DEPLETION OF CATECHOLAMINE BY RESERPINE IN THE INNERVATED AND DENERVATED SUBMAXILLARY GLANDS OF RATS

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Shimamoto et al. (1-3) in this laboratory have shown that the sympathetic stimulation of the submaxillary gland in dog, though produces a transiently mucinous secretion per se, depresses the profuse watery secretion caused by stimulation of the chorda tympani or intravenous injection of pilocarpine. The submaxillary gland contains a relatively large amount of noradrenaline (4). Yamawaki (5) has shown that the profuse, spontaneous flow of the submaxillary saliva begins to manifest in association with sedation caused by reserpine in an unanesthetized dog, and is abolished by sectioning of the chorda tympani. However, the spontaneous flow of saliva turns to a decrease at the time when the endogenous catecholamines in the brain, heart and adrenal glands are maximally depleted (6). Accordingly, it is obscure whether the spontaneous flow of saliva caused by reserpine is conditioned by the amine depletion in the brain or submaxillary gland.

The catecholamine depleting effect of reserpine on the adrenal medulla in the rat is reported to be significantly reduced by the splanchnic denervation (7). In the current experiments the catecholamine depleting effect of reserpine on the innervated and acutely or chronically denervated submaxillary glands in rats was comparatively studied in an attempt to confirm the mode of sympathetic innervation on the reserpine effect. In addition, the changes in the level of the brain noradrenaline caused by reserpine in the intact rats and rats subjected previously to the resection of the superior cervical ganglion were studied in order to know what extent the brain noradrenaline was maintained by the sympathetic nerve originating from the ganglion.

METHODS

Male albino rats of Wistar strain, weighing 200 to 350 g, were used. The animals were maintained on the commercial diet and water ad libitum in an individual cage at the room temperature of 22±2°C. The uni- or bilateral cervical ganglia were aseptically extirpated under anesthesia with intraperitoneal injection of 40 mg/kg of pentobarbital sodium. The sham operated animals were subjected to controls. Ten, 24 and 48 hours and 9 days after the respective surgical operations the animals were killed by decapitation, and the brain and submaxillary glands were isolated for the determination of
tissue catecholamine as described below. The number of animals used for one surgical
procedure was 4 to 10.

The single intraperitoneal dose of reserpine, 0.5 mg/kg, which caused the depletion
of the submaxillary amine by about 90% in the sham operated rat was selected to study
the effect of the sympathetic denervation on the reserpine effect in the submaxillary
gland. The respective groups of the operated rats consisted of 6 to 8 in number were
killed by decapitation at 10 and 24 hours after reserpine, and the isolated brain and
submaxillary gland were subjected to the assay of tissue noradrenaline.

The brain excluding the cerebellum was divided into the cortex and brain stem.
The weighed tissues from two animals were pooled and homogenized with 0.4 N HClO4.
The extract was purified on Dowex 50W×2 column and the content of noradrenaline
was determined photofluorometrically following the methods of Bertler et al. (8) and of
Higuchi (6).

RESULTS

1. Effects of bilateral superior cervical ganglionectomy

a) Submaxillary gland

Table 1 and Fig. 1 show the levels of noradrenaline in the submaxillary gland at
0, 10, 24 and 48 hours and also 9 days after the bilateral superior cervical ganglionecto-
my and the sham operation. The ganglionectomy but not the sham operation produced
a considerable degree of miosis and ptosis which manifested next day after the opera-
tion and lasted until sacrifice of the animals. Though the level of the submaxillary
amine increased by 18% at 10 hours, it decreased by 82% at 24 hours and was completely
depleted at 48 hours after the ganglionectomy. At 9 days after the surgical operation
the gland showed still a complete loss of the amine. On the other hand, the levels of
the submaxillary amine in the sham operated rats showed slight but nonsignificant re-
duction at 10, 24 and 48 hours after the ganglionectomy.

<table>
<thead>
<tr>
<th>Hours after</th>
<th>Sham operation</th>
<th>Bilateral ganglionectomy</th>
<th>% Change from sham operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ganglionectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.87±0.26* (5)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>10</td>
<td>1.67±0.20 (3)</td>
<td>1.99±0.17 (4)</td>
<td>+17.7</td>
</tr>
<tr>
<td>24</td>
<td>1.60±0.12 (3)</td>
<td>0.30±0.07 (3)</td>
<td>−82.3</td>
</tr>
<tr>
<td>48</td>
<td>1.72 (2)</td>
<td>0.00 (2)</td>
<td>−100</td>
</tr>
<tr>
<td>9 (days)</td>
<td></td>
<td>0.00 (4)</td>
<td>−100</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the number of observation.
* Mean±S.E.M. (pg/g).
% Change : + Increase, — Decrease.

b) Brain stem and cortex

Neither ganglionectomy nor sham operation affected significantly the levels of nor-
adrenaline in the brain stem (Table 2 and Fig. 2) and the brain cortex (Table 3 and
FIG. 1. Noradrenaline content in rat submaxillary glands after superior cervical ganglionectomy. Vertical lines represent standard error of the mean.

TABLE 2. Effect of superior cervical ganglionectomy on the noradrenaline content of the brain stem.

<table>
<thead>
<tr>
<th>Hours after ganglionectomy</th>
<th>Sham operation</th>
<th>Bilateral ganglionectomy</th>
<th>% Change from sham operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.55 ± 0.02* (5)</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>10</td>
<td>0.49 ± 0.01 (3)</td>
<td>0.49 ± 0.03 (4)</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0.56 ± 0.06 (3)</td>
<td>0.55 ± 0.03 (3)</td>
<td>-1.8</td>
</tr>
<tr>
<td>48</td>
<td>0.54 (2)</td>
<td>0.52 (2)</td>
<td>-</td>
</tr>
<tr>
<td>9 (days)</td>
<td>0.50 ± 0.01 (4)</td>
<td>——</td>
<td>-9.0</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M. (µg/g).

Figures in parentheses and % Change represent the same as in Table 1.

FIG. 2. Noradrenaline content in rat brain stem after superior cervical ganglionectomy. Vertical lines represent standard error of the mean.
Table 3. Effect of superior cervical ganglionectomy on the noradrenaline content of the brain cortex.

<table>
<thead>
<tr>
<th>Hours after ganglionectomy</th>
<th>Sham operation</th>
<th>Bilateral ganglionectomy</th>
<th>% Change from sham operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.25±0.01* (5)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>0.28±0.03 (3)</td>
<td>0.27±0.02 (4)</td>
<td>−5.6</td>
</tr>
<tr>
<td>24</td>
<td>0.25±0.00 (3)</td>
<td>0.29±0.03 (3)</td>
<td>+16.0</td>
</tr>
<tr>
<td>48</td>
<td>0.28 (2)</td>
<td>0.24 (2)</td>
<td></td>
</tr>
<tr>
<td>9 (days)</td>
<td>--</td>
<td>0.22±0.01 (4)</td>
<td>−12.0</td>
</tr>
</tbody>
</table>

* Mean±S.E.M. (μg/g)
Figures in parentheses and % Change represent the same as in Table 1.

Fig. 3. Noradrenaline content in rat brain cortex after superior cervical ganglionectomy.
Vertical lines represent standard error of the mean.

Although the level in the brain cortex at 24 hours showed an increase by 16% of unknown origin.

2. Effects of the unilateral superior cervical ganglionectomy
Table 4 shows the noradrenaline levels in the right and left submaxillary gland,

Table 4. Noradrenaline content in rat submaxillary glands, brain stem and brain cortex at 24 hours after bilateral and left superior cervical ganglionectomy.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Sham operation</th>
<th>Bilateral ganglionectomy</th>
<th>Left ganglionectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submaxillary glands</td>
<td>R 1.60±0.12* (3)</td>
<td>0.30±0.07 (3)</td>
<td>1.99±0.03 (3)</td>
</tr>
<tr>
<td></td>
<td>L 0.36±0.06 (3)</td>
<td>0.55±0.03 (3)</td>
<td>0.34±0.03 (3)</td>
</tr>
<tr>
<td>Brain stem</td>
<td>0.25±0.00 (3)</td>
<td>0.29±0.03 (3)</td>
<td>0.51±0.04 (3)</td>
</tr>
<tr>
<td>Brain cortex</td>
<td></td>
<td></td>
<td>0.25±0.02 (3)</td>
</tr>
</tbody>
</table>

* Mean±S.E.M. (μg/g).
In the sham operation and bilateral ganglionectomy, the glands on the right and left side were pooled.
brain stem and brain cortex at 24 hours after the sham operation, and left and bilateral ganglionectomies. Sham operation did again not affect the levels. The decrease in the noradrenaline level of the denervated left gland by 78% approximated to that (82%) of the bilaterally denervated gland. On the other hand, the innervated right gland showed a significant increase by 20% in the noradrenaline level. The noradrenaline levels in the brain stem and cortex were not affected by the unilateral ganglionectomy.

3. Effects of reserpine on the innervated and denervated submaxillary gland, brain stem and cortex

Table 5 and Figs. 4, 5 and 6 show the noradrenaline levels in the submaxillary gland, brain stem and cortex of the sham operated and bilaterally ganglionectomized rats at 10 hours after the administration of 0.5 mg/kg of reserpine. The depletion of the amine in the submaxillary gland and, brain stem and cortex of the sham operated rat were 92, 31 and 57%, respectively. The depletion of the tissue amine after reserpine treatment in the rat at 10 hours after the bilateral ganglionectomy when the amine level in the denervated gland showed some increase were 90, 43 and 68%, respectively. Thus,

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Sham operation Controls</th>
<th>Reserpine</th>
<th>% Change</th>
<th>Bilateral ganglionectomy Controls</th>
<th>Reserpine</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submaxillary glands</td>
<td>1.67±0.20* (3)</td>
<td>0.13±0.04 (3)</td>
<td>-92.3</td>
<td>1.99±0.17 (4)</td>
<td>0.21±0.02 (3)</td>
<td>-89.3</td>
</tr>
<tr>
<td>Brain stem</td>
<td>0.49±0.01 (3)</td>
<td>0.34±0.02 (3)</td>
<td>-50.6</td>
<td>0.49±0.03 (4)</td>
<td>0.28±0.02 (3)</td>
<td>-42.9</td>
</tr>
<tr>
<td>Brain cortex</td>
<td>0.28±0.03 (3)</td>
<td>0.12±0.02 (3)</td>
<td>-57.2</td>
<td>0.27±0.02 (4)</td>
<td>0.09±0.01 (3)</td>
<td>-67.9</td>
</tr>
</tbody>
</table>

* Mean±S.E.M. (µg/g).
Figures in parentheses indicate the number of observation. % Change : — Decrease.

**Fig. 4.** Noradrenaline content of submaxillary glands at 10 and 24 hours after reserpine (0.5 mg/kg) into sham operated and denervated rat.

S : Sham operation. S+R : Reserpine into sham operation.
D : Denervation. D+R : Reserpine into denervation.
Vertical lines represent standard error of the mean.
FIG. 5. Noradrenaline content of brain stem at 10 and 24 hours after reserpine (0.5 mg/kg, i.p.) into sham operated and denervated rat.

Vertical lines represent standard error of the mean.

FIG. 6. Noradrenaline content of brain cortex at 10 and 24 hours after reserpine (0.5 mg/kg, i.p.) into sham operated and denervated rat.

Vertical lines represent standard error of the mean.

**TABLE 6.** Noradrenaline content of rat tissues at 24 hours after the intraperitoneal injection of 0.5 mg/kg of reserpine into sham operated and ganglionectomized rats.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Sham operation Controls</th>
<th>Reserpine</th>
<th>% Change</th>
<th>Bilateral ganglionectomy Controls</th>
<th>Reserpine</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submaxillary glands</td>
<td>1.60 ± 0.12*(3)</td>
<td>0.16 ± 0.00 (4)</td>
<td>-90.0</td>
<td>0.30 ± 0.07 (3)</td>
<td>0.15 ± 0.04 (4)</td>
<td>-50.0</td>
</tr>
<tr>
<td>Brain stem</td>
<td>0.56 ± 0.06 (3)</td>
<td>0.34 ± 0.07 (4)</td>
<td>-39.3</td>
<td>0.55 ± 0.03 (3)</td>
<td>0.37 ± 0.07 (4)</td>
<td>-32.7</td>
</tr>
<tr>
<td>Brain cortex</td>
<td>0.25 ± 0.00 (3)</td>
<td>0.11 ± 0.02 (4)</td>
<td>-56.0</td>
<td>0.29 ± 0.03 (3)</td>
<td>0.16 ± 0.03 (4)</td>
<td>-44.9</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M. (μg/g).

Figures in parentheses indicate the number of observation.
there was no significant difference in the noradrenaline depletion by reserpine between the innervated and denervated submaxillary gland and brain tissues. However, the administration of the same dose of reserpine at 24 hours after the bilateral ganglionectomy when the submaxillary amine was depleted by 82%, produced only reduction by 50% exhibiting the remaining amount of noradrenaline by 0.15 μg/g (Table 6 and Figs. 4, 5 and 6). Almost the same amount of remaining noradrenaline which was likely to be resistant to reserpine effect was observed in the submaxillary gland at 10 hours after the bilateral ganglionectomy. However, the remaining and reserpine-resistant noradrenaline in the gland disappeared at 48 hours after the ganglionectomy, as described above.

**DISCUSSION**

The superior cervical ganglionectomy in the rat resulted in a slight increase in the level of the submaxillary noradrenaline at 10 hours followed with profound decrease by 82% and almost 100% at 24 and 48 hours after the operation. The results differ somewhat from those presented by Benmiloud et al. (9). They showed that the submaxillary noradrenaline in the rat began to decline 8 hours after the sympathetic denervation and complete depletion was attained at 24 hours. Weiner et al. (10) demonstrated that a complete depletion of noradrenaline in the brown adipose tissue in rat was attained at 24 hours after the sympathetic denervation, though the level of the tissue amine did not change until 8 hours after the operative procedure. The similar time-course of the noradrenaline decrease was demonstrated in the adipose tissue in mice by Shideman et al. (11). In the present experiments the level of the submaxillary amine did not decline but rather slightly increased at 10 hours after the denervation. The similar degree of increase in the noradrenaline level in the innervated submaxillary gland was observed at 24 hours after the contralateral superior cervical ganglionectomy. However, the mechanism responsible for the accumulation of the amine in the denervated and innervated submaxillary glands is unknown.

The active release of the endogenous noradrenaline by sympathetic nerve impulses is well known. In the acutely decentralized sympathetic nerve supplying the vasculatures of the skeletal muscle in cat, Rossel et al. (12) postulated that about 10,000 impulses were needed to cause the release of the endogenous noradrenaline to the same extent as the spontaneous release of the amine in the innervated nerve. In this connection, it is possible to deduce that the surgical block of the nerve impulse results in accumulation of the endogenous noradrenaline within 10 hours after the ganglionectomy.

The complete disappearance of noradrenaline in the submaxillary gland at the later stage of denervation is due to the degeneration of the postganglionic fibers. The uptake of the circulating catecholamine by the innervated sympathetic structures was shown by Whitby et al. (13), Marks et al. (14) and Hattori (15). A marked decrease in uptake of the exogenously administered noradrenaline-3H by the chronically denervated submaxillary gland was shown by Hertting et al. (16), Burn and Rand (17) and Strömblad et al. (4). Recently, Fujiwara et al. (18) in this laboratory have demonstrated histochemically
in rats that noradrenaline fluorescence in the submaxillary gland is markedly increased by the infusion of noradrenaline and reduced by the sympathetic denervation. Thus, the decreased uptake of the circulating noradrenaline by the denervated gland is likely to be responsible, at least in some part, for the decrease of endogenous noradrenaline. The superior cervical ganglionectomy did not affect the noradrenaline level in the brain stem, while that in the brain cortex was slightly increased at 24 hours after the surgical operation.

The intraperitoneal administration of 0.5 mg/kg of reserpine depleted profoundly the endogenous noradrenaline in the innervated as well as in the acutely denervated submaxillary glands. Carlsson et al. (19) demonstrated that the noradrenaline-depleting effect of reserpine differed strikingly according to tissues and the heart was most sensitive to reserpine. In this respect, the submaxillary gland is also one of the most sensitive tissues to reserpine. The catecholamine-depleting effect of reserpine within 10 hours after treatment showed little difference between the innervated and denervated glands, while the percent depletion caused by reserpine at 24 hours after the ganglionectomy was much lower than that in the innervated gland, though the noradrenaline level in the denervated gland was markedly reduced. The result indicates that the depletion of catecholamine at least within 10 hours after reserpine treatment does not concern with the central effect, and that reserpine depletes the submaxillary noradrenaline by the peripheral mechanism. Kroneberg and Schümann (7) and Holzbauer and Vogt (20) in the adrenal glands and Sedvall and Thorson (21) in the skeletal muscle demonstrated that the catecholamine-depleting effect of reserpine is reduced by acute sympathetic denervation. These results are inconsistent with the present results on the salivary gland.

As described above, the percent depletion of noradrenaline in the submaxillary gland caused by reserpine at 24 hours after the ganglionectomy was much less that in the innervated gland. Reserpine could not produce further depletion of the submaxillary amine profoundly reduced by the denervation. Therefore, the degenerating process in the sympathetic postganglionic fibers is likely to interfere with the reserpine effect. The residual amount of the submaxillary amine at 24 hours after reserpine treatment was almost the same in the innervated and denervated glands. The residue is likely to be located at some storage site highly resistant to the attack of reserpine (22, 23).

SUMMARY

The effects of the superior cervical ganglionectomy and reserpine on the noradrenaline levels in the submaxillary gland, brain stem and brain cortex were studied in rats.

The superior cervical ganglionectomy resulted in a slight increase in the noradrenaline level of the submaxillary gland at 10 hours followed with depletion by 82% at 24 hours, and by 100% at 48 hours and 9 days after the surgical operation.

The intraperitoneal administration of 0.5 mg/kg of reserpine depleted the submaxillary amine to almost the same extent in the innervated as well as in the acutely denerv-
vated glands. However, the markedly reduced amount of the submaxillary amine at 24 hours after the ganglionectomy was less depleted by reserpine.

The same ganglionectomy did not affect the noradrenaline level in the brain stem but slightly elevated that in the brain cortex at 10 hours after the operation. The depleting responses of the brain noradrenaline to reserpine did not differ between the innervated and denervated animals.

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