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Evaluation of nicotinic receptor of pedunculopontine tegmental nucleus in central cardiovascular regulation in anesthetized rat

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ABSTRACT

Objective(s): Cholinergic neurons are important neurons in the Pedunculopontine tegmental nucleus (PPT). In this study, nicotinic receptor of the PPT in central cardiovascular regulation in the anesthetized rat was evaluated.

Materials and Methods: Saline, acetylcholine (Ach; doses: 90 and 150 nmol), hexamethonium (Hexa; doses: 100 and 300 nmol) and higher doses of Hexa (300 nmol) + Ach (150 nmol) microinjected into the PPT. The femoral artery was cannulated and cardiovascular responses were continuously recorded by a power lab system. After injection of drugs, peak changes of mean arterial pressure (ΔMAP), systolic blood pressure (ΔSBP) and heart rate (ΔHR) calculated and compared with saline group.

Results: The ΔSBP and ΔMAP significantly decreased by two doses of Ach (P<0.05 to P<0.001) but ΔHR did not change. Two doses 100 (P<0.05) and 300 nmol (P<0.01) of Hexa significantly increased ΔHR but did not alter the ΔMAP or ΔSBP. Co-injection of Hexa + Ach significantly strengthened the ΔHR induced by Hexa alone (P<0.01) but did not affect ΔMAP or ΔSBP.

Conclusion: These results indicate that nicotinic receptor of the PPT has an inhibitory effect on ΔHR with no significant effect on ΔMAP or ΔSBP.

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Introduction

Pedunculopontine tegmental (PPT) is a heterogeneous nucleus in the brain stem, which has numerous functions such as control of movement, regulation of sleep (1-3), memory (4), modulation of pain (2, 5) and regulation of autonomic functions (3, 6). Histochemical and anatomical studies show that the PPT has some connections with numerous areas in the brain including basal ganglia (globus pallidus, subthalamic and substantia nigra) (3, 7-10), limbic system (3, 8, 11, 12) and lower brain stem nuclei (3, 6, 13).

Cardiovascular effects of the PPT nucleus have also been evidenced (14, 15). Topchiy *et al.* reported that chemical stimulation of nucleus by dl-homocysteic acid or bicuculline increased blood pressure (BP) and changed baroreceptor reflex function (4, 14, 15). In a recent study, we showed that blockade synthesis of nitric oxide in the PPT elicits BP and heart rate (HR) (16). A cholinergic projection from the PPT to the rostral ventrolateral medulla (RVLM), an integrative area in cardiovascular adjustment, has been reported (15, 17). Also, the anatomical connection has been found between the PPT and other cardiovascular centers, such as periaqueductal gray matter (PAG), nucleus tractus solitarius (NTS), cuneiform nucleus (CnF) and raphe nuclei (5,17, 18). Several neuronal populations have been identified in the PPT nucleus, the most well-known of them are cholinergic neurons that are concentrated in its caudal portion and form the 5th cholinergic cell groups (Ch5) of the central nervous system (19).

The role of the cholinergic neurons of the brain in controlling cardiovascular activity has been shown in numerous studies (20-22). For example, it is reported that cholinergic neurons of the RVLM have stimulating effect on the cardiovascular system (17, 23).

Cholinergic system has both muscarinic and nicotinic receptors. Nicotinic Ach receptors (NAChRs) are heterogeneous families of the ion channels that are present in some areas of central and peripheral nervous system (24-27). These receptors are permeable to sodium, potassium, and calcium and are fast response in addition to other effective properties (28, 29).

The presence of NAChRs in cardiovascular centers and their role in cardiovascular regulation is well defined (21, 22, 30-32). The NAChRs in the PPT are also identified (19, 33), but the role of these receptors in cardiovascular activities is unknown. Therefore, in this study, the probable role NAChRs of the PPT in cardiovascular activities was investigated.

Materials and Methods

Animals

Forty eight male Wistar rats were used in this study (250–270 g, Mashhad, Iran). After anesthesia with urethane (1.4 g/kg, IP), the femoral artery was cannulated with a polyethylene catheter (PE-50). To prevent blood clotting, the catheter was filled with heparinized saline (50 units /ml). Cardiovascular parameters (mean arterial pressure (MAP), systolic blood pressure (SBP) and HR) recorded by a pressure transducer connected to a power lab system (ID instrument, Australia). The peak changes (Δ) of parameters induced by drugs were achieved and

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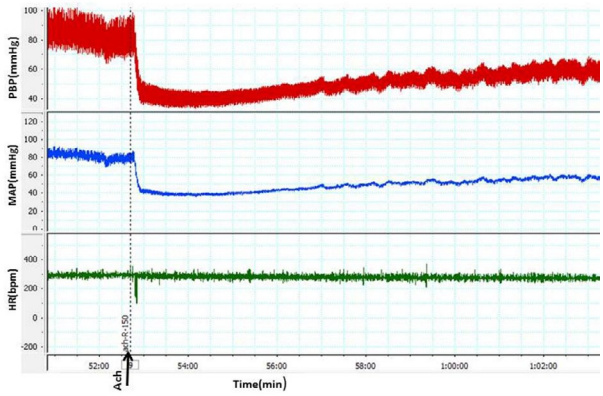

Figure 1. A sample recording of cardiovascular parameters induced by injection of Acetylcholine (Ach) into the Pedunculopontine tegmental nucleus (PTT); the time of injection is indicated by an arrow

compared with changes of saline. All experiments were conducted in accordance with the protocols approved by Ethical Committee of Mashhad University of Medical Sciences (ID: 900894).

Drugs

The acetylcholine hydrochloride (Ach), hexamethonium (Hexa, a nonselective nicotinic antagonist) and urethane, which were used in this study, were provided by sigma chemical company, USA. The solvent of reagents was saline.

Microinjection

The injection of drug into the nucleus was performed based on the previous study (34). After head fixation of the animal in a stereotaxic frame (Stoelting, USA), a hole was created in the skull over the PPT according to the atlas of Paxinos and Watson (AP: -7.6–8.5 mm, Lateral: ±1.7–2.2 mm and vertical: 5.5–6.2 mm (16, 35). Drugs were injected (100 –150 nl) by a micropipette that was connected to an injector syringe (Stoelting, USA) and introduced into the PPT (36).

Groups

Animals were divided into six groups as follow:

1. Control group; saline injected into the PPT.
2, 3. Ach groups; two doses of Ach (90 or 150 nmol, separately) injected into the PPT
4, 5. Hexa groups: two doses of Hexa (100 or 300 nmol, separately) injected into the PPT
6. Hexa + Ach group; co-injection of the highest doses of Hexa (300 nmol) and Ach (150 nmol) into the PPT

Histology

After completing experiments, the rats were sacrificed with a high dose of anesthetic drug. Brains perfused firstly with 100 ml of 0.9% saline following 100 ml of 10% formalin. The brains were then removed from the skull and placed in formalin 10% for 24 hours. A serial section with a thickness of 60 microns was prepared and the injection site was approved according to rat brain atlas of Paxinos and Watson using a light microscope (35).

Data analysis

Values were expressed as mean ± SEM. The peak

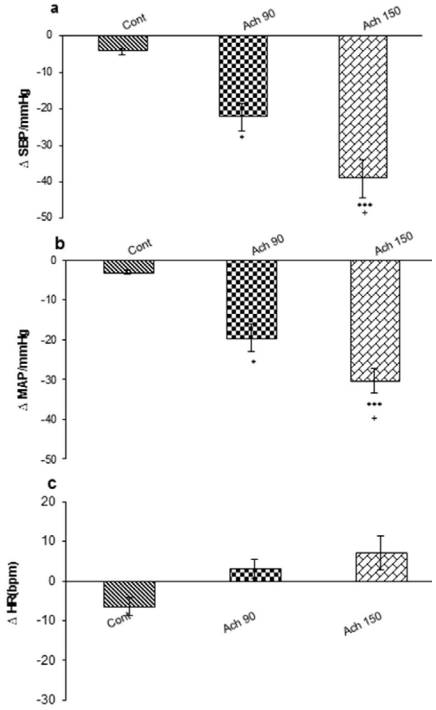

Figure 2. Effects of two doses of Acetylcholine (Ach; 90 and 150 nmol) microinjection into the Pedunculopontine tegmental nucleus (PTT) on cardiovascular parameters
One-way ANOVA with Tukey's post hoc test was used for statistical analysis
* indicate changes induced by microinjection of Ach compared to control (*; $P<0.05$, ***; $P<0.001$)
+ Show changes induced by dose 150 compared to dose 90 nmol (independent-samples t- test) ; n=8
ΔMAP: Maximal changes of mean arterial pressure; ΔSBP: Maximal changes of systolic blood pressure; ΔHR: Maximal changes of heart rate

changes in SBP, MAP and HR were provided and analyzed by one-way ANOVA followed by a Tukey's *post hoc* test and compared with control. Independent-samples *t*-test was used for comparing two groups. The data was significant when the *P*-value was $P<0.05$.

Results

Effect of microinjections of saline into the PPT on cardiovascular responses

In this group, the basal cardiovascular parameters were recorded and then saline was injected into the PPT. Microinjection of saline did not change MAP (baseline: 114.42±1.98 mm Hg and after: 109.38±2.33 mm Hg), SBP (baseline: 126.12±4.04 mm Hg and after: 121.6±4.14 mm Hg) or HR (baseline: 325.68±12.2 beats per min (bpm) and after: 313.35±14.65 bpm; n=8).

Effect of microinjections of Ach into the PPT on cardiovascular responses

In this experiment, two doses of Ach (90 and 150 nmol) were injected into the nucleus. The SBP and MAP in all doses of Ach decreased, but HR did not change. The changes of BP and HR after injection of 150 nmol Ach are depicted in Figure 1. As indicated, all doses of Ach significantly decreased ΔSBP compared to saline (-22.19±4.8 and -39.01±5.2, mm Hg *vs* -4.22±1.8 mm Hg, $P<0.05$ and $P<0.001$ respectively, n= 8; Figure 2a). The changes of SBP at 150 nmol of Ach was also significantly higher compare to lower dose (90 nmol) ($P<0.05$).

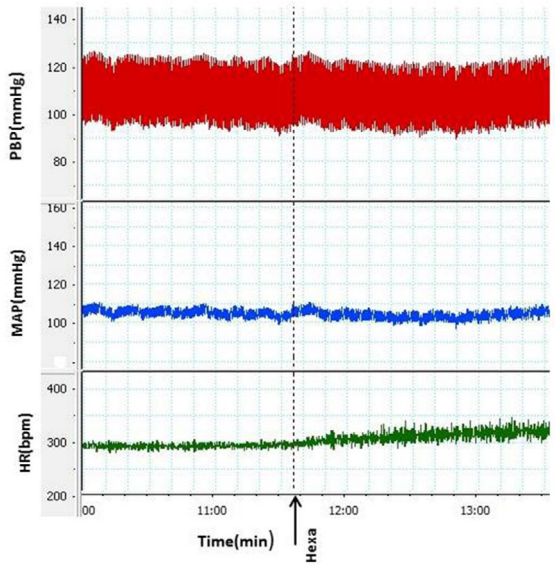

Figure 3. A sample of recording of cardiovascular parameters induced by injection of Hexa into the PPT nucleus; the time of injection is indicated by an arrow

Peak changes of MAP induced by both doses of Ach also significantly reduced compared with control group (-17.8±4.54 and -30.27±3.18, mm Hg *vs* -3.04±1.6 mm Hg, $P<0.05$ and $P<0.001$, respectively n= 8; Figure 2b).

The peak changes of HR after microinjection of both doses of Ach were not significant compared with the control group (3.17±2. 4 bpm and 7.01±2.34 bpm, respectively *vs*. -5.39±2.2 bpm, n= 8; Figure 2c).

Effect of microinjections of Hexa into the PPT on cardiovascular responses

To determine the role of nicotinic receptor of the PPT on basal cardiovascular parameters, two doses of Hexa were injected into the nucleus. The effect of higher dose of Hexa has been indicated in Figure 3. As demonstrated, the 100 and 300 nmol doses of Hexa had no significant effect on ΔSBP (3.4±1.6 and 6.5±3.6 mm Hg, respectively vs saline: -4.22±1.8 mm Hg; Figure 4 a) and ΔMAP (2.3± 0.8 and 4.6±2.4 mm Hg, respectively *vs* saline: -3.04±1.6 mm Hg, n=8; Figure 4 b) in comparison with the control group. The maximal ΔHR was significantly increased by both doses of Hexa (10.3±5.6 bpm and 14.3±3.90 bpm *vs* control: -5.39±2.2 bpm, $P<0.05$ and $P<0.01$, respectively; n=8; Figure 2c).

Effect of co-injection of Hexa and Ach into the PPT on cardiovascular responses

In this experiment, to better understand the cardiovascular effect of the nicotinic receptor in the PPT, the highest dose of Hexa (300 nmol) was microinjected before the highest dose of Ach (150 nmol). A sample of cardiovascular parameters recording after drugs injection was shown in Figure 5. The maximal ΔSBP and ΔMAP in Ach+Hexa group were not significantly attenuated compare to Ach alone (ΔSBP: Ach + Hexa: 31.7±3.6 *vs* Ach: 39.1±5.2 and ΔMAP: Ach + Hexa; -26.6±4.2 *vs* Ach: -30.3±3.18; Figure 6 a, b).

The maximal ΔHR in Hexa (300 nmol) group was significantly higher than controls (14.3±3.90 beats/min *vs* control: -5.39±2.2 beats/min, $P<0.01$) and this effect

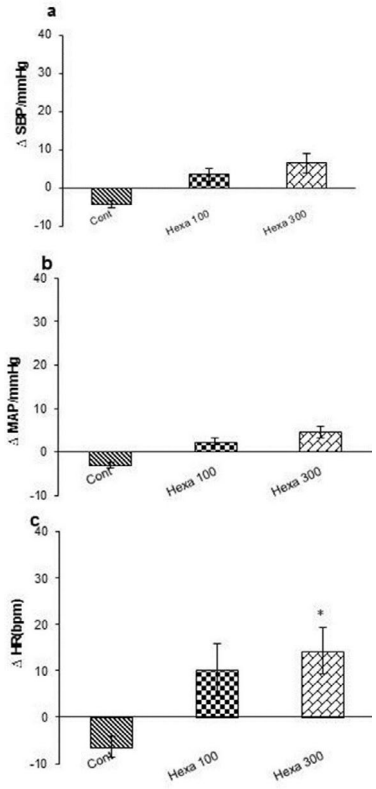

Figure 4. Effects of two doses of Hexa (100 and 300) microinjected into the PPT nucleus on cardiovascular parameters
One-way ANOVA with Tukey's post-hoc test was used for statistical analysis
* indicate changes induced by microinjection of Hexa compared to control (*; $P<0.05$, **; $P<0.01$); n =8
ΔMAP: Maximal changes of mean arterial pressure; ΔSBP: Maximal changes of systolic blood pressure; ΔHR: Maximal changes of heart rate

was increased in the presence of Ach (Hexa + Ach: 33.2 ± 4.1 beats/min vs Hexa alone: 14.3±3.90 beats/min; $P<0.01$). The maximal ΔHR in Hexa + Ach group was also significant compare to the control (Hexa + Ach: 33.2 ± 4.1 beats/min vs control:-5.4 ± 2.2 beats/min, $P<0.001$) and Ach groups (Hexa + Ach: 33.2 ± 4.1 beats/min vs Ach: 7.01 ± 2.34 beats/ min, $P<0.001$; Figure 6c).

Discussion

The cardiovascular effects of nicotinic receptor of the PPT nucleus were evaluated in this study. We found that MAP and SBP decreased when Ach injected into the PPT, but HR did not change. Microinjection of Hexa alone did not affect baseline MAP and SBP but significantly increased HR. Co-injection of Hexa and Ach did not significantly affect ΔMAP and ΔSBP induced by Ach but significantly increased tachycardia induced by Hexa.

Previous studies have documented that BP and HR are regulated by brain cholinergic neurons (37, 38).The effects of these neurons in brain areas are different. For example, injection of Ach into the RVLM can elicit MAP and HR (39, 40), while it reduces MAP when injected into the CnF nucleus (38). In consistent with these results, our study also indicates that Ach in the PPT decreased BP but did not change HR.

The responsible mechanism(s) for cardiovascular effects of cholinergic neurons in the PPT has not been defined. Because the PPT has important cholinergic projections

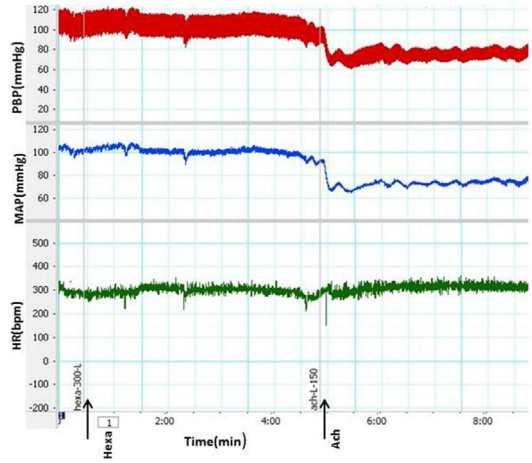

Figure 5. A sample of recording of cardiovascular parameters induced by co-injection of Hexa + Ach into the PPT nucleus; the time of injection is indicated by an arrow

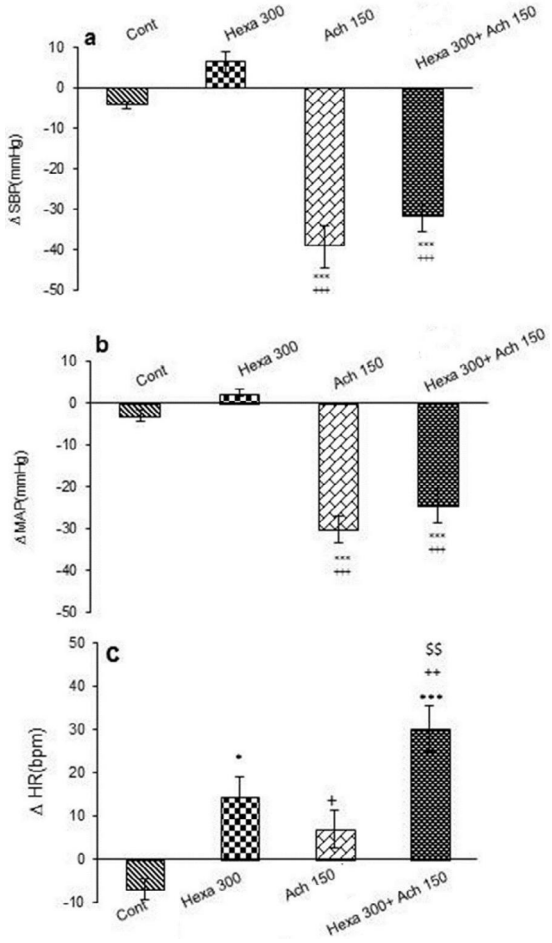

Figure 6. Effect of co-injection of higher doses of Hexa (300 nmol) and Ach (150 nmol) into the PPT nucleus on cardiovascular parameters
One-way ANOVA with Tukey's post-hoc test was used for statistical analysis
* indicate changes induced by co-injection of Hexa + Ach compared to control **; P<0.01, ***; P<0.001)
+ show changes induced by co-injection of Hexa + Ach compared to Hexa alone (++; P<0. 01)
$ show changes induced by co-injection of Hexa + Ach compared to Ach 150 ($$$; P<0.001)
ΔMAP: Maximal changes of mean arterial pressure; ΔSBP: Maximal changes of systolic blood pressure; ΔHR: Maximal changes of heart rate. n= 8

to several brain areas including the areas involved in central cardiovascular regulation (14, 15), we assumed that this projection probably precipitates in the cardiovascular response of the PPT nucleus.

Immunohistochemical and anatomical studies reported a relation between the PPT and the RVLM (17). Padly *et al.* indicated that this connection is cholinergic projection involved in the cardiovascular effect of the PPT (15). A decrease in BP after microinjection of Ach into the PPT was observed in the present study and therefore, it is conceivable that cardiovascular effect of Ach is mediated via inhibition of cholinergic PPT -RVLM pathway. In consistent with our result, it is reported that microinjections of carbachol into the guinea-pig brain slice hyperpolarized and reduced activity of cholinergic PPT neurons (41).

Electrophysiological studies also indicated that there are three types (I, II and III) of neurons in the PPT (42), of which the type II shows the maximal response to carbachol and serotonin (17). Carbachol is well-known that increases conductance of K^+ to induce hyperpolarization (41). In the present study, it is possible that Ach may activate this type of neurons to hyperpolarize cholinergic projection to the RVLM. Hyperpolarization of cholinergic neurons leads to hypotension by decreasing the activity of vasomotor neurons in the RVLM.

Beside direct projection to RVLM, the PPT neurons have been found to project to other areas related to BP including the PAG nucleus (18). Additionally, microinjection of Ach into the lateral ventral portion of the PAG (vlPAG) was reported to decrease BP (43). Since the PAG has a projection to the RVLM (44), it is possible that the effect of Ach in the PPT, which was observed in the present study, is polysynaptic and mediated through PPT-PAG-RVLM pathway; however, it needs to be more investigated in the future.

On the other hand, a relation between the PPT nucleus and the CnF in control of locomotion has been reported (45). It is also indicated that Ach decreased BP when microinjected into the CnF (36). Due to the similarity of the Ach action in the PPT with the CnF and considering the fact that the PPT is an integrative area (14), the same cardiovascular effect of cholinergic system might be suggested in both nuclei to adjust cardiovascular activity in conditions such as movement and sports. However, more studies are needed to verify this hypothesis.

In another experiment, we evaluated the role of Hexa (a nicotinic antagonist) on the cardiovascular system of the PPT. Hexa increased HR with no significant effect on basal BP. Therefore, it is conceivable that the effect of Ach on BP in the PPT is mediated by muscarinic receptor (44). Muscarinic receptors are important receptors of brain cholinergic system and the cardiovascular effects mediated by muscarinic receptor have been indicated in numerous experiments (38, 44, 46). Therefore, the presence of muscarinic receptors in the PPT (47) may confirm this idea.

An increment in HR after microinjection of the highest dose of Hexa that was observed in the present study might be considered as an inhibitory effect of nicotinic receptors in the PPT on HR. The responsible mechanism(s) for this effect of Hexa on HR has not been

well-determined. However, a relation between PPT and NTS has been shown (18). NTS nucleus has been reported to be a primary integrative area in central cardiovascular regulation to control baroreflex and chemoreflex functions (44). The presence of nicotinic receptor in the NTS (44) and its inhibitory effects on cardiovascular response let us suggest that the effect of Hexa on HR might be related to projection from PPT to the NTS. However, it needs to be more examined.

Furthermore, the PPT has known to have central command regulation (15). We hypothesized that in the anesthetized condition, which was performed in the present study, the release of Ach in this nucleus is low. Therefore, in another group, we co-injected Hexa with Ach into the PPT. Our result showed that hypotensive effect of Ach did not alter by co-injection of Hexa + Ach, but tachycardia induced by Hexa was significantly augmented. The exact mechanism of this effect is unknown. It might be assumed that this effect is mediated by both baroreflex activity and nicotinic receptor. In the cardiovascular system, hypotension has been reported that reflexively induces an increment in HR. However, a decrease in BP in Ach group was not accompanied by a change in HR. Considering these results, it seems that injection of Ach has two effects on HR. Firstly, HR was reflexively increased via induction of hypotension and secondly, Ach decreased HR through the effect on the nicotinic receptor. On the other hand, tachycardia induced by the baroreflex function is balanced by bradycardia induced by activation of nicotinic receptor; therefore, HR did not change. However, ΔHR was increased when Hexa + Ach was co-injected, which might be due to nicotinic receptor blockade. In addition, the hypotensive effect of Ach that was observed in the present study might be followed by an increase in HR via baroreflex activity. Therefore in Hexa + Ach group, HR was augmented to be higher than Hexa alone.

Conclusion

Our results in this study indicated that the PPT cholinergic neurons could inhibit cardiovascular parameters but only their effect on HR is achieved by nicotinic receptor. It is conceivable that the effect of these neurons on BP is mediated by muscarinic receptor.

Acknowledgment

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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