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EFFECT OF SODIUM ION ON ATRIAL NATRIURETIC FACTOR RELEASE FROM RAT HYPOTHALAMIC FRAGMENTS

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Summary

The effects of Na ion and choline choride on the release of atrial natriuretic factor (ANF) and growth hormone-releasing factor (GHRF) from rat hypothalamic fragments including the organum vasculosum of the lamina terminalis (OVLT) were examined in vitro. Although the release of ANF was stimulated by Na ion, choline chloride, and glucose in concentration-dependent manners, the release was more sensitive to a change in concentration of Na ion than to those of choline chloride and glucose. On the other hand, the change in Na ion concentration did not affect the release of GHRF. It can be therefore proposed that Na ion is the first candidate controlling ANF release from the brain tissue and that ANF in the hypothalamus and/or OVLT may play some role in the regulation of the Na ion and water balance in the central nervous system.

ANFs were first isolated from human and rat atrial extracts, and their amino acid sequences have been characterized (1-4). Several recent studies have revealed that ANF-positive neurons and fibers are widely distributed in the brain, such as in the pons, hypothalamus, preoptic area and OVLT (5-9). This distribution pattern of ANF in the brain indicates that ANF plays some different roles as neurotransmitter or neuromodulater in the central nervous system. We and others have recently found that ANF is releasable from the rat hypothalamus in vitro (10,11). These results suggest that ANF may play some role in the control of pituitary hormone secretion. However, the action of ANF on the pituitary is still controversial. Concerning the effect of ANF on the secretion of proopiomelanocortin (POMC)-derived peptides from the pituitary, we reported that ANF suppressed the basal and corticotropin-releasing factor-induced secretion of POMC-derived peptides from the rat pituitary in vitro (12). Conversely, other groups found no effect of ANF on adrenocorticotropin (ACTH) secretion (13,14). In addition, the stimulatory effects of ANF on the secretion of ACTH from the rat pituitary (15) and α-melanocyte stimulating hormone from neurointermediate lobes of the frog pituitary were reported (16).

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Further, several lines of evidence such as the inhibitory effects of ANF on vasopressin release (17-19) and drinking (20,21), in addition to its presence in the OVLT (8), have suggested that ANF in the brain may be involved in the regulation of fluid and electrolyte balance, because the OVLT is known to possess osmoreceptors (22). This study was therefore designed to reveal the effect of Na ion on the release of ANF from hypothalamic fragments including the OVLT in the rat.

MATERIALS AND METHODS

Incubation of rat hypothalamic fragments

The brains were obtained from male Wistar rats, weighing approximately 200 to 250 g, after decapitation and placed in a dish kept on ice. Hypothalamic fragments were immediately dissected out from an area 1.5 mm lateral to the midline at the anterior border of the optic chiasma anteriorly and the anterior border of the mammillary bodies posterioly, and the depth of the fragments was approximately 2.5 mm. Each hypothalamic fragment was transferred into a well containing 1.0 ml of cold Krebs Ringer bicarbonate buffer (120 mM NaCl, 24 mM $NaHCO_3$, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4 and 1.2 mM $MgSO_4$ 7 H_2O) supplemented with 0.2 % glucose and 0.1 % bovine serum albumin (BSA) (KRBGA), and the wells were kept on ice until all hypothalamic fragments had been dissected out. The wells were then placed in a water bath saturated with an atmosphere of 5 % CO_2 and 95 % oxygen at 37 °C, and the fragments were incubated for 60 min. After 60 min of the preincubation, each hypothalamic fragment was gently transferred into another well containing 1.0 ml of KRBGA composed of 50 μM bacitracin and different concentrations of Na ion such as 124, 134, 144, 154, 164 and 174 mM Na ion and incubated for 60 min. The concentrations of Na ion were changed by decreasing or increasing NaCl concentration in KRBGA containing 144 mM Na ion. In another experiment, hypothalamic fragments were incubated in 1.0 ml of KRBGA supplemented with 10, 20 or 30 mM pharmacologically inactive choline chloride and 50 μM bacitracin. They were also incubated in 1.0 ml of KRBGA supplemented with 360, 720 or 1080 mg/dl glucose and 50 μM bacitracin. The effect of incubation temperature on ANF release from the hypothalamus was examined. After 60 min of the preincubation, hypothalamic fragments were incubated in 1.0 ml of KRBGA containing 144 mM or 164 mM Na ion at 4°C, 25°C or 37°C for 60 min. In order to test the effect of incubation time on ANF release in another experiment, hypothalamic fragments were incubated with 1.0 ml of KRBGA containing 144 mM or 164 mM Na ion at 37°C for 20, 40 or 60 min. After the incubation, the fragment was removed from each well and 50 μl of 0.1 M phosphate buffer saline supplemented with 0.5 % Tween 20, 6 % monoethanolamine and 1 % BSA was added into each well to improve recovery of ANF in the media. The medium was then aspirated off and centrifuged at 3000 rpm at 4 °C for 30 min. The supernatant was frozen at -20 °C until ANF and GHRF determination.

Radioimmunoassays for ANF and GHRF

The amounts of ANF and GHRF in media were determined by their respective specific radioimmunoassay as described previously (23,24).

Statistical analysis

The data were subjected to analysis of variance and multiple comparisons were performed by Duncan's new multiple range test. The number of wells ranged from 7 to 27.

RESULTS

The amounts of ANF and GHRF released from one hypothalamic fragment into control KRBGA containing 144 mM Na ion for 60 min of incubation were 113.5 ± 3.4 pg/ml and 128.1 ± 10.7 pg/ml, respectively. The amounts of ANF released from hypothalamic fragments incubated in 124, 134, 154, 164 and 174 mM Na ion-containing KRBGA were 96.4 ± 7.4 %, 96.7 ± 7.9 %, 134.6 ± 13.8 %, 153.7 ± 11.0 % and 197.5 ± 19.8 % that of the control, respectively, as shown in Figure 1. On the other hand, the release of GHRF was not influenced by Na ion concentration as shown in Figure 1.

Figure 2 shows the effect of osmotic pressure on the release of ANF from the hypothalamic fragments. The release of ANF was stimulated by the rise of osmotic pressure due to an increase in choline chloride concentration at the osmotic pressure of 355 mOsm/kg. The release of ANF was also increased by the rise of osmotic pressure due to an increase in glucose concentrations. However, the amount of ANF released in response to a rise of osmotic pressure due to an increase in Na ion was significantly ($P < 0.01$) greater than that induced by a rise of osmotic pressure due to an increase in choline chloride or glucose concentrations.

The effect of incubation temperature on ANF release was shown in Figure 3. The amount of ANF released was larger in the incubation at 25°C and 37°C than that in the incubation at 4°C. Further, the amount of ANF released into the medium containing 164 mM Na ion was significantly larger than that into the 144 mM Na ion-containing KRBGA at 25°C and 37°C whereas no significant difference of ANF release was found between the incubation in 144 mM Na ion-containing KRBGA and the incubation in 164 mM Na ion-containing KRBGA at 4°C.

Figure 4 shows the effect of incubation time on ANF release. The amount of ANF released increased in a time-dependent manner. The release of ANF from the hypothalamic fragments incubated in 164 mM Na ion-containing KRBGA was significantly greater than that from the hypothalamic fragments incubated in 144 mM Na ion-containing KRBGA for 40 min and 60 min of incubation whereas no difference of ANF release was found between the incubation in 144 mM Na ion-containing KRBGA and the incubation in 164 mM Na ion-containing KRBGA for 20 min of incubation.

DISCUSSION

The present study has shown that the release of ANF is stimulated by Na ion, choline chloride and glucose and that the release is much more sensitive to the change in Na ion concentration than to those in choline chloride and glucose. The results indicate that Na ion itself rather than osmotic pressure plays a greater role to stimulate the release of ANF and that the stimulatory effect of Na ion on ANF is temperature- and time-dependent. It has also been found that the release of GRF, one of the hypothalamic peptides, is not influenced by the change in Na ion concentrations, suggesting that the effect of Na ion is specific for the release of ANF and not for that of GRF. This result therefore excludes a possibility that ANF detected in the incubation media in response to high concentrations of Na ion might have leaked from ANF-neurons destroyed by high osmotic pressure.

The origin of ANF released from the hypothalamic fragments is unclear because the tissue fragments used in this study included the OVLT and preoptic area, in which ANF-positive neurons exist. ANF-positive neurons are widely distributed in various areas, such as the OVLT, preoptic area, hypothalamus and pons in the brain (5-9). Among such ANF-positive neurons, those in the OVLT

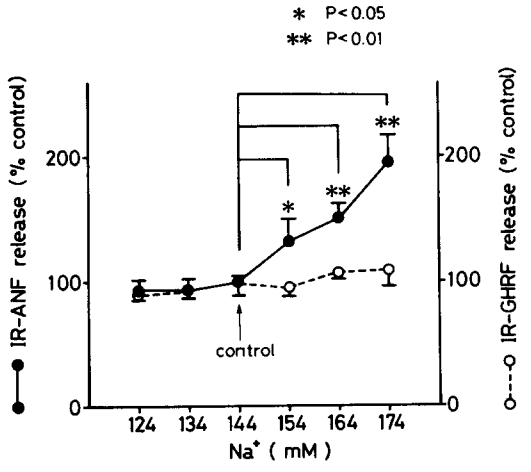

Figure 1. Effect of Na ion on ANF and GHRF release from rat hypothalamic fragments. The results are expressed as % of the controls (the amounts of ANF and GHRF released into KRBGA containing 144 mM Na ion). The number of wells in each group varied from 10 to 27.

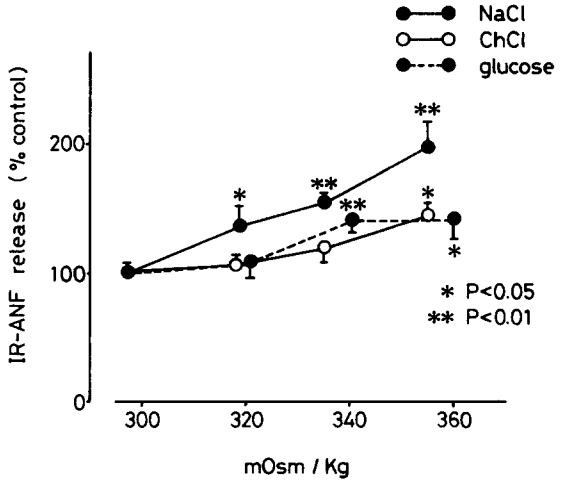

Figure 2. Effect of osmotic pressure on ANF release. The results are expressed as % of the control (the amount of ANF released into KRBGA containing 144 mM Na ion). The number of wells in each group varied from 7 to 14.

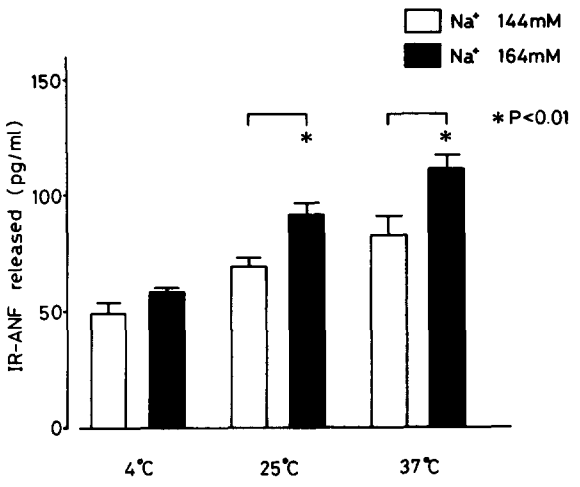

Figure 3. The effect of incubation temperature on the release of ANF from the hypothalamic fragments incubated in 144 mM and 164 mM Na ion-containing media. The number of wells in each group was 7.

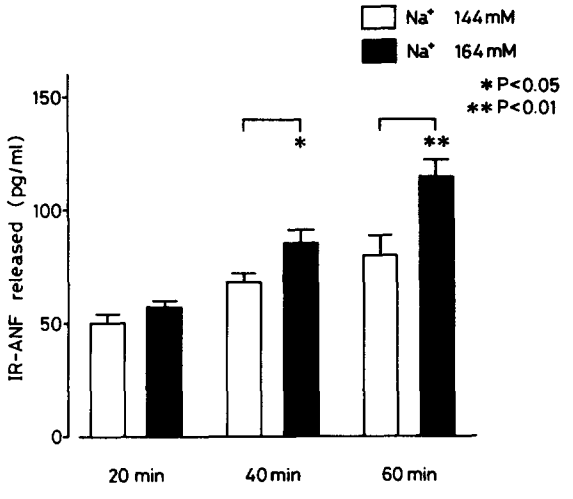

Figure 4. The effect of incubation time on the release of ANF from the hypothalamic fragments incubated in 144 mM and 164 mM Na ion-containing media. The number of wells in each group was 7.

are of special interest as to the physiological significance of ANF, because the OVLT is considered to contain osmoreceptors from findings that lesions in this area suppress osmotic stimulation-induced drinking and induce significant plasma osmolarity elevation in dogs (22). Drinking behavior and vasopressin release are induced by an increase in plasma osmolarity while ANF release from the brain is stimulated by an increase in Na ion concentration as found in this study. ANF suppresses dehydration- and hemorrhage-induced vasopressin secretion (17-19). Furthermore, intracerebroventricular administration of ANF inhibits dehydration- and angiotensin II-induced water drinking in the rat (20,21). Thus these reports combined with the present results suggest that ANF in the brain may function as a neurotransmitter or neuromodulator in the counterregulatory system that controls vasopressin release and water drinking when plasma osmotic pressure is elevated.

ANF acts on the kidney to increase urinary water and sodium excretion (25), and to suppress plasma renin activity (26-28), and on the adrenal to inhibit aldosterone secretion (29-31). The most probable site from which ANF is released to act on these tissues is the atrium. ANF release from the atria into the circulating blood is stimulated by elevation of the right atrial pressure and volume (32,32), and Na loading (34). It has recently been reported that the sensitivity of the atria to a change in the concentration of Na ion in terms of ANF release is markedly greater than to that of choline chloride (35,36). This phenomenon of the release of one peptide, that exists in both the central nervous system and peripheral tissue being controlled by the same stimuli was also found in the case of somatostatin release from the hypothalamus and pancreas, since the release of the peptide from the two tissues is increased by the same stimuli, such as growth hormone (37,38) and free fatty acids (39,40).

It is unlikely that ANF released from the hypothalamus and/or OVLT plays some role in the peripheral tissues after entering into the systemic circulation, since the content of ANF in the hypothalamus is extremely low compared with that in the atria (5). ANF released from the hypothalamus and/or OVLT is therefore thought to act in the brain. The physiological significance of ANF in the brain is not clear at present. However, it is suggested from the results of this study and other reports mentioned above that ANF-neurons in the brain and ANF-producing atrial cells possess similar receptors, and that the peptide is presumably involved in the control mechanism for water and electrolyte balance in both the brain and the peripheral tissues.

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