Brain Research 1652 (2016) 43–52

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Central mechanism of the cardiovascular responses caused by L-proline microinjected into the paraventricular nucleus of the hypothalamus in unanesthetized rats

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ARTICLE INFO

Keywords:
L-Proline
Paraventricular nucleus of the hypothalamus (PVN)
Cardiovascular response
Ionotropic glutamate receptors
Oxide nitric

ABSTRACT

Previously, we reported that microinjection of L-proline (L-Pro) into the paraventricular nucleus of the hypothalamus (PVN) caused vasopressin-mediated pressor responses in unanesthetized rats. In the present study, we report on the central mechanisms involved in the mediation of the cardiovascular effects caused by the microinjection of L-Pro into the PVN. Microinjection of increasing doses of L-Pro (3–100 nmol/100 nL) into the PVN caused dose-related pressor and bradycardic responses. No cardiovascular responses were observed after the microinjection of equimolar doses (33nmol/100nL) of its isomer D-Proline (D-Pro) or Mannitol. The PVN pretreatment with either a selective non-NMDA (NBQX) or selective NMDA (LY235959 or DL-AP7) glutamate receptor antagonists blocked the cardiovascular response to L-Pro (33 nmol/100 nL). The dose-effect curve for the pretreatment with increasing doses of LY235959 was located at the left in relation to the curves for NBQX and DL-AP7, showing that LY235959 is more potent than NBQX, which is more potent than DL-AP7 in inhibiting the cardiovascular response to L-Pro. The cardiovascular response to the microinjection of L-Pro into the PVN was not affected by local pretreatment with N^{ω}-Propyl-L-arginine (N-Propyl), a selective inhibitor of the neuronal nitric oxide synthase (nNOS), suggesting that NO does not mediate the responses to L-Pro in the PVN. In conclusion, the results suggest that ionotropic receptors in the PVN, blocked by both NMDA and non-NMDA receptor antagonists, mediate the pressor response to L-Pro that results from activation of PVN vasopressinergic magnocellular neurons and vasopressin release into the systemic circulation.

1. Introduction

The nonessential amino acid L-proline (L- Pro) attains criteria to be considered as a neurotransmitter in the central nervous system (CNS) of mammals: it is synthesized and stored in nerve terminals (Yoneda and Roberts, 1982); it is released by Ca^{2+}-dependent mechanism (Bennett et al., 1972; Mulder and Snyder, 1974; Snyder et al., 1973); its exogenous application reproduces nerve responses (Felix and Kunzle, 1974; Zarzecki et al., 1975), producing excitatory effects on interneurons of the spinal cord or inhibitory on Purkinje cells in the cerebellar cortex of cats (Ault et al., 1987; Felix and Kunzle, 1976; Zarzecki et al., 1975), suggesting excitatory or inhibitory actions depending on the type of stimulated neuron; and, finally, it is inactivated by a Na^{+}-dependent neuronal uptake (Hauptmann et al., 1983). Moreover, L-Pro transporter is located in suprabulbar glutamatergic neurons of the rat brain, suggesting a possible synaptic role for L-Pro in CNS excitatory pathways (Fremeau et al., 1992; Velaz-Faircloth et al., 1995). There is further evidence that L-Pro transporter protein is located in synaptic vesicles, in the presynaptic axon terminal forming asymmetric excitatory synapses in the forebrain of rats (Renick et al., 1999). Binding studies performed on synaptic membranes of rat brain showed that L-Pro binds in a saturable manner, suggesting the presence of receptors for L-Pro in rat brain (Greene et al., 1986; Negron et al., 1988; Ortiz et al., 1989). Immunohistochemical studies revealed several groups of strongly labeled L-Pro-containing neurons in hypothalamic and medullary nuclei (Takemoto and Semba, 2006), which are structures known to modulate the cardiovascular system.

Previously, cardiovascular effects of L-Pro in supramedullary structures were observed when L-Pro was microinjected into the third ventricle (3 V) of unanesthetized rats, being mediated by vasopressin release into the systemic circulation (Lopes-Azevedo et al., 2012). Because the walls of the 3 V are located close to the paraventricular nucleus of the hypothalamus (PVN), which is involved in neuroendocrine and autonomic regulation (Swanson and Kuypers, 1980; Swanson and Sawchenko, 1980), it was proposed that the vasopressin-mediated

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http://dx.doi.org/10.1016/j.brainres.2016.09.046
Received 29 April 2016; Received in revised form 30 August 2016; Accepted 28 September 2016
Available online 29 September 2016
0006-8993/

pressor response of L-Pro injected into the 3 V could be mediated, at least in part, by cells located in or near the PVN (Lopes-Azevedo et al., 2012). To test this hypothesis, L-Pro was microinjected into the PVN of unanesthetized rats, in a dose of 33 nmol/100nL, which is 20-fold lower than that causing similar vasopressin-mediated cardiovascular responses when injected into the 3 V (600 nmol/500 nL) (Lopes-Azevedo et al., 2012).

The PVN comprises a complex neuronal network that besides the magnocellular portion is also constituted by a parvocellular portion, which can be subdivided into pre-autonomic and neuroendocrine portions (Swanson and Kuypers, 1980; Swanson and Sawchenko, 1980). The magnocellular neuroendocrine cells (MNCs) are responsible for the synthesis of vasopressin, a potent vasoconstrictor agent (Swanson and Sawchenko, 1983). MNCs in the PVN send efferent projections to the neurohypophysis, which secretes vasopressin into the bloodstream (Cunningham et al., 2004; Renaud and Bourque, 1991), and also interact with intrinsic PVN neural circuitry to regulate the autonomic and/or neuroendocrine mechanisms (Busnardo et al., 2009; Ferguson et al., 2008; Son et al., 2013). Preautonomic parvocellular cells send efferent projections to sympathetic nuclei in the brainstem and spinal cord that are responsible for the neural control of cardiovascular tone (Badoer, 1996; Kuypers and Maisky, 1975; Pyner et al., 2001; Ranson et al., 1998; Shafton et al., 1998).

Although there is strong evidence about the pressor effects of the microinjection of L-Pro into the PVN, there are no studies on the central mechanisms mediating these responses. L-Glutamate (L-Glu) is the most abundant excitatory neurotransmitter in the central nervous system (CNS). Binding and in situ hybridization studies revealed a high density of metabotropic and ionotropic glutamate receptors as well as the expression of mRNA for several subunits of these receptors throughout the PVN (Herman et al., 2000; Meeker et al., 1994). Injection of L-Glu into the PVN has been reported to cause increase in blood pressure and heart rate (Busnardo et al., 2009; Martin and Haywood, 1992). The cardiovascular responses evoked by L-Glu injected into the PVN were blocked after intravenous treatment with the ganglion blocker pentolinium, indicating an autonomic mediation of these responses (Busnardo et al., 2009). Moreover, the tachycardic response to L-Glu in the PVN was reversed into a bradycardic response after PVN pretreatment with the NMDA antagonist LY2359595 (2 nmol/100 nL) (Busnardo et al., 2009). This observation suggests that activation of NMDA glutamatergic receptors in the PVN pre-autonomic parvocellular portion mediates the cardiac component of the cardiovascular response to L-Glu, which is associated with activation of the sympathetic nervous system. Furthermore, when PVN NMDA receptors were blocked, PVN stimulation with L-Glu now caused pressor and bradycardic responses that were blocked by local treatment with the non-NMDA antagonist NBQX or intravenous treatment with the V1-vasopressin receptor antagonist dTyrAVP (Busnardo et al., 2009), suggesting that non-NMDA glutamatergic receptor stimulation in the PVN magnocellular portion mediates pressor response by vasopressin release to systemic circulation.

There is evidence that L-Pro may activate both NMDA and non-NMDA-subtypes of ionotropic glutamate receptors, because selective ionotropic-receptor antagonists were shown to attenuate L-Pro effects (Ault et al., 1987; Henzi et al., 1992; Pace et al., 1992). However, differences in the profile of inhibition of L-Glu and L-Pro effects by antagonists of ionotropic receptors suggest the existence of selective prolinergic receptors (Leone and Gordon, 1989; Pawloski-Dahm and Gordon, 1992; Takemoto, 2001; Lopes-Azevedo et al., 2013). Therefore, more studies are needed to elucidate the mechanism of

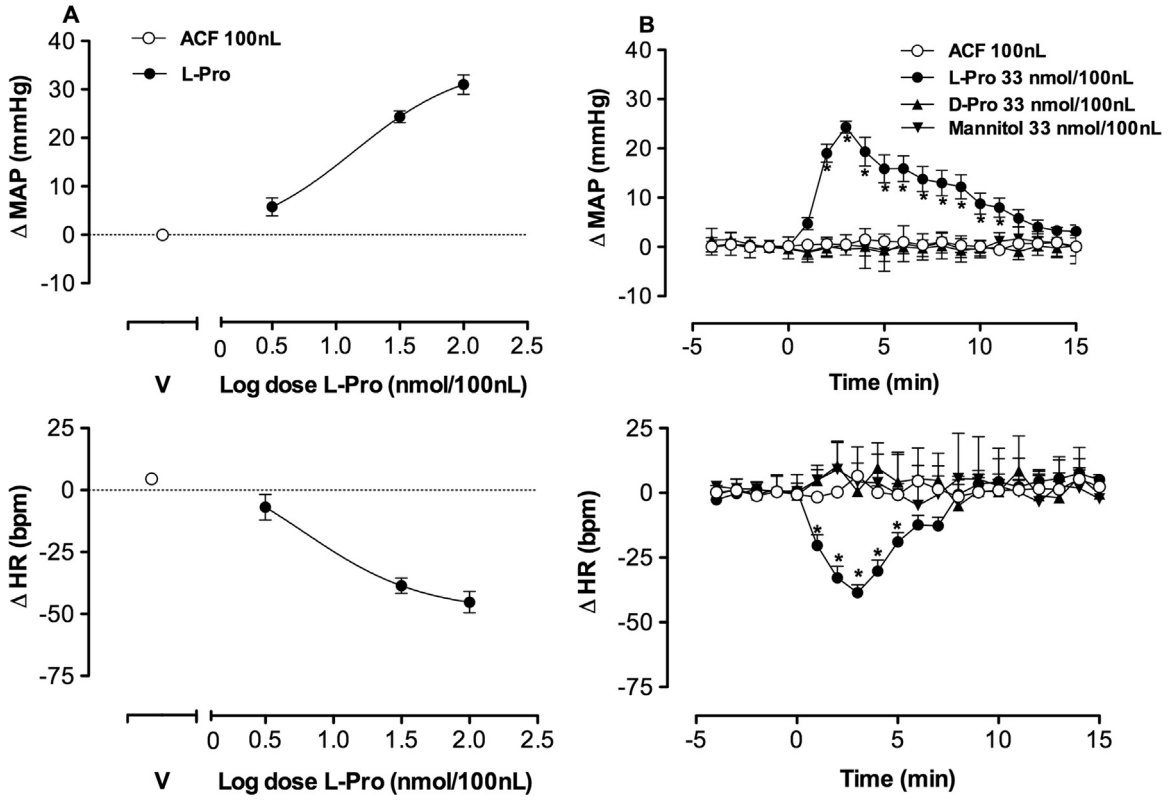

Fig. 1. A) Dose-effect curves for L-proline (L-Pro), 3 (n=6), 33 (n=6), 100 (n=5) nmol/100 nL, and the effect of artificial cerebrospinal fluid (ACF, 100nL, n=5) microinjected into the PVN of unanesthetized rats on the mean arterial pressure (ΔMAP) and heart rate (ΔHR). Circles represent means and bars the SEM. Dose-effect curves were generated by nonlinear regression analysis (ΔMAP, R^2=0.89 and ΔHR, R^2=0.77) of the peaks of the cardiovascular effects, which were observed three minutes after the microinjection of L-Pro; **B)** Time-course of the effect of ACF (100nL; n=5), L-Pro (33 nmol/100 nL; n=6), D-Pro (33 nmol/100 nL, n=5) or Mannitol (33 nmol/100 nL; n=5) microinjected into the PVN on mean arterial pressure (ΔMAP) and heart rate (ΔHR). Microinjections of ACF, L-Pro, Mannitol or D-Pro were made at time 0. Points represent the mean and bars the SEM; * $P < 0.05$, two-way ANOVA, applying the Bonferrroni's correction for multiple comparisons between the effects of microinjections of L-Pro (33 nmol/100 nL) and ACF.