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Original article

$GABA_A$ receptor in the Pedunculopontine tegmental (PPT) nucleus: Effects on cardiovascular system

Hosein Pasandi[a], Saman Abbaspoor[b], Mohammad Naser Shafei[c,*], Mahmoud Hosseini[d], Abolfazl Khajavirad[a,c]

[a] Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Departments of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
[c] Neurogenic Inflammation Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Background: The pedunculopontine tegmental (PPT) nucleus is a heterogeneous nucleus with several functions including cardiovascular regulation. The presence of $GABA_A$ receptor has been shown in the PPT. Therefore, the cardiovascular effects of this receptor were examined.
Methods: Rats were divided into: Control; Muscimol; Bicuculline (BMI); Hexamethonium (Hexa) + BMI and Atropine + BMI groups. The femoral vein and artery were cannulated for drug administration and recording of cardiovascular parameters, respectively. Muscimol (a $GABA_A$ agonist; 1.5 and 2.5 nmol), BMI (a $GABA_A$ antagonist; 0.1 and 0.2 nmol) were stereotaxically microinjected into the PPT. To evaluate the peripheral cardiovascular mechanisms of $GABA_A$ receptors, Hexa (a ganglionic blocker; 10 mg/kg) and atropine (a muscarinic receptor antagonist; 1 mg/kg) were intravenously (*iv*) injected before BMI (0.2 nmol). The average changes of mean arterial pressure (ΔMAP), systolic blood pressure (ΔSBP) and heart rate (ΔHR) in different intervals were calculated and compared both within and between case group and control group (repeated measures ANOVA). The peak changes in each group were also calculated and compared with those of the control group (independent sample *t*-test).
Results: Both doses of BMI significantly increased ΔMAP, ΔSBP and ΔHR compared to control, while the only higher dose of muscimol significantly decreased ΔSBP. *Iv* injection of Hexa significantly attenuated ΔMAP, ΔSBP and ΔHR responses induced by BMI but atropine did not affect.
Conclusions: Our results demonstrate that $GABA_A$ receptor of the PPT has a tonic inhibitory effect on the cardiovascular system and its peripheral effect mostly is mediated by sympathetic system.

Introduction

Pedunculopontine tegmental nucleus (PPT) is a heterogeneous area with different populations of cholinergic, gamma amino-butyric acid (GABA)ergic and glutamatergic neurons [1,2]. The PPT has two distinct parts: pars dissipata and pars compacta. The dominant neurons in pars dissipata are GABAergic and in pars compacta are cholinergic and glutamatergic [1,2]. The PPT has two cholinergic and non-cholinergic projections that connect this nucleus with several areas including basal ganglia, ventral and lateral hypothalamus, ventral tegmental area (VTA), nucleus basalis, amygdala [3–7] and different nuclei in the brain stem including rostral ventrolateral medulla (RVLM), nucleus tractus solitarius (NTS) [8] and spinal cord [4].

The PPT is involved in several functions such as initiation and control of gait [9], reward and behavioral responses [10], wakefulness, rapid eye movement (REM) sleep [11] and autonomic regulation [8].

The role of the PPT in cardiovascular regulation was also reported previously. For example, microinjection of glutamate into the PPT increased blood pressure [8] and its nitrergic system decreased cardiovascular parameters [12]. There is also evidence that the PPT has projection to the RVLM, the main center of cardiovascular regulation [8,13].

The GABAergic system and its $GABA_A$ receptor is a well-known inhibitory system in the central nervous system and its effect on

* Corresponding author.
E-mail address: Shafeimn@mums.ac.ir (M.N. Shafei).

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central cardiovascular regulation has been reported in several studies [14–16]. The cardiovascular effect of $GABA_A$ receptor in RVLM, NTS, paraventricular nucleus of hypothalamus (PVN) and horizontal limb of the diagonal band of Broca (hDB) has been reported [14–18].

Also, microinjection of bicuculline methiodide (BMI), a $GABA_A$ antagonist into the RVLM [16], PVN [19] and hDB [20] has been shown to evoke changes in cardiovascular parameters that confirm inhibitory effect of $GABA_A$ receptor in those areas.

Presence of the $GABA_A$ receptor in the PPT and its involvements in several functions such as locomotion [5], REM sleep [5] and reward [21] has been shown. Due to the involvement of the PPT in cardiovascular regulation and presence of $GABA_A$ receptor in this nucleus, in the present study, we evaluated the possibility cardiovascular responses $GABA_A$ receptor of the PPT. In addition, hexamethonium (Hexa; a ganglionic blocker) and atropine (a muscarinic receptor antagonist) were injected intravenously (*iv*) before microinjection of BMI, to assess the possible peripheral cardiovascular mechanism of $GABA_A$ receptor in the PPT.

Materials and methods

Animals and surgery

Experiments were done on 56 male Wistar rats (250–280 g). The animals were anesthetized with urethane (1.4 g/kg; *ip*) and supplementary doses (0.7 g/kg). The left femoral vein and artery were cannulated for drug administration and recording of cardiovascular parameters, respectively. The femoral artery was then connected to a pressure transducer and cardiovascular parameters were recorded by a Power Lab system (ADinstruments, Bella Vista, NSW, Australia). For microinjecting of the drugs, the animals were fixed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) and a small hole was drilled over the PPT according to the rat brain atlas of Paxinos and Watson (AP: 7.6–8.5 mm caudal to bregma, L: 1.7–2.2 mm lateral to the midline; H: 5.5–6.2 mm ventral from the bregma [22].

Drugs and microinjection

The drugs used in the experiments were urethane (anesthesia, Sigma-USA), muscimol (a $GABA_A$ agonist, Sigma, USA), bicuculline methiodide (BMI, a $GABA_A$ antagonist, Sigma, USA), atropine (Atr; a muscarinic receptor antagonist, Sigma, USA) and hexamethonium hydrochloride (Hexa; a ganglionic nicotinic receptor blocker, Sigma, USA). All drugs were dissolved in saline. BMI and muscimol were microinjected into the PPT and Hexa and Atr were injected intravenously. Microinjection was carried out by a single barreled 35–45 μm internal diameter micropipette that was connected through a PE-10 tube to an injection syringe [23]. The micropipette was carefully introduced into the PPT using stereotaxic apparatus and injection performed by a manual microinjector.

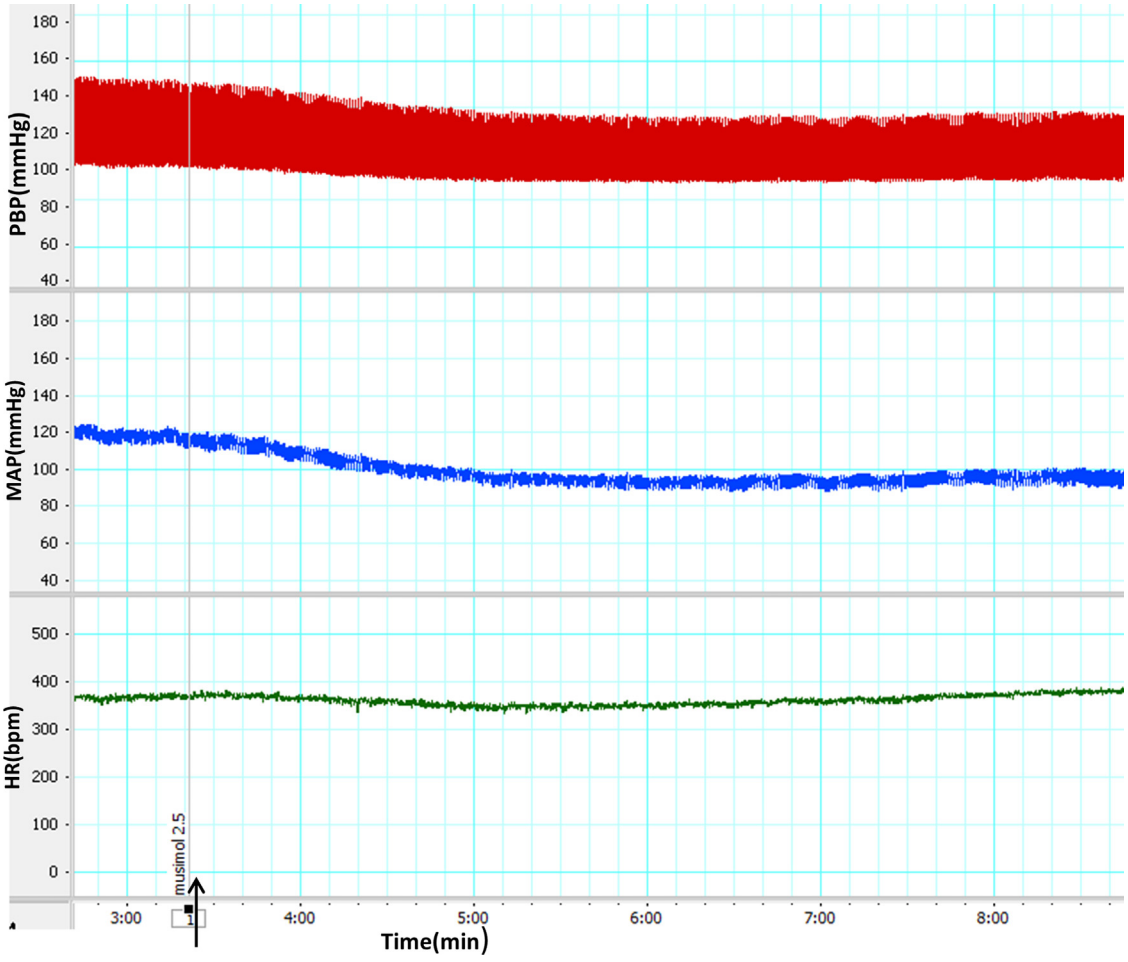

Fig. 1. Recording sample of changes in blood pressure and heart rate after microinjection of muscimol (2.5 nmol) into the PPT.