Role of Brain Ouabainlike Compound in Central Nervous System–Mediated Natriuresis in Rats

Kaoru Yamada, Atsuo Goto, Hiroshi Nagoshi, Chen Hui, Masao Omata

Abstract Intracerebroventricular infusion of artificial sodium-rich cerebrospinal fluid induces increases in blood pressure and urinary sodium excretion. To examine the role of brain ouabainlike compound in these central nervous system–mediated responses, we evaluated the effects of prior intracerebroventricular injection of the Fab fragments of digoxin-specific antibody (Digibind, 10 mg/mL, 10 μL) on changes in blood pressure and urinary sodium excretion after intracerebroventricular infusion of high-sodium (323 mmol/L, 150 μL/kg per 15 minutes) cerebrospinal fluid in anesthetized rats. Antiouabain action of Digibind was revealed by the inhibition of a contractile response to ouabain in guinea pig aorta. Similar significant increases in blood pressure were found in rats that received preinjection of Digibind (n=14) compared with control rats that received injection of saline (n=5) or normal sheep IgG (n=8). In rats pretreated with Digibind the natriuretic responses to central high sodium were significantly diminished by 68% (P<.05) or 82% (P<.05) compared with rats treated with saline or normal IgG, respectively. In contrast, Digibind did not affect either pressor or natriuretic responses to intracerebroventricular angiotensin II (600 ng/30 μL per 10 minutes). These data indicate that Digibind significantly inhibits increases in renal sodium excretion in response to high central sodium and suggest that brain ouabainlike compound may be involved in central nervous system–mediated natriuresis with nonpressor mechanisms. (Hypertension. 1994;23[part 2]:1027-1031.)

Key Words • ouabain • natriuresis • central nervous system

Infusion of hypertonic sodium chloride (NaCl) solution into the brain ventricle induces in most animal species various cardiovascular and renal responses, including increases in arterial blood pressure (BP) and urinary sodium excretion (UN.V).1,4 The stimulus for these responses may be sodium rather than hyperosmolality because other hypertonic solutions, eg, mannitol, do not produce comparable responses. Presumably, specific sodium-sensitive receptors, which primarily are influenced by the sodium concentration of the cerebrospinal fluid (CSF), are located in close proximity to the third brain ventricle and mediate the effects of central high NaCl.8 However, it is still unclear how periventricular sodium receptors are being activated and/or regulated.

Recently, Huang and coworkers6 have focused on the roles of ouabainlike compound (OLC) in the central nervous system (CNS) and provided evidence that brain OLC may participate in the sympathoexcitatory and pressor effects of intracerebroventricular (ICV) hypertonic NaCl. Actually, OLC exhibiting Na^+,K^+-ATPase inhibitory activity has been extracted and partially purified from the mammalian brain, hypothalamus, and CSF.7,9 Furthermore, the presence of OLC has been demonstrated by immunohistochemical methods in the hypothalamus of rat and macaque.11 We undertook the present study to determine whether brain OLC is involved in the natriuretic response to increases in central sodium. For this, we evaluated the effects of ICV preinjection of Fab fragments of anti-digoxin antibody (Digibind) on the changes in urine volume (UV) and urinary electrolyte excretion after ICV infusion of artificial CSF with a high sodium concentration (H-CSF) in anesthetized rats.

Methods Antiouabain Action of Digibind

The effect of Digibind on the contractile response to ouabain was evaluated using thoracic aorta from male guinea pigs (350 g).12 All procedures were in accordance with the guidelines of the Animal Experiment Committee of the University of Tokyo. The connective tissue was removed and the vessel cut into ring preparations 3 mm long. Preparations were suspended in 30-mL jacketed organ baths containing 15 mL Krebs-Henseleit solution kept at 37°C and bubbled with a mixture of 5% CO₂ and 95% O₂. Changes in isometric tension were recorded by means of a force transducer connected to a polygraph. During a stabilizing period of 1 hour the basal tension was adjusted to 1 g. Then repeated contractions were produced by 50 mmol/L KCl until the responses were reproducible. Digibind (20 mg/mL) dissolved in physiological saline solution (PSS, 153 mmol/L NaCl) or PSS alone was added to the bath, and the contraction to 100 or 250 nmol ouabain was examined 30 minutes later.

Effects of Intracerebroventricular Digibind on CNS-Mediated Responses

Experiment 1

Male Wistar rats weighing 250 to 280 g were maintained on normal rat chow and tap water and were used for all experiments. PE-50 polyethylene catheters were introduced with rats under pentobarbital anesthesia (30 mg/kg IP) into the carotid artery for monitoring of arterial BP. The bladder was cathe-

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terized (PE-50) for urine sampling. A skin incision was made to expose the upper part of the skull, and a 1-mm hole was drilled through the left parietal bone, 0.5 mm posterior to the bregma and 1.5 mm lateral to the mid sagittal suture. A stainless steel cannula was introduced stereotaxically into the left lateral ventricle (4.5 mm below the skull surface) using a stereotaxic apparatus (Takahashi Inc). Direct arterial BP was monitored throughout the experiment. After it was confirmed that rats were stable, urine collection was started. Urine was collected during six consecutive periods (U1 through U6, corresponding to periods 1 through 6, 15 minutes each) from 30 minutes before (periods 1 and 2) the ICV injection of Digibind or saline. Data from periods 1 and 2 served as basal values. Rats were divided into two groups. In the Digibind group (n=5), 10 µL of Digibind solution (10 mg/mL in PSS) was injected in 10 seconds into the left lateral brain ventricle at the beginning of period 3. In the control group (n=5), 10 µL of PSS was injected. Fifteen minutes after the injection, ICV infusion of H-CSF was performed through the cannula over 15 minutes (period 4) at a rate of 150 µL/kg per 15 minutes. H-CSF was composed of (mmol/L) NaCl 323, KCl 3, CaCl2 1.3, MgCl2 0.8, NaHCO3 25, and Na2HPO4 1 as well as 2.5 g/L glucose, pH 7.4. Urine collection was continued until 30 minutes after the end of H-CSF infusion (periods 5 and 6). Urine volume was measured gravimetrically. Urinary sodium and potassium concentrations were determined by flame photometry.

**Experiment 2**

In the second experiment the control group (n=8) received ICV injection of normal sheep IgG (10 mg/mL in PSS, Sigma Chemical Co) instead of PSS. Another group of rats (n=9) received ICV injection of the same amount of Digibind as in experiment 1. Otherwise, the experimental protocol was the same as in experiment 1.

**Experiment 3**

In the third experiment ICV infusion of angiotensin II (Ang II) instead of H-CSF was performed at a rate of 600 ng/30 µL per 10 minutes during period 3. The effects of ICV injection of Digibind (n=7) on the cardiovascular and renal responses induced by ICV infusion of Ang II were similarly examined. The control group (n=7) received ICV injection of normal sheep IgG.

**Statistical Analysis**

The significance of differences in mean values within and between groups was determined with ANOVA for repeated measures followed by Duncan’s multiple range test. Values are given as mean±SEM; a value of P<.05 was taken as significant.

**Results**

In control preparations 100 or 250 nmol ouabain caused a gradual contraction of guinea pig aorta (Fig 1). The contractile response to 100 or 250 nmol ouabain was totally or partially diminished in vessel preparations pretreated with Digibind, respectively.

In all experiments, BP, UV, UN,V, and urinary potassium excretion (Un,V) remained unaltered during 15 minutes (period 3) after ICV administration of saline, normal sheep IgG, or Digibind compared with periods 1 and 2. BP reached a maximum value 2 to 3 minutes after the start of H-CSF infusion, and a significant rise in BP still persisted 15 minutes after the end of infusion. In experiment 1, H-CSF significantly increased BP from 113±2 to 126±2 mm Hg (maximum response) in the saline group and from 105±5 to 117±6 mm Hg in the Digibind group (P<.05 for both). In experiment 2, BP increased from 110±4 to 120±4 mm Hg in the IgG group and from 109±2 to 118±7 mm Hg in the Digibind group after ICV infusion of H-CSF (P<.01 for both). Thus, the magnitude of the pressor response was not different between the Digibind group and control groups.

Figs 2 and 3 show the effects of Digibind pretreatment on UV, Un,V, and Ur,V induced by ICV infusion of H-CSF. UV increased significantly from 72.6±8.8 ([U1+U2]/2) to 184.9±38.1 (U5, P<.05) µL per 15 minutes and from 86.9±11.0 to 214.3±48.1 µL per 15 minutes (P<.05) in the saline and normal IgG groups, respectively. The CNS-induced increases in UV of the Digibind group were from 74.4±7.5 to 113.9±21.0 µL per 15 minutes in experiment 1 and from 111.0±23.3 to 135.9±12.8 µL per 15 minutes in experiment 2 and were significantly inhibited in the Digibind group compared with each control group (Figs 2 and 3, P<.05 for both).

Un,V also increased significantly, from 4.1±1.6 to 36.4±3.7 µmol per 15 minutes (US, P<.05) and from 3.6±0.8 to 32.9±12.0 µmol per 15 minutes (US, P<.05) in the saline and IgG groups, respectively. ICV preinjection of Digibind significantly inhibited CNS-induced natriuresis by 68% (from 5.3±2.2 to 15.2±8.1 µmol per 15 minutes in experiment 1, P<.01) and by 82% (from 9.7±4.9 to 15.2±3.3 µmol per 15 minutes in experiment 2, P<.05) compared with the respective controls. Ur,V also increased after ICV infusion of H-CSF, but the kaliuretic response was not affected by Digibind pre-treatment (Fig 3).

In experiment 3, ICV Ang II infusion raised blood pressure from 116±5 to 131±5 mm Hg (maximum response) in the normal IgG group and from 114±5 to 125±5 mm Hg in the Digibind group (P<.01 for both). Thus, Digibind treatment did not affect the pressor response to Ang II. UV, Un,V, and Ur,V increased

**Fig 1.** Traces show effects of pretreatment with Digibind on contractile response to ouabain in guinea pig thoracic aorta. Responses to 250 (a, b) or 100 (c, d) nmol ouabain were compared in the absence (a, c) or presence (b, d) of 20 mg Digibind. Digibind was dissolved in 1 mL physiological saline solution (PSS).
Fra 2. Bar graphs show effects of intracerebroventricular (ICV) preinjection of Digibind on diuresis induced by ICV infusion of high-sodium cerebrospinal fluid (H-CSF). Urine was collected during six consecutive 15-minute periods (U₁ through U₆). ICV injection of Digibind (a, b), normal sheep IgG (b), or saline (a) was performed at the beginning of period 3. ICV infusion of H-CSF was performed over 15 minutes during period 4. Changes in urine volume are shown as multiples of the average value during periods 1 and 2. Values are mean±SEM. *P<.05 compared with basal value (U₁, U₂) in each group. Statistical significance between groups is shown at the top of each panel.

Fra 3. Bar graphs show effects of intracerebroventricular (ICV) preinjection of Digibind on natriuresis (left) and kaliuresis (right) induced by ICV infusion of high-sodium cerebrospinal fluid. Changes in urinary electrolyte excretion are shown as multiples of the average value during periods 1 and 2. Urine-collection periods are shown as U₃ through U₆. Values are mean±SEM. *P<.05, **P<.01 compared with basal value (U₁, U₂) in each group. Statistical significance between groups is shown at the top of each panel.

Discussion

Natriuresis in response to ICV stimulation with hypertonic NaCl was first demonstrated by Andersson et al. The natriuretic response on ICV stimulation has been found in anesthetized and conscious animals. Subsequent studies revealed that this natriuretic response still persisted after renal denervation, after removal of both adrenals, during infusion of a large amount of arginine vasopressin, and when renal arterial BP was kept constant. CNS-induced natriuresis appears to be mediated by pressor and sympathoexcitatory mechanisms. Nonpressor mechanisms appear to be mediated by bloodborne natriuretic factors distinct from atrial natriuretic peptide, aldosterone, or Ang II. Although the release of OLC or Na⁺,K⁺-ATPase inhibitor is reportedly precipitated by the elevation of sodium levels in the brain ventricle, our previous results with the use of intravenous Digibind suggest that circulating OLC may be an unlikely candidate as a natriuretic factor.

A recent observation that brain OLC is closely associated with pressor and sympathoexcitatory effects of central hypertonic NaCl prompted us in this study to examine the role of brain OLC in CNS-mediated natriuresis. We also used Digibind, which is a commercial preparation of Fab fragments raised in sheep immunized with digoxin-human serum albumin and is able to reverse the signs and symptoms of digitalis intoxication. Recent findings suggest that Digibind may also recognize the structure of ouabain and inhibit its action. In agreement with these observations, Digibind dose dependently inhibited the contraction of guinea pig aorta to ouabain in the present study. Although Digibind has been found to be remarkably specific, it appears that sufficient amounts of Digibind more or less recognize cardiotonic steroids possessing a cis-trans-cis configuration. Digibind could also recognize OLC, as OLC may actually be ouabain or its isomer, as suggested by Hamlyn et al. Our assumption was that pretreatment with ICV Digibind would affect the natriuretic response to ICV H-CSF if OLC is actually involved in CNS-induced natriuresis.

In the present study infusion of H-CSF into the lateral brain ventricle induced significant increases in BP, UV, U₅V, and U₆V. ICV Digibind, which was administered 15 minutes before ICV infusion of H-CSF,
significantly inhibited the increases in UV and UV/V. The results obtained with sheep IgG controls were not different from those obtained with saline alone, indicating that the effect of Digibind was not due to a nonspecific action of sheep IgG. Because the pressor response was not affected by preinjection of ICV Digibind, our present finding suggests that brain OLC, which is recognized by Digibind, may participate at least with nonpressor mechanisms in CNS-mediated natriuresis. In our previous experiment intravenous preinjection of Digibind at a dosage 500 times larger than that of ICV Digibind did not affect CNS-induced natriuresis.12 Although distribution volume and the elimination rate of Digibind may be quite different between the two administration routes (intravenous versus ICV), our present results suggest that brain OLC may be more important as a modulator of CNS-induced natriuresis than circulating OLC.

Contrary to the observations of Huang and coworkers,6 we could not find inhibitory effects of Digibind on the pressor response to central high sodium. This discrepancy may be due to some difference in experimental conditions. First, we used anesthetized rats because of the difficulty of collecting urine in conscious rats. Second, we did not use a vasopressin antagonist to exclude the effects of vasopressin. The animals in the present study might have elevated circulating levels of vasopressin at the beginning of the study because of the potent vasodilatation induced by pentobarbital anesthesia and perhaps because the modest anesthetic dose might have permitted psychostimulation of vasopressin release. Although we tried to make the fluid volume infused during operation and experiments as small as possible, the changes in volume status might have affected responses, including diuresis. Third, in our study the solutions placed in the ventricular system closely resembled normal CSF, except with regard to NaCl, thus minimizing the side effects reported to occur when the concentrations of other ions are altered.18 Fourth, we compared the responses among different groups of rats as opposed to those in the same rats before and after Digibind treatment to exclude the possible tachyphylaxis.19

Although our present results point to a contribution of brain OLC to CNS-induced natriuresis, the mechanisms by which brain OLC mediates its effects are mostly speculative. Because OLC inhibits Na+,K+-ATPase, OLC is likely to alter sodium-receptor interaction or sodium ion transport directly or indirectly through the inhibition of Na+,K+-ATPase. It is well known that the choroid plexus Na+,K+-ATPase system has a primary function in the formation of CSF.20 Furthermore, a close relation between excitation of the periventricular sodium receptor and Na+,K+-ATPase activity has been suggested.21,22 ICV infusions of glucose and dextrose, which have been shown to inhibit Na+,K+-ATPase activity, block basal arginine vasopressin release and induce pronounced water diuresis.23 Many studies have examined the central hypertensive effect of ouabain,19,24 but few were concerned with changes in sodium and fluid balance. Gutman et al25,26 indicated that sodium excretion in the urine was markedly increased after implantation of ouabain in the lateral hypothalamus. It is possible that brain OLC may regulate how kidneys excrete sodium and/or HCl by neural and/or humoral modulation, but its exact mechanism remains to be determined.

ICV infusion of Ang II induces CNS-mediated responses similar to those of central high NaCl. Moreover, a close relation of Ang II with CSF sodium concentrations has been suggested in these responses.27 However, in the present study Digibind did not affect either pressor or natriuretic responses to central Ang II. Because the magnitude of the natriuretic response to Ang II was much smaller than that induced by H-CSF, further investigations are clearly needed before we can conclude that brain OLC plays no role in CNS-mediated responses to central Ang II.

In conclusion, our observation indicates that ICV preinjection of Digibind significantly inhibited the natriuretic but not pressor responses to ICV infusion of H-CSF in anesthetized rats. These results support the view that brain OLC, which is recognized by Digibind, may play an important role with nonpressor mechanisms in CNS-induced natriuresis in rats.

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