Modulation of the levels of ouabain-like compound by central catecholamine neurons in rats

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Abstract Catecholamine regulates the Na^+,K^+-ATPase activity in the central nervous system and the Na^+,K^+-ATPase has been shown to have endogenous ligands (ouabain-like compound; OLC). To examine the relationship between OLC and central adrenergic neurons, we evaluated the effects of central sympathectomies with intracerebroventricular (i.c.v.) injection of 6-hydroxydopamine (6-OHDA; 250 μg) on brain and plasma OLC levels and brain catecholamine levels. In centrally sympathectomized rats, hypothalamic OLC content and plasma OLC level were significantly decreased by 90% ($P < 0.01$) and 70% ($P < 0.01$), respectively, in accordance with reduced brain norepinephrine content compared with control rats pretreated by i.c.v. injection of vehicle (ascorbic acid). On the other hand, peripheral sympathectomy with a similar manner did not affect plasma OLC level at all. These findings suggest that central adrenergic neurons may be involved in the synthesis and/or release of circulating OLC.

Key words: Ouabain-like compound; Catecholamine; 6-Hydroxydopamine; Na^+,K^+-ATPase

1. Introduction

It is believed that central adrenergic neurons are involved in the initiation and development of experimental hypertension. The destruction of central adrenergic neurons by intracerebroventricular (i.c.v.) infusion of 6-hydroxydopamine (6-OHDA) interferes with the development of nearly all types of experimental hypertension [1–3]. Na^+,K^+-ATPase has a major role in the regulation of neural excitability and energy metabolism and it has recently been shown that there are three isoforms of the catalytic (α) subunit of Na^+,K^+-ATPase that differ in their sensitivity to inhibition by cardiac glycosides [4]. In the rat, α1 has low affinity for ouabain, whereas α2 and α3 have high affinities for ouabain [5]. As α2 and α3 isoforms are predominant in the rat brain and the ouabain binding site of Na^+,K^+-ATPase has been shown to have endogenous ligands (ouabain-like compound; OLC) [6,7], the role of brain OLC in cardiovascular regulation have attracted much interest [8,9].

Huang and coworkers provided evidence that brain OLC may participate in the sympathoexcitatory and pressor effects of i.c.v. infusion of hypertonic saline solution [8]. We have recently shown that brain OLC may be involved in central nervous system (CNS)-mediated natriuresis in rats [10]. We undertook the present study to examine the interaction between brain OLC and central adrenergic neurons. For this, we evaluated the effects of central and peripheral sympathectomy on tissue and plasma OLC levels in rats.

2. Materials and methods

2.1. Central sympathectomy

Male Wistar rats were centrally sympathectomized by i.c.v. injections of 6-OHDA. Male Wistar rats weighing 300 to 330 g ($n = 11$) were anesthetized with pentobarbital and were placed in a Kopf stereotaxic apparatus. A burr hole was made approximately 0.5 mm posterior to the bregma and 1.5 mm lateral to the midsagittal suture. A stainless steel cannula was introduced stereotaxically into the left lateral ventricle (4.5 mm below the skull surface). 6-OHDA (250 μg) in 20 μl physiological saline solution containing 0.1% ascorbic acid was injected into the left lateral ventricle via a 25 μl Hamilton syringe. Control rats ($n = 9$) received 20 μl physiological saline solution containing 0.1% ascorbic acid. Twenty four hours after the injection the animals were killed by decapitation. Trunk blood was collected into a tube containing EDTA-2Na. Plasma OLC was measured by radioimmunoassay for ouabain. The brain was removed and was dissected into the following regions: right and left hypothalamus and left cortex [11]. Right and left hypothalamus were used for the measurements of Na^+,K^+-ATPase activity and OLC content, respectively. Norepinephrine content of left cortex was measured to determine the validity of sympathectomy.

2.2. Peripheral sympathectomy

Peripheral sympathectomy was performed by a single intravenous injection of 6-OHDA. 6-OHDA (66 mg) in 0.8 ml of physiological saline solution containing 0.1% ascorbic acid was injected into the tail vein of 8 male Wistar rats weighing 300 g under conscious and restrained condition. Control rats ($n = 10$) received 0.8 ml of physiological saline solution containing 0.1% ascorbic acid. Twenty-four hours later rats were killed by decapitation to determine plasma OLC level and the content of OLC in heart, kidney and adrenals.

2.3. Measurement of OLC

The tissue samples were homogenized with 5 vols. of distilled water and the homogenates were centrifuged at 15,000 rpm for 60 min. The resulting supernatant and the plasma sample (1.0 ml) mixed with the equal volume of 0.1% TFA solution were applied to Sep-Pak C_{18} cartridge that had been activated with methanol and equilibrated with distilled water. After complete washing with 30 ml of distilled water, OLC was eluted with 3 ml of 25% acetonitrile in water. The eluent was evaporated and assayed for OLC based on radioimmunoassay for ouabain as described in our previous report [12].

2.4. Measurement of Na^+,K^+-ATPase activity

Preparation of microsomes from cerebral cortex and assay of Na^+,K^+-ATPase activity were performed according to the method described by Donaldson et al. [13]. Briefly, a heavy microsomal fraction was prepared from cerebral cortex and was preincubated with 50 mM Tris-HCl buffer containing 2 mM $MgCl_2$, 100 mM NaCl, and 20 mM KCl (pH 7.6). Tris-ATP was added to the mixture (final concentration: 2.5 mM) and the reaction was terminated after 10 min by the addition of trichloroacetic acid. Inorganic phosphate (P_i) released was measured by the ascorbic acid method [14] and was expressed as mg P_i released in 10 min per μg of protein.

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2.5. Statistics

The data are expressed as mean ± S.E.M. and were analyzed by the unpaired Student's *t*-test.

3. Results

A summary of the effect of central sympathectomy on measured parameters is shown in Table 1. The norepinephrine and epinephrine levels in the cerebral cortex were significantly lower in centrally sympathectomized rats compared to those in control rats. The dopamine levels were not significantly different between the two groups of rats. The hypothalamic OLC content in centrally sympathectomized rats was 0.8 ± 0.2 pmol/g and was markedly lower than that in control rats (8.0 ± 1.8, $P < 0.01$; Fig. 1). The plasma OLC level was also significantly lower in centrally sympathectomized rats compared to that in control rats (74 ± 19 vs. 243 ± 47 pmol/l, $P < 0.01$; Fig. 2). Na^+,K^+-ATPase activity of the hypothalamus was higher in centrally sympathectomized rats compared to that in control rats, but the difference was not significant (6.2 ± 1.1 vs. 3.6 ± 1.6 mg P_i/10 min/mg protein; NS). As shown in Table 2, intravenous injection of 6-OHDA did not influence the OLC levels of plasma and three organs examined.

4. Discussion

Injection of 6-OHDA into a brain ventricle or the cisterna magna causes degeneration of central adrenergic nerve terminals without affecting peripheral adrenergic nerves [15]. I.c.v. injection of 6-OHDA has been shown to prevent development of hypertension in one-kidney one-clip hypertensive and one-kidney DOCA-salt hypertensive and young spontaneously hypertensive rats [1,2]. Further, Huot et al. have reported that i.c.v. injection of 6-OHDA markedly attenuated the development of reduced renal mass hypertension, prevented inhibition of vascular Na^+,K^+-ATPase pump activity in these animals, and prevented appearance of a circulating sodium transport inhibitor in their plasma [3].

OLC exhibiting Na^+,K^+-ATPase inhibitory activity has been extracted and partially purified from mammalian brain, hypothalamus and cerebrospinal fluid [6,7,16]. Recently, Tymiak et al. have isolated a ouabain isomer from bovine hypothalamus

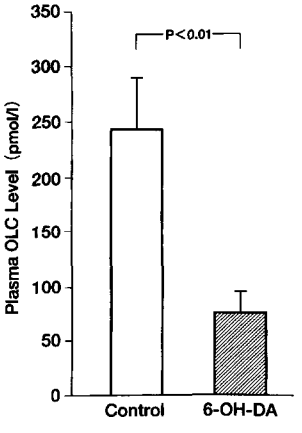

Fig. 1. Hypothalamic ouabain-like compound (OLC) content in control rats ($n = 9$) and centrally sympathectomized rats by intracerebroventricular injection of 6-hydroxydopamine (6-OHDA; $n = 11$).

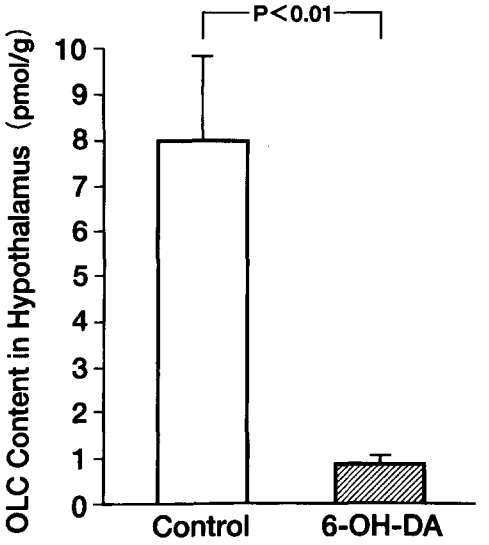

Fig. 2. Plasma ouabain-like compound (OLC) level in control rats ($n = 9$) and centrally sympathectomized rats by intracerebroventricular injection of 6-hydroxydopamine (6-OHDA; $n = 11$).

[17]. Furthermore, the presence of OLC has been demonstrated by immunohistochemical methods in hypothalamus of rat and macaque [18]. We have previously shown that i.c.v. preinjection of the Fab fragment of digoxin-specific antibody (Digibind) significantly inhibited increases in renal sodium excretion in response to high central sodium and suggested that brain OLC may be involved in CNS-mediated natriuresis [10].

In the present study, noradrenaline measurement indicates that central sympathectomies were effective. Central sympathectomy by i.c.v. injection of 6-OHDA markedly reduced not only the OLC content in hypothalamus but also plasma OLC level. On the other hand, peripheral sympathectomy did not affect plasma OLC level. These findings suggest that central adrenergic neurons may be involved in the synthesis and/or release of OLC in rats. Furthermore, it appears that circulating OLC derives from the central nervous system.

We could not find significant change in the Na^+, K^+-ATPase activity in hypothalamus. Swann et al. have shown that acute noradrenergic stimulation in vivo increased and 6-OHDA lesions decreased Na^+,K^+-ATPase activity in brain [19] and that

Table 1
Effects of central sympathectomy on measured parameters

	Control ($n = 9$)	6-OHDA ($n = 11$)	p
Norepinephrine content in cerebral cortex (ng/g)	368 ± 84	102 ± 15	<0.05
Epinephrine content in cerebral cortex (ng/g)	474 ± 204	83 ± 15	<0.05
Dopamine content in cerebral cortex (ng/g)	855 ± 125	621 ± 101	NS
OLC content in hypothalamus (pmol/g)	8.0 ± 1.8	0.8 ± 0.2	<0.01
Plasma OLC level (pmol/l)	243 ± 47	74 ± 19	<0.01
Na^+,K^+-ATPase activity in hypothalamus (μg P_i/10 min/mg protein)	3.6 ± 1.6	6.2 ± 1.1	NS

Values are given as means ± S.E.M. OLC = ouabain-like compound; 6-OHDA = 6-hydroxydopamine.

Table 2
Plasma and tissue OLC levels in control and intravenously 6-OHDA infused rats

	Control (n = 10)	6-OHDA (n = 8)	p
Plasma (pmol/l)	60 ± 5	65 ± 23	NS
Adrenals (pmol/g)	2.77 ± 0.17	2.98 ± 0.47	NS
Kidney (pmol/g)	0.21 ± 0.03	0.22 ± 0.09	NS
Heart (pmol/g)	0.72 ± 0.30	0.14 ± 0.03	NS

Values are given as means ± S.E.M. OLC = ouabain-like compound; 6-OHDA = 6-hydroxydopamine.

repeated noradrenergic stimulation for 3 weeks increased ouabain binding to rat cortex membrane [18]. In the rat brain, two isoforms (α2 and α3) with high affinity to ouabain exist [5]. In this context, it is supposed that the decrease of OLC in hypothalamus may increase the Na^+,K^+-ATPase activity in hypothalamus. OLC content and Na^+,K^+-ATPase activity were measured using different parts of hypothalamus in this study. Significant change might be detected if we could measure these two parameters using the same material. In order to evaluate the exact relationship between OLC and Na^+,K^+-ATPase activity, we surely need to measure the each isoform specific Na^+,K^+-ATPase activity.

In conclusion, our observation indicates that central sympathectomy acutely decreased OLC in hypothalamus and plasma, but that peripheral sympathectomy did not influence plasma OLC level. These results support the view the brain OLC may be modulated by adrenergic neurons and may be the source of circulating OLC. Our finding provides also indirect evidence for an endogenous nature of brain OLC.

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