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Dopamine-beta-hydroxylase activity in adrenal gland and spleen of rats after fasting and cold exposure*

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Summary. Fasting (48 h) results in dopamine-beta-hydroxylase (DBH) release both in adrenal gland and spleen, suggestive of an increase in the activity of these organs. Cold exposure (48 h) produces a dissociation of the sympathoadrenal response. When both stimuli are simultaneously employed, the DBH response suggests the preponderance of the response to fasting. Plasma DBH is decreased in all groups studied, this could be due to its half-life and the splenic DBH depletion.
Key words. Rat, adrenal; rat, spleen; adrenal, rat; spleen, rat; fasting; cold exposure; dopamine-beta-hydroxylase.

The sympathetic nervous system and adrenal medulla regulate physiological responses to stress and adverse environmental conditions. The secretion of catecholamines and noradrenaline-forming enzyme, dopamine-beta-hydroxylase (DBH) occurs by exocytosis[2]. Several studies have attempted to demonstrate that DBH activity could serve as an index of sympathetic activity[3,4]. In the present study, we have evaluated whether DBH activity in rat adrenal gland, spleen and plasma could be modified by short-term cold exposure and brief fasting.

Material and methods. Male Wistar rats (300–350 g) were used in all experiments. All animals were housed in individual cages for 48 h and divided into 4 groups: 1) Control (n = 8); 2) cold exposure (n = 8): 48 h in a cold room at 4 °C; 3) fasting (n = 8) and 4) fasting plus cold exposure (n = 8). After 48 h, rats were anesthetized with ethyl ether and 1 ml of heparinized blood was obtained by cardiac puncture. Blood samples were collected in chilled tubes and spun at 5000 × g for 10 min at 4 °C. The plasma was stored at −20 °C until it was used for DBH assay. 0.1 ml of blood was used for the glucose determination, as described by Werner et al.[5]. Adrenal glands and spleen were removed and homogenized with an ice-cold 5 mM Tris-HCl solution, pH 7.3, containing 0.2% Triton X-100; after centrifugation (26,000 × g for 10 min), 5-µl (adrenal) and 10-µl (spleen) portions of the supernatants were taken for DBH assay[5]. DBH activity in plasma and in tissues was assayed by a sensitive procedure using tyramine as substrate[4]. In order to obtain an optimal enzymatic activity and overcome the effect of endogenous inhibitors[7], the following copper concentrations were selected: 16.6 µm/tube (adrenal), 33.3 µm/tube (spleen) and 47.6 µm/tube (plasma). In the DBH assay, the optimal plasma volume and the optimal tyramine concentration were 25 µl and 0.645 mM, respectively. The concentration of octopamine standards was 0.2 nm/tube. All samples, blanks and standards were assayed in duplicate in the cold (4 °C). The variability between replicates was 5.6% for adrenal, 4.9% for spleen and 4.1% for plasma. The adequate inactivation of enzyme inhibitors was further tested by adding to a duplicate of each sample a known amount of a partially purified bovine adrenal DBH. Using these aliquots of tissue homogenates and plasma, the recoveries were always greater than 90%; data were not corrected for recoveries. To evaluate the significance of a difference between mean values, Student's t-test was used. A p-value < 0.05 was considered to indicate a significant difference.

Results and discussion. In the present work DBH activity was studied in rat adrenal gland, spleen and plasma after cold exposure and/or fasting for 48 h. The spleen was chosen because of its rich sympathetic innervation.

Fasting for 48 h produces a significant decrease in DBH content of both organs (table) as a result of DBH release, suggesting an increase in the activity of these organs. It is well established that hypoglycemia increases the activity of adrenal medulla[8]. Therefore, the significant decrease of plasma glucose levels in this group (table) could be partly responsible for the increase in adrenal release of DBH. Plasma DBH activity, after fasting, is significantly decreased compared to control (table). This situation can be explained by the following observations; first, DBH in plasma is derived mainly from the sympathetic nerves rather than the adrenal medulla[9]; second, some stimuli that produce activation of the sympathoadrenal system cause

	DBH activity			
	Adrenal	Spleen	Plasma	Plasma glucose
Control	1.368 ± 0.196	0.114 ± 0.004	9.715 ± 0.478	103.670 ± 2.210
Cold exposure	2.306* ± 0.220	0.066* ± 0.007	7.819* ± 0.286	117.980* ± 4.250
Fasting	0.875* ± 0.083	0.015* ± 0.002	6.450* ± 0.290	66.240* ± 1.440
Cold exposure plus fasting	0.899* ± 0.064	0.036* ± 0.0035	6.730* ± 0.150	60.840* ± 1.550

The results are expressed as the mean ± SEM for 8 animals. DBH activity is expressed in nmoles of octopamine/mg of tissue (adrenal and spleen) and nmoles of octopamine/ml of plasma/h. Plasma glucose is expressed as mg/100 ml. * $p < 0.05$ compared to control.

the depletion of tissue DBH content and this depletion can be maintained while the stimulus is continued[2,10], and third, the half-life of the enzyme in plasma is now estimated to be about 7 h, using homologous rat DBH[11]. Accordingly, it can be suggested that fasting for 48 h depletes the DBH content in adrenal medulla and spleen. Released DBH should increase in plasma after the stimulus for a few hours, and later clear; because of the persistence of the stimulus (48 h) and the maintained depletion, plasma DBH levels decrease below the basal value.

After 48 h of cold exposure, the adrenal DBH content is significantly increased; on the contrary, the splenic DBH content is significantly decreased (table). These results suggest, on the one hand, that the adrenal activity is diminished with increase of its DBH content as a result of an impaired release, and on the other hand, that splenic stimulation gives rise to depletion of DBH, although this is less than is seen in the fasting group (table). These results agree with previous reports showing preferential sympathetic nervous system activation (increased plasmatic norepinephrine), whereas the adrenal medulla is only marginally affected after cold exposure[12]. Plasma DBH is also significantly decreased, but less than in the fasting group (table), which is logical since spleen depletion in the cold is smaller than in fasting and, moreover, adrenal exocytosis seems to be practically nonexistent.

Finally, when both stimuli are simultaneously employed, DBH activity is significantly decreased both in adrenal gland and spleen. Plasma glucose and plasma DBH activity are decreased too, both significantly when compared to controls and in a similar way to that seen in the fasting group (table), which suggests, in this situation, the preponderance of the effect of fasting on the sympathoadrenal response.

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Biochemical changes in some acrosomal enzymes of spermatozoa during maturation

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Summary. Goat spermatozoal hyaluronidase and acrosin show significantly increased activities during transition from caput to cauda epididymis. The activity of alkaline phosphatase decreases during spermatozoal transport through epididymis.
Key words. Goat, male reproductive organs; epididymis, caput; epididymis, cauda; testicular fluid; spermatozoa, maturation; enzymes, acrosomal.

Mammalian spermatozoa acquire their motility and fertilizing ability during their transport through the epididymal tract and in the presence of an epididymal fluid environment of special composition[2]. In this process of maturation, changes of spermatozoal motility[3], morphology[4], membrane properties[5], and of acrosomal enzymes, namely phosphatase[6], proacrosin[7] and surface ATPase[8], and other spermatozoal constituents[9] occur during the transition through epididymis. However, almost nothing is known about the biochemical changes that occur in the content of hydrolytic enzymes present in the sperm acrosome during epididymal maturation. In the present investigation, changes in the activities of acrosomal enzymes namely phosphatases, hyaluronidase and acrosin, during maturation of spermatozoa in different regions of epididymis, were demonstrated.

Materials and methods. Male reproductive organs of sexually mature goats were collected from the slaughter house immediately after slaughtering and were brought to the laboratory. The cauda and caput portions of the epididymis were carefully removed and were minced by a fine scalpel[10]. The rete testicular fluid, containing spermatozoa, was collected following the method of Voglmayr et al.[11]. The sperm suspensions (in saline) were centrifuged at 6000 × g for 15 min at 4 °C. The resultant sperm pellets were washed thrice with saline and resuspended in 0.5% Triton ×100 for 30 min at 37 °C[12]. After extraction, the supernatants were collected by centrifugation of the sperm suspension at 10,000 × g for 10 min and were used to assay the phosphatases and hyaluronidase. Acid and alkaline phosphatase activities were determined according to the method of Michell et al.[13] using para-nitrophenyl phosphate (Merck, Germany) as substrate. Hyaluronidase activity was determined following the method of Zaneveld et al.[14] using hyaluronic acid (Sigma) as substrate.

For assay of acrosin, spermatozoa were extracted with 10% glycerol-HCl, pH 2.8, containing 50 mM benzamidine (Sigma) for estimation of total activity. After centrifugation at 27,000 × g for 30 min at 4 °C, extracted supernatant was dialyzed (using Spectrapor 1) against 0.001 M HCl, pH 3.0 to