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Research Report

Role of angiotensin II type 1 receptors in the subfornical organ in the pressor responses to central sodium in rats ☆

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ABSTRACT

Central infusion of Na^+-rich artificial cerebro-spinal fluid (aCSF) activates the brain renin–angiotensin system and causes sympatho-excitatory and pressor responses. We evaluated the role of the subfornical organ (SFO) and angiotensin II type 1 (AT_1) receptors in the SFO in mediating the central Na^+-induced pressor response. In conscious Wistar rats, intra SFO infusions of Na^+-rich aCSF containing 0.45 and 0.6 M Na^+ at 10 nl/min or injection of angiotensin II (Ang II) at 80 ng increased blood pressure (BP) by 15–22 mmHg, whereas mannitol with the same osmolarity as the Na^+-rich aCSF had no effects. Intra SFO infusion of the AT_1 receptor blocker candesartan abolished the pressor response induced by intra SFO administration of Na^+-rich aCSF or Ang II. Intra cerebro-ventricular (icv) infusion of Na^+-rich aCSF (0.3 M Na^+) at 3.8 μl/min for 10 min increased BP by 15–20 mmHg. Electrolytic lesion of the SFO attenuated these BP increases by 50–70%. Intra SFO infusion of candesartan also prevented 50% of these pressor responses. These data suggest that SFO neurons are indeed sensitive to Na^+, the SFO is a major – but not only – site in the brain to sense an increase in CSF [Na^+], and activation of AT_1 receptors in the SFO mediates the SFO component of the Na^+-induced pressor response.

1. Introduction

Neural mechanisms play a major role in the development of salt-induced hypertension. In Dahl salt-sensitive (S) rats, Na^+-entrance from blood into the cerebro-spinal fluid (CSF) is enhanced (Amin et al., 2009) and high salt intake increases CSF [Na^+] (Huang et al., 2004; Nakamura and Cowley, 1989). In normotensive rats, intra cerebro-ventricular (icv) infusion of Na^+-rich artificial (a) CSF causes sympatho-excitation and hypertension, whereas icv infusion of equi-osmotic solutions of e.g. sucrose or mannitol does not (Andersson et al., 1972; Buñag and Miyajima, 1984; Huang and Leenen, 1996; Huang et al., 2004). These findings suggest that an increase in the CSF [Na^+] may activate Na^+-sensitive neurons and thereby

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elicits sympatho-excitatory and pressor responses. The salt-induced sympatho-excitation and hypertension in Dahl S on high salt or Wistar rats with icv infusion of Na^+-rich aCSF can be prevented by icv infusion of an angiotensin II type 1 (AT_1) receptor blocker (Huang and Leenen, 1996, 1998; Huang et al., 1998; Rohmeiss et al., 1995b) indicating that AT_1 receptor stimulation in the central nervous system (CNS) mediates the CSF Na^+-induced increase in blood pressure (BP). Ang II containing nerve fibers and terminals are found throughout the brain, in areas with and without a blood–brain barrier (Oldfield et al., 1989). AT_1 receptors are also distributed widely in the CNS (Lenkei et al., 1998; Mendelsohn et al., 1984), in the forebrain especially in the subfornical organ (SFO), organum vasculosum lamina terminalis (OVLT), median preoptic nucleus (MnPO) and paraventricular nucleus (PVN).

The SFO is one of the circumventricular organs which lack the blood brain barrier and is exposed to both blood and CSF. Electrolytic lesions of the lamina terminalis including the SFO, MnPO and OVLT fully block pressor responses to icv infusion of hypertonic saline (May et al., 2000). Intra SFO injection of losartan largely prevents pressor responses to icv injection of hypertonic saline (Rohmeiss et al., 1995a). However, an intra SFO injection of 1 μl of 0.2 M NaCl did not increase BP (Camargo et al., 1984). Effects of infusions and/or higher [Na^+] were not studied. We hypothesized that SFO neurons are Na^+ sensitive and Na^+ induced local angiotensin II (Ang II) release (Doris, 1988; Qadri et al.,1994) and AT_1 receptor activation causes pressor responses.

In the present study, we tested in conscious Wistar rats (1) the BP and heart rate (HR) responses to intra SFO infusion of Na^+-rich aCSF and injection of Ang II with and without intra SFO infusion of the AT_1 receptor blocker candesartan; (2) the BP and HR responses to intra SFO infusion of mannitol with the same osmolarity as the Na^+-rich aCSF; (3) effects of electrolytic lesion of the SFO on the BP and HR responses to icv infusion and injection of Na^+-rich aCSF and of Ang II; and (4) the BP and HR responses to icv infusion and injection of Na^+-rich aCSF or Ang II before and after intra SFO infusion of candesartan.

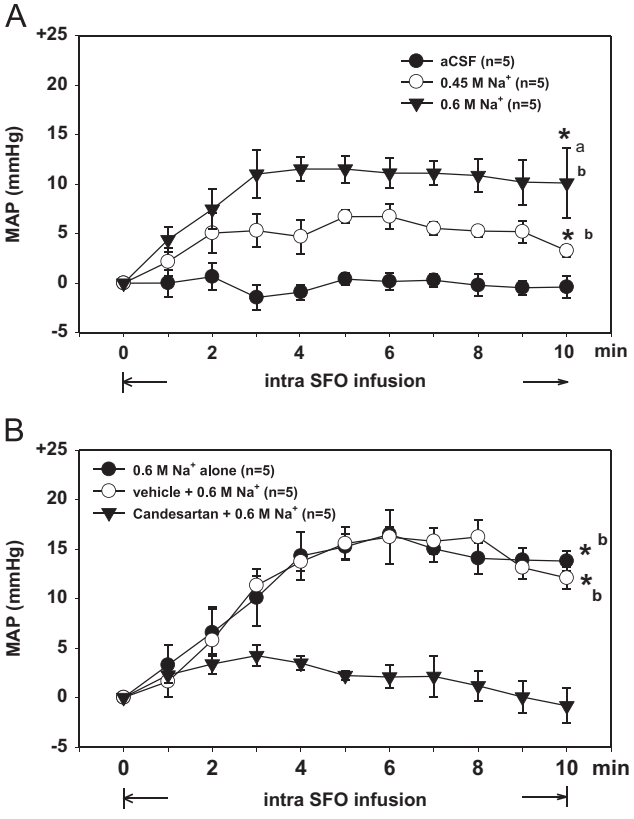

Fig. 1 – (A) Changes in MAP by intra SFO infusion of aCSF or Na^+-rich aCSF containing 0.45 or 0.6 M Na^+ ($n=5$/group). (B) Changes in MAP by intra SFO infusion of Na^+-rich aCSF containing 0.6 M Na^+ before or after intra SFO infusion of candesartan, or vehicle ($n=5$/group). Values are means ± SE, analyzed by one-way ANOVA for areas under the curve, and one way repeated-measures ANOVA. *$p<0.05$, vs. aCSF in panel A or candesartan+0.6 M Na^+ in panel B. a: $p<0.05$, vs 0.45 M Na^+ in panel A. b: $p<0.05$, vs baseline.

2. Results

2.1. AT_1 receptor blockade and responses to Na^+-rich aCSF in the SFO

Intra SFO infusion of aCSF or of Na^+-rich aCSF containing 0.3 M Na^+ at 10 nl/min did not increase BP. Na^+-rich aCSF containing 0.45 and 0.6 M Na^+ at 10 nl/min increased BP gradually, and BP reached a plateau within 3 min with maximal increases of 5±1 and 11±2 mmHg, respectively (Fig. 1A). HR did not change significantly (data not shown). No behavioral changes were observed during these infusions. Infusion of Na^+-rich aCSF with 0.6 M Na^+ at 10 nl/min outside the SFO ($n=5$), or into the third ventricle ($n=6$) did not change BP significantly (maximal response: +3±2 or −1±1 mmHg, respectively, NS vs baseline for both). Intra SFO infusion of 0.85 M mannitol did not change BP (98±7 vs 99±7 mmHg at baseline, NS), while subsequent infusion of Ang II at 80 ng increased BP by 17±3 mmHg ($p<0.05$, vs baseline).

Infusion of candesartan or vehicle did not alter BP (data not shown). In rats with or without intra SFO vehicle, Ang II at 80 ng injected in the SFO increased MAP similarly by 22±1 or 21±3 mmHg. Intra SFO vehicle did not affect responses to intra SFO infusion of aCSF with 0.6 M Na^+ as compared to 0.6 M Na^+ alone (Fig. 1B). In contrast, pressor responses to aCSF with 0.6 M Na^+ were abolished by candesartan (Fig. 1B), and pressor responses to Ang II were fully blocked (+0±3 vs 22±1 or 21±3 mmHg of control, $p<0.05$ for both).

2.2. Electrolytic lesion of the SFO and responses to icv Na^+-rich aCSF and Ang II

There were no significant differences in body weight and baseline BP and HR in rats with a SFO lesion versus sham lesion.

Icv infusion of Na^+-rich aCSF with 0.3 M Na^+ at 3.8 μl/min gradually increased BP with a maximal increase of 27±2 mmHg at 4–5 min in rats with sham lesion (Fig. 2A). The extent of the pressor responses in rats with the SFO lesion was significantly smaller, with a maximal increase of 14±2 mmHg ($p<0.05$, versus sham). Icv injection of Na^+-rich aCSF increased BP rapidly to maximal increase within 1 min and the increase subsided soon after the injection. The maximal increase in BP was markedly attenuated by 72% in