The Effect of Progesterone and Oestradiol on Rat Uterine Monoamine Oxidase Activity

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It appears that rat uterine monoamine oxidase (EC 1.4.3.4) activity may be under hormonal control (G. G. S. Collins, J. Pryse-Davies, M. Sandler & J. Southgate, unpublished work). Groups of 30 rats were injected on 4 consecutive days with oestradiol and progesterone (3.3mg/kg subcutaneously); a third group of untreated animals was used as controls. The enzyme activities of whole uterine homogenates were measured with [14 C]tryptamine, [14 C]dopamine (3,4-dihydroxy[14 C]phenethylamine), [14 C]tyramine and [14 C]benzylamine as substrates (Robinson, Lovenberg, Keiser & Sjoerdsma, 1968); the relative rates of oxidation were 1.00, 2.21, 4.05 and 26.32 by homogenates from untreated rats. Compared with these values, the activity of uteri from progesterone treated animals was higher but differed according to the substrate employed. In contrast, oestradiol pretreatment decreased monoamine oxidase activity. Michaelis constants were not invariably altered by hormonal pretreatment, the effect again being substrate-dependent. The pH optimum with kynuramine (Kraml, 1965), tyramine and dopamine as substrates was between 7.5 and 8.0, whereas with benzylamine a second peak of activity occurred at pH 6.5.

The inhibitory effect of oestradiol could be mimicked *in vitro* and appeared to be competitive with the substrate. In contrast with rat liver (Hawkins, 1952), addition of FAD (0.01–1mM) caused an increase in uterine monoamine oxidase activity to up to 180% of control values.

Uterine monoamine oxidase from the three groups of rats was solubilized by using a modification of the method described by Youdim & Sandler (1967). Electrophoresis on 5% polyacrylamide gel (Youdim, Collins & Sandler, 1968) separated the enzyme into three bands of activity, one migrating towards the anode (MAO₁) and two towards the cathode (MAO₂ and MAO₃). The activity of each band was measured with kynuramine as substrate; of the three bands the activities of MAO₁ and MAO₂ were similar in each group, whereas that of MAO₃ prepared from oestradiol-treated rats was 17% and that from progesterone-treated rats 184% of the same band from untreated animals. In addition, this steroid-sensitive band was more heat-stable than either MAO₁ and MAO₂. The bands of activity appear to represent a polymeric series having molecular weights of about 175 800, 371 500 and 676 100.

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Mixed Lipolytic Activities of an Enzyme Preparation from the Pancreas

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Lipolytic enzymes from various tissue sources have been shown to aggregate with lipid to form high-molecular-weight complexes (Sarda, Maylie, Roger & Desnuelle, 1964; Gelotte, 1964; Downey & Andrews, 1965; Schnatz & Cortner, 1967; Okuda & Fujii, 1967, 1968). These lipoprotein complexes can be delipidized to leave enzyme subunits of much lower molecular weight, which can be reaggregated to higher-molecular-weight forms by incubation and/or sonication with lipid.

In the present study a high-molecular-weight lipolytic preparation, demonstrating the above characteristics, was obtained from acetone-dried pig pancreas powder (Miller, 1969). Chromatography of the preparation on Sephadex G-200 showed that the lipolytic activity was associated with a protein fraction excluded from the gel and eluted at the void volume of the column, and therefore having a molecular weight greater than 800 000. It also formed an insoluble complex with polyvinylpyrrolidone, thus indicating its lipoprotein nature (Cornwell & Kruger, 1961). This high-molecular-weight fraction with lipolytic activity was capable of hydrolysing both the cholesteryl esters in a plasma substrate and triglyceride emulsion in the free or albumin-activated state.

This high-molecular-weight preparation could be converted by acetone or ether extraction into a delipidized product, which on chromatography gave a second protein peak of much smaller molecular weight (15 000–20 000). This molecule also showed the same substrate specificity as the high-molecular-weight parent molecule.

Incubation of this low-molecular-weight preparation with a triglyceride emulsion at 37°C resulted in the production of a fraction that was excluded from Sephadex G-200 and whose substrate specificity and showed it to have lipolytic activity characteristic of cholesterol esterase and triglyceridase.

Since the parent high-molecular-weight preparation, the low-molecular-weight preparation and the reconstituted high-molecular-weight preparation all demonstrated the lipolytic activities associated with cholesterol esterase and triglyceridase, it is postu-