PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184**, 102–106 (1987)

β-Adrenergic and Muscarinic Receptors of Parotid Gland following Maintenance of Rats on Liquid Diet (42452)

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Abstract. Parotid gland of adult rats maintained exclusively on liquid (milk) diet for 7 or 13 days showed a 25% reduction in number of β-adrenoceptors, and after 21 days, the reduction was 33%; with maintenance of rats on Metrecal for 7 days, the decrease was 24% for female rats and 22% for male rats. The decrease in number of muscarinic receptors after 7 or 13 days on milk was 32%, and 38% after 21 days; the decreases for rats on Metrecal for 7 days were 32% for females and 35% for males. In rats maintained on liquid (milk) diet for 7 days, and then denervated by unilateral removal of the parasympathetic and sympathetic innervations to parotid there were decreases of 39–42% in number of β-receptors and 50–52% in muscarinic receptors at 6 or 14 days after denervation (maintenance on liquid diet for 13 and 21 days, respectively) from those of innervated glands of chow-fed rats; denervated glands of rats on chow diet showed the same reduction. Thus, it was concluded that absence of neurally mediated glandular activity, imposed by either diet or surgical removal of the nerves, caused marked decreases in number of both β-adrenergic and muscarinic receptors, but that the presence of the nerves, even though inactive (liquid diet), provided a trophic influence that prevented the more marked decreases seen in the absence (surgical removal) of the nerves.

Denervation of rat salivary glands is reported to result in a decrease in number of muscarinic receptors (1). The size of the glands is also decreased following denervation (2) but the change in receptor number is not attributed solely to the decrease in gland size (1). Since an increase in receptor number follows glandular enlargement induced by increased autonomically mediated activity (3–5), it seemed possible that the decrease in receptor density with denervation might stem from the absence of neurally mediated glandular activity. However, it is also possible that changes in receptor number are the consequences of the denervation and not the absence of neural stimulation of the gland. Therefore, receptor density of parotid glands of rats maintained on an all-liquid diet were examined; under these conditions, the autonomic pathways to the gland are intact but inactive (6, 7). The results were compared with the effects induced by surgical removal of the autonomic nerves to determine if changes were induced when the nerves were present but inactive as well as when they were physically absent.

Materials and Methods. Long–Evans rats 4 months of age were maintained on solid chow and water *ad libitum* until introduction of experimental manipulations. Some animals were maintained on a liquid diet consisting exclusively of milk; others were on a liquid diet of Metrecal. It was previously established that milk, Metrecal, or pulverized solid chow suspended in water can serve interchangeably as a liquid diet to produce salivary gland atrophy (6, 7). Under ether anesthesia, one superior cervical ganglion and a portion of the auriculotemporal nerve on the same side were removed surgically. Some of these animals were maintained on the normal solid chow diet, whereas other animals were maintained on the liquid (milk) diet. The period on milk diet was varied and included maintenance on milk for 7, 13, or 21 days. The length of the denervation period was 6 or 14 days. Under Nembutal anesthesia (ip, 1%) both parotid glands were rapidly removed, and weighed separately; parts of the glands were placed in 10 m*M* Tris–HCl buffer for subsequent membrane isolation and assays for cyclic nucleotides and receptor densities. The same tissue samples were used for cyclic GMP and cyclic AMP determinations. In brief, the gland homogenates were deproteinized by the addition

0037-9727/87 $1.50

of an equal volume of 10% TCA and the insoluble protein subsequently was removed by centrifugation at 10,000 rpm for 10 min in a Sorval HB-4 rotor (Dupont Company, Wilmington, DE) at 4°C. The TCA was removed from the aqueous phase by repeated washing (four to six times) with 4 vol of water-saturated ether (8).

Cyclic AMP levels were measured according to the method of Gilman (9) using a kit assay system (Amersham Corp., Arlington Heights, IL). Assays were performed on fresh ground preparations with 50 μl of sample in duplicate reactions.

The measurements of cyclic GMP concentration were performed using an assay kit purchased from Amersham Corporation (two replicates were used for each measurement). The sample (100 μl) was mixed with 50 μl of ^{3}H cyclic GMP in an assay tube containing Tris–HCl buffer (10). Finally, 50 μl of specific antiserum was added to each sample and incubated for 90 min at 4°C. The reaction was terminated by the addition of ammonium sulfate. The samples were centrifuged (10,000g for 10 min), the supernatant was decanted, and the pellet was resuspended in 1 ml of water. The samples were then placed in scintillation cocktail and counted after dark adaptation by a Beckman LS211 liquid scintillation counter (Beckman, Palo Alto, CA) using the tritium channel.

Gland homogenates for both [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB) and [^{3}H]-dihydroalprenolol ([^{3}H]DHP) (Dupont NEN, North Billerica, MA) binding were prepared by centrifugation at 20,000g for 30 min (4°C). The pellet containing the membrane fraction was resuspended in 100 vol of 10 mM Tris–HCl buffer, pH 7.6, containing 4 mM $MgCl_2$ and 100 μM dithiothreitol. Membranes were resuspended by a combination of vortex vibration followed by Dounce homogenization. Protein concentrations were subsequently determined by a modification of the Lowry protein assay using bovine serum albumin as standard (11). Binding of [^{3}H]QNB and [^{3}H]DHP were linearly dependent on membrane concentration within this dilution of both the parotid and submandibular glands. Binding assays were performed in duplicate using 1.0 ml of diluted membrane and 1.0 nM [^{3}H]QNB or [^{3}H]DHP. This concentration of [^{3}H]QNB and [^{3}H]DHP has been found to be above saturation levels for parotid cell membranes as described by Ludford and Talamo (12). The reaction mixture was incubated for 90 min at 37°C, and terminated by the addition of 3 ml ice-cold 0.9% NaCl. Quantitation of binding was performed by precipitation of membranes from the above slurry onto glass fiber filters, washed three times in 5 vol of cold PBS, and counted for radioactivity by liquid scintillation. Nonspecific binding was determined by the inclusion of 1.0 μM atropine 10 min prior to the addition of labeled QNB or 1.0 μM propranolol prior to [^{3}H]DHP addition.

Results. β-Adrenoceptors of parotid gland of rats maintained on liquid diet of milk were reduced from levels of glands of chow-fed controls when DHP binding per milligram of membrane protein was measured. The reduction was 25% after 7 or 13 days on the milk diet; by 21 days, the reduction with milk was 33%. Unilateral removal of the parasympathetic and sympathetic innervation to parotid of liquid (milk) diet-fed rats at 6 or 14 days prior to receptor assays resulted in a 39–42% reduction in β-receptor density, when comparison was made to innervated parotid of chow-fed rats; the reduction was virtually the same in parotid of chow-fed rats when the parasympathetic and sympathetic nerves were removed 6 days before receptor assays.

The data in Table 1 show the changes in density of muscarinic receptors of parotid gland after maintenance of rats on a milk diet. After 7 or 13 days on milk diet, there was a 32% reduction when comparison was made to chow controls; after 21 days on this diet, the reduction was 38%. A 50–52% reduction was observed at 6 or 14 days after denervation in rats fed either liquid or solid chow diet.

The data in Table II are presented to compare the effects of a liquid diet of milk with those of another liquid diet, Metrecal (powder in water). Reductions from chow controls in number of β-adrenergic and muscarinic receptors of parotid gland of female rats on Metrecal for 7 days were 24 and 32%, respectively, and thus these changes are very similar to those induced by the liquid milk diet. Moreover, the changes in male rats were very similar to those in female rats (22% in β adrenoceptors, and 35% in muscarinic receptors,

TABLE I. DENSITY OF β-ADRENERGIC AND MUSCARINIC RECEPTORS OF NEURALLY INTACT AND DENERVATED PAROTID GLAND OF RATS FED ONLY LIQUID OR SOLID DIET

Diet and condition	Receptor density: β Adrenergic [^{3}H]DHP Binding (f mol/mg mem. prot.)	Receptor density: Muscarinic [^{3}H]QNB Binding (f mol/mg mem. prot.)	Cyclic nucleotides: AMP (p mol/mg prot.)	Cyclic nucleotides: GMP (p mol/mg prot.)	Gland wt (mg)
Chow, Inn. (10)	122 ± 1.9	185 ± 2.2	7.4 ± 0.6	6.7 ± 0.2	211 ± 10.0
MI-7 days, Inn. (10)	91 ± 0.7	126 ± 1.9*	5.4 ± 0.3*	6.7 ± 0.4	119 ± 4.0*
MI-13 days, PxSx-6 days } (10)	71 ± 2.2*	88 ± 1.7*	7.0 ± 0.4	6.4 ± 0.3	85 ± 4.3*
MI-13 days, Inn. } (10)	92 ± 1.2*	125 ± 2.7*	6.8 ± 0.1	6.5 ± 0.2	110 ± 1.8*
MI-21 days, PxSx-14 days } (10)	73 ± 2.2*	92 ± 1.9*	6.7 ± 0.4	6.1 ± 0.2	84 ± 4.0*
MI-21 days, Inn. } (10)	82 ± 0.9*	115 ± 2.6*	6.6 ± 0.3	6.3 ± 0.2	108 ± 3.5*
MI-21 days, PxSx-14 days; Refed chow-2 days } (7)	81 ± 2.0*	95 ± 1.2*	7.3 ± 0.5	6.4 ± 0.1	82 ± 6.4*
MI-21 days, Inn.; Refed chow-2 days } (7)	113 ± 6.5	133 ± 2.8*	9.7 ± 1.2*	7.2 ± 0.2	175 ± 10.0*
Chow, PxSx-6 days (7)	78 ± 3.0*	98 ± 2.0*	6.5 ± 0.6	6.8 ± 0.3	113 ± 15.0*

Note. Values are means ± SE. Numbers in parentheses equal number of rats. *Values differ significantly from Chow, Inn. ($P < 0.01$). MI = rats maintained on liquid diet of milk for number of days indicated. PxSx = part of one auriculotemporal nerve and a superior cervical ganglion were removed and then maintained on the same dietary regimen for the number of days indicated.

respectively) (Table II). The rats show similar body weights on solid or liquid diet (Table II).

To show that the change in receptor density with liquid (milk) diet was actually the result of neural inactivity, rats denervated and maintained on liquid (milk) diet were refed for 2 days with solid chow. The data in Table I show that when the innervation was absent, refeeding with chow had no effect on receptor density and the numbers remained at the same low levels seen with liquid diet alone or with complete denervation. However, with the fully innervated gland of rats on liquid (milk) diet, introduction of solid food for only 2 days caused an increase in masticatory activity and resulted in an increase in β-adrenoceptors to 93% of control chow levels, and an increase in muscarinic receptors to 72% of control chow levels.

Cyclic AMP concentrations of parotid gland of rats on liquid diet showed a 27% reduction from glands of chow-fed controls (Table I) only when the period of maintenance on liquid diet did not exceed 7 days. There were no decreases under the other conditions described. There was, however, an increase in cyclic AMP concentration in glands of milk-fed rats when refed with chow diet for 2 days. Concentration

TABLE II. DENSITY OF β-ADRENERGIC AND MUSCARINIC RECEPTORS OF PAROTID GLAND OF MALE AND FEMALE RATS FED ONLY LIQUID (METRECAL) OR SOLID DIET

Diet and sex of rat	Receptor density: β-Adrenergic [^{3}H]DHP binding (f mol/mg mem. prot.)	Receptor density: Muscarinic [^{3}H]QNB binding (f mol/mg mem. prot.)	Gland wt (mg)	Rat wt (g)
ME, female (6)	89 ± 0.7	122 ± 0.3	90 ± 3	198 ± 12
Chow, female (6)	117 ± 1.3	180 ± 2.9	208 ± 17	205 ± 13
ME, male (6)	90 ± 0.5	121 ± 0.6	122 ± 7	380 ± 20
Chow, male (6)	116 ± 0.3	187 ± 0.2	201 ± 15	371 ± 25

Note. Values are means ± SE. Numbers in parentheses equal number of rats. Rats were maintained on Metrecal (ME) for 7 days, or on solid chow (Chow).

of cyclic GMP was not changed under any conditions described.

Discussion. Present data show that both β-adrenergic and muscarinic receptors of rat parotid gland are reduced from 25 to 52% from that of normally innervated glands when autonomically mediated glandular activity is absent. The removal of such neural influences was effected by maintenance of rats on an all-liquid diet (7), by surgical removal of the parasympathetic and sympathetic nerves (complete denervation) to the gland (2), or by a combination of liquid diet and complete denervation. The reduction was more marked with complete denervation than it was with liquid diet alone. With liquid diet, the volley of neural impulses from both parasympathetic and sympathetic nerves to the gland is assumed to be absent, due to the elimination of masticatory activity (6, 13–15); however, some residual trophic effects of the intact innervation must remain since the reduction in receptor number was greater when the innervation was absent. A decrease in receptor number has been shown previously to occur following denervation (1) and these authors suggested that the decrease was not solely a reflection of the decrease in size of the gland, since the magnitude of the two changes was not similar (1). Present data support this view and furthermore show that the absence of neurally mediated activity is primarily responsible for the receptor changes.

Levels of cyclic AMP of rat parotid gland were reduced when the period of maintenance on liquid diet did not exceed 10 days, but not when the period on liquid diet exceeded this. No change in cyclic AMP was observed with complete denervation, and levels of GMP were unchanged with either liquid diet or complete denervation. Only with the reintroduction of solid chow to rats on liquid diet was there an increase in cyclic AMP; this is assumed to be related to the sudden increase in level of neurotransmitter (10, 16). Receptor levels were increased nearly to those seen in glands of chow-fed rats with the reintroduction of solid chow to rats on liquid diet. The increase in neurally mediated activity associated with this maneuver also results in an increase in mitotic activity of the parotid gland (17), and an increase in size of the gland and its acinar cells (17). When the two branches of the innervation were absent, the reintroduction of solid food had no effect, levels of receptors remained low, and gland size was unchanged. The data thus suggest that it is the increase in neurally mediated glandular imposed with the return to solid food that causes these changes.

The data also show that there were no significant differences between male and female in the percentage reduction of receptors, both β-adrenergic and muscarinic. Moreover, both sexes showed similar weights on liquid Metrecal and solid chow; similar reductions in size of the parotid gland were also effected in both sexes by the liquid diet of Metrecal. Thus, a difference in kind of nutrients in the diets had no influence in the effects induced by the liquid diet. Furthermore, cyclic fluctuations in hormones of female rats apparently had no important influence since the receptor changes in male and female rats were so similar.

This work was supported by Grant DE 02110 from the National Institutes of Health, Bethesda, Maryland 20205. The authors acknowledge the excellent technical assistance of Herman Forrest, Gloria Robinson, and Sue Al-Zahid.

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Received June 11, 1986. P.S.E.B.M. 1987, Vol. 184.
Accepted October 13, 1986.