Br. J. Pharmac. (1969), **36**, 561–570.

Studies of the effect of natural and synthetic polypeptide type ergot compounds on a peripheral vascular bed

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1. Six ergot alkaloids were tested for their effect on vascular resistance and for α-adrenergic blocking activity on the innervated perfused hind limb of the dog. The results were compared with those obtained earlier for three compounds of the ergotamine group.

2. Ergostine, dihydroergostine, l-methylergostine and dihydroergocristine resembled ergotamine, dihydroergotamine and l-methylergotamine in eliciting vasoconstriction at low vascular resistance and vasodilatation at high vascular resistance. The changeover occurred at the following "inversion points": ergostine and dihydroergostine as with ergotamine and dihydroergotamine at about 4 R.U.; l-methylergostine as with l-methylergotamine at about 2·3 R.U.; dihydroergocristine at about 1·9 R.U. [1 R.U. = 1 resistance unit = 1 mm Hg/ml. per min.]

3. l-methyldihydroergocristine consistently elicited vasodilatation (for initial vascular resistances down to 1·3 R.U.) and 5′-methylergoalanine always caused vasoconstriction (for initial values up to 5·8 R.U.).

4. Ergostine and 5′-methylergoalanine had the most powerful vasoconstrictor effect, which was of the same order of magnitude as that of ergotamine. Dihydroergostine, like dihydroergotamine, was considerably less active. Both l-methylergostine and l-methylergotamine elicited only weak vasoconstriction. Moreover, when the initial vascular resistance exceeded the critical inversion value, they elicited only weak vasodilatation. Dihydroergocristine and l-methyldihydroergocristine had the least effect on vascular resistance.

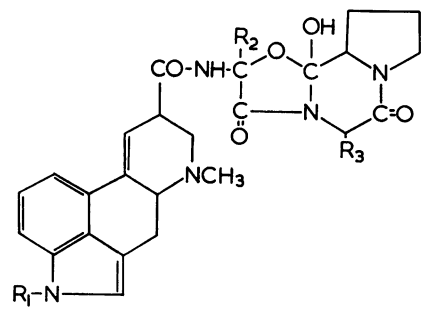
5. The increase in vascular resistance by noradrenaline was inhibited in a dose-dependent manner by all the ergot alkaloids investigated. Ergostine, 5′-methylergoalanine and ergotamine had the greatest α-adrenergic blocking activity and l-methylergostine, l-methyldihydroergocristine and l-methylergotamine the weakest. The activity of dihydroergostine, dihydroergocristine and dihydroergotamine fell between these two extremes.

6. No correlation was found between the qualitative effect of these ergot alkaloids on vascular resistance (inversion point) and (*a*) their quantitative effect on this parameter or (*b*) their α-adrenergic blocking activity. Determination of the inversion point thus provides additional pharmacological information on the vasoactive properties of the ergot alkaloids.

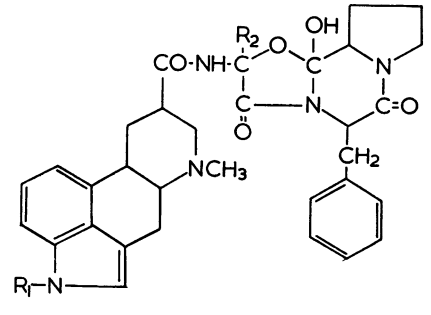
Ergot alkaloids exert various effects on the circulation by action at different sites (Rothlin, 1923; Rothlin 1946/47; Rothlin & Cerletti, 1949; Konzett & Rothlin, 1953; Cerletti & Berde, 1969). In addition to their greater or lesser central effect on vascular tone, the alkaloids mainly exert an α-adrenergic blocking action and a direct effect on vascular tone, this latter effect varying within wide limits for the different alkaloids. With the aim of elucidating the action on the peripheral circulation, three ergot alkaloids—namely, ergotamine, dihydroergotamine and l-methylergotamine—were investigated in previous studies on the perfused hind limb of the dog (Aellig, 1967). This preparation permits an assessment of purely peripheral activity in a mainly cutaneous and muscular vascular bed. All three substances displayed both vasoconstrictor and vasodilator properties in this vascular bed, and the nature of the vascular reaction did not depend upon the dose of alkaloid but upon the pre-existing vascular resistance. In the first part of the present study, three more ergot substances—namely, ergostine, dihydroergostine and l-methylergostine—were tested using this same test preparation. Since all three likewise showed vasoconstrictor and vasodilator activity, three further ergot alkaloids were included in the study which were conspicuous for their pronounced vasoconstrictor (5′-methylergoalanine) or vasodilator (dihydroergocristine and l-methyldihydroergocristine) properties in other tests (Fig. 1).

Methods

Both hind limbs of the dog were perfused *in situ* at constant and identical flow rates with blood taken from the abdominal aorta and fed into the femoral arteries



	R_1	R_2	R_3
Ergotamine	H	CH_3	CH_2—
l-Methyl-ergotamine	CH_3	CH_3	CH_2—
Ergostine	H	CH_2-CH_3	CH_2—
l-Methyl-ergostine	CH_3	CH_2-CH_3	CH_2—
5′-Methyl-ergoalanine	H	CH_3	$CH\langle^{CH_3}_{CH_3}$



	R_1	R_2
Dihydroergotamine	H	CH_3
Dihydroergostine	H	CH_2-CH_3
Dihydroergocristine	H	$CH\langle^{CH_3}_{CH_3}$
l-Methyl-dihydroergocristine	CH_3	$CH\langle^{CH_3}_{CH_3}$

FIG. 1. Structural formulae of the ergot alkaloids investigated and of those alkaloids of the ergotamine group with which they are compared.

by means of a peristaltic pump operating at a constant rate. The perfusion pressure recorded in each of the femoral arteries was then a measure of the vascular resistance in the given hind limb. The ergot alkaloid under investigation was administered as an intra-arterial infusion during 25 min to one limb only. Changes in the vascular resistance of this limb, corrected for any fluctuations in the untreated (control) limb, were taken to represent the peripheral vascular effects of the ergot alkaloids. Small spontaneous pressure fluctuations generally followed a parallel course in the two limbs. Hence by taking into account changes occurring in the control limb, it was possible to determine the actual change of blood pressure induced by these substances. It was assumed that the effect of the alkaloid was, for all practical purposes, confined to the vascular bed of the treated limb. A check was kept on possible systemic effects by recording the mean aortic pressure and heart rate during the experiment. The peripheral α-adrenergic blocking effect of the ergot alkaloids was investigated by short infusions of noradrenaline (5 min) in each femoral artery in turn, before and after the infusion of ergot alkaloid. Differences between the vasoconstrictor effect of noradrenaline on the test limb corrected for alterations in noradrenaline sensitivity in the control limb during the same period were a measure of the α-adrenergic blocking properties of the ergot alkaloid. The method has been described fully elsewhere (Aellig, 1967), hence only the essential details will be given here.

Animals

Mongrel dogs of either sex, body weight 10–18 kg, were used.

Anaesthesia

Anaesthesia was induced with thiopentone (25 mg/kg intravenously) followed by intubation and administration of 1·1–1·5% halothane in oxygen. Succinylcholine was given by intravenous infusion into a jugular vein (30 μg/kg per min in glucose) as a muscle relaxant.

Blood pressure measurement

The blood pressures were measured by means of Statham Pressure Transducers (Model P 23 AC). The aortic pressure was recorded via a polyethylene catheter inserted through an exposed brachial artery into the aorta. The pressures in the perfused femoral arteries were measured via side arms in the perfusion system.

Perfusion

A Harvard peristaltic pump (Model 600–1200 VDC) was employed for perfusion. The flow rate was generally 4–5 ml./min per kg body weight. To prevent blood clots from forming in the extracorporeal circulation, heparin was constantly added to the perfused blood to give a concentration of 0·217 i.u. heparin/ml. blood.

Dosage

The ergot alkaloids were administered in such a way that their effect was confined to the test limb. The dose of noradrenaline was adjusted so as to bring about a 25–50% increase in resistance. Generally some 10–40 μg of L-noradrenaline base was administered per ml. of perfused blood.

Substances

The ergot alkaloids were employed in the following forms: ergostine base, dihydroergostine methanesulphonate (0·86), 1-methylergostine hydrogen tartrate (0·8), 5′-methylergoalanine sulphate (0·87), dihydroergocristine methanesulphonate (0·86), 1-methyldihydroergocristine methanesulphonate (0·87), ergotamine tartrate (0·88), dihydroergotamine methanesulphonate (0·87), 1-methylergotamine tartrate (0·89). (The figures given in brackets are conversion factors for calculating the weight of free base.)

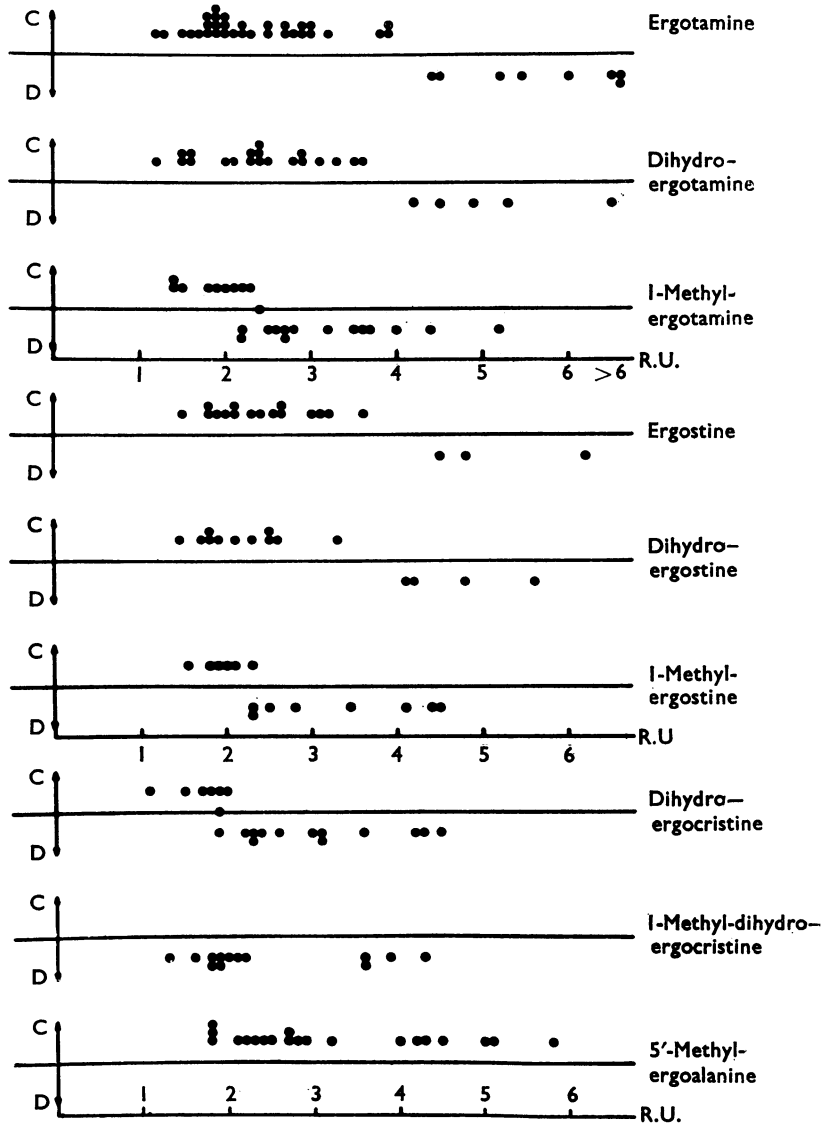


FIG. 2. Dependence of the nature of the vascular reaction upon the pre-existing vascular resistance. C, Constriction; D, dilatation; 1 R.U., 1 resistance unit = 1 mm Hg/ml. per min. Results for ergotamine, dihydroergotamine and 1-methylergotamine after Aellig (1967).

Results

Qualitative effect on vascular resistance (nature of the vascular reaction) as a function of the initial vascular resistance

Figure 2 shows the initial resistance (perfusion pressure/blood flow) in resistance units (1 R.U. = 1 mm Hg/ml. per min = 8×10^4 dyn sec cm^{-5}) for all the experiments and indicates whether vasoconstriction (C) or vasodilatation (D) occurred. The magnitude of the observed effect was not taken into account. In addition to the ergot alkaloid infusions administered as described above, this figure also includes the results obtained on administering substances to the control limb at the end of the experiments. Here, the perfusion flow rate was reduced in some cases to afford a higher resistance. These infusions in the control limb merely served to determine the qualitative nature of the reaction at a given initial level of resistance; they could not, however, yield quantitative data, because no untreated "control" limb was available.

The vasoconstrictor effect of ergostine, dihydroergostine, 1-methylergostine and dihydroergocristine at low vascular resistance was found to become a vasodilator effect once the vascular resistance rose to a certain level, the "inversion point".

The inversion points for the three substances of the ergostine group were of the same order as the inversion points for the corresponding alkaloids of the ergotamine group (that is, approximately 4 R.U. for ergostine and dihydroergostine and for ergotamine and dihydroergotamine; similarly they were approximately 2·3 R.U. for 1-methylergostine and 1-methylergotamine).

Of the other three ergot alkaloids investigated only dihydroergocristine showed an inversion point. It was situated at approximately 1·9 R.U., which was considerably lower than the inversion point for dihydroergotamine and dihydroergostine. The two other substances did not display ambivalent activity: 1-methyldihydroergocristine elicited vasodilatation in all cases (at initial vascular resistance values down to 1·3 R.U.), 5′-methylergoalanine invariably caused vasoconstriction (at initial vascular resistance values up to 5·8 R.U.). The vascular activity of dihydroergocristine and 1-methyldihydroergocristine in doses which did not affect the control limb was so low that it was only possible to decide whether they caused vasoconstriction or vasodilatation at the given level of vascular resistance by giving an infusion at a higher dose rate (5 μg/kg per min) in the control limb at the end of the experimental procedure. One experiment in this series is particularly interesting: at an initial vascular resistance of 1·9 R.U. (the inversion point) infusion of dihydroergocristine 5 μg/kg per min elicited no change in perfusion pressure.

Quantitative effect on vascular resistance as a function of the dose administered

The percentage change in initial vascular resistance brought about by infusing ergot alkaloids is shown in Fig. 3 for all the experiments. From the mean values the dose-effect curves were constructed by Askovitz's method (1957). In the doses employed, dihydroergocristine and 1-methyldihydroergocristine elicited only an insignificant change in vascular resistance. For this reason, mean values and dose effect curves are not given for these compounds. (Infusion of these substances in higher doses did elicit an effect on the vascular resistance, but also affected the control limb and aortic pressure.)

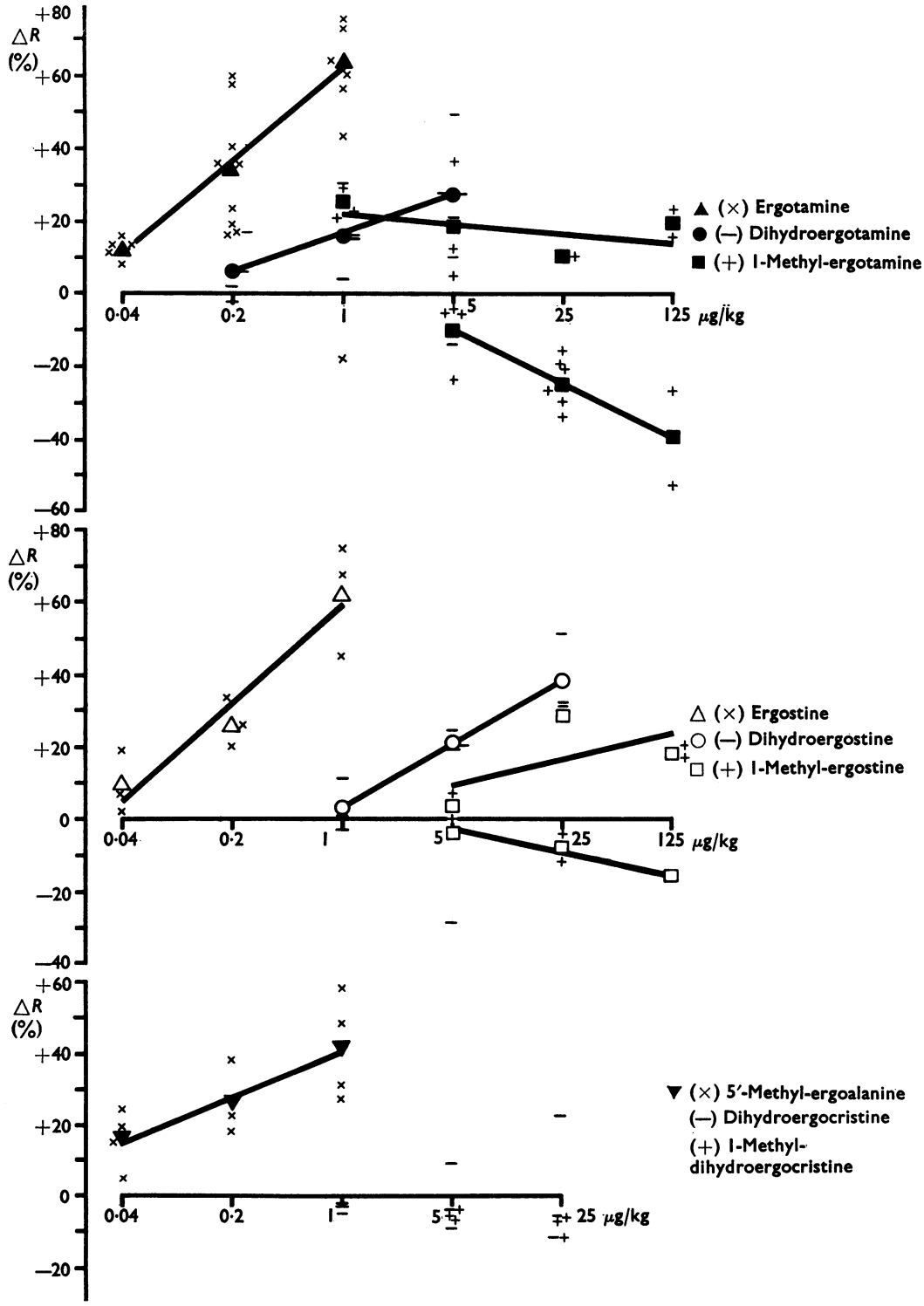


FIG. 3. Dose-dependence of the magnitude of the change in vascular resistance (ΔR[%]) elicited by intra-arterial infusion of ergot alkaloids. ▲●■△○□▼, Mean values of all experiments at the given dose-level, leading to vasoconstriction or vasodilatation. ×−+, Results of the individual experiments. Results for ergotamine, dihydroergotamine and 1-methylergotamine after Aellig (1967).

Well defined dose dependence was observed for the vasoconstrictor actions of ergostine, dihydroergostine and 5′-methylergoalanine. The vasoactivity of ergostine and dihydroergostine was of the same order of magnitude as that of the corresponding alkaloids of the ergotamine group. Here, too, the dihydrogenated compound was appreciably less potent. As in the previous study, the 1-methylated compound was the least active and the results obtained with it showed the greatest scatter. But this compound was the only one for which it was possible to construct a dose-effect relation for vasodilator activity, although the relation has a very flat slope. Owing to the higher " inversion points " of the other substances, vasodilatation was induced in too small a number of experiments to permit a relationship to be plotted.

The vasoconstrictor activity of 5′-methylergoalanine was of the same order of magnitude as that of ergotamine and ergostine. However, the dose-effect curve is rather flatter.

In ten control experiments, the animals were given a 25 min infusion of physiological saline 0·5 ml./min instead of an ergot alkaloid infusion. The maximum changes in vascular resistance which it produced were relatively small: mean +2·8%, with a standard deviation of ±4·9% (individual readings: +6, +3, +7, −3·5, +6·5, −2, +5·5, +7·5, +4, −6%).

α-Adrenergic blocking activity, as tested by inhibition of noradrenaline vasoconstriction

The percentage change elicited by the ergot alkaloids in the vasoconstrictor response to noradrenaline infusions is shown in Fig. 4 for all the experiments (dose-effect relations constructed by Askovitz's method (1957)). In the ten control experiments using physiological saline instead of the ergot alkaloids, the mean change in the response of intra-arterial noradrenaline infusions was +2·0%, with a standard deviation of ±6·7% (individual values: +5, −10, +6, +4, +10, +0·5, +2, ±0, +10, −8%).

A dose-dependent α-adrenergic blocking action was observed with all the alkaloids investigated. The magnitude of the blocking activity was of the same order of magnitude for the corresponding compounds in the ergostine and ergotamine series. Ergostine and ergotamine were the most powerful, and 1-methylergostine and 1-methylergotamine the least potent. It will be seen that the dose effect relations for the 1-methylated compounds are relatively flat and that there were considerable individual differences in the sensitivity of the different animals. Of the alkaloids investigated in this study, only ergostine showed any tendency to reinforce the vasoconstrictor action of noradrenaline (one out of the three experiments at a dose level of 0·04 μg/kg).

At 1 μg/kg 5′-methylergoalanine was roughly equipotent with ergostine and ergotamine in its α-adrenergic blocking activity; but in none of the experiments did it reinforce noradrenaline vasoconstriction.

Dihydroergocristine inhibited the effect of the noradrenaline infusions to about the same extent as dihydroergostine and dihydroergotamine, while 1-methyldihydroergocristine was approximately as potent as 1-methylergostine and 1-methylergotamine.

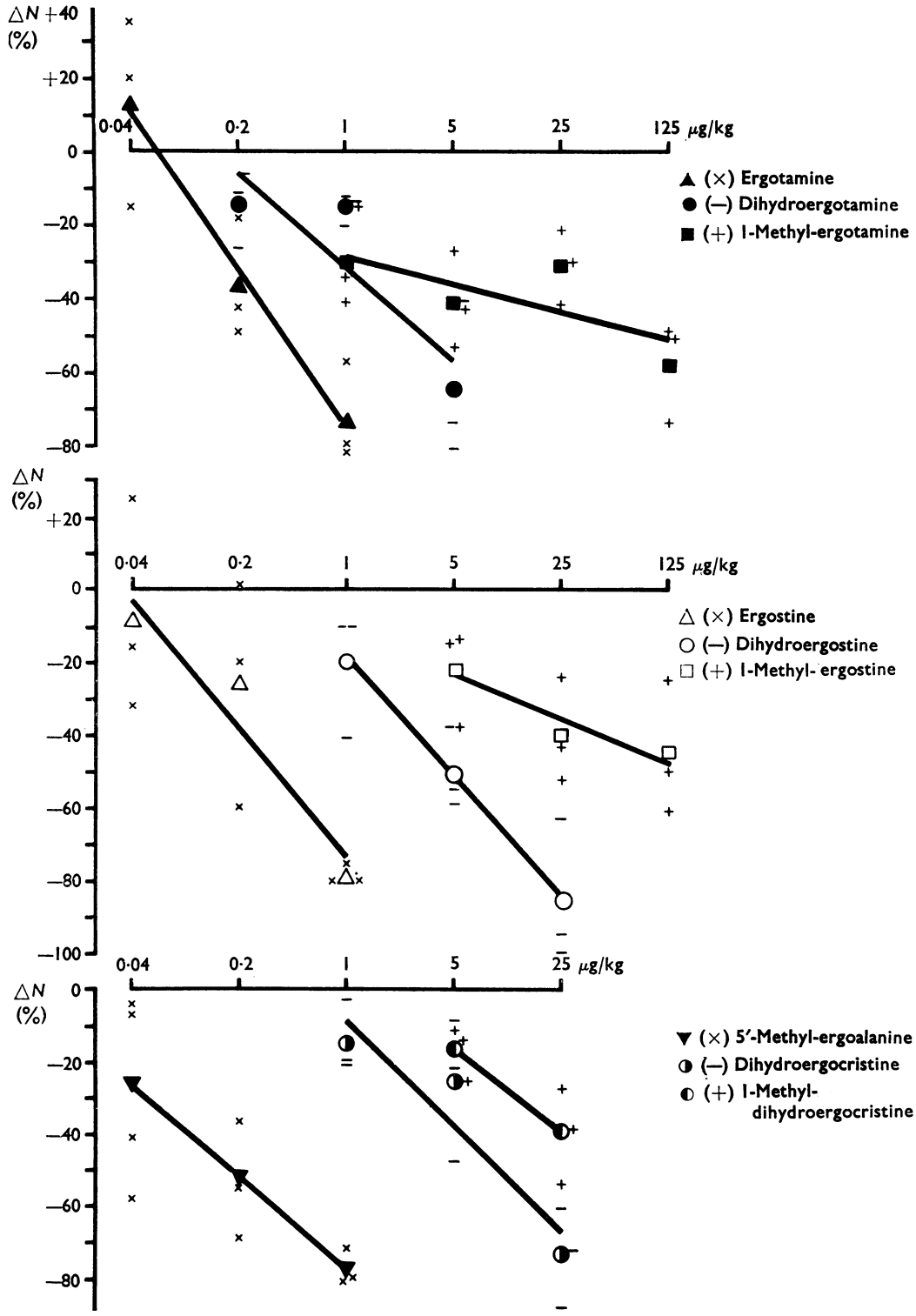


FIG. 4. Effect of intra-arterial infusion of ergot alkaloids on the vasoconstrictor action of intra-arterial infusion of noradrenaline. ▲●■△○□▼◑◐, Mean values of all experiments at the given dose-level. × — +, Results of the individual experiments. Results for ergotamine, dihydroergotamine and l-methylergotamine after Aellig (1967).

Discussion

As far as their qualitative effect on the vascular resistance of the innervated, perfused hind limb of the dog is concerned, four of the six ergot alkaloids investigated in this series of experiments displayed the same vasoconstrictor and vasodilator properties that had previously been described for ergotamine, dihydroergotamine and 1-methylergotamine (Aellig, 1967). Like ergotamine and dihydroergotamine, ergostine and dihydroergostine elicited vasoconstriction in all experiments at a vascular resistance below 4 R.U.: at higher vascular resistances they caused vasodilatation. The inversion point for 1-methylergostine was 2·3 R.U., as for 1-methylergotamine. The three alkaloids of the ergostine group resemble those of the ergotamine group in this respect, whereas the two compounds of the ergocristine group tested were characterized by their more marked vasodilator properties. Nevertheless, it was still possible to determine an inversion point for dihydroergocristine (at 1·9 R.U.), but 1-methyldihydroergocristine caused vasodilatation in all experiments. By contrast, 5′-methylergoalanine elicited a rise in vascular resistance in all experiments.

The magnitude of the change in vascular resistance is independent of the pre-existing vascular resistance, as was shown for alkaloids of the ergotamine group. However, the experiments showed that the vasoconstrictor effects of ergostine, ergotamine, dihydroergostine, dihydroergotamine and 5′-methylergoalanine and the vasodilator effects of 1-methylergostine and 1-methylergotamine, are dose-dependent.

In the investigation of α-adrenergic receptor blockade, the decline in α-adrenergic blocking activity of the ergotamine group was in the order: parent compound, its product of dihydrogenation in position 9–10, and the 1-methylated compound. The same order of decline in blocking activity was also found in the alkaloids investigated in this study. In the ergostine group, ergostine itself was the most potent inhibitor of vasoconstriction induced by noradrenaline infusion, while 1-methylergostine was the weakest. In the ergocristine group, dihydroergocristine had a more powerful α-adrenolytic effect than 1-methyldihydroergocristine. In drawing any qualitative conclusions, account should be taken of the considerable scatter of the individual values due to the varying sensitivity of the individual animals. Nevertheless it is clear that the α-adrenergic blocking activity of corresponding alkaloids of the ergostine and the ergotamine group are of the same order of magnitude. The activity of dihydroergocristine is of the same order as that of dihydroergostine and dihydroergotamine, while the activity of 1-methyldihydroergocristine is in the same range as that of 1-methylergostine and 1-methylergotamine. It is interesting to note that methylation of ergostine and ergotamine in position 1 leads to a more than 25-fold decrease in α-adrenergic blocking activity (measured at 40% inhibition of noradrenaline vasoconstriction), whereas methylation of dihydroergocristine in position 1 brings about a barely 5-fold decrease of activity. Experiments on the cat nictitating membrane and on the isolated perfused cat spleen (Salzmann, Pacha & Weidmann, 1967) demonstrated that small doses of ergotamine potentiated the action of noradrenaline. The reinforcement of the vasoconstrictor effect of noradrenaline observed in our experiments with ergostine (one out of three experiments at a dose level of 0·04 μg/kg) and previously with ergotamine (two out of three experiments at a dose level of 0·04 μg/kg), while not statistically significant owing to the small number of experiments, does nevertheless

appear to lend support to the findings on the cat nictitating membrane and perfused spleen.

The inversion point and the magnitude of the vasoconstrictor effect of the ergot alkaloids investigated are independent of each other. Ergostine and ergotamine have the same inversion point as dihydroergostine and dihydroergotamine. However, the dihydrogenated alkaloids are considerably weaker than the parent compounds in their effect on vascular resistance. The increase in vascular resistance elicited by 5′-methyl-ergoalanine is roughly the same as that observed with ergostine and ergotamine infusions. But whereas ergostine and ergotamine had an inversion point at 4 R.U. 5′-methylergoalanine invariably elicited vasoconstriction at initial vascular resistance levels up to 5·8 R.U.

The same applies to the relationship between the α-adrenergic blocking activity of the ergot alkaloids and their inversion point. Ergostine and ergotamine inhibited the pressor effect of noradrenaline more powerfully than dihydroergostine and dihydroergotamine, yet all four had the same inversion point. On the other hand, the α-adrenergic blocking action of ergostine and ergotamine was no more powerful than that of 5′-methylergoalanine, which invariably caused vasoconstriction. Dihydroergocristine possessed a more potent α-adrenolytic effect than 1-methylergostine and 1-methylergotamine, which had a higher inversion point. Dihydroergocristine was, however, a more potent α-adrenergic blocking agent than 1-methyldihydroergocristine, which elicited vasodilatation in all experiments. In the light of these findings, no correlation can be found between the vasoconstrictor and vasodilator effects of the ergot alkaloids and their α-adrenergic blocking activity.

The inversion point determined by the method described provides additional pharmacological information on vaso-active properties of individual ergot alkaloids.

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(*Received January* 22, 1969)