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VESICULAR NORADRENALINE STORES IN PERIPHERAL NERVES OF THE RAT AND THEIR MODIFICATION BY TRANYLCYPROMINE

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1 Vesicular noradrenaline stores were compared in the heart, salivary gland and vas deferens of the rat.

2 Noradrenaline storage vesicles in nerve terminals of different organs differed with respect to the amount of noradrenaline they contain in the endogenous store (content), the amount of exogenous noradrenaline they can take up from the circulation (uptake) and the amount of noradrenaline they contain when they are saturated (total storage capacity).

3 The data suggest that the vesicles in the salivary gland and vas deferens are almost completely filled with transmitter while, in the heart, the vesicular store is filled to only 55% of its total capacity.

4 The monoamine oxidase inhibitor, tranylcypromine, was found to increase not only the size of the endogenous store but also the size of the unfilled store.

Introduction

The major storage site for noradrenaline is in vesicles which can be seen under the electron microscope and isolated by differential centrifugation. The vesicles can take up and store noradrenaline from the neuronal cytoplasm as it accumulates as a result of monoamine oxidase (MAO) inhibition or after injection of exogenous noradrenaline, for example (Wegmann & Kako, 1961; Stanford, unpublished observations). Although this suggests that the store is not normally completely filled with transmitter, there is also some evidence that the vesicular store can be saturated since a second injection of exogenous noradrenaline does not cause a further increase in vesicular transmitter concentration (Fillenz & Howe, 1971). We therefore decided to investigate the effects of MAO inhibition on the size of the unfilled stores in different organs of the rat.

In order to be able to make simultaneous measurements on the size of the endogenous store ('content') and the amount of noradrenaline contained in the vesicles when they are saturated ('total storage capacity') in the same animals, we gave intravenous injections of [^{3}H]-noradrenaline. The noradrenaline taken up from the circulation could then be distinguished by the tritium label. The vesicular noradrenaline content was calculated by subtraction of the amount taken up from the total storage capacity. The ratio of content: capacity $\times$ 100 (percentage saturation) gives an index of the extent to which the endogenous noradrenaline store fills the vesicles under normal conditions. These parameters were compared both in control animals and after tranylcypromine (TCP) administration in three organs of the rat: the heart, the salivary gland and vas deferens.

Methods

Male Sprague-Dawley rats weighing 120–160 g were used. Injections of 0.9% w/v NaCl solution (saline) or [^{3}H]-noradrenaline were made via the tail vein. The animals were killed by cervical dislocation. Tissues were prepared, subjected to differential centrifugation and the noradrenaline content of the vesicular pellet estimated as described in previous papers (Fillenz & West, 1974). An aliquot of the eluate from the alumina column used for the separation of noradrenaline was taken for estimation of [^{3}H]-noradrenaline (Fillenz, Howe & West, 1976). Proteins were measured by the method of Lowry, Rosebrough, Farr & Randall (1951). The *t* test was used to test for differences between means.

Drugs

Tranylcypromine sulphate was a gift from Smith, Kline & French. The solution for injection of noradrenaline contained (−)-noradrenaline (Sigma), pentolinium tartrate (May & Baker), phentolamine (Rogitine; CIBA), ascorbic acid as an antioxidant and (−)-[7-^{3}H] noradrenaline (sp. act. 10.9 Ci/mmol) obtained from the Radiochemical Centre, Amersham (Fillenz *et al.*, 1976). All drugs were dissolved in saline.

Results

Comparison of vesicular noradrenaline stores in sympathetically innervated tissues

In Table 1 are compared the endogenous noradrenaline content, noradrenaline uptake, total storage

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Table 1 Comparison of vesicular noradrenaline stores in peripheral organs of the rat

	Heart	*Salivary gland*	*Vas deferens*
Content	37.5 ± 1.8	48.6 ± 5.8*	310.7 ± 37.5*
(μg/g protein)	$n = 79$	$n = 12$	$n = 13$
Uptake	30.0 ± 1.3	5.3 ± 0.9*	3.45 ± 0.9*
(μg/g protein)	$n = 79$	$n = 12$	$n = 13$
Total storage capacity	66.6 ± 2.4	53.9 ± 6.2*	314.1 ± 37.3*
(μg/g protein)	$n = 79$	$n = 12$	$n = 13$
% saturation	55.2 ± 1.6	89.8 ± 1.7*	98.5 ± 0.6*
	$n = 79$	$n = 12$	$n = 13$

Values expressed as μg NA /g protein in vesicular pellet and show mean ± s.e.mean.
* $P < 0.05$ for difference between means when compared with heart.

capacity and percentage saturation of the heart, salivary gland and vas deferens. In heart and salivary gland, the vesicular noradrenaline content estimated in the present experiments was similar to that found in previous experiments where noradrenaline content was measured directly. Although the total storage capacity of these two organs was similar, the uptake of exogenous noradrenaline was much lower in the salivary gland, suggesting that the sizes of the unfilled stores were different. The results show a percentage saturation of only 55.2% for the heart but of 89.8% for the salivary gland. The difference between these two values was statistically significant.

In the vas deferens, the mean value for vesicular noradrenaline content (shown in Table 1) was higher than that recorded previously. This may reflect variation between batches of animals, or that [^{3}H]-noradrenaline uptake was restricted owing to the poor vascularisation of this organ. If uptake were impeded, this would lead to an overestimation of the size of the endogenous store and of the percentage saturation of the vesicles. Experiments *in vitro* suggest that this may be the case and that the vesicles in this organ are normally 90% saturated (unpublished observations).

Effect of tranylcypromine on the percentage saturation of noradrenaline storage vesicles in heart

Since TCP causes a 33% rise in vesicular noradrenaline content in the heart (Table 2), we were interested to know whether this rise was sufficient to saturate the vesicular noradrenaline store in this organ. Six rats were therefore given an injection of [^{3}H]-noradrenaline, 18 h after TCP administration. The storage parameters of these animals were compared with those of [^{3}H]-noradrenaline-injected controls. A group of six animals that received only TCP were included in the series.

If TCP causes noradrenaline accumulation sufficient to fill the vesicles, no uptake of exogenous [^{3}H]-noradrenaline would be expected. However, if the size of the stores increases but does not saturate the vesicles, they will still take up noradrenaline from the circulation but the uptake would be expected to be lower than in the simultaneous controls.

The results of this study are shown in Table 2. The value for vesicular noradrenaline content in the control animals was similar to that recorded in Table 1 and there is clear agreement between noradrenaline concentration in TCP-injected animals whether this

Table 2 Vesicular noradrenaline (NA) storage after tranylcypromine administration

	Content (μg/g protein)	*Uptake* (μg/g protein)	*Total storage capacity* (μg/g protein)	*% saturation*
Control (+[^{3}H]-NA)	45.8 ± 3.6	40.8 ± 4.1	86.6 ± 4.8	53.1 ± 3.5
Tranylcypromine (+[^{3}H]-NA)	63.2 ± 3.9*	42.8 ± 5.4	106.1 ± 7.6*	60.2 ± 2.8
Tranylcypromine alone	60.7 ± 3.9*			

Values expressed as μg NA /g protein in pellet and show mean ± s.e.mean. For each group $n = 6$.
* $P < 0.05$ for difference between tranylcypromine and control means.

is measured directly or estimated by subtraction after [^{3}H]-noradrenaline injection. Although TCP caused a 35% increase in vesicular noradrenaline content in these experiments (a value close to that recorded previously), there was no significant difference between [^{3}H]-noradrenaline uptake in control and TCP pretreated animals. This finding was reflected by the statistically significant increase in total storage capacity in the TCP pretreated group. The results show that despite the 35% increase in noradrenaline content, the percentage saturation was increased by only 13%.

Does tranylcypromine cause an increase in total storage capacity?

There are several possible explanations for the increase in total storage capacity found in the TCP-injected animals (Table 2) including a direct action of TCP itself on the vesicular store or an increase in the vesicular store as a result of the stress of the [^{3}H]-noradrenaline injection which is only apparent when MAO is inhibited.

To determine whether the stress of the injection was the cause of the increase, the study of the changes in the storage parameters caused by TCP was repeated but including a group of control animals and a group of TCP pretreated animals, both injected with saline 30 min before the injection of [^{3}H]-noradrenaline.

Table 3 shows that for both control and TCP-injected animals, the noradrenaline content was lower than usual, but TCP still caused a 69% increase in vesicular noradrenaline levels. There was no statistically significant difference between the uptake in any of the three groups of animals. This was reflected in the increased storage capacity in both the TCP and TCP plus saline-injected animals when compared with the controls. There were no differences between any of the vesicle parameters in the TCP and TCP plus saline-injected animals.

Discussion

It has been known for several years that noradrenaline, when injected into the circulation, is taken up by noradrenergic nerves (Wegmann & Kako, 1961). It has also been shown that after an injection of exogenous noradrenaline, the noradrenaline content of the storage vesicles increases to a level that seems constant, at least in the heart (Fillenz & Howe, 1971). The present experiments confirm that labelled noradrenaline is taken up and bound within storage vesicles; the amount taken up presumably being limited by the capacity of the vesicles to store the excess transmitter.

Noradrenaline in nerves is thought to be divided into two functional pools: a small release pool and a larger storage pool which is not easily mobilized for release (Swedin, 1972; Glowinski, 1973). The mechanisms underlying this subdivision are not understood but the release pool, at least, is thought to be located within vesicles that discharge the transmitter by exocytosis (reviewed by Fillenz, 1977). The amount of noradrenaline available for release may then depend on the extent to which the vesicles are filled with transmitter. The present experiments suggest that this may be different for each organ and that the vesicles in the heart, on average, have only 55% of their available store filled with noradrenaline, while those in the salivary gland and vas deferens are filled to over 90% of their total capacity.

This finding is supported by the results of a comparative electron microscopic study on the dimensions of the vesicle cores in heart and vas deferens (Fillenz & Pollard, 1976) which showed that the cross-sectional areas of the cores of the vesicles in these organs have unimodal distributions but the peaks of the histograms favour a larger area in the vas deferens than in the heart. It seems that the vesicles in the nerve terminals from each organ represent a heterogenous distribution with respect to the extent to which they are filled with noradrenaline but there

Table 3 Effect of saline injection on noradrenaline (NA) storage in tranylcypromine pretreated animals

	Content (μg/g protein)	*Uptake* (μg/g protein)	*Total storage capacity* (μg/g protein)
Controls (saline + [^{3}H]-NA injected)	23.4 ± 2.5 $n = 8$	20.4 ± 2.4 $n = 8$	43.8 ± 4.1 $n = 8$
Tranylcypromine ([^{3}H]-NA injected)	39.6 ± 4.1* $n = 9$	22.6 ± 4.9 $n = 9$	62.2 ± 8.6* $n = 9$
Tranylcypromine (saline + [^{3}H]-NA injected)	39.0 ± 3.2* $n = 9$	20.2 ± 3.2 $n = 9$	59.3 ± 5.7* $n = 9$

Values show mean ± s.e.mean expressed as μg NA /g protein in pellet.
* $P < 0.05$ for difference between tranylcypromine-injected animals and controls.

are more completely filled vesicles in the vas deferens and salivary gland than in the heart. This may have some effect on the amount of transmitter released after each nerve impulse in these different tissues.

The second part of the study examined the effect of MAO inhibition on the vesicular noradrenaline stores in the heart. We have shown that TCP, a well-known MAO inhibitor, causes a 33% rise in vesicular noradrenaline content in this organ. We therefore examined the possibility that the accumulation of noradrenaline after TCP administration is sufficient to saturate the vesicular store in this organ. The effect this would have on the release pool may have some bearing on the hypertensive actions of TCP (reviewed by Atkinson & Ditman, 1965). The results showed that although vesicular noradrenaline content was increased by 35% after TCP administration, the percentage saturation was increased by only 13%. The possibility that the small rise in percentage saturation is a result of exchange of the injected [^{3}H]-noradrenaline with unlabelled noradrenaline in the endogenous store in the TCP pretreated animals is unlikely in view of the similarity between the estimated noradrenaline content of these animals and the measured noradrenaline content of the tissues of animals injected with TCP alone. Furthermore, the fluorometric measurement of noradrenaline shows that the total storage capacity of TCP pretreated animals is increased by over 22%. It seems that although noradrenaline content is increased by TCP, the size of the unfilled store is also increased.

Experiments where estimation of vesicular noradrenaline content and total capacity were preceded by saline injection suggest that the increase in total capacity is a direct action of TCP and not a consequence of the stress of the intravenous injection. Although the physiological significance of this rise is not known, we have encountered other experimental situations where this has occurred. After 4 h cold-stress, for instance, there is a transient, but statistically significant increase of 22% in the total vesicular storage capacity in heart (Fillenz, Stanford & Benedict, 1978). These findings suggest that TCP may have effects on noradrenaline stores that are not a direct result of MAO inhibition and the subsequent accumulation of noradrenaline.

Reprint requests to S.C.S.

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