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LYSOSOMAL ENZYMES IN THE BOVINE ADRENAL GLAND

A COMPARISON OF MEDULLA AND CORTEX

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Abstract—Acid ribonuclease, acid deoxyribonuclease, cathepsin, acid phosphatase and β-glucuronidase activity were measured in 0·3 M sucrose homogenates of bovine adrenal medulla and cortex. Significant amounts of each of these enzymes were associated with the mitochondrial fractions of each tissue. Sucrose density gradient centrifugation of the mitochondrial fractions showed that the acid hydrolases were present in particles distinct from mitochondria and, in medulla, distinct from chromaffin granules. Each of the hydrolases exhibited an acid pH optimum.

Acid hydrolase activity of homogenates and of density gradient fractions was increased after several freeze–thaw cycles, upon incubation at 50° for 10 min, upon incubation at 37°, pH 9·0, for 15 min, after exposure to 0·1% Triton X-100, and with sonication for 60 sec. It was concluded from these data that in both cortex and medulla the acid hydrolases are associated with a population of subcellular organelles that possess the characteristics of lysosomes.

STIMULATION of the perfused adrenal gland with acetylcholine-like drugs causes the secretion of catecholamines,[1] adenine nucleotides and their derivatives[2] and protein.[3–8] The protein secreted from the bovine adrenal gland consists of chromogranin A,[4–8] the major soluble protein of chromaffin granules,[6] as well as other soluble proteins of chromaffin granules.[7] Hydrolytic enzymes typical of those associated with lysosomes are also secreted from the perfused bovine adrenal gland.[9]

The presence of lysosomes in the adrenal cortex,[10] in the adrenal medulla,[11–13] and in phaeochromocytoma[14] has been demonstrated by histochemical methods. Lysosomal enzymes have also been shown by biochemical methods to be present in both portions of the gland.[15–18] Smith and Winkler[18] showed that six hydrolytic enzymes in the bovine adrenal medulla were found in organelles distinct from both mitochondria and chromaffin granules, and were in all likelihood associated with lysosomes. A similar investigation of the lysosomes of adrenal cortex, however, has not been reported. A quantitative comparison of lysosomal enzymes of medulla and cortex is presented here, and the results of this study are used in the following paper[19] in which the secretion of acid hydrolases from the isolated bovine adrenal gland is examined.

MATERIALS AND METHODS

Fractionation of homogenates. Adrenal glands were removed from cows within 15 min after death, and stored in ice until they were used. The cortex and medulla were

separated and each placed in several volumes of chilled 0·3 M sucrose. The following operations were carried out at temperatures near 0°. The tissue was minced with a knife blade, then homogenized in 3 vol. of 0·3 M sucrose in two stages with a Potter–Elvehjem homogenizer. In the first stage, three strokes were made with a loose-fitting Teflon pestle (total clearance, 0·08 mm) and in the second stage the tissue was rehomogenized with three strokes using a Teflon pestle with a total clearance of 0·04 mm.

The homogenates were centrifuged at 550 *g* for 15 min. The resulting supernatants (cytoplasmic extract) were centrifuged at 12,500 *g* for 20 min to give the sediments (large-granule fractions[18]) and the postlarge-granule fraction supernatants. In some experiments this supernatant was centrifuged at 120,000 *g* for 60 min to give a microsomal pellet and a high-speed supernatant.

The large-granule fractions were further fractionated by density gradient centrifugation. For medulla this was accomplished with the use of a sucrose gradient method similar to that described by Smith and Winkler,[18] except that the gradient consisted from bottom to top of 1 ml of 2·2 and 3·5 ml each of 1·6, 1·5, 1·4 and 1·3 M sucrose. Centrifugation was carried out in the Spinco swinging-bucket type 25·3 rotor at 100,000 *g* for 120 min. The cortex large-granule fraction was resuspended in 3 vol. of 0·3 M sucrose. One ml of this suspension was centrifuged in the Spinco swinging-bucket type 25·3 rotor at 100,000 *g* for 180 min over a gradient consisting from bottom to top of 1·5 ml each of 2·0, 1·8, 1·7, 1·6, 1·4, 1·3, 1·2, 1·1, 1·0 and 0·9 M sucrose. After centrifugation the tubes were cut with a Spinco tube cutter to give 6 fractions (see Fig. 1). The fractions were dialyzed for 16 hr against hypotonic Tris-sodium succinate buffer,[18] pH 5·9 (I = 0·05). Fractions were diluted with this same buffer before they were assayed for enzyme activity.

Analytical methods. Catecholamines were assayed by the colorimetric method of von Euler and Hamberg,[20] using citrate–phosphate buffer at pH 6·0,[21] and are expressed as μmoles of epinephrine. Protein was precipitated by trichloracetic acid [final concentration of 5%, (w/v)], and measured by the microbiuret method according to Goa.[22] Protein assays were standardized with bovine serum albumin.

Acid phosphatase (APase), and β-glucuronidase (β-GLUCase) activities were measured by the methods of Gianetto and de Duve,[23] and cathepsin (CATH) activity was measured by the procedure of Anson.[24] Acid ribonuclease (RNase) and acid deoxyribonuclease (DNase) activities were measured according to Smith and Winkler,[18] except that acid ribonuclease activity was measured at pH 5·2 instead of 5·5 since acid ribonuclease activity in perfusates from isolated bovine adrenal glands was measured at this pH in order to minimize the activity of alkaline ribonuclease present in the perfusates.[1] This pH is slightly more acid than the pH optimum of the enzyme, which is 5·5–5·6. Studies at pH 5·2 and 5·5 indicate that with both cortex and medulla acid ribonuclease, the enzyme activity is 10 to 15 per cent lower at the more acid pH. The final concentrations of the buffers used for the assay of enzyme activities at different pH values were 0·07 M acetate for deoxyribonuclease (pH 3·7–5·7), 0·07 M Tris-sodium succinate for ribonuclease (pH 4·4–6·5), 0·14 M acetate for β-glucuronidase (pH 4·0–6·4), and 0·07 M citrate for cathepsin (pH 2·8–6·0); sodium hydroxide was used to adjust the pH of the buffers. Acid phosphatase activity was measured in 0·136 M Tris-acetate (pH 2·4–6·5). Fumarase activity was measured according to Racker,[25] and lactate dehydrogenase activity by the procedure of

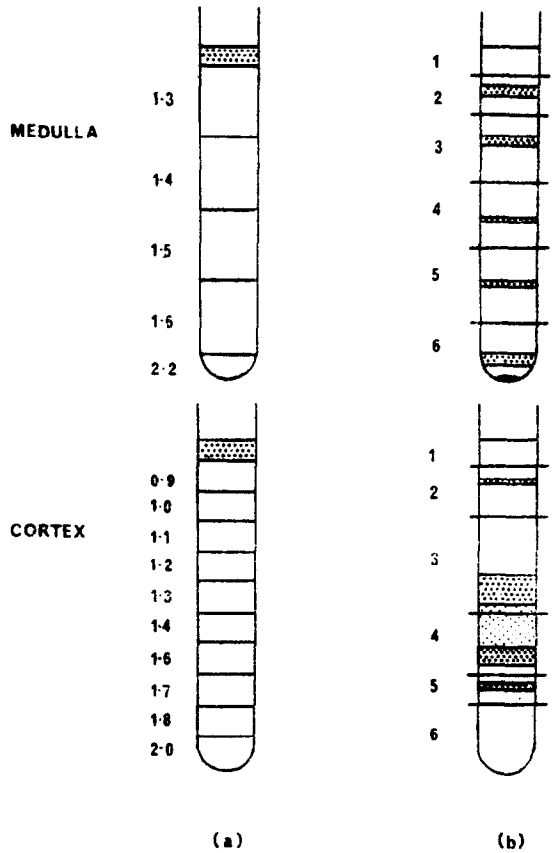

FIG. 1. Sucrose gradient centrifugation of medulla and cortex large-granule fractions. (a) Composition of the gradients before centrifugation. Figures refer to the molarity of sucrose. (b) Appearance of the tubes after centrifugation at 100,000 *g* for 120 min for medulla and 100,000 *g* for 180 min for cortex. The horizontal lines show the positions at which the centrifuge tubes were cut; the fractions are labeled 1 to 6.

Wroblewski and LaDue.[26] Fumarase and LDH were assayed at 24°. Other enzyme assays were performed at 37°. Enzyme activity is expressed as μmoles of substrate transformed per unit of time under the conditions of assay. Enzyme activities were linear with respect to time of incubation and to the amount of enzyme added to the assay mixture. Appropriate substrate and enzyme blanks were determined for each assay and the assays were performed in duplicate. For the experiments in which inhibition of acid deoxyribonuclease was examined, appropriate inhibitor blanks were run in parallel.

Latency experiments. Sonication was performed with an MSE ultrasonic disintegrator at the maximal output setting. In some experiments samples were subjected to homogenization with the Willems Polytron homogenizer at a setting of 4. Freeze–thawing was done by rapid immersion of the samples in a slurry of solid CO_2 and acetone, followed by thawing at 10°. In some experiments 1 vol. of 1% (v/v) aqueous Triton X-100 was added to 9 vol. of the assay mixture; an equal volume of distilled

water was added to controls. Liberation of bound enzyme activity was measured in either of two ways: (a) aliquots of treated and untreated control samples were assayed directly using a 10-min incubation period, or (b) treated and untreated samples were centrifuged at 15,000 *g* for 20 min, and an aliquot of the resulting supernatant was assayed.

Chemicals. Highly polymerized calf thymus DNA (substrate for acid deoxyribonuclease), bovine hemoglobin type I (substrate for cathepsin, phenolphthalein glucuronic acid sodium salt (substrate for β-glucuronidase) and DL-β-glycerophosphate disodium salt pentahydrate (substrate for acid phosphatase) were obtained from Sigma Chemical Co., St. Louis, Mo.; yeast RNA (substrate for acid ribonuclease) from Calbiochem, Los Angeles, Calif.; L-malic acid (substrate for fumarase) and pyruvic acid sodium salt (substrate for lactate dehydrogenase) from Eastman Organic Chemicals, Rochester, N. Y.

RESULTS

Acid hydrolases of homogenates. The total activities of five typical lysosomal hydrolases in sucrose homogenates of medulla and cortex are shown in Table 1.

TABLE 1. ACID HYDROLASE ACTIVITIES IN HOMOGENATES OF BOVINE ADRENAL MEDULLA AND CORTEX*

Acid hydrolases	Medulla	Cortex
Acid ribonuclease	1·18 ± 0·20 (5)	0·72 ± 0·09 (8)
Acid deoxyribonuclease†	0·51 ± 0·10 (5)	0·15 ± 0·02 (6)
Cathepsin	0·91 ± 0·17 (4)	1·30 ± 0·28 (8)
Acid phosphatase†	0·26 ± 0·05 (4)	0·70 ± 0·12 (6)
β-Glucuronidase‡	0·07 ± 0·01 (5)	0·26 ± 0·08 (9)

* The units of activity are μmoles of substrate converted per minute per gram tissue. The figures are means ± S.E. The number of experiments is shown in parentheses.
† $P < 0·02$ for medulla vs. cortex.
‡ $P < 0·05$ for medulla vs. cortex.

Latent enzyme activity of the homogenates was released by lysis in hypotonic buffer prior to analysis. Activity of acid deoxyribonuclease is higher in medulla than in cortex, whereas acid phosphatase and β-glucuronidase activities are higher in cortex.

Centrifugation. Significant portions of the acid hydrolase activities of the cytoplasmic extracts were associated with the large-granule fraction (Table 2). Fumarase activity was also associated with the large-granule fraction. For medulla, 69·3 ± 11·6 ($n = 2$) per cent, and for cortex 67·0 ± 1·6 ($n = 3$) per cent of the fumarase activity of the cytoplasmic extract was sedimented with the large-granule fraction. Total recovery of fumarase was 91·0 ± 8·9 per cent for medulla, and 82·3 ± 5·6 per cent for cortex. Only a small portion of the cytoplasmic extract lactate dehydrogenase activity was sedimentable; 5·9 per cent for medulla ($n = 1$; total recovery, 88·4 per cent) and 7·6 per cent for cortex ($n = 1$; total recovery, 94·2 per cent).

The particulate nature of the hydrolytic enzymes was further investigated by subjecting the large-granule fractions to sucrose density gradient centrifugation (Fig. 1). In both tissues an acid hydrolase-rich fraction was separated from the main

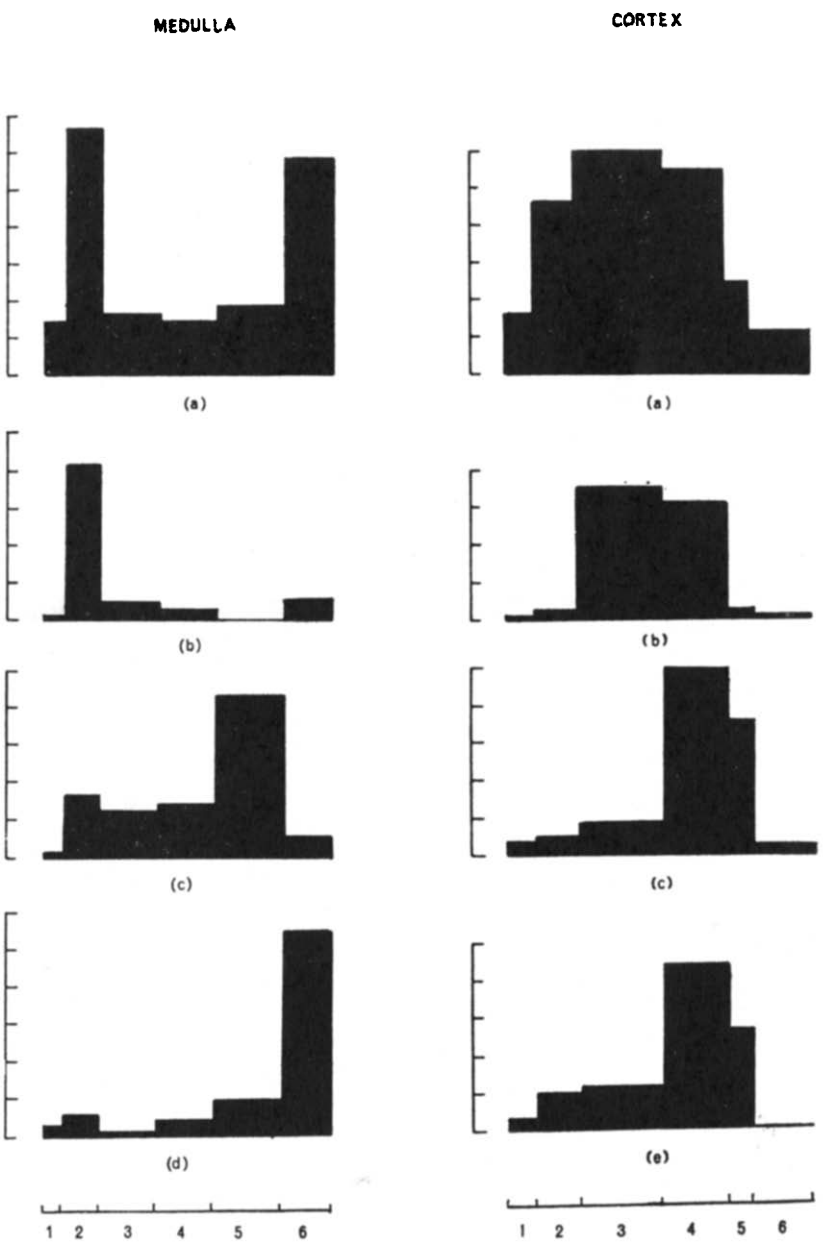

FIG. 2. Analysis of fractions obtained by sucrose density gradient centrifugation of medulla and cortex large-granule fractions. The abscissa is divided according to the volumes of the fractions and the numbers refer to the fractions of the gradient illustrated in Fig. 1. The ordinates are divided into artitrary units per ml, and the value of 1 arbitrary unit is given below. The values for the enzymes are expressed in μmoles substrate per hour per ml of fraction. (a) Protein (1 mg/ml); (b) fumarase (56·8/ml for medulla, 28·4/ml for cortex); (c) acid ribonuclease (2·0/ml for medulla, 1·0/ml for cortex); (d) catecholamines (1·0 μmole/ml); (e) β-glucuronidase (0·1/ml). Recoveries of material put on the gradients ranged between 65·3 per cent for medulla acid ribonuclease and 90·8 per cent for cortex acid ribonuclease.

TABLE 2. SEDIMENTATION AND RECOVERY OF ACID HYDROLASES OF BOVINE ADRENAL MEDULLA AND CORTEX*

Tissue	Fraction	RNase	DNase	CATH	APase	β-GLUCase
Medulla	$\frac{\text{LGF}}{\text{Cytoplasmic extract}} \times 100$	83·5 ± 9·1 (4)	84·7 ± 9·4 (3)	45·4 ± 8·6 (3)	32·1 ± 3·4 (4)	66·7 ± 3·0 (3)
	$\frac{\text{LGF} + S_2}{\text{Cytoplasmic extract}} \times 100$	107·7 ± 10·1 (4)	104·8 ± 10·6 (3)	94·3 ± 4·9 (3)	72·2 ± 8·1 (4)	100·4 ± 1·6 (3)
Cortex	$\frac{\text{LGF}}{\text{Cytoplasmic extract}} \times 100$	81·8 ± 14·0 (4)	79·7 ± 5·1 (3)	46·7 ± 7·4 (3)	57·9 ± 7·0 (3)	59·3 ± 6·0 (5)
	$\frac{\text{LGF} + S_2}{\text{Cytoplasmic extract}} \times 100$	96·6 ± 18·6 (4)	95·3 ± 5·6 (3)	86·3 ± 9·0 (3)	108·7 ± 10·2 (3)	91·7 ± 3·6 (5)

* Cytoplasmic extracts, large-granule fractions (LGF) and postlarge-granule fraction supernatants (S_2) were obtained from homogenates as described in the Methods section. The figures are means ± S.E. The number of experiments is shown in parentheses.

TABLE 3. RELATIONSHIPS BETWEEN ACID HYDROLASES OF BOVINE ADRENAL GLAND*

Enzyme activity ratios	Medulla	Cortex
RNase/DNase	2·99 ± 0·12	4·01 ± 0·47
RNase/CATH†	2·19 ± 0·24	0·88 ± 0·16
RNase/APase‡	25·27 ± 2·76	3·34 ± 1·00
RNase/β-GLUCase‡	47·49 ± 2·90	15·00 ± 2·32

* Enzyme activities were measured in lysosomal fractions obtained by density gradient centrifugation of medulla and cortex large-granule fractions. The figures are means ± S.E. The number of assays ranged between 5 and 8.
† $P < 0·01$ for medulla vs. cortex.
‡ $P < 0·001$ for medulla vs. cortex.

mitochondria-containing fraction, indicated by the distribution of fumarase (Fig. 2). For medulla, the distribution of acid ribonuclease is also different from that of chromaffin granules, indicated by the pattern of catecholamine distribution. The fraction rich in acid hydrolase activity is found in the 1·5–1·6 M sucrose region for medulla and 1·6–1·7 M sucrose band for cortex.

Total activity for each hydrolytic enzyme was measured in the acid ribonuclease-rich fraction (cut 5) from each tissue (Table 3). The ratios of acid ribonuclease activity to that of cathepsin, acid phosphatase and β-glucuronidase for medulla and cortex, respectively, are significantly different.

Acid hydrolase pH optima. Each of the 5 acid hydrolases in the acid hydrolase rich density gradient fractions was assayed over a range (see Methods section) of acid pH values (Table 4). Each enzyme exhibited an optimal activity at an acid pH. The activities of the acid phosphatases from both tissues were demonstrable over a fairly broad pH range.

TABLE 4. pH OPTIMA FOR ACID HYDROLASES OF BOVINE ADRENAL GLAND*

Acid hydrolases	Medulla	Cortex
Ribonuclease	5·6	5·5–5·6
Deoxyribonuclease	4·6–4·7	4·6–4·7
Cathepsin	3·2 (shoulder, 4·6)	3·2 (shoulder, 4·6)
Acid phosphatase	3·5–4·5	3·5–4·5
β-Glucuronidase	4·8	4·8

* Acid hydrolase activities in lysosomal fractions obtained by sucrose density gradient centrifugation of large-granule fractions were measured with the buffers described in the Methods section.

TABLE 5. ACID HYDROLASE LATENCY IN HOMOGENATES OF BOVINE ADRENAL MEDULLA AND CORTEX*

Tissue	Enzyme	Treatment		Activity in treated / Activity in control
Medulla	β-GLUCase	Exposure to hypotonic media	(a)	3·29
		Sonication	(a)	2·55
	DNase	Triton X-100, 0·1%	(b)	7·1
	RNase	Triton X-100, 0·1%	(a)	24·3
		Heat, 55°; 10 min		17·8
Cortex	β-GLUCase	Exposure to hypotonic media	(a)	3·14
		Sonication	(a)	2·65
	DNase	Triton X-100, 0·1%	(b)	2·7
	RNase	Freeze/thaw, four times in 0·3 M sucrose	(a)	10·2
		Heat; 55°, 10 min	(b)	4·6

* Samples were assayed for enzyme activity either by (a) direct assay of the homogenate after the treatment indicated, utilizing a 10-min incubation period, or (b) by analysis of the supernatant obtained after centrifugation at 15,000 g for 20 min of the treated homogenate. The figures represent the ratio of free-enzyme activity after treatment to that of untreated samples.

Latency of acid hydrolases. Acid hydrolase activities of medulla and cortex homogenates were increased after exposure to conditions that release enzyme activity from an inactive or bound form (Table 5). The acid hydrolases in large-granule fractions of both medulla and cortex also exhibited latency. Figure 3 shows the effect of sonication on the liberation of β-glucuronidase activity from the large-granule fractions of medulla and cortex. Additional means of demonstrating acid hydrolase latency in the large-granule fractions are summarized in Table 6. In all cases enzyme activity was greater after exposure of the sample to lytic conditions.

Inhibition of acid deoxyribonuclease. The effect of sample dilution upon acid deoxyribonuclease activity of cortex cytoplasmic extract, resuspended large-granule fraction, and postlarge-granule fraction supernatant is shown in Fig. 4. Enzyme activity is linear with respect to the amount of large-granule fraction present in the incubation mixture, whereas for the cytoplasmic extract and the postlarge-granule fraction supernatant the activity falls off at the highest concentration of sample. The effect of sample dilution on acid deoxyribonuclease activity is also evident from the deoxyribonuclease activities and recoveries given in Table 7. Acid deoxyribonuclease activity increases after 10-fold dilution of the homogenates. A corresponding increase

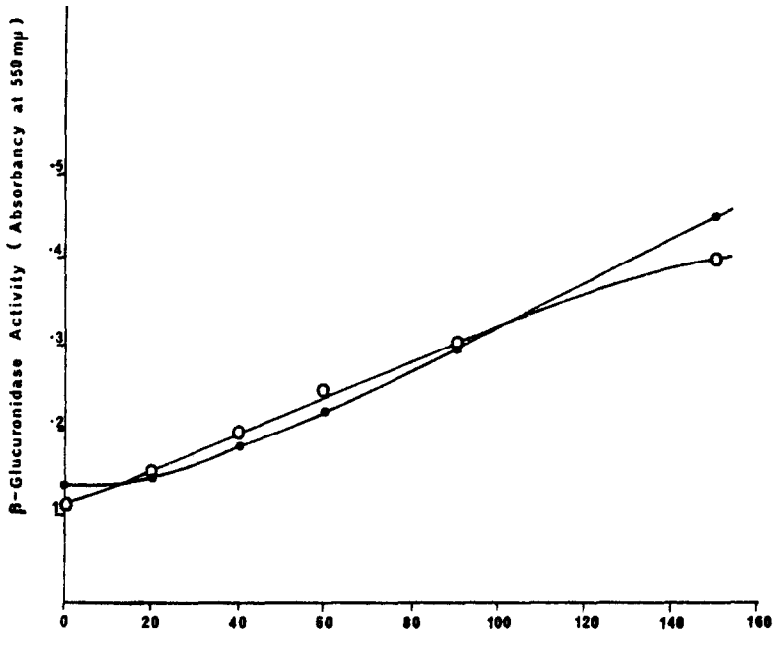

FIG. 3. The effects of sonication on the level of free β-glucuronidase activity in medulla and cortex large-granule fractions. ○——○, medulla; ●——●, cortex. Medulla and cortex large-granule fractions were resuspended in 3 vols. of 0·3 M sucrose and sonicated with an MSE ultrasonic disintegrator. At the end of each period of sonication 0·5 ml aliquots of the suspension were withdrawn and assayed for β-glucuronidase activity at 37° utilizing a 10-min incubation period. The optical density of the phenolphthalein formed during the 10-min incubation period is given on the ordinate.

TABLE 6. ACID HYDROLASE LATENCY IN LARGE-GRANULE FRACTIONS OBTAINED FROM BOVINE ADRENAL MEDULLA AND CORTEX*

Tissue	Enzyme	Treatment		Activity in treated / Activity in control
Medulla	RNase	Triton X-100, 0·1%	(b)	20·0
		Heat, 55°, 5 min	(b)	16·7
		Freeze/thaw, four times (in hypotonic medium)	(b)	14·3
		Incubation at pH 9·0 for 15 min at 37°	(a)	3·3
	β-GLUCase	Triton X-100, 0·1%	(a)	2·6
	DNase	Sonication, 60 sec	(b)	16·7
	CATH	Triton X-100, 0·1%	(b)	10·5
		Polytron homogenization (30 sec)	(b)	7·3
Cortex	RNase	Freeze/thaw, four times	(b)	5·3, 10·0
		Heat; 55°, 5 min	(b)	4·6
		Incubation at pH 9·0 for 15 min at 37°	(a)	12·7
	DNase	Sonication, 60 sec	(b)	10·6
	CATH	Triton X-100, 0·1%	(b)	4·2
		Polytron homogenization	(b)	10·5
	APase	Triton X-100, 0·1%	(a)	14·5, 8·2
	β-GLUCase	Freeze/thaw, four times	(b)	5·6, 5·6
		Triton X-100, 0·1%	(a)	4·8

* Samples were assayed for enzyme activity either by (a) direct assay of the homogenate after the treatment indicated, utilizing a 10-min incubation period, or (b) by analysis of the supernatant obtained after centrifugation at 15,000 *g* for 20 min of the treated homogenate. The figures represent the ratio of free-enzyme activity after treatment to that of untreated samples.

was not observed after dilution of the large-granule fractions. Furthermore, the sum of acid deoxyribonuclease activity in the large-granule fractions and in the postlarge-granule fraction supernatants was much greater than that of the cytoplasmic extracts for undiluted samples; the recovery was close to 100 per cent after dilution.

The results presented in Table 7 and in Fig. 4 indicate that there is a nonsedimentable inhibitor of acid deoxyribonuclease present in the tissue, and that inhibitor activity is lost upon sufficient dilution. Accordingly, deoxyribonuclease activity in the large-

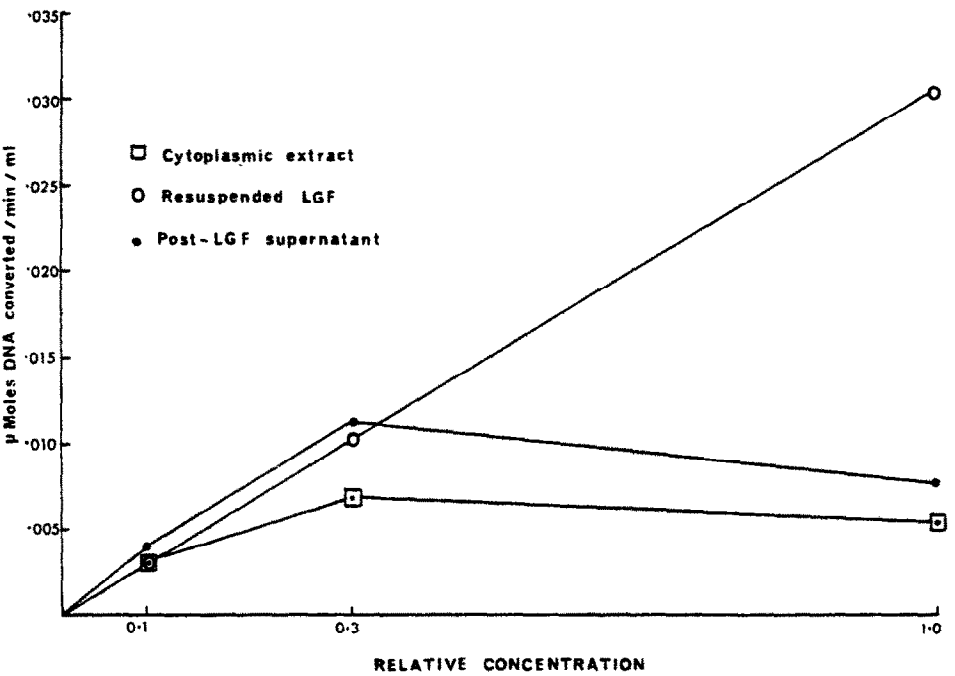

FIG. 4. The effect of dilution on acid deoxyribonuclease activity in fractions of adrenal cortex homogenates. The large-granule fraction was resuspended in 3 vols. of distilled water and subjected to repeated freeze–thawing and then centrifuged at 15,000 *g* for 20 min. The sediment was resuspended in 2 vols. of water, subjected to additional freezing and thawing, and centrifuged at the same *g* force. The combined supernatants were diluted with water to give a concentration corresponding to 1 g of cortex per ml. The cytoplasmic extract, the lysate of the large-granule fraction and the post-large-granule fraction supernatant were diluted (shown on the abscissa) with water; 0·1 ml was assayed for acid deoxyribonuclease activity by the procedure described in the Methods section.

granule fraction was measured in the presence of various amounts of high-speed supernatant (Fig. 5). Addition of increasing amounts of high-speed supernatant (prepared from a 0·3 M sucrose homogenate of cortex, as described in the Methods section) produced an increasing degree of enzyme inhibition. A moderate degree of stimulation was often observed at low inhibitor concentrations. Incubation of the enzyme in the presence of the resuspended high-speed pellet did not cause inhibition.

TABLE 7. ACTIVITY AND RECOVERY VALUES OF ACID DEOXYRIBONUCLEASE OF BOVINE ADRENAL MEDULLA AND CORTEX*

Tissue	Fraction	DNase activity in undiluted samples	DNase activity after 10-fold dilution
Medulla	Act/g, Homogenate	0·19 ± 0·04 (3)	0·51 ± 0·10 (5)
	Act/g, LGF	1·11 ± 0 (2)	1·13 ± 0·11 (9)
	$\frac{\text{LGF}}{\text{Cytoplasmic extract}} \times 100$	156·1 ± 11·2 (3)	84·7 ± 9·4 (3)
	$\frac{\text{LGF} + S_2}{\text{Cytoplasmic extract}} \times 100$	249·6 ± 69·4 (4)	104·8 ± 10·6 (3)
Cortex	Act/g, Homogenate	0·04 ± 0·02 (3)	0·15 ± 0·02 (5)
	Act/g, LGF	0·35 ± 0·00 (2)	0·34 ± 0·06 (6)
	$\frac{\text{LGF}}{\text{Cytoplasmic extract}} \times 100$	1080·3 ± 819·8 (4)	77·9 ± 5·1 (3)
	$\frac{\text{LGF} + S_2}{\text{Cytoplasmic extract}} \times 100$	1091·8 ± 816·6 (4)	95·3 ± 5·6 (3)

* Homogenates, cytoplasmic extracts, large-granule fractions (LGF) and postlarge-granule fraction supernatants (S_2) were obtained as described in the Methods section. The figures are means ± S.E. The number of experiments is shown in parentheses.

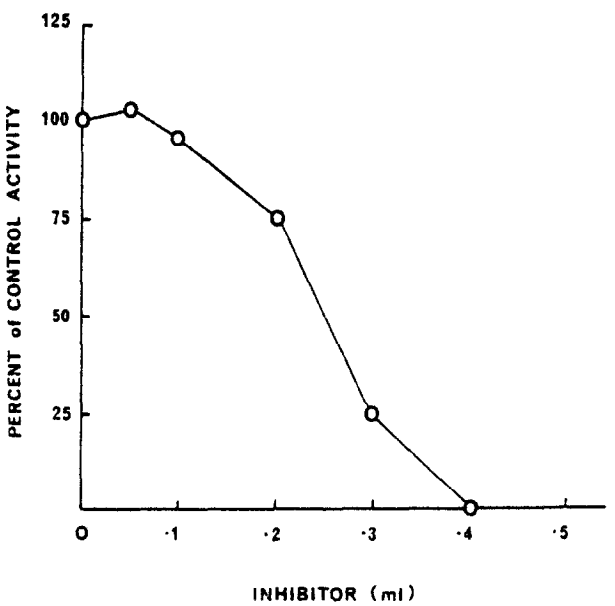

FIG. 5. Inhibition of cortex lysosomal acid deoxyribonuclease activity by the cortex high-speed supernatant. Inhibition (shown on the ordinate) is expressed as per cent of the acid deoxyribonuclease activity obtained in the absence of inhibitor. The amount (ml) of inhibitor solution added to the incubation mixture is shown on the abscissa. Details of the experiment are given in the text.

DISCUSSION

The isolated bovine adrenal gland perfused with physiological salt solution has been used extensively to study biochemical and pharmacological mechanisms of catecholamine secretion.[3-5, 7-9, 27-30] It has been shown in several laboratories that protein is among the secretion products obtained after stimulation of the gland with acetylcholine or related drugs.[3-5, 7-9] The major portion of the protein secreted is from chromaffin granules,[7] although stimulation also induces release of enzymes similar to those found in lysosomes.[9]

The 5 acid hydrolases, ribonuclease, deoxyribonuclease, cathepsin, acid phosphatase and β-glucuronidase, are demonstrable in homogenates of medulla and of cortex. The activities of acid ribonuclease, acid deoxyribonuclease, cathepsin and acid phosphatase of medulla are less than the corresponding values reported by Smith and Winkler;[18] medulla β-glucuronidase activity was similar to their value. However, the relationships between ribonuclease activity and the activities of the other enzymes in the homogenates (with the exception of β-glucuronidase), are similar to the corresponding ratios calculated from the data of Smith and Winkler.[18]

The relative activities of the acid hydrolases in cortex differed from the activities in medulla. In cortex the activities of ribonuclease and deoxyribonuclease were less, and cathepsin, acid phosphatase and β-glucuronidase activities were greater than in medulla. Total adrenal cathepsin activity, estimated from the data for medulla and cortex shown in Table 1, is about 1 μmole/min/g. This value is close to the value of 1·07 calculated from the data of Todd and Trikojus[31] for whole bovine adrenal gland.

The enzyme activity ratios shown in Table 3 for the acid hydrolase-rich fractions obtained by density gradient centrifugation also reflect the differences in enzyme activities between medulla and cortex. The respective ratios of acid ribonuclease activity to the activities of cathepsin, acid phosphatase and β-glucuronidase are appreciably different in medulla and cortex. These differences provide a basis for comparison to the corresponding ratios for perfusates from isolated adrenal glands.[19]

The finding that lysosomal enzyme activities in medulla and cortex are different is not surprising in view of the wide variation in acid hydrolase levels among other tissues.[32] A difference in acid hydrolase activity between bovine adrenal medulla and cortex was also shown by Malmstrom and Glick,[15] who found arylsulfatase activity to be higher in medulla than in cortex.

Each of the acid hydrolases of medulla and cortex was sedimentable at a gravitational force sufficient to sediment a major portion of the mitochondria in the tissue. The presence of acid hydrolases in the large-granule fraction could not merely reflect contamination by cytoplasm, since only 6–8 per cent of the lactate dehydrogenase of cytoplasmic extracts was found in this fraction. The relative amount of activity which sedimented varied among the enzymes. For medulla the acid nucleases sedimented most readily, and acid phosphatase sedimented to the smallest extent; for cortex, the acid nucleases were most extensively sedimented and cathepsin the least. Similar results for these same enzymes in medulla have been reported previously; the proportion of cytoplasmic extract acid hydrolase activity obtained in the large-granule fraction after similar centrifugation ranged between 47% for acid phosphatase and 81% for acid deoxyribonuclease.[18] Similar studies of the corresponding enzymes of rat liver cytoplasmic extracts give values between 42·4% for β-glucuronidase and 77·9% for cathepsin.[33]

The distribution after gradient centrifugation of the large-granule fraction acid hydrolases is consistent with the presence of a population of acid hydrolase-rich particles distinct from mitochondria, and, for medulla, distinct from chromaffin granules. The distribution patterns of medulla fumarase, a marker for mitochondria and of catecholamines, associated with chromaffin granules, are clearly different from that of acid ribonuclease. The separation of large-granule components obtained by gradient centrifugation is similar to that reported by Smith and Winkler.[18] Separation of mitochondria from acid hydrolase-containing particles also was obtained after gradient centrifugation of the cortex large-granule fraction, although the separation was not as complete as with medulla. It was possible, however, to obtain a fraction relatively rich in ribonuclease activity and low in fumarase activity.

The acid hydrolases in homogenates and large-granule fractions of both medulla and cortex displayed latency. The degree of activation varied among enzymes, and varied also with the method employed to liberate latent activity. The variability is not unexpected, since some of the same factors that affect the degree of sedimentability of the acid hydrolases will also influence their apparent latency.[34]

The results of differential and density gradient centrifugation, the results of the latency studies, and the existence of acid pH optima for the large-granule fraction acid hydrolases indicate that the five acid hydrolases are most likely associated with lysosomes. This conclusion is in agreement with studies reported earlier for medulla,[18] and supports the histochemical evidence for the occurrence of lysosomes in adrenal medulla[11-13] and cortex.[10]

The observation that acid deoxyribonuclease activities in homogenates of medulla and cortex increase after dilution, as well as the effect of dilution on the recovery of deoxyribonuclease activity in the fractions obtained by centrifugation of cytoplasmic extracts, indicates that there exists in these tissues an inhibitor of deoxyribonuclease. Aliquots of either the postlarge-granule fraction supernatant or of the high-speed supernatant inhibit lysosomal deoxyribonuclease. Inhibitors of deoxyribonucleases have been reported to occur in a variety of systems. Deoxyribonuclease inhibition by various proteins has been reported for yeast[35] and for mammalian systems,[36, 37] while inhibition of deoxyribonuclease by ribonucleic acid was reported to occur in bacterial systems[38-41] and for acid deoxyribonuclease of hog spleen.[42] An investigation of the chemical nature of the inhibitor of adrenal cortex acid deoxyribonuclease is in progress.

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