Sex related fibrinolytic activity in rat tissues

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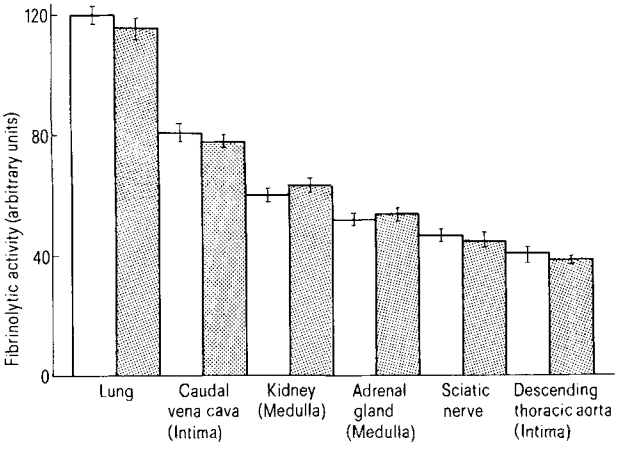
Summary. Histochemical study of many organs of male and female adult Wistar rats of the same age showed no difference in fibrinolytic activity (plasminogen activator activity) between the sexes.

Administration of sex hormones affects the fibrinolytic activity of some human[1,2] and animal[3,4] tissues. Oestrogens influence cell proliferation not only in 'target' tissues but also in other tissues, although this influence is less marked[5]. There is a correlation between cell proliferation and tissue fibrinolytic activity[6]. Furthermore, it has been shown in animals and humans that many enzymes exhibit different activity in blood vessels of the male and female[7], and it is well established that tissue fibrinolytic activity is mainly related to the vascular endothelium. The present study was undertaken to discover possible differences in tissue fibrinolytic activity between the sexes.

Materials and methods. 10 male and 10 female Wistar rats of the same age (11–12 weeks) were killed by a blow on the head, always at the same time of the day (09.00–10.00 h). Female rats were killed at different stages of the oestrus cycle (which lasts 4–5 days), taking into account vaginal smears in correlation with ovarian and uterine changes[8]. Tissue specimens (aorta-thoracic and abdominal-, caudal vena cava, heart, lung, trachea, kidney, urinary bladder, adrenal, liver, spleen, oesophagus, rectum, sciatic nerve, diaphragm, tongue, gastrocnemius muscle and skin) were removed immediately and frozen and stored at −20 °C or below. Specimens from male and female animals were examined histochemically in matched samples under the same experimental conditions (tissue sections from the same anatomical area of each organ, same batch of fibrinogen, etc.). 45 sections (7 µm) from each specimen were examined with Todd's fibrin slide technique as follows: Frozen sections were collected on precleaned microscope slides and briefly dried in air. They were then covered with a film of fibrin by spreading and mixing 60 µl of a solution of 0.7% plasminogen-rich bovine fibrinogen in phosphate buffer (prepared by ammonium sulphate precipitation after Brakman and Astrup[9]) and 10 µl of a solution of bovine thrombin (Leo Pharmaceuticals, Denmark) in saline (20 NIH units/ml) over an area of 2.5 × 4 cm. After clot stabilization for 20 min at 10–15 °C, the slides were incubated at 37 °C in a moist incubator for periods of time ranging from 10 to 30 min for lung, kidney, adrenal, sciatic nerve, aorta and caudal vena cava, from 10 to 60 min for heart, trachea, urinary bladder, oesophagus, rectum, diaphragm, tongue, gastrocnemius muscle and skin and from 30 to 180 min for spleen and liver. After fixation in 10% formaldehyde and staining with Harris' haematoxylin, the slides were mounted with glycerin jelly. Clear foci or areas in the haematoxylin-stained fibrin indicate fibrinolysis caused by activation of the plasminogen in the bovine fibrin film by an activator located to the corresponding area of the tissue section. To test for nonspecific protease activity, some slides were also prepared with plasminogen-free fibrinogen (Poviet, Organon-Teknika, Oss, The Netherlands). The fibrinolytic activity of each section (on plasminogen-rich fibrin slides) was evaluated according to the following grades[1]: grade I, foci of lysis well damarcated; grade II, areas of lysis, often confluent; grade III, lysis covering the entire section. 1, 2 and 3 points were allotted to grades I, II, and III, respectively. The sum of the points scored for each set of the slides was taken as a measure of the fibrinolytic activity of the specimen. The results were analysed statistically by the Wilcoxon's test.

Results. The highest overall fibrinolytic activity (plasminogen activator activity) was found in lung, kidney (medulla), caudal vena cava (intima), adrenal (medulla), descending thoracic aorta (intima) and sciatic nerve; the lowest was found in spleen and liver, while the activity was intermediate in the remainder of the organs examined, with marked differences in the different layers of each organ. Control sections covered with plasminogen-free fibrin showed little or no lysis, indicating that the activity reported above was caused by a plasminogen activator. No significant difference was found between the sexes in the fibrinolytic activity of the tissues examined ($p > 0.05$). The variation in the activity between specimens of the same organ from male and female animals was within the range of the variation found between specimens of the same organ in the same sex (figure).

Discussion. No sex difference in tissue fibrinolytic activity was found even in organs such as the kidney, lung, liver and heart, which are sites of preferential accumulation of administered oestrogens[10] ('target' organs are not included in the comparison) and which show a specific binding to these hormones[11–14]. In addition, the lung is an organ in which plasminogen activator activity is influenced by injected oestrogens[4]. Interestingly, there is also no sex difference in the plasma fibrinolytic activity in Wistar rats[15]. A sex difference in the human blood fibrinolytic activity was noticed by some[16], but not by other investigators[17,18]. Furthermore, no significant difference between men and women in the fibrinolytic response to venous occlusion (release of plasminogen activator) was observed[19]. Human blood fibrinolytic activity is also unaffected by the oestrus cycle[18]. It seems that normally circulating sex hormones (except during pregnancy) have no obvious influence on tissue fibrinolytic activity, with the exception of the genital system[3]. The periodic change in the fibrinolytic activity in endometrium and vaginal epithelial cells during the oestrus cycle of rat[3] represents a specific influence of sex hormones on tissue fibrinolysis. The fibrinolytic activity of large human veins is significantly reduced throughout pregnancy[20], returning to the normal early in the puerperium[21]. The marked and multiple hormonal changes during preg-



Mean fibrinolytic activity in tissues of the male (open columns) and female (hatched columns) rat.

nancy, as well as the high concentration of inhibitors of fibrinolysis in the placenta[22], might be responsible for the observed decrease in the blood fibrinolytic activity mainly in the last months of pregnancy with a rapid return to its nonpregnant level after delivery[23].

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Variation in the lactase dehydrogenase activity of the esophagus[1]

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Summary. Quantitative assay and electrophroetic study of lactate dehydrogenase (LDH) from various tissues of the opossum esophagus were performed. On the basis of expression of the LDH isozymes, we concluded that the smooth muscle of the body of the esophagus carry on more anaerobic glycolysis than the striated muscle. The smooth muscle of the gastroesophageal junction carry on both anaerobic as well aerobic glycolysis.

Histochemical and biochemical studies have shown that metabolism in muscle tissues varies according to muscle types and their pattern of physical activity[2-5]. Recent studies have shown physiological difference in the response of the different segments of esophageal muscle strips to hypoxia[6,7], indicating possible metabolic differences. The purpose of the present investigation was to study the glycolytic metabolism of different segments of esophageal muscle by analyzing the relative activity of the isozymes of lactate dehydrogenase (LDH).

Materials and methods. Studies were performed on both male and female opossums *(Didelphis virginiana)* due to structural and functional similarity of the esophagus in this species to that of man[8]. The animals were anesthetized by i.p. injection of 150 mg/kg of sodium barbital. Under anesthesia, the animals were sacrificed by left thoracotomy and puncture of the heart. The entire esophagus was carefully dissected out with a rim of the stomach and the whole specimen was placed in icecold 0.9% sodium chloride solution. After cleaning the outer surface, the esophagus was opened by an incision from the lesser curvature side of the stomach. Samples were obtained from the following areas: a) pure smooth muscle zone of the body of the esophagus, b) pure striated muscle zone of the body of the esophagus, c) junctional area between the striated and smooth muscle zones, and d) smooth muscle from the gastroesophageal junction. Additionally, samples were also taken from esophageal mucosa and the diaphramatic muscle tissue. The tissues were homogenized separately in cold homogenizing butter (0.15 M KCl, 0.25 M K_2HPO_4, pH 8.54). Crude tissue extracts were obtained by collecting the supernatant following centrifugation at 10,000 × g in refrigerated condition. The crude extracts from these various tissues were then subjected to vertical starch fel electrophoresis. Following electrophoresis, the gel slice was stained for LDH as described by Shaw and Prasad[9]. Spectrophotometric assay of LDH was done by measuring the change in absorbance at 340 nm due to reduction of NAD^+ in a Unicam DB spectrophotometer. The assay mixture contained 2.5 ml and 0.2 M Tris-HCl buffer pH 8.0, 77.5 mM lactate, 5.6 mM NAD^+ and 0.1 ml of crude extract at 25 °C. 1 unit of enzyme is defined as the amount of enzyme producing the conversion of μM of NAD per min. Protein was determined according to Lowry et al.[10].

Results and discussion. Electrophoretic study of muscle extracts showed that the striated muscle of the body of the esophagus, esophageal mucosa, diaphragm striated muscle and the muscle from the gastroesophageal junctional area have all 5 bands of LDH, although the esophago-gastric junctional muscle have very faint LDH-1 as compared to others (figure). The extracts of smooth muscle of the body of the esophagus and the junction of striated and smooth muscle had only 4 molecular forms of LDH. LDH-1 was absent in these 2 tissues. The quantitative measurement of LDH (table) showed that the striated muscle tissues had higher LDH activity as compared to smooth muscle areas.

The enzyme LDH is involved in the glycolytic metabolism. Extensive study on this enzyme has shown that the A and B polypeptides in this enzyme are significantly different in their K_m values with respect to various NAD analogues and in thermostability[11]. It has been also shown that subunit B of LDH is preponderant in tissues with abundant oxygen