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# STIMULATION OF PAROTID CELL GLUCOSE OXIDATION

## ROLE OF ALPHA$_1$-ADRENERGIC RECEPTORS AND CALCIUM MOBILIZATION

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**Abstract**—Epinephrine stimulated glucose oxidation in isolated rat parotid cell aggregates through alpha- and beta-adrenergic mechanisms. The alpha-adrenergic component appeared to be of the alpha$_1$-receptor subtype as evidenced by inhibition studies with selective antagonists. Calcium mobilization in the presence of the ionophore A23187 was as capable as epinephrine in eliciting this response. Epinephrine stimulation of glucose oxidation was, however, only partially dependent on extracellular calcium. Only the upper 50% of the maximal ($10^{-5}$ M) epinephrine response required external calcium, since stimulation by $10^{-6}$ M epinephrine, which elicits only about 40% of the maximal ($10^{-5}$ M epinephrine) response, was independent of external calcium. Furthermore, no external calcium dependence was observed for pure alpha- or beta-adrenergic stimulation alone, which comprised about 60 and 40%, respectively, of the maximal epinephrine response. Thus, adrenergic mediated parotid cell glucose oxidation may proceed by different mechanisms depending on the extent and nature of cellular stimulation.

Much recent work has focused on the elucidation of mechanisms by which the alpha-adrenergic system regulates cellular functions [1–6]. The rat parotid gland provides an excellent model for studying such actions since both secretory processes [1, 7, 8] and energy production [9] are under direct alpha-adrenergic control. Although considerable information exists regarding the various biochemical events which mediate alpha-adrenergic secretion [1–3, 6–8], only a few reports concerning the corresponding mechanisms for control of glucose metabolism have been presented [9, 10].

For example, it is known that stimulation of potassium release from these glands is mediated through the alpha$_1$-adrenergic receptor subtype [8]. Both alpha$_1$ and alpha$_2$ receptors are present on parotid cells [8], but the receptor subtype responsible for regulation of glucose metabolism has not been identified. Also, stimulation of potassium release is apparently related to increased phospholipid turnover and associated calcium mobilization [7] whereas the role of these processes in controlling glucose oxidation remains to be determined.

Since exocrine secretion is an energy requiring process [3], it was decided to more closely examine alpha-adrenergic regulated energy metabolism in isolated parotid cell aggregates.

### MATERIALS AND METHODS

*Animals.* Three- to six-month-old male Wistar rats were used in this study. All animals were obtained from the Gerontology Research Center (NIA) colony. The Genterology Research Center is fully accredited by the America Association for Accreditation of Laboratory Animal Care. Animals were maintained on NIH Purina laboratory chow and water *ad lib.* until they were killed.

*Chemicals and supplies.* Chromatographically purified collagenase (type CLSPA Lot No. W2J452, sp. act. 400 units/mg) was purchased from the Worthington Biochemical Corp., Freehold, NJ. Bovine testicular hyaluronidase (type 1-S, sp. act. 270 NF units/mg), bovine serum albumin (Fraction V), calf thymus DNA, (-)-epinephrine bitartrate and yohimbine hydrochloride were obtained from the Sigma Chemical Co., St. Louis, MO. (±)-Propranolol hydrochloride was purchased from Ayerst laboratories, New York, NY. Prazosin hydrochloride was a gift from Pfizer Laboratories, New York, NY. Rauwolscine was from Roth (Karlsruhe, F.R.G.) and 2-[β-(hydroxyphenyl)-ethyl-aminomethyl]-tetralone (HEAT) from Beiersdorf Ltd. (Hamburg, F.R.G) Hyamine hydroxide was from Amersham, Chicago, IL, and A23187 from Calbiochem, La Jolla, CA. All other chemicals were the highest grade commercially available. Tubes used for glucose oxidation assays were obtained from Falcon (2059), Oxnard, CA, and plastic center wells from Kontes, Vineland, NJ.

*Radioactive isotopes.* D-[U-$^{14}$C]Glucose (sp. act. 0.23 Ci/mmole) was obtained from Schwarz/Mann, Orangeburg, NY.

*Preparation of parotid cell aggregates.* All rats were killed between 9:30 and 10:00 a.m. by cervical dislocation. Parotid glands were removed quickly, trimmed of connective tissue, fat and lymph nodes, and minced finely with iridectomy scissors. Enzy-

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matically dispersed parotid cell aggregates were prepared as previously described [11]. Briefly, parotid tissue minces were digested with collagenase and hyaluronidase at concentrations of 96 units/ml and 0.19 mg/ml, respectively, in 10 ml of complete Hanks' balanced salt solution for 60 min at 37° with constant shaking (100 cycles/min) under an atmosphere of 95% $O_2$–5% $CO_2$. After enzymatic digestion, resulting parotid cell aggregates were washed once with Hanks' balanced salt solution without glucose containing 4% bovine serum albumin, and twice with Hanks' balanced salt solution without glucose containing 0.022% bovine serum albumin (incubation medium). In some experiments, calcium was omitted from the medium and 0.2 mM ethyleneglycolbis (amino-ethylether) tetra-acetate (EGTA) added for all steps subsequent to digestion.

*Assessment of glucose oxidation.* Aggregates were divided into aliquots and suspended in 0.45 ml of incubation medium. After a 10-min preincubation, 25 μl of an adrenergic agonist and/or antagonist solution was added, except where otherwise indicated. Agonists and antagonists were dissolved, immediately before use, in incubation medium containing 2 mM ascorbic acid to prevent oxidation. After 12 min of incubation with agonist and/or antagonist, 25 μl of 4 mM glucose solution containing 0.5 μCi of D-[U-$^{14}$C]glucose was added. Incubations were performed for 40 min at 37° at a shaking rate of 60 cycles/min under an atmosphere of 95% $O_2$–5% $CO_2$ in a rubber stoppered tube with a disposable plastic center well, to which 200 μl of hyamine hydroxide had already been added. The incubation was terminated by the injection of 0.5 ml of 3 N perchloric acid. The tubes were shaken for an additional 60 min at 37° to trap $CO_2$, as bicarbonate, in the hyamine hydroxide. The hyamine hydroxide containing [$^{14}$C]bicarbonate was assessed for radioactivity in a liquid scintillation counter. Under these incubation conditions, glucose was oxidized to $CO_2$ at a constant rate for at least 60 min, both in the presence and absence of agonists and antagonists. Individual values were corrected for the amount of protein, or DNA, per sample.

*Measurement of protein and DNA content.* Protein concentration was determined according to the method of Lowry *et al.* [12] using bovine serum albumin as a standard. DNA content was determined by the diphenylamine method as modified by Richards [13] using calf thymus DNA as a standard.

## RESULTS

*Alpha-adrenergic receptor subtype specificity.* Epinephrine-stimulated glucose oxidation in the rat parotid gland has been shown previously to be concentration dependent with a maximum at $10^{-5}$-$10^{-4}$ M and half-maximum around $10^{-6}$ M [9]. About 60% of the response is alpha adrenergic and 40% beta adrenergic as determined from inhibition studies with propranolol and phenoxybenazmine [9]. In the present study, the alpha-adrenergic component was further characterized by selective inhibition using the alpha$_1$ antagonist, prazosin, and the alpha$_2$ antagonist, yohimbine. Figure 1 shows that about 45% of the glucose oxidation stimulated by $10^{-5}$ M

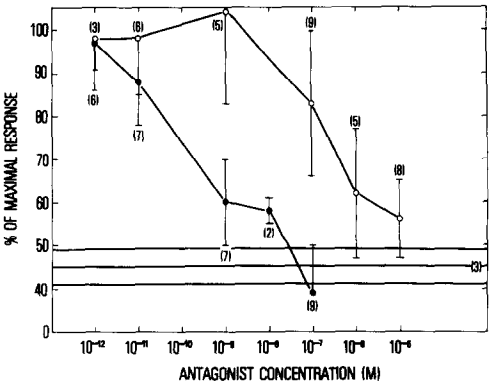


Fig. 1. Effects of prazosin, yohimbine and propranolol on stimulation of parotid cell glucose oxidation by $10^{-5}$ M (-)-epinephrine. Parotid cell aggregates were exposed to $10^{-5}$ M (-)-epinephrine with or without propranolol at $3 \times 10^{-5}$ M, and prazosin or yohimbine at the indicated concentrations. Values represent the means ± standard errors for the numbers of experiments indicated in parentheses. Key: (●——●) prazosin; and (○——○) yohimbine. The continuous horizontal lines represent the (-)-epinephrine response remaining in the presence of $3 \times 10^{-5}$ M propranolol (mean ± standard error).

(-)-epinephrine was inhibited by $3 \times 10^{-5}$ M (±)-propranolol. Of the remaining 55% (the alpha-adrenergic component), a marked, preferential sensitivity for inhibition by prazosin, as opposed to yohimbine, was displayed. Half-maximal inhibition of the alpha-adrenergic response was exerted by prazosin at approximately $10^{-10}$ M and by yohimbine at about $5 \times 10^{-7}$ M, thus suggesting an alpha$_1$-adrenergic mechanism. The alpha$_1$-adrenergic nature of the response was further verified by use of HEAT and rauwolscine which are believed to be, respectively, alpha$_1$ [14] and alpha$_2$ [15] antagonists. In five separate experiments, half-maximal inhibition of alpha-adrenergic stimulated glucose oxidation occurred at about $10^{-8}$ M for HEAT and $10^{-5}$ M for rauwolscine (data not shown).

*Role of calcium in alpha-adrenergic stimulated glucose oxidation.* It has been shown previously that exposure of parotid cells to (-)-epinephrine elicits a rapid stimulation of calcium efflux, indicative of cellular calcium mobilizing processes (e.g. Refs. 6 and 7). Since such calcium mobilization is required for many alpha-adrenergic responses [4, 5, 7, 15], the role of calcium in parotid cell glucose oxidation was examined. Table 1 shows that, with various medium

Table 1. Effect of calcium on rat parotid cell glucose oxidation

| Experimental condition | % Stimulation above basal* |
|---|---|
| $10^{-5}$ M Epinephrine | 138.6 ± 19.2 |
| A23187 (20 μg/ml) | |
| +0.8 mM $CaCl_2$ | 81.2 ± 15.6 |
| +3.8 mM $CaCl_2$ | 168.0 ± 25.7 |
| +11.8 mM $CaCl_2$ | 120.2 ± 20.1 |

* Values are the means ± standard errors of five individual experiments in which all four conditions were examined.

Table 2. Effect of calcium on alpha- and beta-adrenergic components of (-)-epinephrine-stimulated glucose oxidation in rat parotid cells*

| | Number of experiments | Percent of maximal stimulation |
|---|---|---|
| $10^{-5}$ M Epinephrine + 1.26 mM $CaCl_2$ | 16 | 100† |
| $10^{-5}$ M Epinephrine, − $CaCl_2$ + 0.2 mM EGTA‡ | 16 | 54.6 ± 3.2 |
| $10^{-5}$ M Epinephrine + $1 \times 10^{-7}$ M prazosin + 1.26 mM $CaCl_2$ | 5 | 46.0 ± 9.9 |
| $10^{-5}$ M Epinephrine + $1 \times 10^{-7}$ M prazosin, − $CaCl_2$ + 0.2 mM EGTA‡ | 5 | 67.2 ± 13.4 |
| $10^{-5}$ M Epinephrine + $3 \times 10^{-5}$ M propranolol + 1.26 mM $CaCl_2$ | 6 | 66.8 ± 3.3 |
| $10^{-5}$ M Epinephrine + $3 \times 10^{-5}$ M propranolol, − $CaCl_2$ + 0.2 mM EGTA‡ | 6 | 72.5 ± 7.8 |
| $10^{-5}$ M Epinephrine + $3 \times 10^{-5}$ M alprenolol + 1.26 mM $CaCl_2$ | 11 | 53.5 ± 13.4 |
| $10^{-5}$ M Epinephrine + $3 \times 10^{-5}$ M alprenolol, − $CaCl_2$ + 0.2 mM EGTA‡ | 11 | 41.9 ± 6.4 |
| $10^{-5}$ M Epinephrine + $1 \times 10^{-7}$ M prazosin + $3 \times 10^{-5}$ M propranolol + 1.26 mM $CaCl_2$ | 4 | 20.0 ± 7.6 |
| $10^{-5}$ M Epinephrine + $1 \times 10^{-7}$ M prazosin + $3 \times 10^{-5}$ M alprenolol + 1.26 mM $CaCl_2$ | 4 | 12.0 ± 5.1 |

* Values represent means ± standard errors for the indicated number of experiments.
† Percent stimulation above basal was 166.2 ± 10.1%.
‡ EGTA alone is capable of increasing glucose oxidation about 10% of maximal levels. Data presented here are not corrected for this effect.

calcium concentrations, the ionophore A23187 (20 μg/ml) elicited a stimulation of glucose oxidation comparable to that exerted by epinephrine at maximal concentrations.

When epinephrine was added to cells which had been prepared and suspended in calcium free medium containing 0.2 mM EGTA, maximal epinephrine stimulation of rat parotid cell glucose oxidation was only about half that achieved in incubations at the normal medium calcium concentration (1.26 mM) (Table 2). Longer exposure of cells to calcium free conditions or higher EGTA concentrations did not reduce the response further (data not shown). EGTA alone (0.2 mM) caused a slight increase in glucose oxidation over basal values (~ 10% of maximum epinephrine response).

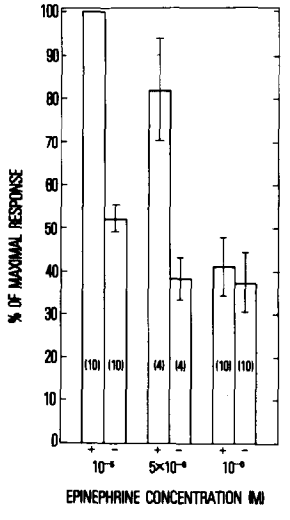


Fig. 2. Effect of (-)-epinephrine concentration on the external calcium dependence of stimulated parotid cell glucose oxidation. Parotid cell aggregates were exposed to indicated concentrations of (-)-epinephrine in the presence of (+) or absence (−) of 1.26 mM $CaCl_2$. Incubations withoug $Ca^{2+}$ also included 0.2 mM EGTA. As noted in the text, EGTA alone is capable of increasing glucose oxidation about 10%. Data here are not corrected for this EGTA effect. Values represent the means ± standard errors for the numbers of experiments indicated in parentheses.

Since epinephrine-stimulated glucose oxidation involves activation at both alpha- and beta-adrenergic receptors [9], an attempt was made to determine whether the external calcium-dependent portion of the response was associated preferentially with one of the two adrenergic systems. Table 2 shows that neither the "pure" alpha (propranolol or alprenolol blocked) nor "pure" beta (prazosin blocked) components of the maximal epinephrine response were dependent on external calcium. In the presence of external calcium, importantly, the inhibition by alpha and beta antagonists was essentially additive.

Another possible explanation for these results would be that the lower 50% of the maximal response was external calcium independent, while the upper 50% was external calcium dependent, regardless of the adrenergic systems utilized. To test this hypothesis, submaximal concentrations of epinephrine were administered to parotid cell aggregates either in the presence or absence of calcium. Figure 2 shows that ~ 45% of the maximal epinephrine stimulated glucose oxidation was external calcium independent, and this absolute amount remained relatively constant at submaximal concentrations of epinephrine. Thus, at $10^{-6}$ M epinephrine which yielded responses only about 40% of those at $10^{-5}$ M epinephrine, essentially no external calcium dependence was apparent.

## DISCUSSION

The findings reported here clearly indicate that

alpha-adrenergic stimulated glucose oxidation in rat parotid cell aggregates is mediated through the $alpha_1$-receptor subtype. In this respect, then, both alpha-adrenergic stimulation of glucose metabolism and secretory processes share a common initiation point [8]. The physiological role of the $alpha_2$-receptor subtype, which comprises about half of the alpha-adrenergic receptor complement of these cells [8], remains to be clearly established. Parotid cell glucose oxidation can also be stimulated through beta-adrenergic receptors, and epinephrine exerts its effect through both alpha- and beta-adrenergic mechanisms [9]. Calcium mobilization appears to mediate many alpha- [4, 5, 7, 16], and some beta- [17, 18], adrenergic responses. This is true, at least in part, for the response studied here since exposure to various calcium concentrations in the presence of the ionophore A23187 stimulated glucose oxidation as well as epinephrine. However, only the upper half of maximal epinephrine-stimulated glucose oxidation appeared to be dependent on external calcium. Neither the pure alpha nor the pure beta component of the response, which comprise roughtly 60 and 40% of the epinephrine effect, respectively [9], was external calcium dependent. Furthermore, $10^{-6}$ M epinephrine, which stimulates glucose oxidation to approximately 40% of maximal levels, also exhibited no external calcium dependence. Interestingly, in a series of experiments studying the metabolic effects of adrenergic stimulation on another salivary gland cell type (rat submandibular), removal of $Ca^{2+}$ from the incubation media did not completely block all responses [19, 20], but preferentially decreased alpha effects.

The external calcium independence of a portion of the epinephrine response in the present study may be due to the mobilization of internal calcium pools. It has been shown previously that alpha-adrenergic stimulation of electrolyte secretion by parotid cells exhibits a biphasic response, the earlier portion of which is independent of external calcium, while the later portion is dependent on external calcium [1]. Similarly, two calcium pools have been identified recently in aorta [21]. Both pools appear to be accessible to mobilization by norepinephrine during contraction, but one is resistant to extensive washing in calcium free, EGTA containing medium [21]. Alternatively, some other intracellular mediator may be responsible for the external calcium independent portion of the epinephrine glucose oxidation response. This, however, seems rather unlikely, especially with respect to $alpha_1$-adrenergic effects, since virtually all $alpha_1$-adrenergic processes described thus far appear to be regulated through calcium mobilization (e.g. Refs. 4, 5, 7 and 16). Clearly, further study of the roles for both external and internal calcium in the $alpha_1$-adrenergic control of glucose oxidation is necessary. In particular, such experiments will be dependent on methods which selectively identify mobilized or immobilized calcium supplies in various cellular and extracellular locations.

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