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ADRENERGIC AND CHOLINERGIC MEDIATED GLUCOSE OXIDATION BY RAT PAROTID GLAND ACINAR CELLS DURING AGING

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Summary: Adrenergic (both α and β) and cholinergic agonists stimulated glucose oxidation in dispersed parotid acinar cells obtained from various aged rats. Basal levels of glucose oxidation were constant over the adult rat life span. Epinephrine and norepinephrine effects on parotid cell glucose oxidation were reduced about 50% in cells from 24 mo rats compared to results with 3 and 12 mo rats. The epinephrine effect was mediated primarily via the α-adrenergic system. In addition, an α-adrenergic agonist (phenylephrine) stimulated glucose oxidation significantly less in parotid cells from 24 mo rats than in those from younger rats. β-Adrenergic (isoproterenol) and cholinergic (carbachol) stimulation did not change with age. These results suggest that an age-related impairment occurs specifically in the α-adrenergic system of rat parotid acinar cells.

Introduction: Adrenergic and cholinergic systems have important roles in the regulation of salivary gland functions (1,2). These systems control the exocrine secretion of water, electrolytes and macromolecules into the forming saliva. Exocrine secretion from salivary glands is energy dependent (3,4).

Earlier reports have demonstrated that glucose oxidation in rat parotid (5) and submandibular glands (5-8) is stimulated by epinephrine (5,8), norepinephrine, pilocarpine (5) and acetylcholine (6,7) at concentrations similar to those eliciting exocrine secretion. However, the pharmacological characteristics of adrenergic effects on glucose oxidation in rat salivary glands are not yet known.

Several age-related morphological changes in rat salivary glands have been reported (9-11) including the presence of unusual pleomorphic mitochondria (11). The metabolic correlates of these morphological observations have not been investigated. Because the secretion of saliva requires energy, and saliva plays an important role in oral physiology (12), we have investigated glucose oxidation,

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as an index of parotid acinar cell energy metabolism, over the entire adult life span of the rat. This tissue provides a good model for aging and neurotransmitter activity studies because of its relatively uniform cellular composition (serous acinar cells). Thus, effects of possible cell population changes are minimized.

Materials and Methods: All animals used in these experiments were male Wistar strain rats, obtained from the Gerontology Research Center (NIA) colony. Rats were fed *ad libitum* until sacrifice. Animals were killed between 9:30 to 10:00 A.M. by a sharp blow to the head and bled via the abdominal aorta. Parotid acinar cells were dissociated by modification of the method of Strittmatter *et al.* (13). Briefly, parotid tissue minces were digested with chromatographically purified collagenase (type CLSPA, Worthington, specific activity; 395 U/mg) and bovine testicular hyaluronidase (type 1-S, Sigma, specific activity; 270 NF units/mg) at a concentration of 96 U/ml and 0.19 mg/ml, respectively, in 5 ml of complete Hanks' balanced salt solution for 60 min at 37° C 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 twice with Hanks' balanced salt solution without glucose containing 4% bovine serum albumin (fraction V, Sigma) and twice with Hanks' balanced salt solution without glucose containing 0.022% bovine serum albumin (incubation medium). Parotid cell aggregates prepared in this way from young and old animals remain viable for at least 6 hr (data not shown). Aggregates were divided into aliquots and suspended in 0.45 ml of incubation medium. After a 10 min preincubation, 25 µl of an adrenergic or cholinergic agonist solution were added except when otherwise indicated. Agonists were dissolved, immediately before use, in incubation medium containing 2 mM ascorbic acid to prevent agonist oxidation. After 12 min of incubation with agonist, 25 µl of 4 mM glucose solution containing 0.5 µCi of D-[U-^{14}C] glucose (Schwarz/Mann, specific activity; 0.23 Ci/mmol) was added. Incubations were performed for 40 min at 37°C at a shaking rate of 60 cycles/min under an atmosphere of 95% O_2 - 5% CO_2 in a rubber stoppered tube (Falcon 2059) with a disposable plastic center well (Kontes), to which 200 µl of hyamine hydroxide (Amersham) 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°C to trap $^{14}CO_2$, 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 into CO_2 at a constant rate for at least 60 min both in the presence and absence of agonists (data not shown). DNA content was determined by the diphenylamine method as modified by Richards (14) using calf thymus DNA (Sigma) as a standard. Protein content was assayed by the method of Lowry *et al.* (15) with bovine serum albumin as a standard.

Epinephrine, norepinephrine, phenylephrine, isoproterenol, all bitartrate form and L-isomer, and carbachol were purchased from Sigma. Phenoxybenzamine HCl and DL-propranolol HCl were obtained from Smith Kline and French Laboratories and Ayerst Laboratories, respectively. All other chemicals used here were the highest grade commercially available.

Data were presented as means ± standard errors (S.E.M.) and the statistical analysis was performed by an unpaired Student's t-test (two-tailed).

Results: As shown in Table 1, no age differences were observed in basal levels of glucose oxidation in rat parotid cell aggregates. This finding was observed

Table 1. Basal levels of glucose oxidation in rat parotid cell aggregates obtained from 3, 12 and 24 mo rats.

Age	Basal Glucose Oxidation	
	nmoles/mg protein/40 min	nmoles/mg DNA/40 min
3 mo	0.40 ± 0.06 (14)	8.0 ± 2 (3)
12 mo	0.35 ± 0.04 (14)	9.1 ± 1.2 (10)
24 mo	0.38 ± 0.06 (11)	7.7 ± 1.2 (9)

Glucose oxidation, protein and DNA content were measured as described under *Materials and Methods*. Each value represents the mean ± S.E.M. for the numbers of rats indicated in parentheses. Triplicate determinations were performed for all assays.

both when data were expressed per mg protein and per mg DNA. Accordingly, all subsequent data on agonist-stimulated glucose oxidation were expressed as percent increase over basal of the glucose oxidized per mg protein.

Figure 1 shows the effects of various epinephrine concentrations on glucose oxidation in parotid cell aggregates obtained from different aged rats. The dose response curves of epinephrine-stimulated glucose oxidation were similar in all age groups examined (3, 12 and 24 mo). A slight stimulatory effect of 10^{-7} M epinephrine was observed in both 3 mo (12 ± 3%) and 12 mo (11 ± 8%), but not in 24 mo rats (1 ± 7%). Substantial elevations of stimulated glucose oxidation were observed between 10^{-6} and 10^{-5} M epinephrine. At 10^{-6}, 10^{-5}, and 10^{-4} M epinephrine, the response of parotid cell aggregates obtained from 24 mo rats was significantly less than that found with parotid cell aggregates obtained from younger (3 and 12 mo) animals.

Since epinephrine is both an α- and β-adrenergic agonist (16), we investigated which adrenergic system was responsible for epinephrine-stimulated glucose oxidation. Initially the effects of specific adrenergic antagonists were studied. As shown in Figure 2, epinephrine (10^{-5} M)-stimulated glucose oxidation was reduced significantly more in the presence of phenoxy-

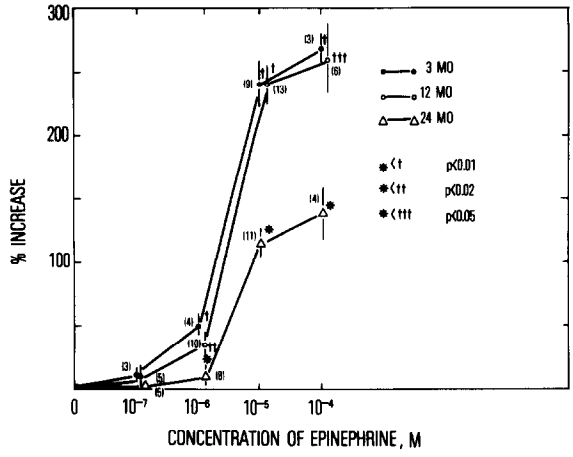

Figure 1. Effects of various concentration of epinephrine on glucose oxidation, expressed as percent increase over basal values, in parotid cell aggregates obtained from 3, 12 and 24 mo rats. Incubations were performed as described in *Materials and Methods*. Each point represents the mean ± S.E.M. for the numbers of rats indicated in parentheses. All determinations were performed in triplicate.

benzamine (3×10^{-5} M), an α-adrenergic antagonist, than in the presence of propranolol (3×10^{-5} M), a β-adrenergic antagonist. This phenomenon was observed in parotid cell aggregates from all age groups examined. Thus, the epinephrine-stimulated glucose oxidation appeared to be primarily an α-adrenergic effect.

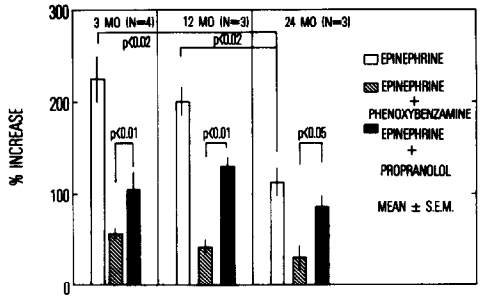

Figure 2. Effects of phenoxybenzamine (3×10^{-5} M) and propranolol (3×10^{-5} M) on epinephrine (10^{-5} M)-stimulated glucose oxidation. Parotid acinar cell aggregates were incubated with phenoxybenzamine or propranolol for 12 min prior to addition of epinephrine. 12 min after epinephrine administration, D-[U-^{14}C] glucose was added. Other experimental conditions were as described in *Materials and Methods*. The results, expressed as percent increase over basal values, are the means of values obtained from the numbers of animals indicated in parentheses. The error bars represent S.E.M. Triplicate determinations were performed for all assays.

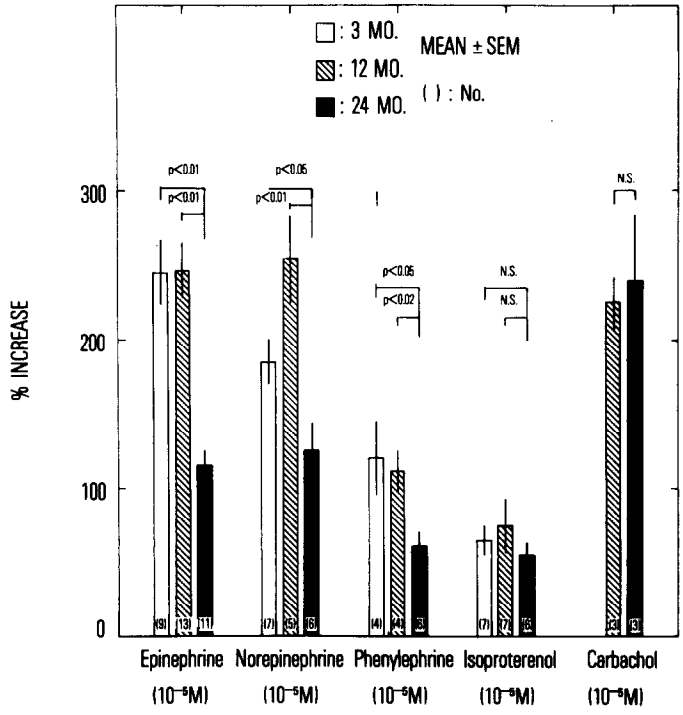

Figure 3. Effects of various agents on glucose oxidation in rat parotid cell aggregates obtained from 3, 12 and 24 mo rats. Incubations were performed as described in *Materials and Methods*. The results, expressed as percent increase over basal values, are the means of values obtained from the numbers of animals given in parentheses. The error bars represent S.E.M. Triplicate determinations were performed for all assays.

To further examine the adrenergic regulation of glucose oxidation, the abilities of several catecholamines to stimulate glucose oxidation were measured. As shown in Figure 3, the relative stimulatory potencies of catecholamines, at a 10^{-5} M concentration, on glucose oxidation in parotid cell aggregates from 3 and 12 mo rats was epinephrine $\geq$ norepinephrine > phenylephrine > isoproterenol. However, in parotid cell aggregates from 24 mo rats, isoproterenol stimulated glucose oxidation was about equal to that of phenylephrine. This was due to a diminished phenylephrine response. At a 10^{-5}M concentration, epinephrine and norepinephrine as well as phenylephrine (a pure α-adrenergic agonist) stimulated glucose oxidation significantly less in parotid cell aggregates from 24 mo rats than in those from younger rats (3 and 12 mo). On the other hand, isoproterenol (a pure β-adrenergic

Table 2. The K_a values and maximal levels of phenylephrine and isoproterenol for stimulation of glucose oxidation in rat parotid cell aggregates obtained from 12 and 24 mo rats.

	K_a for Glucose Oxidation (μM)	Maximal Stimulatory Effects on Glucose Oxidation (% Increase)
Phenylephrine		
12 mo (N=3)	4.4 ± 1.4	93 ± 11†*
24 mo (N=3)	4.7 ± 1.7	39 ± 5††
Isoproterenol		
12 mo (N=3)	3.2 ± 1.9	62 ± 15**
24 mo (N=3)	3.1 ± 1.8	65 ± 16

*Percent stimulation at 10^{-3} M phenylephrine

**Percent stimulation at 10^{-4} M isoproterenol

†>††, $P<0.01$

The effects of various concentrations (10^{-7}-10^{-3}M) of phenylephrine and isoproterenol on glucose oxidation were examined as described in *Materials and Methods*. Substantial elevations of stimulated glucose oxidation between 10^{-5} and 10^{-6} M for both agents, and a slight increase between 10^{-5} and 10^{-3} M for phenylephrine and between 10^{-5} and 10^{-4} M for isoproterenol were observed in both age groups. The maximal levels were achieved at 10^{-3}M for phenylephrine and at 10^{-4} M for isoproterenol in both age groups. The K_a values represent the concentrations of each agonist causing half maximal stimulation (17,18). Each value represents the mean ± S.E.M. for three experiments. Triplicate determinations were performed in each assay.

agonist)- and carbachol (a cholinergic agonist)-stimulated glucose oxidation did not change with age. The K_a values and the maximal levels of both phenylephrine and isoproterenol for stimulation of glucose oxidation were determined in parotid cell aggregates from 12 and 24 mo rats (Table 2).

The K_a values for phenylephrine and isoproterenol did not change with age. However, the maximum stimulatory effect of phenylephrine, but not of isoproterenol, in parotid cell aggregates from 24 mo rats was significantly less than that of 12 mo rats.

Discussion: The data presented here demonstrate that exocrine secretion from the rat parotid gland is accompanied by marked metabolic changes. Both adrenergic and cholinergic agonists stimulated glucose oxidation in rat parotid acinar cells. The adrenergic effects were mediated by both α- and β-adrenergic systems. Two types of experiments support this conclusion; 1) specific α(phenoxybenzamine)- and β(propranolol)-adrenergic antagonists each inhibited epinephrine-stimulated glucose oxidation (Fig. 2). 2) specific α(phenylephrine)- and β(isoproterenol)-adrenergic agonists were able to stimulate glucose oxidation independently (Fig. 3).

In cells from younger (3 and 12 mo) rats, both specific antagonist (Fig. 2) and agonist experiments (Fig. 3) suggested that adrenergic stimulation was principally mediated via the α-adrenergic system. This was not so clear with cells from aged (24 mo) rat parotid. While antagonist experiments (Fig. 2) seem to imply a greater α-adrenergic potency in stimulating glucose oxidation, as in cells of younger animals, specific agonist stimulation studies (Fig. 3, Table 2) suggested that α- and β-adrenergic stimulation of glucose oxidation were approximately equivalent in cells from aged rats. This apparent discrepancy between the antagonist and agonist experiments in aged rats might be due to different age effects on parotid cell responsiveness to agonists *vs* antagonists.

Basal levels of glucose oxidation were the same in parotid acinar cells from rats of all age groups examined. However, epinephrine (Figs. 1-3)- and norepinephrine (Fig. 3)-stimulated glucose oxidation in parotid cell aggregates from 24 mo rats was significantly less than that of cells from 3 and 12 mo rats. A reduced stimulation of glucose oxidation by parotid cells from aged rats was also observed following treatment with phenylephrine,

a specific α-adrenergic agonist (Fig. 3, Table 2). There was, however, no age-related change in the ability of parotid cells to oxidize glucose following treatment with isoproterenol, a specific β-adrenergic agonist (Fig. 3, Table 2). Furthermore, the K_a values for phenylephrine and isoproterenol did not change with age. In addition, cholinergic effects on glucose oxidation in rat parotid cells remained intact throughout the adult rat life span (Fig. 3). These results suggest a rather specific change in metabolic responsiveness, following α-adrenergic stimulation, occurs in rat parotid acinar cells with increased age. Such an alteration in neurotransmitter reponse could have marked physiological sequelae.

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