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A comparison of the sensitivities of innervated and denervated rat vasa deferentia to agonist drugs

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Summary

1. One vas deferens of a rat was denervated by stripping away the serous coat; the other vas was left intact as a control. One week later the sensitivity *in vitro* of both vasa deferentia to noradrenaline, adrenaline, dopamine, oxymetazoline or acetylcholine was measured.

2. Log concentration response curves for the mean responses of both vasa from a group of four rats for each drug were plotted. Denervated vasa were more sensitive than control vasa to noradrenaline (16-fold), to adrenaline (8-fold), to dopamine (2-fold) and to oxymetazoline (2-fold). Denervated vasa were more sensitive to acetylcholine over the lower half of the concentration range only.

3. It is concluded that these results support the theory that at least part of the increased sensitivity of denervated smooth muscle to catecholamines is due to an abolition or reduction of the neuronal uptake process. There is also a small non-specific increase in sensitivity.

Introduction

The hypogastric nerve of the rat contains many ganglion cells situated close to the serous coat of the vas deferens; most of the fibres of the nerve are therefore preganglionic (Graham, Al Katib & Spriggs, 1968). From the ganglion cells short postganglionic nerves reach the vas deferens and travel in the serous coat to send fibres down into the muscle layers. Cutting the hypogastric nerve trunk does not denervate the vas deferens (Sjöstrand, 1965), but stripping away the serous coat interrupts the short postganglionic fibres which then degenerate to leave denervated smooth muscle which is supersensitive to noradrenaline (Birmingham, 1970). We have now compared the sensitivity of innervated and denervated rat vasa deferentia to several agonist drugs to determine the degree to which this supersensitivity is specific for noradrenaline.

Methods

Male rats (200–400 g) were anaesthetized with ether and one vas deferens was denervated (Birmingham, 1970) by stripping away the serous coat and mesenteric

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attachments ; the other vas was left intact as a control. One week later the rats were killed by stunning and bleeding and both vasa deferentia were removed. For each rat the two vasa were suspended side by side in 20 ml of Krebs solution at 32° C bubbled with 95% oxygen and 5% carbon dioxide. Contractions were recorded on smoked paper with isotonic frontal writing levers giving 5-fold magnification ; the load on the tissue was 0·5 g. Agonist drugs were added to the bath and left for 1 min ; the drug was washed out with several changes of bath fluid and the interval between the doses was at least 5 min.

For each pair of innervated and denervated vasa deferentia the responses were recorded to successive doubling of the concentration of an agonist drug from the threshold value for the most sensitive vas to the concentration producing a maximum response from the least sensitive vas. The vasa from four rats were used for each drug.

The drugs tested were (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, dopamine hydrochloride, oxymetazoline hydrochloride and acetylcholine chloride. Final bath concentrations of the drugs are expressed in molarity.

Results

For each vas deferens the responses to concentrations of an agonist drug were expressed as a percentage of the maximum response. Log concentration response curves were plotted from the means of the results from four rats for each drug. Regression lines with confidence limits were calculated for the linear portion of each log concentration response curve and the dose limits for innervated and for denervated vasa were used to measure the statistical significance of log concentration response curve shifts.

Noradrenaline

The curve for the denervated vasa was to the left of and parallel to that for the innervated vasa (Fig. 1). The increase in sensitivity was statistically significant ($P<0{\cdot}001$) and was about 16-fold.

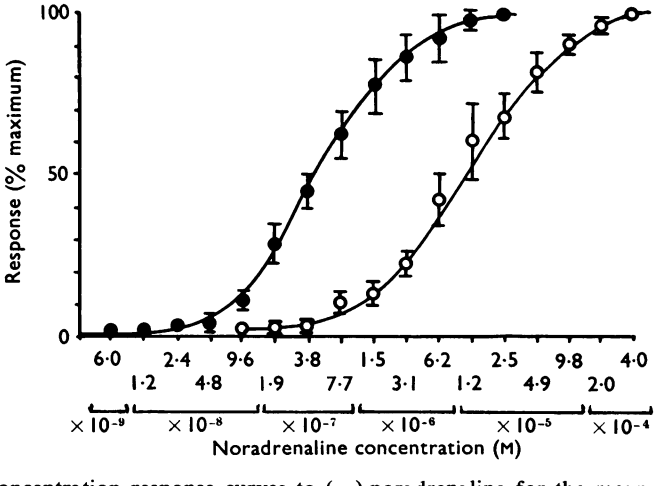

FIG. 1. Log concentration response curves to (−)-noradrenaline for the mean response of the vasa deferentia from four rats ; the standard errors of the means are shown as vertical bars. Ordinate: contraction height as a percentage of the maximum response. Abscissa: final bath concentration (M) of noradrenaline on a log scale. Normal control vasa, ——○——; vasa stripped of serous coat 8 days previously, ——●——.

Adrenaline

When (−)-adrenaline was the agonist the curve for the denervated vasa was again to the left of that for control vasa (Fig. 2). The denervated vasa were about 8 times more sensitive than the control vasa ($P<0.001$).

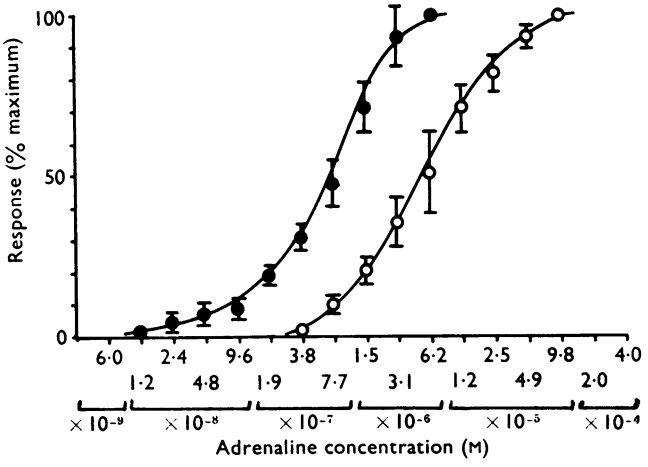

FIG. 2. Log concentration response curves to (−)-adrenaline for the mean responses of the vasa deferentia from four rats; the standard errors of the means are shown as vertical bars. Ordinate: contraction height as a percentage of the maximum response. Abscissa: final bath concentration (M) of adrenaline on a log scale. Normal control vasa, ——○——; vasa stripped of serous coat 8 days previously, ——●——.

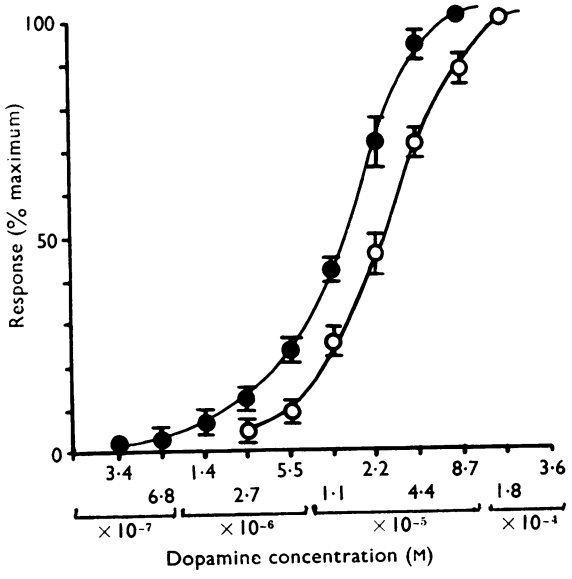

FIG. 3. Log concentration response curves to dopamine for the mean responses of the vasa deferentia from four rats; the standard errors of the means are shown as vertical bars. Ordinate: contraction height as a percentage of the maximum response. Abscissa: final bath concentration (M) of dopamine on a log scale. Normal control vasa, ——○——; vasa stripped of serous coat 8 days previously, ——●——.

Dopamine

There was a small shift to the left for the curve for the denervated vasa when dopamine was tested (Fig. 3). The increase in sensitivity was about 2-fold ($P<0{\cdot}05$).

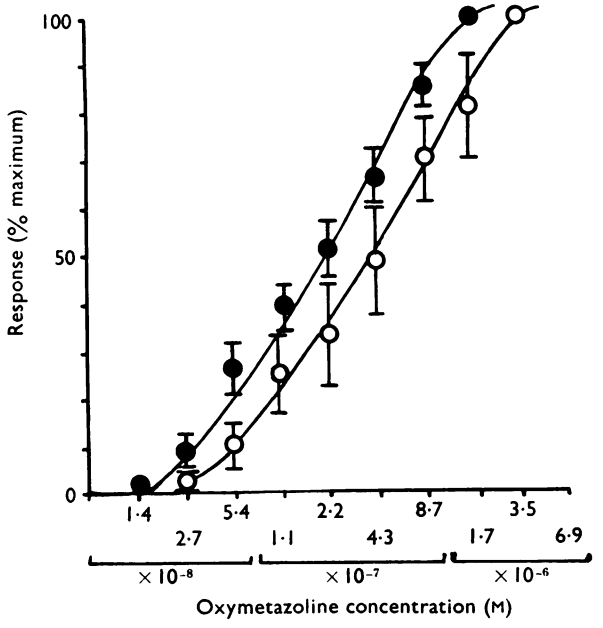

FIG. 4. Log concentration response curves to oxymetazoline for the mean responses of the vas deferentia from four rats; the standard errors of the means are shown as vertical bars. Ordinate: contraction height as a percentage of the maximum response. Abscissa: final bath concentration (M) of oxymetazoline on a log scale. Normal control vasa, ——○——; vasa stripped of serous coat 8 days previously, ——●——.

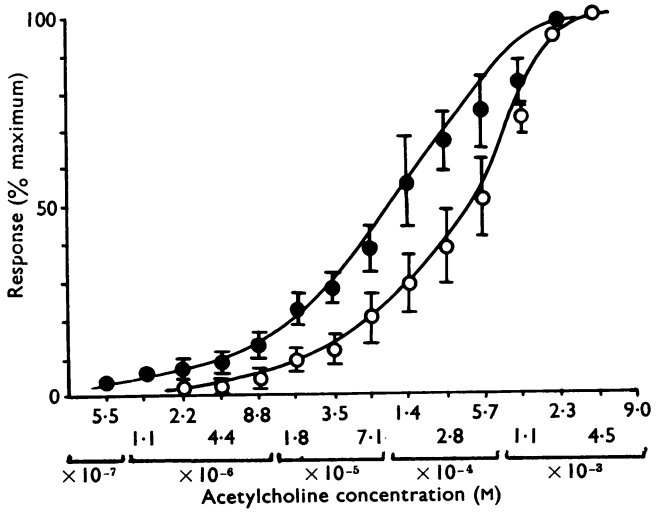

FIG. 5. Log concentration response curves to acetylcholine for the mean responses of the vasa deferentia from four rats; the standard errors of the means are shown as vertical bars. Ordinate: contraction height as a percentage of the maximum response. Abscissa: final bath concentration (M) of acetylcholine on a log scale. Normal control vasa, ——○——; vasa stripped of serous coat 8 days previously, ——●——.

Oxymetazoline

The curve for the denervated vasa was to the left of that for the innervated vasa (Fig. 4), the shift was statistically significant ($P<0.05$) and the increase in sensitivity was about 2-fold.

Acetylcholine

With acetylcholine as the agonist the curve for the denervated vasa was also to the left of that for the control vasa (Fig. 5). The shift was not maintained at high concentrations ; at lower concentrations it was statistically significant ($P<0.01$).

Discussion

Stripping the vas deferens of its serous coat by an *in vivo* operation interrupts the short postganglionic nerve terminals so that they then degenerate (Birmingham, 1970). The smooth muscle becomes denervated and does not respond to transmural electrical stimulation with pulses of short duration, does not respond to tyramine, has no noradrenaline content, has no visible nerve terminals on fluorescence microscopy and is supersensitive to noradrenaline. These findings applied to guinea-pig and to rat vas deferentia ; the increased sensitivity to noradrenaline was established by 24 h and did not increase thereafter.

The present results for the rat show that the increased sensitivity of denervated vasa to catecholamines was in the order noradrenaline>adrenaline>dopamine. The 16-fold increase in sensitivity to noradrenaline is in agreement with the 17-fold increase found by Tsai, Denham & McGrath (1968) for the cat denervated nictitating membrane tested *in vitro*. The increases in sensitivity to adrenaline (8-fold) and to dopamine (2-fold) compared with that to noradrenaline seem to support the theory that at least part of the supersensitivity of denervated tissues to catecholamines is due to an abolition or reduction of the neuronal uptake process. Iversen (1967) stated for the rat isolated heart that " . . . adrenaline was taken up by the saturable mechanism responsible for the uptake of noradrenaline. The two amines competed for uptake and the affinity of the process for adrenaline was less than half that for noradrenaline ". If this is also true of the vas deferens then it would be expected that the increase in sensitivity after denervation would be greater for noradrenaline than for adrenaline. The still smaller increase in sensitivity to dopamine also implies that dopamine uptake in the vas deferens is quantitatively less important than that of noradrenaline or adrenaline.

We also compared the sensitivity of innervated and denervated vasa to oxymetazoline which was reported by Mujic & Van Rossum (1965) as being the most potent of a group of imidazoline compounds which they found to be " specific directly acting alpha-sympathomimetic drugs ". Our results confirmed the potency of oxymetazoline which on innervated vasa was about 10 times as potent as noradrenaline. Denervation only slightly increased the sensitivity of the vas to oxymetazoline, suggesting that oxymetazoline has little affinity for the neuronal uptake process.

The change in sensitivity of denervated vasa to acetylcholine was qualitatively different from that to the other drugs tested. The concentration of acetylcholine producing a maximum response was the same for denervated and innervated vasa,

but at lower doses the denervated vasa were significantly more sensitive. This is in contrast to the findings for guinea-pig vasa (Birmingham, 1970) in which denervated and innervated vasa showed the same sensitivity to acetylcholine over the whole dose-range.

The explanation for this species difference may lie in the suggestion that the cholinergic element to the innervation of the vas deferens is quantitatively more pronounced in the rat than in the guinea-pig (Graham *et al.*, 1968). It has been shown for the rat that denervation of one vas deferens significantly decreases acetylcholinesterase activity compared with that of the normal innervated vas (B. Jarrott, personal communication). In these circumstances, it would be expected that the vas with the less cholinesterase would be the more sensitive to acetylcholine and that the difference in sensitivity would be less pronounced at high acetylcholine concentrations.

It has been reported (Bentley & Sabine, 1963 ; Thoa & Maengwyn-Davies, 1968) that simply stripping away the serous coat after removing the vas deferens from the animal increases the sensitivity of the vas to drugs by facilitating access to the smooth muscle cells. It may be that some of the increase in sensitivity to noradrenaline and to adrenaline found after denervation can be accounted for on this basis. Birmingham (1970) has shown for the guinea-pig that the small increase in sensitivity of the acutely stripped vas is insufficient alone to account for the much larger increase in sensitivity of the chronically denervated vas. However, the absence of a serous coat may explain why for each drug tested, the mean log dose curve for denervated vasa was always to the left of that for innervated vasa even though the shift was small.

Again, this non-specific supersensitivity, shown in some degree to all the drugs as evidenced by the shifts of the curves to the left, may be similar to the first type of supersensitivity discussed by Trendelenburg and his co-workers (Trendelenburg, 1966 ; Langer, Draskoczy & Trendelenburg, 1967) called decentralization supersensitivity, which is of moderate degree and non-specific. A non-specific decentralization supersensitivity has recently been demonstrated for the guinea-pig vas deferens after removal of the hypogastric nerve (Westfall, 1969).

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REFERENCES

BENTLEY, G. A. & SABINE, J. R. (1963). The effects of ganglion-blocking and postganglionic sympatholytic drugs on preparations of the guinea-pig vas deferens. *Br. J. Pharmac. Chemother.*, **21**, 190–201.

BIRMINGHAM, A. T. (1970). Sympathetic denervation of the smooth muscle of the vas deferens. *J. Physiol. Lond.*, **206**, 645–661.

GRAHAM, J. D. P., AL KATIB, H. & SPRIGGS, T. L. B. (1968). The isolated hypogastric nerve–vas deferens preparation of the rat. *Br. J. Pharmac. Chemother.*, **32**, 34–45.

IVERSEN, L. L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves.* Cambridge: Cambridge University Press.

LANGER, S. Z., DRASKOCZY, P. R. & TRENDELENBURG, U. (1967). Time course of the development of supersensitivity to various amines in the nictitating membrane of the pithed cat after denervation or decentralization. *J. Pharmac. exp. Ther.*, **157**, 255–273.

MUJIC, M. & VAN ROSSUM, J. M. (1965). Comparative pharmacodynamics of sympathomimetic imidazolines; studies on intestinal smooth muscle of the rabbit and the cardiovascular system of the cat. *Archs int. Pharmacodyn. Thér.*, **155**, 432–449.

SJÖSTRAND, N. O. (1965). The adrenergic innervation of the vas deferens and the accessory male genital glands. *Acta physiol. scand.*, **65**, suppl. 257.

THOA, N. B. & MAENGWYN-DAVIES, G. D. (1968). The guinea-pig isolated vas deferens: a method for increasing sensitivity to drugs. *J. Pharm. Pharmac.*, **20**, 873–876.

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

TSAI, T. H., DENHAM, S. & MCGRATH, W. R. (1968). Sensitivity of the isolated nictitating membrane of the cat to norepinephrine and acetylcholine after various procedures and agents. *J. Pharmac. exp. Ther.*, **164**, 146–157.

WESTFALL, D. P. (1969). The effect of decentralisation and reserpine-treatment on the sensitivity of the isolated vas deferens of the guinea-pig to stimulant drugs. *Br. J. Pharmac.*, **37**, 514–515*P*.

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