The Relationship of Epinephrine Oxidation to Rat Parotid Metabolism and Secretion *in Vitro*[1] (35529)

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(Introduced by J. A. Pittman, Jr.)

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The rat parotid *in vitro* has been utilized by several investigators as a model system for the study of biochemical events involved in enzyme secretion (1, 2). Epinephrine and pilocarpine initiate the discharge of exportable protein into the incubation medium and produce alterations in metabolism. There has been some suggestion that the effects attributed to pilocarpine *in vitro* may be produced by stored endogenous catecholamines (1) released by this compound. It is well known that epinephrine rapidly undergoes metabolic alterations in biological systems (3) and has been reported to be consumed during incubation with rat parotid slices (1). Conversion of epinephrine to an oxidation product, adrenochrome, was also suggested in the authors' laboratory by the appearance of a pink color in the medium during incubation with rat parotid tissue.

Adrenochrome is not believed to be an *in vivo* metabolite of catecholamines but is formed in *in vitro* systems (3). This metabolite rather than intact epinephrine appears to be responsible for the stimulation of glucose oxidation in thyroid slices (4, 5) and may also be responsible for a similar epinephrine effect on sheep liver, kidney, and testicular slices. Adrenochrome apparently produces this stimulation by acting as an autooxidizable electron carrier for TPNH and DPNH oxidase systems in thyroid (4, 5) and in liver (6). This alternative means of oxidizing reduced pyridine nucleotides could uncouple oxidative phosphorylation and might reduce the energy level of the tissue. Epinephrine stimulation of enzyme secretion by rat parotid *in vitro* is accompanied by an increase in oxidative processes but a depression of energy-requiring processes suggesting an uncoupling effect and the possibility that adrenochrome might be responsible for the changes observed. If adrenochrome produces effects on metabolism or secretion, the value of the *in vitro* parotid preparation as a model system would be greatly reduced.

The present investigation is concerned with an assessment of the role played by oxidative metabolites of epinephrine in producing the effects on metabolism and enzyme secretion by rat parotid *in vitro* which have been attributed to epinephrine. Particular attention has been given to adrenochrome which is a major metabolite in the *in vitro* system.

Materials and Methods. Three male Sprague-Dawley rats (200–250 g) were sacrificed by exsanguination for each experiment. The parotid glands were removed, freed of extraneous tissue, then placed on numbered squares on buffer-moistened filter paper in covered, iced petri dishes. The glands from each animal were prepared individually and kept separated at all times. The tissues were minced with scissors and portions of 40 ± 2 mg were incubated, in triplicate, in a total of 3.0 ml Krebs–Ringer bicarbonate buffer with the appropriate additions. Tissue from each animal was used to prepare one vessel of each triplet in each experiment. All nonradioactive substances were dissolved in Krebs–Ringer bicarbonate buffer before addition to the medium. Radioactive glucose and leucine (New England Nuclear) were added in 0.025 ml of H_2O; and controls were corrected accordingly. Incubation was carried

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out in stoppered flasks with a removable center well at 37° in 95% oxygen–5% CO_2 at a shaking rate of 120/min.

The methods used to determine glucose oxidation, amino acid oxidation, amino acid incorporation into protein, and α-amylase secretion were identical to those previously described (2). Ascorbic acid, adrenochrome, epinephrine as the bitartrate, and iproniazid as the phosphate salt were purchased from Sigma.

TABLE I. Effects of 30-min Preincubation on Epinephrine Stimulation of Glucose-6-^{14}C Oxidation.[a]

Additions		Glucose-6-^{14}C oxidized (dpm $^{14}CO_2$/100 mg of tissue)
Before preincubation	After preincubation	
None	Glucose-6-^{14}C	5375 ± 659
Epinephrine	Glucose-6-^{14}C	20,348 ± 1094
None	Glucose-6-^{14}C Epinephrine	20,897 ± 1540

[a] The values shown are means ± SE of 3 experiments, each performed in triplicate. Preincubation was for 30 min in Krebs-Ringer bicarbonate buffer with 4 μmoles of glucose/3.0 ml prior to the addition of 0.25 μCi of glucose-6-^{14}C and incubation for 1 hr. Epinephrine, when added, was 5 μg/ml.

TABLE II. Effects of Bisulfite, Ascorbic Acid and Iproniazid on Epinephrine Stimulation of Glucose-6-^{14}C Oxidation.[a]

Additions to medium	Glucose-6-^{14}C oxidized (dpm $^{14}CO_2$/100 mg of tissue)
None	10,566 ± 1265
Na bisulfite	10,318 ± 987
Ascorbic acid	10,171 ± 1174
Epinephrine	37,746 ± 4707
+ Na bisulfite	32,413 ± 3767
+ Ascorbic acid	34,151 ± 3987
None	7176 ± 721
Iproniazid	6862 ± 531
Epinephrine	20,447 ± 1687
+ Iproniazid	21,121 ± 1971

[a] The values shown are means ± SE of 3 experiments, each performed in triplicate. Epinephrine was present at 5.0 μg/ml, sodium bisulfite at 10^{-3} M, ascorbic acid at 5.6×10^{-4} M and iproniazid at 5×10^{-3} M. Incubation was for 60 min.

Results. The oxidation of epinephrine to adenochrome by rat parotid *in vitro* was suggested by the appearance of a pink color in the incubation medium. Spectrophotometric analyses of the medium at 0 time and after incubation also demonstrated a marked increase in UV absorbance and a slight shift in the absorption spectrum. These changes characterize the oxidation of epinephrine to adrenochrome (5).

Rat parotid preparations were preincubated 30 min before the addition of glucose-6-^{14}C as shown in Table I. Preincubation with epinephrine to produce high adrenochrome levels prior to incubation did not result in a stimulation of glucose-6-^{14}C oxidation greater than the approximately 400% observed when epinephrine was added after preincubation. Thus the magnitude of the increase did not appear to be directly related to the concentration of adrenochrome suggesting that epinephrine rather than an oxidation product was responsible for the effects observed.

The oxidation of epinephrine to adrenochrome has been shown to be inhibited by sodium bisulfite and ascorbic acid (5). The results presented in Table II demonstrate that concentrations of these two reducing agents, sufficient to produce 90–100% inhibition of adrenochrome formation and a marked inhibition of the epinephrine stimulation of glucose-1-^{14}C oxidation by thyroid slices (5), had no effect on the magnitude of the epinephrine stimulation of glucose-6-^{14}C oxidation by rat parotid. Stromblad (7) has reported that salivary glands possess an unusually high monamine oxidase activity, an enzyme producing oxidative deamination of epinephrine. Iproniazid is a potent inhibitor of monamine oxidase (8), but, as shown in Table II, had no effect on glucose oxidation or the magnitude of the response to epinephrine.

If adrenochrome formed by the oxidation of epinephrine in the *in vitro* system was responsible for the stimulation of α-amylase secretion or the metabolic alterations observed, adrenochrome should mimic the actions of epinephrine. The results presented in

TABLE III. Effects of Epinephrine and Adrenochrome on the Metabolism of Rat Parotid *in Vitro*.[a]

Additions to medium	α-Amylase secreted (units/100 mg of tissue)	Glucose-6-^{14}C oxidized (dpm $^{14}CO_2$/100 mg of tissue)	Leucine-U-^{14}C oxidized (dpm $^{14}CO_2$/100 mg of tissue)	Leucine-U-^{14}C incorporated into protein (dpm/mg of protein)
None	52 ± 7	6238 ± 780	94,567 ± 4434	3038 ± 172
Epinephrine	88 ± 8	25,198 ± 3693	185,953 ± 11,357	555 ± 43
Adrenochrome	52 ± 6	6976 ± 934	96,123 ± 3849	3070 ± 221

[a] The values shown are means ± SE of 3 experiments, each performed in triplicate. Epinephrine was present at 5 μg/ml, adrenochrome at 2.5 μg/ml, glucose-6-^{14}C at 0.25 μCi/4.0 μmoles/3.0 ml and leucine-U-^{14}C at 0.25 μCi/2.0 μmoles/3.0 ml. Incubation time was 60 min.

Table III demonstrate that epinephrine stimulated α-amylase secretion, as well as glucose oxidation and leucine oxidation, but caused a marked depression of leucine incorporation into proteins. In contrast, adrenochrome had no measurable effect on either α-amylase secretion or parotid metabolism. No effect on glucose oxidation was obtained with concentrations of adrenochrome from 0.025 to 5.0 μg/ml and the results presented in Table III were obtained with concentrations of adrenochrome that could be produced by a conversion of approximately 50% of the epinephrine initially present. On the basis of these results, it is highly unlikely that epinephrine must be metabolized to adrenochrome or other oxidation products prior to producing the alterations in α-amylase secretion and metabolism observed with rat parotid *in vitro*.

Discussion. The recent report by Ueha *et al.* (2) from this laboratory demonstrated that epinephrine and pilocarpine stimulation of enzyme secretion *in vitro* was accompanied by an increase in oxidative metabolism but by a decrease in energy-requiring processes such as protein and nucleotide synthesis. Subsequent studies (9, 10), demonstrated that chemical initiation of α-amylase secretion was independent of the alterations previously observed in metabolism. The latter were probably side-effects of the initiators and were not related to the discharge of enzyme protein.

The present results indicate that prior transformation to adrenochrome or other oxidation products is not required for epinephrine or endogenously released catecholamines to affect metabolism and secretion. Consequently the effects of epinephrine and pilocarpine on rat parotid preparations are not due to the formation of a nonphysiological oxidative metabolite of epinephrine. Support for this conclusion is also provided by the earlier observation that pilocarpine, which may release endogenous catecholamine, was a more potent stimulator of parotid oxidation than epinephrine, although no evidence for adrenochrome formation was observed with this substance.

The evidence presented did demonstrate that adrenochrome was produced during incubation of a rat parotid mince with epinephrine but this oxidation product had no measurable effects. It is obvious that if an uncoupling effect was produced by adrenochrome it was not sufficient to produce a compensating increase in the oxidation of glucose or amino acids.

The initiation of enzyme secretion by rat parotid *in vitro* appears to be mediated by cAMP. Epinephrine apparently stimulates the formation of cAMP which then initiates the events which result in the discharge of enzyme protein. The absence of any effect of adrenochrome on enzyme secretion strongly suggests that this compound has little or no effect on the adenyl cyclase system.

Summary. Epinephrine conversion to adrenochrome by rat parotid *in vitro* was verified by the appearance of a pink color in the medium and an increase in UV absor-

bance. Preincubation with epinephrine to increase adrenochrome concentration during incubation produced no alteration in the magnitude of catecholamine stimulation of glucose-6-^{14}C oxidation. Inhibition of epinephrine conversion to adrenochrome or of degradation by monamine oxidase also had no effect on the response to this catecholamine. Although epinephrine produced pronounced stimulation of α-amylase secretion, glucose-6-^{14}C oxidation, leucine-U-^{14}C oxidation, and inhibition of leucine-U-^{14}C incorporation into rat parotid protein, no measurable effects were produced by adrenochrome. The present results strongly indicate that epinephrine rather than adrenochrome or other oxidation products is primarily responsible for the alterations in α-amylase secretion and metabolism observed with rat parotid *in vitro*.

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