Biochemical Pharmacology, Vol. 18, pp 2032–2035. Pergamon Press. 1969. Printed in Great Britain

Distribution of an epinephrine-oxidizing enzyme in mammalian tissues*

(*Received* 12 *November* 1968; *accepted* 31 *January* 1969)

PREVIOUS reports described an enzyme from bovine uterine muscle which oxidized epinephrine to adrenochrome and resulted in the formation of an actomyosin adenosine triphosphatase (ATPase) inhibitor.[1–4] Adrenochrome was identified as an intermediate in the reaction from spectral analyses[3] and chromatographic data.[4] The ATPase inhibitor was isolated by paper chromatography and tentatively identified as a zwitterion, indole isomer of adrenochrome (ultraviolet absorption spectrum and tentative structure presented in reference 3). Both uterine[1] and cardiac[3] actomyosin ATPase were sensitive to the inhibitor. Isoproterenol and norepinephrine were also substrates for the enzyme, while tyrosine, dihydroxy-L-phenylalanine and reduced cytochrome C were not oxidized.[3]

The present report describes the distribution of the epinephrine-oxidizing enzyme in various tissues. Quantitative extraction of enzyme was preferred for these comparisons rather than enzyme purity. The following procedure is based on the known characteristics of the bovine uterine enzyme.[3,4] The tissues were frozen prior to being extracted. The thawed tissues were rinsed with cold, glass distilled water. Homogenization (3·5 min) was carried out with 12 ml of 0·3 M KCl, 0·15 M in KH_2PO_4 and 0·15 M in K_2HPO_4 (pH 6·5), per gram of tissue.† The temperature was maintained at 0°–5° for the entire procedure. The homogenate was centrifuged at 11,000 *g* for 30 min; 4 ml of the supernatant solution was dialyzed overnight against 32 ml water to yield ionic strength (μ) 0·1. This ionic strength caused quantitative precipitation of the bovine uterine enzyme;[4] actomyosin is also precipitated at this ionic strength. The mixture was removed from the dialysis sack into a 30-ml conical, graduated centrifuge tube and centrifuged at 1500 *g*. The supernatant solution was discarded. The precipitate was washed with appropriate volumes of 0·1 M KCl to dilute the phosphate concentration of the precipitate at least 1000-fold. The precipitate was recovered after each wash by centrifugation at 1500 *g* and dissolved finally in a volume of 1·0 ml, 1 M in KCl. A 0·6-ml aliquot was taken for the epinephrine oxidase assay and represented the extract from 200 mg tissue.

In the case of intestinal tissue from all species studied, extract from less tissue (50–100 mg) was represented in the oxidase assays. This dilution was necessary in order to determine the initial velocity of the very active ileal extracts. For the same reason, only 33 mg tissue was represented in the rat uterine assay. The other exceptions were in cardiac and skeletal muscle. Because of the large precipitate volumes obtained from these tissues, the precipitates were dissolved in a final volume of 2·0 ml. The 0·6-ml aliquot taken for the oxidase assays represented the extract from 100 mg tissue.

Virgin, young adult animals were used as a source of uterine tissue in each species tested. The rat was sacrificed in early diestrus. Animals of either sex were used at random as a source for the other tissues. In the assays of tracheal muscle, only the small segment of smooth muscle connecting the C rings of cartilage was extracted. The tracheae of ten guinea pigs (400–500 *g* body weight) were required to obtain enough tissue for one assay. The ileal segments were sectioned starting at a point approx. 15 cm from the ileocecal junction. The combined ventricles were extracted for the heart assays, the leg muscle of the rabbit for the skeletal muscle assay, whole brain of each species tested, and transverse slices through cortex and medulla for the kidney assays.

Details of the spectrophotometric assay of epinephrine oxidation to adrenochrome at the pH optimum for the bovine uterine enzyme have been described previously.[3] The assay is based on the increase in the absorbance of the reaction mixtures at 485 mμ, an absorption maximum of adrenochrome. Table 1 presents the results for the levels of the epinephrine-oxidizing enzyme in various tissues. The rates of epinephrine oxidation (Table 1) are corrected for the autoxidation of epinephrine ($\Delta E_{485\ m\mu}$/min = 0·050) under identical conditions, but in the absence of enzyme.

The highest level of epinephrine oxidase was found in the rat uterus. In comparison, rabbit uterus was essentially devoid of activity and guinea pig uterus contained intermediate amounts. These observations may provide some basis for the fact that the inhibition of the rat uterus by epinephrine

* Supported by a grant from the New York Heart Association.

† Kontes conical test-tube homogenizer, matching surfaces ground glass.

is one of the most sensitive bioassays for this compound; the guinea pig uterus is also inhibited by epinephrine while, in comparison, the rabbit uterus is stimulated. The uniformly high oxidase activity observed for the ileum of the three species tested would, likewise, be consistent with the inhibitory effect of epinephrine on intestinal muscle.[6, 7] The smooth muscle of the guinea pig trachea also contained moderate amounts of the epinephrine oxidase (Table 1). The efficacy of epinephrine as a bronchodilator is well known and formed the basis for the assay of epinephrine and other bronchodilator drugs using guinea pig tracheal rings.[8] Muscles in which epinephrine causes an increased contractility (heart and skeletal muscle) and nonmuscular tissues (liver, kidney and brain) showed uniformly low levels of the epinephrine oxidase. In cardiac muscle, it is possible that the activity

TABLE 1. EPINEPHRINE-OXIDIZING ACTIVITY OF TISSUE EXTRACTS*

Tissue	Species	Δ $E_{485\,m\mu}$/min/g tissue†	Yield of μ, 0·1, precipitate/g tissue (mg N)	Δ $E_{485\,m\mu}$/min/75 μg N/ml‡
Uterus	rat	105·66	1·59	4·966
	rabbit (1)	0·11	1·83	0·005
	rabbit (2)	0·23	2·66	0·006
	guinea pig	3·25	2·36	0·103
Ileum	rat	51·05	1·73	2·211
	rabbit	20·14	0·95	1·590
	guinea pig	12·89	0·77	1·250
Tracheal muscle	guinea pig	2·80	0·72	0·290
Heart	rat	0·49	5·39	0·007
	rabbit	0·25	4·79	0·004
	guinea pig	0·36	3·95	0·007
Skeletal muscle	rabbit	0·17	5·47	0·002
Liver	rabbit	0·35	0·29	0·092
	guinea pig (1)	0·22	0·25	0·065
	guinea pig (2)§	0·34	1·36	0·019
Kidney	rabbit	0·14	0·38	0·028
	guinea pig	0·23	0·65	0·027
Brain	rat (1)	0·12	0·07	0·129
	rat (2)§	0·10	0·21	0·036
	rabbit	0·03	0·03	0·071
	guinea pig	0·35	0·09	0·284

* The tracheal muscle value represents the pooled tissue from the tracheae of ten guinea pigs (see text). Each of the other values represents the results from a single animal.

The reaction mixtures for the oxidase assays contained, in a final volume of 1·2 ml: Tris-HCl buffer, pH 9·70, 150 μmoles; KCl, 600 μmoles; μ, 0·1, precipitate protein from 200 mg tissue, except as noted in text; adrenochrome bisulfite addition compound, 320 mμmoles; *l*-epinephrine-*d*-bitartrate, 2·4 μmoles. Final pH, 9·5; temp., 25°. The oxidation of epinephrine to adrenochrome by the bovine uterine enzyme was shown to be an autocatalytic reaction. The addition of catalytic amounts of crystalline adrenochrome, or the bisulfite addition compound of adrenochrome, to the reaction mixture before the addition of epinephrine eliminated the latent period.[3] The bisulfite addition compound does not interfere with the spectrophotometric assay of epinephrine oxidation, since it has no absorbance at 485 mμ.[5]

† Absorbance changes compared on the basis of extract from 1 g tissue in a volume of 1 ml of reaction mixture.

‡ Oxidase activity calculated per 75 μg of μ, 0·1, precipitate N; this was the concentration of N used for assays with bovine uterine enzyme.[3] Proportionality of oxidase activity to protein concentration was verified with extracts of rabbit ileum and rat uterus.

§ After sedimentation (1500 *g*) of the μ, 0·1, precipitated protein from liver and brain extracts, the supernatant solutions were slightly turbid. For these animals the protein was sedimented at 18,000 *g*.

which is expressed may be associated with vascular smooth muscle; the coronary arteries are predominantly dilated by epinephrine.[9]

A comment is necessary regarding the extraction of ileal tissue. The washing of the ileal segments after they had been frozen and thawed resulted in an almost complete sloughing off of the mucosa, leaving a thin, translucent sheet of muscle. The data in Table 1 represent the values for the muscle layers in the three species studied. In the rabbit ileum, an adjacent segment of ileal tissue was washed before being frozen and, when thawed, was transferred directly to the homogenizer. The oxidase value for this sample which included both mucosa and muscle was (Δ $E_{485\,m\mu}$/min/g tissue) 7·94, compared with 20·14 for the muscle alone (Table 1). It can be concluded that in the rabbit ileum the enzyme is concentrated in the muscle. In this connection, the studies with bovine uterus were carried out with only the myometrium.[3, 4]

Sedimentation of the μ, 0·1, precipitated protein at 18,000 *g* instead of at 1500 *g* (Table 1; liver of guinea pig No. 2 and brain of rat No. 2) did not appreciably affect the total activity per gram of tissue. The yield of μ, 0·1, protein was increased in both instances, but the additional protein was largely inactive, since it diluted the activity when compared on the basis of 75 μg N per ml (Table 1).

The assays of oxidase activity of the various tissue extracts (Table 1) were compared at pH 9·5, since the bovine uterine enzyme showed optimal activity at that pH.[3] In a separate experiment, using another rabbit, an extract of ileal muscle was assayed at pH 9·2 and pH 7·4. The assay procedure was identical except that the temperature was 37°. The values for epinephrine oxidation (Δ $E_{485\,m\mu}$/min/g of tissue) were 23·26 and 0·61 for the pH 9·2 and pH 7·4 assays respectively. This relationship between pH and activity is the same as that observed for the bovine uterine enzyme.[3] The absorbance change at pH 7·4 represented oxidation of epinephrine to adrenochrome at the rate of 0·14 μmole per min per g of tissue; at pH 9·2 the oxidation rate was 5·35 μmoles per min (E_{mol} adrenochrome, 485 $m\mu$ = 4350[3, 10]).

It was expected that some protein would be precipitated from the brain extracts at μ, 0·1, since the presence of an actomyosin-like protein from rat and cat brain has been recently reported.[11] The control of conformational changes in membranes has been suggested as a possible role for brain actomyosin. It is of interest that the epinephrine oxidase activity of the brain protein per unit of N (Table 1) was, on the average, the highest of the nonmuscular tissues studied. One can only speculate about the possible physiologic importance of the epinephrine-oxidizing activity of brain extracts in relation to the effect on brain actomyosin ATPase.

Extracts from rabbit ileum and guinea pig trachea were tested for capacity to form ATPase inhibitor from epinephrine. Still another rabbit was used as a source of ileal tissue for this experiment, and tracheal muscle was obtained from a second pool of 10 guinea pigs. The details of the assay of ATPase inhibitor formation were presented previously[3, 4] and will only be summarized here. After the μ, 0·1, precipitated protein was dissolved in KCl solution (final KCl concentration, 1 M), the extract was dialyzed against water to yield a final KCl concentration of approximately 6×10^{-5} M. Epinephrine was incubated with an aliquot of the dialyzed extract in the presence of O_2 by using a buffer system (pH 8·5) of low ionic strength. The reactions were stopped by addition of HCl (final concentration, 0·001 N), flushing the reaction mixtures with 95% N_2–5% CO_2, and lowering the temperature to 0°. The precipitated protein was removed by centrifugation at 35,000 *g* and an aliquot of the supernatant solution was chromatographed on paper (ascending in organic phase of 4:1:5, *n*-butanol:glacial acetic acid: water). The chromatograms of the oxidation products of epinephrine (Fig. 1) demonstrated the presence of adrenochrome (R_f 0·47) and the ATPase inhibitor (R_f 0·16). The chromatograms are the same as those obtained with the products of the oxidation of epinephrine by the purified bovine uterine enzyme.[4]

In a previous report,[3] the epinephrine-oxidizing enzyme from bovine uterus was distinguished from a salivary gland enzyme which oxidizes epinephrine to adrenochrome,[12] mammalian tyrosinase, cytochrome C oxidase, peroxidase and ceruloplasmin. Thus far, the enzymic activity observed in the present study for the extracts of the various tissues appears similar to the bovine uterine ezyme in regard to solubility characteristics, pH-activity profile, and isomerization of adrenochrome to the ATPase inhibitor.

Lundholm[13] has associated the vasodilating effects of epinephrine with production of lactic acid and CO_2. Relaxation of other smooth muscles by epinephrine was also accompanied by a stimulation in carbohydrate metabolism and lactic acid production. Lactic acid showed direct relaxing effects on smooth muscle.[13] Bülbring[14] has proposed another mechanism for the inhibitory effects of epine-

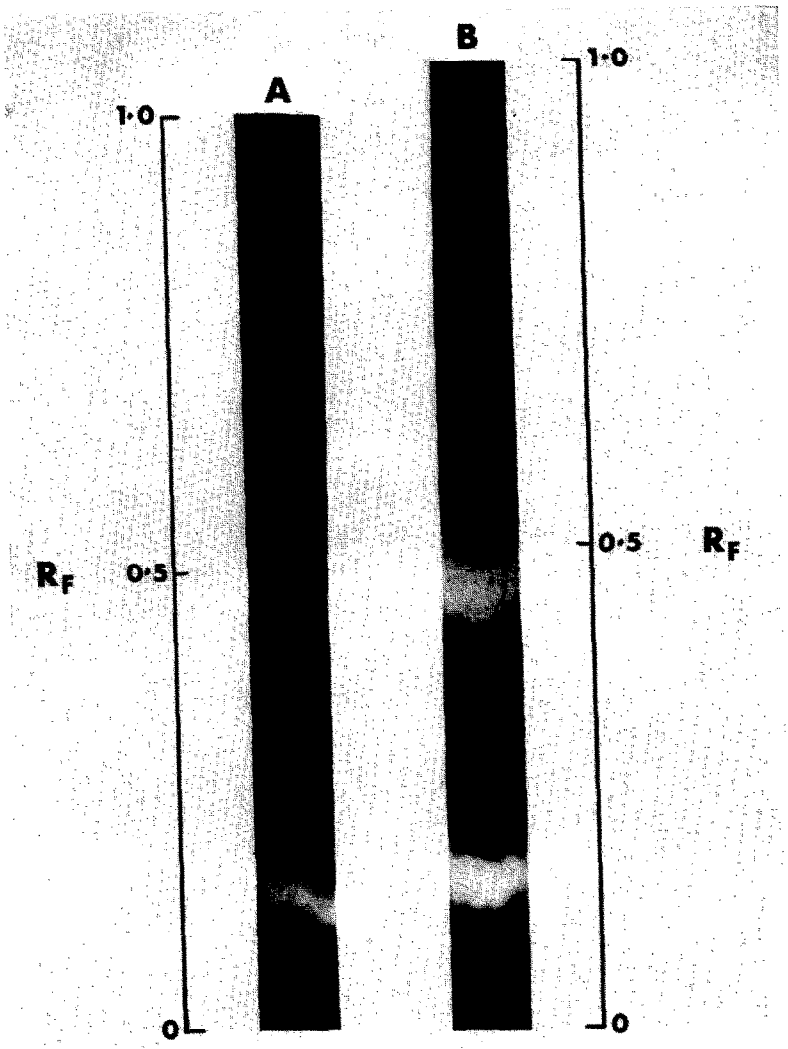

FIG. 1. Chromatograms of oxidation products of epinephrine, photographed while being irradiated with ultraviolet light (320–400 mμ). A, products from reaction catalyzed by guinea pig tracheal enzyme; B, same for rabbit ileal muscle enzyme. The reaction mixtures contained, per ml: $NaHCO_3$, 1 μmole; μ, 0·1, precipitate protein from 83 mg tissue; *l*-epinephrine-*d*-bitartrate, 0·25 μmole. Incubation period, 10·5 min; temp., 37·5°; pH, 8·5. Each chromatogram (1·5 cm in width) represents products from 0·2 ml of reaction mixture.

phrine on smooth muscle. It was suggested that increased production of adenosine triphosphate by epinephrine may stimulate the active extrusion of sodium from the cell, with a resulting hyperpolarization of the membrane.[14] It must still be established whether the metabolic and muscle-relaxing effects of epinephrine have a causal or parallel relationship.

The results of the present study demonstrated relatively high concentrations of an epinephrine-oxidizing enzyme in muscles which are relaxed by epinephrine. An actomyosin ATPase inhibitor was formed as a product of the oxidation of epinephrine by extracts of guinea pig trachea and rabbit ileum (Fig. 1). In studies now in progress, extracts from the uteri of five rats, sacrificed in various stages of the estrous cycle, all showed the capacity to form the ATPase inhibitor from epinephrine.

These findings lend support to our hypothesis of a possible relationship between the oxidation of epinephrine to an actomyosin ATPase inhibitor in certain smooth muscles and the negative inotropic effects of the hormone.[1, 3, 4]

Acknowledgement—Credit for the photographic recording of the ultraviolet-fluorescent chromatograms is due to Paul J. Showstark, RBP, Director of Bio-photography, Beth Israel Hospital, Boston, Mass.

Department of Pharmacology,
New York Medical College,
Flower and Fifth Avenue Hospitals,
New York, N.Y., U.S.A.

MARIO A. INCHIOSA, JR.
IRMINA B. RODRIGUEZ

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Biochemical Pharmacology, Vol. 18, pp 2035–2038. Pergamon Press. 1969. Printed in Great Britain

Differential inhibitor sensitivities of thymidine phosphorylases from *E. coli* and mammalian tissues

(*Received* 18 *February* 1969; *accepted* 18 *March* 1969)

THYMIDINE phosphorylase (EC 2.4.2.4) catalyzes the phosphorolytic cleavage of thymine- or uracil-deoxyribonucleosides into free base and deoxyribose-1-phosphate. The enzyme can be easily dis-