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# Effect of Isoproterenol on Calcium, Protein, and Electrolytes of Rat Submaxillary Gland.* (32423)

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The marked effect of isoproterenol on calcium content of rat submaxillary gland(1) is an opportunity to learn something of the mechanism of storage and release of Ca. Two hypotheses have been considered as mechanisms to explain the high submaxillary gland Ca: (a) a specific one-way transfer mechanism(2) or (b) the presence of a binding substance(3). Loss of Ca after stimulation could occur by reversal of the transfer mechanism, change of pH, or by extrusion of the binding substance.

*Methods.* Male Long-Evans rats, 100-150 g, were prepared by once daily injection of ascending doses of isoproterenol(1). An injection-free period of 40-48 hours was then allowed before rats were used either as controls or for reinjection of 10 mg/kg isoproterenol. Rats were killed 2 hours after drug injection. Analytical methods were as follows: sialic acid(4), amylase(5), Mg(6), $CO_2$(7), intracellular pH using inulin as indicator of extracellular space(8), inulin (9), Na and K by direct flame photometry, total P(10), or as described previously(1-3). Statistical summaries are arithmetic mean ± standard error of the mean.

*Results. Effect of isoproterenol on gland electrolytes.* Reinjection of 10 mg/kg of isoproterenol 40 hours after the last injection of an ascending series of the same drug produced the alterations in electrolytes shown in Table I. Significant changes in addition to the marked loss of Ca previously reported(1) include a fall in Mg and a rise in Na and Cl.

TABLE I. Effect of Isoproterenol on Submaxillary Gland Electrolytes ($\mu$M/g water).*

| | Ca | Mg | Na | K | Cl | P | $CO_2$ |
|---|---|---|---|---|---|---|---|
| Control | 28.5 ±1.4 | 27.0 ±1.0 | 42.8 ± .9 | 137.5 ±1.2 | 62.7 ±1.0 | 152. ±4. | 21.8 ±1.2 |
| Isoproterenol | 2.3 ± .1 | 13.1 ± .7 | 53.0 ±2.9 | 143.0 ±3.0 | 72.5 ± .9 | 144. ±2. | 20.4 ±1.3 |
| % Change | −92 | −51 | +24 | +4 | +16 | −5 | −6 |

* All determinations, except $CO_2$, made on each gland from 6 rats in control (n—12) and 5 rats in experimental (n—10). $CO_2$ determinations made on individual glands from additional 16 rats.

Gland protein N, total N, and sialic acid content fall significantly in relation to total P but much less than Ca (Table II). In another experiment, gland amylase was found to be 91 ± 6 units/g wet weight after isoproterenol but only 13 ± 2 units/g in control rats.

TABLE II. Gland N, Protein N, Sialic Acid, and Ca (per mM total P).*

| | Total N, mg | Protein N, mg | Sialic acid, $\mu$M | Calcium, $\mu$m |
|---|---|---|---|---|
| Control | 252 ± 17 | 144 ± 5 | 46 ± 4 | 131 ± 4 |
| Isoproterenol | 190 ± 12 | 102 ± 7 | 22 ± 2 | 23 ± 5 |

* Determinations done on pooled glands from each rat (n=4 for both groups).

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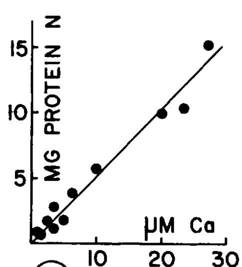
TABLE III. Saliva Electrolytes.*

| Drug & time | Ca | Mg | K | Na | Cl | P | $CO_2$ | Amylase, units | Flow rate, mg/min |
|---|---|---|---|---|---|---|---|---|---|
| | μM | | | | | | | | |
| Isoproterenol | | | | | | | | | |
| 0 - 20 min | 36.0 | 25.3 | 136.5 | 29.7 | 16.7 | 2.0 | 75 | 17 | 4.2 |
| 135 - 240 min | 5.1 | 10.3 | 121.5 | 45.3 | 31.5 | 1.2 | 61 | 63 | 1.3 |
| Pilocarpine | | | | | | | | | |
| 0 - 10 min | 1.26 | .59 | 33.6 | 24.0 | 21.7 | .91 | 50 | 1.33 | 62 |
| 58 - 83 min | .77 | .25 | 27.9 | 32.7 | 22.7 | .46 | 46 | .76 | 22 |

* Values per g of saliva. Results for $CO_2$ and amylase are from separate experiments.

*Saliva electrolytes and protein.* Saliva electrolytes in typical experiments after isoproterenol or pilocarpine injection are shown in Table III. Not only do the individual ion concentrations differ with the two drugs, but after isoproterenol, the sum of cations is almost 200 μEq/g higher than the sum of anions. After pilocarpine, the sum of cations is approximately equal to the sum of anions.

Sialic acid and protein were determined in other experiments and the results in relation to Ca concentration are shown in Fig. 1 and 2. Protein was directly proportional

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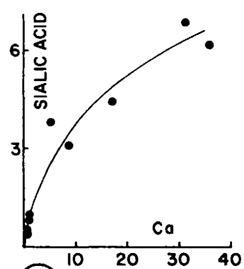


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FIG. 1. Relation of Ca to protein concentration/g of saliva after isoproterenol or pilocarpine.

FIG. 2. Relation of sialic acid to Ca concentration (μM/g) in saliva after isoproterenol or pilocarpine.

to Ca concentration whereas sialic acid was not. Ca binding by isoproterenol or pilocarpine stimulated saliva ranged from 0.19-0.51 μM Ca/mg N when dialyzed against solutions containing 1.0 to 2.2 mM Ca/liter. Saliva dialyzed against 0.003 mM Ca/liter contained 0.035 μM or less of bound Ca per mg N indicating that essentially all of the Ca in saliva was dialyzable. Accuracy of determining Ca after dialysis at this low Ca-free concentration was insufficient to indicate whether binding no longer obeyed the mass law.

*Intracellular pH.* Submaxillary gland intracellular pH was determined either by intravenous or subcutaneous injection of the indicator substances. When the indicators were injected subcutaneously, gland intracellular pH in control rats was 7.08 and 7.11 and in isoproterenol treated rats was 7.29 and 7.23. When the indicators were injected intravenously, gland pH in control rats was 6.66 and 6.77 and in isoproterenol treated rats was 7.15 and 7.16. The indicated increase in pH in the gland in isoproterenol treated rats reflected a greater entry of dimethyloxazolidinedione into the glands of these rats and this enhanced entry might have come about by other than pH related distribution.

*Discussion.* Results are consistent with the hypothesis that the elevated gland Ca in rat submaxillary gland is a storage form related in some way to protein. Loss of cation without similar loss in inorganic anion (Table I), loss of gland protein along with Ca loss (Table II), and appearance of protein in saliva in direct proportion to Ca concentration (Fig. 1) all agree with this hypothesis. A possible role of glycoproteins was indicated by the loss in sialic acid from the gland after isoproterenol. Although the concentration of sialic acid in saliva is related to Ca, the relationship is not so direct as for protein. Ca binding by the protein of saliva was of similar magnitude to Ca binding by gland homogenates(3).

Change in intracellular pH, if real, was in the opposite direction to that which would be expected to reduce Ca binding(3).

*Summary.* The fall in Ca in rat submaxil-

lary gland after isoproterenol injection is at least twice the fall in Mg, sialic acid, or protein. The relationship of Ca to protein in rat saliva is linear. The data are consistent with the hypothesis that Ca is held at high concentrations in salivary glands by means of binding.

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## Adenosine Phosphate of Rat Salivary and Lacrimal Glands *in vitro*.* (32424)

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The transfer of Ca *in vitro* in rat salivary and lacrimal glands is increased by both parasympathetic and sympathetic stimulants(1). This effect of stimulants occurs in relatively large pieces of gland and is only abolished by Na-lack, by high concentrations of metabolic inhibitors, or at 1°C. In the present study, the concentration of adenosine phosphates has been measured after incubation to determine the role of such substances in the transfer process.

*Methods.* Glands were removed under ether anesthesia from Wistar strain male rats maintained on Purina feed and water *ad lib* or from hooded male rats fed a mixed green diet. Glands were incubated for 2 hours with slow agitation in conical flasks gassed with 5% $CO_2$ in $O_2$ in an incubation medium made up in glass distilled water and containing the following (mM/l); 110 NaCl, 3 KCl, 0.1 $MgCl_2$, 1.0 $CaCl_2$, 0.5 $KH_2PO_4$, 25 $NaHCO_3$, 5 dextrose, pH 7.4. Experiments were ordinarily done in groups of 4 using one kind of gland. The experimental groups were usually divided as follows: immediately frozen gland, gland incubated in normal medium, and 2 groups of glands incubated with different concentrations of inhibitor or other altered conditions. Adenosine phosphates and inorganic phosphate ($P_i$) were separated by paper chromatography(2) and estimated by phosphate analysis(3,4) or by UV absorption(5).

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*Results and discussion.* Table I gives the concentrations of adenosine phosphates and $P_i$ in parotid, submaxillary or lacrimal glands for various conditions. After 2 hours incubation, glands had ATP concentrations ranging from 30-80% of that found in immediately frozen glands with the following exception. In glands cut perpendicularly to the greatest dimension the ATP concentration was only 13%. Pieces prepared in this way were approximately 4 mm thick whereas submaxillary gland cut perpendicularly to the smallest dimension or lacrimal and parotid glands as removed from the rat were approximately 2 mm thick. Thus, the system for maintaining ATP functioned as well in pieces up to 2 mm in thickness as in slices approximately 0.5 mm in thickness.

The addition of $5 \times 10^{-5}$ M DNP reduced ATP concentration to approximately ⅔ whereas increasing DNP to $2 \times 10^{-4}$ M reduced ATP to ⅕ of the level found after incubation without inhibitor. These results correlate well with effects of DNP on $Ca^{45}$ activation by acetylcholine in these glands.