

Effect of nitrendipine, a calcium antagonist, on cell volume in rat salivary glands after isoproterenol stimulation

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Summary. Four days of isoproterenol injections induced a marked enlargement of the rat parotid and submandibular glands reflected in significant increases in the absolute and relative wet and dry weight of the glands. The enlargement in parotid gland was attributable at least in part to cellular hypertrophy inasmuch as the average volume per cell of acinar cells increased. In contrast, the average volume of acinar cells in the submandibular gland was decreased as compared to that of control. It is likely that hyperplasia in both groups accounts in part for the enlargement. The slow calcium channel is unlikely involved in the isoproterenol-induced stimulation of the gland, inasmuch as the calcium channel antagonist did not modify the enlargement of the parotid or submandibular glands.

Key words: Isoproterenol, Calcium antagonist, Salivary glands, Cell volume, Stereology

Introduction

Isoproterenol, a beta adrenergic agonist, stimulates massive enlargement of the parotid and submandibular glands (Novi and Baserga, 1971; Klingman and McKay, 1973; Hopper et al., 1980). Although morphometry has been employed to quantitate acute effects of isoproterenol-induced enlargement in the parotid (Schneyer, 1969), previous studies of chronic treatment have reported only relative data (Schneyer, 1962; Klingman and McKay, 1973). The data must in turn be related to some absolute quantity, frequently the average volume of the cell, which has not been reported. One of the objectives in the present study therefore was to measure the average volume of parenchymal cells in

the salivary glands of rats injected with isoproterenol.

Isoproterenol injection provides a useful model to study growth, differentiation and secretion in response to stimulation (Schneyer and Shackelford, 1963; Sahara et al., 1984; Argent and Arkle, 1985). Calcium regulates many cellular processes, including growth and differentiation (Terman and Gunter, 1983; Keith et al., 1985). Calcium channel antagonists modulate cellular calcium entry by binding to the slow calcium channel located in the plasmalemma and, at least in the heart, modulate injury induced by isoproterenol (Yeager and Whitehurst, 1982). A second objective of the present study was therefore to determine whether the calcium antagonist nitrendipine (Antman et al., 1980) modifies the stimulatory effects of isoproterenol on salivary glands.

Materials and methods

Sixty female rats weighing between 140 and 150 gm. were obtained from the Holtzman Breeding Co., Madison, WI (USA) and acclimatized to the laboratory for one week before the experiment was begun. Animals received tap water and Purina lab chow *ad libitum*. Animals were divided into groups ($n = 15$ rats/group): group 1, control, receiving vehicle for nitrendipine days 1 to 6 and vehicle for isoproterenol days 3 to 6; group 2, isoproterenol, days 3 to 6; group 3, nitrendipine, days 1 to 6; group 4, nitrendipine, days 1 to 6, isoproterenol, days 3 to 6. The vehicle for nitrendipine was polyethylene glycol 400 diluted 1:10 in sterile 0.9% sodium chloride, whereas the vehicle for isoproterenol was sterile distilled water. Injections of nitrendipine (kindly provided by Dr. Alexander Scriabine, Miles Institute for Preclinical Pharmacology, New Haven, CT) (5 mg/kg body weight) were given intraperitoneally twice daily. Isoproterenol (Sigma Chemical Co., St. Louis, MO) (10 mg/kg) was given intramuscularly twice daily into the gluteal musculature. All injections for rats were delivered alternately to the right or left sides of the

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peritoneum or gluteal muscles.

Animals were anesthetized with pentobarbital immediately before sacrifice; animals were sacrificed 18 hr. after the last injection. Parotid and submandibular glands were excised, trimmed of nonparenchymatous tissue and weighed. One member of each gland pair was cut into 1 mm slices and fixed in 3% glutaraldehyde in 0.1 M phosphate, pH 7.2. After rinsing in several changes of 0.1 M phosphate (pH 7.4) glands were postfixed in 1% osmium tetroxide in 0.1 M phosphate, pH 7.2. The tissue was processed as described previously (Nickerson et al., 1969) and embedded in a mixture of Epon 812 Araldite (Mollenhauer, 1964). One μm sections were cut from 4 blocks for each of 5 animals in each group and stained with 1% toluidine blue in 1% sodium borate. For calculation of the average cell volume, two photographs were recorded from sections of four blocks for 5 animals in each group and enlarged to 1260X. Counts of nuclei from cells of parotid and submandibular glands were made on the photographs. The largest and smallest axes of each acinar cell nucleus were measured and averaged to calculate the mean nuclear diameter. The distribution of nuclear diameters was corrected for missing small profiles by the Giger-Riedwyl method (DeHoff and Rhines, 1961; Weibel, 1979) and the mean particle diameter (D) calculated (Weibel and Bolender, 1973). Volume density (V_v) of acinar parenchymal cells was determined using a plastic overlay with 100 points arranged in a square configuration. The average acinar cell volume (V_{cell}) was obtained by dividing the volume density of acinar cells (V_v) by the numerical density per volume (N_v) (Weibel and Bolender, 1973).

The wet weight of the other member of each gland pair was recorded and the gland dried to constant weight at 100° C. Data was expressed as the mean $\pm$ standard error of the mean. Means were compared using Student's *t* test.

Results

Wet and dry weights

The absolute and relative wet weights of salivary glands in the isoproterenol groups as compared to controls were increased significantly (Table 1). Nitrendipine treatment did not affect the isoproterenol-induced increase in absolute or relative wet weights inasmuch as highly significant increases in both parotid and submandibular gland weights were observed in groups treated with isoproterenol, whether alone or in combination with nitrendipine, as compared to the control. Weights for glands treated with nitrendipine alone were comparable to those of the control groups.

The mean absolute dry weight of salivary glands treated with isoproterenol as compared to control glands was nearly quadrupled in the case of parotid and tripled for the submandibular gland (Table 1).

Nitrendipine did not prevent or modify the isoproterenol-induced increase in absolute dry weights of either gland type. Dry weights for glands after nitrendipine alone were comparable to those of controls.

Average cell volume

The mean cellular volume of parotid acinar cells after isoproterenol (Fig. 1) was elevated (1.8x) above that of controls or 1.9x greater than the nitrendipine (Fig. 2) group (Table 1). Nitrendipine (Fig. 3) did not prevent or inhibit the isoproterenol-induced increase in volume. In the submandibular gland, the mean cellular volume of the control group was 1.5x and nitrendipine alone (Fig. 4) was 1.4x greater than that of animals treated with isoproterenol (Fig. 5) (Table 1). Nitrendipine (Fig. 6), at least in part, ameliorated this response, although the difference was not statistically significant.

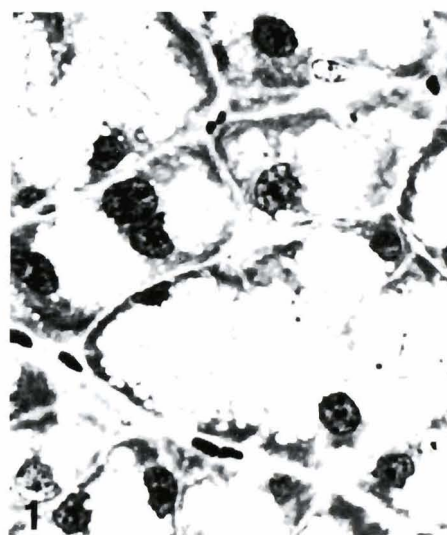


Fig. 1. Parotid acinar cells in group treated with isoproterenol. Mean volume = 2450 μm^3 . Toluidine blue stain. $\times 750$

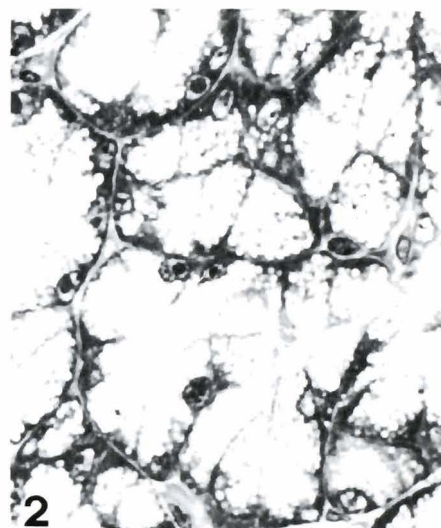


Fig. 2. Parotid acinar cells in group treated with nitrendipine. Mean volume = 1300 μm^3 . Toluidine blue stain. $\times 750$

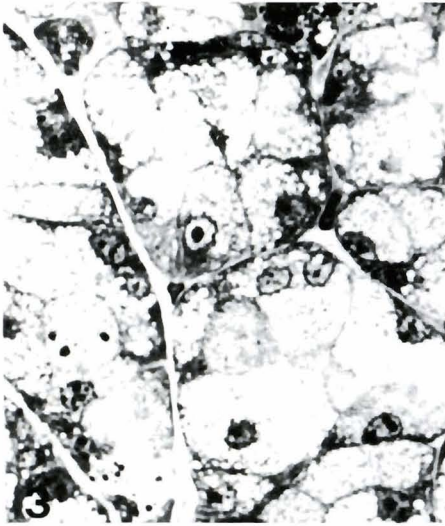


Fig. 3. Parotid acinar cells in group treated with nitrendipine in combination with isoproterenol. Mean volume = $2020 \mu\text{m}^3$. Toluidine blue stain. $\times 750$

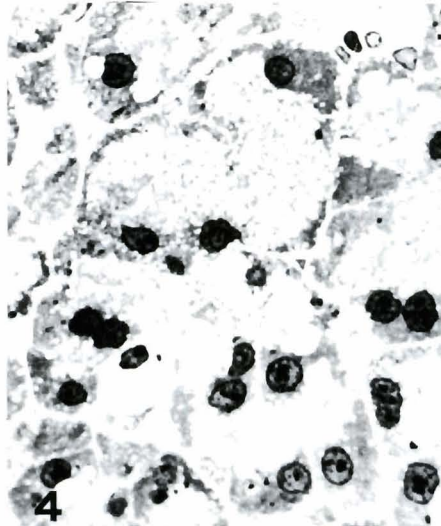


Fig. 4. Submandibular acinar cells in group treated with nitrendipine. Mean volume = $1310 \mu\text{m}^3$. Toluidine blue stain. $\times 750$

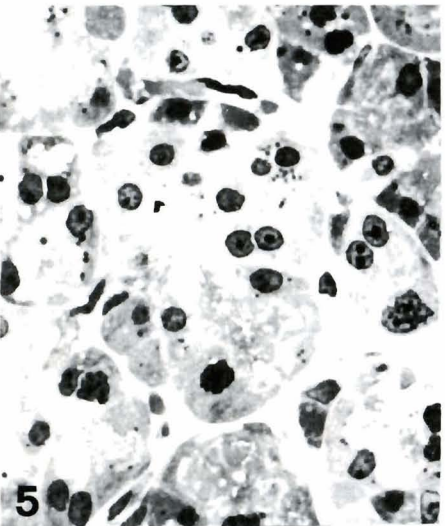


Fig. 5. Submandibular acinar cells in group stimulated by isoproterenol. Mean volume = $917 \mu\text{m}^3$. Toluidine blue stain. $\times 750$

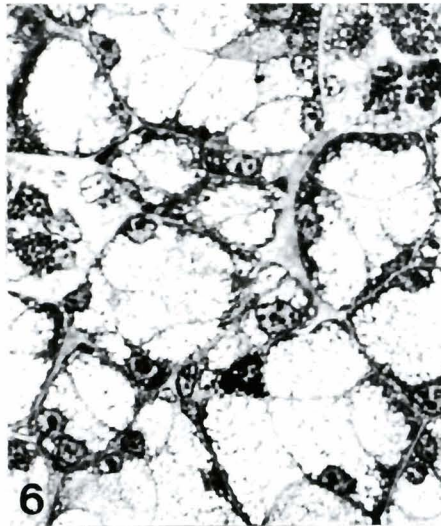


Fig. 6. Submandibular acinar cells in group treated with nitrendipine in combination with isoproterenol. Mean volume = $1190 \mu\text{m}^3$. Toluidine blue stain. $\times 750$

Table 1. Effect of Isoproterenol or Nitrendipine alone or in combination on wet and dry weights and average cell volume of parotid and submandibular glands

Gland	Control	Isoproterenol	Group Nitrendipine	Nitrendipine + Isoproterenol
Parotid				
Wet Weight				
Absolute (mg)	102 ± 16^1	468 ± 47^4	$115 \pm 17^{\text{NS}}$	$522 \pm 22^{4,\text{NS}}$
Relative (mg/100g BW)	55 ± 9	268 ± 27^4	$61 \pm 10^{\text{NS}}$	$294 \pm 14^{4,\text{NS}}$
Dry Weight (mg)	33 ± 4	128 ± 11^4	$40 \pm 5^{\text{NS}}$	$134 \pm 6^{4,\text{NS}}$
Cell volume (μm^3)	1384 ± 15	2450 ± 53^2	$1300 \pm 94^{\text{NS}}$	$2020 \pm 42^{2,\text{NS}}$
Submandibular				
Wet Weight				
Absolute (mg)	290 ± 11	889 ± 39^4	$302 \pm 11^{\text{NS}}$	$947 \pm 42^{4,\text{NS}}$
Relative (mg/100g BW)	151 ± 6	465 ± 20^3	$153 \pm 7^{\text{NS}}$	$534 \pm 22^{4,2}$
Dry Weight (mg)	76 ± 3	225 ± 8^4	$73 \pm 3^{\text{NS}}$	$243 \pm 12^{4,\text{NS}}$
Cell Volume (μm^3)	1360 ± 34	917 ± 49^2	$1310 \pm 41^{\text{NS}}$	$1190 \pm 34^{2,\text{NS}}$

¹ Mean $\pm$ SEM; first superscript is comparison to control; second superscript is comparison of isoproterenol and nitrendipine + isoproterenol groups; NS = not significant.

² $p < 0.05$

³ $p < 0.01$

⁴ $p < 0.001$

Discussion

In the present study four days of injections with isoproterenol induced marked enlargement of parotid gland as reflected in increases in glandular absolute and relative wet weight and dry weight, similar to the observation of Schneyer (1962). The enlargement of the gland is attributable in part to hyperplasia and in part to hypertrophy of the acinar cells reflected in an increase in average volume of the acinar cells in the present study. As reported by Baserga (1970), isoproterenol stimulates mitosis of acinar cells under conditions similar to those employed in the present study.

In contrast to the parotid gland the average cell volume of the acinar cells of the submandibular gland showed a slight, but significant decrease in size, suggesting that hyperplasia at least at this stage (4 days) plays the predominant role in inducing the enlargement. Although an explanation for the difference in response to isoproterenol between the two glands is not readily apparent, one possible explanation involves calcium. A major question arises as to whether isoproterenol induced hypertrophy and hyperplasia are effected by increases in cytoplasmic calcium which arises from an influx of extracellular calcium or from redistribution of sequestered intracellular pools. If the mechanism of isoproterenol induced hypertrophy and hyperplasia involves calcium influx through the long lasting (slow) calcium channel, then the use of nitrendipine, which blocks the influx of calcium through the voltage dependent channel, would be expected to interfere with isoproterenol induced hypertrophy and hyperplasia. By all parameters studied, including quantitative morphometry, nitrendipine neither blocked nor ameliorated the hypertrophy and hyperplasia which resulted from stimulation of rat salivary glands by isoproterenol. This would support the conclusion that isoproterenol induced hypertrophy and hyperplasia are not effected through the slow calcium channel. The stimulation may instead be mediated through a redistribution of intracellular calcium. In partial support of this hypothesis, Simpson and Spicer (1973), using the pyroantimonate technique, reported that isoproterenol affects nuclear and cytoplasmic redistribution of cations in parotid acinar cells.

Another consideration regarding the inter-relationships between isoproterenol, nitrendipine and the calcium channel is the differential sensitivity to the dihydropyridine molecule found among various tissues. Bean and colleagues (1986) detected a profound difference in the binding of nitrendipine molecules between vascular smooth muscle and cardiac muscle. Thus the salivary glands may not be responsive to blockade of the slow calcium channel.

A mechanism not related to calcium may also explain the pathogenesis of the isoproterenol-induced enlargement. It has been suggested that polyamines play a role in isoproterenol induced stimulation.

Ornithine decarboxylase (ODC) and polyamine concentrations are highest during rapid growth, differentiation and replication, and are thought to play regulatory roles in protein and nucleic acid biosynthesis. In rat salivary glands, the growth promoting effects of isoproterenol have been linked to increases in ODC activity and polyamine levels at 4-20 hours after injection (Inoue et al., 1975; Blume et al., 1985). Isoproterenol induces increased levels of putrescine and spermidine as well as hypertrophy in cardiac muscle. Difluoromethylornithine, an irreversible inhibitor of ODC, reverses the isoproterenol-induced elevation in putrescine levels and partially attenuates the increase in spermidine and the hypertrophic response as well (Bartolome et al., 1982).

Thus isoproterenol-induced stimulation of the parotid gland produced cellular hypertrophy, whereas a slight decrease in cell volume was observed in the submandibular gland. The mechanism for the difference between the two glands is unknown and not likely mediated through the slow calcium channel. The pathogenesis of the cellular hypertrophy after 4 days of stimulation will serve as the basis for further investigation.

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