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Further evidence for the dissociation of digoxin-like immunoreactivity from Na^+, K^+-ATPase inhibitory activity

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Summary. The effects of adrenalectomy or nephrectomy, carried out one hour previously, on the levels of endogenous digitalis-like factors were determined in rat plasma. Factors were assayed by digoxin-like immunoreactivity and direct Na^+, K^+-ATPase inhibitory activity. Digoxin-like immunoreactivity significantly decreased one hour after bilateral ablation of adrenals, while Na^+, K^+-ATPase inhibitory activity remained unaltered. There were no changes in either activity one hour after bilateral nephrectomy. These results suggest that digoxin-like immunoreactivity may be derived from the adrenal gland or under adrenal control and the major substances detected by digoxin-like immunoreactivity and direct Na^+, K^+-ATPase inhibitory activity may be different.
Key words. Endogenous digitalis-like factor; digoxin-like immunoreactivity; Na^+, K^+-ATPase inhibitor; adrenalectomy.

The notion that endogenous inhibitors of the sodium pump exist, and bind to the cardiac glycoside binding site on Na^+, K^+-ATPase, has been a source of much controversy[1, 2]. Although considerable work has been carried out, the exact nature, structure and production site of the inhibitors are not as yet known. Moreover, because of the lack of specific assay methods, a variety of different procedures have been used to detect such endogenous digitalis-like factors (EDLF). It is possible that each procedure may detect a completely different substances. Indeed, we have indicated that the major substances detected by digoxin-like immunoreactivity and direct Na^+, K^+-ATPase inhibitory activity are totally different at least in rat plasma[3].

Recent findings have pointed to the possibility that digoxin-like immunoreactivity is closely associated with the adrenal gland[4–8]. In the present study, we determined the effects of adrenalectomy or nephrectomy carried out only one hour before assay on plasma levels of EDLF, assayed by digoxin-like immunoreactivity and Na^+, K^+-ATPase inhibitory activity, to gain further insight into the tissue source of EDLF.

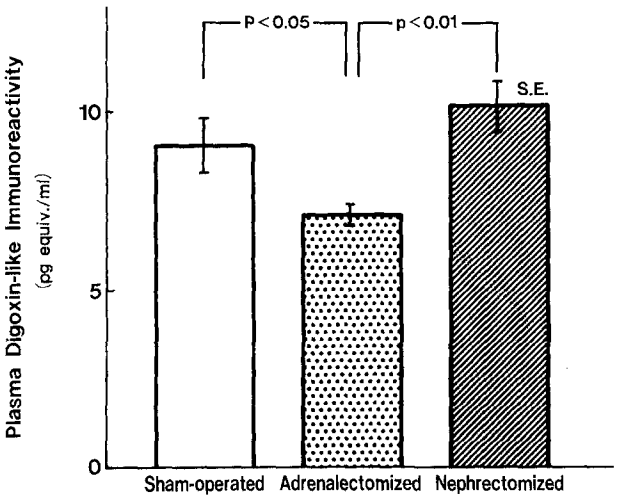
Materials and methods

Male Sprague-Dawley rats under pentobarbital anesthesia (40 mg/kg b.wt, i.p.) were used in this experiment. Bilateral adrenalectomy was performed through dorsal incisions in 8 rats. Bilateral nephrectomy was performed in another 8 rats, also through dorsal incisions. They were compared to 8 sham-operated controls.

A PE-50 catheter was inserted to the right carotid artery and direct blood pressure was recorded. A blood sample was obtained from the catheter into a heparinized syringe 60 min after the completion of the operation. Arterial blood was immediately chilled and centrifuged at 3000 rpm for 5 min. 5 ml of plasma was mixed with 10 ml of methanol and the mixture was kept at 4 °C for 16 h. After filtration through filter paper, the filtrate was evaporated and lyophilized. The resulting residue was dissolved in 8 ml of distilled water and the solution was applied to Amberlite XAD-2 (3 ml). After washing with 30 ml of distilled water, EDLF was eluted with 8 ml of methanol. The eluent was evaporated and the residue was redissolved in 0.5 ml of distilled water. EDLF was determined by digoxin-like immunoreactivity and Na^+, K^+-ATPase inhibitory activity according to the methods described in detail previously[3]. The data are expressed as mean ± SE. Group comparisons were made by analysis of variance and differences between two groups were analyzed by the unpaired Student's t-test.

Results

Body weight, mean blood pressure and hematocrit were not different among the three groups (334 ± 8, 329 ± 9



The digoxin-like immunoreactivity in the sham-operated, adrenalectomized and nephrectomized rats (n = 8, each). Digoxin-like immunoreactivity significantly decreased one hour after adrenalectomy, but did not change after nephrectomy.

and 325 ± 13 g, 114 ± 6, 109 ± 5 and 119 ± 6 mmHg, 43.8 ± 0.8, 45.3 ± 0.8 and 46.8 ± 0.4% in sham-operated, adrenalectomized and nephrectomized rats, respectively). The plasma digoxin-like immunoreactivity was significantly lower in the adrenalectomized rats than in the sham-operated rats (7.09 ± 0.29 vs 9.07 ± 0.77 pg digoxin equivalents/ml; $p < 0.05$). The plasma digoxin-like immunoreactivity in the nephrectomized rats was 10.14 ± 0.71 pg digoxin equivalents/ml and was not different significantly from that in the sham-operated rats. On the other hand, Na^+, K^+-ATPase inhibitory activities did not differ significantly among the three groups (75.7 ± 3.6, 76.5 ± 3.8 and 80.9 ± 3.0% in sham-operated, adrenalectomized and nephrectomized rats, respectively).

Discussion

Many researchers have chosen to use digoxin-like immunoreactivity to study EDLF on the premise that the digoxin antibody may be able to recognize the endogenous ligand of the digitalis receptor. Actually, immunoreactive digoxin-like factors have been found in body fluids from subjects with a variety of physiological and pathological conditions, including uremia [9], liver disease [10], during pregnancy [11] and in the neonatal period [12]. The nature and source of the immunoreactive material are still unclear, but several observations have pointed to the adrenal as a potential source of the material [4–8]. The adrenals contain the greatest amount of digoxin-like immunoreactivity [8, 13], and the plasma digoxin-like immunoreactivity has been shown to decline 48–96 h after adrenalectomy [5]. Moreover, Doris and colleagues have observed that adrenal glands from several species, and cells of the murine adrenocortical tumor cell line Y-1, release an immunoreactive digoxin-like material into a serum-free incubation medium [14, 15]. Together, these lines of evidence suggest that the adrenal gland may be a source of digoxin-like immunoreactivity.

In the current study, performed 1 h before the assay, adrenalectomy but not nephrectomy performed at the same time, significantly decreased digoxin-like immunoreactivity in rat plasma. This findings support the contention that digoxin-like immunoreactivity is of adrenal origin or under strict adrenal control, and suggest that such an activity has a rather short half-life in the circulation. In contrast, Na^+, K^+-ATPase inhibitory activity remained unaltered one hour after bilateral adrenalectomy. It is possible that this discrepancy may be caused by different sensitivities of these two procedures to EDLF. However, Pernollet and colleagues have also reported that there was no significant reduction in the plasma-induced inhibition of Na^+, K^+-ATPase activity four days after adrenalectomy [5]. We have previously pointed to the possibility that digoxin-like immunoreactivity and Na^+, K^+-ATPase inhibitory activity may detect totally different substances in rat plasma, in view of their opposite behavior during acute saline infusion [3]. The distinct effects of adrenalectomy on digoxin-like immunoreactivity and Na^+, K^+-ATPase inhibitory activity in this study also suggest that the major substances measured by these two methods in rat plasma are not the same.

We have recently isolated two EDLF from human urine using a combination of chromatographic systems including reverse-phase high performance liquid chromatography (HPLC) [16]. On analysis of rat plasma with the reverse-phase HPLC, digoxin-like immunoreactivity and direct Na^+, K^+-ATPase inhibitory activity did not necessarily coincide (our unpublished observations). This is also the case with human plasma and urine [17, 18]. These facts clearly indicate the dissociation of digoxin-like immunoreactivity from digoxin-like biological activity, at least in crude samples. This does not mean that genuine EDLF with digitalis-like biological activities do not exist in the mammalian body. Actually, potential candidate substances have been purified to homogeneity in several laboratories [19–22].

In our previous communication we reported that Na^+, K^+-ATPase inhibitory activity significantly increased in response to acute saline infusion, and this was considered to reflect the natriuretic EDLF. The capacity to inhibit the Na^+, K^+-ATPase has been reported to rise 48 h after nephrectomy [5] or in renal failure [23]. The inability of nephrectomy to enhance the Na^+, K^+-ATPase inhibitory activity in this study may be explained by the difference either in the time that elapsed following nephrectomy, or in the extraction methods.

In conclusion: adrenalectomy performed only one hour before assay noticeably reduced digoxin-like immunoreactivity in rat plasma, while direct Na^+, K^+-ATPase inhibitory activity remained unchanged. These results suggest that digoxin-like immunoreactivity may be derived from the adrenal gland, and provide another piece of

evidence for the dissociation of digoxin-like immunoreactivity and digitalis-like biological activity (Na^+, K^+-ATPase inhibitory activity).

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Studies on the thyroid in transgenic mice expressing the genes for human and bovine growth hormone

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Summary. The thyroid glands of transgenic mice (TM) expressing the genes for human (*h*) and bovine (*b*) growth hormone (GH) were studied. The percentages of larger follicles in *h*GH TM of either sex were significantly greater than in the corresponding normal littermates, and follicles ranging up to 350 µm in diameter were present in male *h*GH TM. In contrast, thyroid follicles were only slightly enlarged in male *b*GH TM, and were unchanged in female *b*GH TM. The serum concentrations of T4 were significantly decreased in male *b*GH TM and not altered in the other groups. Serum concentrations of T3 were slightly, but significantly increased in female *h*GH TM and female *b*GH TM, but were unaffected in male TM of either type. Since the principal difference between these foreign GHs in rodents is the additional lactogenic activity of human GH, these results may indicate that the effects of prolactin can influence the development of the thyroid.
Keywords. Thyroid; transgenic mouse; human growth hormone; thyroid hormones.

Transgenic mice (*Mus musculus*) expressing the genes for the human (*h*) or bovine (*b*) growth hormones (GH) fused with the mouse methallothionein (mMT) promoter show marked stimulation of body growth. These transgenic mice (TM) synthesize the foreign GHs at several ectopic sites, especially in liver and kidney, and have measurable amounts of heterologous GH in the peripheral circulation[1-3]. The incorporation of the GH genes in TM is permanent and is passed to their progeny as a dominant trait.

Underdeveloped thyroid glands in mutant dwarf mice with inherited lack of prolactin and GH suggest a role for GH and possibly for prolactin in the development of the thyroid[4]. Since in rodents *b*GH shows purely somatotrophic effects, whereas *h*GH has, in addition, a prolactin-like action[5,6], TM expressing the genes for these hormones offer the opportunity to examine further the role of GH and prolactin in the development of the thyroid.

Materials and methods

Transgenic mice. Transgenic mice of both types were originally produced by microinjection of gene constructs (mMT/GH) into pronuclei of fertilized eggs, as described previously[3,7]. All TM used for the present study were derived from one fertile *h*GM TM, male and one fertile *b*GH TM male produced in the original experiments. These founder males and their male transgenic progeny were mated with B6C3F1 hybrid females (Jackson Laboratory, Bar Harbor, ME). Their offspring, normal mice