J. Pharm. Pharmac., 1967, **19**, 705–708 Received June 16, 1967

On the disposition of [^{3}H]metaraminol in the rat salivary gland*

O. ALMGREN AND B. WALDECK

The left salivary glands of rats were sympathetically denervated or decentralized. In some experiments the excretory ducts of the left submaxillary and sublingual glands were ligated to produce atrophy of the acinar cells. The rats received [^{3}H]metaraminol (^{3}H-MA) intravenously and were killed at various time intervals thereafter. The amount of ^{3}H-MA in the salivary glands was determined. ^{3}H-MA was taken up and retained in the intact gland, but disappeared rapidly from the denervated one, indicating that ^{3}H-MA is taken up and stored in the adrenergic neuron. Decentralization resulted in a decreased turnover of the amine, especially during the first 18 hr, which supports the view that metaraminol is released by nerve activity. The ability of the salivary gland to take up ^{3}H-MA was diminished by glandular atrophy, and the disappearance of the ^{3}H-MA so taken up was delayed.

METARAMINOL is an analogue of noradrenaline and it is similarly taken up by the adrenergic neuron and released by nerve stimulation and by drugs (Andén, 1964; Crout, Alpers & others, 1964; Shore, Busfield & Alpers, 1964) but it is not attacked by monoamine oxidase and catechol-*O*-methyl transferase.

By using [^{3}H]-labelled metaraminol, its fate in tracer amounts has been studied (Carlsson & Waldeck, 1965). We have now examined the fate of [^{3}H]metaraminol (^{3}H-MA) in the rat salivary gland after denervation, decentralization or ligation of the excretory ducts, to gain further insight into the mechanisms of amine uptake and release in adrenergic nerves.

The submaxillary gland of the rat has a relatively rich supply of adrenergic nerves and is easily available for surgical procedures. Ligation of the excretory duct of the gland causes a weight reduction of about 65% due mainly to a reduction of the cytoplasm in the acinar cells. Fluorescent microscopic pictures indicate that the network of noradrenaline nerve terminals is much denser after duct ligation while the adrenergic ground plexus of the blood vessels is not noticeably different from the innervated organ. The noradrenaline content of the atrophied gland, per unit weight, is almost double that found in the intact organ (Andén, Norberg & Olson, 1966). It has been suggested that extraneuronal binding sites might be of importance for the efficient uptake of monoamines (e.g. tyramine and noradrenaline) in the adrenergic nerves of the rat salivary gland, and that glandular atrophy might result in the loss of such binding sites leading to a reduced uptake capacity (Almgren, Andén & Waldeck, 1965).

From the Department of Pharmacology, University of Göteborg, Sweden.

* Part of this report was presented in a discussion remark at the "International Symposium on Mechanisms of Release of Biogenic Amines" in Stockholm, February 21–24, 1965 (Almgren, 1966).

Experimental

MATERIALS AND METHODS

Sprague-Dawley rats of either sex were used. In one group the left submaxillary and sublingual glands were denervated by excising the superior cervical ganglion. In a second group decentralization was effected by removing about 1 cm of the cervical sympathetic trunk proximal to the superior ganglion, and in a third group the excretory ducts were ligated causing the glands to atrophy. In all animals the operations were performed on the left side, the right side serving as a control. In some experiments both ligation of the excretory ducts and decentralization were performed.

The ganglionectomized animals were left 10 days to allow adrenergic nerves to degenerate. After decentralization the animals had 5 days to recover from the operation. After duct ligation, maximal atrophy developed in 14 days. At the end of the above time intervals the rats were given 0·01 mg/kg of (±)-^{3}H-MA via a tail vein and maintained in an environmental temperature of 30°. At different time intervals following drug administration the animals were killed by a blow on the head. The submaxillary and sublingual glands of each side were removed, weighed and extracted in perchloric acid. After ion-exchange chromatography the ^{3}H-MA content of the eluates was determined in a liquid scintillation counter (Carlsson & Waldeck, 1965).

Results

The results are presented on a semilogarithmic graph (Fig. 1). In the intact gland ^{3}H-MA was rapidly taken up and then disappeared in two distinct phases, both following an exponential course. Ten min after the injection of the amine about 8 ng of ^{3}H-MA per gland was found. Eighteen hr later about 2·5 ng was left, the half-life of the amine during this initial phase being about 12 hr. Between 18 and 144 hr the release was much slower. During this period the half-life was approximately 2·5 days.

In the postganglionically denervated gland about 2 ng of ^{3}H-MA was found 10 min after its injection; it then disappeared rapidly at a single exponential rate. The half-life was only about 3 hr and after 18 hr the amine was almost completely lost.

The level of ^{3}H-MA found in the decentralized gland 10 min after the injection of the amine, 6 ng/gland, was not significantly lower than in the intact gland. The amine disappeared at a single exponential rate with a half-life of about 4 days.

The atrophied gland showed a markedly reduced capacity to take up ^{3}H-MA. Only about 3 ng was found after 10 min, i.e. one third of the corresponding control value. As in the control gland the amine disappeared exponentially in two phases. It may also be noted that in both cases the change in the rate of turnover occurred at about 18 hr after the injection of the amine. The half-life of the amine during the first 18 hr

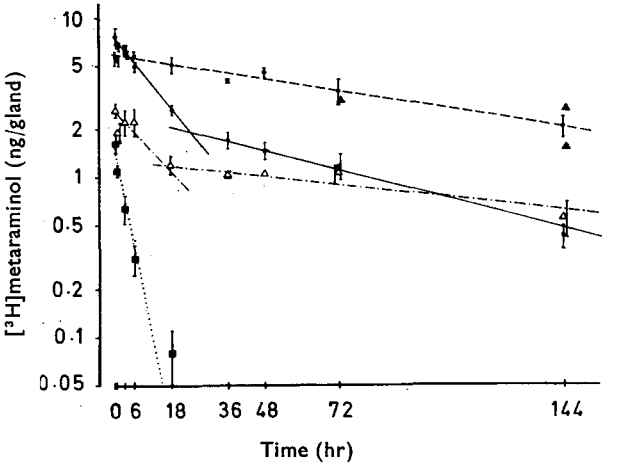

FIG. 1. Disposition of [³H]metaraminol in the rat salivary gland. [³H]-Metaraminol 0·01 mg/kg was given i.v. to rats whose left submaxillary and sublingual glands had been either denervated (■ - - - - ■), decentralized (○ — — ○) or atrophied by ligation of the excretory ducts (△ — · — · — △). ▲ Denotes decentralized and atrophied. The right glands served as controls (●——●). At various time intervals after the injection the animals were killed and the amount of [³H]metaraminol in the salivary glands was determined. The symbols denote the means of 6–18 (controls), 3–4 (denervated), 4–8 (decentralized), and 2–5 (atrophied) experiments. Symbols without indication of the s.e.m. denote single values.

was approximately 12 hr, while the half-life after that time was about 6 days, which is much longer than in the control gland. When the atrophied gland was decentralized the ³H-MA level 72 and 144 hr after the injection was of the same order of magnitude as in a gland only decentralized.

Discussion

The submaxillary gland of the rat was able to take up and concentrate ³H-MA. The ability to retain the ³H-MA taken up was lost after sympathetic denervation indicating that adrenergic nerves are responsible for the storage of this amine, thus giving further support to the conclusions reached by Andén (1964) and Shore & others (1964). During the first 18 hr the disappearance of ³H-MA from the intact salivary gland was more rapid than that previously observed in the mouse heart, although not as rapid as from the femoral muscle of this animal (Carlsson & Waldeck, 1965).

When the nerve impulse flow to the gland was blocked by decentralization, the rapid decrease of ³H-MA during the first 18 hr after its injection was markedly reduced, whereas the difference in turnover rate between the decentralized and the intact gland after that time was less marked. Crout & others (1964) found that metaraminol was more easily released by nerve stimulation 2 hr rather than 17–20 hr after its injection. It would

thus appear that the early decrease of ^{3}H-MA in the intact gland is due mainly to nerve activity. After about 18 hr, however, the amine seems to be less available to nerve impulses, possibly due to a movement from an "available" to a "less available" pool. It is hard to evaluate to what extent loss of the (+)-form of the amine may contribute to the rapid initial decrease (Shore & others, 1964).

In the atrophied gland the accumulation of ^{3}H-MA was markedly reduced. One possible cause of this reduced uptake could be a decreased blood flow through the atrophied gland. This possibility, however, does not seem likely since studies on the distribution of ^{22}Na in the atrophied salivary gland made earlier (Almgren & others, 1965), indicate that alterations in the blood flow cannot be of major importance. Moreover, as seen in the microscope, there seem to be no visible changes in the distribution of the blood vessels after glandular atrophy. A more likely reason for the reduced uptake capacity would be loss of extraneuronal binding sites facilitating the uptake of amines by the adrenergic nerve fibres (Almgren & others, 1965). The turnover rate in the atrophied gland during the first 18 hr did not appreciably differ from that of the intact gland, while after that time it was markedly lower. Since both curves show the same rapid, initial decrease in contrast to that of the decentralized gland, it is difficult to believe that the nerve impulse flow could be changed. This is also supported by the fact that decentralization of an atrophied gland causes a large increase of the ^{3}H-MA level 72 and 144 hr after the injection. The data, however, do not permit a conclusion whether this is due to an increased uptake of the amine or to a delayed disappearance.

No certain explanation can be offered at present for the markedly reduced turnover rate of ^{3}H-MA in the atrophied gland from 18–144 hr after the injection. It is possible that the increased density of nerve terminals seen after glandular atrophy results in a more efficient recapture of the amine.

Acknowledgements. This research has been supported by grants from the Swedish State Medical Research Council (B67-14X-155-03A) and from the Medical Faculty, University of Göteborg. For skilful technical assistance we are indebted to Mrs. Inger Börjesson, Miss Ingrid Weigner and Mrs. Evelin Öst.

References

Almgren, O. (1966). In *Mechanisms of Release of Biogenic Amines*, editors Euler, U. S. von, Rosell, S. & Uvnäs, B. Oxford: Pergamon.
Almgren, O., Andén, N.-E. & Waldeck, B. (1965). *Life Sci.*, **4**, 121–126.
Andén, N.-E. (1964). *Acta pharmac. tox.*, **21**, 260–271.
Andén, N.-E., Norberg, K.-A. & Olson, L. (1966). *Acta physiol. scand.*, **66**, 501–507.
Carlsson, A. & Waldeck, B. (1965). *Acta pharmac. tox.*, **22**, 293–300.
Crout, J. R., Alpers, H. S., Tatum, E. L. & Shore, P. A. (1964). *Science, N.Y.*, **145**, 828–829.
Shore, P. A., Busfield, D. & Alpers, H. S. (1964). *J. Pharmac. exp. Ther.*, **146**, 194–199.