Effect of Isoproterenol on Calcium Metabolism in Rat Salivary Gland.* (29418)

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Recent reports(1-6) describe a marked effect of isoproterenol on the size of salivary glands in rats and mice. The concomitant administration of dihydrotachysterol causes, in addition, a visible deposition of Ca salts in the salivary glands(7). These reports suggested the following study on the effect of isoproterenol on Ca metabolism in rat salivary glands.

Methods. Male Wistar rats obtained commercially, weighing from 150-250 g, have been used for all experiments. For acute experiments, isoproterenol was injected subcutaneously at the indicated time prior to autopsy of the rat. Control rats were not injected. Rats were killed by chloroform inhalation. For determining the chronic effects of isoproterenol, rats were injected subcutaneously twice daily beginning at a dose of 1 mg/kg. The dose was then doubled at each injection until 10 mg/kg was being given. The low initial dose was necessary to avoid occasional deaths in rats given higher doses initially. Saliva was collected from the combined submaxillary-sublingual glands and Ca was determined as previously described(8). Statistical summaries are arithmetic mean ± standard error of the mean. Probability was calculated by Fisher's t-test(9).

Results. Acute effect of isoproterenol. Injection of 10 mg/kg of isoproterenol caused a marked loss of Ca from the submaxillary and parotid glands (Table I). The loss of Ca was maximal at the end of 2-4 hours and reaccumulation of Ca began after this time. Significant increases in water content occurred in submaxillary gland at 15 and 30 minutes and in parotid gland in all samples from 30 minutes on. A significant increase in gland weight accompanied the increase in water content of the submaxillary gland at 15 minutes. Submaxillary gland weight fell significantly if 2 doses each of 10 mg/kg were given 2 hours and 1 hour prior to autopsy. Sublingual glands, which contained 12.5 ± 1.5 μEq Ca/g, and extra-orbital lacrimal gland, which contained 6.2 ± 0.3 μEq Ca/g were not significantly affected.

The duration of the acute response was determined after 10 mg/kg isoproterenol was given to rats which had been injected with 7 mg/kg in fractional doses over the previous 48 hours (Fig. 1, 2). The zero time rats, which were autopsied at the time the 10 mg/kg dose was injected, already showed some effects on weight and Ca content of glands as compared to controls (Table I). The further fall in parotid gland Ca concentration subsequent to injection of 10 mg/kg isoproterenol returned to the level found in control

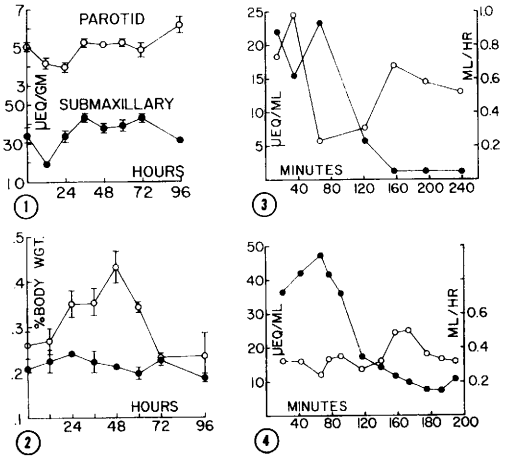

FIG. 1 and 2. Ca concentration (μEq/g) and weight of parotid (○) and submaxillary (●) glands. All rats injected with 1, 2, and 4 mg/kg isoproterenol at 12-hour intervals. Twenty-four hours later, zero time rats were autopsied and remaining rats were injected with 10 mg/kg isoproterenol. Time after 10 mg/kg dose and 2-4 rats at each time.

FIG. 3. Effect of isoproterenol, 10 mg/kg at zero time and again at 127 minutes, on Ca in saliva (●) and flow rate/g gland (○) in normal rat.

FIG. 4. Effect of isoproterenol, 10 mg/kg at zero time, on Ca in saliva (●) and flow rate/g gland (○) in rat injected for 10 days with isoproterenol, 10 mg/kg twice daily.

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TABLE I. Acute Effect of Isoproterenol on Rat Glands.

Time†	Submaxillary Ca, μEq/g	Submaxillary Gland/BW, %	Submaxillary Dry/Wet, %	Parotid Ca, μEq/g	Parotid Gland/BW, %	Parotid Dry/Wet, %
0 (8)	18.2 ± .9	.126 ± .004	25.3 ± .3	6.0 ± .5	.184 ± .020	29.4 ± .8
15 (6)	8.2* ± .7	.156* ± .007	20.0* ± .3	4.7 ± .3	.170 ± .014	25.9 ± 1.9
30 (8)	5.9* ± .4	.123 ± .004	22.2* ± 1.2	3.2* ± .2	.161 ± .004	21.2* ± 1.7
60 (4)	3.7* ± .1	.112 ± .009	24.3 ± .7	2.7* ± .1	.161 ± .023	22.6* ± 1.2
120 (6)	3.2* ± .2	.115 ± .007	23.1 ± 1.4	2.7* ± .1	.147 ± .009	23.1* ± 1.3
60, 120 (4)	3.1* ± .1	.100* ± .006	24.4 ± 1.1			
240 (4)	3.0* ± .1	.116 ± .006	21.3 ± 1.3	3.0* ± .2	.146 ± .009	23.3* ± .8
480 (4)	3.6* ± .6	.118 ± .005	23.8 ± .9	3.7* ± .2	.162 ± .007	24.0* ± .8

* Significantly different from control ($P < .05$).
† Time in minutes after 10 mg/kg isoproterenol subcutaneously. No. of rats in parentheses.

rats (Table I) only after 96 hours. At 96 hours, the increase in submaxillary gland Ca concentration was only beginning to disappear. The weights of submaxillary and parotid glands were always higher than non-injected controls (Table I), with parotid gland showing the greatest rise.

Chronic administration of isoproterenol. Repeated twice daily subcutaneous administration of isoproterenol produced a marked increase in weight and Ca content of submaxillary glands and an increase in weight of parotid glands in rats autopsied 24 hours after the last injection (Table II). Sublingual and extra-orbital lacrimal glands were not affected. Injection of isoproterenol 2 hours prior to autopsy (5‡, 10‡, Table II) in chronically injected rats caused approximately the same percent loss of Ca from submaxillary gland as occurred in normal rats when compared to rats autopsied 24 hours after the last injection (5, 10, Table II). Although the glands of these reinjected rats appeared to decrease in weight as compared to rats autopsied 24 hours after the last injection (5, 10, Table II), the difference was not statistically significant.

Effect of isoproterenol on Ca in saliva. The average isoproterenol-stimulated saliva flow rate in these experiments (Fig. 3, 4) was 0.45 ml/g gland per hour as compared to 3.7 ml/g gland in experiments with pilocarpine (8). On the other hand, the initial saliva Ca concentration in the previously untreated rat (Fig. 3) was 22.1 μEq/ml as compared to an average initial concentration of 3.2 μEq/ml after pilocarpine injection(8). The high concentration of Ca in saliva persisted for more than 2 hours and then fell to a stable concentration averaging 1.2 μEq/ml. The stable Ca concentration after pilocarpine averaged 0.93 μEq/ml(8).

In the experiment shown in Fig. 3, the Ca secreted in saliva during the period of elevated saliva Ca concentration was found to be 3.46 μEq. At the stable concentration

TABLE II. Effect of Chronic Administration of Isoproterenol on Rat Glands.

Days†	Submaxillary Ca, μEq/g	Submaxillary Gland/BW, %	Submaxillary Dry/Wet, %	Parotid Ca, μEq/g	Parotid Gland/BW, %	Parotid Dry/Wet, %
Control (8)	18.2 ± .9	.13 ± .01	25.3 ± .3	6.0 ± .5	.18 ± .02	29.4 ± .8
3 (4)	28.5* ± 2.5	.25* ± .02	25.7 ± .4	3.7* ± .1	.40* ± .02	27.3 ± .6
5 (4)	30.3* ± 1.4	.56* ± .14	24.7 ± .7	3.7* ± .1	.68* ± .05	26.0* ± .2
5‡ (4)	5.0* ± .5	.24* ± .04	21.7* ± .1	2.9* ± .1	.52* ± .01	20.4* ± .3
10 (4)	33.7* ± 1.0	.64* ± .09	26.9 ± .1	4.1* ± .2	1.23* ± .15	26.1* ± .5
10‡ (4)	5.1* ± 1.0	.47* ± .08	21.1* ± .2	2.7* ± .1	.96* ± .21	21.6* ± .2
20 (4)	36.0* ± 2.6	.99* ± .12	27.4 ± .4	4.8 ± .1	.98* ± .23	27.4 ± .2

* Significantly different from control ($P < .05$).
† No. of days isoproterenol injected. Determinations 24 hr after last injection except ‡ which were reinjected with 10 mg/kg isoproterenol 120 minutes prior to autopsy. No. of rats in parentheses.

(1.2 μEq/ml), the volume of saliva secreted during this period would have contained 0.31 μEq Ca. Thus, 3.15 μEq of excess Ca were secreted during the initial transient phase. At termination of the experiment, the gland from which saliva was collected was found to contain 1.27 μEq Ca and to weigh 0.256 g. The initial gland Ca calculated from the sum of the excess Ca in saliva and the final gland Ca was 17.3 μEq/g. This is the same Ca content as is found in untreated control rats (Table I).

When saliva was collected from a rat which had been injected with isoproterenol for 10 days prior to the experiment, the initial Ca concentration in saliva was even higher than in a normal rat (Fig. 4). Ca concentration in the saliva never reached the low level found in rats injected with isoproterenol or pilocarpine. However, the gland from which saliva was collected contained only 5.2 μEq/g of Ca at the end of experiment or the same amount as was found in other reinjected chronically treated rats (5‡, 10‡, Table II). The difference in total content of Ca between the control gland taken out prior to injection of isoproterenol and the gland removed at the end of experiment was 19.1 μEq. Only 17.3 μEq of Ca appeared in the saliva indicating that the saliva collection was not complete, that a part of the gland Ca escaped from the gland by other means, or that the initial Ca content of the gland used for saliva collection had been less than that present in the control gland.

Discussion. Isoproterenol injection caused even more striking depletion of Ca in submaxillary glands of rats than occurs with pilocarpine injection(8). In fact, the final Ca content of the submaxillary gland was less than the 4.0 μEq/g found in kidney(10). The greater depletion of Ca by isoproterenol probably results from a greater loss of secretory granules after administration of sympathomimetic agents than after parasympathomimetic agents(11). Depletion of Ca in lacrimal glands and sublingual glands did not occur although the amount of Ca in sublingual glands was two-thirds that found in submaxillary glands and the amount of Ca in extra-orbital lacrimal glands was the same as in parotid glands.

Repeated administration of isoproterenol induced a rapid increase in both weight and calcium content in submaxillary glands and in weight of parotid glands but no change in other glands. The Ca content of submaxillary gland reached a plateau at approximately twice the initial level whereas the size of the gland continued to increase in the second 10 days after beginning isoproterenol injection. In contrast, the weight of parotid gland did not increase in the second 10 days of isoproterenol administration. The rapid loss of Ca from the submaxillary gland (5‡, 10‡, Table II) indicates that the Ca was held in a readily mobilizable form.

The secretion of Ca in saliva after isoproterenol injection was chiefly remarkable for the great ratio between saliva Ca and the ultrafiltrable Ca of serum. If Ca in the isoproterenol-stimulated saliva is present in ionic form, then a barrier to free diffusion of Ca out of the duct system must be present. Another possibility which might account for the great excess of Ca in saliva in these experiments is the presence of a Ca-binding substance in the saliva which prevents diffusion. Such a substance could be liberated from secretory granules at the same time Ca is lost from the gland.

Conclusions. (1) A single injection of isoproterenol reduces submaxillary gland Ca to one-sixth and parotid gland Ca to one-half of the initial level, or to concentrations comparable to those present in other soft tissues. (2) Repeated twice daily subcutaneous administration of isoproterenol increases the weight of submaxillary and parotid glands by up to 6-fold and the Ca content of submaxillary gland to double the initial concentration. (3) Isoproterenol-stimulated submaxillary gland saliva initially contains about 20 μEq Ca/g in normal rats and approximately twice this amount in rats chronically injected with isoproterenol.

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1. Selye, H., Veilleux, R., Cantin, M., *Science,*

1961, v133, 44.
2. Brown-Grant, K., *Nature*, 1961, v191, 1076.
3. Schneyer, C. A., *Am. J. Physiol.*, 1962, v203, 232.
4. Schneyer, C. A., Shackelford, J. M., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 320.
5. Selye, H., Veilleux, R., Cantin, M., *Growth*, 1961, v25, 243.
6. Wells, H., *Am. J. Physiol.*, 1962, v202, 425.
7. Selye, H., *Calciphylaxis*, Univ. of Chicago Press, Chicago, 1962, p159.
8. Dreisbach, R., *Am. J. Physiol.*, 1959, v196, 645.
9. Batson, H. C., *An Introduction to Statistics in the Medical Sciences*, Burgess Publishing Co., Minneapolis, 1960, p16.
10. Dreisbach, R. H., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 555.
11. Junqueira, L. C. U., *Salivary Glands and their Secretions*, Pergamon Press, New York, 1964.

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Effects of Maternal Intake of Nicotine on Fetal and Newborn Rats.* (29419)

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Administration of nicotine to pregnant animals has been shown to produce capillary damage in the placenta in dogs(1), skeletal defects of the offspring and decrease of litter size in mice(2), vertebral anomalies in chicks (3), and postponement of the appearance of the first litter(4) and lighter weight offspring in rats(5). Administration of nicotine to nursing rat mothers is associated with a poor neonatal weight gain(5) and an increase in neonatal mortality(5,6). Geller(7) gave pregnant rats nicotine doses less than 15% of those used by Nishimura and Nakai in mice (2) and produced no fetal abnormalities. This difference may be explained on the basis of dosage and species differences; however, the current status of this problem is unsettled.

Acute administration of nicotine results in a rise in serum free fatty acids in dogs and humans(8). Smokers, when compared with non-smokers, have been shown to have increased serum lipoproteins(9) and cholesterol (9,10,11) and to produce babies of lighter weight(12). The effect of nicotine on fetal lipids has not been investigated.

In this study, a nicotine solution in place of water was given to rats throughout pregnancy and up to the time of weaning in order to determine its effect on fetal and neonatal growth and mortality. Measurements of neonatal brain and liver lipid and cholesterol content were made as a preliminary investigation of the effect of nicotine on fetal lipid metabolism.

Materials and methods. Two experiments were carried out using virgin Sprague-Dawley female rats which were mated to males of the same strain. The body weight at the start of pregnancy ranged from 237 to 305 g. Conception was dated from the time of the appearance of the vaginal plug. The experimental groups were given a solution of .05 mg/ml nicotine as the sole source of drinking water from time of conception throughout pregnancy and the control animals were given distilled water. The solution and water were given *ad lib* in graduated drinking tubes and daily intake was recorded. All animals were given Purina Lab Chow *ad lib*.

In Experiment I, 8 rats given nicotine and 9 control rats were studied. Three of the experimental animals received nicotine only for the last 14 days, 6 days and 4 days of pregnancy respectively. Within a few hours of birth the newborn rats were weighed and then killed by decapitation. The brains and livers were promptly dissected free of other tissue, weighed, and frozen. Lipid and nitro-

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