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The Endogenous Digitalis-like Factor

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The endogenous digitalis-like immunoreactive factor (DLF) was discovered independently by Gruber *et al.* (1979, 1980) and in our laboratory (Schreiber *et al.* 1980, 1981), in both cases in animals with a cardiac overload and blood volume expansion or hypertension. Before that, however, there had been evidence of the existence of a natriuretic hormone (Clarkson *et al.* 1970). Subsequently many authors who studied this hormone found that endogenous inhibitors of Na,K-ATPase do exist (Buckalew and Nelson 1974, Kramer and Gonick 1974, Hillyard *et al.* 1976). It has actually been demonstrated that they are present in the brain (Fishman 1979) or in the hypothalamus (Haupert and Sancho 1979). The question of the identity of this (peptide ?) natriuretic hormone has not yet been resolved. Although our hypothesis of the identity of the natriuretic hormone with an ACTH fragment (Schreiber *et al.* 1982) has not been confirmed, the natriuretic activity has been demonstrated in α-MSH and β-MSH (Hradec and Horký 1979). Furthermore, Gruber and Callahan (1989) showed that γ-MSH (an analogue of ACTH 4 - 10) possesses a high natriuretic (as well as pressor and cardiostimulative) activity.

This minireview is confined to two questions: the site of origin and the chemical nature of DLF, with special reference to the adrenal cortex. In our original studies, we proposed from the correlation between the growth reaction of the heart and the adrenal cortex that the endogenous digitalis-like factor is produced in the adrenals (Schreiber *et al.* 1981a). In addition to the above data concerning the brain (Fishman 1979) and hypothalamus (Haupert and Sancho 1979), Takahashi *et al.* (1986) suggested the adenohypophysis as the site of origin. DLF was also demonstrated in the plasma of patients with acromegaly (Deray *et al.* 1987). These latter authors, however, concluded their study with the statement that "further investigations are required to evaluate the role of the pituitary-adrenal axis in the control of the circulating digitalis-like factor".

Several authors have compared digitalis-like immunoreactivity in various tissue extracts. Castaneda-Hernandez and Godfraind (1984) found the highest activity in the adrenals (but did not ascribe it to a steroid), followed by the kidneys, brain and heart (the heart displayed the weakest activity, while that of the kidneys and brain was almost the same). Štolba *et al.* (1985) found the highest activity in the kidneys, a somewhat lower activity in the brain and definitely less in the heart (they did not examine the adrenals). The finding of DLF in the kidneys (see also Hillyard *et al.* 1976) is further supported by our observation (Schreiber *et al.* 1990) of the

accumulation of radioactivity from labelled antidigitalis antibodies in the kidneys. In addition, Schwartzman *et al.* (1985) found that renal cytochrome P450-related arachidonate metabolite inhibited Na,K-ATPase. It would not be illogical if DLF were to be formed in the kidneys (as an "autocrine" natriuretic factor), but also only accumulated in the kidneys (as the target tissue).

The multitude of suggestions concerning the chemical nature of DLF is bewildering. Ascorbic acid (Ng *et al.* 1985 – also with a review of the earlier literature), non-esterified fatty acids (Wauquier *et al.* 1986), unidentified neutral lipid fractions (Kim *et al.* 1980), bile acids (Toseland *et al.* 1988), lysophospholipids together with non-esterified fatty acids (Kelly *et al.* 1986) and an unidentified amino-glyco-steroid (Cloix *et al.* 1983) have all been cited as the "structure" of DLF. The reader will no doubt have noticed how frequently the lipid hypothesis appears.

Among the known steroids, chlormadinone acetate (Kim *et al.* 1980, LaBella *et al.* 1985, Hnatowich and LaBella 1985) and in cord serum cortisone (Diamandis *et al.* 1985) have been named as potential DLF. Dehydroepiandrosterone sulphate (Vasdev *et al.* 1985) and the spironolactone derivative canrenone (Hannaert *et al.* 1985) are further candidates. The question of whether any of the above substances is physiological DLF is still unanswered.

What, therefore, is the evidence that DLF (if it exists) is formed in the adrenals? Our original findings of digitalis-like immunoreactivity in rat (Schreiber *et al.* 1981b) and rabbit (Schreiber 1981c) adrenal fractions were evidently due to cross-immunoreactivity of various steroid hormones in the digitalis immunoanalysis. This is likewise borne out by the effect of a number of synthetic steroid hormones on $^{86}Rb^{+}$ influx into human erythrocytes, i.e. inhibition of the sodium pump similar to the effect of digitalis glycosides (Schreiber *et al.* 1981d). Further preliminary evidence has come from many laboratories (Solomon 1984, Roulston and Trail 1986, Yamada *et al.* 1986, Pernollet *et al.* 1986, Shilo *et al.* 1987). The last-named authors observed raised urinary excretion of DLF after ACTH and a decrease after dexamethasone. In our laboratory (Horký *et al.* 1989), no changes were observed in the DLF level in human plasma after either ACTH or dexamethasone. On the other hand, Doris (1989) found a decrease in the DLF level in rat blood after adrenalectomy.

The following data on the molecular weight of DLF have been published to date (the presumed structures are given in brackets), 431 (a steroid – Cloix *et al.* 1985), 336 (a steroid – Inagami and Tamura 1988), 550 (a peptide – Horacio *et al.* 1988), 680 (a peptide – Kramer 1989), 600 (a peroxide different from prostaglandins – Masugi *et al.* 1988).

Some obscurities in findings on the structure of the putative DLF can perhaps be explained by new observations by Doris and colleagues. In the first place among all organs studied Doris (1988) found the highest incidence in the adrenals and a lower incidence in the liver. Further studies brought unexpectedly important results. Rat adrenals incubated *in vitro* (Doris and Stocco 1989) release DLF into the medium and this is not inhibited by aminoglutethimide (an inhibitor of steroidogenesis which inhibits the splitting of the cholesterol side chain and the formation of pregnenolone, but not the formation of DLF) and the amount of DLF released into the medium during the first hours of incubation actually rises in the presence of aminoglutethimide. Doris et al (1989) also found that the release of DLF into the medium of mouse adrenocortical tumour Y–1 cultures was not

inhibited by the 17-α-hydroxylase inhibitor SU-10603 or by the 3-β-hydroxysteroid dehydrogenase inhibitor cyanoketone. This means that neither inhibition of splitting off of the cholesterol side chain, nor inhibition of the further phases of steroidogenesis, prevent the release of DLF. DLF could thus be a cholesterol derivative different from the known steroid hormones (formed from cholesterol *via* pregnenolone after the side chain had been split off). At present, the active fractions (HPLC) are not homogeneous. If Doris's hypothesis (Doris and Stocco 1989, Doris *et al.* 1989) of DLF as a derivative of cholesterol were to be confirmed by conclusive structural identification, it might explain both of the above emphatic identifications of DLF with various lipids and of the possibility of cross immunoreactivity of different steroid hormones. In our earlier reports, DLF activity moved in chromatographic fractions which might have been cholesterol derivatives (which we were not looking for) and on one occasion we even arrived at an actual mass spectrometric identification: β-sitosterol (not published, because authentic β-sitosterol did not display DLF activity).

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