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AN ELECTROPHYSIOLOGICAL ANALYSIS OF THE EFFECTS OF AMINE-UPTAKE BLOCKERS AND α-ADRENOCEPTOR BLOCKERS ON ADRENERGIC NEUROMUSCULAR TRANSMISSION

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1 An electrophysiological study has been made of the effects of either blocking noradrenaline (NA) uptake or α-adrenoceptors on conduction in adrenergic preterminal axons and on NA release.

2 The excitatory junction potential (e.j.p.) evoked by a single stimulus increased slightly in duration (maximum 20%) in the presence of high concentrations of desipramine or cocaine ($\geqslant$ 1 μg/ml) but there was no change in the spontaneous miniature excitatory junction potential (m.e.j.p.s); the single compound preterminal action potential was decreased in amplitude by a maximum of 10%. The e.j.p., m.e.j.p. and the terminal action potential were not altered by lower concentrations of these drugs ($<$ 1 μg/ml).

3 The increased decline of the e.j.p. amplitude observed during the first few hundred impulses at high frequencies (10 Hz) in the presence of desipramine or cocaine was accompanied by a similar decline in the amplitude of the preterminal compound action potential, suggesting that the latter gave rise to the former.

4 These observations suggest that the action on post-synaptic α-adrenoceptors of NA released by single impulses is terminated by diffusion, and that any NA which is subsequently taken up into nerves is metabolized.

5 All the α-adrenoceptor blocking drugs tested reversed the normal depression in e.j.p. amplitude observed during the first few hundred impulses at high frequencies to facilitation; this was unaccompanied by any changes in the preterminal compound action potential.

6 α-Adrenoceptor blocking drugs did not alter the potentiating effect which a conditioning impulse had on the amplitude of the e.j.p. evoked by a subsequent test impulse. The facilitated release of NA during trains of impulses was quantitatively predicted in terms of the addition of the individual potentiations introduced by each impulse in the train.

7 It is suggested that if there is an auto-inhibition of NA release, then it is unlikely that the pre- and post-synaptic α-adrenoceptors are identical.

Introduction

Smooth muscles which receive a sympathetic innervation can take up exogenous noradrenaline (NA) (Axelrod, 1964) due to the presence of uptake processes in the membranes of both nerve (Hillarp & Malmfors, 1964; Taxi & Droz, 1966) and muscle cells (Gillespie & Muir, 1970) which may be selectively blocked by drugs (Iversen, 1963, 1965; Lightman & Iversen, 1969). During nerve stimulation much of the NA released does not diffuse from the muscle but is taken up by nerve and muscle cells where it is at least in part metabolized (Langer, 1970; Langer, Stefano & Enero, 1972; Dubocovitch & Langer, 1973). However, it is not clear whether the action of NA released by single nerve impulses onto muscle receptors is terminated by these uptake processes (Iversen, 1971) or simply by diffusion from the varicose synapses into the extracellular space of the muscle (Bennett, 1972). It is also uncertain to what extent the NA taken up by the nerve terminals during stimulation becomes available for re-release by subsequent impulses (Blakeley, Brown & Geffen, 1969; Bennett, 1973a). An, electrophysiological study of these problems is described in the first part of this paper.

Phenoxybenzamine, which blocks both the

neuronal (Avakian & Gillespie, 1968) and muscle cell (Eisenfeld, Axelrod & Krakoff, 1967) uptake of NA, increases the release of NA from nerve terminals (de Potter, Chubb, Putt & de Schaepdryver, 1971; Cripps, Dearnaley & Howe, 1972) by reversing the usual depression in transmitter output per impulse observed after the first few impulses (Bennett, 1973b) to a facilitation (Bennett, 1973a). It has therefore been suggested that the NA released from nerve terminals acts on presynaptic α-receptors to in part inhibit the release of the transmitter by subsequent nerve impulses (Farnebo & Hamberger, 1971; Langer, Adler, Enero & Stefano, 1971; Kirpekar & Puig, 1971; Enero, Langer, Rothlin & Stefano, 1972; Starke, 1972; Starke & Schümann, 1972). The second part of this paper describes a study of the effects of drugs which block α-adrenoceptors on NA release.

Methods

Transmission

The isolated vas deferens of the mouse was used in all the intracellular studies of adrenergic transmission. The experimental arrangements and techniques, as well as the method of normalizing and graphing the amplitude changes of the e.j.p. during trains of impulses were the same as that described in the preceding paper (Bennett & Middleton, 1975).

Conduction

Isolated bundles of preterminal sympathetic axons (Malmfors, 1965) from the guinea-pig vas deferens were used in all studies of action potential conduction. These bundles were dissected free from the serosal surface of the vas deferens in the organ bath, at their point of entry into the superficial smooth muscle bundles (Merrillees, Burnstock & Holman, 1963); such axons are already varicose and contain synaptic vesicles (Bennett, 1972). Each end of an approximately 2 mm length of preterminal-axon bundle was taken up into fine glass-capillary suction electrodes, one of which was used to stimulate and the other to record the preterminal compound action potential.

Drugs

The following drugs were added directly to the organ bath where specified, generally to a final concentration of 10 μg/ml, and a delay of 0.5 h was allowed for equilibration before electrophysiological measurement began: desipramine hydrochloride (Geigy); cocaine; phentolamine hydrochloride (Regitine, CIBA); dibenamine hydrochloride (Smith, Kline & French); dihydroergocristine (Sandoz); dihydroergocornine (Sandoz).

Results

The effect of blocking neuronal uptake on the release of noradrenaline by a single nerve impulse

If the neuronal uptake of NA is as important for terminating the action of NA on α-adrenoceptors after its release from nerve terminals, as cholinesterase is for terminating the action of acetylcholine at the endplate (Fatt & Katz, 1951, 1952), then inhibiting neuronal uptake should greatly increase both the amplitude and time course of the

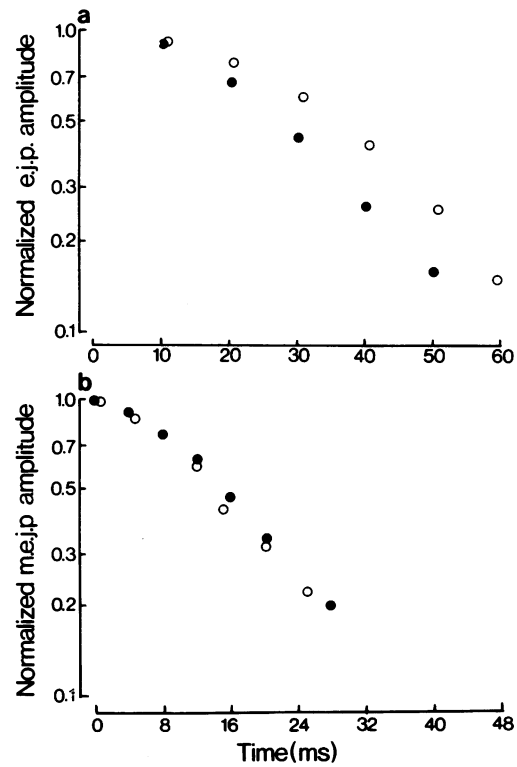

Figure 1 Time course of decline of the excitatory junction potential (e.j.p.) (a) and the m.e.j.p. (b) in the presence of amine-uptake blockers. (●), Controls; (○), desipramine (3 μg/ml). The s.e. mean was less than 5% of the mean for each point ($n > 10$).

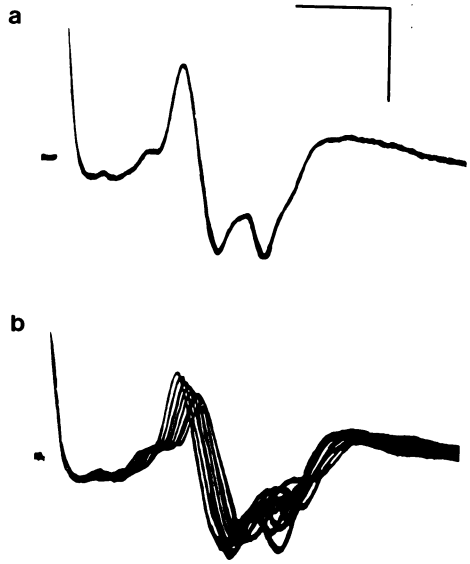

Figure 2 Change in characteristics of successive preterminal compound action potentials before (a) and during (b) exposure of a preparation to desipramine (3 μg/ml). Stimulation frequency 10 Hz. Horizontal calibration, 4 ms; vertical calibration, 100 μV.

spontaneous miniature excitatory junction potential (m.e.j.p.) due to transmitter release at close contact varicosities on smooth muscle cells (Bennett, 1972, 1973c) as well as the excitatory junction potential (e.j.p.) due to evoked transmitter release. The m.e.j.p. was unaffected by either desipramine (⩽ 3 μg/ml)' or cocaine (⩽ 10 μg/ml) (Figure 1b), suggesting that the action of this spontaneous transmitter release is terminated by diffusion.

The duration of the e.j.p. but not the amplitude was increased by about 20% (Figure 1a) in the presence of desipramine (3 μg/ml) and this was accompanied by a decline in amplitude and increase in duration of the preterminal compound action potential (Figure 2) of about 10% (88% ± 1.3%, $n = 6$). If this latter increase is due to the temporal dispersion of the action potentials in the preterminal axons (see below), then as the smooth muscle syncytium receives a multiple and distributed innervation from these axons (Bennett, 1972), the temporal dispersion is liable to account for the increase in duration of the e.j.p. It is unlikely that blocking neuronal uptake has simply enhanced the effect of muscle cell uptake, which then inactivates the transmitter, as blocking both neuronal uptake (desipramine 3 μg/ml) and muscle uptake (normetanephrine 10 μg/ml) simultaneously did not increase the duration of the e.j.p. over that by blocking neuronal uptake alone.

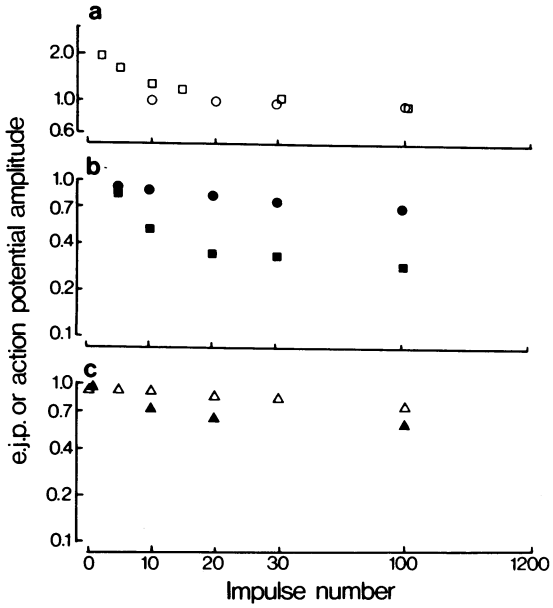

Figure 3 The decline of the compound action potential in a sympathetic preterminal nerve bundle entering the vas deferens muscle and in the excitatoty junction potential (e.j.p.) during the first 1200 impulses at 10 Hz. (a) Control: (○) compound action potential, (□) e.j.p. amplitude; (b) in the presence of desipramine (3 μg/ml): (●) compound action potential, (■) e.j.p. amplitude; (c) in the presence of cocaine (10 μg/ml): (△) compound action potential, (▲) e.j.p. amplitude. Both the amplitude of the compound action potential and that of the e.j.p. were normalized to their respective values at the beginning of stimulation. The s.e. mean was less than 10% of the mean for each e.j.p. point ($n > 6$) and less than 8% of the mean for each compound action potential point ($n > 8$).

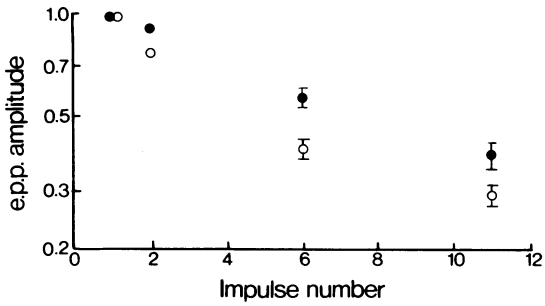

Figure 4 The decline in the amplitude of the endplate potential (e.p.p.) during the first 12 impulses at 100 Hz. (●), Control; (○), desipramine 3 μg/ml. The amplitude of successive e.p.ps is normalized to the first e.p.p. in the train. Vertical bars show s.e. mean ($n > 13$).

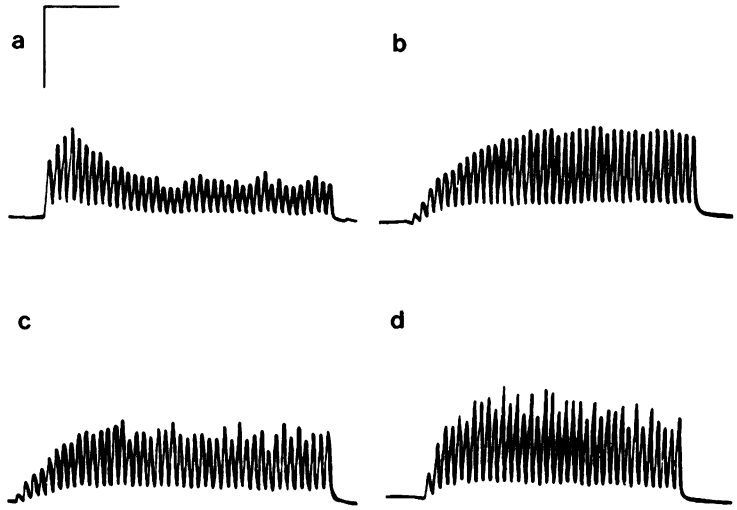

Figure 5 The effect of α-adrenoceptor blocking drugs on the amplitude of the excitatory junction potential (e.j.p.) during short trains of impulses at high frequency (10 Hz). (a) Control; (b) dihydroergocornine; (c) dibenamine; (d) phentolamine; all drug concentrations, 10 μg/ml. Calibrations: vertical, 10 mV; horizontal, 1 second.

The effect of blocking neuronal uptake on the release of noradrenaline by trains of nerve impulses

It has been reported that blocking the neuronal uptake of NA with desipramine ($\geqslant$ 3 μg/ml) or cocaine ($\geqslant$ 10 μg/ml) leads to a depression in the amplitude of successive e.j.ps in a short train (Bennett, 1973a). If it is assumed that these drugs have effects specific to their uptake blocking capacities at these concentrations, then these results suggest that normally the NA released by each impulse must be taken up and immediately re-released by subsequent impulses. This assumption has been examined by determining the effects of these drugs on the compound action potential in preterminal axons (Figure 2). Both desipramine and cocaine caused a sequential decline in the amplitude of the compound action-potential during a short train of impulses (Figure 3b and c) compared with control trains (Figure 3a) and this was accompanied by an increase in duration of the action potential. The decline reached between 30% and 50% of the control in a few hundred impulses and followed a similar time course to that of the decline in the e.j.p. (Figure 3). During continual stimulation over minutes there was no further change in the amplitude of the compound action potential following that in the first few hundred impulses, compared with controls, and the amplitude of the e.j.p. following the first few hundred impulses declined at the same rate in the presence of the drugs as in the controls (Bennett, 1973a). These observations suggest that the rapid decline in the e.j.p. during short trains in the presence of these concentrations of desipramine and cocaine is due to a decline in the nerve action potential. At lower concentrations of these drugs there is no difference in the amplitude changes, if any, in the e.j.p. or the compound action potential which accompany continual stimulation, compared with controls.

A study was made of the effects of these drugs on transmission at the endplate in order to determine whether the decline in amplitude of the compound action potential in the preterminal nerves is due to a decrease in amplitude and conduction time of the action potential in individual fibres, which would lead to a decrease in transmitter release from individual terminals (Hagiwara & Tasaki, 1958), or to complete failure of conduction in a proportion of the fibres present in the nerve (Chang & Chuang, 1972). At low frequencies ($<$ 50 Hz) of stimulation of the phrenic nerve in the well oxygenated rat hemidiaphragm there was no difference (at the 1% level of significance) in the amplitude of the endplate potential (e.p.p.) in the presence of desipramine (3 μg/ml) compared with controls (Bennett, 1973), whereas at high frequencies ($>$ 50 Hz) there was (Figure 4). This increased depression of the e.p.p. in the presence of desipramine was accompanied by an increase in latency, suggesting that the action of the drug is to slow conduction

and decrease the amplitude of the action potential in the nerve terminal during trains of impulses, as it does the action potential in skeletal muscle (Anderson, 1973).

The effect of blocking α-adrenoceptors on the release of noradrenaline by a single nerve impulse

There was a significant decrease in the amplitude of the e.j.p. in the presence of α-adrenoceptor blockers at a concentration of 10 μg/ml (phenoxybenzamine; phentolamine; dibenamine; dihydroergocornine; dihydroergocristine), the relationship between the duration of the stimulus strength and the amplitude of the e.j.p. decreasing from a control value of 170 V/s (Bennett & Middleton, 1975) to 35 V/s, although there was no change in the amplitude of the preterminal compound action potential (see also Kao & McCullough, 1972).

The effect of blocking α-adrenoceptors on the release of noradrenaline by trains of nerve impulses

It has been reported that phenoxybenzamine reverses the normal depression in the amplitude of successive e.j.ps in a short train at high frequencies to facilitation (Bennett, 1973a). The same effect is produced by the other α-adrenoceptor blocking drugs enumerated above (Figure 5), the normal decrease in successive e.j.ps after the first few being replaced by a facilitation in which the e.j.ps continue to grow in amplitude until a steady-state NA release-rate is reached (Figure 6). There is no alteration in the amplitude of the preterminal compound action potential in the presence of α-adrenoceptor blocking agents during these changes in the amplitude of the e.j.p. occurring during short trains, so that the enhanced amplitude of the e.j.p. is presumably due to an increase in NA release from the nerve terminals.

Although the α-adrenoceptor blocking drugs greatly enhanced the steady-state e.j.p. amplitude reached during short trains of impulses (Figure 7) compared with controls, the amount by which a conditioning impulse potentiated the amplitude of the e.j.p. due to a subsequent test impulse at different intervals was approximately the same in the presence of any of the α-adrenoceptor blockers as it was in their absence (Figure 8). The amplitude and time course of decay of this facilitation following a single impulse was used to predict the growth of facilitation which occurred during trains of impulses in the presence of the α-adrenoceptor blockers (for theory, see methods section). The time course of growth of the e.j.ps during a train (Figure 6) and the steady-state e.j.p. amplitude reached at low frequencies (Figure 7) were predicted on this basis.

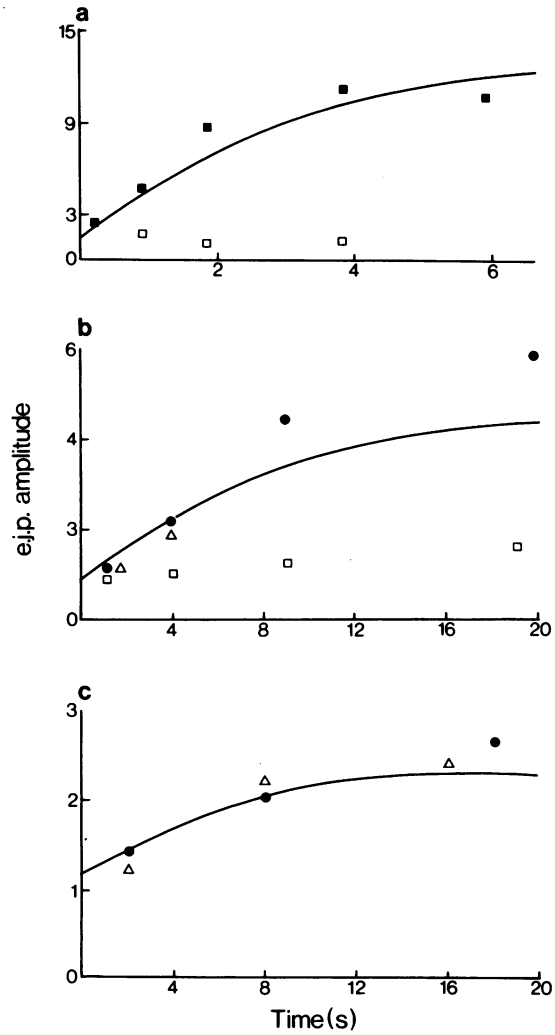

Figure 6 Effect of α-adrenoceptor blocking drugs on the growth of the excitatory junction potential (e.j.p.) during short trains of impulses at different frequencies: (a) 5 Hz; (b) 1 Hz; (c) 0.5 Hz. (●), Dihydroergocornine (10 μg/ml); (■), dihydroergocristine (10 μg/ml); (△) and (□), controls. The e.j.p. is normalized to the first impulse in the train. Curves are the predicted relationships for the growth of the e.j.p. given in Bennett (1973b). The s.e. mean was less than 20% of the mean for each point ($n > 3$).

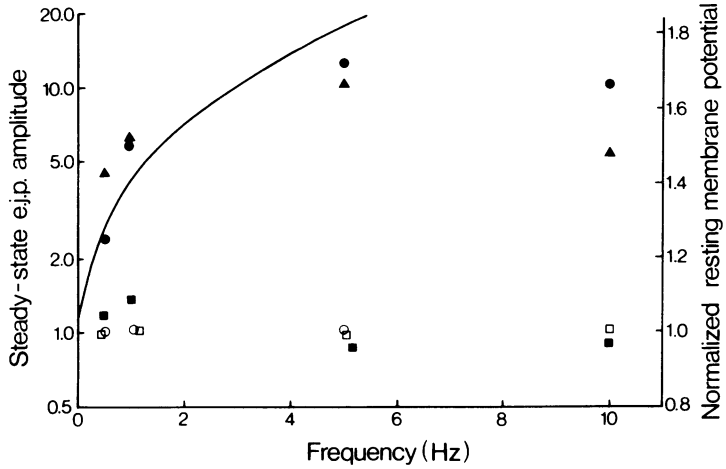

Figure 7 Effect of α-adrenoceptor blocking drugs on the relationship between the amplitude of the steady-state excitatory junction potential (e.j.p.) reached during a short train of impulses and the frequency of stimulation as well as on the resting membrane potential between successive e.j.ps when the e.j.p. steady-state has been reached. (■), (▲) and (●) are respectively the control steady-state e.j.p. amplitude, and that in the presence of dihydroergocristine (10 μg/ml) and dihydroergocornine (10 μg/ml); the steady-state e.j.p. is normalized to the first impulse in the train. (□) and (○) give the values of the membrane potential between successive e.j.ps after the steady-state e.j.p. amplitude had been reached in control and dihydroergocornine (10 μg/ml) treated preparations respectively; in each case the value of the membrane potential has been normalized to that found before stimulation commenced. The curve is the predicted relationship for the steady-state e.j.p. amplitude given in Bennett (1973b). The s.e. mean was less than 12% of the mean for each point ($n > 17$).

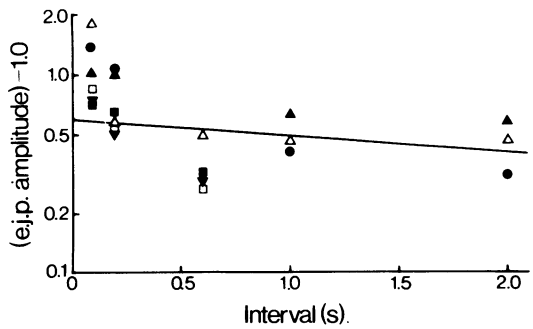

Figure 8 Effect of a conditioning impulse on the amplitude of an excitatory junction potential (e.j.p.) evoked by a subsequent test impulse at different intervals in the presence of α-adrenoceptor blocking drugs. (□) and (△), Control from two series of experiments; (■), dibenamine; (●), dihydroergocornine; (▲), dihydroergocristine; (♦), phentolamine. All drug concentrations 10 μg/ml. The ordinate scale is the test e.j.p. amplitude normalized to the conditioning e.j.p. amplitude less 1.0. The s.e.m. can was less than 10% of the mean for each point ($n > 16$). The line indicates a time constant of 6 s (see Bennett, 1973b).

Discussion

The termination of the effect of noradrenaline released by single nerve impulses

It has been suggested that amine-uptake plays a role analagous to that of cholinesterase at the motor endplate in terminating the action of the released transmitter (Iversen, 1971); however, uptake blockers have only a small effect on the e.j.p. compared with the effect of anticholinesterase on the endplate potential (Fatt & Katz, 1951, 1952). There is no change in the characteristics of the e.j.p. in the mouse vas deferens in the presence of concentrations of desipramine (1 μg/ml) or cocaine (1 μg/ml) (Holman, 1967) which are known to produce an over 60% inhibition of noradrenaline uptake (Iversen, 1965). As the preterminal action potential is unaffected at these concentrations, it is unlikely that a potentiation of the e.j.p. due to blocking NA uptake has been masked by a decrease of NA release due to changes in the preterminal action potential.

At a higher concentration of desipramine (3 μg/ml) there is about a 20% increase in the duration of the e.j.p., but little change in its amplitude, whilst cocaine produces a similar increase in duration as well as a diminution in amplitude of the e.j.p. (Holman, 1967); similar effects of cocaine have been observed on the e.j.p. of the guinea-pig vas deferens (Bell, 1966; see, however, Bennett, 1972). These drugs, even at these high concentrations, do not change the time course of the m.e.j.p. due to the spontaneous release of NA from the nerve terminals, indicating that their effect on the evoked e.j.p. is likely to be either on the nerve terminal action potential or the mechanism of evoked transmitter secretion. The observation that a single preterminal compound action potential is increased in duration with higher concentrations of drugs suggests that the slight increase in duration of the e.j.p. is due to an increase in temporal dispersion of the action potentials invading the different nerve terminals which supply the smooth muscle syncytium.

It seems likely that the NA released by a single impulse is simply inactivated by diffusing from a point of high concentration at the close-contact varicosities to a much lower concentration in the extracellular space (Bennett, 1972), as seems to be the case for acetylcholine released at preganglionic nerve terminal varicosities (Ogston, 1955; Burke, 1956). Having ceased to exert a physiological effect it may then be taken up from the extracellular space by neuronal or muscle cell uptake (Bennett, 1972), this then subserving a role with respect to NA similar to that which the cholinesterase located throughout structures in sympathetic ganglia has for the acetylcholine released within the ganglia (Bennett & McLachlan, 1972).

The effect of blocking neuronal uptake on the release of noradrenaline by trains of impulses

The increased decline in amplitude of the e.j.p. during the first few hundred impulses of a high frequency (10 Hz) train in the presence of desipramine (3 μg/ml) or cocaine (10 μg/ml) (Bennett, 1973a) is paralleled by a decline in amplitude and an increase in duration of the preterminal compound action potential, which suggests that the latter gives rise to the former. It is likely, in view of the effects of desipramine on the endplate potential, that the changes in characteristics of the sympathetic preterminal compound action potential are due to a decrease in conduction velocity and amplitude of the action potential in individual nerve fibres, and that this is the main reason for the rapid decline in the e.j.p. observed during continual stimulation in the presence of amine-uptake blockers. The alternative suggestion (Bennett, 1973a) that the rapid decline in the e.j.p. in the presence of amine-uptake blockers might be due to their uptake blocking capacity *per se* seems unlikely in the light of the present observations.

If the decline in e.j.p. amplitude in the presence of amine-uptake blockers is entirely due to effects of the drugs on the nerve impulses, then these drugs do not affect the release of NA during over 20 min of stimulation of the sympathetic nerves at 10 Hz. This suggests that any neuronal uptake of the released NA in this organ does not contribute to the subsequent release of the transmitter under these conditions.

During stimulation of more than 20% of the intramural sympathetic nerves at 10 Hz for 20 min in the presence of neuronal uptake blockers there is no change in the resting membrane potential of muscle cells between individual e.j.ps. Thus the increase in NA concentration in the extracellular spaces of the muscle during this pattern of stimulation is not sufficient to exert a physiological effect on α-adrenoceptors, either because it can quickly diffuse from this thin walled muscle (Bennett, 1972) or because it has been taken up into the muscle cells and metabolized (Dubocovitch & Langer, 1973).

The uptake of NA and its subsequent release by nerve impulses in a short train is difficult to reconcile with the vesicle hypothesis for it would require that the NA taken up in some way enhances the NA content of the vesicles which are immediately available for release, thus effectively increasing the quantal size during stimulation. The present results suggest an alternative explanation, namely that the NA taken up is metabolized.

The effect of blocking α-adrenoceptors on the release of noradrenaline by impulses

The facilitation in amplitude of the e.j.p. during the first few hundred impulses in the presence of α-adrenoceptor blockers was unaccompanied by any changes in the preterminal action potentials, indicating that these drugs were probably enhancing the release of NA from the nerve terminals (Enero *et al.*, 1972). The reversal of the normal depression in the e.j.p. during a short/high-frequency train to facilitation in the presence of the α-adrenoceptor blockers, is similar to that observed in the presence of reserpine (Bennett & Middleton, 1974); in both cases the growth of facilitation has the time course and reaches a steady-state amplitude which would be expected if the facilitation due to each impulse in the train simply adds, according to the theory previously described (Bennett, 1973b). However, the dif-

ferences between the effects of the α-adrenoceptor blocking drugs and reserpine, is that the former lead to a greatly enhanced release of NA during a short train as the facilitation they introduce is not accompanied by a simultaneous depression in the quantity of NA released by the first impulse in the train (Bennett & Middleton, 1974). Nevertheless, the quantitative similarity in the facilitatory effects introduced by both the α-adrenoceptor blockers and reserpine suggests that each acts at a common point in modifying the secretory process during trains of impulses.

If the NA released by nerve impulses normally acts back on these presynaptic α-adrenoceptors located on the nerve terminal, to inhibit its own release, then it is likely that these receptors are different from the post-synaptic α-adrenoceptors. The facilitatory effect of the α-adrenoceptor blockers during a train was clear when the interval between successive e.j.ps was as great as 500 ms; during this interval the membrane potential had returned to its normal resting value (Figure 7) indicating that NA was not present in sufficient concentrations to exert a post-synaptic effect, although it is proposed that during this period it is exerting a presynaptic action. These observations can be reconciled with the suggestion that there is an auto-inhibition of NA release, if the dissociation rate constant of the presynaptic α-adrenoceptors is different from the post-synaptic α-adrenoceptors. That the two receptors are not identical is also indicated by the differencies in the potencies of phenoxybenzamine in blocking them (Dubocovich & Langer, 1974).

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