Cardiovascular responses to apelin-12 injection into the ventral medullary surface in rats

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(Received September 25, 2001 ; Accepted December 21, 2001)

Abstract : We examined cardiovascular responses to the injection of apelin-12 (AP12) into the ventral medullary surface (VMS) in Wistar rats anesthetized with ethyl carbamate and pentobaribital sodium. We found that the injection of AP12 into the VMS induced pressor and tachycardiac responses. With stepwise increases in the dose (1nmol/kg. 5 nmol/kg. 10nmol/kg), the pressor and tachycardiac responses increased dose-dependently. The present results suggest that apelin peptides may play some roles in central cardiovascular regulation.

Key words : Apelin-12, Ventral medullary surface, Rostral ventrolateral medulla, Pressor response, Tachycardiac response.

INTRODUCTION

Apelin is a peptide that was recently isolated from bovine stomach extracts and demonstrated to be an endogenous ligand for the human orphan receptor APJ [1]. Several investigators have started to study the physiological functions of apelin peptide. Zou et al. reported that apelin peptides block the entry of human immunodeficiency virus (HIV)[2]. Lee et al. reported that intravenous injection of an apelin peptide homologue in rats elicited depressor response, while intra-peritoneal injection of the apelin peptide homologue induced an increase in drinking behavior[3]. More recently Tatemoto et al. reported that apelin-like immunoreactivity was detected in endotheria of small arteries in various organs, and that the intravenous (i.v.) injection of apelin peptides induced depressor response[4]. They suggested that the depressor response to i.v. injection of apelin peptides might be induced via NO production by the action of apelin to NO synthase[4]. Takayama et al. reported that the i.v. injection of apelin-12 (AP12, one of apelin peptide homologues) induced much greater depressor response in spontaneously hypertensive rats (SHR) than in normotensive Wistar Kyoto rats (WKY)[5]. So far, however, effects of apelin peptides on the central nervous system have not been examined. Very recently we found that the ventral medullary surface (VMS) are immunoreactive for apelin-like peptides (unpublished results). These urged us to examine the cardiovascular responses to the injection of AP12 into VMS. We found that the injection of AP12 into the VMS induced tachycardiac and pressor responses, and it was suggested that the apelin peptides may play some roles in the central regulation of circulation.

MATERIALS AND METHODS

Experiments were done on 8-10-week old male Wistar rats (250-280g). The rats were anesthetized with ethyl carbamate (Wako Pure Chemicals: initially 720mg/kg, intraperitoneally (i.p.); thereafter 60 mg/kg, i.p. every hour) and pentobarbital sodium (Abbott: 30mg/kg, i.p.; thereafter 5mg/kg, i.p. every

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hour). The trachea was cannulated with vinyl tubing (2 mm in internal diameter). Femoral artery and vein were cannulated with polyethylene catheters (0.58 mm, internal diameter). Systemic arterial pressure (SAP), mean arterial pressure (MAP) was measured with a pressure transducer (TP-400T, Nihon Kohden, Japan) connected to carrier amplifier (AP-601G, Nihon Kohden, Japan), and heart rate (HR) computed from pulse pressure by a cardiotachometer (AT-601G, Nihon Kohden, Japan). SAP, MAP and HR were recorded on a thermal array recorder (RTA-1100M, Nihon Kohden, Japan). Rectal temperature was maintained at 37℃ with an infrared heat lamp. The head of the rats was placed in a stereotaxic frame flexed at 10°. The occipital bone was removed, and part of the floor of the fourth ventricle was exposed and covered with 0.9% NaCl solution containing 1.0% agar to prevent evaporative cooling of the brain surface. A 10-μl Hamilton microsyringe was filled with freshly prepared AP12 solution in 0.9% NaCl solution. The tip of the Hamilton syringe needle was placed on the dorsal surface of the medulla, 1.0 mm rostral to the obex. Then the tip was advanced 3.4-4.0 mm ventrally to the VMS, and a volume of 1.0μl solution of AP12 was injected over a period of 12s. Under these conditions, AP12 injected was confirmed to diffuse upon the VMS[6]. AP12 was also administered into the femoral vein through the cathter.

RESULTS

Fig. 1 shows an example of the cardiovascular responses elicited after microinjection of AP12 into the VMS. With stepwise increases in the dose, the pressor and tachycardiac responses increased dose-dependently. This certified that the neurons responsible for the cardiovascular responses were not desensitized by the repetitive injections of the peptide. As shown in Fig. 1A, the threshold of the cardiovascular responses was as low as 1 nmol/kg. The time courses of pressor response to AP12

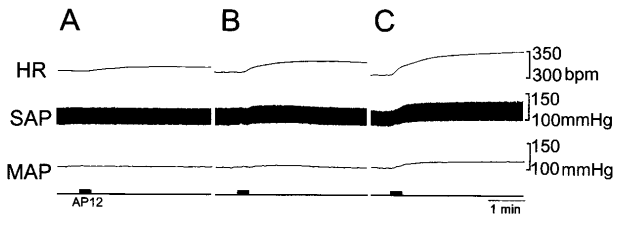

Fig. 1. Cardiovascular responses to increasing doses of apelin-12 (AP12) injected into the ventral medullary surface of Wistar rat. Abbreviations: bpm, beats per minutes; HR, heart rate; MAP; mean arterial pressure; SAP, systemic arterial pressure. Dose of AP12: A, 1 nmol/kg; B. 5 nmol/kg; C, 10 nmol/kg.

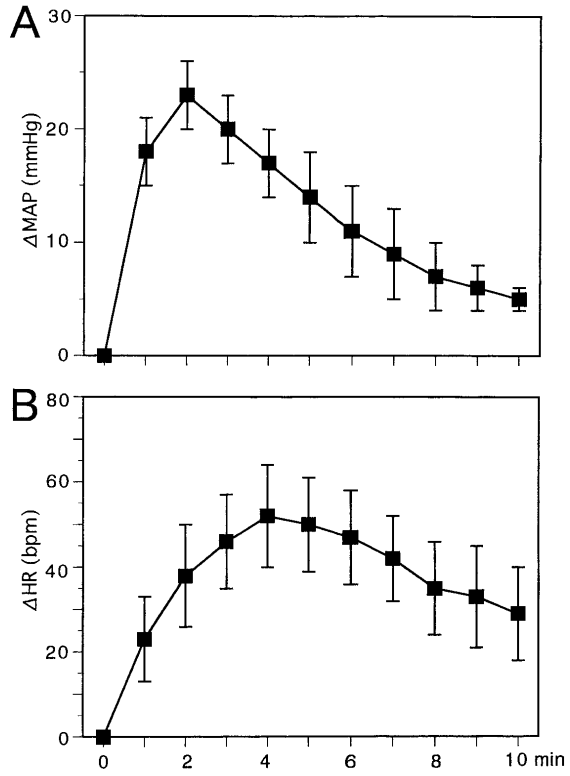

Fig. 2. Time courses of cardiovascular responses elicited by the injection of AP12 (10 nmol/kg) into the ventral medullary surface of Wistar rat. Abbreviations are described in legend of Fig. 1.

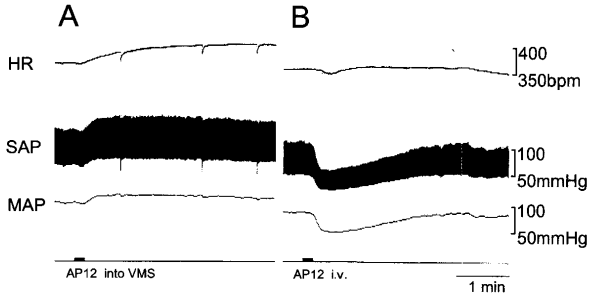

Fig. 3. Differences in cardiovascular responses to injection of AP12 into ventral medullary surface (A) and into femoral vein (B). Dose of AP12: 10 nmol/kg. Abbreviations: VMS: ventral medullary surface; other abbreviations are described in legend of Fig. 1.

injection into VMS were shown in Fig. 2. Maximal increase in MAP was 23±7mmHg (n=5) at 2 min after the injection of AP12, and the maximal increase in heart rate was 52±12 bpm at 4 min after the injection of AP12. The increased arterial pressure returned to the normal level within 5-10 min after the injection of AP12, whereas the increased heart rate was continued as long as 30 min after AP12 injection.

Recently, we reported that the intravenous injection of AP12 in rats elicited depressor responses via the mechanism of nitric oxide involvement[4]. In this study we found that the injection of AP12 into the VMS elicited pressor response along with tachycardia. Fig. 3 shows diametrically opposite responses to AP12 injection into the VMS and peripheral vein. Fig. 3A shows the pressor and tachycardiac responses to AP12 injection into the VMS, whereas Fig.3B shows the depressor response elicited by i.v. injection of AP12, without any change in heart rate,.

DISCUSSION

It has been well known that the central cardiovascular regulatory neurons are located in the rostral ventrolateral medulla (RVLM) closely situated to the VMS[7]. The pressor- and tachycardiac-response neurons are located in the rostral ventolateral medulla[8]. In this study we found that AP12 injected into the VMS induced pressor and tachycardiac responses. The present results implicated that there must be apelin peptide receptors in the neurons along the VMS which may project to the cardiovascular center neurons in the RVLM that, when activated, may initiate the stimulation of pressor- and tachycaridiac-neurons in the RVLM. Another implication is that there must be apelin peptide receptors in the medulla oblongata which are accessible from the VMS and which, when activated, initiate the stimulation of pressor- and tachycaridiac-neurons in the RVLM.

It must be noted that the pressor and tachycardiac responses to central injection of AP12 was completely opposite to the cardiovascular responses to peripheral i.v. injection of AP12. It has been known that neuroactive peptides such as substance P[9], enkephalin[10], bradykinin[11,12], neurotensin[13], vasoactive intestinal polypeptides[14] and angiotensin II[12] are pressor agents when administered centrally to conscious rats. On the other hand, these peptides were also found to cause hypotension after peripheral administration. Peripheral vasodilatation and central action of vasoconstriction by these peptides indicate a complete compartmentalization of central VMS route versus peripheral i.v. route of administration. Since the effects are opposite, the possibility of leakage from central to peripheral is unlikely except at the highest dose level. Peptides with central pressor effects have been shown to actively increase sympathetic stimulation[15]. Thus, it is plausible that AP12 bears similar mechanism as such peptides to induce the pressor and tachycardiac responses, though detailed mechanism on the central action of AP12 is needed to be elucidated.

In conclusion, we suggest that AP12 may play some roles in central cardiovascular regulation.

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