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EFFECT OF SYMPATHETIC DENERVATION ON THE 5-HYDROXYTRYPTAMINE LEVELS OF THE SUBMAXILLARY GLANDS OF RATS*

GERDA I. KLINGMAN and JACK D. KLINGMAN

Department of Biochemical Pharmacology, School of Pharmacy and Department of Biochemistry, School of Medicine, State University of New York at Buffalo, Buffalo, N.Y. 14214, U.S.A.

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Abstract—Sympathetic denervation of submaxillary glands of rats by means of superior cervical ganglionectomy resulted in significant increases of the 5-hydroxytryptamine concentration and total 5-hydroxytryptamine content. Sympathetic denervation reduced significantly the weight of the glands but this decrease was less marked than the previously observed reduction in gland weight following immunosympathectomy. These observations indicated that absence of sympathetic innervation may be responsible for the previously observed increase of the 5-hydroxytryptamine levels in a number of peripheral tissues of rats following immunosympathectomy.

PREVIOUS investigations have shown that the 5-hydroxytryptamine levels in some areas of the gastrointestinal tract of immunosympathectomized mice[1–3] and in the submaxillary glands, heart and cecum of immunosympathectomized rats[4] were increased. An increase in the mast cell populations of the intestinal tract has been held responsible for the more intense fluorescence characteristic for 5-hydroxytryptamine observed in the intestinal tract of immunosympathectomized rats.[5] Thompson[2] and Thompson and Campbell[3] presented evidence that the elevated 5-hydroxytryptamine levels in some areas of the intestinal tract from immunosympathectomized mice seemed to be the result of increased concentrations of 5-hydroxytryptamine per argentaffin cell rather than of a larger cell population. In our previous study[4] statistically significant increases of the 5-hydroxytryptamine levels were noted in the submaxillary glands, heart and cecum. In other tissues, such as the small intestine, lungs, uterus and liver, a mean increase occurred which was not statistically significant.

It was proposed that the increased 5-hydroxytryptamine levels of some peripheral tissues following immunosympathectomy is a secondary effect of sympathetic denervation.[4] This postulate is tested in the present investigation.

The postganglionic sympathetic outflow to the submaxillary glands of rats is almost entirely via the superior cervical ganglion and the right gland and the left gland are almost exclusively innervated by the respective superior cervical ganglion. The submaxillary glands lend themselves to complete sympathetic denervation by means of unilateral and bilateral superior cervical ganglionectomies.

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METHODS

Male and female Sprague–Dawley rats weighing between 120 and 200 g (before surgery) were used in the present study. Sympathetic denervation of the submaxillary glands was achieved by unilateral and bilateral superior cervical ganglionectomies. The animals were anesthetized with 25–35 mg/kg of pentobarbital sodium, preceded by 0·6 mg/kg of propantheline bromide and 8 mg/kg of chlorpromazine hydrochloride. These drug solutions were administered intraperitoneally (i.p.). The neck was shaved and sponged with 70% alcohol. Both superior cervical ganglia were removed from six rats, the right superior cervical ganglion from seven rats and the left superior cervical ganglion from nine rats. Sham operations were performed on control littermates and on the contralateral control ganglia. The animals were sacrificed 3–12 weeks after surgery by exsanguination under pentobarbital sodium anesthesia (35–45 mg/kg, i.p.).

The submaxillary glands were dissected, weighed on a Mettler balance and homogenized in a Duall tissue grinder (Kontes, Size A or B). The 5-hydroxytryptamine was extracted by the method of Bogdanski *et al.*[6] and measured on an Aminco–Bowman spectrophotofluorometer at 295/540 mμ (uncorrected wavelengths). The norepinephrine and epinephrine were extracted by a modification[7] of the method of Shore and Olin[8] and measured spectrophotofluorometrically by a modification[9] of the method of von Euler and Floding.[10] After bilateral superior cervical ganglionectomy the concentrations of the amines of the heart were also determined.

RESULTS

The right and the left submaxillary glands from control rats were similar in weight, 5-hydroxytryptamine, norepinephrine and epinephrine concentrations (Table 1).

TABLE 1. CONCENTRATIONS OF 5-HYDROXYTRYPTAMINE (5-HT), NOREPINEPHRINE (NE) AND EPINEPHRINE (E) IN THE RIGHT AND LEFT SUBMAXILLARY GLANDS OF EIGHT CONTROL RATS* (BODY WEIGHT 273 ± 26 g†)

Submaxillary glands	Weight (mg)†	5-HT	NE	E	Ratio‡ (mg/g)†
		(ng/g)†			
Right	231 ± 18	1110 ± 110	1250 ± 100	90 ± 20	0·861 ± 0·037
Left	230 ± 17	1010 ± 70	1300 ± 130	110 ± 20	0·858 ± 0·043

* Six female and two male rats.
† ± Standard error.
‡ Submaxillary gland/body weight.

After bilateral superior cervical ganglionectomy, the submaxillary glands were smaller in weight, the gland / body weight ratio was significantly reduced and the 5-hydroxytryptamine concentration was increased to 133 per cent of the littermate control values. The norepinephrine concentration was decreased to barely measureable quantities (Table 2).

After right ganglionectomy no residual norepinephrine was discernable in any of the seven glands while after left ganglionectomy residual norepinephrine was detected

as small peaks characteristic of norepinephrine at 405/510 mμ. The 5-hydroxytryptamine concentration nearly doubled in the denervated glands compared to the contralateral innervated control glands. The total 5-hydroxytryptamine content of the denervated glands was also increased. Due to the relatively large variations in the 5-hydroxytryptamine concentration and total content of innervated and denervated

TABLE 2. CONCENTRATIONS OF 5-HYDROXYTRYPTAMINE (5-HT), NOREPINEPHRINE (NE) AND EPINEPHRINE (E) IN THE SUBMAXILLARY GLANDS AND HEARTS OF SEVEN CONTROL RATS AND THEIR SIX BILATERAL SUPERIOR CERVICAL GANGLIONECTOMIZED LITTERMATES

	Submaxillary glands		Hearts	
	Control	Ganglionecto-mized	Control	Ganglionecto-mized
No. of rats	7	6	7	6
Body weights (g)*	251 ± 14	259 ± 19	251 ± 14	259 ± 19
Tissue weights (mg)*	461 ± 16	414 ± 23†	802 ± 33	791 ± 47
Ratios‡ (mg/g)*	1·866 ± 0·103	1·618 ± 0·096§	3·226 ± 0·070	3·068 ± 0·092
5-HT (ng/g)*	790 ± 90	1050 ± 70	220 ± 30	190 ± 30
NE (ng/g)*	990 ± 70	60 ± 40‖	600 ± 50	560 ± 40
E (ng/g)*	30 ± 10	50 ± 10	20 ± 10	30 ± 0

* ± Standard error.
† $P < 0·05$ (*t*-test in paired experiments).[11]
‡ Wet weight of tissue, mg/body weight, g.
§ $P < 0·01$ (*t*-test in paired experiments).[11]
‖ $P < 0·001$ (*t*-test in nonpaired experiments).[11]

glands, some of the data summarized in Table 3 (denervated left glands) were statistically nonsignificant. Denervation resulted in reduced gland weights; this reduction was statistically significant after left ganglionectomy. However, the ratios of gland weights to body weights (mg/g) were statistically different after either right or left superior cervical ganglionectomy.

Pooling of the data from the right and left denervated and from the right and left contralateral innervated glands revealed a significant increase of the 5-hydroxytryptamine concentration and of the total 5-hydroxytryptamine content (Table 4). In fourteen of the sixteen unilaterally ganglionectomized rats, the denervated glands were smaller than the contralateral innervated glands.

DISCUSSION

The results of the present investigation demonstrated that the absence of sympathetic innervation may be related to the increased 5-hydroxytryptamine concentrations of some peripheral tissues of immunosympathectomized rats[4] and portions of the intestinal tract of immunosympathectomized mice.[1-3] It is not known whether the absence of peripheral sympathetic innervation is directly responsible for the increased 5-hydroxytryptamine levels or indirectly responsible by altering some biochemical or functional properties of the tissue.

It is of interest to note that the magnitude of the increased 5-hydroxytryptamine concentrations of the submaxillary glands after unilateral superior cervical ganglionectomy and after immunosympathectomy were quantitatively similar; after right or left ganglionectomy the 5-hydroxytryptamine increased to 193 and 196 per cent of

TABLE 3. CONCENTRATIONS OF 5-HYDROXYTRYPTAMINE (5-HT), NOREPINEPHRINE (NE) AND EPINEPHRINE (E) IN THE SUBMAXILLARY GLANDS OF RATS AFTER RIGHT OR LEFT UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY

Ganglionectomy	No. of rats	Body weight (g)*	Gland	Gland weights (mg)*	5-HT (ng/g)*	NE (ng/g)*	E (ng/g)*	5-HT (ng/gland)*	Ratio† (mg/g)*
Left	9	260 ± 7	Left	199 ± 5‡	2000 ± 450§	100 ± 20‖	90 ± 20	400 ± 100§	0·752 ± 0·027¶
			Right (control)	221 ± 4	1150 ± 100	1860 ± 100	150 ± 30	260 ± 20	0·833 ± 0·024
Right	7	309 ± 29	Right	237 ± 12§	1850 ± 260‡	0	0	390 ± 40**	0·788 ± 0·953††
			Left (control)	252 ± 15	960 ± 110	1980 ± 160	100 ± 40	250 ± 30	0·836 ± 0·046

* ± Standard error.
† Ratio of gland weight/body weight, mg/g.
‡ $P < 0·01$ (*t*-test in nonpaired experiments).[11]
§ $P < 0·05$ (*t*-test in nonpaired experiments).[11]
‖ $P < 0·001$ (*t*-test in nonpaired experiments).[11]
¶ $P < 0·01$ (*t*-test in paired experiments).[11]
** $P < 0·02$ (*t*-test in nonpaired experiments).[11]
†† $P < 0·05$ (*t*-test in paired experiments).[11]

TABLE 4. CONCENTRATION AND TOTAL CONTENT OF 5-HYDROXYTRYPTAMINE OF THE SUBMAXILLARY GLANDS OF SIXTEEN RATS AFTER RIGHT OR LEFT UNILATERAL SUPERIOR CERVICAL GANGLIONECTOMY (BODY WEIGHTS 281 ± 14*)

	Gland weights (mg)*	5-hydroxytryptamine		Ratio† (mg/g)*
		(ng/g)*	(ng/lobe)*	
Control gland (right or left lobe)	235 ± 8	1070 ± 80	250 ± 20	0·834 ± 0·023
Ganglionectomy (right or left lobe)	216 ± 7	1850 ± 260‡	390 ± 50§	0·768 ± 0·012‖

* ± Standard error.
† Ratio of gland weight/body weight, mg/g.
‡ $P < 0.01$ } (*t*-test in nonpaired experiments).[11]
§ $P < 0.05$ }
‖ $P < 0.001$ (*t*-test in paired experiments).[11]

control values, respectively, and to 194 per cent of control values after immunosympathectomy.[4] It seems relevant that the most marked and consistent elevations of 5-hydroxytryptamine after immunosympathectomy occurred in the submaxillary glands and the heart;[4] these two tissues are nearly completely sympathectomized by the Nerve Growth Factor (NGF)-antiserum treatment.[7, 12]

We were unable to explain the less marked increase of 5-hydroxytryptamine observed after bilateral superior cervical ganglionectomy (Table 2). It may be that the variation of the 5-hydroxytryptamine concentrations from rat to rat, even among littermates, was responsible for this apparently less pronounced effect of bilateral ganglionectomy.

Superior cervical ganglionectomy resulted in a small reduction in the weight of the denervated submaxillary glands (6–10 per cent; Tables 2, 3 and 4). This decrease was statistically significant after bilateral and unilateral (left) superior cervical ganglionectomy. A similar decrease (average, 8 per cent) has been reported by Nordenfelt.[13] The observation that the reduction of the gland weight was more pronounced after immunosympathectomy[4] than after surgical denervation may be explained on the basis that the NGF-antiserum contained, in addition to the NGF-antibody, antibodies against other components of the submaxillary glands. Another possibility to explain this may be connected with the difference in the ages of the rats subjected to immunosympathectomy and ganglionectomy. Immunosympathectomy was performed within 6 days after birth while ganglionectomy was performed in rats 43 days old or older. Sympathetic denervation of a smooth muscle or a gland before it is fully developed may result in different effects than denervation of a mature tissue.

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