Br. J. Pharmac. (1974), 51, 441-446

INVESTIGATIONS ON THE MODE OF ACTION OF ERGOTAMINE IN THE ISOLATED FEMORAL VEIN OF THE DOG

E. MÜLLER-SCHWEINITZER & E. STÜRMER

Department of Pharmacology, Biological and Medical Research Division, Sandoz Ltd, Basle, Switzerland

1 Experiments on spiral strips cut from the femoral vein of dogs suspended in Krebs-Henseleit solution were carried out.

2 Ergotamine caused stimulation in concentrations about 350 times lower than noradrenaline (ED_{50} of ergotamine = 2.2×10^{-9} M; ED_{50} of noradrenaline = 7.6×10^{-7} M), but the maximal responses to ergotamine were only about one third those to noradrenaline.

3 The pA_2 value of ergotamine against noradrenaline was 8.8.

4 The effects of ergotamine can be blocked by prior administration of phentolamine. The pA_2 value for phentolamine against ergotamine was 6.8 and the pA_2 value for phentolamine against noradrenaline was 7.5.

5 It is concluded that the stimulant action of ergotamine on smooth vascular muscle probably is mediated mainly via α-adrenoceptors.

Introduction

Dale (1906) considered that the vasoconstriction produced by ergotoxine is due to a direct effect on smooth muscle. Similarly, after Stoll isolated ergotamine in 1918 (1945), its stimulant effect in many different test preparations was regarded as a direct effect on smooth muscle cells (Rothlin & Brügger, 1945; Rothlin, 1946). This view was supported by reports of Biörck (1947), King (1947) and Nickerson (1949) that *in vivo*, ergotamine maintained a stimulant effect on blood pressure after treatment of animals with dibenamine. Using the technique of selective receptor protection described by Furchgott (1954) on strips of rabbit aorta, Innes (1962) first demonstrated that the stimulant action of ergotamine on the smooth vascular muscles is mediated at least in part by α-adrenoceptors. These findings were largely confirmed by other studies on isolated canine veins (Guimarães & Osswald, 1969), perfused hind limb of the dog (Osswald, Guimarães & Garret, 1970), isolated spleen and nictitating membrane of the cat (Salzmann, Pacha, Taeschler & Weidmann, 1968) and on vertebrate melanophores (Goldman & Hadley, 1970). All these results indicate that ergotamine exerts its agonistic and antagonistic effects via α-adrenoceptors.

Using isolated strips of vein, we set out to study in more detail the peripheral mechanism of action of ergotamine. Part of this work was communicated to the Union of Swiss Societies for Experimental Biology (Müller-Schweinitzer & Stürmer, 1972).

Methods

Mongrel dogs of either sex were anaesthetized by rapid intravenous injection of pentobarbitone (50 mg/kg) and killed by intravenous injection of 100 cm^3 air. The femoral veins were carefully dissected out and immediately put into Krebs-Henseleit solution (mM): NaCl 118; KCl 4.7; $MgSO_4 . 7H_2O$ 1.2; $CaCl_2 . 2H_2O$ 2.5; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 11) at 37° C, slipped over a Plexiglass rod, dissected free of adhering adipose and connective tissue and cut into spiral strips about 3 mm wide. Three cm segments of these strips were suspended in 10 ml organ baths containing Krebs-Henseleit solution at 37° C and constantly aerated with a mixture of 95% oxygen and 5% carbon dioxide.

The tension of the strips was recorded isometrically with an electromechanical transducer (Statham model UC 3) and a potentiometric recorder. At the beginning of the experiments, the strips were stretched to an initial tension of 0.5 grams. During the first hour this declined to about 0.2 g and then remained constant throughout the experiment.

Three or four cumulative dose-response curves (van Rossum, 1963) were first established with

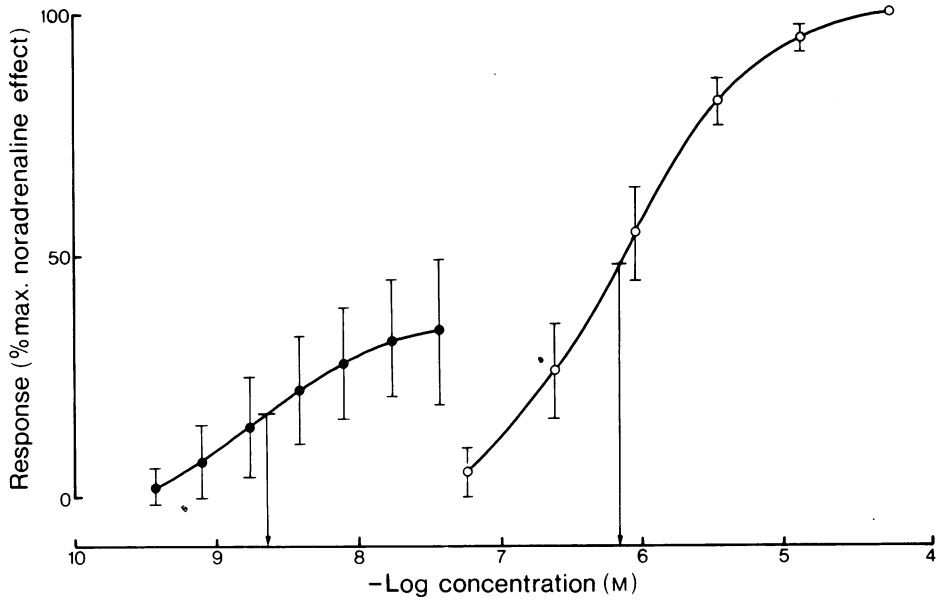

Fig. 1 Cumulative dose response curves for ergotamine (●) and noradrenaline (○). ED_{50} is represented by an arrow in each case. Organ: femoral vein of the dog. Recording: isometric. Vertical bars indicate s.e. mean.

noradrenaline at intervals of 60-90 min, until the reproducibility of the effects indicated a consistent response by the venous strips. In each case the next concentration was added when the response to the preceding dose had developed fully.

The antagonists were added 15 min before the first administration of the agonist. Prolongation of the pretreatment to 30 min did not increase the inhibition. The compounds were dissolved just before use. The effects were expressed as a percentage of the maximal response to noradrenaline before adding the antagonist. Six organ baths were used in parallel and the experiments were so arranged that each test was carried out once in each organ bath.

In each series of experiments vein strips from six dogs of either sex were used. The mean age of the dogs was 5 (2-9) years and their mean body weight was 30 (15-40) kg. Substances used were noradrenaline hydrogen tartrate (Hoechst), ergotamine tartrate (Sandoz), phentolamine (Ciba), dibenamine, phenoxybenzamine (Smith Kline and French), thymoxamine (Goedecke). The stated concentrations all refer to the base.

Results

Dose-response curves for ergotamine and noradrenaline

In a preliminary series of tests, the stimulant effect of ergotamine was compared with that of noradrenaline. When the strips were responding consistently, dose-response curves were established on each strip first for noradrenaline and then for ergotamine. Responses of the vein strips to ergotamine were slower than those to noradrenaline and took about three times longer to develop fully. Preliminary studies had shown that cumulative dose-response curves were not significantly different from those constructed from the effects of single doses of ergotamine, excluding progressive desensitization of the preparations by ergotamine.

The effects of ergotamine were expressed as percentages of the maximal response to noradrenaline for each strip. Figure 1 shows that ergotamine is effective in much lower concentrations than noradrenaline, the ED_{50} being 7.6×10^{-7} M for noradrenaline and 2.2×10^{-9} M for ergotamine.

However, the maximal response to ergotamine is only 30% of the maximal response to noradrenaline. Thus, compared with noradrenaline, ergotamine behaves like a partial agonist.

α-Adrenoceptor blockade by ergotamine

A second series of experiments was carried out to study the α-blocking activity of ergotamine on vein strips from six dogs. Addition of ergotamine 15 min before stimulating the vascular smooth muscle caused a shift of the log dose response

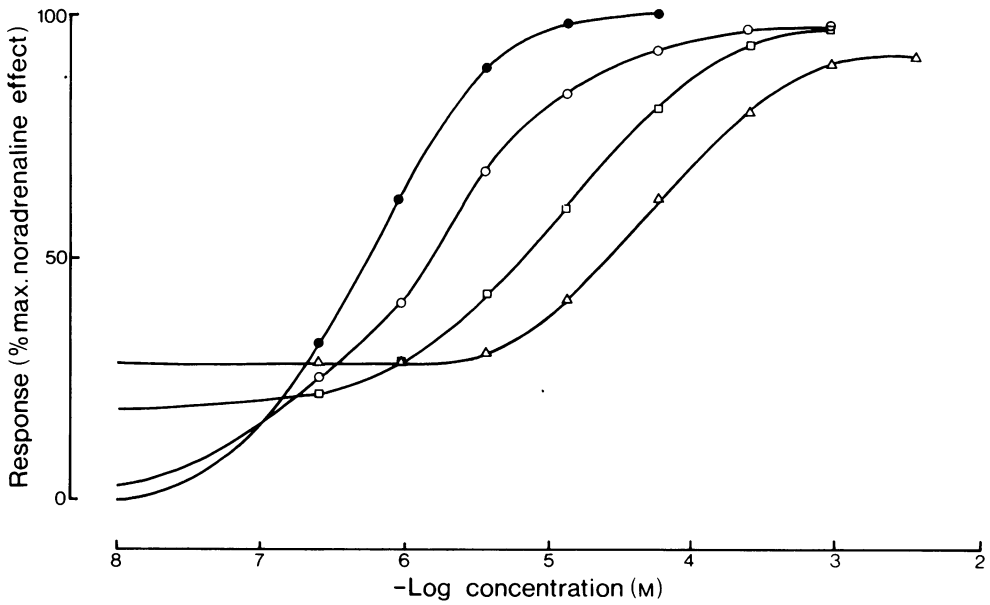

Fig. 2 Cumulative dose-response curves for noradrenaline. Organ: femoral vein of the dog. Recording: isometric. (●) control curve (n = 18); 15 min after ergotamine in the final concentrations of $10^{-8.76}$ (○), $10^{-7.76}$ (□) and $10^{-6.76}$ M (△). n = 6 for each curve.

curves for noradrenaline to the right without depressing the maximal response to any noticeable extent (Figure 2). At the same time the baseline was progressively raised due to the addition of ergotamine. These curves correspond qualitatively to curves described by van Rossum (1963) for the combined action of a full agonist and a partial agonist (competitive dualist). But, whereas theoretically all dose-response curves should cross at the same point, our curves in Fig. 2 do not do so exactly. An empirical pA_2 value of 8.8 for ergotamine-noradrenaline was calculated from the shift of the log dose-response curves at the level of 50% response of the control curve.

Blockade of the effects of ergotamine by phentolamine

In the third series of experiments the influence of the α-blocker phentolamine on dose-response curves for ergotamine and noradrenaline was investigated.

Cumulative dose-response curves of ergotamine with and without phentolamine are shown in Figure 3. Phentolamine caused a shift of the log dose-response curves of ergotamine to the right coupled with an increase in the maxima of the curves. By contrast the effect of phentolamine on noradrenaline curves was a simple shift to the right (Figure 4). From the shifts at the 50% effect levels of the control curves a pA_2 value of 7.5 for noradrenaline *versus* phentolamine and a pA_2 value of 6.8 for ergotamine *versus* phentolamine was calculated.

Additional experiments showed that the vascular smooth muscle stimulating effect of ergotamine could also be inhibited by pre-treatment with other α-adrenoceptor blocking drugs such as the competitive antagonist thymoxamine and the non-competitive antagonists phenoxybenzamine and dibenamine.

Discussion

Cumulative dose-response curves obtained with spiral strips from femoral veins of dogs showed that ergotamine is active in about 350 times lower concentrations than noradrenaline. However, it took three times longer with ergotamine than with noradrenaline for the rise in tone to develop fully. This could be due partly to differences in molecular size and hence different diffusion rates of the two compounds.

An ED_{50} of 7.6×10^{-7} M was calculated for noradrenaline, whilst the ED_{50} for ergotamine was 2.2×10^{-9} M. On the other hand, maximal responses to ergotamine in our experiments were only about one third those of noradrenaline. Thus, compared with noradrenaline, ergotamine is slow-acting and behaves as a partial agonist.

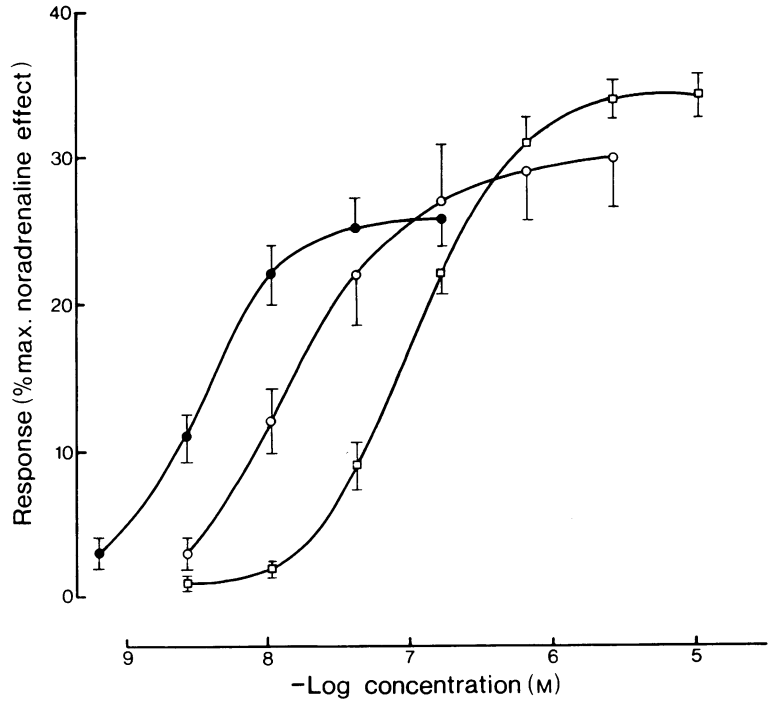

Fig. 3 Cumulative dose-response curves for ergotamine. Organ: femoral vein of the dog. Recording: isometric. (●) control curve (n = 12); 15 min after phentolamine in the final concentrations of $10^{-6.45}$ (○) and $10^{-5.45}$ M (□). n = 6 for each curve. Vertical bars indicate s.e. mean.

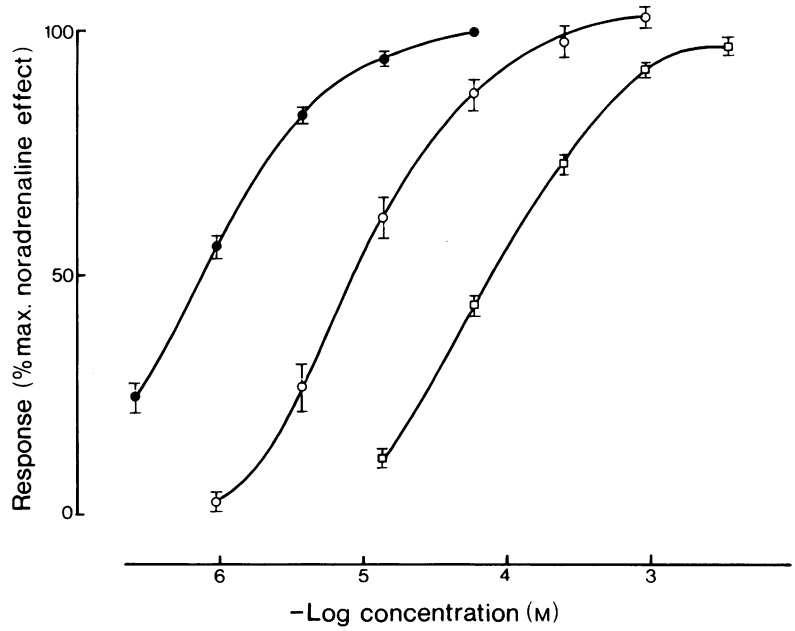

Fig. 4 Cumulative dose-response curves for noradrenaline. Organ: femoral vein of the dog. Recording: isometric. (●) control curve (n = 12); 15 min after phentolamine in the final concentrations of $10^{-6.45}$ (○) and $10^{-5.45}$ M (□). n = 6 for each curve. Vertical bars indicate s.e. mean.

The combination of increasing concentrations of ergotamine with noradrenaline produced a series of log dose-response curves starting from progressively higher baselines but with similar maxima and showing approximate parallelism in the upper reaches. These curves correspond in general shape to the theoretical curves calculated by van Rossum for the interaction of a full agonist and a partial agonist (competitive dualist). They thus support the idea that the action of ergotamine is due, at least in part, to stimulation followed by block of α-adrenoceptors. These findings are in line with former observations on rabbit aortic strips (Innes, 1962), canine veins (Guimarães & Osswald, 1969), perfused hind limb of the dog (Osswald *et al.*, 1970), isolated spleen and nictitating membrane of the cat (Salzmann *et al.*, 1970). Since in our experiments thymoxamine, phenoxybenzamine and dibenamine also caused inhibition of dose-response curves of ergotamine, the discrepancy with earlier reports from Biörck (1947), King (1947) and Nickerson (1949) describing no serious impairment of the stimulant effect of ergotamine on blood pressure in cats and dogs after pretreatment with dibenamine cannot be explained from the results reported here.

If two agonists act on the same receptor they can be expected to show the same pA_2 values if they are tested with the same competitive antagonist (Arunlakshana & Schild, 1959). We have tested in this way the effect of phentolamine using noradrenaline and ergotamine as agonists. We found that the pA_2 value phentolamine-ergotamine was 6.8 and the pA_2 value phentolamine-noradrenaline was 7.5. Whilst these differences could indicate different receptors it seems more likely that they can be explained by secondary effects connected with the long-lasting and relatively stable drug receptor bond produced by ergotamine. It would not be expected that under such conditions mass action rules which are valid for rapidly established equilibrium would strictly apply. Furthermore the different intercepts of dose-response curves for noradrenaline in combination with increasing concentrations of ergotamine and the increased maximal responses to ergotamine in combination with phentolamine suggest that ergotamine may also have other effects on vascular smooth muscle such as effects on 5-hydroxytryptamine receptors or stimulation of prostaglandin synthesis, as recently shown for dihydroergotamine (Müller-Schweinitzer, 1973), which could account for some of the discrepancies observed.

The authors wish to express their appreciation to Professor H.O. Schild for his advice and critical review of this manuscript.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48-58.

BIÖRCK, G. (1947). Studies on the action of dibenzyl-β-chlorethylamine. *Acta physiol. scand.*, **14**, 174-185.

DALE, H.H. (1906). On some physiological actions of ergot. *J. Physiol., Lond.*, **34**, 163-206.

FURCHGOTT, R.F. (1954). Dibenamine blockade in strips of rabbit aorta and its use in differentiating receptors. *J. Pharmac. exp. Ther.*, **111**, 265-284.

GOLDMAN, J.M. & HADLEY, M.E. (1970). Direct agonistic and antagonistic effects of ergotamine on vertebrate melanophores. *Arch. int. Pharmacodyn.*, **183**, 239-246.

GUIMARÃES, S. & OSSWALD, W. (1969). Adrenergic receptors in the veins of the dog. *Eur. J. Pharmac.*, **5**, 133-140.

INNES, I.R. (1962). Identification of the smooth muscle excitatory receptors for ergot alkaloids. *Br. J. Pharmac. Chemother.*, **19**, 120-128.

KING, T.O. (1947). A comparative study of sympatholytic drugs. *Fedn. Proc.*, **6**, 345.

MÜLLER-SCHWEINITZER, E. & STÜRMER, E. (1972). Investigations on the mode of action of ergotamine in the isolated femoral vein of the dog. *Experientia*, **28**, 743.

MÜLLER-SCHWEINITZER, E. (1973). Investigations on the mode of action of dihydroergotamine in human saphenous and dog saphenous and femoral veins. *Arch. Parmac.*, **279**, Suppl. R44.

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

OSSWALD, W., GUIMARÃES, S. & GARRET, J. (1970). Influence of propranolol and ICI 50,172 on the cardiovascular actions of catecholamines as modified by ergotamine. *J. Pharmac. exp. Ther.*, **174**, 315-322.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves (II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters). *Arch. int. Pharmacodyn.*, **143**, 299-330.

ROTHLIN, E. (1946). Zur Pharmakologie des Sympathicolyticums Dihydroergotamin DHE 45. *Schweiz. Med. Wschr.*, **76**, 1254-1259.

ROTHLIN, E. & BRÜGGER, J. (1945). Quantitative Untersuchungen der sympathikolytischen Wirkung genuiner Mutterkornalkaloide und derer Dihydroderivate am isolierten Uterus des Kaninchens. *Helv. Physiol. Acta*, **3**, 519-535.

SALZMANN, R., PACHA, W., TAESCHLER, M. &

WEIDMANN, H. (1968). The effect of ergotamine on humoral and neuronal actions in the nictitating membrane and the spleen of the cat. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **261**, 360-378.

STOLL, A. (1945). Ueber Mutterkornalkaloide. *Helv. chim. Acta.* **28**, 1283-1308.

(Revised January 3, 1974)