cardiorespiratory HDA evoked response. These microinjections were located in the parabrachial nucleus, internal part (n = 1), sagulum nucleus (n = 1) and paracollicular tegmentum (n = 1) (Fig. 2D) and were not included in the statistical study.

MCPG microinjected into the lPB (n = 7) (Fig. 2D) produced, 4 min after its administration, a significant increase in inspiratory time ($p<0.01$) without significant changes in expiratory time and respiratory rate. The microinjection of MCPG within the lPB did not produce changes in the cardiovascular parameters (Table 4).

The cardiorespiratory response evoked to HDA stimulation remained unchanged compared with control stimulations after the microinjection of MCPG within the lPB: respiratory rate from 41.9 ± 9.1 rpm to 48.3 ± 9.6 rpm, blood pressure from 34.9 ± 3.0 mm Hg to 36.4 ± 2.7 mm Hg and heart rate from 30.0 ± 2.5 bpm to 27.2 ± 7.1 bpm (Fig. 3A, B, C respectively and Table 4).

3.3. HDA stimulations before and after mPB microinjections

PBS microinjected into the mPB (n = 4) did not produce, 4 min after its administration, changes in resting blood pressure (from 107.2 ± 1 to 105.4 ± 1.1 mm Hg), heart rate (from 377.4 ± 10 to 365.8 ± 11.2 bpm) or respiratory rate (from 94.8 ± 5.1 to 98.2 ± 5.4 rpm).

The microinjection of PBS (n = 5) within the mPB failed to affect the HDA evoked cardiorespiratory response: respiratory rate, from 39.9 ± 11.7 rpm to 38.4 ± 12.8 rpm; blood pressure, from 32.3 ± 7.7 mm Hg to 33.9 ± 8.4 mm Hg and heart rate, from 38.7 ± 9.0 bpm to 37.8 ± 10.5 bpm (Fig. 4A, B, C respectively).

3.3.1. Kynurenic acid microinjection

Only locations from histological verified sites of microinjection within the mPB region were considered for statistical procedures. In some experiments (2 rats) the microinjection of kynurenic acid did not produce changes neither in resting cardiorespiratory parameters nor in the intensity of the cardiorespiratory HDA evoked response, these microinjection were located in the superior cerebellar peduncle (n = 1) and the mesencephalic trigeminal nucleus (n = 1) (Fig. 2A) and were not included in the statistical study.

Kynurenic acid microinjected into the mPB (n = 8) (Fig. 2A) produced, 4 min after its administration, an increase in resting blood pressure ($p<0.05$), however, no significant changes were observed in heart rate. Respiratory rate was decreased ($p<0.05$) due to an increase of expiratory time ($p<0.05$) (Fig. 1C, D and Table 1).

HDA electrical stimulation before and after the microinjection of kynurenic acid into the mPB evoked similar respiratory responses. The amplitude of the respiratory response to HDA stimulation after the microinjection of kynurenic acid remained unchanged compared with control stimulations (from 26.6 ± 7.7 rpm to 33.4 ± 6.1 rpm) (Figs. 1C, D, 4A and Table 1).

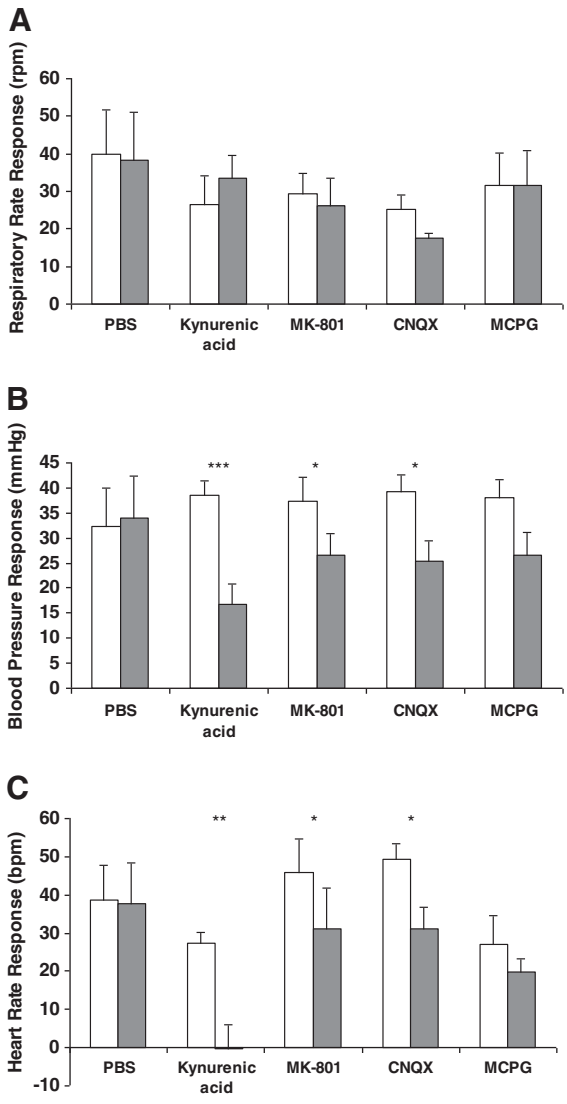

Fig. 4. Changes in the intensity of the cardiorespiratory response evoked on HDA stimulation (respiratory rate response in respirations per minute — rpm, blood pressure response — mm Hg and heart rate response in beats per minute — bpm) before (empty bar) and after (filled bar) microinjection of PBS, kynurenic acid, MK-801, CNQX and MCPG within the mPB. *$p<0.05$, **$p<0.01$, and ***$p<0.001$.

Kynurenic acid into the mPB decreased the cardiovascular response evoked to HDA stimulation. The increase in blood pressure was still present although the amplitude was smaller than before kynurenic acid (from 38.5 ± 2.9 mm Hg to 16.7 ± 4.2 mm Hg, $p<0.001$, Figs. 1C, D, 4B and Table 1). On the other hand, kynurenic acid microinjection, abolished the increase in heart rate evoked by HDA stimulation (from 27.4 ± 4.5 bpm to −0.3 ± 6.3 bpm, $p<0.01$, Figs. 1C, D, 4C and Table 1).

3.3.2. MK-801 microinjection

As said before only locations from histological verified sites of microinjection within the mPB region were considered for statistical procedures. In 2 rats the microinjection of MK-801 did not produce changes neither in resting cardiorespiratory parameters nor in the intensity of the cardiorespiratory HDA evoked response, these microinjection were located in the parabrachial nucleus, central part at the level of interaural 0.3 mm ($n=1$, not shown in Fig. 2B) and the superior cerebellar peduncle ($n=1$) (Fig. 2B). These experiments were not included in the statistical study.

MK-801 microinjected into the mPB ($n=7$) (Fig. 2B) produced, 4 min after its administration, a significant increase in inspiratory time ($p<0.05$) without significant changes in expiratory time and respiratory rate. Neither was observed changes on resting cardiovascular parameters (Table 2).

The microinjection of MK-801 into mPB did not produce changes on respiratory response to HDA stimulation. No changes were observed in the tachypnea evoked to HDA stimulation (from 29.3 ± 5.6 rpm to 26.2 ± 7.4 rpm, Fig. 4A and Table 2). The amplitude of blood pressure and heart rate response was significantly smaller (from 37.4 ± 4.8 mm Hg to 26.5 ± 4.3 mm Hg and from 46.0 ± 8.6 bpm to 31.2 ± 10.7 bpm respectively, $p<0.05$ in both cases) after the MK-801 microinjection within mPB (Fig. 4B, C and Table 2).

3.3.3. CNQX microinjection

In all cases, only locations from histological verified sites of microinjection within the mPB region were considered for statistical procedures. No changes were observed in resting cardiorespiratory parameters or during HDA stimulation when CNQX microinjections were delivered to sites outside the mPB, within the mesencephalic trigeminal nucleus ($n=2$) and supratrigeminal nucleus ($n=1$) (Fig. 2C). These experiments were not included in the statistical study.

CNQX microinjected into the mPB ($n=7$) (Fig. 2C) produced, 4 min after its administration, a significant increase in blood pressure ($p<0.001$). No changes were observed in heart rate response and on resting respiratory parameters after the microinjection of CNQX within the mPB (Table 3).

The microinjection of CNQX into mPB did not produce changes on respiratory response to HDA stimulation. The amplitude of the respiratory response to HDA stimulation after the microinjection of CNQX remained unchanged compared with control stimulations (from 25.2 ± 3.7 rpm to 17.4 ± 1.5 rpm, Fig. 4A and Table 3). The increase in blood pressure and heart rate was still present although the amplitude was smaller than before CNQX microinjection (from 39.2 ± 3.3 mm Hg to 25.3 ± 4.2 mm Hg and from 49.4 ± 3.9 bpm to 31.1 ± 5.6 bpm respectively, $p<0.05$ in both cases, Fig. 4B, C and Table 3).

3.3.4. MCPG microinjection

As said before only locations from histological verified sites of microinjection of MCPG within the mPB region were considered for statistical procedures. No changes were observed in resting cardiorespiratory parameters or during HDA stimulation when MCPG microinjections were delivered to sites outside the mPB region, within the supratrigeminal nucleus ($n=2$) (Fig. 2D). These experiments were not included in the statistical study.

MCPG microinjected into the mPB ($n=7$) (Fig. 2D) produced, 4 min after its administration, a significant increase in inspiratory time ($p<0.05$) without significant changes in expiratory time and respiratory rate. Moreover, the microinjection of MCPG within the mPB produced an increase in blood pressure and heart rate ($p<0.05$ in both cases) (Table 4).

The cardiorespiratory response evoked to HDA stimulation remained unchanged compared with control stimulations after the microinjection of MCPG within the mPB: respiratory rate, from 31.6 ± 8.5 rpm to 31.5 ± 9.3 rpm; blood pressure, from 38.1 ± 3.5 mm Hg to 26.5 ± 4.6 mm Hg, and heart rate, from 27.1 ± 7.6 bpm to 19.7 ± 3.6 bpm (Fig. 4A, B, C respectively and Table 4).

4. Discussion

The primary observation of this paper indicates that the pattern of the cardiorespiratory response evoked from HDA, is modified by the microinjection of different glutamate antagonists into the PBc. Kynurenic acid microinjected into the lPB and mPB abolished the tachycardia and decreased the pressor response to HDA electrical stimulation. The respiratory response was only abolished when kynurenic acid was microinjected into the lPB. Second, MK-801 or CNQX microinjection into the lPB reduced the pressor response and abolished the tachycardia

evoked to HDA stimulation. A decrease in the pressor response and tachycardia was also observed with the microinjection of MK-801 or CNQX into the mPB. MCPG microinjection within the lPB or mPB failed to produce changes in HDA evoked cardiovascular responses. Finally, the tachypnea evoked on HDA stimulation was not affected with the microinjection of MK-801, CNQX or MCPG in lPB or mPB.

These results suggest that, in our experimental conditions, ionotropic glutamate receptors located within the lPB region are involved in both, the respiratory and cardiovascular evoked responses from the HDA, whereas ionotropic glutamate receptors located in mPB are only involved in the modulation of the cardiovascular response. On the contrary, metabotropic glutamate receptors, in our experimental conditions, seem not to be involved in the cardiorespiratory response evoked to HDA stimulation.

4.1. Methodological considerations

In the present study we chose only electrical stimulation to activate the HDA. Electrical stimulation activates both neurons and fibers of passage. However, previous studies have demonstrated that either electrical or chemical stimulation of the HDA induces similar cardiorespiratory responses (Hilton and Redfern, 1986). Electrical stimulation allowed us to tightly regulate the onset and offset of HDA activation. We selected the most usual stimulation parameters used in previous studies (Dawid-Milner et al., 1995, 2001, 2003; Diaz-Casares et al., 2009; Lara et al., 1994, 2002; Silva-Carvalho et al., 1993).

Blockade of glutamate receptors located in the PBc was performed by unilateral microinjections of kynurenic acid (glutamate receptor unspecific antagonist), MK-801 (subtype NMDA glutamate receptor specific antagonist), CNQX (subtype non-NMDA glutamate receptor specific antagonist) and MCPG (antagonist of metabotropic glutamate receptors). The usual volume of our injections was 50 nl given in 5 s. Volumes and injection times were selected according with the observations of similar studies (Dutschmann and Herbert, 1998).

Another methodological consideration for the present study is that our experiments were performed in spontaneously breathing animals. Previous studies demonstrated that microinjections of muscimol within the PBc markedly altered baseline respiratory timing, including increasing the time of expiration and reducing baseline respiratory frequency (Diaz-Casares et al., 2009) This change in respiratory control may have independently altered baroreflex function.

4.2. Cardiorespiratory responses to HDA stimulations after PBc microinjections

This study deals with the notion that neurones located within the PBc play a role in the cardiorespiratory response evoked from HDA. We have previously shown that the stimulation of cell bodies located within the PBc resembles the cardiovascular response elicited by HDA electrical stimulation, thus evoking tachycardia and hypertension (Lara et al., 1994). Moreover, to confirm the interactions between these cardiorespiratory regions, recently we have carried out microinjections of muscimol (a GABA agonist) into the main subdivisions within the lPB and mPB (Diaz-Casares et al., 2009). Muscimol microinjected into the lPB abolished the respiratory and decreased the cardiovascular response to HDA electrical stimulation whereas the microinjection of muscimol within the mPB changed only the cardiovascular response elicited by HDA stimulation. These results demonstrated that lPB is involved in both, the respiratory and cardiovascular evoked responses from the HDA whereas mPB is only involved in the modulation of the cardiovascular response.

As glutamate plays a crucial role in parabrachial neurotransmission, to evaluate the possible participation of PBc glutamate receptors in the modulation of the cardiorespiratory response evoked to HDA stimulation, microinjection of kynurenic acid, MK-801, CNQX and MCPG, were made into the main subdivisions within the PBc (lPB and mPB).

We have first analyzed the effects of PBS, kynurenic acid, MK-801, CNQX and MCPG microinjections on resting cardiorespiratory parameters, to follow with the effects on the cardiorespiratory response evoked to HDA stimulation.

4.2.1. Resting conditions

PBS microinjections within the lPB or mPB did not produce changes in cardiorespiratory parameters. The microinjection of kynurenic acid, MK-801 and CNQX within the lPB produced, 4 min after its administration, an increase in blood pressure and heart rate. No cardiovascular changes were observed when MCPG was microinjected into the lPB. The microinjection of kynurenic acid and CNQX within the mPB also produced an increase in blood pressure but without significant changes in heart rate. An increase in blood pressure and heart rate was observed when MCPG was microinjected into the mPB. No cardiovascular changes were observed when MK-801 was microinjected into the mPB.

The primary observation of these results is that two different patterns of heart rate responses can be induced by inhibiting parabrachial glutamate receptors. The effect depends on the exact location of the different subnuclei, indicating the complexity of the PBc on the modulation of heart rate at rest.

An interesting result is that the applications of glutamate receptor antagonists seem to have identical effects on resting cardiovascular variables as the effects of glutamate given to the same areas (Chamberlin and Saper, 1992; Lara et al., 1994). Such discrepancies between the effects of glutamate and its receptor antagonists could be due to the complexity of the PBc with respect to neuronal types, glutamate receptor subtypes and interaction or neuronal projections. We need further investigation to solve the possible mechanism underlying this contradictory evidence.

In addition, our results show that, 4 min after the microinjection of kynurenic acid within the mPB or lPB, a decrease in respiratory rate due to an increase in expiratory time was observed. The microinjection of MK-801, CNQX and MCPG within the mPB or lPB did not produce changes in respiratory rate, although, in some groups, an increase of inspiratory time was observed. There is a clear evidence that stimulation with glutamate of discrete cell groups within the PBc evoke a decrease in respiratory rate due to an increase in expiratory time (Dawid-Milner et al., 2003; Lara et al., 2002). Chamberlin and Saper (1994) using very small chemical stimuli, described three types of respiratory responses within the mPB, Kölliker-Fuse nucleus and its ventral–lateral boundaries, suggesting a complex organization of the PBc modulating inspiratory, postinspiratory and expiratory mechanisms.

Our results support the suggestions of Chamberlin and Saper. The integrity of the PBc seems to be needed to produce the expiratory facilitatory response, since PBc glutamatergic inhibition with kynurenic acid produced the same effect as glutamate activation, a decrease in respiratory rate due to an increase in expiratory time. However, no changes were observed in the respiratory rate response after the microinjection of glutamate specific antagonists (MK-801, CNQX and MCPG) within the PBc. The increase in inspiratory time observed in some of these groups was sometimes compensated with a decrease in expiratory time. Since inspiratory time is shorter and its duration is more constant than expiratory time at rest the probability of finding significant differences in this parameter increases, but its influence on respiratory rate is less effective than the changes in expiratory time. Thus is common to find changes in inspiratory time with no differences in respiratory rate.

In previous reports it has been demonstrated that the activation of lPB somata with glutamate evokes an inspiratory facilitatory response (Lara et al., 2002). The response consisted of an increase of respiratory rate due to a decrease in expiratory time. In our study the inhibition of glutamate receptors located within the PBc with kynurenic acid was able to produce the opposite effect. Kynurenic acid decreased respiratory rate due to an increase in expiratory time.

The fact that kynurenic acid decreases respiratory rate but that this effect is not observed with specific glutamate receptor antagonists suggests that glutamate receptors are involved in respiratory rate at rest. An integration of all types of receptors is necessary for this effect, thus suggesting the complexity of the PBc in respiratory regulation. Further research is necessary to solve this question.

4.2.2. Effects on the cardiovascular response to HDA stimulation

The cardiovascular response evoked during the stimulation of the HDA was modified after the microinjection of kynurenic acid into the lPB and mPB. In both regions, the increase in blood pressure was reduced and the tachycardia observed during HDA stimulation was abolished.

These results confirm that HDA-evoked cardiovascular responses are partially mediated through a glutamatergic activation of lPB and mPB regions. Thus, our second aim was to determinate the contribution of glutamate ionotropic subtypes (NMDA and non-NMDA) and metabotropic receptors in this process.

MK-801 and CNQX microinjection into the lPB produced a decrease in the pressor response and abolished the tachycardia evoked by HDA stimulation. Similarly, the microinjection of MK-801 and CNQX within the mPB also reduced pressor and heart rate responses. No changes were observed in the cardiovascular response after the microinjection of MCPG within the lPB or mPB.

The intensity of HDA stimulations before and after microinjections is constant, as a result the increase in blood pressure or heart rate reaches the maximal possible value for that intensity. CNQX and MK801 microinjections increased blood pressure and heart rate values at rest. During HDA stimulation maximal cardiovascular values were reached, thus producing an attenuation of the response. This kind of response suggests a potential ceiling effect. With kynurenic acid, this is not the case. The attenuation of the response to HDA stimulation is real. These results suggest that the cardiovascular response to HDA electrical stimulation is modulated by activation of ionotropic glutamate receptors. The effectiveness of the modulation is depending on the distribution of these receptors within the PBc. In our experimental conditions, lPB appears to exert a more efficient modulation on HDA evoked cardiovascular response than mPB.

The cardiovascular response evoked during HDA stimulation, seems to be due to a direct activation of neurones from the rostral ventrolateral medulla (RVLM) that send direct projections to the sympathetic preganglionic neurons of the intermediolateral cell column of the spinal cord (IML) (Lovick, 1992; van der Plas et al., 1995; Verberne and Guyenet, 1992). The activity of the RVLM can be also modulated via indirect projections.

Our observations support previous results suggesting that changes in heart rate and blood pressure evoked from 'defense' regions of the brain may use separate efferent pathways (Fontes et al., 2001). This idea is also supported by Nosaka and colleges who reported that dlPAG-evoked changes in blood pressure were attenuated after blockade of the PBc (Nosaka et al., 1993).

Thus, the cardiovascular response evoked by HDA stimulation could be partially mediated by "direct" projections to the RVLM and by indirect projections via the PBc which are activated by ionotropic glutamate receptors.

The PBc is essential for a full expression of the heart rate response that accompanies the activation of the baroreceptor reflex (Diaz-Casares et al., 2009). The decrease in the intensity of the cardiovascular response evoked from HDA stimulation after kynurenic microinjection could be due to an inhibition of tonic excitatory inputs from neurons of the lPB-mPB on inhibitory mechanism of the baroreceptor reflex at the level of the NTS (Dawid-Milner et al., 1995). The observation that the decrease in the pressor response is associated with the decrease and/or the abolishment of the tachycardia suggests this hypothesis.

As described before lPB appears to exert a more efficient modulation on HDA evoked cardiovascular responses than mPB. The expression of glutamate receptors within the lPB is quite different from the remaining PBc subnuclei, lPB presents a specific pharmacological profile. Non-NMDA receptors in the internal lPB are dominated by GluR4 subunits (Chamberlin and Saper, 1995) characterized by a high sensitivity for glutamate. There is also evidence that the external and internal lPB express specific subunits of NMDA receptors different to that of mPB (Guthmann and Herbert, 1999b). NMDA receptors can be quite different with respect to their physiological and pharmacological channel properties, such as differences in glutamate affinity and glycine sensitivity, crucial coagonist for glutamate efficacy (Kemp and Leeson, 1993), in calcium currents and deactivation kinetics as well as other single-channel characteristic (Feldmeyer and Cull-Candy, 1996). NMDA receptors of lPB are composed of NR2A and NR2B subunits which are characterized by high affinity for glutamate and long mean open time. NMDA receptors located within the mPB are composed of NR2D subunits, which exhibit low affinity for glutamate (Feldmeyer and Cull-Candy, 1996; Guthmann and Herbert, 1999b).

Immunocytochemical studies demonstrate the presence of glutamate metabotropic receptors in the PBc which might induce a variety of second messenger cascades (Guthmann and Herbert, 1999a). Our experiments show no modulation of glutamate metabotropic receptors on the cardiovascular response to HDA stimulation. However MCPG microinjected within the mPB produced an increase of blood pressure and heart rate at rest thus indicating a role for glutamate metabotropic receptors on cardiovascular regulation. These results need also further investigation.

Our results provide new data to suggest that the pressor response evoked during the stimulation of the HDA may involve a glutamate ionotropic receptor activation and recruitment of neurons in both the lPB and mPB subdivisions producing an indirect activation of the sympathetic preganglionic neurons of the IML. The inhibition of the baroreceptor response seems to be more dependent on lPB glutamatergic ionotropic activation than mPB since the tachycardia associated with the pressor response is only abolished with microinjections within the lPB.

4.2.3. Effects on the respiratory response to HDA stimulation

The respiratory response evoked by HDA stimulation was abolished after the microinjection of kynurenic acid into the lPB. On the contrary, no changes were observed in the respiratory response after kynurenic acid microinjection within the mPB.

These data suggest that glutamate receptors located into the lPB are involved in the modulation of the respiratory response evoked to HDA stimulation.

Previous studies have demonstrated the involvement of the lPB as part of the neuronal pathways involved in the respiratory response evoked on HDA stimulation. Microinjections of the GABA agonist muscimol within the lPB have similar effects to kynurenic microinjections (Diaz-Casares et al., 2009); the tachypnea observed during HDA stimulation was abolished. This observation gives a role for the described lPB afferent connections from several hypothalamic nuclei involved in the defense reaction (Moga et al., 1990a).

The importance of lPB in integrating tachypneic evoked responses from supraencephalic regions is also suggested by Hayward et al. who obtained similar results when blocking glutamate receptors with kynurenic acid microinjected within the lPB during the stimulation of the dorsal periaqueductal gray (dPAG) (Hayward et al., 2004), one of the so called secondary 'brain defense regions'.

There are indications that HDA stimulation may facilitate the chemoreceptor reflex at specific cells located within the NTS (Silva-Carvalho et al., 1993). These neurons are activated by HDA-NTS direct excitatory connections and are also the main targets of excitatory inputs from the lPB (Felder and Mifflin, 1988). Our results suggest that glutamate activates these excitatory inputs . The inhibition of the activation of these lPB projections with kynurenic acid leads to the abolishment of the tachypnea evoked on HDA stimulation.

However, no changes in the respiratory response to HDA stimulation are observed after the blockade of ionotropic and metabotropic glutamate receptors in lPB. We speculate that there has to be an interaction between cells with different subtypes of glutamate receptors to perform this effect. Interactions of NMDA and non-NMDA receptors have been demonstrated in other regions of the brain (Barnard, 1997). Neurons with NMDA and non-NMDA type glutamate receptors have been localized in a number of sites related to respiratory control, including in the lPB and mPB (Alheid et al., 2004). This striking result needs further investigation.

4.3. Final consideration

The results of our study demonstrate for the first time that PBc glutamate receptors are involved in the cardiorespiratory response evoked during HDA stimulation.

lPB glutamate receptors mediate both the cardiovascular and respiratory responses whereas mPB glutamate receptor modulate only the cardiovascular response evoked by HDA stimulation. lPB subnuclei involved in these cardiorespiratory effects include the crescent, ventral, central and external. No effects were seen with microinjections located in the internal subnucleus of the lPB. The response is so specific that regions as close as the internal or external lPB nuclei, separated by microns, show no effects or attenuations in the intensity of the cardiorespiratory responses. All microinjections within the mPB, including external mPB had an effect. There is evidence that glutamate receptors of neurons in the region of the PBc are essential mediators for the descending pathways of the cardiovascular sympathetic and respiratory drive. Our results raise the possibility that although these direct projections exist, the impact of these projections on overall cardiorespiratory function is highly dependent on convergent inputs from specific subnuclei of the lPB region.

Our observations also suggest that alternate pathways, outside the PBc, possibly direct projections to the RVLM, are also involved in HDA-evoked changes in arterial pressure (Lovick, 1992; van der Plas et al., 1995; Verberne and Guyenet, 1992), thus supporting that changes in heart rate and blood pressure evoked from 'defense' regions of the brain may travel via separate pathways (Fontes et al., 2001). The study must be extended to contribute to clarify the importance of the integrated control of both pontine regions and those defense regions on autonomic functions.

Acknowledgments

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