Br. J. Pharmac. (1970), **39**, 110–120.

Nonspecific supersensitivity of the guinea-pig vas deferens produced by decentralization and reserpine treatment

D. P. WESTFALL*

Department of Pharmacology, University of Oxford

Summary

1. The sensitivity of the guinea-pig vas deferens to noradrenaline, histamine, methylfurmethide and potassium was examined *in vitro* following decentralization and reserpine treatment.

2. One day after decentralization or administration of reserpine (1·0 mg/kg daily) the sensitivity of the vas deferens was not increased. After 5 days' treatment the muscle was supersensitive to all four stimulants.

3. The magnitude of the sensitivity increase to an individual drug was the same following both chronic reserpine treatment and decentralization. However, the degree of supersensitivity differed for the four stimulants. The order of potentiation was noradrenaline>histamine>methylfurmethide> potassium.

4. The magnitude of the supersensitivity was inversely correlated with the slope of the dose-response curves to the four agonists. The dose-response curve to potassium had the steepest slope, followed in order by methylfurmethide, histamine and noradrenaline.

5. A hypothesis is presented to account for the inverse relationship between the slope of the dose-response curve and the degree of supersensitivity which follows reserpine treatment or decentralization.

Introduction

The supersensitivity of the nictitating membrane of the spinal cat which results from pretreatment with reserpine resembles that caused by decentralization (preganglionic denervation). Following both procedures the increase in sensitivity to noradrenaline is of similar magnitude (Fleming & Trendelenburg, 1961) and exhibits a similar time course of development (Fleming, 1963a). In addition, the supersensitivity is nonspecific in that it occurs not only to noradrenaline but to acetylcholine (Trendelenburg & Weiner, 1962), potassium (Fleming, 1963b) and barium (Morrison & Fleming, 1967). The common features have led to the concept that reserpine treatment and decentralization produce supersensitivity by a similar mechanism (Trendelenburg, 1963).

* J. H. Burn fellow. Present address: Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

Tsai, Denham & McGrath (1968) were unable to demonstrate supersensitivity of the isolated nictitating membrane following either reserpine treatment or previous decentralization. However, reserpine induced supersensitivity has been demonstrated in other tissues *in vitro*, for example in rabbit aortic strips (Hudgins & Fleming, 1966) and perfused guinea-pig hearts (Westfall & Fleming, 1968). To date, supersensitivity following decentralization has not been observed *in vitro*. The apparent difference between the two procedures, when studied *in vitro*, may simply indicate that the phenomenon has not been investigated in an appropriate experimental system or it may indicate a difference in the mechanism by which decentralization and reserpine treatment produce supersensitivity. The present investigation was designed to test these alternatives by using the guinea-pig vas deferens, a tissue which is readily adaptable to experiments *in vitro* and is amenable to both decentralization and reserpine treatment. A preliminary account has been presented to the British Pharmacological Society (Westfall, 1969).

Methods

The experiments were performed with isolated, desheathed vasa deferentia from guinea-pigs ranging in weight between 250 and 500 g. Tissues were set up in isolated organ baths of 10 ml capacity and bathed in modified Krebs solution of the following composition (mM): Na^+ 137 ; K^+ 5·9 ; Ca^{++} 2·5 ; Mg^{++} 1·2 ; Cl^- 134 ; HCO_3^- 15·5 ; $H_2PO_4^-$ 1·2 ; glucose 11·5.

The solution was maintained at 37° C and continually gassed with 97% oxygen and 3% carbon dioxide. Contractions of the smooth muscle were recorded via a strain gauge and a potentiometric recorder. Approximately 0·2 g resting tension was applied to the tissue. One hour was allowed for equilibration before the tissue was exposed to drugs. Full dose-response curves were obtained by a stepwise increase in concentration. Doses were applied at 4 min intervals, each dose being washed out before the next. Unless otherwise indicated, only one dose-response curve was obtained on each muscle. The response to a given dose was plotted as % of the maximum response obtained.

The guinea-pigs were of three groups: (1) no treatment (control) ; (2) pretreatment with reserpine by intraperitoneal injection (1·0 mg/kg daily) for either 1 or 5 days ; (3) animals in which 1–2 cm of both hypogastric nerves were removed at a distance of about 4 cm from the vas deferens 1, 5 or 10 days previously. The animals were anaesthetized with ether and operated under aseptic conditions. Sjöstrand (1962) has demonstrated that the section of the hypogastric nerve constitutes a preganglionic denervation (that is, decentralization) of the vas deferens.

Smooth muscle stimulants used in this study were (−)-noradrenaline bitartrate, 5-methylfuryltrimethylammonium iodide (methylfurmethide), histamine dihydrochloride and potassium chloride. Methylfurmethide and histamine were diluted in saline solution while noradrenaline was diluted in saline containing 0·01% ascorbic acid. Potassium chloride was dissolved in distilled water. The antagonists atropine sulphate and phentolamine mesylate were added directly to the Krebs solution. Reserpine was prepared from the dry powder according to the formulation of Martindale (1958). All drug concentrations are expressed in terms of the free base.

Statistical analysis of the data included Student's t test, Duncan's multiple range test and least squares analysis according to Snedecor (1956). The 0·05 level of probability was considered significant.

Results

Characteristics of the response

Noradrenaline, methylfurmethide and histamine produced dose-dependent contractions of the vas deferens which reached maximum within 30 to 60 s and then quickly declined. Upon washing the muscle relaxed to the initial tension.

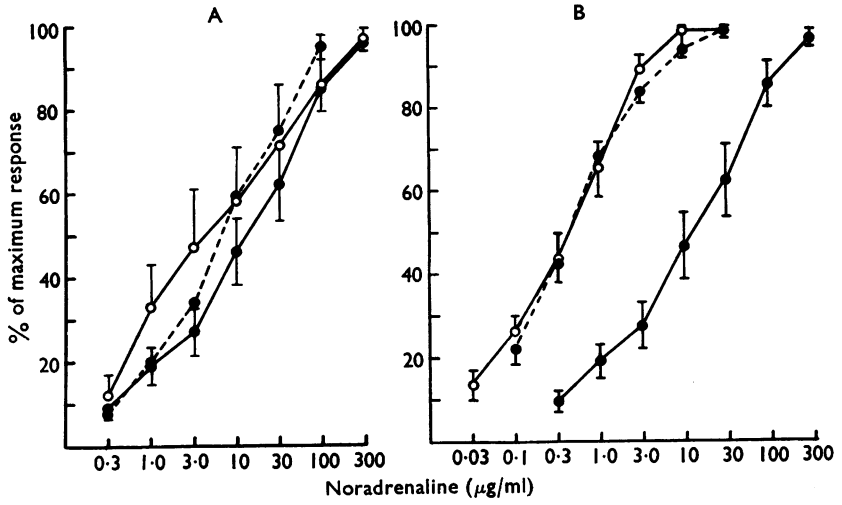

FIG. 1. Mean dose-response curves to noradrenaline in untreated (●——●), reserpine-treated (○——○) and decentralized (●– – –●) vas deferens. A, 1 day following decentralization or reserpine. B, 5 days following decentralization or reserpine treatment. Vertical bars represent standard errors. See Table 1 for number of experiments.

TABLE 1. *Geometric mean ED50* values (with 95% confidence intervals in brackets) in untreated, reserpine-treated and decentralized vas deferens*

Treatment	Noradrenaline (μg/ml)	*n*	Methylfurmethide (μg/ml)	*n*	Histamine (μg/ml)	*n*	Potassium (μg/ml)	*n*
None (Control)	11·99 (9·62–15·0)	15	0·438 (0·297–0·645)	15	30·69 (23–40)	9	1·12 (0·93–1·34)	10
Reserpine (1 day)	4·35 (3·21–5·92)	8	0·192 (0·162–0·227)	8				
Reserpine (5 days)	0·42 (0·32–0·55)†	10	0·066 (0·057–0·077)†	10	1·85 (1·35–2·52)†	6	0·79 (0·63–0·99)†	6
Decentralization (1 day)	6·90 (5·08–9·35)	8	0·259 (0·218–0·306)	8				
Decentralization (5 days)	0·41 (0·29–0·58)†	10	0·071 (0·060–0·084)†	8	2·19 (1·61–3·00)†	6	0·73 (0·58–0·91)†	6
Decentralization (10 days)	0·88 (0·58–1·41)†	4						

*ED50, Concentration producing a contraction which is 50% of maximum for the drug in an individual experiment.
† Significantly different from control ($P<0·01$).
n, Number of experiments.

Potassium ions also produced a dose-dependent contraction but the maximum was reached in 5 to 10 s. The response quickly fell off, but the muscle did not always relax to the original level even with repeated washing, if potassium had been allowed to remain in contact with the tissue for 60 s. If the potassium was washed out of the bath after 15 s, the tissue relaxed to the initial level. For this reason contact time was 15 s for potassium and 60 s for the other agonists.

The dose-response curves obtained with the four stimulants had different slopes. The mean regression coefficients, calculated by the method of least squares from the individual dose-response curves (20 to 80% response region) were as follows: noradrenaline, 34 ; histamine, 45 ; methylfurmethide, 51 ; potassium, 102 (expressed as % per 10-fold change).

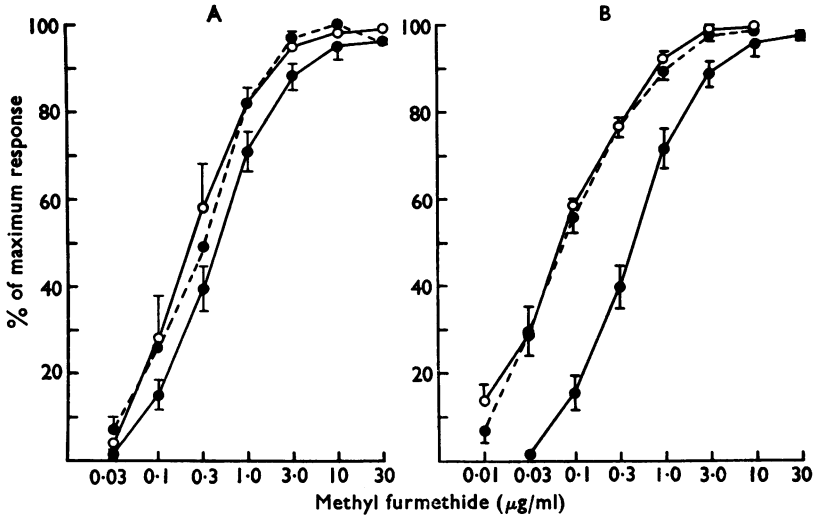

FIG. 2. Mean dose-response curves to methylfurmethide in untreated (●——●), reserpine-treated (○——○) and decentralized (●– – –●) vas deferens. A, 1 day following decentralization or reserpine. B, 5 days following decentralization or reserpine treatment. Vertical bars represent standard errors. See Table 1 for number of experiments.

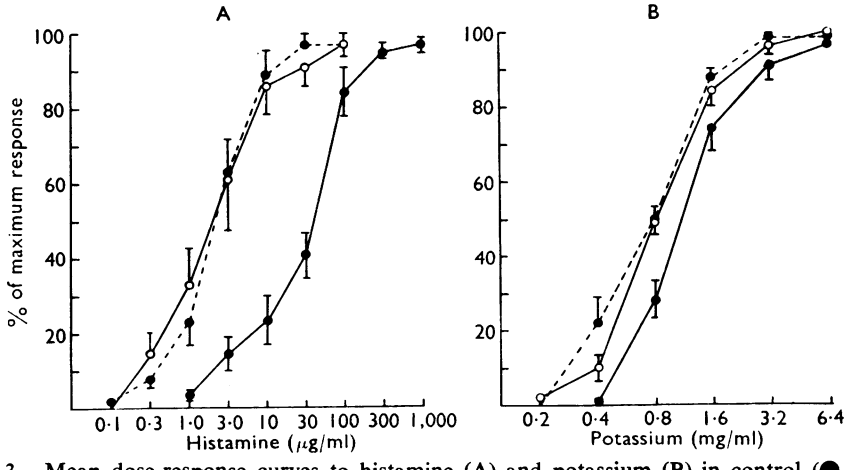

FIG. 3. Mean dose-response curves to histamine (A) and potassium (B) in control (●——●), 5 day reserpine-treated (○——○) and 5 day decentralized (●– – –●) vas deferens. Vertical bars represent standard errors. See Table 1 for number of experiments.

The absolute maximum contraction of the muscle to all the agonists was of similar magnitude. Neither reserpine treatment nor decentralization altered the maximum response.

Sensitivity changes

Figure 1a shows mean dose-response curves to noradrenaline in untreated, 1 day reserpine treated and 1 day decentralized preparations. Although the dose-response curves obtained following these procedures lie slightly to the left of the control curve they are not significantly different at the ED50 level (Table 1). However, when the experiments were conducted after 5 days of reserpine treatment or 5 days following decentralization there was a significant shift to the left of the dose-response curves (Fig. 1b). The increase in sensitivity to noradrenaline, determined by the ratio of the geometric mean ED50 values (Table 1), was of similar magnitude (29 fold) after 5 days' decentralization and 5 days' reserpine administration. The supersensitivity was maximal, since 10 days after decentralization there was no greater increase in sensitivity (Table 1). Treatment of guinea-pigs with a ten-fold lower dose of reserpine (0·1 mg/kg daily for 5 days) failed to produce supersensitivity.

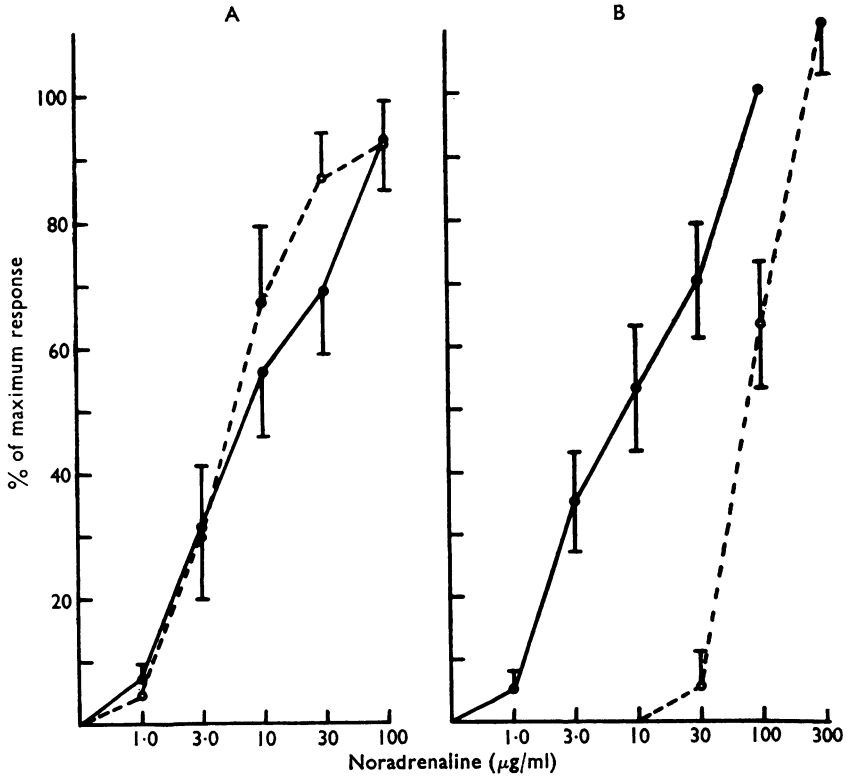

FIG. 4. Effect of phentolamine on the response of the vas deferens to noradrenaline. A, Two consecutive dose-response curves (——, 1 ; – – –, 2) to noradrenaline ($n=4$). B, Dose-response curves to noradrenaline in the absence (——) and in the presence (– – –) of phentolamine (10^{-6} g/ml) ($n=4$). Vertical bars represent standard errors.

Similar findings were obtained when methylfurmethide, a cholinomimetic agent, served as the agonist. After only 1 day of treatment the sensitivity of the vas deferens was not significantly increased (Fig. 2a). But, as with noradrenaline, if the treatment was extended to 5 days the dose-response curves were shifted to the left, indicating supersensitivity (Fig. 2b). The mean ED50 values were reduced to the same extent, 6·2 fold by decentralization and 6·5 fold by reserpine treatment (Table 1).

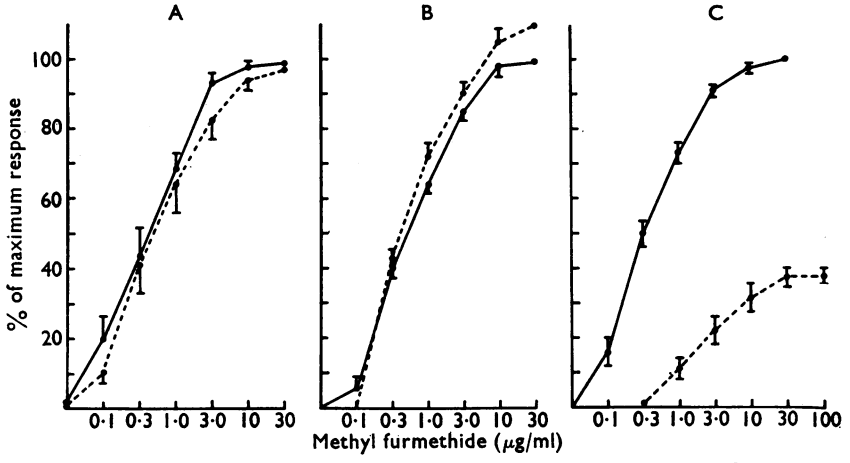

FIG. 5. Effect of phentolamine and atropine on the response of the vas deferens to methylfurmethide. A, Two consecutive dose-response curves (——, 1 ; – – –, 2) to methylfurmethide ($n=4$). B, Dose-response curves to methylfurmethide in the absence (——) and in the presence (– – –) of phentolamine (10^{-6} g/ml) ($n=4$). C, Dose-response curves to methylfurmethide in the absence (——) and in the presence (– – –) of atropine (10^{-9} g/ml) ($n=4$). Vertical bars represent standard errors.

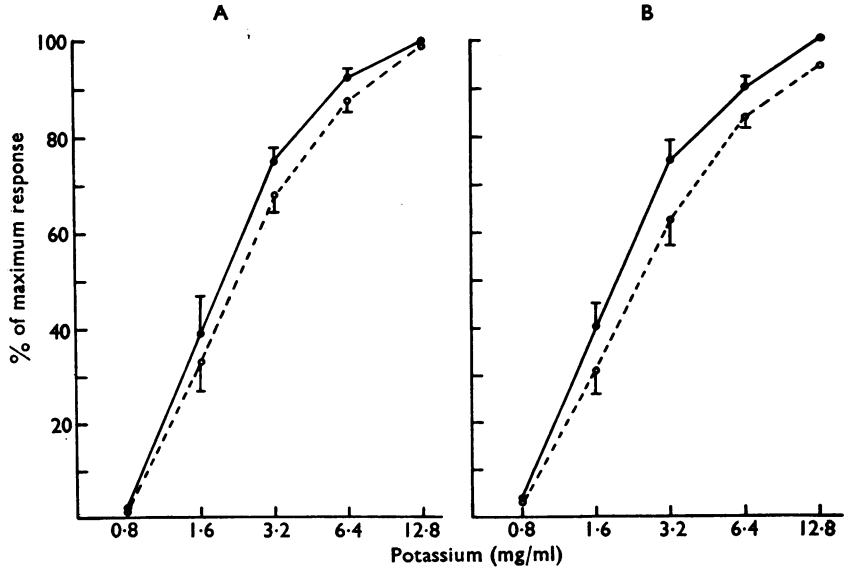

FIG. 6. Effect of phentolamine and atropine on the response of the vas deferens to potassium. A, Two consecutive dose-response curves (——, 1 ; – – –, 2) to potassium ($n=4$). B, Dose-response curves to potassium in the absence (——) and in the combined presence (– – –) of phentolamine (10^{-6} g/ml) and atropine (10^{-9} g/ml) ($n=4$). Vertical bars represent standard errors.

The vas deferens also exhibited supersensitivity to histamine and potassium following chronic decentralization or administration of reserpine (Fig. 3). The increase in sensitivity was approximately 15 fold to histamine and 1·5 fold to potassium (Table 1).

Specificity of agonist action

In order to determine whether the stimulants produced their effects by independent mechanisms, experiments with antagonists were conducted. In these experiments two dose-response curves to an agonist were obtained on each muscle. The responses for the second curve were obtained after a one hour interval and the magnitude was calculated as percentage of the maximum response obtained in the first curve. In some tissues the second curve was obtained without the addition of an antagonist in order to assess the repeatability of the dose-response curves. In the other tissues an antagonist was added 20 min before, and remained in the bathing solution while the second dose-response curve was obtained.

Figure 4 shows dose-response curves to noradrenaline obtained in this fashion. Without the addition of an antagonist the second dose-response curve does not

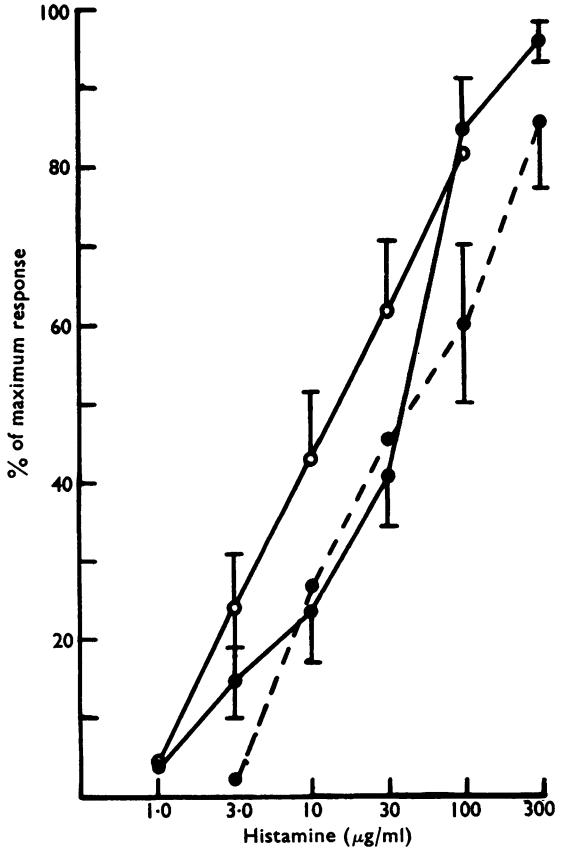

FIG. 7. Effect of phentolamine and atropine on the response of the vas deferens to histamine. Mean dose-response curves to histamine in the absence of antagonists ($n=9$) (●——●), in the presence of phentolamine (10^{-6} g/ml) ($n=4$) (●– – –●), and in the presence of atropine (10^{-9} g/ml) ($n=4$) (○——○). Vertical bars represent standard errors.

appreciably differ from the first (Fig. 4a). In the presence of phentolamine (10^{-6} g/ml) the second dose-response curve to noradrenaline is shifted significantly to the right, indicating an effective antagonism (Fig. 4b).

Figure 5 shows results obtained with methylfurmethide. With no antagonist, the dose-response curves were repeatable (Fig. 5a). Phentolamine (10^{-6} g/ml) was without effect (Fig. 5b), while atropine (10^{-9} g/ml) antagonized the response of the vas deferens to methylfurmethide (Fig. 5c).

The responses to potassium were tested in a similar manner. Figure 6 shows that the dose-response curve to potassium obtained in the combined presence of atropine (10^{-9} g/ml) and phentolamine (10^{-6} g/ml) was not significantly altered.

The influence of antagonists on the response to histamine was also examined. However, the experimental design differed from that described for the other three agonists. In preliminary experiments it was observed that the responses to a second series of doses of histamine were depressed. The reason for this may be the exposure to extremely high concentrations of histamine required to reach the maximum response in the first curve, which may depress subsequent responses It was believed more valid, therefore, to compare experiments in which only one dose-response curve to histamine was obtained on each muscle.

Figure 7 shows mean dose-response curves in untreated tissues and in tissues exposed to either atropine (10^{-9} g/ml) or phentolamine (10^{-6} g/ml) for 20 min before and during exposure to histamine. Although the dose-response curves obtained in the presence of atropine and phentolamine deviate somewhat from the control the ED50 values are not significantly different, indicating a lack of antagonism.

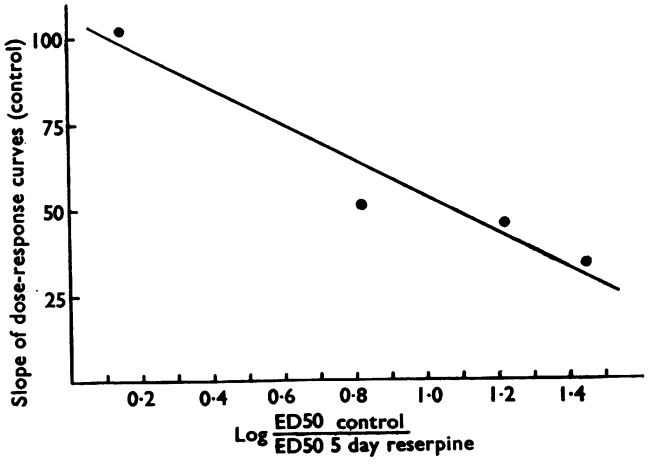

FIG. 8. Relationship between the slope of dose-response curves and the magnitude of supersensitivity. Ordinate: mean slope of the dose-response curves in normal preparations. Abscissa, magnitude of supersensitivity expressed by the ratio of log $\frac{\text{ED50 control}}{\text{ED50 5-day reserpine}}$. The points represent the values for the four stimulants noradrenaline, histamine, methylfurmethide and potassium. The regression line, fitted by the method of least squares, has a correlation coefficient of 0·989.

Correlation between magnitude of supersensitivity and slopes of dose-response curves

Figure 8 indicates that there was a significant relationship between the magnitude of supersensitivity and the slope of the dose-response curve. The slope of the dose-response curve to the agonist in control tissues was plotted against the supersensitivity to the agonist after chronic reserpine treatment $\left(\log \frac{\text{ED50 control}}{\text{ED50 treated}}\right)$. The resulting regression line has a correlation coefficient of 0·989 ($P<0·05$) and an inverse relationship. In other words, the degree of supersensitivity in a group of stimulants decreases as the slope of the dose-response curves increases. Since the supersensitivity after decentralization was the same as that after reserpine treatment, the regression line of slope versus supersensitivity was virtually identical.

Discussion

The supersensitivity of the vas deferens which results from pretreatment with reserpine resembles that caused by decentralization and can be demonstrated *in vitro*. Following both procedures, the increase in sensitivity develops with a similar time course, is of equal magnitude to a given agonist and is nonspecific. The findings indicate that there is no basic difference in the mechanism by which reserpine treatment and decentralization cause supersensitivity. Furthermore, since this phenomenon is exhibited *in vitro* the supersensitivity cannot be explained by a change occurring in the whole animal such as an altered blood flow to the tissue.

To term such a supersensitivity " nonspecific " requires that the agonists act by different mechanisms. This is the case in the vas deferens. The dose schedule of the reserpine treatment (1·0 mg/kg daily for 5 days) was more than adequate to reduce endogenous noradrenaline to undetectable levels (Sjöstrand, 1962), yet it did not reduce the responses to methylfurmethide, histamine and potassium. This finding, together with the demonstration that phentolamine, in a dose sufficient to antagonize exogenous noradrenaline, did not antagonize the other three agonists is strong evidence that they are not acting via an adrenergic mechanism. Similarly, the finding that the responses to histamine and potassium were not antagonized by atropine, while the response to methylfurmethide was, is evidence against a cholinergic mechanism.

Emmelin (1961) pointed out that chronic interruption of tonic nerve impulses between the central nervous system and the target organ, which reduced the activity of the tissue, is essential for the development of nonspecific supersensitivity. This criterion is fulfilled in the present experiments since both reserpine treatment and decentralization reduce the activity of the vas deferens. Trendelenburg (1966) has suggested that a postsynaptic mechanism is responsible for a supersensitivity which requires time for development and is nonspecific. In the light of the nonspecific nature of supersensitivity, this postsynaptic mechanism probably involves some change in the physiological state of the responding cells probably at the level of the cell membrane (Fleming, 1963b ; Hudgins and Fleming, 1966 ; Green & Fleming, 1967).

If the supersensitivity is nonspecific one might expect that the degree of sensitivity change would be the same regardless of the stimulus. This is clearly not the

case in the vas deferens. The magnitude of the sensitivity change was greatest for noradrenaline followed in order by histamine, methylfurmethide and potassium. One possibility to explain the greater degree of supersensitivity to noradrenaline is that there is some additional factor affecting the sensitivity to this agent over and above that which is causing a generalized supersensitivity. For example, there could be a " spread of receptors " for the neurotransmitter as has been found in denervated skeletal muscle (Axelsson & Thesleff, 1959). This would imply two distinct ranges of supersensitivity, one for the neurotransmitter and its analogues, and another for the nonspecific agonists. This suggestion appears unlikely, however, since in the vas deferens the degree of supersensitivity is different for all four agonists.

An alternative possibility is that the degree of supersensitivity is related to the slope of the dose-response curve (Fig. 8); that is, for a heterogeneous group of stimulants the shift of the dose-response curve becomes less as the slope becomes steeper. This relationship occurs in tissues other than the vas deferens. In rabbit aortic strips, Hudgins & Fleming (1966) reported reserpine-induced supersensitivity whose magnitude was greatest for noradrenaline, less for acetylcholine and least for potassium. Visual inspection of their dose-response curves reveals that the slope was greatest for potassium, less for acetylcholine and least for noradrenaline.

One possibility to explain the relationship between slope and shift of dose-response curves is that the slope is determined by the relative contribution of two pathways both of which lead to a contraction. If drug A, which utilizes primarily pathway 1, has a steep slope then one would expect that drugs B, C and D, each possessing a greater contribution from pathway 2, to have slopes which were less and less steep. If the change which results in supersensitivity involves both pathways, but pathway 2 more than 1, the order of potentiation would be D>C>B>A.

Evidence for a dual pathway leading to contraction does exist. One generally accepted pathway is that a drug produces contraction by depolarizing the cell membrane, thus allowing entry of ions, of which Ca^{2+} is important. However, Evans, Schild & Thesleff (1958) have demonstrated that stimulants can evoke contraction of smooth muscle in the absence of depolarization. Edman & Schild (1962) suggested that this alternate pathway involves mobilization of membrane bound calcium which becomes available for the contraction process. Thus, while both pathways require calcium, the mechanism by which the two utilize calcium differs. Hinke (1965) has shown that the contractions of vascular smooth muscle induced by potassium and by noradrenaline are both calcium dependent, but rely on a different source of calcium. The calcium utilized by potassium is mobile and dependent on the extracellular calcium concentration, while noradrenaline utilizes a firmly-bound calcium fraction. Hudgins & Weiss (1968) confirmed these findings and demonstrated that histamine was intermediate between potassium and noradrenaline in its dependence on extracellular calcium for producing contraction.

In the context of the proposed hypothesis, a drug which produces contraction primarily by depolarization (potassium) would have a dose-response curve with a steep slope while a drug which also mobilizes membrane bound calcium (noradrenaline) would have a dose-response curve with a shallow slope. Supersensitivity would be expected to produce an increased efficiency in both pathways but preferentially in the mechanism responsible for mobilizing bound calcium. The

result would be supersensitivity to both agents but to a greater extent to noradrenaline. The response to histamine, being intermediate, would be potentiated more than potassium but less than noradrenaline.

Such a model, although speculative, accounts for the results obtained in the vas deferens and is consistent with the hypothesis that nonspecific supersensitivity results from an alteration at the level of the cell membrane.

The author wishes to thank Professor Edith Bülbring for her encouragement and advice. This research was supported by the Medical Research Council. The author is grateful for a travel grant from the Wellcome Trust.

REFERENCES

AXELSSON, J. & THESLEFF, S. (1959). A study of supersensitivity in denervated mammalian skeletal muscle. *J. Physiol., Lond.*, **147**, 178–193.

EDMAN, K. A. P. & SCHILD, H. O. (1962). The need for calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus. *J. Physiol., Lond.*, **161**, 424–441.

EVANS, D. H. L., SCHILD, H. O. & THESLEFF, S. (1958). Effects of drugs on depolarized plain muscle. *J. Physiol., Lond.*, **143**, 474–485.

EMMELIN, N. (1961). Supersensitivity following "pharmacological denervation". *Pharmac. Rev.*, **13**, 17–37.

FLEMING, W. W. (1963a). A comparative study of supersensitivity to norepinephrine and acetylcholine produced by denervation, decentralization and reserpine. *J. Pharmac. exp. Ther.*, **141**, 173–179.

FLEMING, W. W. (1963b). Changes in the sensitivity of the cat's nictitating membrane to norepinephrine, acetylcholine and potassium. *Biochem. Pharmac.*, **12**, suppl., 202.

FLEMING, W. W. & TRENDELENBURG, U. (1961). The development of supersensitivity to norepinephrine after pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **133**, 41–51.

GREEN, R. D., III & FLEMING, W. W. (1967). Agonist-antagonist interactions in the normal and supersensitive nictitating membrane of the spinal cat. *J. Pharmac. exp. Ther.*, **156**, 207–214.

HINKE, J. A. M. (1965). Calcium requirements for noradrenaline and high potassium ion contraction in arterial smooth muscle. In *Muscle*, ed. Paul, W. M., Daniel, E. E., Kay, C. M. and Monckton, G., pp. 269–285. New York: Pergamon Press.

HUDGINS, P. M. & FLEMING, W. W. (1966). A relatively nonspecific supersensitivity in aortic strips resulting from pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **153**, 70–80.

HUDGINS, P. M. & WEISS, G. B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmac. exp. Ther.*, **159**, 91–97.

MARTINDALE, W. (1958). *Extra Pharmacopoeia*. London: Pharmaceutical Press.

MORRISON, J. M., JR. & FLEMING, W. W. (1967). The nonspecific supersensitivity of the cat nictitating membrane. *Pharmacologist*, **9**, 234.

SNEDECOR, G. W. (1956). *Statistical Methods*. Ames: Iowa State College Press.

SJÖSTRAND, N. O. (1962). Effect of reserpine and hypogastric denervation on the noradrenaline content of the vas deferens and seminal vesicle of the guinea-pig. *Acta physiol. scand.*, **56**, 376–380.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **15**, 225–276.

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

TRENDELENBURG, U. & WEINER, N. (1962). Sensitivity of the nictitating membrane after various procedures and agents. *J. Pharmac. exp. Ther.*, **136**, 152–161.

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. & FLEMING, W. W. (1968). The sensitivity of the guinea pig pacemaker to norepinephrine and calcium after pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **164**, 259–269.

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

(*Received December* 15, 1969)