Extracellular Release of Lysosomal Phospholipases from the Perfused Adrenal Gland

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Schneider (1968*a,b*) has found that carbamoylcholine, which evokes the release of catecholamines from the adrenal medulla, also causes the release of several acid hydrolases from the perfused bovine adrenal gland. The relative activities of the acid hydrolases released from the gland were similar to those of the enzymes in lysosomes of bovine adrenal medulla (for review see Smith & Winkler, 1969) and so it is likely that the enzymes are released from the lysosomes of the chromaffin cell. These particles contain two enzymes of the phospholipase A type (Smith & Winkler, 1968) that have different pH optima and positional specificities (Winkler, Smith, Dubois & van den Bosch, 1967). Experiments have been carried out to see whether the lysosomal phospholipases A₁ and A₂ are released from the adrenal medulla.

Bovine adrenal glands were perfused with Tyrode's solution and perfusates were collected for 5 min. periods before and during perfusion with carbamoylcholine (Schneider, Smith & Winkler, 1967). The perfusates were analysed for acid deoxyribonuclease activity and for phospholipase A activity with [³²P]phosphatidylethanolamine as substrate (Smith & Winkler, 1968). Enzyme activities are expressed as nmoles/hr. at 37°.

Perfusates collected during control periods from five adrenal glands contained (in 1 min.) 38 ± 8 units of acid deoxyribonuclease activity, 2.1 ± 0.7 units of phospholipase A activity (pH 4.4) and 1.5 ± 0.3 units of phospholipase A activity (pH 6.5). The amount of enzymic activity was higher in perfusates collected during perfusion with carbamoylcholine: the ratio of deoxyribonuclease activity in stimulation-period perfusates to that in control-period perfusates was 4.1 ± 0.6 ; the corresponding ratios for phospholipase A activity were 2.8 ± 0.3 (pH 4.4) and 2.6 ± 0.2 (pH 6.5).

The following observations suggest that the phospholipases released from the gland come from the lysosomes of the adrenal medulla. The effect of pH on the activity was the same as that on the activity of the lysosomal enzymes (Smith & Winkler, 1968), and the activity optimum at pH 4.4 preferentially removed the 1-acyl residue of phosphatidylcholine, whereas that optimum at pH 6.5 preferentially removed the 2-acyl residue, just as was found for the lysosomal enzymes (Winkler *et al.* 1967). In stimulation-period perfusates the ratio of deoxyribonuclease activity to phospholipase A₁ activity was 27 and that of deoxyribonuclease activity to phospholipase A₂ activity was 40; the

corresponding ratios in lysosomes of the adrenal medulla are 30 and 49.

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Lipid Composition at Different Depths in the Cow Snout Epidermis

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In the absence of glucose skin cells degrade endogenous phospholipid (Yardley & Godfrey, 1964; V. J. W. Long & H. J. Yardley, unpublished work). This phenomenon may be important in keratinization since the concentration of glucose in epidermis is only 35–67% of the blood concentration (Halprin, Ohkawara & Adachi, 1967), presumably varying from high values in the basal layer to low values in the upper epidermis. If this is so, the phospholipid content of epidermis should decrease from the basal layer to the stratum corneum.

The cow snout was chosen as a source of thick epidermis. After an area of skin that was free of sebaceous glands had been washed, six slices of epidermis were removed with a Castroviejo Keratotome (Blank, Rosenberg & Sarkany, 1961). Lipids were extracted from the skin slices by the method of Folch, Lees & Sloane-Stanley (1957) and separated by t.l.c. The following lipid classes were obtained: phospholipids, free sterols, free fatty acids, triglycerides and sterol and wax esters. The silica gel, containing the phospholipids, was analysed for phosphorus and the other compounds were assayed colorimetrically after elution from the silica.

A typical experiment gave the following results. Proceeding from the basal layer, the phospholipid content increased at first and then decreased steadily from a maximum of 0.44 µg. of P/mg. of epidermis to 0.09 µg. of P/mg. in the stratum corneum. The sterol content was fairly constant throughout the lower epidermis (2.6–4.1 nmoles/mg. of skin) but greater in the granular layer and stratum corneum (5.7 nmoles/mg.). The triglyceride content of the lower slices of epidermis was small and constant at approx. 0.2 nmoles/mg., but the two upper layers had a much higher content (1.2 and 2.8 nmoles/mg.). The fatty acids gave a similar pattern;

the lower layers of the epidermis contained 0.6 nmole/mg., whereas the two upper layers contained 1.8 and 3.9 nmoles/mg. of skin.

These changes in lipid composition may be attributed to temperature differences and the supply of oxygen or glucose. The increase of triglycerides in the upper layers may be due to an increase in phosphatidate phosphohydrolase activity caused by starvation of glucose. The activity of this enzyme was found to be rate-limiting in liver homogenates from fed rats and to be increased in starved rats (Vavrečka, Mitchell & Hübscher, 1968). The increase of fatty acids in the upper layers of skin may be due to increased phospholipase activity.

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Comparison of Steroid Sulphate and Free Steroids as Substrates of a Crystalline Oxidoreductase

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3 β -Hydroxypregn-5-en-20-one sulphate is now reported to be a substrate for crystalline preparations of 20 β -hydroxy steroid-NAD oxidoreductase (EC 1.1.1.53) from strains of *Streptomyces* (Hübener, Sahrholz, Schmidt-Thomé, Nesemann & Junk, 1959). Kinetic constants for this sulphate [K_m (substrate) 20 μ M with 145 μ M-NADH; K_m (NADH) 4.5 μ M with 100 μ M-substrate; V_{max} 4.0 μ moles/min. $\times$ mg. of enzyme at pH 5.2 at 25°] were similar to those for the corresponding acetate [K_m (substrate) 18 μ M; K_m (NADH) 4.9 μ M; V_{max} 2.7 μ moles/min $\times$ mg. of enzyme] and 4-en-3-one, progesterone [K_m (substrate) 22 μ M; K_m (NADH) 4.6 μ M; V_{max} 20 μ moles/min. $\times$ mg. of enzyme]. 3 β -Hydroxypregn-5-en-20-one itself behaved anomalously, undergoing only partial reduction, and it appeared to inhibit competitively (K_i 34 μ M) the reduction of pregn-4-ene-3,20-dione. This anomalous behaviour was not characteristic of 3 β -hydroxy-5-ene compounds in general. For example, 17 α ,21-dihydroxypregn-4-ene-3,20-dione [K_m (substrate) 7 μ M; V_{max} 8.9 μ moles/min. $\times$ mg. of enzyme] and 3 β ,17 α ,21-trihydroxypregn-5-en-20-one [K_m (substrate) 9 μ M; V_{max} 3.7 μ moles/min. $\times$ mg. of enzyme] behaved normally.

To investigate whether the sulphate and related free steroid substrates reacted at the same centre, the total initial rates of reaction were determined for various concentrations of each of the three possible binary mixtures of 3 β -hydroxypregn-5-en-20-one sulphate, acetate and pregn-4-ene-3,20-dione. In every case the possibility that the substrates reacted independently was excluded. However, the rates of reaction of the mixtures were lower than would result from simple mutual competitive inhibition (Pocklington & Jeffery, 1969).

The similar substrate behaviour of the ionic sulphate and hydrophobic acetate of 3 β -hydroxypregn-5-en-20-one suggests that the region of C-3 is not involved in enzyme-substrate interactions. Steroids and steroid sulphates occur widely, and some general implications of the findings are discussed.

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Stimulation by Glucose of Steroidogenesis from Acetate by the Superovulated Rat Ovary *in vitro*

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Glucose stimulates the incorporation *in vitro* of [14 C]acetate into sterols and steroids by slices of superovulated rat ovary; this effect appears to involve the provision of neither carbon nor energy and occurs in the absence of measurable pentose-cycle activity (Flint & Denton, 1969). Since malate, pyruvate, lactate and isocitrate also stimulate steroidogenesis from acetate the possibility has been considered that these effects are the result of an increased availability of substrates for NADP-malate dehydrogenase and NADP-isocitrate dehydrogenase. These enzymes may play a more important role than glucose 6-phosphate dehydrogenase in the production of NADPH for steroidogenesis in this tissue.

The total activities of NADP-malate dehydrogenase and NADP-isocitrate dehydrogenase are both about four times that of glucose 6-phosphate dehydrogenase (which is present at a total activity of 10.8 units/g. wet wt.), and their K_m values for NADP $^{+}$ are 0.24 and 0.48 respectively of that of glucose 6-phosphate dehydrogenase. All three enzymes are cytoplasmic.