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# SHORT COMMUNICATIONS

## Digitalis glycoside-like biological activity (inhibition of $^{86}Rb^+$ uptake by red blood cells *in vitro*) of certain steroids and other hormones

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Digitalis glycoside-like immunoreactivity was observed in the blood of rats with a cardiac overload [1, 2] and with dried thyroid-induced hyperthyroidism [3, 4], in the blood of dogs with expanded plasma volume [5, 6] and ouabain-like immunoreactivity in the blood of the toad *Bufo marinus* [7]. Digitalis-like biological activity (inhibition of $Na^+$–$K^+$ ATPase) was observed in human blood [8–11], in bovine hypothalamus [12] and in guinea-pig brain [13]. These findings all suggest the possible existence of an endogenous factor with digitalis-like activity. In the blood of dogs with expanded plasma volume, the activity is present in the low molecular weight peptide fraction [5, 6] and it is probably caused by the presence of peptidic natriuretic hormone. Digoxin-like immunoreactivity is also present in the fractions of lipid extracts of rat [14] and rabbit [15] adrenals, however, together with digitalis-like biological activity (inhibition of $Na^+$–$K^+$ ATPase). In the present experiments we therefore tried to detect digitalis-like biological activity in some of the steroid hormones. Special attention was paid to certain 14-hydroxy derivatives of steroid hormones, since the presence of a 14-OH group—as well as the C-17 lactone ring—is a prerequisite for the cardiotropic activity of the cardenolides. In all, 19 steroids were examined and, among the non-steroid hormones, thyroxine (since digoxin-like immunoreactivity appears in the blood of thyroid treated rats) and ACTH (since digoxin-like immunoreactivity appears in the blood of adrenalectomised rats) were tested.

Steroids and thyroxine were dissolved in 50% ethanol and from this solution, by serial dilution with physiological saline, final concentrations of 50, 100, 150, 200 and 250 nmol/ml were obtained. Synthetic $\beta^{1-24}$ACTH (Synacthen, Ciba) was diluted to final concentrations of 5.6, 11.2, 22.5, 45 and 90 nmol/ml. Digitalis-like biological activity was measured in 0.1 ml samples by Lowenstein's method [8, 9], as the inhibition of $^{86}Rb^+$uptake by red blood cells *in vitro*.

The results are shown in Fig. 1. Among corticosteroids and congeners tested, only aldosterone displayed slight activity. Among the androgens, etiocholanolone displayed slight and 5-$\alpha$-androstanedione, dehydroepiandrosterone, 14-OH testosterone and 14-OH androstenedione a distinct biological activity. Natural oestrogens had no effect, but their 14-OH derivatives were highly active. Calculated on a molar basis, 14-OH oestradiol displayed biological activity of the same order as digitoxigenin and of a higher order than digoxigenin. 14-OH estrone was less but still highly active. Thyroxine was ineffective, but synthetic $\beta^{1-24}$ACTH displayed significant activity.

The results primarily show that the surprisingly high biological activity of 14-OH oestradiol and the other 14-OH derivatives merit further study of its possible physiological significance. In another communication [16] we report digoxin-like immunoreactivity of some of the tested steroids (especially of the 14-OH derivatives, from which maximum immunoreactivity, reaching 3% of the immunoreactivity of digoxigenin, was found with 14-OH oestradiol) and of ACTH. The results thus suggest the possible existence of two groups of substances with digitalis-like activity. The activity present in the blood of volume-expanded dogs [5, 6] and in the brain [12, 13] is probably caused by a peptidic natriuretic hormone which could be similar to ACTH or might be a fragment of the ACTH molecule. However, the activity present in the fractions of rat [14] and rabbit [15] adrenals is evidently caused by steroids. We do not yet know whether 14-OH steroids are present in the active fractions. Mammalian tissues are capable of 14-hydroxylation of the steroid molecule [17] and the endogenous factor which possibly participates in the regulation of cardiac growth, and for which we suggested the name endocardin or endocardiotonin [1-4, 14, 15, 18] might well be one of the 14-OH steroids.

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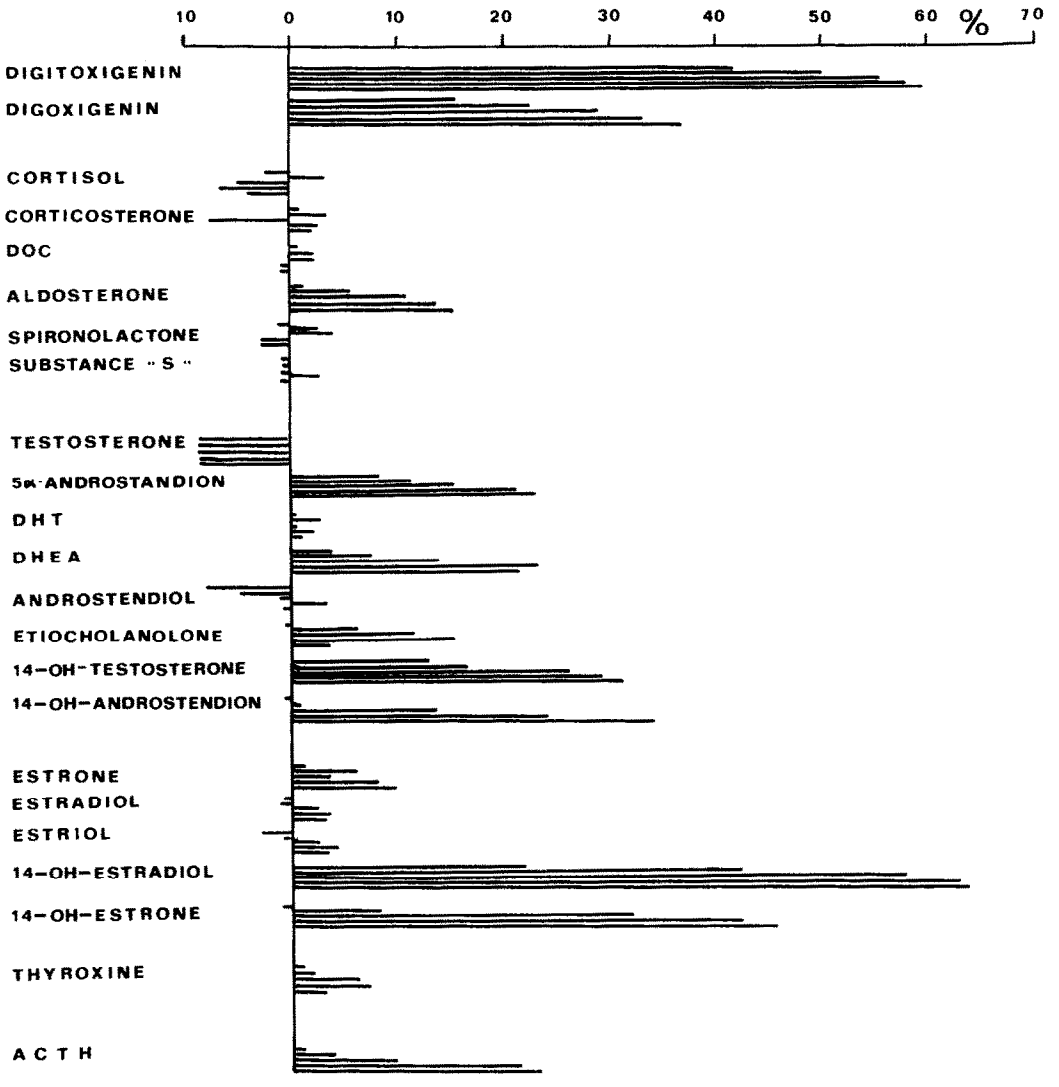


Fig. 1. Percentual inhibition of the influx of $^{86}Rb^{+}$ into red blood cells *in vitro* as a test of digitalis-like biological activity. Minus values denote activation of influx: the only steroid which consistently activated the influx was testosterone, although the activation was dose-unrelated in this dose range. It is interesting to note here that the stimulatory effect of testosterone on $K^{+}$ influx into prostatic cells was reported [19]. Cardenolide and steroid concentrations were 50, 100, 150, 200 and 250 nmol/ml, and ACTH concentrations 5.6, 11.3, 22.5, 45 and 90 nmol/ml. The five horizontal bars along the level of each substance correspond to five concentrations tested (means from three to four determinations done in two runs on two preparations of RBC; the indices of precision $\lambda$ ranged from 0.21 to 0.48). Abbreviations: DOC, deoxycorticosterone; substance S, 11-deoxycortisol; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; ACTH = $\beta^{1-24}$ ACTH.

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