Editorial

Endogenous Digitalislike Factor: An Update

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This issue of Hypertension Research contains the review and original articles presented at the International Symposium on Natriuretic and Digitalis-Like Factors held in Chitose, Hokkaido, Japan, on August 24, 1999. The symposium was the satellite meeting of the 9th International Conference on the Na/K-ATPase and Related ATPases, which was held in Sapporo, Hokkaido. At the symposium, it became clear that ouabain is the most promising candidate for a circulating hormone to regulate a number of physiological functions, including hypertension, and that other minor substances may also exist as endogenous digitalislike factors. Most of the symposium contributors submitted papers to this journal. I am going to summarize briefly the research history and current research results on endogenous digitalislike factors (EDLF).

Identification of Endogenous Digitalis

It has been more than 40 years since researchers suggested that there exists an endogenous digitalis in mammalian species (1). Hamlyn et al. (2) isolated ouabain from a large amount of human plasma and they claimed that ouabain is the endogenous digitalislike factor (EDLF) that had long been sought. However, other EDLFs such as digoxinlike factors (3), bufodienolide derivatives (4-6), and others have been postulated to be the candidate. So far, because of the significant hypertensinogenic actions of ouabain, it seems to be the most probable candidate to fill the putative roles of EDLFs such as water-electrolytes balance maintenance, vasoconstriction, inotropic action on the heart, and hypertension. Endogenous digoxin may also exist.

It has been suggested that digoxinlike immunoreactive substances are closely related with hypertension, heart failure, and renal failure. However, because digoxin administered chronically does not elevate blood pressure in humans, it may not be the cardinal endogenous digitalis for blood pressure regulation. Rather, digoxin is found to decrease blood pressure. This points to endogenous ouabain as a possible novel hypertensinogenic hormone. However, there are still concerns to address before reaching this conclusion. One major concern is that ouabain is present in our diets of plant origin (7) and seems to be incorporated in such organs as the adrenal gland, kidney, and brain. In fact, the distribution of ouabainlike and digoxinlike immunoreactivity of high concentration is restricted in those organs; immunohistochemistry with antidigoxin and anti-ouabain antibody have revealed that the immunoreactivity is restricted in neurons of the hypothalamic (8, 9) and medullary nuclei (10) in the brain. In the adrenals, we have found immunoreactivity in the adrenal medulla (11), but others have claimed to find it in the adrenal cortex (12). In any case, digitalislike immunoreactivity found not only in the brain but also in the adrenals could be of plant origin. However, because plasma concentrations and hypothalamic content of ouabainlike immunoreactivity are markedly reduced by intracerebroventricular (ICV) injections of 6-hydroxydopamine in comparison to amounts in sham-operated rats (13), the existence of endogenously formed ouabain, at least in the brain, is highly probable. Furthermore, some hyperten-
sive models such as DOCA-salt rats (14) and 1k, 1c renal-vascular hypertensive rats (15) show an increased level of ouabainlike immunoreactivity (OLI) in the urine and plasma. However, because plant ouabain incorporated in the adrenal can be released in response to stimuli (16), even the ouabain of extrinsic origin may behave as endogenous hormones.

Because the plasma ouabain concentration is as low as a few nmol/l, the level may be too low to play the physiological role in vivo. In fact, the concentration needed for pharmacological actions is more than 10 times the plasma concentration, based on my experiences. At the symposium, however, Kimura et al. noted that the plasma levels of ouabain in pathophysiological conditions in humans are almost the same levels as the concentrations needed to induce hypertension by ouabain in rats (17).

**Physiological and Pathophysiological Roles of Endogenous Digitalis**

Plasma concentration may not be the determinant of whether blood pressure rises, but locally elevated and acting ouabain may be essentially involved in blood pressure regulation. We have found that long-term treatment with high sodium diets increases turnover rates of digoxinlike immunoreactivity in the hypothalamus (8). ICV injections of ouabain elevate blood pressure by acting on the hypothalamus (18). Yamada et al. (19) report that ICV infusions of Digibind™ (Fab fragments of anti-digoxin antibody) lower blood pressure in reduced renal mass-saline hypertension in rats, and Huang and Leenen (20) report the same result in ouabain-induced hypertensive rats, where sympathetic inhibition is involved mainly as the hypotensive mechanism. Ouabain-induced hypertension may be caused by the action on the central nervous system rather than by the actions on the peripheral vessels and the heart. Furthermore, digoxin may be hypertensinogenic endogenous digitalis acting locally in the central nervous system by increasing both sympathetic outflow and pituitary hormone release, according to the finding demonstrated by Leenen et al. (20, 21).

Long-term treatment with ouabain actually increases blood pressure in rats (17). ICV injections of ouabain elevate blood pressure, too (18), by increasing sympathetic outflow. Therefore, ouabain is hypertensinogenic, although the underlying mechanism is not fully understood. Digoxin is also a potent inhibitor for Na/K-ATPase. Long-term treatment with digoxin does not increase blood pressure but may decrease it (18). The explanation for this discrepancy is also becoming clear, which is, in part, that ouabain and digoxin differently bind to Na/K-ATPase subunit isoforms and express messenger RNA for the isoform differently (22). These different features of ouabain and digoxin may lead to the opposite physiological actions, hypertension and hypotension. However, because plasma digoxin levels elevate with rising blood pressure.

**Fig. 1. Schematic drawing of the putative roles of endogenous digitalislike factors on sodium metabolism and hypertension.**
Another clue in the discussion of hypertensinogenic digitalis may be PST2238 (17β-(3-furyl)-5β-androstan-3β, 14β, 17α-triol), which is a digitoxigenin derivative. PST2238 displaces ouabain from the Na/K-ATPase receptor in vitro and is a potent antihypertensive compound in Milan hypertensive rats (24), reduced renal mass hypertensive rats (25), and ouabain-dependent (26) experimental models of hypertension. Although it has not yet been described in published research, PST2238 is effective in lowering blood pressure even in some selected patients with essential hypertension. Early Phase 2 clinical studies are currently underway in essential hypertensive patients in Europe. PST2238 may be a novel antihypertensive agent that will open a new field in hypertension research.

**Identification of Endogenous Ouabain**

From our experiences, we know that the adrenal contains ouabainlike immunoreactivity abundantly (14), and adrenomedullary cells are stained by monoclonal antiouabain antibody (11). The OLI content in the cortex from normal human adrenals is lower than that in the medulla (27). During surgery to remove a pheochromocytoma, we found that the plasma OLI level elevated in parallel with plasma norepinephrine levels (27). The OLI content in the removed pheochromocytoma was higher than that in the adrenal cortex and medulla. Therefore, we assumed that adrenomedullary cells produce endogenous ouabain. We characterized the OLI produced by cultured PC12 cells, which are of rat pheochromocytoma origin. Since bovine serum may contain ouabain, the culture medium was starved from serum for a significant period. OLI was purified from culture supernatant with 5 steps of column chromatography. Finally, the main peak of the OLI substance was analyzed by liquid chromatography/mass spectrometry. The OLI was found to be identical to authentic ouabain (28). Since OLI content was increased with the addition of progesterone in the culture medium dose-dependently, we believe OLI is produced endogenously in the PC12 cells. This is the simplest way to identify whether or not the substance is produced endogenously in mammalian cells, and this is why we are convinced that ouabain is actually produced in vivo with progesterone as the substrate.

Perrin et al. (12) recently reported that the OLI concentration in adrenocortical cultured cells increases with the addition of pregnenolone and progesterone. Shimojo et al. (29, 30) reported that adrenal 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts cortisol to cortisone and corticosterone, is present in the adrenal medulla in rats. Especially, 11β-HSD-1 mRNA is abundant in the whole adrenal, adrenal medulla, and cortex. These findings do not directly indicate the steroid genesis, but they do indicate that the interconversion of steroid hormones is actually possible in the rat adrenal medulla. Very recently, Isobe et al. (31) found the expression of 17α-hydroxylase messenger RNA in human pheochromocytoma tissue. The level of expression correlated with that of messenger RNA coding phenylethanolamine-N-methyl transferase (PNMT). Therefore, there remains the possibility that the adrenal medulla is one of the steroid-producing organs. However, when we consider the above findings, we believe that progesterone may act as the substrate for OLI production in PC12 cells.

Recently, Schneider et al. (32) isolated another digitalislike substance, a proscillaridin A-like factor, from the bovine adrenal gland. They also report that OLI is present in the same tissue (32). Recently we found that ouabain was isolated from culture supernatant from PC12 cells in the presence of progesterone. The amount of OLI in the culture supernatant of PC12 cells was small (0.5-1.5 ng/mg protein) but not less than that in adrenocortical cells (about 1 ng/mg protein) (33). Recently, Lichtstein et al. (34) reported that the digitalislike compound from rat adrenal cells is synthesized from hydroxycholesterol and that the amount of the substance is about 200 pmol/g wet weight.

**The Site of Endogenous Digitalis Production**

The site of production and secretion of OLI has not yet been definitely determined. In our previous studies, OLI seemed to present in the adrenal medulla and the central nervous system, particularly in the hypothalamo-pituitary axis (9, 14). Namely, our previous data suggested that the OLI in plasma is exclusively derived from the nervous tissue but not from adrenocortical tissue in rats (13). Schonauer and his colleagues recently found a novel sodium pump inhibitor other than ouabain, which was found in the hypothalamus and the adrenal in rats and cows (32, 35). Moreover, we (18), Leenen and his colleagues (36), and Yamada et al. (37) reported the effect of ouabain on the central nervous system. On the other hand, Hamlyn’s group and others have demonstrated that adrenocortical cells are the origin of ouabainlike factor (OLF) (12, 33, 38, 39).

Another area of research is to search for the physiological stimuli that release OLF. Unfortunately, conflicting results have been published. Recently, Laredo et al. (33, 40) demonstrated that ACTH and angiotensin II stimulate the secretion of an endogenous ouabainlike factor from bovine adrenal cells. On the other hand, Butt et al. (41) report that in a clinical study ACTH does not regulate endogenous ouabain secretion, assayed as ouabainlike materials by radioimmunoassay in men. Elucidation of the metabolic pathway responsible for the synthesis of ouabain in mammalian cells is definitely

(23), digoxinlike immunoreactivity may be a good index for hypertension.
necessary for understanding the pathophysiology of essential hypertension and other disease states related to electrolyte imbalances.

Acknowledgements

This symposium was made possible by the generous financial support of Pfizer Japan Co. Ltd.

References