

Does Digoxin-Like Immunoreactivity Really Represent the Natriuretic Hormone?

A.		Goto
K.		Yamada
M.		Ishii
T.		Sugimoto

Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Japan

Atsuo Goto, MD, Second Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113 (Japan)

Dear Sir,

The possibility that endogenous inhibitors of the sodium pump exist and bind to the cardiac glycoside receptor on Na⁺, K⁺-ATPase has been a source of much interest. The wide distribution of the high affinity binding site for the cardiac glycoside in normal tissues strongly support this speculation. There is increasing recognition that such endogenous digitalis-like factors may act as 'natriuretic hormones' by modulating sodium pump activity in response to extracellular volume expansion and play roles in the pathogenesis of hypertension or chronic renal failure [1, 2].

Many investigators have measured digoxin-like immunoreactivity to identify the putative digitalis-like factors on the assumption that biological substances which bind to the same receptors as drugs may compete with antibodies specific for the drugs. However, it has become evident that the use of antidigoxin antiserum in radioimmunoassay (RIA) systems to detect digitalis-like factors may give some erroneous findings and difficulties in the interpretation of results [3]. Many steroids give false-positive results for digoxin, as do many lipids and bile acids. Recent findings indicate the dissociation of digoxin-like immunoreactivity from digitalis-like biological activity [3-5].

We have recently purified to homogeneity two distinct digitalis-like factors from human urine based on [³H]ouabain displacing activity from intact human erythrocytes [6, 7]. The polar compound was eluted off the C18 reverse phase column with 18% acetonitrile and cross-reacted very weakly with specific antidigoxin antibody. On the other hand, the less polar compound was eluted at 31% acetonitrile and showed a prominent digoxin-like immunoreactivity.

In this preliminary communication, we report the different behavior of these two digitalis-like factors in re-

High salt

1

100

50 Retention time (min)

Fig. 1. Effects of a salt intake on urinary excretion of two distinct digitalis-like factors. Urine (500 ml) was analyzed by reverse-phase high-performance liquid chromatography on a D-ODS-5 column (YMC; 2.0 × 25 cm) with a linear gradient of acetonitrile in water (0–50%) over 100 min at 10 ml/min. This was one of the chromatographic systems used for the complete isolation of these compounds [6, 7]. One-minute fractions were collected, lyophilized, concentrated to 1/20 of their original volume and assayed for the capacity to inhibit [³H]ouabain binding to human erythrocytes. The polar and less polar digitalis-like factors were reproducibly eluted off the column with 18 and 31% acetonitrile in water, respectively. Representative results from a male normotensive subjects are illustrated.

sponse to chronic alterations in dietary salt intake. Urine samples were obtained from seven normotensive subject (average age: 42 years) after 5 days on a low-salt diet (51 mmol/day) and after 5 days on a high-salt diet (342 mmol/day). Urine was fractionated by reverse-phase

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Clerico A: Digoxin-like immunoreactivity, endogenous cardiac glycoside-like factor(s) and natriuretic hormone: more than a hypothesis. *J Nucl Med Allied Sci* 1987;31:341–356.

Yamada K, Goto A, Ishii M, et al: Dissociation of digoxin-like immunoreactivity and Na⁺, K⁺-ATPase inhibitory activity in rat plasma. *Experientia* 1988;44:992–993.

Boero R, Guarena C, Berto IM, et al: Erythrocyte Na,K pump activity and arterial hypertension in uremic dialyzed patients. *Kidney Int* 1988;34:691–696.

Goto A, Yamada K, Ishii M, et al: Purification and characterization of human urine-derived digitalis-like factor. *Biochem Bio-phys Res Commun* 1988;154:847–853.

Goto A, Yamada K, Ishii M, et al: Urinary sodium pump inhibitor raises cytosolic free calcium concentration in rat aorta. *Hypertension* 1989; in press.

Shilo L, Adawi A, Solomon G, et al: Endogenous digoxin-like immunoreactivity in congestive heart failure. *Br Med J* 1987;295:415–416.

Valdes R, Hagberg JM, Vaughan E, et al: Endogenous digoxin-like immunoreactivity in blood is increased during prolonged strenuous exercise. *Life Sci* 1987;42:103–110.

Gault MH, Vasdev SC, Longerich LL, et al: Plasma digitalis-like factors(s) increase with salt loading. *N Engl J Med* 1983; 309:1459.

Kramer HJ, Heppe M, Weiler E, et al: Further characterization of the endogenous natriuretic and digoxin-like immunoreacting activities in human urine: effects of changes in sodium intake. *Renal Physiol* 1985;8:80–89.

Scott PJ, Little PJ, Bobik A: Circulating digoxin-like immunoreactivity in renal hypertensive rabbits: lack of modulation by alterations in dietary sodium intake. *J Hypertens* 1988;6: 205–209. high-performance liquid chromatography and the frac- 3 tions corresponding to the elution positions of these substances were selectively and separately collected. The levels of digitalis-like factors were determined by an inhibitory effect on [³H]ouabain binding to human erythrocytes (radioreceptor assay) and by a digoxin-like immunoreactivity.

Urinary excretion of the polar digitalis-like factor markedly increased from 1.8 ± 0.5 < SD > to 69.2 ± 27.4 pmol ouabain equivalents/day in parallel with the increment in the dietary salt intake ($p < 0.001$). The polar 7 compound actually behaved as might be expected for a 'natriuretic hormone'. However, the change in the urinary level of this compound was not reflected by a digoxin-like immunoreactivity.

In contrast, urinary excretion of the less polar digita- 9

lis-like factor remained unaltered during changes in salt intake. The levels of this factor determined by radioreceptor assay and by RIA for digoxin averaged 1.5 ± 0.5 and 11.7 ± 3.5 on a low-salt intake and 1.9 ± 0.8 pmol ouabain equivalents/day and 14.0 ± 5.6 ng digoxin equivalents/day on a high-salt intake, respectively (N.S.).

These observations clearly indicate that the digoxin-like immunoreactive, digitalis-like factor in human urine may not represent a genuine 'natriuretic hormone'. Digoxin-like immunoreactive substances are reportedly increased in plasma or urine from patients with renal failure, liver failure or heart failure, from newborn infants, from pregnant women and from normal subjects during exercises [3, 8, 9]. However, the effects of salt loading on digoxin-like immunoreactivity in plasma or urine are not necessarily consistent [10–12]. Our results may explain these discrepancies. We conclude that estimation of the 'natriuretic hormone' solely based on digoxin-like immunoreactivity may be misleading and should be regarded with caution.

References

de Wardener HE, Clarkson EM: Concept of a natriuretic hormone. *Physiol Rev* 1985;65:658–759.

Haddy FJ: Endogenous digitalis-like factor or factors. *N Engl J Med* 1987;316:621–623. Tokyo 113 (Japan)