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# NORADRENALINE RELEASE FROM ISOLATED MUSCLES OF THE NICTITATING MEMBRANE OF THE CAT

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SUMMARY

1. When the splanchnic nerves were stimulated in the cat for periods of up to 2 hr, the amount of adrenaline taken up by the nictitating membrane was very small; the highest figure was 0·37 $\mu$g/g, corrected for losses.

2. The noradrenaline (NA) stores of the isolated medial muscle of the nictitating membrane were labelled with [$^3$H]NA, and field stimulation of single muscles was carried out in a small organ bath. A stimulation period of 5 min with supramaximal shocks delivered at a frequency of 25/sec produced a mean release of NA into the bath fluid of $0{\cdot}46 \pm 0{\cdot}13$ $\mu$g/g (uncorrected). Phenoxybenzamine, 10 $\mu$g/ml., increased this figure by a factor of 2–3·6. When corrections were made for the amount of transmitter metabolized before it could be collected, 47 % of the tissue content of NA were shown to be released during the 5 min stimulation of the normal membrane, and about 55 % in the presence of phenoxybenzamine. Prolonged stimulation of a phenoxybenzamine-treated membrane was apt to lead to loss of tissue stores of NA.

3. Calculation of the degree of labelling of the NA in the tissue, and of NA released by stimulation, indicated that about 21 % of endogenous NA had been replaced by labelled compound both in the tissue and in the overflow.

4. Transmitter release per pulse was estimated by applying 720–1330 shocks at frequencies of 4 or 6/sec to membranes exposed to phenoxybenzamine. Mean release of NA per shock and per g tissue was 1·3 ng (corrected for losses). This represents $3{\cdot}5 \times 10^{-4}$ of the tissue content determined at the end of the experiment.

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## INTRODUCTION

By labelling the nerves in the isolated smooth muscles of the nictitating membrane of the cat with tritiated noradrenaline, and estimating the radioactive compounds released into the medium, Langer (1970) was able to show that, on nerve stimulation, a proportion of the transmitter escapes in the form of metabolites. The purpose of the present paper was to combine assays of total catecholamines with those of the radioactive compounds in order to determine:

(1) the contribution circulating catecholamines might make to the stores in the tissue,

(2) the proportion of noradrenaline exchanged by the incubation method adopted,

(3) any difference which might exist between the proportion of radioactive noradrenaline stored in the tissue and released as transmitter, and

(4) the amount of transmitter released per impulse and the relation it bears to the amount stored.

## METHODS

*Stimulation of isolated muscles of the nictitating membrane.* Single muscles of the nictitating membrane were dissected out from adult cats anaesthetized with pentobarbitone (40 mg/kg I.P.); noradrenaline (NA) was labelled by incubating the muscles for 30 min with 30 $\mu$c [$^3$H]DL NA (2 c/m-mole) dissolved in 3 ml. Krebs solution. An isolated muscle was suspended in a bath and washed for 90 min, the time required for spontaneous release of radioactive compounds to level off. When phenoxybenzamine was used, the tissue was incubated for 30 min in a solution of the hydrochloride 10 $\mu$g/ml. ($3 \times 10^{-5}$ M). Field stimulation was carried out using as anode (upper electrode) a Pt wire of 0·6 mm diameter. Compounds released on electrical stimulation of the muscle were separated by paper chromatography and estimated in a liquid scintillation spectrometer (for all further details see Langer, 1970).

*Stimulation of splanchnic nerves.* Cats were anaesthetized with ether followed by chloralose, 80 mg/kg, given I.V. The splanchnic nerves were divided at their point of emergence from the diaphragm and the distal ends placed on shielded electrodes. Biphasic stimuli of 0·5 msec duration were applied from a Grass stimulator at a frequency of 10/sec; the voltage was gradually increased till it was supramaximal. Blood pressure was recorded by transducer from a femoral artery. Stimulation was continued for periods of 90–120 min with regular interruptions, 5 min stimulation being followed by 1 min rest. At the end of the experiments, the two muscles of each nictitating membrane were dissected out and the catecholamines estimated.

*Assay of total catecholamines.* Muscles of the nictitating membrane were extracted with acid ethanol and chromatographed in phenol-HCl as described (Vogt, 1952, 1953; Langer, 1970). The regions of the chromatogram containing NA or adrenaline were eluted with 0·4% $NaH_2PO_4$ and assayed on the blood pressure of the pithed, pronethalol treated rat (Vanov & Vogt, 1963). In those experiments in which the NA of the membrane had been labelled, one tenth of the eluate was added to 12 ml. of Bray (1960) solution and counted in the scintillation counter; the bio-assay was carried out on the remainder.

Estimation of released noradrenaline required some modification of the procedure adopted (Langer, 1970) when only radioactivity was measured. Two thirds of total organ bath fluid which had been in contact with the tissue for 5 min were collected, and three such samples (7·5 ml.) were combined both for control and stimulation periods. This was done irrespective of the duration of stimulation which never exceeded 5 min. It had in the past been found that NA did not continue to appear in the bath fluid after such stimulation for longer than 15 min. Therefore all the released NA was combined into one 15 min sample which it was possible to bio-assay. The bath fluid was evaporated in two portions of 3·75 ml. each, and each residue was extracted and applied separately to chromatography paper on adjoining 4 cm lanes. This method avoided overloading of the paper with salts. For elution of each compound, a single piece of paper was cut out which contained the material from the combined half samples. Nine tenths of the eluate from the NA region was used for bio-assay, and one tenth for counting. In some experiments metabolites were estimated by measuring the radioactivity of the appropriate regions of the chromatogram.

Mean recoveries of adrenaline were 62%, of NA 57% for tissue and 52% for incubation fluid (bio-assay after elution). The recoveries showed little scatter.

## RESULTS

*Uptake of circulating adrenaline in vivo.* These experiments were carried out with the object of finding out whether adrenaline released from the adrenal medulla was taken up to an appreciable degree by the nictitating membrane. If this happened, estimation of NA only, either in the tissue or released by stimulation, might give an incomplete picture of transmitter release. Strömblad & Nickerson (1961) found quantities of adrenaline of the order of 1 $\mu$g/g in the heart and submaxillary gland of adult rats injected I.M. with 1 mg adrenaline; when endogenous circulating adrenaline was increased by stimulating the adrenal medulla with insulin, Strömblad (1961) saw smaller increases, up to 0·16 $\mu$g/g in the heart and 0·24 $\mu$g/g in the submaxillary gland. In the present experiments, supramaximal electrical stimulation of the peripheral ends of the splanchnic nerves was carried out for 1·5–2 hr, in an attempt at mimicking the maximal discharge from the adrenal medulla which might occur naturally. Effectiveness of the stimuli, which were delivered for periods of 5 min followed by an interval of 1 min, was assessed by their effect on the blood pressure. The blood pressure base line fell during the course of the experiments, but the rise in pressure produced by each period of stimulation either remained the same throughout or decreased by not more than 40%. Because of the suggestion (Gillis, Schneider, van Orden & Giarman, 1966) that uptake of catecholamines might be reduced during relatively fast activity in the nerve, the sympathetic chains were cut in the neck to prevent impulses from travelling down to the membrane (Expts. 2–4).

In the first experiment, in which splanchnic stimulation was carried out

TABLE 1. Catecholamine content (μg/g fresh tissue, uncorrected for losses) of muscles from the cat's nictitating membrane isolated after prolonged stimulation of the splanchnic nerves

| Expt. no. | Tissue | Duration of stimulation (min) | Noradrenaline | | Adrenaline | | | Comment |
|---|---|---|---|---|---|---|---|---|
| | | | Total (ng) | μg/g | Total (ng) | μg/g | % total catechol-amines | |
| 1 | Two medial muscles | 70 | 100 | 2·2 | < 2·5 | < 0·045 | < 2·4 | — |
| | Two inferior muscles | 70 | 130 | 2·0 | < 2·5 | < 0·039 | < 1·9 | |
| 2* | Two medial muscles | 120 | 250 | 3·8 | 6·7 | 0·09 | 2·6 | — |
| | Two inferior muscles | 120 | 237 | 3·2 | 15·0 | 0·23 | 6·0 | |
| 3* | Two medial muscles | 125 | 150 | 2·6 | 10·0 | 0·17 | 6·3 | Cat eviscerated |
| | Two inferior muscles | 125 | 80 | 1·5 | 7·5 | 0·14 | 8·6 | |
| 4* | Both muscles of right eye | 120 | 100 | 1·7 | 5·0 | 0·09 | 4·8 | Cat eviscerated |
| | Both muscles of left eye | 120 | 133 | 2·3 | 5·0 | 0·09 | 3·6 | |

* The cervical sympathetic chains were cut in the neck.

for 70 min (Table 1), the presence of adrenaline in the nictitating membrane could not be ascertained ($< 0{\cdot}045$ $\mu$g/g uncorrected). In the next cat, after stimulation of the splanchnic nerves for 2 hr, adrenaline was detectable in the membranes, but its share in the total catecholamine content was only 3% in the medial and 6% in the inferior muscles. The remaining two experiments were carried out on eviscerated cats with a view to further increasing the concentration of circulating adrenaline. The adrenaline content of the membranes remained small, the highest percentage obtained was 8·6%. The tissue content (Expts. 2–4) lay between 0·09 and 0·23 $\mu$g/g uncorrected, or 0·15 and 0·37 corrected for losses. This is 3 to 6 times as much as the highest (approximate) concentration determined fluorimetrically by Kirpekar, Cervoni & Furchgott (1962) in muscles of the nictitating membranes of normal cats. There was thus a small uptake of circulating adrenaline, but even under conditions of maximal (not drug-induced) sympathetic activity the effect is so small as to be irrelevant to estimations of transmitter release.

*Release of noradrenaline.* Preliminary experiments with stimulation periods lasting 5 min had shown that, in order to obtain enough NA in the bathing fluid for bio-assay, the frequency of stimulation had to be no less than 25/sec. This frequency was, therefore, used for the experiments recorded in Table 2. At this frequency the mechanical response was not sustained and usually declined by 30% during the last 2 min of stimulation.

In Expts. 1–5 (Table 2), the absolute amount of NA found in the bathing fluid after stimulation of the medial muscle from the right eye ranged from 6·8 to 28·9 ng (uncorrected); this corresponded, per g tissue, to $0{\cdot}46 \pm 0{\cdot}13$ $\mu$g (mean ± S.E. of mean). In cats 1–4, the medial muscle of the other eye was used to measure release under the influence of phenoxybenzamine 10 $\mu$g/ml. Release of NA was 2 to 3·6 times that of the right muscle.

Spontaneous release of NA was also increased after phenoxybenzamine; values (which could only be obtained for the labelled fraction) were approximately doubled; in nc per 15 min and g tissue, the [$^3$H]NA increased from $134 \pm 24$ (mean ± S.E. of mean) to $224 \pm 33$.

In Expt. 6 (Table 2), the cat had been pretreated with pargyline (100 mg/kg). The amount of NA released was high but did not exceed the upper limit of normal release.

Table 2 allows the calculation of the fraction of the total NA stores released by 5 min stimulation at 25 shocks/sec. For the untreated membrane, mean content (Expts. 2–5) was 2·7 $\mu$g/g and mean release 0·46 $\mu$g/g. To obtain actual outflow, the figure 0·46 $\mu$g/g has to be corrected for the metabolites formed from released transmitter under these conditions (Langer, 1970); they amount to 64% or 0·82 $\mu$g/g, and the transmitter overflow is therefore 1·28 $\mu$g/g.

TABLE 2. Release of total and labelled NA from single medial muscles of the nictitating membrane by field stimulation (25 shocks/sec for 5 min). Increase of release by phenoxybenzamine. Values not corrected for losses. While the muscle of the right eye served as control, that of the left eye was incubated in phenoxybenzamine (10 μg/ml.) for 30 min before stimulation

| | Right medial muscle (untreated) | | | | | | Left medial muscle (after phenoxybenzamine) | | | | | | Release after phenoxybenzamine (% of control) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Release | | | Tissue content | | | Release | | | Tissue content | | | | |
| Expt. no. | NA (μg/g) | [$^3$H]NA (μc/g) | [$^3$H]NA (μg/g) | NA (μg/g) | [$^3$H]NA (μc/g) | [$^3$H]NA (μg/g) | NA (μg/g) | [$^3$H]NA (μc/g) | [$^3$H]NA (μg/g) | NA (μg/g) | [$^3$H]NA (μc/g) | [$^3$H]NA (μg/g) | Total NA | [$^3$H]NA |
| 1 | 0·32 | — | — | — | — | — | 0·64 | — | — | — | — | — | 200 | — |
| 2* | 0·17 | 1·36 | 0·052 | 2·5 | 7·68 | 0·29 | 0·61 | 4·23 | 0·16 | 2·6 | 9·48 | 0·36 | 363 | 310 |
| 3 | 0·33 | 1·25 | 0·095 | 1·9 | 7·40 | 0·56 | 1·09 | 3·41 | 0·26 | 1·2 | 3·44 | 0·26 | 322 | 274 |
| 4 | 0·92 | 2·62 | 0·200 | 3·9 | 4·97 | 0·38 | 1·88 | 5·14 | 0·38 | 1·7 | 3·75 | 0·29 | 204 | 192 |
| 5 | 0·56 | 1·88 | 0·143 | 2·6 | 8·64 | 0·66 | — | — | — | — | — | — | — | — |
| 6† | 0·92 | 2·54 | 0·194 | 2·8 | 6·48 | 0·49 | — | — | — | — | — | — | — | — |

* Muscle incubated with solution of specific activity of 4·45 c/m-mole, twice the activity used in the other experiments.

† Cat injected with pargyline (100 mg/kg) 18 hr before the experiment.

Stimulation thus released an average of 47·5 % of the NA content of the membrane as determined at the end of the experiment.

In muscles incubated with phenoxybenzamine, this fraction was somewhat higher; it can be calculated from Expts. 2–4 by using the contralateral membrane as an approximate guide for the original NA content; mean content was 2·6 μg/g, mean release 1·19 μg/g. After phenoxybenzamine, a smaller correction (of 16 %) is required for the formation of metabolites (Langer, 1970); the corrected value for release is 1·42 μg/g, a single period of stimulation having thus released about 55 % of the stores.

TABLE 3. Total and labelled NA. Comparison of amounts contained in medial muscle of the nictitating membrane and released by nerve stimulation

| | [$^3$H]NA as % of total NA | | | |
|---|---|---|---|---|
| | Untreated | | Phenoxybenzamine* | |
| Expt. no. | Released | Tissue content | Released | Tissue content |
| 2 | 29·8 | 11·6 | 26·5 | 14·1 |
| 3 | 28·1 | 30·1 | 23·8 | 21·6 |
| 4 | 21·8 | 9·8 | 20·9 | 16·6 |
| 5 | 25·6 | 25·9 | — | — |
| 6† | 21·1 | 17·6 | — | — |
| 7 | — | — | 19·9 | 23·5 |
| 8 | — | — | 14·4 | 15·5 |
| 9 | — | — | 26·7 | 23·8 |
| 10 | — | — | 19·6 | 30·3 |
| 11‡ | — | — | 11·2 | 24·5 |
| Mean ± S.E. of mean | 25·3 ± 1·7 | 19·0 ± 3·9 | 20·4 ± 1·9 | 21·2 ± 1·9 |

* Exposure to phenoxybenzamine (10 μg/ml.) for 30 min. Frequency of stimulation 25 shocks/sec in Expts. 2–6 (see Table 1), and 6 shocks/sec in Expts. 7–11 (see Table 4).

† Cat given pargyline 100 mg/kg on day preceding experiment.

‡ Membrane decentralized 75 days previously.

It is evident from the low NA content of the tissue in Expts. 3 and 4, in which release was very high, that under the influence of phenoxybenzamine stimulation causes a rapid loss of NA stores from the tissue.

Table 3, compiled from the data of Tables 2 and 4, shows the degree of labelling achieved by the technique of incubation. Labelled NA is expressed as percent of total NA.

The proportion of labelled to total NA was not always the same in tissue and in the fluid obtained after stimulation. In Expts. 2 and 4, the released NA had a greater activity than the NA left in the tissue, and the reverse

TABLE 4. Release of noradrenaline from the medial muscle of the nictitating membrane exposed for 30 min to phenoxybenzamine 10 μg/ml. Field stimulation at 4 and 6 shocks/sec. Values not corrected for losses

| Expt. no. | Frequency and duration of stimulation | No. of shocks | Release | | | Total content (μg/g) | |
|---|---|---|---|---|---|---|---|
| | | | Total NA (ng/membrane) | Total NA (μg/g) | [$^3$H]NA (μg/g) | Total NA | [$^3$H]NA |
| 7 | 6/sec 3·7 min | 1330 | 18·9 | 0·89 | 0·179 | 3·0 | 0·71 |
| 8 | 4/sec 5 min | 1200 | 12·8 | 0·46 | 0·065 | 2·0 | 0·31 |
| 9 | 6/sec 2 min | 720 | 20·3 | 0·98 | 0·263 | 2·7 | 0·63 |
| 10 | 6/sec 2 min | 720 | 10·1 | 0·35 | 0·067 | 1·6 | 0·47 |
| 11* | 6/sec 3 min | 1080 | 11·7 | 0·40 | 0·045 | 1·25 | 0·31 |
| Mean ± S.E. of mean | | — | 14·8 ± 2·0 | 0·62 ± 0·13 | 0·123 ± 0·042 | — | — |

* Membrane decentralized 75 days previously.

was true of Expts. 10 and 11. However, the average labelling of released NA and tissue NA was not significantly different, about 21 % of endogenous NA having been replaced by labelled NA. Exposure of the muscles to phenoxybenzamine did not modify the degree of labelling of released transmitter.

The experiments of Table 4 served the purpose of estimating the amount of transmitter released per shock. Frequency and duration of stimulation were kept to the minimum compatible with a valid bio-assay of released NA. Frequency of stimulation was either 4 or 6 shocks/sec and total

TABLE 5. Noradrenaline released per shock from the medial muscle of the nictitating membrane exposed for 30 min to phenoxybenzamine 10 $\mu$g/ml.

| Expt. no. | NA output per shock (ng/g tissue) | Tissue content ($\mu$g/g) |
|---|---|---|
| 7 | 1·34 | 5·3 |
| 8 | 0·77 | 3·5 |
| 9 | 2·74 | 4·7 |
| 10 | 0·96 | 2·8 |
| 11* | 0·75 | 2·2 |
| Mean 7–11 | 1·31 ± 0·21 | 3·7 ± 0·58 |
| Mean 7–10 | 1·45 ± 0·45 | 4·1 ± 0·57 |

Values are corrected for losses.
* Membrane decentralized 75 days previously.

number of shocks ranged from 720 to 1330; phenoxybenzamine was used to increase overflow by inhibiting both reuptake and metabolism of NA. At the frequencies used, the mechanical response of the membrane is well sustained in the absence of phenoxybenzamine.

The results of five such experiments are shown in Tables 4 and 5. The mean release of NA per g tissue (not corrected for losses) was 0·62 $\mu$g; the individual results were not clearly related to the number of pulses applied. One of the experiments (no. 11) was carried out on a decentralized nictitating membrane, and since the release observed fell within the normal range, the experiment was included in the means.

In Table 5, the figures for release are calculated per shock and corrected for losses. Mean release per shock and per g tissue was 1·3 ng; this represented $3{\cdot}5 \times 10^{-4}$ of the tissue content as determined at the end of the experiment. If the last experiment is excluded from the series because the membrane had been decentralized, the fraction remains the same.

## DISCUSSION

The adrenaline content of a single muscle from a nictitating membrane of a normal cat was found to be below the threshold of the method employed (2·5 ng). After sufficiently prolonged stimulation of the distal ends of the severed splanchnic nerves it was possible to detect small amounts of adrenaline ranging from 5 to 15 ng and representing a few percent of the total catecholamines of the muscle. In contrast to the higher blood concentrations one could elicit by injection of drugs or of adrenaline itself, the conditions chosen would produce the highest concentrations of circulating adrenaline likely to occur under natural conditions in a roused or stressed animal. In these circumstances, increase of circulating noradrenaline would accompany that of adrenaline, and since noradrenaline is taken up preferentially, the experiments do not prove that uptake of circulating catecholamines never plays more than a negligible part in maintaining the noradrenaline stores of the tissue. They do, however, show that in experiments on transmitter release the part played by adrenaline can be ignored.

The figures for the noradrenaline content of the isolated muscles of the nictitating membrane agree well with those found by other authors. The range of 2·6–6·6 $\mu$g/g (figures corrected for losses) is little different from that of 2·4–4·3 reported by Kirpekar *et al.* (1962). Table 1 shows a tendency for the medial muscle to have a higher concentration than the inferior muscle as was also found by Trendelenburg, Draskóczy & Pluchino (1969).

The ratio of labelled to total NA in the overflow and in the tissue was determined in experiments in which the frequency of stimulation was 25/sec, since with lower frequencies NA release into the bathing fluid was below the threshold of the bioassay. In spite of a certain amount of variation, mean labelling of tissue NA, and of released NA, were not significantly different. This shows that the prolonged period of incubation and washing employed here produced a fairly homogeneous labelling within the sympathetic nerves.

At a frequency of 25/sec, phenoxybenzamine increased overflow of noradrenaline by a factor of 2–3. This increase has been shown to be largely due to prevention of metabolism (Enero & Langer, 1970). Furthermore, there was rapid exhaustion of the NA stores of the tissue, indicating that synthesis did not keep pace with utilization in isolated muscles exposed to this concentration of phenoxybenzamine. This loss of NA from the tissue may be one, or even the main, reason for the rapid decline in overflow reported (Blakeley, Brown & Geffen, 1964) when the perfused spleen of the cat is stimulated repeatedly after exposure to phenoxybenzamine.

In order to obtain as precise an estimate as possible of the actual amount of transmitter released per pulse, experiments were conducted at frequencies of 4–6 shocks/sec at which release is better maintained than at 25/sec. Reuptake and metabolism were prevented by phenoxybenzamine as before. The result, calculated per single pulse, was a release of 1·3 ng/g tissue. To this must be added 16% which escape detection by bio-assay since they are present in the form of metabolites. The corrected release per pulse is, therefore, about 1·5 ng/g. Expressed as a fraction of tissue content, the mean of $3{\cdot}5 \times 10^{-4}$ arrived at in the last paragraph of Results has to be increased to $4{\cdot}0 \times 10^{-4}$. This figure differs greatly from the ratio of $2 \times 10^{-5}$ obtained (Folkow, Häggendal & Lisander, 1967) by stimulating the nerves to the perfused calf muscle of the cat and estimating NA in the venous effluent. The lower proportion obtained by the Swedish workers led them to the conclusion that each pulse released only a few percent of the NA contained in a single vesicle from a nerve terminal. Stjärne, Hedqvist & Bygdeman (1969) repeated the experiments of Folkow *et al.* (1967) using shorter stimulation periods, and reported values which were three times larger. However, the ratios obtained by us on the isolated nictitating membrane are about 20 times greater than those of Folkow *et al.* (1967), and, taking into account the low accuracy of such calculations, would be compatible with the view that each pulse releases the content of at least one whole vesicle.

The reason for the discrepancy could well lie in the fact that the transmitter is trapped and metabolized, even in the presence of substances such as phenoxybenzamine or other inhibitors of uptake and metabolism, on the long way from the synapse to the vein. Direct evidence in favour of this interpretation was obtained in experiments on the rabbit ear artery (I. de la Lande & M. Vogt, to be published).

Two factors might further modify slightly, and in opposite directions, the figure for release as a fraction of tissue content. Since the stimulation period was, of necessity, fairly prolonged, it is likely to have caused some decline in release during the final stages of stimulation, so that the real fraction might be slightly higher. On the other hand, a small loss of stored NA may have occurred during the stimulation period in spite of the fact that it was much shorter than that of 7500 pulses used in the experiments in which loss of NA from the tissue was evident. A decrease of tissue stores would inflate the value for release as a fraction of tissue content. In the present context, neither of these factors is of practical importance.

The fraction of tissue NA released per pulse was also estimated in the isolated uterine arteries of the guinea-pig (Bell & Vogt, 1971). In this organ, the endings of the sympathetic fibres lie in the adventitia, and the diffusion barrier from synapse to bath fluid must be small and keep the loss

of transmitter on its way to the bath to a minimum. A figure of $2{\cdot}2 \times 10^{-4}$ was obtained which is not very different from that of $3{\cdot}5 \times 10^{-4}$ (see p. 167).

Nearly the same ratio is arrived at when calculations are carried out for the cat spleen, using data from Haefely, Hürliman & Thoenen (1965). In the presence of phenoxybenzamine and cocaine, and with a frequency of stimulation of 4/sec, 5 ng transmitter were released per pulse, or 0·5 ng/g. The NA content of the spleen being 1·5 $\mu$g/g, the fraction of the store released per pulse was $3{\cdot}3 \times 10^{-4}$.

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