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THE EFFECTS OF RELEASE AND DEPLETION OF ENDOGENOUS NORADRENALINE ON THE TRANSMISSION OF IMPULSES IN THE MOUSE VAS DEFERENS

I. MARSHALL, P.A. NASMYTH & N.B. SHEPPERSON

The Department of Biochemical and Experimental Pharmacology, St Mary's Hospital Medical School, Paddington, London W2

1 The effects of endogenous noradrenaline released by tyramine and the influence of depletion of the tissue noradrenaline with reserpine and/or α-methyl-*p*-tyrosine on the twitch responses of the field-stimulated mouse vas deferens have been studied.

2 Tyramine (10–40 μM) inhibited the twitch responses to field stimulation and failed to produce a contraction. The inhibition decreased as the rate of stimulation increased.

3 The inhibition produced by tyramine was antagonized by cocaine (10 μM) and by yohimbine (10 nM), which indicated that it was produced by released noradrenaline acting on presynaptic α-adrenoceptors.

4 Depletion of the tissue noradrenaline by 39% by blockade of the synthesis of noradrenaline with α-methyl-*p*-tyrosine, was without effect on the twitch response but it reduced the inhibitory effect of tyramine.

5 Depletion of the tissue noradrenaline by 96.5% with reserpine alone and by 99.4%, with a combination of reserpine and α-methyl-*p*-tyrosine, reduced the twitch responses by approximately 66% and virtually abolished the inhibition produced by tyramine. It also increased the rate of decline of the responses when the tissue was continuously stimulated. The remaining twitch was not antagonized by phenoxybenzamine (15 μM).

6 Residual twitches were bigger in tissues depleted by 99.4% than in those depleted by only 96.5%. This difference was eliminated in the presence of yohimbine (128 nM).

7 It is concluded that inhibition of the twitch responses by tyramine is produced by stimulation of presynaptic α-adrenoceptors and that the twitch response is associated with stimulation of the sympathetic neurone, but that it is not mediated by postsynaptic α-adrenoceptors.

Introduction

Ambache & Zar (1971) were the first to suggest that noradrenaline was not the motor transmitter in the guinea-pig vas deferens and their suggestion was supported by von Euler & Hedqvist (1975). The experiments of Jenkins, Marshall & Nasmyth (1976; 1977) indicated that noradrenaline had an inhibitory rather than a motor role in the mouse vas deferens. However, Jones & Spriggs (1975) using different stimulus parameters, concluded that noradrenaline was the motor transmitter in this species.

In 1972, Ambache, Dunk, Verney & Zar examined the effect of releasing endogenous noradrenaline with tyramine in the vasa of guinea-pigs, rats and rabbits, and found that this inhibited the twitch responses to field stimulation in all three species, but in the rat vas only, the drug was capable of causing a contraction. Both the inhibitory effect of tyramine on the twitch response in all three species and its motor effect in the rat vas was blocked by phenoxybenzamine or phentolamine. Depletion of the noradrenaline content of the tissue by pretreatment with reserpine blocked the contraction to tyramine in the rat vas, but only reduced the twitch responses. It seemed possible that the twitch responses were only reduced because the synthesis of noradrenaline could still take place and that this synthesis was sufficient to maintain a reduced response. With this possibility in mind, the experiments of Ambache *et al.*, (1972) have been repeated in the mouse vas and, additionally, noradrenaline synthesis has been blocked with α-methyl-*p*-

tyrosine. A preliminary account of some of these results has appeared elsewhere (Marshall, Nasmyth & Shepperson, 1977).

Methods

Isolation and stimulation of the vas deferens

Male T.O. strain mice (20 to 30 g) were killed by a blow on the head and exsanguinated. The whole of the vas deferens was excised and placed in a Petri dish containing Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaH_2PO_42H_2O$, 1.2, $NaHCO_3$ 25.0 and glucose 11.0. Magnesium was omitted because, in agreement with Hughes, Kosterlitz & Leslie (1975), it was easier to obtain larger responses in its absence. The vas was dissected under a magnifying glass, freed from blood vessels and all mesenteric connections and then suspended in a 2.0 ml organ bath between gutter electrodes as described by Birmingham & Wilson (1963) except that the distance between the electrodes was approximately 6.0 mm. A tension of 500 mg was applied to the tissue and field stimulation was provided by a Grass S48 stimulator at 64 V, which was the highest voltage that could be applied to the tissue without causing overload cut-out. The frequency, duration of stimulation and the pulse width employed were varied as indicated in the text. The responses obtained with all the combinations of stimulus parameters used were abolished by tetrodotoxin (0.63 μM) indicating that they were mediated only by the stimulation of nerve fibres. The responses were recorded isometrically on a Grass polygraph via a Grass FT03 transducer. In measurements of twitch responses, a mean of three observations in each of at least four different tissues is reported in each case. Where necessary, significance of difference was tested by Student's *t* test and the number of tissues, not the number of observations was used as the value for *n*.

Blockade of noradrenaline synthesis and depletion of noradrenaline stores

To block the activity of tyrosine hydroxylase, which is the rate limiting enzyme in the synthesis of noradrenaline (Levitt, Spector, Sjoerdsma & Udenfriend, 1965), L-α-methyl-*p*-tyrosine (200 mg/kg) was given intraperitoneally 4, 2 and 1 h before the animals were killed.

To deplete the tissue stores of noradrenaline, reserpine (5 mg/kg s.c.) was given 48 h and (2.5 mg/kg i.p.) 24 h before the animals were killed. In some animals the treatment with reserpine and α-methyl-*p*-tyrosine was combined.

Estimation of tissue noradrenaline

The procedure employed was a modification of the method of Henry, Starman, Johnson & Williams (1975) in which tissue noradrenaline is converted to radioactive adrenaline. This procedure made possible the measurement of the very small amounts of noradrenaline that could be obtained from the diminutive vas deferens of the mouse. The method differed from that of Henry, Starman, Johnson & Williams in that the samples were incubated for 40 min and the reaction was stopped by the addition of 2.0 ml of 2 M Tris buffer (pH 8.6). Phenylethanolamine *N*-methyl transferase was prepared by the method of Axelrod (1962). *S*-adenosyl methionine was supplied by the Radio Chemical Centre Amersham (specific activity 1 mCi/ml, 25 mCi/mg).

The radioactive adrenaline formed from the tissue noradrenaline in the reaction was counted by scintillation spectroscopy in a Packard 3300 counter for 10 min. A set of noradrenaline standards was included in each assay. Results are expressed as the amount of noradrenaline per g of tissue in each of at least 14 animals.

Drugs

The following drugs were used: cocaine hydrochloride; L-α-methyl-*p*-tyrosine hydrochloride (Aldrich); phenoxybenzamine hydrochloride (Dibenyline, Smith Kline & French) tyramine hydrochloride (Sigma) and yohimbine hydrochloride (Sigma).

The reserpine was prepared for injection as described in Martindale, Extra Pharmacopoeia 26th ed. p. 813, except that glycol 400 was substituted for polysorbate 80.

Drugs were added to the bath with a micrometer syringe in volumes which never exceeded 0.05 ml. The figures in the text represent the final concentrations in the bath.

Results

Effects of tyramine on the mouse vas deferens

Concentrations of tyramine up to 40 μM produced no contraction of the mouse vas deferens. However, at half this concentration, the twitch obtained in response to field stimulation (2.0 ms, 0.2 Hz) was inhibited by 68% by the sixth pulse, the first stimulus being applied 30 s after the addition of tyramine to the bath. The inhibition of the response became progressively less as the frequency of stimulation increased, until, at 16 Hz, it was only 2.0% (Figure 1). At frequencies of stimulation in excess of 1.0 Hz, the twitches were no longer discrete and so the effects

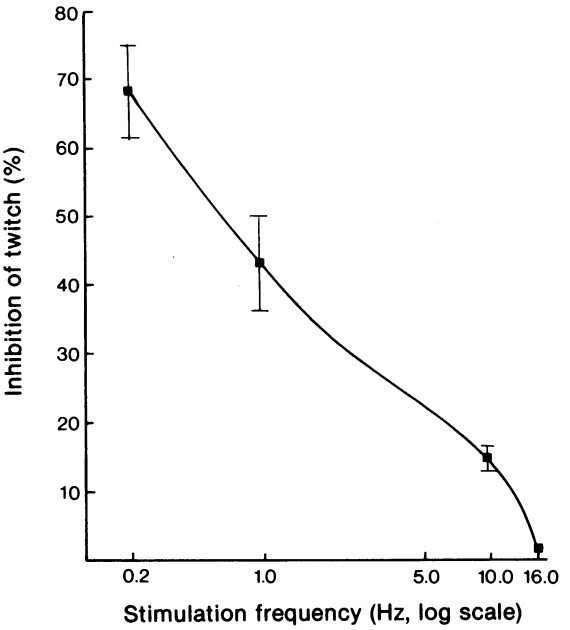

Figure 1 The inhibitory effect of tyramine (20 μM) on the twitch response of the field-stimulated mouse vas deferens at different frequencies of stimulation. Where no standard error is shown, it is contained within the point (n = 4 in all cases).

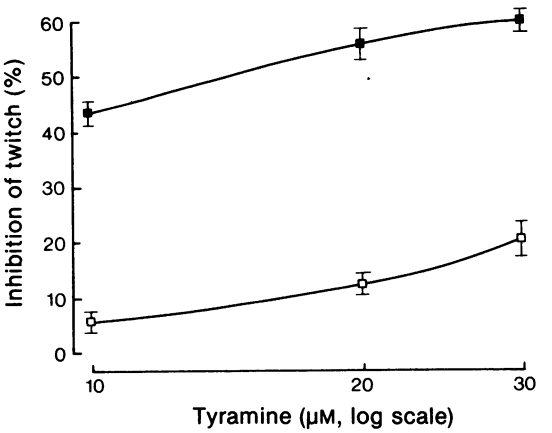

Figure 2 Antagonism by cocaine (10 μM) of the inhibitory effect of tyramine on the twitch response of the field-stimulated mouse vas deferens. Tyramine alone (■), n = 4; tyramine in the presence of cocaine (□), n = 4.

of tyramine were measured when the 'fused twitches' reached a maximum. This occurred within 50 pulses at 5.0 Hz and within 120 pulses at 10 and 16 Hz. The control response from which the inhibition was calculated was determined by using the same stimulus parameters in the absence of tyramine.

With a constant frequency of 0.2 Hz and a pulse width of 2.0 ms, which were the parameters which gave the greatest inhibition in the experiment described above, tyramine produced an increasing inhibition as its concentration in the bath was raised from 10 to 30 μM. The addition of cocaine (10 μM) to the Krebs solution bathing the tissue shifted the dose-response curve far to the right (Figure 2) and the inhibitory effect was reduced. When yohimbine (10 nM) was added to the bath 2 min before tyramine the inhibitory response to the latter drug was shifted to the right by one log unit (Figure 3).

Depletion of tissue noradrenaline

The noradrenaline content of the mouse vas deferens was found to be 9.95 ± 0.4 μg/g (n = 18) and that of the heart 0.41 ± 0.03 μg/g (n = 6). Treatment with α-methyl-*p*-tyrosine to antagonize noradrenaline synthesis by inhibition of tyrosine hydroxylase, reduced the noradrenaline content of the vas to 6.1 ± 0.5 μg/g (n = 19) and that of the heart to 0.27 ± 0.03 μg/g (n = 9). Reserpine pretreatment reduced the noradrenaline content of the vas deferens to 0.35 ± 0.03 μg/g (n = 15) and the combination of reserpine and α-methyl-*p*-tyrosine treatment reduced the content further to 0.06 ± 0.01 μg/g (n = 14). The effect of this combined treatment on the noradrenaline content of the heart was to reduce it to an unmeasurable amount (<0.02 μg/g) because the volume of perchloric acid needed to extract it was relatively large.

Effect of noradrenaline-depletion on twitch responses of the mouse vas

The reduction of the noradrenaline content of the vas to 61.3% of normal, by inhibition of noradrenaline synthesis with α-methyl-*p*-tyrosine, had no significant effect on the response to electrical stimulation (64 V, at pulse widths varying from 0.25 to 2.0 ms) (Figure 4). The reduction in the tissue noradrenaline content to 3.5 ± 0.06% of normal by pretreatment with reserpine, or to an even greater extent by pretreatment with reserpine and α-methyl-*p*-tyrosine, diminished the twitch response by approximately 66%. Curiously, however, at the higher pulse widths of 1.5 and 2.0 ms, the combination of reserpine and α-methyl-*p*-tyrosine appeared to be less effective than reserpine alone in diminishing the twitch response, even though it produced a greater depletion of the tissue noradrenaline. The difference between treatments was not significant at 1.5 ms, but was at 2.0 ms (P < 0.05). When yohimbine (128 nM) was added to the bath 2 min before stimulation started, the responses of the tissues

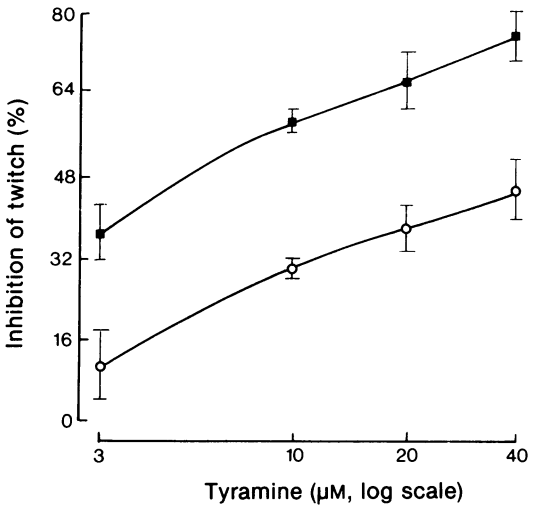

Figure 3. The antagonism by yohimbine (10 nM) of the inhibitory effect of tyramine on the twitch response of the field-stimulated mouse vas deferens. Tyramine alone (■) $n = 4$; tyramine in the presence of yohimbine (○) $n = 4$.

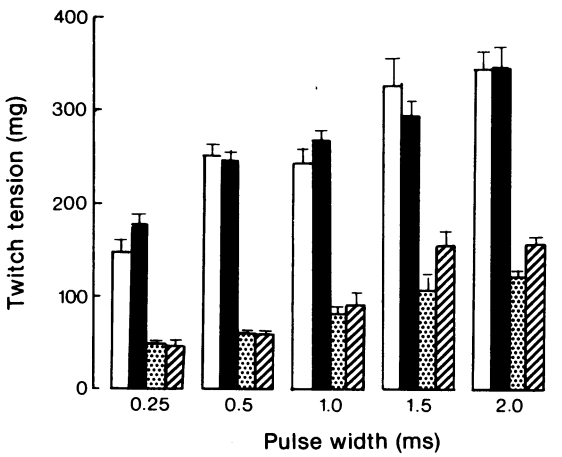

Figure 4 Reduction of the twitch response to field stimulation of the mouse vas deferens (ordinate scale) by depletion of the tissue noradrenaline. Open columns: control ($n = 10$); solid columns: α-methyl-*p*-tyrosine ($n = 12$); stippled columns: reserpine ($n = 10$); hatched columns: reserpine plus α-methyl-*p*-tyrosine ($n = 12$). Tissue noradrenaline was depleted by 38.7% by pretreatment with α-methyl-*p*-tyrosine alone, by 96.5% by pretreatment with reserpine alone and by 99.4% by reserpine and α-methyl-*p*-tyrosine combined. Note that at pulse widths above 1.0 ms the reduction was less in the more completely depleted tissues. The difference was significant ($P < 0.05$) when a 2.0 ms pulse width was used.

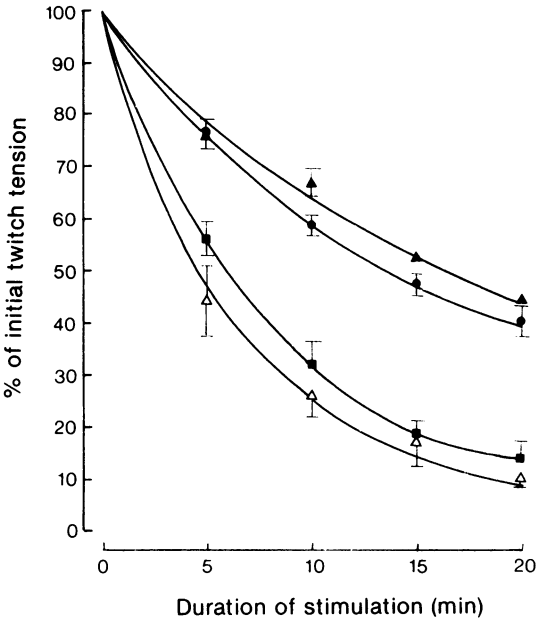

Figure 5 The influence of depletion of the noradrenaline content of the mouse vas deferens on the rate of decline of the twitch response to field stimulation when the tissue was stimulated continuously for 20 min (64 V, 2.0 ms, 0.1 Hz). Controls (normal noradrenaline) (●), $n = 9$; α-methyl-*p*-tyrosine pretreated (noradrenaline 61.3% of normal) (▲), $n = 6$; reserpine pretreatment (noradrenaline 3.5% of normal) (△), $n = 4$; reserpine plus α-methyl-*p*-tyrosine pretreatment (noradrenaline 0.6% of normal) (■), $n = 9$. Where no standard error is shown, it is contained within the point.

from animals treated with reserpine alone were potentiated, whilst those of tissues from animals treated additionally with α-methyl-*p*-tyrosine were not affected. Thus yohimbine eliminated the difference in the responses between these two types of noradrenaline-depleted tissues.

When the vas was stimulated continuously for 20 min (64 V, 2.0 ms, 0.1 Hz) there was a gradual decline in the tension developed at each stimulus (Figure 5). The rate of decline was initially faster in vasa from animals pretreated with reserpine, or α-methyl-*p*-tyrosine plus reserpine, but after 5 min the rate of decline paralleled that in control vasa. The rate of decline in the response of continuously stimulated vasa was unaffected by pretreatment with α-methyl-*p*-tyrosine alone and as in the experiments which measured the effect of noradrenaline depletion on twitch tension at various pulse widths, the tension developed did not differ significantly from the controls. In those treated with reserpine and α-methyl-*p*-tyrosine however, the twitch tension was reduced as before and though the

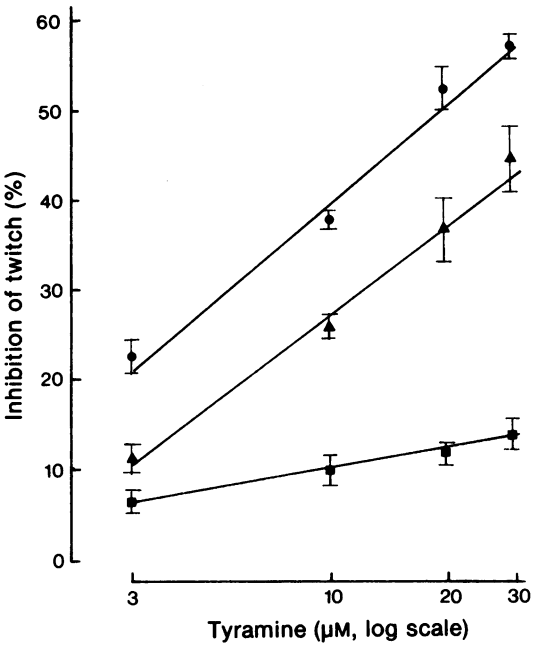

Figure 6 The influence of depletion of the noradrenaline content of the mouse vas deferens on the inhibition by tyramine of the twitch response to field stimulation. Control (no depletion) (●), $n = 8$; α-methyl-*p*-tyrosine pretreated (noradrenaline 61.3% of normal) (▲), $n = 4$; reserpine plus α-methyl-*p*-tyrosine pretreated (noradrenaline 0.6% of normal) (■), $n = 4$.

same trend for the diminution to be less in those treated with reserpine and α-methyl-*p*-tyrosine together than in those treated with reserpine alone was observed, in no instance was it significant in these experiments.

The diminished twitches and their increased rate of decline in the first 5 min of continuous stimulation were unaffected by the α-adrenoceptor blocking agent phenoxybenzamine, even at the high concentration of 15 μM.

Tissue noradrenaline depletion and the effects of tyramine

Depletion of the tissue noradrenaline with α-methyl-*p*-tyrosine alone caused a significant shift in the inhibitory response curve to tyramine ($P < 0.02$), but it remained parallel to the control curve (Figure 6). The figure also shows that depletion with reserpine plus α-methyl-*p*-tyrosine almost abolished the inhibitory effect of tyramine ($P < 0.001$) so that the curve was no longer parallel with the control. The effect of depletion with reserpine alone was identical with that obtained with reserpine plus α-methyl-*p*-tyrosine and is not therefore shown in the figure.

Discussion

In an endeavour to resolve the controversy surrounding the role of noradrenaline as the motor transmitter in the mouse vas deferens, two approaches have been made. The first was to determine the effect of tyramine on the twitch response to field stimulation since its effects are produced by releasing noradrenaline (Burn & Rand 1958; 1960). The second was to deplete the tissue of noradrenaline, determine the effect of this procedure on the response of the tissue to stimulation of its nerve fibres and observe the influence of the procedure upon the previously established effects of tyramine.

In agreement with the observations of Ambache *et al.* (1972) in the vasa of guinea-pigs and rabbits, tyramine produced no contraction of the tissue in doses up to 40 μM. In much lower doses it inhibited the responses to field stimulation. That this inhibition was due to released noradrenaline seemed very likely, since it was reduced considerably when uptake into the neurone was inhibited by cocaine. The inhibition was practically abolished by depletion of the tissue noradrenaline with either reserpine or reserpine plus α-methyl-*p*-tyrosine. That the inhibition produced by tyramine was due to stimulation of presynaptic α-adrenoceptors is very likely since it was antagonized by yohimbine, which antagonizes these receptors at much lower concentrations than are required to antagonize actions at postsynaptic α-adrenoceptors (Starke, Borowski & Endo, 1975; Marshall, Nasmyth, Nicholl & Shepperson, 1978). Also typical of the inhibition of the output of transmitter produced by stimulation of presynaptic α-adrenoceptors was the fact that the inhibition was frequency-dependent, being greatest at low and least at high frequencies of stimulation. It has been established then, by the first approach, the tyramine produces its effect almost certainly by releasing endogenous noradrenaline. The effect of released noradrenaline is inhibitory; it does not produce a contraction, at least in the concentration which can be achieved by the action of tyramine. These effects agree with those observed for exogenous noradrenaline in the mouse vas (Jenkins *et al.*, 1976; 1977).

Inhibition of the synthesis of noradrenaline by pretreating the animals with α-methyl-*p*-tyrosine only, reduced the noradrenaline content of the tissue by 38.7% in the vas and by 33.4% in the heart. This had no significant effect on the twitch response, but significantly antagonized the inhibitory effect of tyramine. Using reserpine alone, Sjöstrand & Swedin (1968) and Gillespie & McGrath (1974) showed that

it was more difficult to deplete noradrenaline in the vas deferens than in the heart though doses of reserpine higher than 1.0 mg/kg were usually effective in both tissues. Sjöstrand & Swedin (1968) thought that this could be due to the properties of the short adrenergic neurones which supply the vas deferens, or less likely to poor circulation to the organ. Gillespie & McGrath (1974) showed that it was more likely to be due to much less frequent stimulation of the vas than the heart in the intact reserpine-treated animals, since stimulation of the sympathetic nerves to the vas, after reserpine treatment increased the depleting effect of the drug. Therefore, to ensure that the maximum possible depletion of the noradrenaline in the vas was obtained in these experiments, a high dose of reserpine was used and the extent of the depletion of noradrenaline in the heart was measured for comparison. Pretreatment with a total of 7.5 mg/kg of reserpine reduced the noradrenaline content of the vas by 96.5%. This reduction was comparable with that obtained in the heart though the amount present in the heart was so much less and the volume of perchloric acid needed to extract it so much more that an accurate measurement of the residual noradrenaline was not possible after reserpine. The combination of reserpine and α-methyl-*p*-tyrosine reduced the noradrenaline content of the vas by 99.4%.

The severe depletion of the tissue noradrenaline reduced the twitch response to field stimulation by 66% and increased the rate at which the twitches diminished when the tissue was continuously stimulated at 0.1 Hz for 20 min. Both of these phenomena seem to link the effect of the transmitter released to the sympathetic neurone and to its noradrenaline content. The reduction in the twitch response is consistent with the observations of Lee (1967), who could detect no decrease in the responses of other sympathetically innervated tissues unless they were depleted by more than 50%.

The reduction in the height of the twitch is also similar to the results reported by Wakade & Krusz (1972) using vasa from reserpine-treated guinea-pigs in which, as in these experiments, the residual response could not be blocked by high concentrations of phenoxybenzamine.

It was curious that vasa in which the noradrenaline content was depleted by 99.4% after pretreatment with reserpine and α-methyl-*p*-tyrosine, gave significantly larger twitch responses when stimulated at a pulse width of 2.0 ms than tissues depleted by only 96.5% with reserpine alone. However, when the presynaptic α-adrenoceptors were blocked by yohimbine the difference disappeared. Thus, when the tissue was depleted by reserpine alone, enough noradrenaline was still released to stimulate presynaptic α-adrenoceptors and inhibit the twitch response. The yohimbine did not increase the responses in the tissues from animals treated with reserpine and α-methyl-*p*-tyrosine, showing that insufficient noradrenaline was released to stimulate the presynaptic α-adrenoceptors when depletion reached 99.4%.

It is concluded that tyramine inhibits the twitch responses of the mouse vas deferens by stimulating presynaptic α-adrenoceptors via released endogenous noradrenaline which does not reach high enough concentrations to contract the tissue. The twitch response itself is associated with stimulation of the sympathetic neurone, but failure of phenoxybenzamine to block it indicates that it is not mediated by postsynaptic α-adrenoceptors.

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References

AMBACHE, N., DUNK, LINDA P., VERNEY, J. & ZAR, M. ABOO (1972). Inhibition of postganglionic motor transmission in vas deferens by indirectly acting sympathomimetic drugs. *J. Physiol.*, **227**, 433–456.

AMBACHE, N. & ZAR, M. ABOO (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.*, **216**, 359–389.

AXELROD, J. (1962). Purification and properties of phenylethanolamine-N-methyl transferase. *J. biol. Chem.*, **237**, 1657–1660.

BIRMINGHAM, A.T. & WILSON, A.B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Br. J. Pharmac. Chemother.*, **21**, 569–580.

BURN, J.H. & RAND, M.J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol.* **144**, 314–336.

BURN, J.H. & RAND, M.J. (1960). The effect of precursors of noradrenaline on the response to tyramine and sympathetic stimulation, *Br. J. Pharmac. Chemother.*, **15**, 47–55.

VON EULER, U.S. & HEDQVIST, P.N. (1975). Evidence for an α and β_2 receptor mediated inhibition of the twitch response in guinea-pig vas deferens by noradrenaline. *Acta physiol. scand.*, **93**, 572–573.

GILLESPIE, J.S. & McGRATH, J.C. (1974). The effect of pithing and of nerve stimulation on the depletion of noradrenaline by reserpine in the rat anococcygeus muscle and vas deferens. *Br. J. Pharmac.*, **52**, 585–590.

HENRY, D.P., STARMAN, B.J., JOHNSON, D.G. & WIL-

LIAMS, R.H. (1975). A sensitive radioenzymatic assay for norepinephrine in tissues and plasma. *Life Sci.*, **16**, 375–384.

HUGHES, J., KOSTERLITZ, H.W., & LESLIE, F.M. (1975). Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *Br. J. Pharmac.*, **53**, 371–381.

JENKINS, D.A., MARSHALL, I. & NASMYTH, P.A. (1976). Is noradrenaline the motor transmitter in the mouse vas deferens? *J. Physiol.*, **254**, 49–50 P.

JENKINS, D.A., MARSHALL, I. & NASMYTH, P.A. (1977). An inhibitory role for noradrenaline in the mouse vas deferens. *Br. J. Pharmac.*, **61**, 649–655.

JONES, M.E.L. & SPRIGGS, T.L.B. (1975). Noradrenaline and motor transmission in the vas deferens of the mouse. *Br. J. Pharmac.*, **53**, 323–331.

LEE, F.L. (1967). The relation between norepinephrine content and response to sympathetic nerve stimulation of various organs of cats pretreated with reserpine. *J. Pharmac. exp. Ther.*, **156**, 137–141.

LEVITT, M., SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S. (1965). Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *J. Pharmac. exp. Ther.*, **148**, 1–8.

MARSHALL, I., NASMYTH, P.A., NICHOLL, C.G. & SHEPPERSON, N.B. (1978). α-Adrenoceptors in the mouse vas deferens and their effects on its response to electrical stimulation. *Br. J. Pharmac.*, **62**, 147–151.

MARSHALL I., NASMYTH, P.A. & SHEPPERSON, N.B. (1977). The effect of depletion of the noradrenaline content on the response of the mouse vas deferens to electrical stimulation and to tyramine. *J. Physiol.*, **265**, 45P.

SJÖSTRAND, N.O. & SWEDIN, G. (1968). Effect of reserpine on the noradrenaline content of the vas deferens and the seminal vesicle compared with the submaxillary gland and the heart of the rat. *Acta physiol. scand.*, **72**, 370–377.

STARKE, K., BOROWSKI, E. & ENDO, T. (1975). Preferential blockade of presynaptic α-adrenoceptors by yohimbine. *Eur. J. Pharmac.*, **34**, 385–388.

WAKADE, A.R. & KRUSZ, J. (1972). Effect of reserpine, phenoxybenzamine and cocaine on neuromuscular transmission in the vas deferens of the guinea-pig. *J. Pharmac. exp. Ther.*, **181**, 310–317.

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