Purification of pig kidney diamine oxidase (histaminase) and in vivo effects on passive cutaneous anaphylaxis and histamine induced bronchoconstriction of guinea-pigs

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Summary. Histaminase (diamine oxidase) was purified from pig kidney using cadaverine-Sepharose affinity chromatography. Pretreatment of guinea-pigs with histaminase caused marked inhibition of the passive cutaneous anaphylaxis. The effect of histaminase on histamine induced bronchoconstriction in vivo was studied. Histaminase significantly reduced the response in guinea-pigs. Histaminase is effective as a potent antihistaminic agent in vivo.

The role of histamine in allergic reactions, especially of the immediate type, has received considerable attention, but most work has concentrated on its mode of secretion from mast cells or basophils. Histaminase (diamine oxidase, EC 1,4,3,6) oxidatively deaminates histamine and aliphatic amines such as putrescine and cadaverine. In the present report we purified the histaminase from pig kidney using a cadaverine-Sepharose, and studied the in vivo effects of histaminase on guinea-pig passive cutaneous anaphylaxis and histamine-induced bronchoconstriction in guinea-pigs.

Materials and methods. Fresh pig kidney cortex (total 5 kg) was homogenized in 5 l of 0.03 M phosphate buffer, pH 7.0 for 3 min in a Waring blender and centrifuged. The supernatant was fractionated with ammonium sulfate (30–60% saturation), followed by dialysis against 0.03 M phosphate buffer, pH 7.0. The dialysate was applied to a DEAE-Sephadex A-50 column (3×40 cm) equilibrated with 0.03 M phosphate buffer. After the column was washed with 0.03 M phosphate buffer, the enzyme was eluted stepwise with 0.07 M and 0.1 M phosphate buffer. The active fractions were combined and concentrated by the addition of ammonium sulfate (60% saturation) and dialyzed[1]. All operations were carried out at 4 °C.

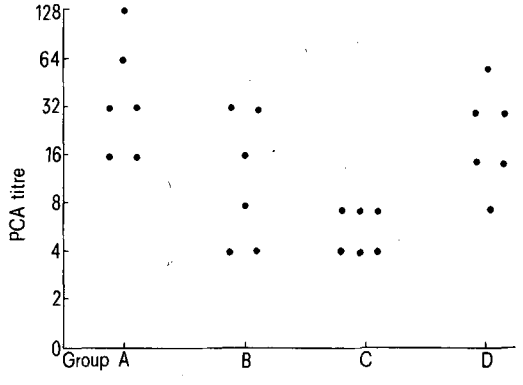

Figure 1. The effects of histaminase on the PCA titre in guinea-pigs. The PCA titres were expressed as the reciprocal of the greatest dilution of antiserum giving a positive reaction. Group A; treated with saline; group B; treated with 3000 units of histaminase 10 min prior to antigen challenge; group C; treated with 10,000 units of histaminase 10 min prior to antigen challenge; group D; treated with 10,000 units of histaminase 30 min prior to antigen challenge.

50 ml of settled cadaverine-Sepharose was added to the histaminase fraction eluted from the DEAE-Sephadex column and the mixture was stirred for 30 min at 4 °C. The entire contents were placed in a 1-l sintered glass funnel and contaminant proteins were eluted with sequential washes of starting buffer containing 1 M NaCl. When the A_{280} of the eluant was less than 0.01, the Sepharose was washed with buffer containing 0.1 m NaCl and then transferred to a 4×10 cm column. Histaminase was then eluted with the same buffer containing heparin (300 units/ml). The column was eluted at a flow rate of 50 ml/h. Histaminase activity was measured by the method of Beaven and Jacobson[2]. Protein concentration was determined by the method of Lowry.

Passive cutaneous anaphylaxis was studied using 24 male guinea-pigs weighing 250 g. The guinea-pigs were divided into 4 groups. 3 groups were treated with histaminase prior to antigen challenge and the other was injected with an equal volume of saline (group A). Dilutions of rabbit anti-bovine serum albumin (BSA) serum in 0.1 ml of saline were injected i.d. into the shaved dorsal skin of guinea-pigs. 3 h later, histaminase (3000 units in group B and 10,000 units in group C) in 1 ml of saline were injected i.v. and 10 min later the animals were injected i.v. with 1 ml of BSA together with 1.0 ml of 0.3% Evans blue saline solution. The animals of group D were injected with histaminase (10,000 units) and 30 min later BSA was challenged. The animals of all groups were killed 45 min later and the reactions on the

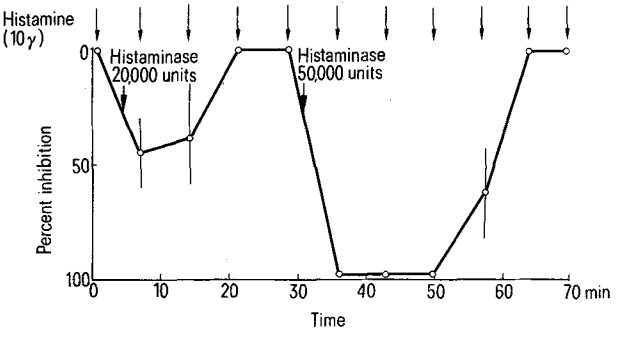

Figure 2. Effects of histaminase on histamine-induced bronchoconstriction. Histaminase inhibition of histamine-induced bronchoconstriction was expressed as percent of maximal bronchoconstriction (n=4).

Purification of pig kidney histaminase

Stage	Protein (mg) (mg)	Total units	Specific activity (units/mg)	Purification (fold)	Yield (%)
Homogenate	112,500	6,300,000	56	1	100
Ammonium sulfate	35,200	5,400,000	153	2.7	86
DEAE Sephadex	4,500	4,600,000	1,033	18.3	73
Ammonium sulfate	2,850	4,100,000	1,493	25.7	65
Cadaverine-Sepharose	9	1,260,000	140,000	2,500	20

inner side of the skin were read. The PCA titres were expressed as the reciprocal of the greatest dilution of serum giving a visible skin reaction. The magnitude of the reaction was expressed by the mean of the long and short diameter of the reaction site.

Histamine-induced airway constriction was studied using 4 guinea-pigs anesthetized with an i.p. injection of pentobarbital sodium (80 mg/kg). For i.v. injections a small polyvinyl chloride catheter was inserted through a skin window into a peripheral vein. Airway resistance was then measured by the overflow technique. The trachea was cannulated and the animal ventilated by means of a Starling miniature respiration pump at a rate of 60 strokes/min. The air overflow from the water valve passed through a pneumotachograph tube connected to a differential air pressure transducer. Changes in overflow were displayed on a recorder. The animal was challenged with various doses of histaminase and histamine. Inhibition of histamine induced bronchoconstriction was recorded as percent of the maximal overflow. Histaminase and histamine were injected within 30 sec.

Results and discussion. The enzyme purification is summarized in the table. With this method histaminase activity was purified 2500-fold from starting materials. The titre of passive cutaneous anaphylaxis was 32 or higher in group A (control) but in group B (3000 units of histaminase) the titre was 32 or lower in all animals. The titre was greatly decreased in group C (10,000 units) to 8 or lower but the effect of histaminase on PCA disappeared within 30 min (group D). The PCA reaction was decreased in size in the animals pretreated with histaminase. At the site injected i.d. with 32 times diluted antiserum the mean diameter of the reaction in group A was 23.5 mm, but in the group B the mean diameter was decreased to 5.5 mm (fig. 1).

I.v. injection of histamine resulted in a marked increase in airway resistance. The time course of airway resistance after i.v. infusions of histaminase and histamine is given in figure 2. 50,000 units of histaminase completely inhibits the histamine-induced bronchoconstriction, but its effect in vivo disappears within 30 min. I.v. infusion of pig kidney histaminase did not cause any allergic side-reaction in guinea-pigs.

Histaminase may be a useful tool in respiratory physiology to distinguish the action of histamine from that of other mediators.

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Effect of streptozotocin diabetes on adenosine-5′-triphosphate, oxygen consumption and steroidogenesis in testis mitochondria from rats

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Summary. The activity of the enzyme cleaving the side-chain of cholesterol (rate limiting step in steroidogenesis) was considerably reduced in experimentally induced diabetes. This result was accompained by both an increase in oxygen consumption and an increase in ATP synthesis. Insulin treatment prevented them.

It is well known that the diabetic state produces reproduction disturbances such as impaired fertility and reduced steroidogenic activity[2-5]. On the other hand it is likely that the respiratory chain and the cholesterol side-chain cleavage enzyme complex of testis mitochondria are linked in some manner, but it is not clear whether energy obtained from substrate oxidation can support both ATP production and mitochondrial steroidogenesis.

We have investigated this problem by studying the metabolism of testis mitochondria from diabetic rats and from diabetic rats treated with insulin.

Material and methods. Male Sprague-Dawley rats, weighing 250–300 g were used in all experiments. Diabetes was induced in 2 groups of animals by i.p. administration of streptozotocin (STZ) solution made in 0.1 M citrate buffer, pH 4.5 at a dose of 40 mg/kg b.wt. 2 days after administration of STZ, 1 group of the diabetic rats was given a protamine zinc insulin s.c. injection at the same time each morning for 28 days, in an amount (1–5 IU) that was adequate for normalizing the diabetic state. The rats were used 30 days after injection of STZ and were killed by decapitation without anesthesia. Testes were removed, decapsulated and homogenized in 250 mM sucrose solution pH 7.4 containing 20 mM KCl and 1 mM EDTA. Mitochondrial ATP synthesis and [4-^{14}C]-cholesterol conversion were determined simultaneously under the same experimental conditions. The mitochondrial preparations were obtained by the method of Dimino et al.[6] and mitochondrial ATP synthesis rate was assayed by measuring the disappearance of inorganic phosphate used for mitochondrial phosphorylation of ADP by trapping the synthesized ATP as glucose-6-phosphate[6,7]. Steroidogenic

Effect of diabetes and insulin treatment on: glycemia; body weight; testicular weight; oxygen utilization, ATP synthesis and cholesterol conversion by testis mitochondria

	Glycemia post prandial serum glucose	Body weight at autopsy (g)	Testes weight (g/100 b. wt)	Oxygen uptake (µl/mg protein × h)	ATP synthesis (µmoles/mg protein × 2 h)	% [4− ^{14}C]-cholesterol conversion/mg protein × 2 h
Control	105± 7	314±15	1.31±0.06	9.7±1.2	96.7± 7.7	6.01±1.7
Diabetic	358±23*	154±13*	0.75±0.07*	18.1±2.9*	156.7±22.6*	3.57±1.4*
D+insulin	115±10	252±14	1.25±0.03	10.9±0.1	124.9±22.2	6.99±0.6

*Significantly different from control rats. Statistical significance is given in the text.