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Crossed digoxin immunoreactivity in chromatographic fractions of rat adrenal extract

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In a series of previous communications we have shown that in the serum of rats with cardiac overload produced by a ligature of abdominal aorta [1, 2] or by thyroid hormone-induced hypermetabolism [3] and accompanied by myocardial hypertrophy an "apparent" presence of digoxin can be demonstrated by homogenous enzymoimmunoassay. There is some indirect evidence [1, 4] that an endogenous cardiotropic hormone is produced by the adrenal cortex, and that this steroid is identical with neither corticosterone [1] nor with aldosterone [5]. In the present experiments we tried to detect "apparent" digoxin immunoreactivity in chromatographic fractions of rat adrenal extract.

In a typical experiment adrenals of 24 normal male Wistar rats (Velaz, Prague) with a wet weight of 571 mg were homogenized in 5.7 ml 70% acetone and the homogenate was extracted 3 times with 5 ml 70% acetone. Then it was filtered through Whatman Phase Separator and the acetone was evaporated. The remaining water phase was mixed with 10 ml of methanol and extracted 3 times with 10 ml Petroleumbenzin Merck (b.p. 60–80°) and the extracts were discarded. The remaining water phase was extracted 3 times with 12 ml dichloromethane and the pooled extracts were evaporated. The dry residues were dissolved in 20 μl of a mixture of chloroform–methanol (1:1) and applied to a thin layer plate (Merck Kieselgel 60F–254, 0.2 mm). The chromatogram was developed 3 times in cyclohexane–isopropanol (7:3) and then once in chloroform–ethanol–water (92:8:0.5). Ten spots were visualized in u.v. light (254 nm), extracted with chloroform–methanol (1:1) and dried, as well as the zones between the spots, where some tailing (especially in zones B, C and D) was present. Halves of the residues were dissolved in physiological saline and the aliquots in duplicate (corresponding each to a quarter of the original weight) were examined for the presence of digoxin immunoreactivity by homogenous enzymoimmunoassay [6] using the EMIT-cad kit (Syva, USA). The spots and zones containing digoxin-like immunoreactivity were applied to the same t.l.c. plate and rechromatographed in the same way. The spots were visualized in u.v. light, extracted and subjected to digoxin enzymoimmunoassay. The experiment was repeated several times with essentially identical results.

A typical chromatogram is shown in Fig. 1. The spots are numbered and the zones between them marked with letters. The numbers on the right represent relative digoxin-like immunoreactivity, expressed here as ΔA (absorbance change) of the enzyme reaction in the homogenous enzymoimmunoassay. Maximum digoxin-like immunoreactivity was present in the sub-aldosterone zone (B) and in the aldosterone spot (No. 4). When these materials were rechromatographed (Fig. 2) with hormonal as well as cardenolide standards the highest digoxin-like immunoreactivity was found in a spot produced by zone B and corresponding to digitoxigenin. The intensity of the spot was quite variable, but maximum digoxin-like immunoreactivity was always here. In some experiments another spot developed from the zone B eluate on rechromatography, with an R_f similar to digoxigenin and again displaying relatively high digoxin immunoreactivity.

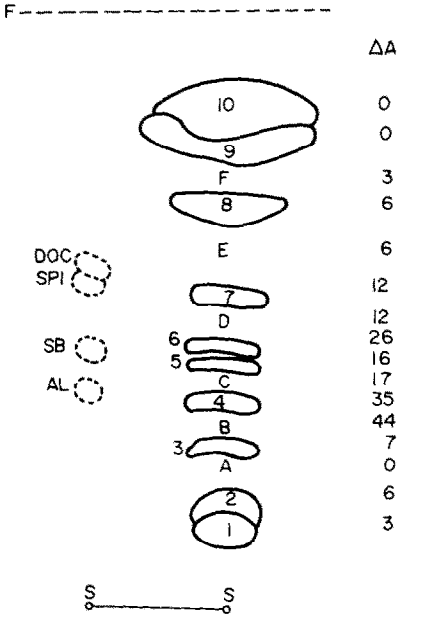

Fig. 1. Scheme of a typical chromatogram of crude extract from 571 mg of rat adrenal tissue. The spots were visualized by quenching (absorption) in u.v. light (254 nm). Abbreviations: S = start, F = front, A = aldosterone standard, SB = corticosterone standard, SPI = spironolactone standard, DOC = deoxycorticosterone standard (all 10 μg). ΔA = absorbance change in the enzyme reaction in homogenous digoxin enzymoimmunoassay (relative value of digoxin-like immunoreactivity): its maximum is in the sub-aldosterone zone B and in the aldosterone spot (No. 4). Some tailing was present in zones B, C and D, which produced the spots seen on rechromatography of these zones (Fig. 2).

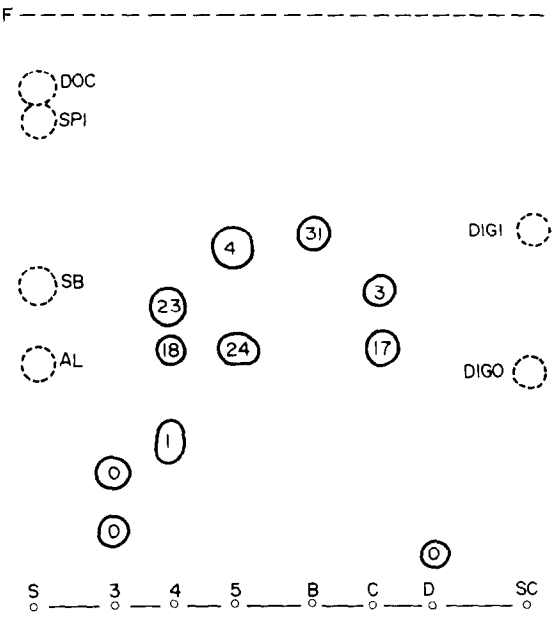

Fig. 2. Scheme of the rechromatography of eluates of spots 3, 4, 5 and zones B, C and D from Fig. 1, now equivalent to 285 mg of rat adrenal tissue. Abbreviations same as in Fig. 1 and: S = start of steroid standards (10 μg each), 3, 4, 5, B, C, D = starts of samples, SC = start of cardenolide standards (10 μg, detectable in this quantity in u.v. light by ·quenching), DIGI = digitoxigenin, DIGO = digoxigenin. The relative digoxin-like immunoreactivity is indicated by numbers (= ΔA) inside the spots. Note: the spots 3, 4 and 5 produced 2 to 3 spots on rechromatography; this was probably caused by partial disintegration of individual steroids by elution, drying and rechromatography.

The results show that several fractions of rat adrenal extract display digoxin-like immunoreactivity. This is evidently caused by a crossed immunoreactivity of adrenal steroids. In other experiments we have shown that the digoxin-like immunoreactivity of rat serum is not correlated with corticosterone [1] or aldosterone [5] levels. Since we do not know the real amount of steroids present in the individual spots and zones of our chromatograms, we cannot say anything about the absolute cross-reactivity of the individual steroids in the digoxin enzymoimmunoassay.

The results suggest that previously observed [1–3] presence of digoxin-like immunoreativity in the blood of rats with cardiac overload and myocardial hypertrophy is probably of adrenal origin but that it is not caused by corticosterone or aldosterone. The hypothetical adrenal cardiotropic steroid could play a role in the development of myocardial hypertrophy in rats with cardiac overload by inducing—as do many other steroid hormones—a proteosynthetic reaction in its target tissue, i.e. the heart. In analogy with the morphine–endorphin group of substances we suggested [7] for it the name endocardin or endocardiotonin. It is noteworthy that "apparent" digoxin immunoreactivity was previously observed [8] in the cord blood: it may be a manifestation of an adrenal reaction to cardiac overload during the parturition.

Laboratory of Endocrinology and Metabolism,
Faculty of Medicine
Charles University
128 21 *Prague*
Czechoslavakia

V. SCHREIBER
J. ŠTĚPÁN
I. GREGOROVÁ
J. KREJČÍKOVÁ

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