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Effects of the inhibition of noradrenaline uptake and synthesis on the maintenance of the response to continuous nerve stimulation in the central artery of the rabbit ear

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Summary

1. The central artery of the rabbit ear was perfused through its lumen *in vitro*, with a constant pressure technique, and stimulated continuously via its periarterial sympathetic nerves at the physiological frequency of 5 Hz.

2. The vasoconstrictor response, which led initially to an almost complete cessation of intraluminal flow, deteriorated steadily over a period of hours. The involvement of presynaptic mechanisms in this effect was indicated by the finding that noradrenaline, administered extraluminally, produced a similar response before the onset of continuous stimulation and at a late stage when the constriction had decreased markedly. In addition, the noradrenaline precursor DOPA, restored the depressed responses towards their original values, indicating that failure involved depletion of mediator for release.

3. Responses to continuous stimulation declined significantly faster after inhibition of tyrosine hydroxylase with α-methyl-*p*-tyrosine. However, inhibition of the uptake of noradrenaline with cocaine did not enhance the decline of the response, even when the sensitization produced by the compound was taken into account.

4. It is concluded that synthesis, along with the mobilization of stored mediator, rather than uptake and re-use of noradrenaline maintain the effector response in the central artery of the rabbit ear stimulated continuously at a frequency within the physiological range.

Introduction

Extracellular noradrenaline is taken up into sympathetic nerve cell cytoplasm at a rapid rate by a specialized membrane transport system (Whitby, Hertting & Axelrod, 1960; Dengler, Spiegel & Titus, 1961; Muscholl, 1961; Iversen, 1963; Malmfors, 1965). This uptake is widely believed to terminate the action of noradrenaline after its release onto effector cells (Hertting, Axelrod & Whitby, 1961; Muscholl, 1961; Rosell, Kopin & Axelrod, 1963; Brown, 1965; Iversen, 1965; Trendelenburg, 1966). However, the relevance of uptake to termination of action, at least in certain preparations, has been questioned recently (Kalsner & Nickerson, 1969a, b, c; Kalsner, 1971a). A particularly attractive feature of the uptake hypothesis is that it provides for the conservation of neurotransmitter substance. It is

generally assumed that the maintenance of sympathetic nerve transmission, and therefore the response during continuous stimulation at physiological frequencies, is dependent on the uptake and re-use of noradrenaline and not to any great extent on the synthesis of new mediator substance (Blakeley & Brown, 1966; Geffen, 1967; Hedqvist & Stjärne, 1969; Bhagat & Zeidman, 1970; Su & Bevan, 1970; Iversen, 1971).

The experiments reported here were designed to assess the roles of uptake and re-use and of synthesis in the maintenance of the response in a preparation of vascular tissue during a prolonged period of continuous stimulation at a physiological frequency. For this purpose, the central artery of the rabbit ear was perfused *in vitro* and stimulated continuously at a moderate frequency of 5 Hz, and the factors involved in the decay of the response with time were assessed.

Methods

Albino rabbits of either sex, between 3·0 and 4·0 kg body weight, were anaesthetized with urethane (6 ml/kg of a 25% solution) given intraperitoneally. A 3–4 cm length of the central artery in the base of each ear was cannulated at both ends with PE 60 tubing and then dissected free of surrounding tissue and placed in a beaker containing oxygenated (95% O_2–5% CO_2) Krebs–Henseleit solution. This surgical procedure required about 30 min and was essentially the same as that described by De La Lande & Rand (1965) and by De La Lande, Frewin & Waterson (1967). The left and right central ear arteries were suspended in individual 30 ml muscle chambers containing Krebs–Henseleit (Krebs) solution (NaCl 115·3 mM, KCl 4·6 mM, $CaCl_2$ 2·3 mM, $MgSO_4$ 1·1 mM, $NaHCO_3$ 22·1 mM, KH_2PO_4 1·1 mM and glucose 7·8 mM) to which the disodium salt of ethylene diamine tetra-acetic acid was added (0·03 mM). The Krebs solution was maintained at 37° C and constantly bubbled with 95% O_2–5% CO_2. The muscle chambers were drained and refilled quickly (washed) with warmed and oxygenated Krebs solution about every 15 min during each experiment.

Each vessel was perfused through its lumen with oxygenated Krebs solution at 37° C by means of a gravity-feed apparatus at a constant pressure of 85 cm of water. The intraluminal fluid did not mix with the fluid bathing the outer surface of the vessel and the absence of leaks was confirmed by monitoring the volume of Krebs in the muscle chambers. The rate of flow of fluid through the lumen of the vessel was recorded by using a modified Gaddum outflow recorder (Andrews, 1952) connected to a piston recorder writing on a slowly revolving kymograph drum. A diagram of the apparatus is provided in Figure 1. The amplitude of each vertical stroke of the writing lever is proportional to the volume of air displaced from the outflow recorder over an 8 s period and thus to the volume of Krebs flowing through the vessel lumen and into the recorder during that same interval.

The procedure used for stimulation of the periarterial sympathetic nerves was described previously by De La Lande & Rand (1965). A set of platinum electrodes was arranged around the proximal end of each artery over the area where the perfusion cannula lay within the artery. The nerves were stimulated at supramaximal voltage with biphasic pulses of 1 msec pulse width delivered by a Grass model S5 stimulator. Supramaximal voltage was determined for each preparation

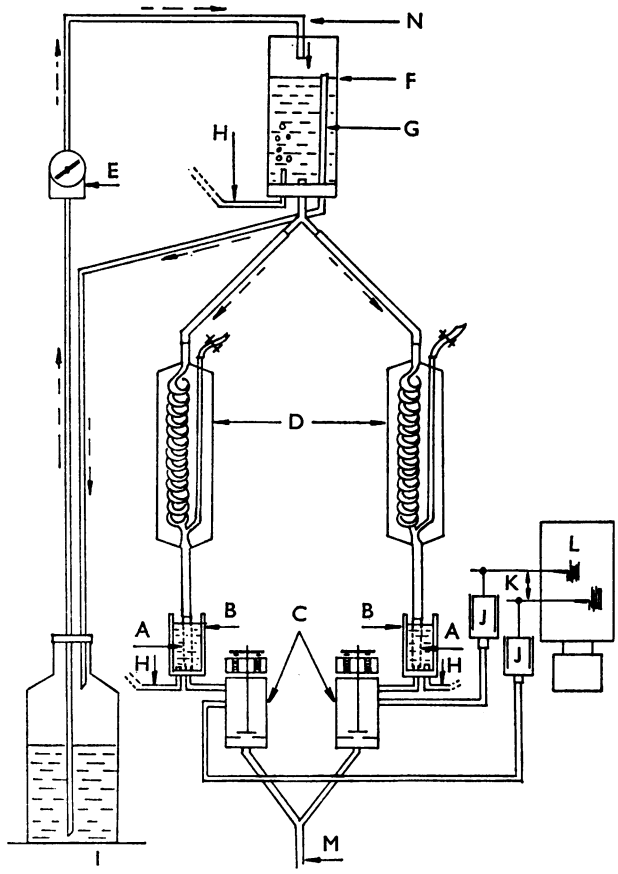

FIG. 1. Diagram of the apparatus for perfusion of the central artery of the rabbit ear. A, artery; B, muscle chamber at 37° C; C, Andrews outflow recorder; D, heat exchangers at 37° C; E, roller pump; F, reservoir of Krebs solution; G, overflow outlet; H, air line (for 95% O_2–5% CO_2); I, container of Krebs solution; J, piston recorder; K, writing lever; L, kymograph drum; M, outlet (to drain) for perfusate and N, inlet for perfusion fluid (Krebs). The dashed arrows indicate the direction of flow of perfusion fluid.

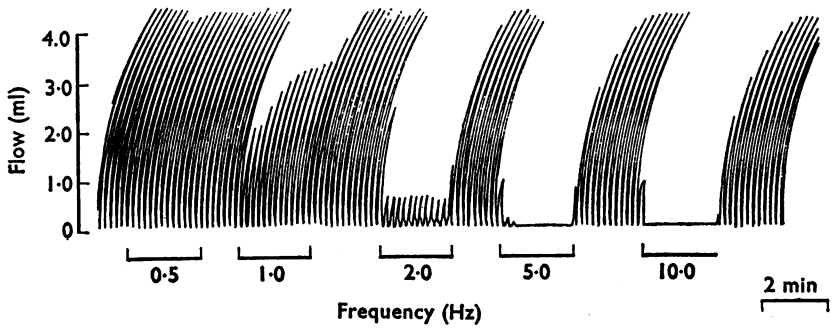

FIG. 2. Responses of the central artery of the rabbit ear to stimulation of the periarterial sympathetic nerves at various frequencies. The duration of stimulation at each frequency is indicated by an enclosed horizontal bar. Vasoconstrictor responses are recorded as reductions in outflow.

by stimulating initially at 5 Hz for 1 min at each test voltage (beginning with 10 V and increasing in 5 V increments) and recording the vasoconstriction produced. A voltage which was about 25% above that which produced the most vasoconstriction was routinely used in all experiments. Frequency–response curves were then obtained in both ear arteries from each rabbit. If one of the preparations did not respond to stimulation at 2 Hz with an inhibition of flow of at least 25%, both preparations were discarded. Mean values of data are presented with their standard errors and were compared by Student's t test. Differences with P values of 0·05 or less were considered significant.

The drugs were cocaine hydrochloride, (±)-α-methyl-*p*-tyrosine, (±)-dihydroxyphenylalanine (DOPA), guanethidine and (−)-noradrenaline bitartrate. These compounds when used were added to the muscle chambers containing the Krebs medium bathing the extraluminal surface of the artery. When cocaine and α-methyl-*p*-tyrosine were used they were kept in the muscle chambers for the duration of the experiment by re-adding them after each wash.

Results

Responses to periarterial nerve stimulation

Typical responses of the central artery of the rabbit ear to periarterial nerve stimulation are shown in Figure 2. Detectable vasoconstriction was observed in most preparations at a frequency of 1·0 Hz and at 5 Hz an intense constriction was usually seen which obliterated flow through the vessel lumen. Evidence that the responses to stimulation were mediated by excitation of the periarterial nerves was obtained with guanethidine which inhibits the release of noradrenaline from sympathetic nerve terminals (Cass & Spriggs, 1961). A 30 min exposure of the ear artery to guanethidine (50 μM), added extraluminally to the muscle chamber, usually abolished responses to stimulation without impairing the vasoconstriction produced by added noradrenaline (0·18 μM). This has also been demonstrated previously by De La Lande & Rand (1965).

The effect of inhibition of noradrenaline synthesis on the maintenance of sympathetic transmission during continuous nerve stimulation

A frequency of 5 Hz was selected for use in experiments designed to assess the importance of transmitter synthesis in maintaining the response during continuous nerve stimulation. This was done because of both the intensity of the response produced and the likelihood that such a rate of stimulation is still within the physiological range of sympathetic nerve discharge (Celander & Folkow, 1953). The procedure adopted for each experiment was first to obtain frequency–response curves for the left and right central ear arteries taken from the same rabbit. Both preparations were stimulated for approximately one minute at each of the test frequencies given consecutively (5, 4, 3, 2, 1 Hz). This was done so that the gradually decreasing vasoconstriction during continuous stimulation of each vessel could be expressed, at any time, in terms of the frequency required to produce an equivalent response prior to the onset of continuous stimulation. One artery of each pair, chosen randomly, was then exposed to α-methyl-*p*-tyrosine (100 μM) and then 30 min later, with the inhibitor of tyrosine hydroxylase still present in the muscle chamber, the frequency–response curves were repeated in both the treated and contralateral control preparations. The results obtained in experiments with 12 pairs of arteries are presented in Figure 3.

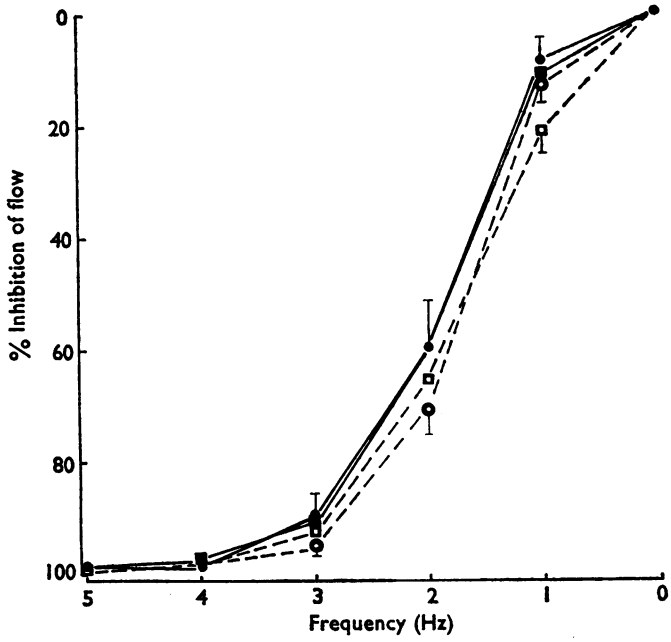

FIG. 3. Frequency–response curves of the central artery of the rabbit ear in the absence and in the presence of α-methyl-*p*-tyrosine. First (●——●) and second (○---○) frequency–response curves in 12 control preparations; and in the absence (■——■) and then in the presence (□---□) of α-methyl-*p*-tyrosine (100 μM) for 30 min in 12 preparations from the contralateral ears. Stimulation was for about one minute at each consecutive test frequency, beginning with the highest (5 Hz). The interval between curves was 30 minutes. Mean values are shown with standard error bars wherever they do not reduce clarity.

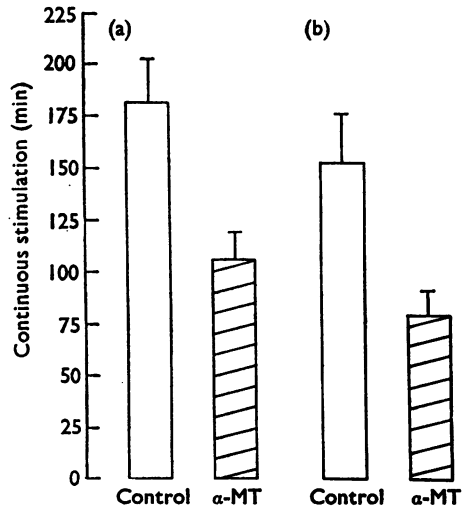

FIG. 4. Effects of α-methyl-*p*-tyrosine on the maintenance of responses to continuous stimulation at 5 Hz in the central artery of the rabbit ear. (a) Mean period of continuous stimulation required for intraluminal flow to increase to 50% of pre-stimulation values, calculated individually in 12 α-methyl-*p*-tyrosine (100 μM)-treated preparations and their contralateral controls; (b) mean period of continuous stimulation required for the vasoconstrictor response to decline (intraluminal flow to increase) to the equivalent of that produced initially at 2 Hz, calculated individually in 12 α-methyl-*p*-tyrosine-treated preparations and their contralateral controls. Mean values are shown with their standard errors. In (a) control vs treated group $P<0.01$ by *t* test for either unpaired or paired data. In (b) control vs treated group $P<0.02$ and $P<0.01$ by *t* test for unpaired and paired data, respectively.

The responses to stimulation did not differ significantly between the first and second frequency–response curves in either group of arteries. After a 15 min interval without stimulation the vessels were stimulated continuously at 5 Hz. The initial peak effect in 12 control arteries and 12 arteries with α-methyl-*p*-tyrosine present was a reduction in the intraluminal flow of $99{\cdot}3 \pm 0{\cdot}4\%$ and $98{\cdot}7 \pm 0{\cdot}5\%$, respectively. The intensity of the vasoconstriction decreased slowly with time. The period of prolonged stimulation required for intraluminal flow to return to 50% of resting values, measured immediately prior to the onset of stimulation, was significantly shorter for the arteries treated with the inhibitor of tyrosine hydroxylase (Fig. 4). It was $181{\cdot}8 \pm 21{\cdot}3$ min for 12 control and $107{\cdot}1 \pm 12{\cdot}9$ min for the contralateral α-methyl-*p*-tyrosine-treated preparations. This difference was significant when determined by either the *t* test for paired or unpaired data. In addition, the vasoconstrictor response declined to the equivalent of that produced initially in each vessel by stimulation at 2 Hz, significantly faster after treatment with α-methyl-*p*-tyrosine. The mean time was only 52·7% of that of the controls (Fig. 4). A typical record showing the gradual decay of the vasoconstrictor response during stimulation is presented in Figure 5.

Evidence that the deterioration of the vasoconstrictor response was not due to some alteration in the effector was the finding that noradrenaline (0·12 or 0·3 μM) added extraluminally, to the muscle chambers, produced an approximately equivalent effect whether it was given prior to continuous stimulation or at a point when the peak response was decreased by more than 50%. Other, more specific evidence that a decrease in the release of noradrenaline from sympathetic nerves was involved in the gradual failure of the constrictor response in both control preparations and in those pretreated with the inhibitor of tyrosine hydroxylase was obtained with the noradrenaline precursor DOPA. This compound was added to the muscle chambers during continuous stimulation, when responses were diminishing rapidly and intraluminal flow was between 50 and 75% of resting values. As shown in Fig. 6, DOPA (50 μM) gradually increased the magnitude of the constriction in both groups of vessels. DOPA had no effect on flow in unstimulated preparations (Fig. 6). The possibility that DOPA potentiated the effects of noradrenaline was ruled out by the observation that dose-response curves to this amine (0·06 to 3·0 μM), added extraluminally, first in the absence and then in the presence of DOPA (50 μM) for 30 minutes, did not differ materially.

Effect of inhibition of noradrenaline uptake on the maintenance of sympathetic transmission during continuous nerve stimulation

Frequency–response curves were obtained before and 15 min after the addition of cocaine (29 μM) to the Krebs solution bathing the extraluminal surface of the ear artery. The results of experiments with 8 pairs of cocaine treated arteries and their contralateral controls are presented in Figure 7. The frequency–response curve was shifted significantly by cocaine (29 μM) indicating a sensitization to the effects of endogenously released amine. For example, stimulation at 2 Hz after cocaine produced a response about equivalent to that seen at 3 Hz before cocaine.

The arteries treated with cocaine, which was kept in the muscle chambers for the duration of the experiment, and their contralateral controls were then stimulated continuously at a frequency of 5 Hz. The initial peak effect was a reduction in intraluminal flow of $99{\cdot}6 \pm 0{\cdot}8\%$ and $99{\cdot}0 \pm 0{\cdot}4\%$, respectively. The period required for the vasoconstrictor response to decay such that intraluminal

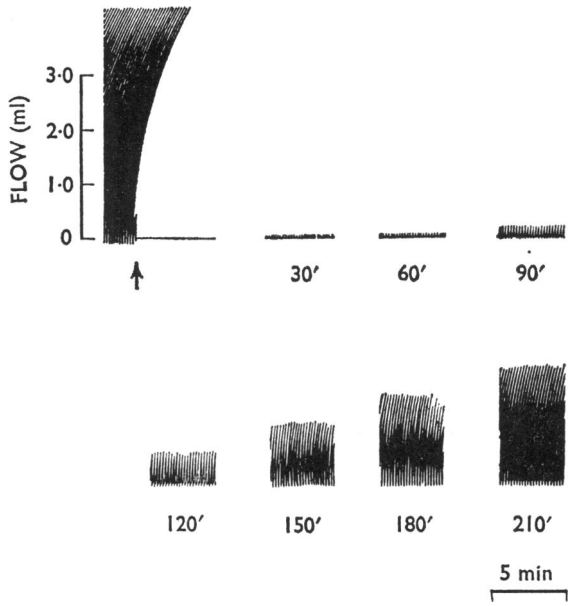

FIG. 5. Decline of the vasoconstrictor response of the central artery of the rabbit ear during a prolonged period of continuous stimulation at 5 Hz. The times indicated are minutes after the start of continuous stimulation, which is shown by an arrow.

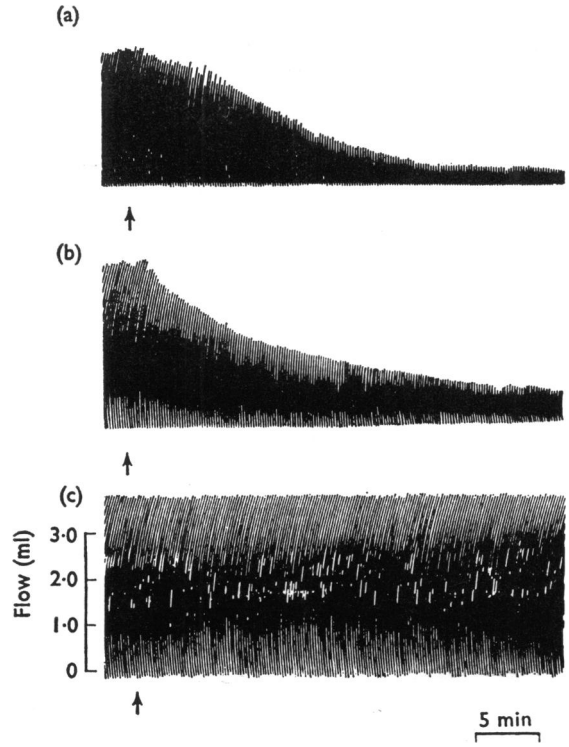

FIG. 6. Effects of DOPA on intraluminal flow in the central artery of the rabbit ear. DOPA (50 μM) was added (arrow) at a late stage during continuous stimulation at 5 Hz in an artery treated with α-methyl-*p*-tyrosine (100 μM) (a) and in an untreated artery (b). (c) Lack of effect of DOPA on intraluminal flow in the absence of nerve stimulation.

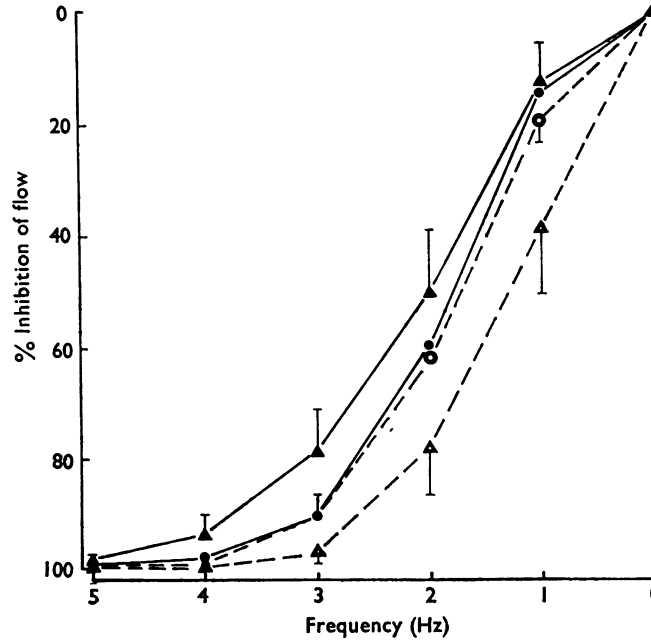

FIG. 7. Frequency–response curves of the central artery of the rabbit ear in the absence and in the presence of cocaine. First (●——●) and second (○- - -○) frequency–response curves in 8 control preparations; in the absence (▲——▲) and then in the presence (△- - -△) of cocaine (2·9 μM) for 15 min in 8 preparations from the contralateral ears. Stimulation was for about one minute at each test frequency consecutively, beginning with the highest (5 Hz). The interval between curves was 15 minutes. Mean values are shown with standard error bars wherever they do not reduce clarity. First frequency curve in the absence of cocaine vs second frequency curve of the same preparations in the presence of cocaine at 3, 2 and 1 Hz $P<0{\cdot}02$, $P<0{\cdot}01$, $P<0{\cdot}01$, by the t test for paired data.

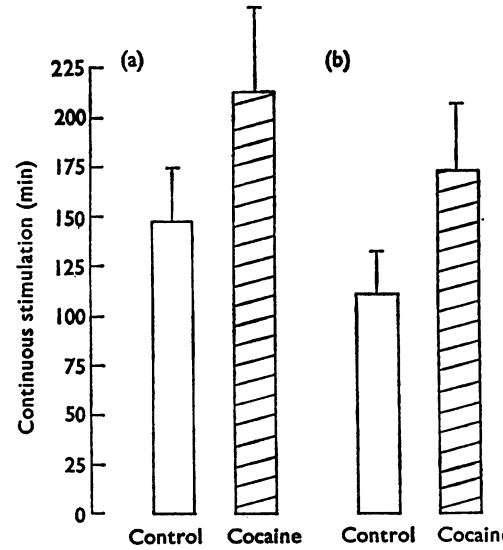

FIG. 8. Effects of cocaine on the maintenance of responses to continuous stimulation at 5 Hz in the central artery of the rabbit ear. a, Mean period of continuous stimulation required for intraluminal flow to increase to 50% of pre-stimulation values, calculated individually in 8 cocaine (29 μM)-treated preparations and their contralateral controls; b, mean period of continuous stimulation required for the vasoconstrictor response to decline (intraluminal flow to increase) to the equivalent of that produced initially at 2 Hz in 8 cocaine-treated preparations and their contralateral controls. Mean values are shown with their standard errors. In (a) and (b) the times for the cocaine-treated group were greater than controls, but not significantly so. In (a) control vs treated group $P<0{\cdot}3>0{\cdot}2$ and $P<0{\cdot}2>0{\cdot}1$ by t test for unpaired and paired data, respectively. In (b) control vs treated group $P<0{\cdot}2>0{\cdot}1$ by t test for either unpaired or paired data.

flow reached 50% of the values recorded in the same preparations with no stimulation, did not differ significantly in control vessels and in those in which uptake was inhibited with cocaine (Fig. 8).

A more accurate assessment of the effects of cocaine required that the sensitization produced by the compound be nullified as a possible factor influencing the results. This was done by expressing the data in terms of the time required for the response of each vessel to decay during continuous stimulation to the equivalent of that observed initially in the second frequency–response curve at 2 Hz (Fig. 7). In the case of the cocaine-treated vessels the second frequency-response curve was obtained in the presence of cocaine. The corresponding times for control and cocaine-treated vessels were $112{\cdot}1 \pm 22{\cdot}2$ min and $173{\cdot}0 \pm 35{\cdot}4$ min, respectively (Fig. 8). These values did not differ significantly when determined by the paired or unpaired t test, and in fact they tended to be greater in the treated group.

Discussion

That synthesis of noradrenaline from tyrosine plays an important role in the maintenance of transmitter release during stimulation at high frequencies has been emphasized recently by several groups of workers (Alousi & Weiner, 1966; Kopin, Breese, Krauss & Weise, 1968; Weiner & Rabadjija, 1968; Kupferman, Gillis & Roth, 1970). However, it is generally considered that at physiological rates of stimulation (no greater than 10 Hz) uptake and re-use of liberated transmitter is the dominant factor supporting transmission and thus the maintenance of the effector response (Blakeley & Brown, 1966; Geffen, 1967; Kopin *et al.*, 1968; Hedqvist & Stjärne, 1969; Kupferman *et al.*, 1970; Su & Bevan, 1970; Iversen, 1971). For example, Su & Bevan suggested that about 80% of the noradrenaline released by stimulation of the rabbit pulmonary artery at 10 Hz is taken up again by the sympathetic nerves. They raised the possibility that 'endogenous norepinephrine after release is normally sequestered and released once more without delay upon subsequent nerve excitation'.

In the experiments reported here the relative importance of synthesis and of uptake and re-use of transmitter during a prolonged period of continuous stimulation at the moderate frequency of 5 Hz was examined in the central artery of the rabbit ear. The gradual failure of the vasoconstrictor response, during several hours of continuous stimulation, appeared to be of presynaptic origin since responses to exogenous noradrenaline were of similar magnitude before stimulation and at a stage when the nerve-induced vasoconstriction was markedly attenuated. Evidence that a deficit in noradrenaline available for release was involved in the deterioration of the response was the finding that the noradrenaline precursor, DOPA, restored the response towards normal.

Failure of the vasoconstrictor response developed in about half the time recorded for control vessels when the synthesis of noradrenaline from tyrosine was inhibited with α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase (Udenfriend, Zaltzman-Nirenberg & Nagatsu, 1965). The addition of DOPA to the muscle chambers when the magnitude of the response was markedly diminished, restored the constriction towards original values confirming that failure involved a lack of noradrenaline for release. Despite the marked effect of α-methyl-*p*-tyrosine, it is un-

likely for several reasons that the conditions used allowed for a complete inhibition of noradrenaline synthesis. Kupferman *et al.* (1970) found that the synthesis of ^{3}H-noradrenaline from ^{3}H-tyrosine in the rabbit pulmonary artery was inhibited only about 50% by a 100 μM concentration of α-methyl-*p*-tyrosine. A higher concentration of the inhibitor could not be used in the present experiments because of its limited solubility in Krebs solution. In addition, the endogenous levels of DOPA and dopamine at the pertinent tissue sites and the way in which they might be mobilized for noradrenaline synthesis when tyrosine hydroxylase is inhibited are not known at present.

A surprising finding was that block of the recapture of noradrenaline by inhibition of the membrane transport system did not accelerate the decline of the response. Cocaine (29 μM) is reported to produce a virtually complete inhibition of noradrenaline uptake (Iversen, 1963), and it clearly enhances the responses of the central ear artery to sympathetic nerve stimulation in the present experiments. The use of a system in these experiments which maintained perfusion pressure constant, rather than outflow, permitted the vessel to constrict and intraluminal flow to decrease drastically during nerve stimulation. Thus, the released amine was probably trapped to a considerable extent in the vicinity of the tissue, and the conditions for uptake should have been optimal. The absence of a significant effect of cocaine indicates that uptake and re-use makes little contribution to the maintenance of the response under the conditions of these experiments.

The possibility was considered that the rate of noradrenaline synthesis is increased during blockade of uptake. However, this could not explain the lack of effect of cocaine. As was shown above, the eventual failure of response in untreated (control) vessels is due to an apparent exhaustion of endogenous noradrenaline and of precursor material for the synthesis of new mediator. If, to take an example, 50% of the noradrenaline released is normally taken up again by nerves and re-used, then, in the presence of cocaine synthesis would have to double to compensate for the lost amine. Thus, the limited supply of substrate for transmitter synthesis in the severed off nerve endings would be exhausted, and failure would occur in half the time of untreated vessels. However, no indication of premature failure was observed in the cocaine-treated vessels and, in fact, they tended to maintain the response for a longer period than did control vessels.

It is also possible that amine which is taken up is stored in a compartment from which it is not available for release during the time course of these experiments (about 3 h). However, an alternate and probably more satisfactory interpretation of the data is that effector cell mechanisms, rather than presynaptic uptake, dominate in terminating the action of noradrenaline in vascular tissue. It was previously proposed that the major mechanism terminating the action of noradrenaline in rabbit aorta is penetration of effector cell membranes followed by enzymatic inactivation (Kalsner & Nickerson, 1969a, b, c ; Kalsner, 1971a, b). Also, evidence has been presented that the sensitization of responses to sympathetic amines by cocaine, which has been used as strong support for the importance of uptake, involves postsynaptic mechanisms (Kalsner & Nickerson, 1969b, c).

Malmfors (1969), using fluorescence microscopy to determine catecholamine levels, reported that inhibition of noradrenaline synthesis produced a much greater depletion of the transmitter in the rat iris stimulated at 10 Hz for 40–80 min than did inhibition of uptake. The present experiments in which the response, rather

than noradrenaline content, was monitored demonstrate that synthesis of mediator, probably along with the mobilization of stored amine, rather than uptake and re-use, maintain sympathetic transmission in the central artery of the rabbit ear.

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REFERENCES

ALOUSI, A. & WEINER, N. (1966). The regulation of norepinephrine synthesis in sympathetic nerves, effect of nerve stimulation, cocaine and catecholamine-releasing agents. *Proc. Nat. Acad. Sci. U.S.A.*, **56**, 1491–1496.

ANDREWS, W. H. H. (1952). A blood outflow recorder. *J. Physiol.*, **117**, 45*P*–46*P*.

BHAGAT, B. & ZEIDMAN, H. (1970). Increased retention of norepinephrine-^{3}H in vas deferens during nerve stimulation. *Am. J. Physiol.*, **219**, 691–696.

BLAKELEY, A. G. H. & BROWN, G. L. (1966). Release and turnover of the adrenergic transmitter: In: *Mechanisms of Release of Biogenic Amines*, ed. U. S. von Euler, S. Rosell & B. Uvnäs. pp. 185–188. Oxford: Pergamon Press.

BROWN, L. (1965). The release and fate of the transmitter liberated by adrenergic nerves. *Proc. Roy. Soc. Lond.*, Series B, **162**, 1–19.

CASS, R. & SPRIGGS, T. L. B. (1961). Tissue amine levels and sympathetic blockade after guanethidine and bretylium. *Br. J. Pharmac. Chemother.*, **17**, 442–450.

CELANDER, O. & FOLKOW, B. (1953). A comparison of the sympathetic vasomotor fibre control of the vessels within the skin and the muscles. *Acta physiol. scand.*, **29**, 241–250.

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. Med. Sci.*, **43**, 639–656.

DE LA LANDE, I. S., FREWIN, D. & WATERSON, G. (1967). The influence of sympathetic innervation on vascular sensitivity to noradrenaline. *Br. J. Pharmac.*, **31**, 82–93.

DENGLER, H. J., SPIEGEL, H. E. & TITUS, E. O. (1961). Effects of drugs on uptake of isotopic norepinephrine by cat tissues. *Nature, Lond.*, **191**, 816–817.

GEFFEN, L. B. (1967). Noradrenaline storage, release and inactivation in sympathetic nerves. *Circulation Res.*, **21**, suppl. III, 57–61.

HEDQVIST, P. & STJÄRNE, L. (1969). The relative role of recapture and of *de novo* synthesis for the maintenance of neurotransmitter homeostasis in noradrenergic nerves. *Acta physiol. scand.*, **76**, 270–283.

HERTTING, G., AXELROD, J. & WHITBY, L. G. (1961). Effect of drugs on the uptake and metabolism of H^3-norepinephrine. *J. Pharmac. exp. Ther.*, **134**, 146–153.

IVERSEN, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523–537.

IVERSEN, L. L. (1965). The inhibition of noradrenaline uptake by drugs. In: *Advances in Drug Research*, ed. N. J. Harper & A. B. Simmonds, Vol. 2, pp. 1–46. London: Academic Press.

IVERSEN, L. L. (1971). Role of transmitter uptake mechanisms in synaptic neurotransmission. *Br. J. Pharmac.*, **41**, 571–591.

KALSNER, S. (1971a). The importance of the uptake mechanism of adrenergic nerves in blood vessels. In: *Physiology and Pharmacology of Vascular Neuroeffector Systems*, ed. J. A. Bevan, R. F. Furchgott, R. A. Maxwell & A. P. Somlyo, pp. 126–129. Basel: S. Karger.

KALSNER, S. (1971b). The mechanism of potentiation of contractile responses to catecholamines by methylxanthines in aortic strips. *Br. J. Pharmac.*, **43**, 379–388.

KALSNER, S. & NICKERSON, M. (1969a). Disposition of norepinephrine and epinephrine in vascular tissue, determined by the technique of oil immersion. *J. Pharmac. exp. Ther.*, **165**, 152–165.

KALSNER, S. & NICKERSON, M. (1969b). Mechanism of cocaine potentiation of responses to amines. *Br. J. Pharmac.*, **35**, 428–439.

KALSNER, S. & NICKERSON, M. (1969c). Effects of a haloalkylamine on responses to and disposition of sympathomimetic amines. *Br. J. Pharmac.*, **35**, 440–455.

KOPIN, I. J., BREESE, G. R., KRAUSS, K. R. & WEISE, V. K. (1968). Selective release of newly synthesized norepinephrine from the cat spleen during sympathetic nerve stimulation. *J. Pharmac. exp. Ther.*, **161**, 271–278.

KUPFERMAN, A., GILLIS, C. N. & ROTH, R. H. (1970). Influence of sympathetic nerve stimulation on conversion of H^3-tyrosine to H^3-catecholamine and on H^3-norepinephrine disposition in rabbit pulmonary artery. *J. Pharmac. exp. Ther.*, **171**, 214–222.

MALMFORS, T. (1965). Studies on adrenergic nerves. The use of rat and mouse iris for direct observations on their physiology and pharmacology at cellular and subcellular levels. *Acta physiol. scand.*, **64**, suppl. 248, 1–95.

MALMFORS, T. (1969). Histochemical studies on release of the adrenergic transmitter by nerve impulses in combination with drugs, especially adrenergic neuron blocking agents. *Pharmacol.*, **2**, 138–150.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Br. J. Pharmac. Chemother.*, **16**, 352–359.

ROSELL, S., KOPIN, I. J. & AXELROD, J. (1963). Fate of H^3-noradrenaline in skeletal muscle before and following sympathetic stimulation. *Am. J. Physiol.*, **205**, 317–321.

SU, C. & BEVAN, J. A. (1970). The release of H^3-norepinephrine in arterial strips studied by the technique of superfusion and transmural stimulation. *J. Pharmac. exp. Ther.*, **172**, 62–68.

TRENDELENBURG, U. (1966). Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **18**, 629–640.

UDENFRIEND, S., ZALTZMAN-NIRENBERG, P. & NAGATSU, T. (1965). Inhibitors of purified beef adrenal tyrosine hydroxylase. *Biochem. Pharmac.*, **14**, 837–845.

WEINER, N. & RABADJIJA, M. (1968). The effect of nerve stimulation on the synthesis and metabolism of norepinephrine in the isolated guinea-pig hypogastric nerve–vas deferens preparation. *J. Pharmac. exp. Ther.*, **160**, 61–71.

WHITBY, L. G., HERTTING, G. & AXELROD, J. (1960). Effect of cocaine on the disposition of noradrenaline labelled with tritium. *Nature, Lond.*, **187**, 604–605.

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