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Research Report

Cardiovascular effects of adrenocorticotropin microinjections into the rostral ventrolateral medullary pressor area of the rat

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ABSTRACT

The presence of adrenocorticotropic hormone (ACTH)-immunoreactive cells and melanocortin (MC) receptors (MC4 and to a lesser extent MC3) has been demonstrated in the medullary reticular formation in the general area where rostral ventrolateral medullary pressor area (RVLM) is located. The importance of RVLM in the regulation of cardiovascular function is well established. Based on these reports, it was hypothesized that ACTH may play a role in the regulation of cardiovascular function. To test this hypothesis, experiments were carried out on artificially ventilated, adult male, urethane-anesthetized and unanesthetized mid-collicular decerebrate rats. The RVLM was identified by microinjections (100 nl) of L-glutamate (L-Glu). Microinjections (100 nl) of ACTH (0.5, 1 and 2 mmol/l) into the RVLM elicited increases in MAP and HR; tachycardic responses were relatively inconsistent. The effects of ACTH were blocked by SHU9119 and agouti-related protein (AGRP). SHU9119 (a synthetic compound) and AGRP (an endogenous peptide) are antagonists for MC4, and to a lesser extent MC3, receptors. The specificity of these antagonists for MC receptors was indicated by their lack of effect on L-Glu responses. Microinjection of ACTH into the RVLM increased the efferent discharge in the greater splanchnic nerve. It was concluded that (1) ACTH exerts excitatory effects on RVLM neurons resulting in pressor and tachycardic responses, (2) these responses were mediated via MC4 and to a lesser extent MC3 receptors in the RVLM, and (3) the pressor effects of ACTH were mediated via sympathetic activation. This is the first report showing central cardiovascular actions of ACTH.

1. Introduction

The presence of pro-opiomelanocortin (POMC) containing neurons has been demonstrated in the anterior pituitary, the arcuate nucleus of the hypothalamus and the nucleus tractus solitarius (NTS) (Bronstein et al., 1992; Gee et al., 1983; Khachaturian et al., 1984; O'Donohue and Dorsa, 1982; Pritchard et al., 2002). POMC is pleiotropic in nature, i.e., site-specific post-translational processing determines which peptide is liberated from it (Guy et al., 1982). Thus, POMC is processed to adrenocorticotropic hormone (ACTH; also known as adrenocorticotropin or corticotrophin) and to alpha-melanocyte stimulating hormone (MSH), respectively, in corticotropic cells of anterior pituitary and melanotropic cells of the intermediate lobe (O'Donohue and Dorsa, 1982). Immunohistochemical studies have shown the presence of ACTH-

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immunoreactive cells in the NTS (Joseph et al., 1983; Palkovits et al., 1987) and lateral reticular formation of the medulla in the general region encompassing the rostral ventrolateral medullary pressor area (RVLM) (Schwartzberg and Nakane, 1983). The importance of RVLM in the regulation of cardiovascular function is well established (Gordon, 1995; Madden and Sved, 2003; Sapru, 2002; Schreihofer and Guyenet, 2002; Willette et al., 1983). Electrophysiological and anatomic evidence shows that sympathoexcitatory neurons located in the RVLM send monosynaptic projections to the intermediolateral cell column (IML) of the thoracolumbar spinal cord (Brown and Guyenet, 1985; Guyenet et al., 1996). It is also well known that RVLM is important in mediating the baroreceptor, chemoreceptor and cardiopulmonary reflex responses (Gordon, 1995; Guyenet et al., 1996, Madden and Sved, 2003; Sapru, 2002; Schreihofer and Guyenet, 2002).

The effects of POMC-derived peptides are mediated through the G-protein-coupled melanocortin (MC) receptors. Five MC receptors (MC1, MC2, MC3, MC4 and MC5) have been identified (Adan and Gispen, 1997, 2000; Griffon et al., 1994). Of these five receptor subtypes, only MC4 and to a lesser extent MC3 receptors have been identified in the central nervous system. The presence of MC4 and MC3 receptors has also been demonstrated in the medullary reticular formation, the general area where RVLM is located (Adan and Gispen, 1997, 2000).

Based on the studies reporting the presence of ACTH-immunoreactive cells (Schwartzberg and Nakane, 1983) and MC receptors (Adan and Gispen, 1997, 2000) in the RVLM, it was hypothesized that ACTH may play a role in the regulation of cardiovascular function in stressful and/or pathological conditions. In this paper, cardiovascular effects of ACTH were investigated. There are no reports in the literature regarding the cardiovascular effects ACTH in the RVLM.

2. Results

2.1. *Concentration response of ACTH*

In this and other series of experiments, the RVLM was always identified by microinjections of L-glutamate (L-Glu, 5 mmol/l), which stimulates neurons but not fibers of passage. The ventral surface of the medulla was exposed, and the confluence of vertebral arteries was used as a reference point for the stereotaxic coordinates in order to identify the RVLM (see Section 4.1 in the Experimental procedures). In order to select appropriate volume of microinjections, ACTH (1 mmol/l) was microinjected into the same site of the RVLM in 100 and 50 nl volumes (n = 5). ACTH–induced increases in mean arterial pressure (MAP) were 32 ± 6.0 and 14 ± 3.3 mm Hg for 100 and 50 nl volumes, respectively ($P < 0.05$). Similarly, ACTH-induced increases in heart rate (HR) were 22 ± 4.9 and 10 ± 5.5 beats/min (bpm), for 100 and 50 nl volumes, respectively ($P < 0.05$). Since the blood pressure (BP) responses to ACTH were significantly greater when the volume of microinjection was 100 nl, this volume was selected for all other microinjections including those of L-Glu. Concentration response of ACTH was studied in the RVLM where microinjections of L-Glu (5 mmol/l) induced pressor (32 ± 4.6 mm Hg) and tachycardic (14 ± 4.0 bpm) responses. The interval between the microinjections of L-Glu and ACTH was at least 5 min. Microinjections of ACTH (0.5, 1, 2 mmol/l) into the RVLM (n = 15, 5 rats for each concentration) elicited increases in MAP (Fig. 1A) and HR (Fig. 1B). Tachycardic responses to ACTH were relatively inconsistent. Maximal pressor responses were elicited by a 1 mmol/l concentration. The onset and durations of the responses to microinjections of ACTH (1 mmol/l) were 5–20 s and 20–25 min, respectively, and the peak effect was observed at 2–3 min. The pressor responses to 1 mmol/l ACTH were significantly larger ($P < 0.05$) than those elicited by 0.5 mmol/l. In these experiments, only one concentration of ACTH was microinjected into the RVLM of each animal.

The interval between different microinjections of ACTH was 40 min in order to avoid tachyphylaxis. Absence of tachyphylaxis to repeated microinjections of ACTH was tested in another group of rats (n = 4). The concentration of ACTH that elicited maximal cardiovascular responses (1 mmol/l) was microinjected into the RVLM at least 3 times, at 40-min intervals. The increases in MAP in response to 3 consecutive microinjections of ACTH (1 mmol/l) were 25.0 ± 2.0, 32.5 ± 4.3 and 28.8 ± 4.3 mm Hg, respectively ($P > 0.05$). The increases in HR in response to these microinjections of ACTH were 10.0 ± 4.1, 12.5 ± 2.5 and 15.0 ± 5.0 bpm, respectively ($P > 0.05$). Thus, no tachyphylaxis of responses was observed with repeated microinjections of ACTH.

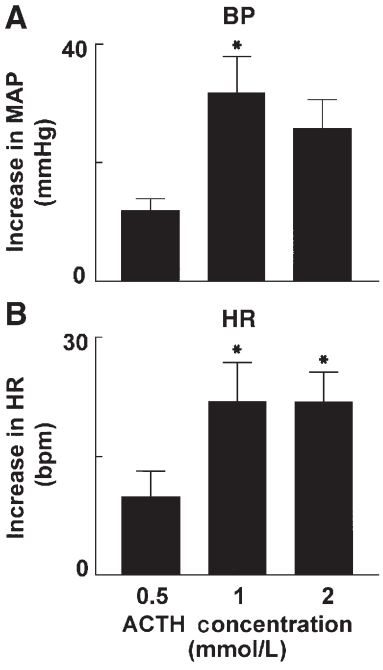

Fig. 1 – Concentration response of ACTH. (A) Microinjections of ACTH at 0.5, 1 and 2 mmol/l into the RVLM (n = 5 for each concentration) elicited increases in MAP: 12 ± 2.0, 32 ± 6.0 and 26 ± 4.8 mm Hg, respectively. (B) Microinjections of ACTH into the RVLM at the same concentrations elicited increase in HR: 10 ± 3.2, 22 ± 4.9 and 22 ± 3.7 bpm, respectively. Bars represent means ± SEM. The volume of all microinjections was 100 nl. *$P < 0.05$ compared to 0.5 mmol/l ACTH. ACTH: adrenocorticotropic hormone; BP: blood pressure; HR: heart rate; MAP: mean arterial pressure.