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DUAL EFFECT OF α-ADRENOCEPTOR ANTAGONISTS IN RAT ISOLATED VAS DEFERENS

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1 In rat isolated vas deferens, the isotonic contractile responses to low doses of noradrenaline or adrenaline were antagonized, and those to high doses were potentiated, by yohimbine, piperoxan, phentolamine and tolazoline. Effects due to intermediate doses were not affected, or were potentiated within about 30 min, following an initial inhibition.

2 The α-adrenoceptor blockers thus caused a shift to the right and an increase of the maximum height of log dose-response curves of α-adrenoceptor stimulants. For a given dose of antagonist, the onset was slower for the potentiating than for the blocking effect.

3 The shift to the right induced by piperoxan and yohimbine on dose-response curves of noradrenaline and adrenaline was analysed with the Schild plot, and the slopes obtained, around 0.3, were lower than expected from receptor theory. When cocaine was used to block neuronal uptake, the slopes were close to 1.0.

4 The increase in maximum response to noradrenaline and adrenaline induced by α-adrenoceptor blockers was dependent on the time of incubation, on the dose of antagonist, and on the initial height of responses to the agonist. A less pronounced potentiation was also obtained when acetylcholine was used as agonist.

5 The findings are explained in terms of receptor theory as being due to a dual effect of α-adrenoceptor antagonists; competitive antagonism proper, which may be disclosed after blockade of neuronal uptake, and an interaction at a different locus, which results in potentiation of the effects of noradrenaline and adrenaline.

Introduction

It is known that yohimbine blocks adrenaline contractions in rat isolated vas deferens (Martins & Valle, 1939) and that antagonism of piperoxan towards (±)-noradrenaline follows receptor theory (Ariëns, 1967). It is apparently contradictory that these antagonists can potentiate the effect of sympathetic stimulation (Ohlin & Strömblad, 1963; Swedin, 1972) and that phentolamine can potentiate the effects of noradrenaline (Barnett, Greenhouse & Taber, 1968), in this preparation.

In the course of experiments performed on the rat vas deferens, it was observed that the reversible α-adrenoceptor antagonists piperoxan, yohimbine, phentolamine or tolazoline antagonized noradrenaline-induced contractions, but the effect was sometimes changed to potentiation within some minutes (Jurkiewicz, Jurkiewicz & Valle, 1971a). Since these findings could help to explain the conflicting reports described above, experiments were undertaken in order to analyse the phenomenon quantitatively.

Methods

Rat isolated vas deferens

Adult albino rats, 5 to 6 months old, were killed with an overdose of ether. The vasa deferentia were dissected from the surrounding tissues and the lumina carefully washed with a solution of the following composition (mM): NaCl 138, KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15, dextrose 5.5, prepared in glass-distilled, deionized water. Each organ was mounted in a 10 ml chamber containing the solution at 30°C, aerated throughout the experiment. Contractions were recorded by means of isotonic levers with 6 × amplification and 1 g load.

Although single doses of active drugs were used in preliminary experiments, most of the work was based on the kymograph recording of cumulative dose-response curves as previously described (Jurkiewicz *et al.*, 1969). Before starting the actual experiments, the tissue was allowed to equilibrate for about 30 min, and 2 or 3 cumulative curves in response to noradrenaline,

adrenaline, or acetylcholine, were obtained at 20 to 60 min intervals, in order to achieve stable responses. Cumulative response curves to barium chloride were also obtained at the beginning and end of most of the assays. Except for barium chloride, only one agonist was used in each preparation. In some of the experiments, the contralateral vas deferens was used as an 'external control preparation' (Furchgott, 1970) in which cumulative curves in response to agonists were determined throughout the experiment, at similar intervals, in order to detect variations in sensitivity of the organ to the active drugs.

Four dose-response curves to an agonist were usually determined at 30 to 60 min intervals, following incubation with a dose of antagonist for periods of 1, 10, 20 and 30 min, in each experiment. In other series of assays the time of incubation with antagonist was constant (30 or 60 min) and 3 different doses of antagonist were used.

Measurement of antagonism and potentiation

In some experiments the degree of antagonism or potentiation was simply measured by the change in contraction height after each dose of antagonist. In general, cumulative curves were assessed by the height of the maximum contraction and by estimating the dose of active drug inducing a 50% effect (ED_{50}). Antagonism induced by a given dose of α-adrenoceptor blocker was estimated by a dose-ratio (ED_{50} after antagonists $\div$ ED_{50} before antagonist) representing an apparent increase of ED_{50}. Potentiation was estimated by the ratio of the maximum response obtainable after, to that obtainable before, the addition of the potentiating drug.

Antagonism and potentiation in terms of receptor theory

In order to verify if antagonism followed receptor theory, plots were performed according to the relation (Schild, 1957), $\log (DR-1)=\log (B/K_B)$, in which DR is the dose-ratio as defined above, B is the molar concentration of antagonist, and K_B is the dissociation constant of the antagonist-receptor complex.

As an aid for the measurement of potentiation, the relative responsiveness ratio (ρ) was also estimated as the quotient between the height of maximum response to the agonist, and the height of the maximum contraction of the vas deferens, arbitrarily taken as that induced by barium chloride (Jurkiewicz, Jurkiewicz, Barros & Valle, 1969).

Statistics

Significance of differences on the height of dose-response curves was analysed according to paired *t* test, slope of the lines on the Schild plot by a linear regression analysis and relations between relative responsiveness and potentiation by a correlation analysis (Snedecor & Cochran, 1967).

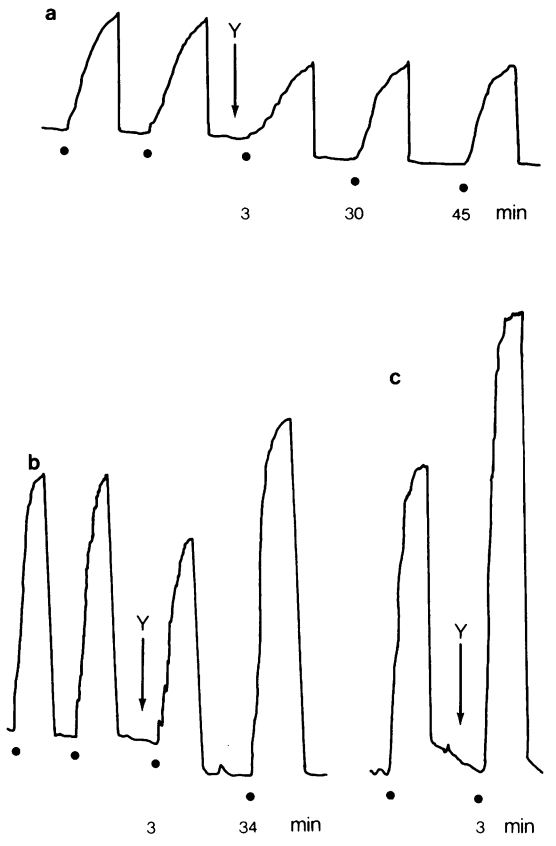


Figure 1 Effect of single doses of noradrenaline (at dots) on three different vasa deferentia (a,b,c), before and at different periods following addition of yohimbine 3×10^{-5} M (Y) to the nutrient solution. Noradrenaline doses: (a) 3×10^{-6} M, (b) 3×10^{-5} M, (c) 1×10^{-4} M. After responses to noradrenaline (45 to 60 s) the Kymograph was stopped for agonist washout. Time in min after introduction of yohimbine solution is indicated. Besides its inhibitory or potentiating effect, yohimbine also induced a slight fall in resting tone.

Drugs

(–)-Adrenaline ((–)-epinephrine bitartrate, Sigma, U.S.A., or Fluka, Germany), (–)-noradrenaline ((–)-arterenol hydrochloride, Sigma, U.SA.), acetylcholine (acetylcholine iodide or chloride, Sigma, U.S.A.), priscoline (tolazoline, Sigma, U.S.A.), piperoxan (chlorhydrate of piperidinomethyl benzodioxane,

Specia, France), yohimbine (yohimbine hydrochloride, Sigma, U.S.A.), regitine (phentolamine, Ciba, Switzerland), and barium chloride (May & Baker, U.S.A.), were used. Stock solutions were maintained frozen and discarded after 15 days. Working solutions of adrenaline and noradrenaline were prepared shortly before the experiments. Except in preliminary experiments, disodium ethylenediaminetetraacetic acid (EDTA, 10 μg/ml) was added to stock and working solutions in order to prevent catalytic oxidation of noradrenaline and adrenaline by traces of heavy metals (Furchgott, 1955).

Results

At a concentration of 3×10^{-5} M, yohimbine induced either a decrease (Figure 1a), a potentiation (Figure 1c) or an inhibition followed by a potentiation (Figure 1b), of the contractile effects of single doses of noradrenaline. In some experiments this concentration of yohimbine did not influence noradrenaline effects at all. Similar results were obtained with piperoxan, 3×10^{-6}M as the antagonist or adrenaline as the agonist.

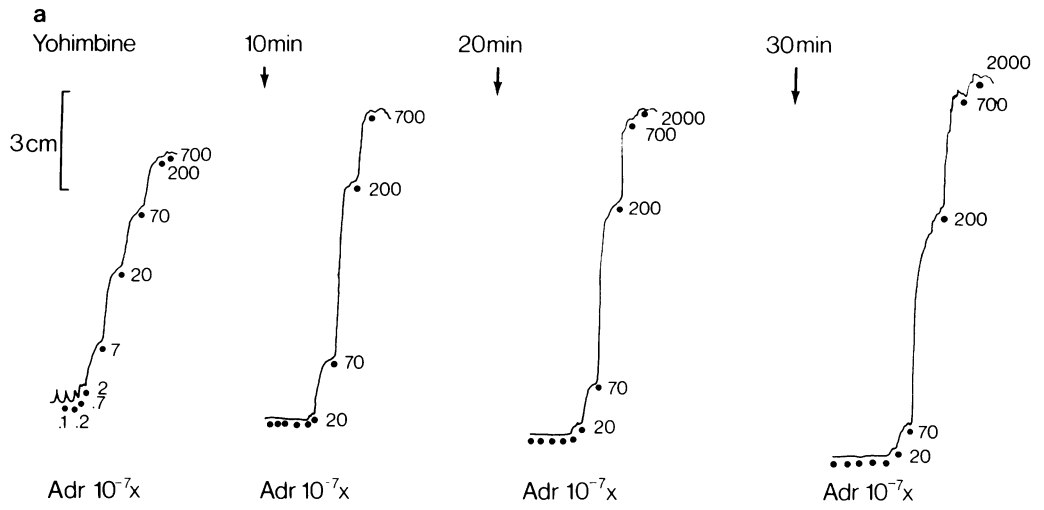
The analysis of changes induced in cumulative dose-response curves show (Figures 2a, b) that 3×10^{-5}M yohimbine in contact for 10, 20 or 30 min induced two clear effects: a reduction in the contractions elicited by the lowest doses of adrenaline (up to 70×10^{-7} M) and an increase in the response to the highest doses of agonist (Figure 2a). As a consequence, the lower part of the log dose-response curve to adrenaline was shifted to the right, and slope of the curve increased, and the maximum of the curve increased from 60% of that for barium chloride to nearly the same as the maximum response to barium (Figure 2b). A response to adrenaline which is insensitive to this concentration of yohimbine can be identified, represented by the point at which the curves for adrenaline in the absence and the presence of antagonist cross. Figure 3 shows that similar potentiations of the maximum response were obtained when the antagonists piperoxan, phentolamine, and tolazoline were used for 10, 20 or 30 minutes. When acetylcholine was used as the agonist, a potentiation of the maximum response could also be obtained, but to a lesser degree. On the other hand, no potentiation or antagonism could be recorded in 8 experiments in which propranolol in doses up to 10^{-7} M, was used instead of the α-antagonists.

Figure 4 shows the potentiation of effects of adrenaline after incubation with a lower dose of yohimbine (3×10^{-6} M), during 1, 10, 20 and 30 min, respectively. The development of potentiation of the maximum effect of the agonist is slower than in Figure 2b in which the effect of a dose 10 times larger of yohimbine was represented. The effect of yohimbine on a range of effects induced by six lower doses of adrenaline is also illustrated. After 1 min no potentiation occurs, but a slight antagonism can be already noticed for all the doses of adrenaline, except the highest (3×10^{-4} M). A slow increase in maximum effect of adrenaline occurs as the incubation with antagonist is prolonged, and as a consequence the initial antagonism shown for the effects of 3×10^{-6} and 10^{-5} M adrenaline is overcome by potentiation. This result corroborates the result shown in Figure 1b, in which an initial decrease of adrenaline effect was followed by potentiation.

Experiments were also performed with higher doses of yohimbine and piperoxan in order to see if the maximum reponse to catecholamines could be increased above that to barium. Although this effect could be obtained in individual experiments, in general the potentiations with 3×10^{-5} M yohimbine and 10^{-5}M piperoxan did not exceed the maximum response to barium. Higher doses induced smaller degrees of potentiation, and with concentrations of 3×10^{-4} or more of either yohimbine or piperoxan, the maximum response to adrenaline or noradrenaline was reduced.

The degree of potentiation for a given dose of antagonist and for a constant period of incubation varied inversely with the initial height of the dose-response curve to the agonist. The contraction obtained by saturation of α-receptors, represented in Figure 5 by the ρ ratio (Jurkiewicz *et al.*, 1969) attains an average value which is about 60% of the maximum contractile capability of the rat vas deferens to barium chloride. The individual ρ values vary about the mean with a minimum of about 25% and a maximum of 75%. Figure 5 shows that the highest potentiations were attained for the lowest dose-response curves to agonist and *vice versa*.

In order to see whether piperoxan and yohimbine actually behave as competitive antagonists, in spite of this potentiation, an analysis was made of changes, induced at equilibrium conditions, on the log dose-response curves to adrenaline and noradrenaline by a series of doses of antagonists. Such an experiment is illustrated in Figure 6 (left) in which the influence of potentiation was eliminated graphically by expressing each set of responses as percentage of its own maximum. The horizontal shift of the noradrenaline dose-response curve produced by a 10-fold increase in yohimbine concentration was less than one quarter of a log unit, though it should be about one log unit. This indicates that as well as potentiation another factor is involved, which modifies the horizontal shift of dose-response curves which would be produced by yohimbine if it was a simple competitive antagonist. Experiments were therefore performed in order to examine the shift of dose-response curves to noradrenaline, after blockade of neuronal uptake (Iversen, 1967) with 10^{-4} M cocaine. Unlike



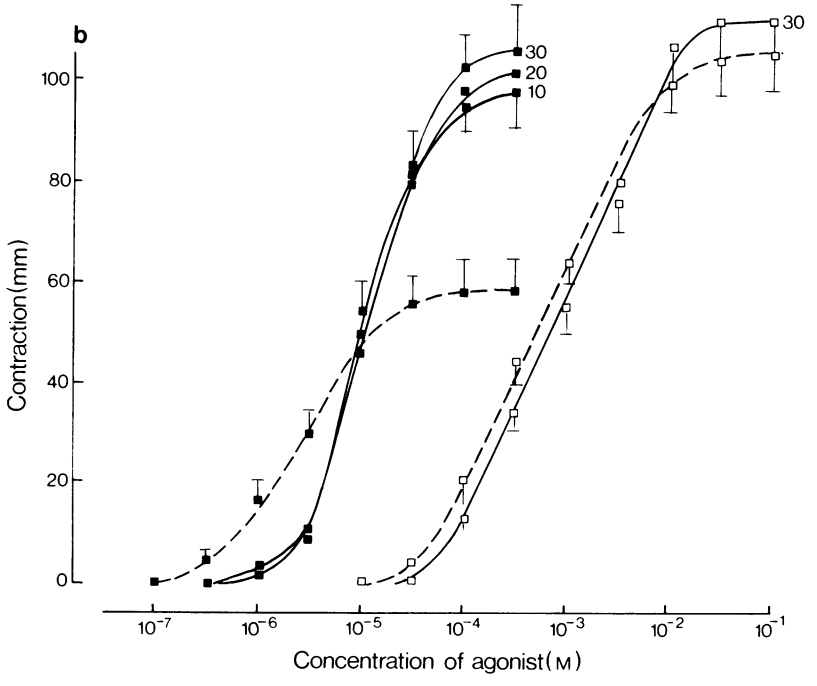


Figure 2 (a) Effect of cumulative molar concentrations of adrenaline (Adr) added at dots before and after 3 different periods of incubation with yohimbine 3×10^{-5} M (at arrows). Interval between curves was 30 to 45 minutes. (b) Mean log dose-response curves from experiments similar to (a). Responses to adrenaline (■); responses to barium (□) were tested at the beginning and end of each assay. Control responses shown by broken lines; responses in presence of yohimbine, complete lines. Time of incubation with yohimbine shown (min) at end of each curve. Points represent means of 15 experiments. Vertical lines show s.e. mean.

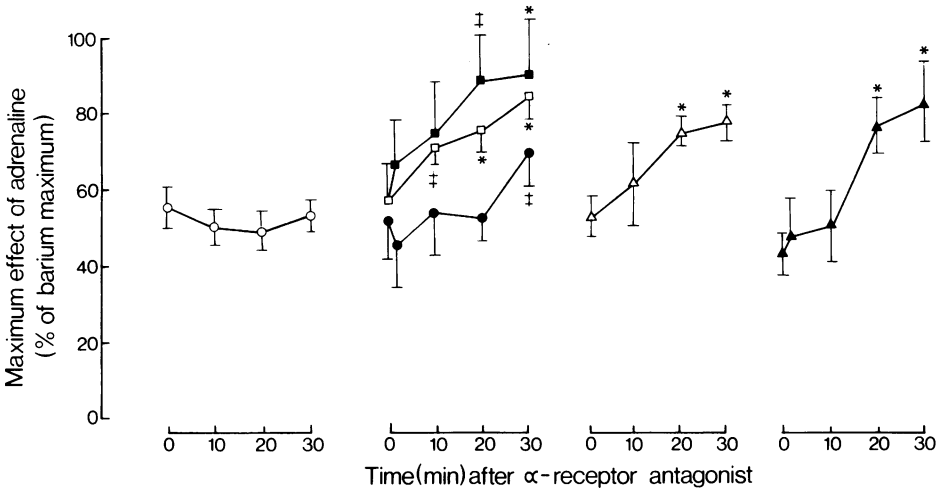


Figure 3 Variation in maximum cumulative effects of adrenaline, from experiments similar to that shown in Figure 2, after different periods of incubation without (○) and with competitive antagonists: piperoxan 3×10^{-6}, 3×10^{-6} and 3×10^{-5} M (●, ■, □), phentolamine 3×10^{-6} M (△) and tolazoline 3×10^{-6} M (▲). Maximum cumulative effect of barium chloride at the beginning of each experiment was taken as 100%. Each point is the mean of 4 to 11 experiments. Vertical lines show mean. ‡ $P < 0.05$ and * $P < 0.01$ compared to respective controls at 0 minutes.

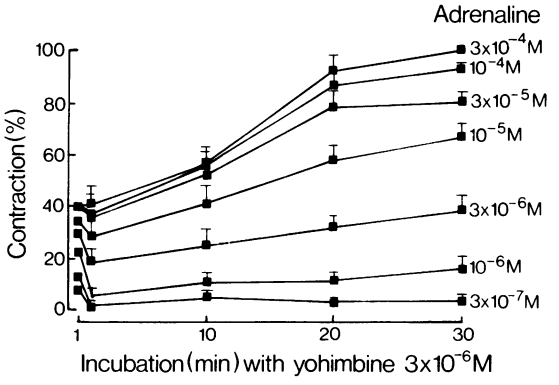


Figure 4 Variation in height of cumulative responses to adrenaline from experiments similar to that shown in Figure 2, after different periods of incubation with yohimbine 3×10^{-6} M. Maximum cumulative response to barium chloride at the beginning of each experiment was taken as 100%. Results are means from 7 experiments. Vertical lines show s.e. mean.

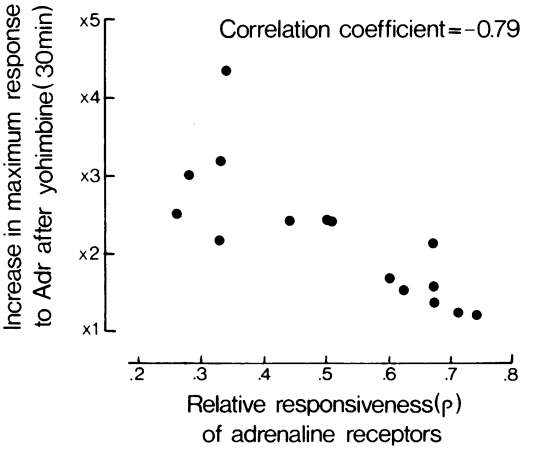


Figure 5 Negative correlation between potentiation induced by yohimbine 3×10^{-5} M, expressed as the ratio of the maximum cumulative responses to adrenaline before and after yohimbine, and the height of adrenaline control responses, expressed as the rho ratio. Each point represents a different experiment. The negative correlation is significant ($P < 0.01$).

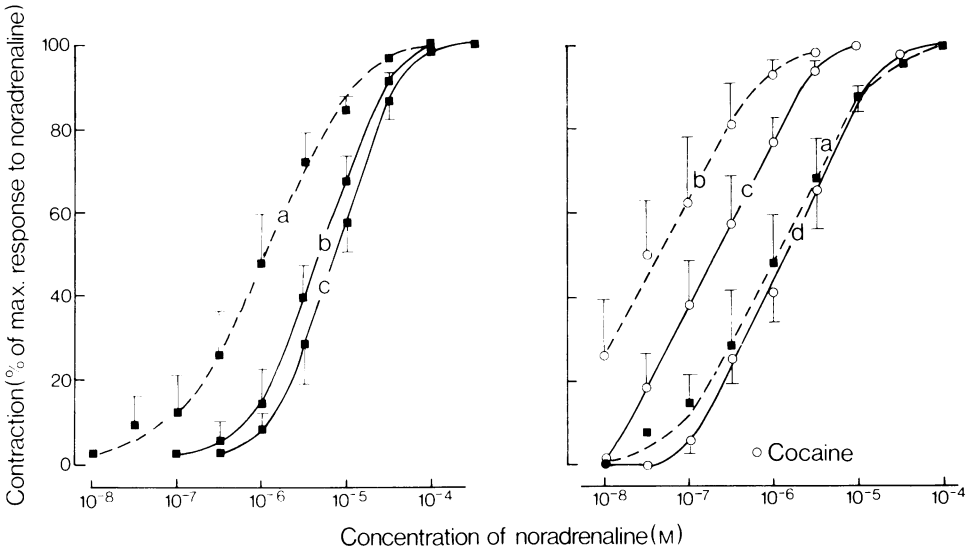


Figure 6 Mean cumulative dose-response curves for noradrenaline. Left panel—(a) control responses, (b) after yohimbine 3×10^{-6} M for 30 min, (c) after yohimbine 3×10^{-5} M for 30 minutes. Right panel—(a) control responses; cocaine 10^{-4} M was then added to the nutrient solution and maintained thereafter, (b) control responses, (c) after yohimbine 3×10^{-6} M for 30 min, (d) after yohimbine 3×10^{-5} M for 30 minutes. The effect of increasing the yohimbine concentration on the antagonism (b and c in the left panel) is greater in the presence of cocaine (c and d in the right panel). Means of 5 experiments. Vertical lines show s.e. mean.

lower and higher doses of cocaine, which can induce respectively an increase and a decrease of maximum effects of agonists (Ursillo & Jacobson, 1965; Kasuya & Goto, 1968), cocaine 10^{-4} M had no significant influence on the maximum response to noradrenaline, although it induced a shift of 1–1.5 log units to the left in the dose-response curve (Figure 6, right). After cocaine treatment the shift induced by a 10-fold increase in antagonist concentration was strikingly larger than before (Figure 6). The relation between the increment of antagonist concentration and the shift in dose-response curves was analysed by the Schild (1957) plot, according to which a slope of 1.0 is expected for the regression lines. Such a slope was obtained with antagonism of either noradrenaline or adrenaline by piperoxane, on cocaine-treated organs (Figure 7). This indicates that the antagonism exerted by the α-antagonists may be classified as competitive in rat vas deferens, though it is modified by potentiation and by the influence of removal mechanisms.

Discussion

It has been shown that so-called reversible α-adrenoceptor antagonists may either antagonize or potentiate the contractile effects of sympathomimetic agonists, depending on the doses and on the time of contact. Furthermore, it was also demonstrated that higher doses of α-blockers fail to cause a corresponding increase in the degree of antagonism (Figures 6 and 7), except when cocaine was simultaneously used. These results, when analysed on the basis of receptor theory, cannot be ascribed to a single mechanism of action of α-receptor antagonists, but indicate that several parts of the adrenaline receptor system are involved.

Adrenaline receptor system in rat vas deferens

Figure 8 pictures the receptor system as a chain of events, leading from drug-receptor interaction to the effect. It has been shown that this system is highly sensitive to the presence of calcium in the nutrient solution (Kasuya & Goto, 1968) and that more than one calcium kinetic compartment is probably involved (Jurkiewicz, Markus & Picarelli, 1975). Agonist concentration at the vicinity of receptors can be reduced by catalytic oxidation by traces of heavy metals present in the nutrient solution (Furchgott, 1955), or by removal mechanisms (Iversen, 1967; Furchgott, 1972). The possibility that sympathomimetic agonists interact with other sites besides the α-adrenoceptors proper cannot be excluded (Ganguly & Bhattacharya, 1969; Janis & Triggle, 1973).

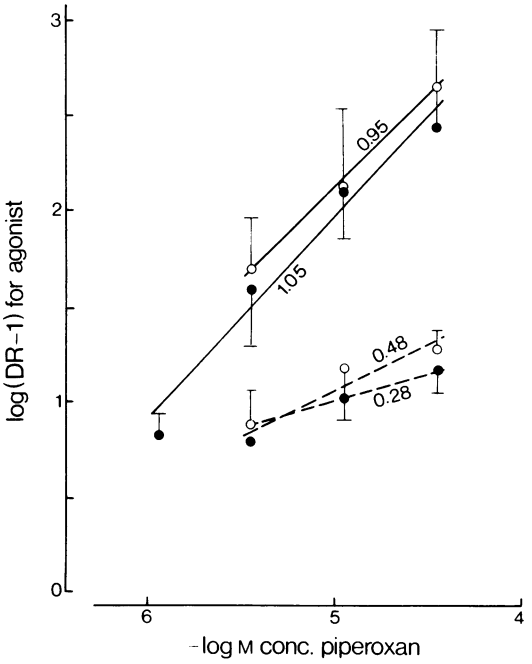


Figure 7 Schild (1957) plot for experiments similar to those shown in Figure 6. Piperoxan was used to antagonise adrenaline (O) and noradrenaline (●) with (full lines) or without (broken lines) 10^{-4} M cocaine. The slopes obtained for regression lines are indicated and were lower than the values expected according to receptor theory, except on cocaine-treated organs. Means of 3 to 5 experiments. Vertical lines show s.e. mean.

Competitive antagonism

According to occupation theory (see for example Ginsborg & Stephenson, 1974), log dose-response curves of agonists can be shifted to the right by reversible competitive antagonists, following Schild's (1957) equation. Shifts similar to those observed here, smaller than expected from theory (Figures 6 and 7), have also been described for other preparations, such as the cat nictitating membrane (Langer & Trendelenburg, 1969), and the guinea-pig trachea (Furchgott, Jurkiewicz & Jurkiewicz, 1973, 1974). A detailed review of the phenomenon was presented by Furchgott (1972). The theoretical approach assumes that a removal mechanism that can be saturated reduces agonist concentration in the biophase. The role of antagonists, by occupying α-receptors, is only to shift the range of doses of agonist up to concentrations high enough to approach saturation of the removal mechanisms. As a consequence, the relative concentration of agonist in the biophase increases and counteracts the shift due to competitive antagonism. For instance, a 10-fold increase of agonist concentration in an organ bath may represent a 20-fold increase in the biophase. When cocaine blocks a source of removal, such as uptake$_1$ (Iversen, 1967; Iversen & Langer, 1969), an increase of agonist concentration in the biophase occurs. This is represented by a leftward shift of the log dose-response curve (Figure 6), and the shifts due to α-receptor antagonism are in accordance with Schild's equation (Figure 7).

The results described here, except for the potentiation, conform to the expectations based on current models for competitive antagonism associated with a

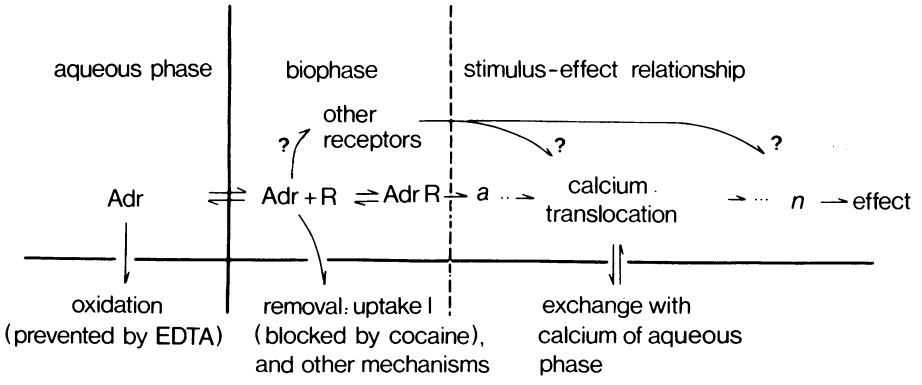


Figure 8 Schematic representation of adrenaline (Adr) receptor system in rat vas deferens, in which R represents α-receptors, AdrR the drug-receptor complex, and *a* . . . *n* the steps leading from drug-receptor interaction to effect (Jurkiewicz, Jurkiewicz & Valle, 1971b). Step *a* represents the stimulus (Ariëns, 1964). Once the stimulus is generated (*a* = efficacy × Adr R), the following steps (stimulus-effect relationship, after dotted line), are expected to occur independently of the drug efficacy. The biophase barrier (Furchgott, 1955), removal mechanisms (Furchgott, 1972), calcium translocation (Jurkiewicz, Markus & Picarelli, 1975) and other sites of interaction (Ganguly & Bhattacharya, 1969; Janus & Triggle, 1973) are indicated.

neuronal uptake (Furchgott, 1972). Other possibilities that could be drawn from isolated experiments, as for instance from Figure 7, that the α-receptor blockers act as non-competitive antagonists because of the low slopes on the Schild plot, or that experiments similar to that shown in Figure 1b are due to desensitization to α-adrenoceptor blockers (Paton, 1967), are rendered unlikely by the overall analysis of our results. For instance, the shift induced on adrenaline curves (Figure 6) does not suggest a non-competitive antagonism, and effects similar to that of Figure 1b cannot be demonstrated by reducing or increasing the dose of agonist (Figures 1a, c).

Potentiation

Kalsner (1974) has recently suggested a classification of sensitization into two types: I, represented by a leftward shift of the log dose-response curve of agonist, and II, represented by an increase in maximum response. The potentiation induced by α-receptor blockers in rat vas deferens may thus be classified as type II sensitization.

In the light of receptor theory, potentiation may be caused by at least two groups of mechanisms (Figure 8): an increase of drug concentration in the vicinity of receptors through blockade of catalytic oxidation or of removal mechanisms, and alterations, either direct or indirect, in phenomena occurring after drug-receptor interaction. Increases of drug concentration in the biophase are bound to induce only type I sensitization (Furchgott, 1972), and may thus be ruled out in the analysis of the action of α-receptor blockers. The possibility remains that such an action is due to events occurring after drug-receptor interaction, though no conclusions can be drawn as to whether it occurs at the level of the stimulus-effect relationship, or on the capability of the drug-receptor complex to generate a stimulus, i.e. on efficacy (Ginsborg & Stephenson, 1974).

Figure 5 illustrates one of the limitations of type II sensitization: even with fixed conditions, as for instance after saturation of receptors with high doses of agonist, the degree of sensitization may vary according to the relative contractile capability of the receptor system. This result corroborates those of Westfall (1970) who found larger potentiations to occur for curves with lower slopes. The fluctuations on maximum effects of adrenaline and other *full* agonists, cannot be ascribed to variations in efficacy, since they could be due to variations in stimulus-effect relationship. Therefore, the ρ ratio is more appropriate to assess the maximum effect of this full agonist, but not of partial agonists, as previously discussed (Jurkiewicz *et al.*, 1969).

Since the initial scanty information concerning potentiation of sympathomimetic effects, including that induced by α-antagonists (Nickerson, 1949; Furchgott, 1955; Emmelin, 1961), several possibilities have been raised to explain the phenomenon (Kirpekar, Cervoni & Furchgott, 1962), mostly based on alterations in steps preceding drug-receptor interaction. In vas deferens, several drugs and procedures, as for example denervation, intervene in the stimulus-effect relationship (Ariëns, 1964; Kasuya, Goto, Hashimoto, Watanabe, Munakata & Watanabe, 1969; Westfall, 1970; Trendelenburg, 1972; Evans, Iwayama & Burnstock, 1973). It has been suggested that in some instances an alteration in the availability of calcium occurs. The view has also been advanced that irreversible α-adrenoceptor antagonists may interact directly with calcium binding sites (Janis & Triggle, 1973); it is known that an increase on the degree of sensitization may be attained by increasing calcium concentration in the aqueous phase from 1.7 to 8.0 mM (Kasuya & Goto, 1968).

The question now arises whether potentiation is unspecific (Fleming, 1971) or is actually related to α-antagonism. Although the different rates of onset of antagonism and potentiation indicate that these are two separate phenomena, the possibility that noradrenaline interacts with a second receptor population cannot be excluded. These are not β-adrenoceptors, since propranolol had no significant effect under our experimental conditions, but α-autoinhibitory sites (Barnett *et al.*, 1968). These sites could be slowly blocked by α-antagonists, indirectly causing a potentiation. The possibility of different types of α-receptors in a smooth muscle preparation has been previously suggested (Dale & Gaddum, 1930; Bentley & Smith, 1967; Furness, 1974) and would help to explain differences observed by application of endogenous and exogenous catecholamines (Swedin, 1972; Pennefather, 1973). The presence of regulatory α-receptors has been indicated in the case of catecholamine release due to nerve activity (Kirpekar, Wakade, Steinsland, Prat & Furchgott, 1972; Starke, 1972; Enero & Langer, 1973). If this is the case, it is difficult to explain why the effects of acetylcholine are also potentiated. The possibility that the potentiation is due to the release of catecholamines by acetylcholine is now under investigation.

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References

ARIËNS, E.J. (ed) (1964). *Molecular Pharmacology, The mode of action of biologically active compounds*, Vol. 1, New York and London: Academic Press.

ARIËNS, E.J. (1967). The structure-activity relationships of *beta* adrenergic drugs and *beta* adrenergic blocking drugs. *Ann. N.Y. Acad. Sci.*, **139**, 606–631.

BARNETT, A., GREENHOUSE, D.D. & TABER, R.I. (1968). A new type of drug enhancement: increased maximum response to cumulative noradrenaline in the isolated rat vas deferens. *Br. J. Pharmac.*, **33**, 171–176.

BENTLEY, G.A. & SMITH, G. (1967). Effects of alpha-adrenergic receptor blocking drugs on the response of vas deferens and arterial muscle to sympathetic drugs and stimulation. *Circulation Res.*, **20**, suppl. III, 101–110.

DALE, H.H. & GADDUM, J.H. (1930). Reactions of denervated voluntary muscle, and their bearing on the mode of action of parasympathetic and related nerves. *J. Physiol.*, **70**, 109–144.

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

ENERO, M.A. & LANGER, S.Z. (1973). Influence of reserpine-induced depletion of noradrenaline on the negative feed-back mechanism for transmitter release during nerve stimulation. *Br. J. Pharmac.*, **49**, 214–225.

EVANS, B., IWAYAMA, T. & BURNSTOCK, G. (1973). Long-lasting supersensitivity of the rat vas deferens to norepinephrine after chronic guanethidine administration. *J. Pharmac. exp. Ther.*, **185**, 60–69.

FLEMING, W.W. (1971). Supersensitivity of the denervated rat diaphragm to potassium: a comparison with supersensitivity in other tissues. *J. Pharmac. exp. Ther.*, **176**, 160–166.

FURCHGOTT, R.F. (1955). The pharmacology of vascular smooth muscle. *Pharmac. Rev.*, **7**, 183–265.

FURCHGOTT, R.F. (1970). Pharmacological characteristics of adrenergic receptors. *Fedn. Proc.*, **29**, 1352–1361.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (Adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines, Handb. exp. Pharmac. N.S.*, Vol. 33, ed. Blaschko, H. & Muscholl, E., pp. 283–335. Berlin and Heidelberg: Springer-Verlag.

FURCHGOTT, R.F., JURKIEWICZ, A. & JURKIEWICZ, N.H. (1973). Analysis of propranolol antagonism to isoproterenol in guinea-pig trachea before and after blockade of active uptake. *Fedn. Proc.*, **32**, 723 Abs.

FURCHGOTT, R.F., JURKIEWICZ, A. & JURKIEWICZ, N.H. (1974). Antagonism of propranolol to isoproterenol in guinea-pig trachea: some cautionary findings. *Bioch. Pharmac.*, Suppl. pt 1, 220–224.

FURNESS, J.B. (1974). Transmission to the longitudinal muscle of the guinea-pig vas deferens: the effect of pretreatment with guanethidine. *Br. J. Pharmac.*, **50**, 63–68.

GANGULY, D.K. & BHATTACHARYA, B.B. (1969). Adrenergic beta receptors in vas deferens. *Arch. int. Pharmacodyn. Thér.*, **185**, 406–412.

GINSBORG, B.L. & STEPHENSON, R.P. (1974). On the simultaneous action of two competitive antagonists. *Br. J. Pharmac.*, **51**, 287–300.

IVERSEN, L.L. (1967). *The uptake and storage of noradrenaline in sympathetic nerves*. London: Cambridge University Press.

IVERSEN, L.L. & LANGER, S.Z. (1969). Effects of phenoxybenzamine on the uptake and metabolism of noradrenaline in the rat heart and vas deferens. *Br. J. Pharmac.*, **37**, 627–637.

JANIS, R.A. & TRIGGLE, D.J. (1973). On the question of subclassification of α-adrenoceptors. *J. Pharm. Pharmac.*, **25**, 263–264.

JURKIEWICZ, A., JURKIEWICZ, N.H., BARROS, G.G. & VALLE, J.R. (1969). Relative responsiveness (ρ) of pharmacological receptor systems in the rat vas deferens. *Pharmacology*, **2**, 89–99.

JURKIEWICZ, N.H., JURKIEWICZ, A. & VALLE, J.R. (1971a). Aumento nos efeitos máximos de alguns agonistas pelo emprego de piperoxane e ioimbina no conduto deferente de rato. *Ciencia e Cultura (São Paulo)*, **23**, 384.

JURKIEWICZ, N.H., JURKIEWICZ, A. & VALLE, J.R. (1971b). Contractile reserve after maximum doses of full agonists on the rat vas deferens. *Pharmacology*, **5**, 129–144.

JURKIEWICZ, A., MARKUS, R.P. & PICARELLI, Z.P. (1975). Effect of full agonists following calcium deprivation in rat vas deferens. *Eur. J. Pharmac.*, **32**, 292–304.

KALSNER, S. (1974). A new approach to the measurement and classification of forms of supersensitivity of autonomic effector responses. *Br. J. Pharmac.*, **51**, 427–434.

KASUYA, Y. & GOTO, K. (1968). The mechanism of supersensitivity to norepinephrine induced by cocaine in rat isolated vas deferens. *Eur. J. Pharmac.*, **4**, 355–362.

KASUYA, Y., GOTO, K., HASHIMOTO, H., WATANABE, H., MUNAKATA, H. & WATANABE, M. (1969). Nonspecific denervation supersensitivity in the rat vas deferens *in vitro*. *Eur. J. Pharmac.*, **8**, 177–184.

KIRPEKAR, S.M., CERVONI, P. & FURCHGOTT, R.F. (1962). Catecholamine content of the cat nictitating membrane following procedures sensitizing it to norepineprhine. *J. Pharmac. exp. Ther.*, **135**, 180–190.

KIRPEKAR, S.M., WAKADE, A.R., STEINSLAND, O.S., PRAT, J.C. & FURCHGOTT, R.F. (1972). Inhibition of the evoked release of norepinephrine (NE) by sympathomimetic amines. *Fedn. Proc.*, **31**, 566 Abs.

LANGER, S.Z. & TRENDELENBURG, U. (1969). The effect of a saturable uptake mechanism on the slopes of dose-response curves for sympathomimetic amines and on the shifts of dose-response curves produced by a competitive antagonist. *J. Pharmac. exp. Ther.*, **167**, 117–142.

MARTINS, T. & VALLE, J.R. (1939). Endocrine control of the motility of the male accessory genital organs. *Endocrinology*, **25**, 80–90.

NICKERSON, M. (1949). The pharmacology of adrenergic blockade. *Pharmac. Rev.*, **1**, 27–101.

OHLIN, P. & STRÖMBLAD, B.C.R. (1963). Observations on the isolated vas deferens. *Br. J. Pharmac. Chemother.*, **20**, 299–306.

PATON, W.D.M. (1967). Adrenergic receptors viewed in light of general receptor theories. *Ann. N.Y. Acad. Sci.*, **139**, 632–644.

PENNEFATHER, J.N. (1973). A comparison of the responses of the isolated vas deferens of the rat to field stimulation and to noradrenaline added cumulatively and non-cumulatively. *Eur. J. Pharmac.*, **23**, 245–250.

SCHILD, H.O. (1957). Drug antagonism and *pAx. Pharmac. Rev.*, **9**, 242–246.

SNEDECOR, G.W. & COCHRAN, G.C. (1967). Statistical methods. 6th ed. Iowa: Iowa State University Press.

STARKE, K. (1972). Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves: evidence for an α-receptor-mediated feed-back inhibition of noradrenaline release. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **275**, 11–23.

SWEDIN, G. (1972). Postnatal development of the mechanical response of the isolated rat vas deferens to nerve stimulation. *Acta physiol. scand.*, **84**, 217–223.

TRENDELENBURG, U. (1972). Factors influencing the concentration of catecholamines at the receptors. In *Catecholamines, Handb. exp. Pharmac. N.S.*, Vol. 33, ed. Blaschko, H. & Muscholl, E., pp. 726–761. Berlin and Heidelberg: Springer-Verlag.

URSILLO, R.C. & JACOBSON, J. (1965). Potentiation of norepinephrine in the isolated vas deferens of the rat by some CNS stimulants and antidepressants. *J. Pharmac. exp. Ther.*, **148**, 247–251.

WESTFALL, D.P. (1970). Nonspecific supersensitivity of the guinea-pig vas deferens produced by decentralization and reserpine treatment. *Br. J. Pharmac.*, **39**, 110–120.

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