J. Physiol. (1973), **229**, *pp.* 515–531
With 12 *text-figures*
Printed in Great Britain

AN ELECTROPHYSIOLOGICAL ANALYSIS OF THE STORAGE AND RELEASE OF NORADRENALINE AT SYMPATHETIC NERVE TERMINALS

By M. R. BENNETT

From the Neurobiology Laboratory, Department of Physiology, University of Sydney, Sydney, N.S.W. 2006, Australia

(*Received 7 September 1972*)

SUMMARY

1. An electrophysiological analysis has been made of the storage and release of noradrenaline (NAd) in the sympathetic nerve terminals of the isolated vas deferens of the mouse. The amplitude of the excitatory junction potentials (e.j.p.s) recorded intracellularly in smooth muscle cells was taken as a measure of the NAd output per impulse from the terminals of sympathetic axons.

2. During short trains of impulses (< 100), the amplitude of the e.j.p. increased with successive impulses at the beginning of a train, and then either continued to increase until a steady-state amplitude was reached (frequencies < 1 Hz), or decreased until a depressed steady amplitude was reached (frequencies > 1 Hz).

3. During trains of impulses lasting for several minutes, the amplitude of the e.j.p. continually declined (frequencies > 1 Hz) until a steady-state amplitude was reached after 8 min of stimulation. This steady-state amplitude is smaller, the higher the frequency of stimulation.

4. During short trains of impulses in the presence of high magnesium solutions, the amplitude of successive e.j.p.s increased until a steady state was reached, no matter what the frequency of stimulation. This growth of the e.j.p. amplitude during a train could be quantitatively predicted in terms of the linear summation of the individual facilitatory effects introduced by each impulse in the train.

5. During trains of impulses lasting for several minutes, in the presence of a NAd synthesis blocker, the amplitude of the e.j.p. continually declined along a curve which could be described as the sum of two exponential components: one with a time constant of 1 min and the other of 10 min.

6. These results suggest that NAd is released from a small pool of transmitter in sympathetic nerve terminals, which is replenished from two stores, which are in turn replenished by the synthesis of new NAd.

INTRODUCTION

The release of noradrenaline (NAd) from nerve terminals by impulses has been studied by assaying the quantity of NAd overflowing into either the venous circulation or incubation medium of an organ during stimulation of its sympathetic nerve supply (Brown & Gillespie, 1957; Langer, 1970). These techniques of estimating the NAd released from nerve terminals have been complicated by the discovery that NAd can be taken up into nerve terminals (Axelrod, 1971; Malmfors, 1965) and muscle cells (Lightman & Iversen, 1969; Clarke, Jones & Linley, 1969). The relationship between the quantity of NAd overflowing from an organ and that released from the nerve terminals during stimulation is therefore uncertain. Furthermore, as it is not known to what degree the transmitter released from the nerve terminals is taken up by them and made available for re-release during stimulation, it is not possible to block neuronal uptake and remain confident that one has not affected the release of the transmitter during stimulation.

A possible means of avoiding these difficulties is to use the amplitude of the junction potential in smooth muscle due to the release of NAd from nerve terminals as a measure of the quantity of NAd released, in much the same way as the amplitude of the synaptic potential at cholinergic junctions has been used to analyse the release of acetylcholine (Elmquist & Quastel, 1965; Mallart & Martin, 1968; Bennett & McLachlan, 1972*a*, *b*). By this means it should be possible to measure directly the NAd released from sympathetic nerve terminals by single impulses, before it has been taken up into nerve or muscle. In the present work, the normal pattern of NAd release from nerve terminals during stimulation is described on the basis of this assumption.

At the amphibian motor end-plate, the first few impulses in a short high-frequency train (> 10 Hz), release successively greater amounts of acetylcholine (ACh), whilst there is a depression in transmitter release by subsequent impulses until a steady-state release rate is reached (Feng, 1941; Eccles & McFarlane, 1949). However high magnesium solutions, which decrease the quantity of ACh released by each impulse, reverse the depression observed after a few impulses to facilitation (del Castillo & Katz, 1954*a*). It has therefore been suggested that normally the first few impulses in a train attempt to release successively greater fractions of ACh from a small pool (Mallart & Martin, 1968), but that this pool is partially depleted after the first few impulses, leading to a depression of transmitter release (Liley & North, 1953; Takeuchi, 1958; Thies, 1965; Elmquist & Quastel, 1965). This pool in preganglionic nerve terminals is maintained partly full during continual stimulation by the mobilization

of ACh from a large single store in the terminal (Bennett & McLachlan, 1972*a*); the store is in turn partly replenished by the synthesis of new ACh (Bennett & McLachlan, 1972*b*). In the present work, a similar study has been made of the compartmentalization of releasable NAd in sympathetic nerve terminals.

METHODS

The isolated mouse vas deferens was used in all experiments. The animals were killed with a cervical fracture, and both vas deferens dissected free and pinned out immediately in a Perspex organ bath. The preparation was bathed in a modified Krebs solution of the following ionic composition (mM): Na 151, K 4·7, Ca 1·8, Mg 1·2, Cl 142, H_2PO_4 1·3, SO_4 1·2, HCO_3 16·3, glucose 7·8, bubbled with a gas mixture containing 95 % O_2, 5 % CO_2. The solution was maintained at 35–37° C and flowed continuously through the bath at 1–3 ml. min^{-1}.

The intramural sympathetic nerves were stimulated with two platinum ring electrodes placed around the vas deferens at about 1 mm apart; the stimulus had a duration of 0·01–0·2 ms duration and 40–50 V amplitude. Intracellular potentials were recorded from the smooth muscle cells with glass micro-electrodes filled with 2 M-KCl, and having resistances of 30–70 MΩ. The signals were led through a high impedance unity gain amplifier, displayed on an oscilloscope and photographed on moving film.

The mouse vas deferens has a comparatively pure sympathetic adrenergic nerve supply (see also Sjostrand, 1965): thus it is denervated with 6-hydroxydopamine (Furness, Campbell, Gillard, Malmfors, Cobb & Burnstock, 1970) which selectively destroys adrenergic nerves (Tranzer & Thoenen, 1968); the evoked junction potentials in the muscle are unaffected by atropine sulphate and the displacement of NAd from the nerve terminals with 5-hydroxydopamine blocks transmission (personal observations).

RESULTS

The resting membrane potential of individual smooth muscle cells was about 60 mV. A single stimulus to the intramural sympathetic nerves gave rise to an excitatory junction potential (e.j.p.) in the muscle cells, which could be graded in amplitude with different strengths of stimulation, indicating that several sympathetic nerve terminals were excited. The strength of the stimulating pulse was adjusted so that the e.j.p. did not increase beyond 10–15 mV during trains of impulses, thus avoiding serious errors due to non-linear summation (Martin, 1955).

For short trains of impulses (< 100) the amplitude of each e.j.p. was normalized with respect to the first e.j.p. in the train; the normalized data for all the sympathetic nerve terminals studied was then pooled. The procedure for long trains of impulses (> 100) was more complicated: first the mean amplitude of 10 e.j.p.s was determined at intervals of about half a minute, and these normalized with respect to the mean e.j.p. amplitude reached after about 100 impulses; this data was then pooled for all the sympathetic nerve terminals studied; the pooled data was then

renormalized with respect to the first e.j.p. in a train by using the relationship between the first e.j.p. and the e.j.p. amplitude reached at about 100 impulses already established for short trains.

Release of NAd from sympathetic nerve terminals by short trains of impulses

Normal release. The amplitude of the e.j.p. increased with successive impulses during a short train at frequencies less than 1 Hz (Fig. 1*A*), until a steady-state e.j.p. amplitude was reached after a few seconds of stimulation. At these frequencies the increase in size of the e.j.p. with successive impulses grows exponentially with a time constant of about 6 s (Fig. 2*a*).

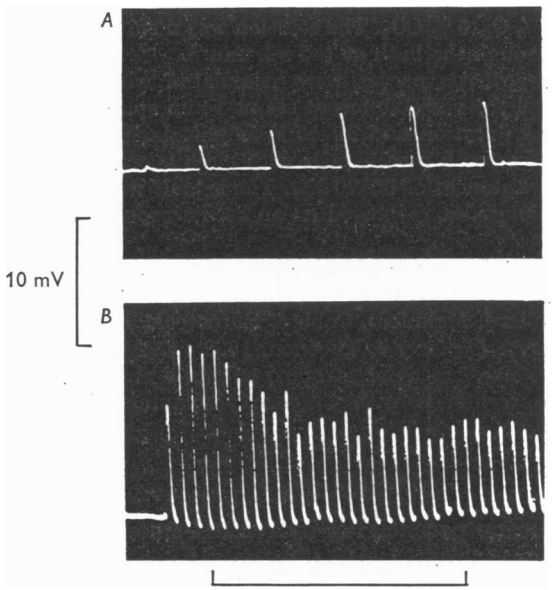

Fig. 1. Changes in amplitude of the e.j.p. during short trains of impulses. *A*, low frequency of sympathetic nerve stimulation (0·5 Hz); only the beginning of the train is shown. *B*, high frequency of sympathetic nerve stimulation (10 Hz). Time calibration: *A*, 8 sec; *B*, 2 sec.

At higher frequencies ($\geqslant$ 1 Hz), successive e.j.p.'s increased with the first few impulses, and then either remained constant or declined with subsequent impulses, until a steady-state e.j.p. amplitude was reached after several seconds of stimulation (Fig. 1*B*). Although the initial increase in size of the e.j.p. at these high frequencies suggested that it would increase exponentially with a time constant similar to that at low frequencies, it departs from this after the first few impulses (Fig. 2*b*), to reach a steady-state amplitude much smaller than that expected for an exponential growth.

The relationship between the frequency of stimulation and the steady-state amplitude reached during a short train of impulses is shown in Fig. 3: the steady-state amplitude at first increases with frequency up to 0·5 Hz and then decreases, until at frequencies above 3 Hz, the steady-state e.j.p. amplitude is similar to that of the first e.j.p. in the train.

Predicted release. The growth of the e.j.p.s during a train at low frequencies can be predicted in terms of the effects which a conditioning

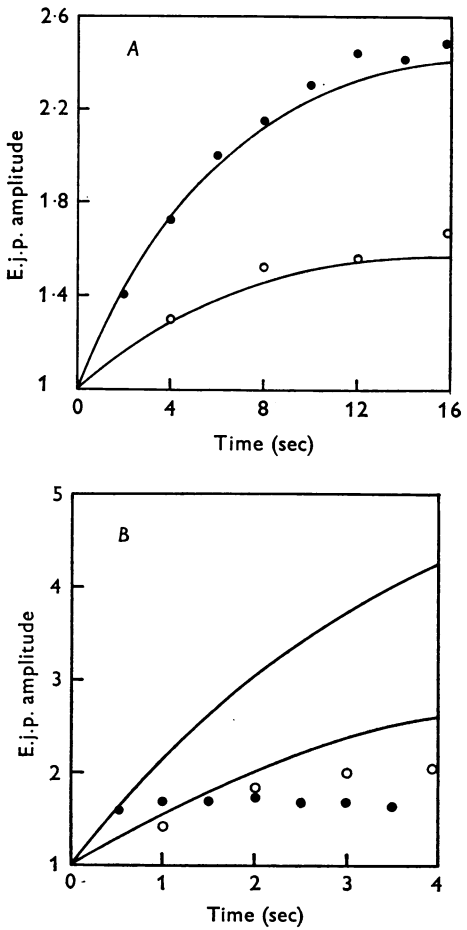

Fig. 2. Changes in amplitude of the e.j.p. during short trains of impulses at different frequencies of sympathetic nerve stimulation. *A*, low frequencies (< 1 Hz): filled circles, 0·5 Hz; open circles, 0·25 Hz. *B*, high frequencies (⩾ 1 Hz): filled circles, 2 Hz; open circles, 1 Hz.

Curve is the predicted relationship, given in the text. S.E. of mean less than 10% of the mean for each point (number of observations, $n > 6$).

impulse has on the amplitude of the e.j.p. evoked by a subsequent test impulse. If the amplitude changes in a test e.j.p., evoked at different intervals after a conditioning impulse, are plotted semilogarithmically as shown in Fig. 4, then the effects of the conditioning impulse can be divided into two components: one of these occurs at short intervals and lasts for about 200 ms, and the other occurs at longer intervals and lasts for several seconds (Fig. 4). The longer of these components follows a single exponential, with a time constant of about 6 s. The facilitated release of ACh at the end-plate can also be described by a two component curve, only in this case the time constants are an order of magnitude smaller than those for adrenergic nerve terminals (Mallart & Martin, 1967).

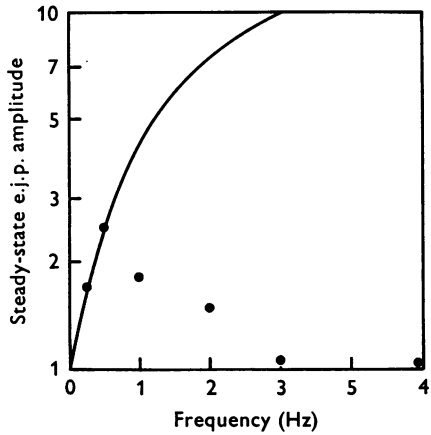

Fig. 3. Relationship between the amplitude of the steady-state e.j.p. reached during a short train of impulses and the frequency of stimulation. Points refer to observations. Curve is the predicted relationship, given in the text. S.E. of mean less than 10 % of the mean for each point ($n > 6$).

If facilitation (f) is defined as $(v-v_0)/v_0$ where v_0 is the amplitude of a conditioning e.j.p., and v is the amplitude of a test e.j.p., then the slow exponential curve in Fig. 4 can be described by the equation $f = f_1 \exp-(b_1 t)$, where f_1 is the facilitation at zero time (equal to 0·6) and b_1 is the rate constant of decay of the curve (equal to 0·17). Furthermore if the successive facilitations introduced by each impulse in a train simply add, then the growth of facilitation during a train is described by the equation $f = k_1(1-\exp(-b_1 t))$, where $k_1 = f_1(\exp b_1 \Delta t - 1)^{-1}$ and Δt is the interval between successive impulses. This interval must not be shorter than 200 ms, otherwise the first component of the facilitation curve (Fig. 4) must be included (for derivation of these equations, see Mallart & Martin, 1967).

The curves in Fig. 2 show the predicted growth in the amplitude of the

e.j.p. during trains of impulses at different frequencies. At frequencies less than 1 Hz there is good agreement between the predicted growth in amplitude of the e.j.p. during a short train and the observed growth (Figs. 2*a* and 3). At higher frequencies, the growth of the e.j.p. at first follows the theoretical curve and then departs from it (Fig. 2*b*) to finish at a much smaller steady-state size than that predicted by the simple addition of successive facilitations in the train (Fig. 3).

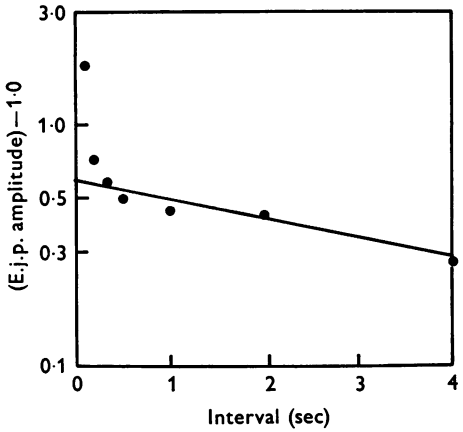

Fig. 4. Effect of a conditioning impulse on the amplitude of the e.j.p. evoked by a subsequent test impulse. Each point gives the mean amplitude of a number of test e.j.p.s (v), each normalized to their respective conditioning e.j.p.s (v_0), at the interval indicated in the abscissa, less 1·0; the ordinate is equivalent to the factor $(v-v_0)/v_0$, given in the text. S.E. of mean less than 10% of the mean for each point ($n > 10$).

Release of NAd from sympathetic nerve terminals by long trains of impulses

The steady-state e.j.p. amplitude reached during short trains of impulses is not maintained if the nerves are stimulated for several minutes at frequencies greater than 1 Hz (Fig 5). The amplitude of the e.j.p. declines continually over 8–10 min (Fig. 5) until a new steady state is reached. This steady state has been observed to last for the longest held impalements of 30 min. The e.j.p. amplitude during this maintained steady state depends on the frequency of stimulation. At 3 Hz it declines to 0·6 times its initial amplitude, whilst at 10 Hz it declines to 0·2 times its initial value (Fig. 5). At frequencies of stimulation equal to or less than 1 Hz, there is no change in the amplitude of the e.j.p. during continual stimulation, after it has reached a steady-state value following the first few seconds of stimulation (Fig. 5).

If the amplitude of the e.j.p. is taken as a measure of the NAd released from the nerve terminals, then Fig. 6 shows that the quantity of NAd

released per unit time is greater the higher the frequency of stimulation, during the first 8 min of continual stimulation; however, after this period, the output reaches a steady state which is the same for all frequencies. Thus, although the steady-state NAd output per impulse reached after continual stimulation decreases with an increase in the frequency of stimulation (Figs. 5 and 7), it does so in such a way that the NAd released

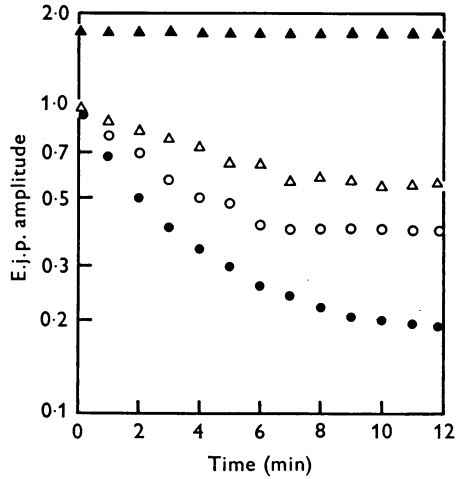

Fig. 5. Decline of the e.j.p. during long trains of impulses at different frequencies of sympathetic nerve stimulation. Filled triangles, 1 Hz: open triangles, 3 Hz: open circles, 5 Hz: filled circles, 10 Hz. S.E. of mean less than 10 % of the mean for each point ($n > 4$).

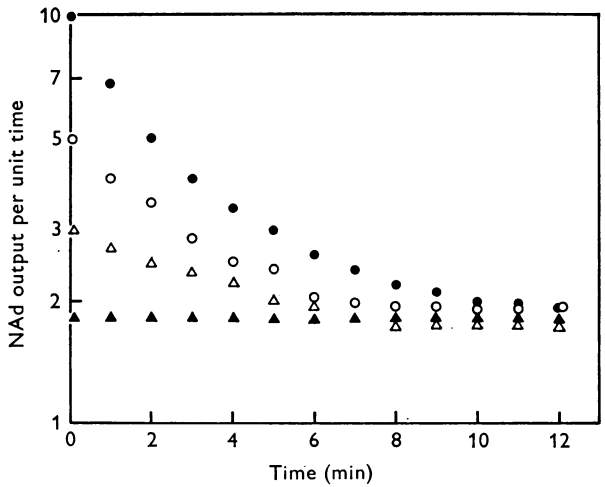

Fig. 6. NAd output per unit time from sympathetic nerve terminals at different frequencies of nerve stimulation. Filled triangles, 1 Hz; open triangles, 3 Hz; open circles, 5 Hz; filled circles, 10 Hz.

per unit time remains constant (Figs. 6 and 7). This is in contrast to the release of ACh at preganglionic nerve terminals, at which the steady-state release per unit time increases with an increase in the frequency of stimulation (Bennett & McLachlan, 1972*b*).

The storage of NAd in sympathetic nerve terminals

The effect of magnesium ions on NAd release. In order to investigate the likelihood that NAd is released from a small pool in sympathetic nerve terminals, analogous to that at cholinergic nerve terminals, the effect of high magnesium solutions on the release of NAd by short trains of impulses was studied. When the vas deferens was incubated in high magnesium

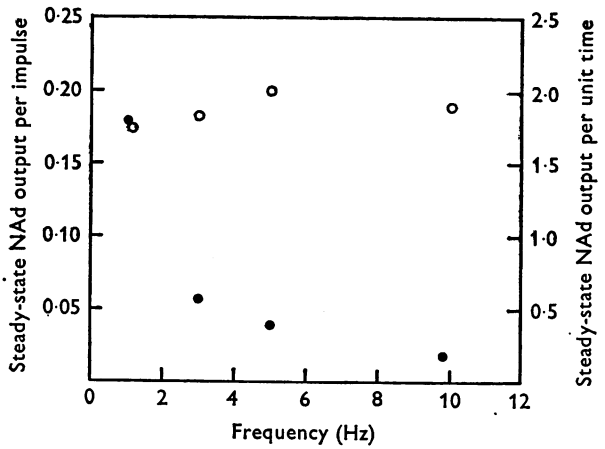

Fig. 7. The dependence of the steady-state NAd output per impulse and per unit time reached during long trains of impulses on the frequency of nerve stimulation. Filled circles give the output per impulse, and open circles give the output per unit time.

solutions (6–10 mM), the amplitude of the e.j.p. was depressed by up to 90 %. However there was no change in the potentiating effect which a conditioning impulse had on the amplitude of the e.j.p. due to a subsequent test impulse. Successive e.j.p.s increased in amplitude during short trains of impulses at all frequencies examined up to and including 10 Hz, until a steady-state facilitated e.j.p. was reached after several seconds of stimulation (Fig. 8). Both the time course of growth of the e.j.p. during these short trains (Fig. 9) and the steady-state e.j.p. amplitude reached (Fig. 10), could be reasonably predicted in terms of the summation of the successive facilitations introduced by each impulse in the train.

This successful prediction of the growth of the e.j.p. during short trains at high frequencies, when the quantity of transmitter released by an impulse

has been reduced, supports the simple concept that NAd is normally released from a small pool which must be replenished from the stores in the terminal if it is not to be depleted.

The effect of blocking the synthesis of NAd on the release of the transmitter. The storage of the bulk of the releasable NAd in the nerve terminals was determined from an examination of the decline in amplitude of the e.j.p.

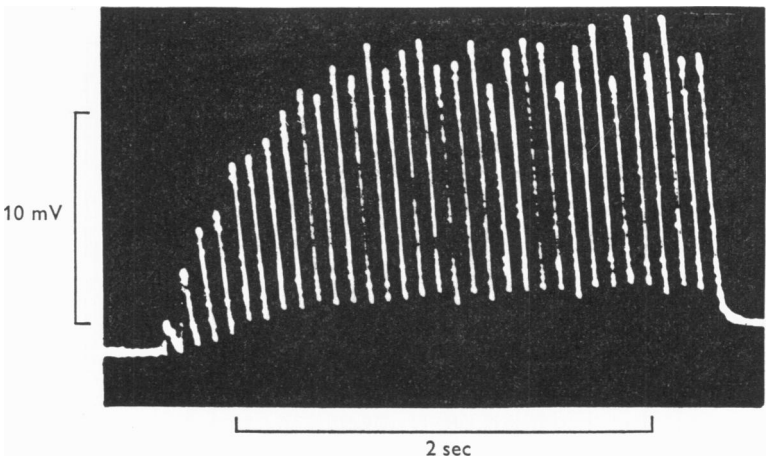

Fig. 8. Changes in amplitude of the e.j.p. during a short train of impulses at 10 Hz in high magnesium solutions (10 mM). Compare with the changes in amplitude of the e.j.p. at 10 Hz in normal magnesium solutions given in Fig. 1*B*.

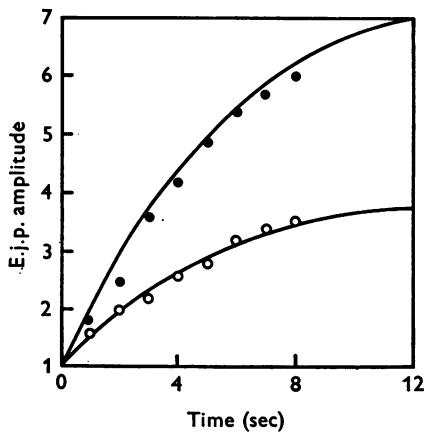

Fig. 9. Changes in amplitude of the e.j.p. during short trains of impulses at different frequencies of sympathetic nerve stimulation, in the presence of high magnesium solutions (10 mM). Filled circles, 2 Hz; open circles, 1 Hz. Curves are the predicted relationships, given in the text. S.E. of mean less than 10% of the mean for each point ($n > 5$).

during continual high-frequency stimulation for over 12 min, when the synthesis of NAd was blocked with either α-methyltyrosine (20 μg ml.$^{-1}$) or 3-iodo-L-tyrosine (2 μg ml.$^{-1}$). Neither of these synthesis blockers affected the amplitude of the e.j.p., compared with a control e.j.p. in the absence of the blockers. Furthermore, blocking synthesis did not affect the e.j.p. amplitude during short trains of impulses.

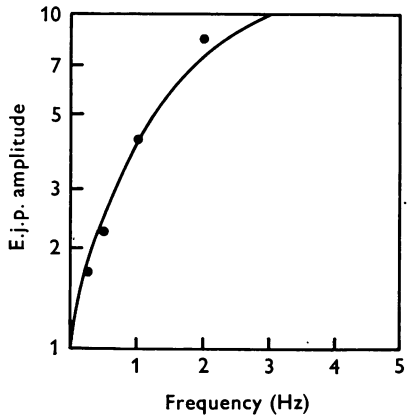

Fig. 10. Relationship between the amplitude of the steady-state e.j.p. reached during a short train of impulses and the frequency of stimulation in high magnesium solutions (10 mM). Curve is the predicted relationship, given in the text. S.E. of mean less than 10% of the mean for each point ($n > 5$).

At 10 Hz, the amplitude of the e.j.p. declined continually during stimulation in the presence of a synthesis blocker (Fig. 11), along a curve which may be described as the sum of two exponentials, one with a time constant of 1 min and the other of 10 min. If, after the first few impulses, it is assumed that the fraction of transmitter released by each impulse remains constant over the 12 min period of stimulation, then these results indicate that the bulk of the releasable transmitter is located in two stores within the terminal. A comparison between the control curve at 10 Hz in the absence of a synthesis blocker (Fig. 5) with that in the presence of a blocker (Fig. 11), shows that synthesis does not normally contribute to release until after the first few minutes of stimulation at this frequency.

As tyrosine is normally taken up into nerve terminals, where it is made available for NAd synthesis, it was possible that the normal decline in the e.j.p. observed during continual stimulation was due to a lack of substrate for the synthesis of NAd. To check if this was the case, the nerve terminals were stimulated continually at 10 Hz in the presence of tyrosine

(20 μg ml.$^{-1}$). There was no change in the rate of decline of the e.j.p. in the presence of tyrosine from that observed without the substrate (Fig. 12), suggesting that the terminals have an adequate supply of tyrosine for the duration of these periods of stimulation.

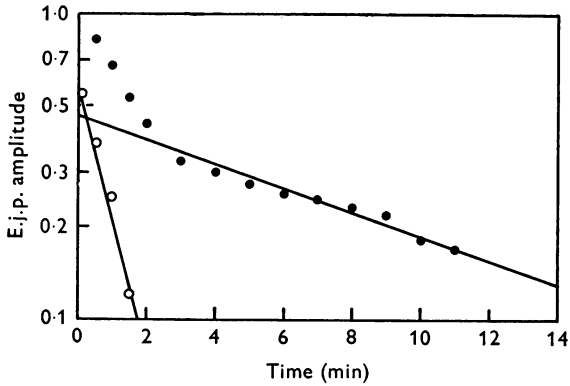

Fig. 11. Decline of the e.j.p. (filled circles) during long trains of impulses at 10 Hz when the synthesis of NAd was blocked with 3-iodo-L-tyrosine (2 μg ml.$^{-1}$). The phase of the decline at times greater than 3 min can be described by a single exponential with a time constant of 10 min; if this is subtracted from the earlier period of decline (3 min), a single exponential (open circles) with a time constant of 1 min is obtained; the filled circles can therefore be described by the sum of two exponentials.

S.E. of mean less than 10% of the mean for each point (n = 4).

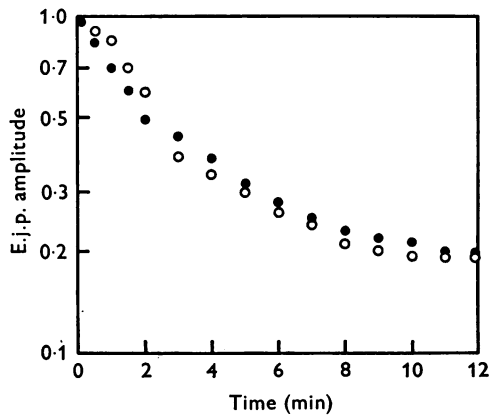

Fig. 12. Decline of the e.j.p. during long trains of impulses at 10 Hz in the presence (filled circles) or absence (open circles) of tyrosine. S.E. of mean less than 10% of the mean for each point (n > 3).

DISCUSSION

The release of NAd by short trains of impulses

The changes in amplitude of the e.j.p. during a short high frequency train of impulses are similar to the changes which occur in the amplitude of the amphibian end-plate potential; thus the first few synaptic potentials are successively greater in size and then subsequently decrease during the train until a steady-state synaptic potential amplitude is reached (Feng, 1941). The phase of facilitation at the end-plate has been quantitatively accounted for in terms of an increase in the quantal content of the end-plate potential (del Castillo & Katz, 1954*b*) and the linear addition of the individual facilitatory effects on transmitter release introduced by each impulse in the train (Mallart & Martin, 1967). The phase of depression is unlikely to be due to a post-synaptic effect, as there is no change in the amplitude of the ACh potential due to the iontophoretic application of the transmitter to the receptors at the end-plate during the phase of depression (Otsuka, Endo & Nonomura, 1962). Furthermore, the depression can be simply described in terms of a reduction in quantal content of the end-plate potential (del Castillo & Katz, 1954*b*; Thies, 1965). These determinations suggest that neuromuscular facilitation and depression are due to changes in the quantity of ACh released from the nerve terminals.

A similar analysis has not been made at sympathetic nerve terminals, so that it is not possible to state unequivocally whether the changes in amplitude of the e.j.p. during facilitation and depression are due to changes in the quantity of NAd released from the nerve terminals or to changes in receptor sensitivity. However, the qualitative similarity between the changes in the e.j.p. and in the end-plate potential during short trains of impulses suggests that changes in e.j.p. amplitude during a train of impulses simply reflect differences in the quantity of NAd released by each impulse in the train.

The release of NAd by long trains of impulses

During long high-frequency trains of impulses in preganglionic cholinergic nerve terminals, the amplitude of the synaptic potential declines during the first 8 min, until a steady-state amplitude is reached which is then maintained (Bennett & McLachlan, 1972*b*). This decline in amplitude of the synaptic potential is due to the partial depletion of the main store of releasable transmitter in the terminals (Bennett & McLachlan, 1972*b*), whilst the steady-state amplitude is maintained by the replenishment of this store with newly synthesized transmitter. The qualitative similarity between the decline in amplitude of these synaptic potentials and of the

junction potential during continual stimulation, suggests that the e.j.p. also decreases as a consequence of the depletion of the stores of releasable NAd in the nerve terminals, and that a steady state is reached when the rate of release of the transmitter equals the rate of synthesis.

It is difficult to assay the small quantities of NAd overflowing from organs during short trains of sympathetic nerve impulses, so that it has not been possible to compare the changes in amplitude of the e.j.p. during these trains with the quantity of transmitter overflowing. However, there have been several studies on the overflow of NAd from organs during continual nerve stimulation which allow a comparison to be made between the changes in amplitude of the e.j.p. and NAd overflow. During the first 8 min of continual stimulation at high frequencies, the amplitude of the e.j.p. declined until a steady-state amplitude was reached which was then maintained; a similar decline occurs in the quantity of NAd which overflows from organs during continual stimulation of their sympathetic nerve supply (Dearnaley & Geffen, 1966; Kopin, Breese, Krauss & Weise, 1968). The e.j.p. size during the steady state reached during continual stimulation at 10 Hz is 0·2 of the initial e.j.p. size; this is the same as the ratio of the rate of NAd overflow from the heart after 6 min of continual stimulation at 10 Hz to the initial overflow rate at the beginning of stimulation (Stjarne & Wennmalm, 1970). These similarities in the changes in e.j.p. amplitude and in the overflow of NAd support the claim that the e.j.p. is a direct measure of the quantity of NAd released per impulse.

The release of NAd from a small pool in sympathetic nerve terminals

The depression in the end-plate potential during a short train of impulses appears to be due to both the depletion of a small pool of transmitter (Liley & North, 1953; Takeuchi, 1958; Thies, 1965), which contains only a small proportion of the releasable ACh in the terminal (Elmquist & Quastel, 1965) as well as a decrease in the fraction of transmitter released from this pool (Betz, 1970; Christensen & Martin, 1970). Thus the depression is reversed to a facilitation when the depletion of the pool is prevented by high magnesium solutions (Mallart & Martin, 1968). Although a quantal analysis of transmitter release (which would establish the size of the pool for sympathetic nerve terminals) is not possible (Bennett, 1972), the qualitative similarity in release and of the effects of magnesium on these terminals and on somatic motor nerve terminals suggests that NAd is also released from a small pool in sympathetic nerve terminals.

The storage of NAd in sympathetic nerve terminals

When the synthesis of ACh in preganglionic nerve terminals is blocked, the amplitude of the synaptic potential decays along a single exponential

with a time constant of about 4 min during continual stimulation at 10 Hz (Bennett & McLachlan, 1972*a*). This suggests that the bulk of the releasable ACh which feeds the pool from which transmitter is released, is contained in a single store, which may be equivalent to the single population of synaptic vesicles in these terminals which store ACh (Grillo, 1966). If the synthesis of NAd in post-ganglionic nerve terminals is blocked, the e.j.p. decays along a curve which may be described by the sum of two exponentials, one with a time constant of 1 min and the other of 10 min. Thus the bulk of the releasable NAd appears to be contained in two stores, which may reside in the two different classes of synaptic vesicles in sympathetic nerve terminals which are known to store NAd (Fillenz, 1971).

The overflow of NAd from the spleen, during continual stimulation of its sympathetic nerve supply in the presence of a synthesis blocker, also declines in two phases (Kopin *et al.* 1968). The first of these two phases of NAd release is not much different from that which occurs before the synthesis blocker is added, suggesting that newly synthesized NAd does not contribute to release at this time. This is in agreement with those studies of perfused organs with [^{14}C]tyrosine or with [^{14}C]noradrenaline which show that little newly synthesized NAd is released during the first few minutes of stimulation at high frequency, but that it is preferentially released during the steady-state release phase (Kopin *et al.* 1968; Stjärne & Wennmalm, 1970). The electrophysiological findings are consistent with these studies.

The present work suggests that NAd is released from a small pool of transmitter in sympathetic nerve terminals, which is partly replenished by the mobilization of NAd from two main stores in the terminal; these stores are replenished in turn by the synthesis of new NAd. It remains to determine to what extent the pool and stores are refilled by the neuronal uptake of NAd released during impulse firing. This is considered in the following paper (Bennett, 1973).

I am indebted to E. McLachlan for helpful comments on the manuscript. This work was supported by the Australian Research Grants Committee.

REFERENCES

AXELROD, J. (1971). Noradrenaline: fate and control of its biosynthesis. *Science, N.Y.* **173**, 598–606.

BENNETT, M. R. (1972). *Autonomic Neuromuscular Transmission.* Monographs of the Physiological Society, no. 30. Cambridge: Cambridge University Press.

BENNETT, M. R. (1973). An electrophysiological analysis of the uptake of noradrenaline at sympathetic nerve terminals. *J. Physiol.* **229**, 533–546.

BENNETT, M. R. & MCLACHLAN, E. (1972*a*). An electrophysiological analysis of the storage of acetylcholine in preganglionic nerve terminals. *J. Physiol.* **221**, 657–668.

BENNETT, M. R. & MCLACHLAN, E. (1972*b*). An electrophysiological analysis of the synthesis of acetylcholine in preganglionic nerve terminals. *J. Physiol.* **221**, 669–682.

BETZ, W. J. (1970). Depression of transmitter release at the neuromuscular junction of the frog. *J. Physiol.* **206**, 629–644.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol.* **138**, 81–102.

CHRISTENSEN, B. N. & MARTIN, A. R. (1970). Estimates of probability of transmitter release at the mammalian neuromuscular junction. *J. Physiol.* **210**, 933–945.

CLARKE, D. E., JONES, C. J. & LINLEY, P. A. (1969). Histochemical fluorescence studies on noradrenaline accumulated by uptake$_2$ in the isolated rat heart. *Br. J. Pharmac. Chemother.* **37**, 1–9.

DEARNALEY, D. P. & GEFFEN, L. B. (1966). Effect of nerve stimulation on the noradrenaline content of the spleen. *Proc. R. Soc.* B **166**, 303–315.

DEL CASTILLO, J. & KATZ, B. (1954*a*). The effect of magnesium on the activity of motor nerve endings. *J. Physiol.* **124**, 553–559.

DEL CASTILLO, J. & KATZ, B. (1954*b*). Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol.* **124**, 574–585.

ECCLES, J. C. & MACFARLANE, W. V. (1949). Actions of anticholinesterases on end-plate potential of frog muscle. *J. Neurophysiol.* **12**, 59–80.

ELMQUIST, D. & QUASTEL, D. M. J. (1965). A quantitative study of end-plate potentials in isolated human muscle. *J. Physiol.* **178**, 505–529.

FENG, T. P. (1941). Local activity around skeletal neuromuscular junctions produced by nerve impulses. *Biol. Symp.* **3**, 121–152.

FILLENZ, M. (1971). Fine structure of noradrenaline storage vesicles in nerve terminals of the rat vas deferens. *Phil. Trans. R. Soc.* **261**, 319–323.

FURNESS, J. B., CAMPBELL, G. R., GILLARD, S. M., MALMFORS, T., COBB, J. L. S., & BURNSTOCK, G. (1970). Cellular studies of sympathetic denervation produced by 6-hydroxydopamine in the vas deferens. *J. Pharmac. exp. Ther.* **174**, 111–121.

GRILLO, M. A. (1966). Electron microscopy of sympathetic tissues. *Pharmac. Rev.* **18**, 387–399.

KOPIN, I. J., BREESE, G. R., KRAUSS, K. R. & WEISE, V. K. (1968). Selective release of newly synthesized norepinephrine from the cat spleen during nerve stimulation. *J. Pharmac. exp. Ther.* **161**, 271–279.

LANGER, S. Z. (1970). The metabolism of [^{3}H]noradrenaline released by electrical stimulation from the isolated nictitating membrane of the cat and from the vas deferens of the rat. *J. Physiol.* **208**, 515–546.

LIGHTMAN, S. L. & IVERSEN, L. L. (1969). The role of uptake in the extraneuronal metabolism of catecholamines in the isolated rat heart. *Br. J. Pharmac. Chemother.* **37**, 638–649.

LILEY, A. W. & NORTH, K. A. K. (1953). An electrical investigation of the effects of repetitive stimulation on mammalian neuromuscular junction. *J. Neurophysiol.* **16**, 509–527.

MALLART, A. & MARTIN, A. R. (1967). An analysis of facilitation of transmitter release at the neuromuscular junction of the frog. *J. Physiol.* **193**, 679–694.

MALLART, A. & MARTIN, A. R. (1968). The relation between quantum content and facilitation at the neuromuscular junction of the frog. *J. Physiol.* **196**, 593–604.

MALMFORS, T. (1965). Studies on adrenergic nerves. *Acta physiol. scand.* **64**, suppl. 248, 1–93.

MARTIN, A. R. (1955). A further study of the statistical composition of the end-plate potential. *J. Physiol.* **130**, 114–122.

OTSUKA, M., ENDO, M. & NONOMURA, Y. (1962). Presynaptic nature of neuromuscular depression. *Jap. J. Physiol.* **12**, 573–584.

SJOSTRAND, N. O. (1965). The adrenergic innervation of the vas deferens and the accessory male genital glands. *Acta physiol. scand.* **64**, suppl. 257.

STÄJRNE, L. & WENNMALM, O. (1970). Preferential secretion of newly formed noradrenaline in the perfused rabbit heart. *Acta physiol. scand.* **80**, 428–429.

TAKEUCHI, N. (1958). The long-lasting depression in neuromuscular transmission of frog. *Jap. J. Physiol.* **8**, 102–113.

THIES, R. (1965). Neuromuscular depression and apparent depletion of transmitter in mammalian muscle. *J. Neurophysiol.* **28**, 427–442.

TRANZER, J. P. & THOENEN, H. (1968). An electron microscopic study of selective, acute degeneration of sympathetic nerve terminals after administration of 6-hydroxy-dopamine. *Experientia* **24**, 155–156.