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EFFECTS OF ADRENOCHROME AND SODIUM PERBORATE ON ISOLATED FAT CELL METABOLISM*

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Abstract—The effects of adrenochrome, epinephrine and sodium perborate on glucose oxidation in isolated white fat cells were studied. Adrenochrome and sodium perborate were found to preferentially stimulate oxidation of [-1-^{14}C]glucose while epinephrine stimulated [-6-^{14}C]glucose oxidation. The stimulation by adrenochrome, epinephrine and sodium perborate of glucose oxidation was not appreciably effected by treating fat cells with trypsin. Treatment of the albumin used with the metal chelator 1,10-phenanthroline decreased but did not abolish the effectiveness of these agents.

Epinephrine in the presence of bovine serum albumin was converted to a pink-colored product which exhibited an absorbance maximum at 300 nm. However, it is unlikely that the effects of epinephrine on D-[-1-^{14}C]glucose oxidation are the result of its conversion to adrenochrome.

Adrenochrome was also found to cause an increase in the accumulation of cyclic AMP over a 10-min incubation period. No effects of adrenochrome were observed on the activity of adenylate cyclase stimulation by glucagon and epinephrine, but adrenochrome did inhibit the stimulation of cyclase activity by fluoride ion. Adrenochrome was active as an inhibitor of phosphodiesterase present in fat cells.

IT HAS been suggested that epinephrine stimulation of glucose oxidation in adipose tissue[1,2] might be due to oxidation products of epinephrine.[3] Adrenochrome, the predominant oxidation product of epinephrine,[4,5] has been shown to stimulate [-1-^{14}C]glucose oxidation in thyroid slices.[6] In isolated thryoid cells adrenochrome has been reported to increase iodine uptake and result in the accumulation of cyclic AMP.[7] Blecher *et al.*[8] concluded that in rat adipocytes the effects of epinephrine on glucose oxidation are independent of its effects on lipolysis. Ho *et al.*[9] reported that adrenochrome antagonized the lipolytic effect of epinephrine on adipose tissue; however, no data were presented and no mention of adrenochrome effects on glucose oxidation was made.

These studies were undertaken to determine if epinephrine was converted to adrenochrome in the presence of isolated fat cells and what effect adrenochrome would have on glucose oxidation in white fat cells. The mild oxidizing agent sodium perborate was included in some experiments to augment the oxidation of epinephrine. However, control experiments revealed that this compound alone increased glucose oxidation. Data are presented concerning this effect of sodium perborate. The effects of adrenochrome on the factors controlling cyclic AMP accumulation in fat cells were also studied.

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MATERIALS AND METHODS

White fat cells were obtained by enzymatic digestion of the parametrial adipose tissue of 130–150 g female rats (Charles River CD strain) fed *ad lib* on laboratory chow.[10] The ratio of 1 g of adipose tissue/3 ml of phosphate buffer containing 2 mg of crude collagenase (*Clostridium histolyticum*) was employed in all experiments. The phosphate buffer also contained: NaCl, 128 mM; $CaCl_2$, 1·4 mM; $MgSO_4$, 1·4 mM; KCl, 5·2 mM; Na_2HPO_4, 10 mM and 3% bovine Fraction V powder (Armour). This buffer was made up fresh daily and the pH adjusted to 7·4 with NaOH. After 1 hr of digestion at 37°, the cells were filtered through nylon chiffon and were washed twice in dilute albumin buffer and resuspended in the same buffer. A 0·2-ml aliquot of the cell suspension was added to 1 ml of 3% albumin buffer containing either 0·5 mM of labeled [-1-^{14}C]glucose or [-6-^{14}C]glucose and the agents to be tested. Glucose conversion to carbon dioxide and triglyceride content of fat cells were determined as previously described.[11] Samples were also removed at the end of the experiments for glycerol analysis.[12] Adenylate cyclase activity[13] and total cyclic AMP[14] determinations were made by previously published methods. Phosphodiesterase activity was measured by previously described methods.[15]

Cyclic AMP-dependent protein kinase was isolated from adipose tissue and purified by the method of Corbin and Krebs.[16] Protein kinase activity was assayed by method B of Corbin *et al.*[17] except that ^{33}P-γ-labeled ATP, prepared from ^{33}P-phosphoric acid[18] was used as substrate.

Epinephrine, adrenochrome and trypsin inhibitor were obtained from Sigma Chemical Co. The epinephrine was converted to the hydrochloride by treatment with an equivalent amount of HCl. Crystalline bovine insulin was a gift of the Eli Lilly Co. and contained less than 0·003% glucagon by weight. Trypsin was obtained from Worthington Biochemicals.

RESULTS

Figure 1 gives the effect of insulin and various other compounds on the stimulation of glucose oxidation. The trypsin-treated cells were obtained by treating fat cells with 1 mg/ml of trypsin for 5 min at 37°. Kono[19] and Czech and Fain[20] have shown that this treatment results in the loss of insulin stimulation of glucose oxidation but does not affect the response to insulin-like agents such as cysteine. These findings are confirmed here and also show that stimulation of glucose oxidation by epinephrine, adrenochrome and sodium perborate was unaffected by trypsin treatment.

Czech and Fain[21] found that treatment of bovine Fraction V albumin with the metal chelator 1,10-phenanthroline and its subsequent removal by Sephadex chromatography resulted in the loss of the insulin-like effect of cysteine while the response to insulin itself was not appreciably altered. Figure 2 presents a log dose response curve for epinephrine and adrenochrome in this albumin. Incubating the cells in this albumin results in a diminished response of epinephrine on [-1-^{14}C]glucose oxidation especially at the 50 μM dose (Fig. 2). The adrenochrome response is somewhat reduced at 50 μM but the high dose of adrenochrome (500 μM) still has a pronounced effect on [-1-^{14}C]glucose oxidation.

Maayan and Ingbar[7] reported that reducing agents such as ascorbic acid and sodium bisulfite blocked the stimulatory effect of epinephrine on the iodine uptake

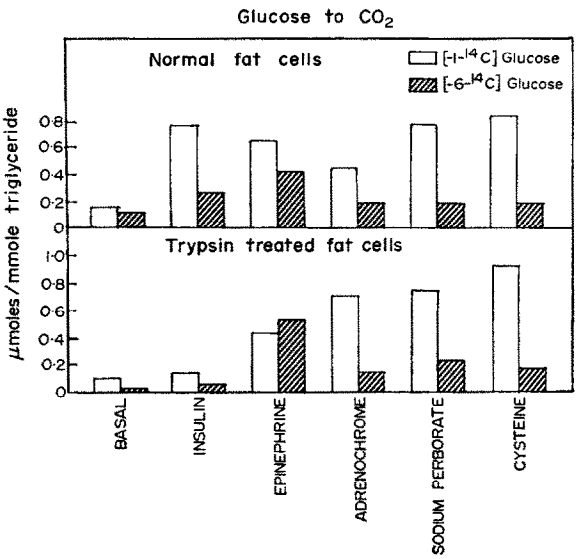

FIG. 1. Effect of various compounds on glucose oxidation in normal and trypsin-treated cells. White adipose tissue was digested for 55 min as is described in the text. To half of the digestion medium was added trypsin (1 mg/ml) and after 5 min trypsin inhibitor (2 mg/ml) was added. A mixture of trypsin and trypsin inhibitor was added to the remaining half of the digestion medium. The cells were washed twice in dilute buffer containing 0·1 mg/ml of trypsin inhibitor and then incubated (24 mg/tube) for 1 hr in 1 ml of buffer containing 3% albumin, 0·5 mM of either [-1-^{14}C]glucose or [-6-^{14}C]glucose and in separate tubes, 240 μunits/ml of insulin, 50 μM epinephrine, 50 μM adrenochrome, 10 μM sodium perborate and 500 μM cysteine. The data are the results of two experiments.

of thyroid cells but had no effect on the stimulation by adrenochrome. Table 1 shows that ascorbate blocked the stimulation of glucose oxidation by epinephrine. Ascorbate decreased the stimulation of glucose oxidation by adrenochrome but did not abolish it. Table 1 also shows that ascorbate inhibited the stimulation by sodium perborate of glucose oxidation.

TABLE 1. EFFECT OF ASCORBATE ON THE STIMULATION OF GLUCOSE OXIDATION BY ADRENOCHROME, EPINEPHRINE AND SODIUM PERBORATE *

Additions	[-1-^{14}C]Glucose to CO_2 (μmoles/g fat cells)		[-6-^{14}C]Glucose to CO_2 (μmoles/g fat cells)	
	Without ascorbate	With Ascorbate (1 mg/ml)	Without ascorbate	With ascorbate (1 mg/ml)
None	0·24	0·27	0·10	0·14
Epinephrine (50 μM)	0·80	0·42	0·69	0·30
Adrenochrome (50 μM)	1·32	0·73	0·46	0·28
Adrenochrome (500 μM)	1·77	0·72		
Sodium perborate (100 μM)	1·59	0·38	0·21	0·19

* Cells were isolated and incubated (25 mg/tube) as described in the text. The results are the means of three experiments.

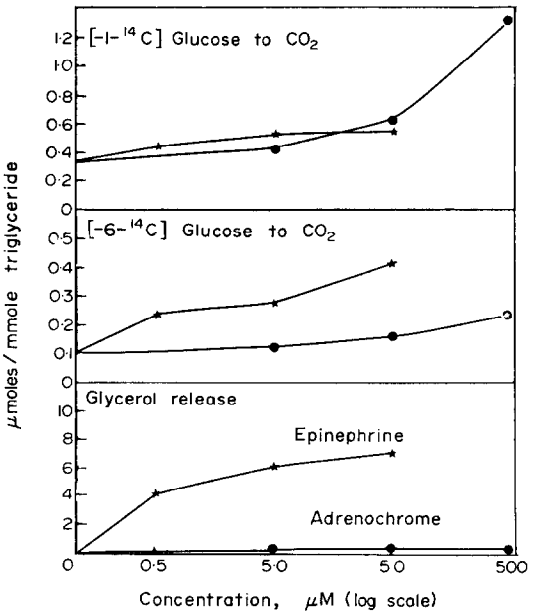

FIG. 2. Log dose response curve of epinephrine and adrenochrome in albumin treated with 1,10-phenanthroline. Thirty ml of 7% albumin in phosphate buffer, pH 7·4, was incubated at 37° for 15 min with 3·3 mM 1,10-phenanthroline. The mixture was then passed through a 55 cm column of Sephadex G-50 which had been equilibrated with phosphate buffer, pH 7·4. The bed volume was 590 ml. The eluted albumin was diluted to 2 per cent with phosphate buffer as estimated by absorbance at 280 nm in a Beckman DU spectrophotometer. The cells were isolated and incubated (29 mg/tube) as described in the text. The data are the mean of two experiments.

The stimulatory effects of epinephrine on glucose oxidation could possibly be due to conversion of epinephrine to adrenochrome. The characteristic pink color of adrenochrome was observed during incubations in which high (50 μM) concentrations of epinephrine were present. The color was intensified by the addition of the mild oxidizing agent sodium perborate.

Table 2 shows that, in the presence of albumin, epinephrine is converted to adrenochrome-like products as measured by the increase in absorbance at 300 nm. There

TABLE 2. APPEARANCE OF ADRENOCHROME-LIKE PRODUCTS IN THE ABSENCE AND PRESENCE OF ALBUMIN*

Time (min)	Epinephrine	Absorbance at 300 nm: Epinephrine + 3% albumin	Absorbance at 300 nm: Epinephrine + fat cells
0	0·14	0·33	0·22
10	0·14	0·36	0·23
30	0·15	0·40	
60		0·44	0·23

* The absorbance of solutions at 300 nm (λ_{max} for adrenochrome, Ref. 4) was determined in a Unicam model SP.800 spectrophotometer. Epinephrine (22·5 μg) was present initially and the increase in absorbance at 300 nm was determined at the times indicated.

was no detectable conversion of epinephrine to adrenochrome in the absence of albumin. The addition of fat cells in the absence of albumin caused only a slight increase in absorbance compared to the increase when albumin was present.

We also examined the effect of adrenochrome on the various factors controlling the level of cyclic AMP in isolated fat cells. Table 3 indicates that 500 μM adrenochrome was capable of raising the cyclic AMP concentration to a level comparable with that of 0·5 μM epinephrine in the presence of theophylline. Table 4 shows the lack of adrenochrome effects on the stimulation of adenylate cyclase by glucagon and epinephrine. However, it is interesting to note that adrenochrome inhibited the stimulation of adenylate cyclase by fluoride ion.

TABLE 3. EFFECT OF ADRENOCHROME ON TOTAL CYCLIC AMP ACCUMULATION*

Additions	Cyclic AMP (nmoles/g fat cells)
None	0·75
Epinephrine (0·5 μM)	2·95
Epinephrine (5·0 μM)	3·95
Adrenochrome (5·0 μM)	0·85
Adrenochrome (50·0 μM)	0·80
Adrenochrome (500·0 μM)	2·60

* Fat cells (30 mg/tube) were incubated for 10 min in the presence of 0·2 mM theophylline. The values are the means of two experiments.

TABLE 4. EFFECT OF ADRENOCHROME ON ADENYLATE CYCLASE*

No additions	+ Glucagon (2 μg/ml)	+ Epinephrine (0·2 mM)	+ Fluoride (10 mM)
	(nmoles/mg protein)		
0·4	5·5	9·3	14·1
	Per cent change ± standard error due to adrenochrome		
Adrenochrome (5 μM)	+8 ± 8	+5 ± 8	−21 ± 3
Adrenochrome (50 μM)	+19 ± 8	−6 ± 15	−40 ± 10

* Fat cell ghosts (113 μg of protein/tube) were incubated for 20 min. The values are the means of four paired replications. The plus values refer to per cent stimulation and negative values to per cent inhibition by adrenochrome.

Adrenochrome (50 μM) did not affect protein kinase activity in the absence of cyclic AMP (Table 5). However, when cyclic AMP was present adrenochrome reduced ^{33}P incorporation.

The data in Fig. 3 indicate that adrenochrome was as effective an inhibitor of phosphodiesterase as was the methyl xanthine theophylline.

DISCUSSION

These data indicate that epinephrine is converted to an adrenochrome-like product in the presence of bovine serum albumin at pH 7·4. The effects of epinephrine on glucose oxidation, however, cannot be explained by its conversion to adrenochrome. From

TABLE 5. EFFECT OF ADRENOCHROME ON THE ACTIVITY OF CYCLIC AMP-DEPENDENT PROTEIN KINASE FROM ADIPOSE TISSUE*

Additions	Without adrenochrome (nmoles of ^{33}P incorporated/mg protein)		With adrenochrome	
	Experiment A	B	Experiment A	B
None	15	10	16	10
Cyclic AMP (0·2 nm)	44	29	40	24
Cyclic AMP (20 nm)	59	31	45	28

* Protein kinase was isolated and assayed as described in the text. The adrenochrome concentration was 50 μM. The data are from two experiments.

Figs. 1 and 2 it is evident that epinephrine and adrenochrome are equipotent in stimulating [-1-^{14}C]glucose oxidation but adrenochrome was unable to stimulate [-6-^{14}C]glucose oxidation to the same extent as epinephrine. Adrenochrome mimics the action of insulin in that it preferentially stimulates [-1-^{14}C]glucose oxidation which is an indication of increased activity of the hexose monophosphate shunt. Epinephrine on the other hand appears to cause a general stimulation of glucose oxidation

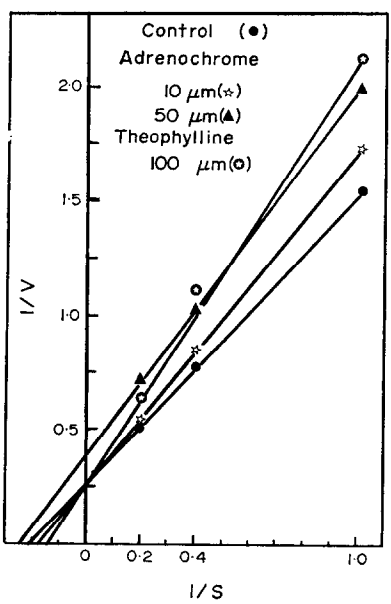

FIG. 3. Effects of adrenochrome and theophylline on phosphodiesterase. The phosphodiesterase activity was determined using the high speed supernatant fraction (approximately 30 μg of protein/tube) obtained after centrifugation of fat cell homogenates. The cyclic AMP concentrations used were 1, 2·5 and 5 μM; V is expressed as nmoles of cyclic AMP hydrolized/mg of protein/10 min. The data are the means of three experiments. The apparent K_m as determined from the plot was 5·6 μM.

by the shunt and glycolytic pathways. The studies with trypsin-treated cells (Fig. 1) indicate that the adrenochrome effect on glucose oxidation is not involved with the specific insulin receptor of fat cells.

The stimulation by the high dose of epinephrine (50 μM) on [-1-^{14}C]glucose oxidation can possibly be explained by epinephrine conversion to adrenochrome. At this concentration of epinephrine, the characteristic pink color of adrenochrome is evident during the incubation period. The failure of lower doses (0·5 and 5·0 μM) of epinephrine to significantly stimulate [-1-^{14}C]glucose oxidation is probably the result of the smaller amount of adrenochrome produced under these conditions and the fact that adrenochrome is inherently unstable in aqueous solutions.[4] The small amount of adrenochrome produced would be rapidly destroyed during the incubation period. The mechanism of the effect of epinephrine on [-6-^{14}C]glucose oxidation is probably secondary to the increased lipolysis due to epinephrine.

The insulin-like effect of sodium perborate (Fig. 1) on glucose oxidation appears to be the result of its ability to act as an oxidizing agent, since in the presence of ascorbate (Table 1) its effect is greatly diminished. Other boron-containing compounds such as borax and borate iron had no effect on glucose oxidation (unpublished results).

The ability of adrenochrome to increase cyclic AMP accumulation (Table 3) can be explained by its ability to act as an inhibitor of phosphodiesterase (Fig. 3). The lack of a lipolytic response by adrenochrome (Fig. 2) is not as easily explained.

Adrenochrome inhibited protein kinase activity by 16 per cent in the presence of 2 pmoles of cyclic AMP. It is possible that this effect could be responsible for the lack of a lipolytic response by adrenochrome. However, it remains to be established that this decrease in protein kinase activity could be responsible for the lack of stimulation of the hormone-sensitive lipase in adipose tissue.

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