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α-Adrenergic agonist and antagonist activity of dihydroergotoxine in rats

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The α-adrenergic activity of dihydroergotoxine had been studied in both pithed and urethane or pentobarbitone anaesthetized rats. In anaesthetized rats, blood pressure effects varied with the anaesthetic agent: hypotension with urethane, hypertension with pentobarbitone. This latter pressor response was a peripheral effect. In pithed rats, the vasopressor response to dihydroergotoxine was reduced competitively by yohimbine, and non-competitively by nifedipine, but not by prazosin or methysergide, showing that the vasoconstriction is mediated by α_2-adrenoceptors. Dihydroergotoxine decreases the tachycardia elicited by stimulation of the cardioaccelerator nerves, this effect being antagonized by yohimbine. It also reduced the pressor response to (−)-phenylephrine. These results indicated that, on the peripheral vascular system of the rat, dihydroergotoxine acts as an α_1-adrenoceptor blocker and an α_2-adrenoceptor agonist.

Dihydroergotoxine (Hydergine) is an ergot preparation composed of the methane-sulphonates of dihydroergocornine, dihydroergocristine, dihydro-α-ergocryptine and dihydro-β-ergocryptine in the ratio 3:3:2:1, which has been found to be of particular therapeutic value in senile cerebral insufficiency.

Several studies have shown that dihydroergotoxine can interfere with at least three types of receptor: α-adrenergic, 5-hydroxytryptaminergic and dopmaminergic receptors (Müller-Schweinitzer & Weidmann 1978; Markstein 1983; Markstein et al 1983). At α-adrenoceptors it is considered essentially as a potent blocking agent (Rothlin 1946; Berde & Stürmer 1978; Markstein et al 1983; Müller-Schweinitzer 1982). However in-vitro, dihydroergotoxine showed partial α-adrenergic agonist activity on both arterial and venous smooth muscle (Müller-Schweinitzer 1982). In the pentobarbitone anaesthetized dog it has been found to increase blood pressure, at least in part by activation of vascular post-junctional α-adrenoceptors (Schmitt et al 1971).

Dihydroergotoxine enhances the noradrenaline release induced by sympathetic nerve stimulation, from the isolated cat spleen, rabbit heart and guinea-pig vas deferens, by blocking presynaptic, α_2-adrenoceptors. (For references see Müller-Schweinitzer & Weidmann 1978.) On saphenous vein strips, however, dihydroergotoxine, diminished the stimulation-induced noradrenaline outflow in a concentration-dependent way indicating prejunctional agonist activity (Müller-Schweinitzer 1982). In experiments in-vivo, dihydroergotoxine appears to inhibit the release of transmitter. For example, it diminished the positive chronotropic response of the heart to nerve stimulation in the pithed cat (Scholtysik 1975), by activation of prejunctional α_2-adrenoceptors (Loewe & Müller-Schweinitzer 1979).

The aim of the present investigation was to evaluate the involvement of α_1- and α_2-adrenoceptors in the vascular effects of dihydroergotoxine in the rat.

MATERIALS AND METHODS

Anaesthetized, intact rats

Male, normotensive Wistar rats (250 g) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1} i.p.) or urethane (1 g kg^{-1} i.p.). The animals were artificially respired via a tracheal cannula, attached to a Harvard Ventilator. Rectal temperature was kept at about 37 °C by placing the animals on a thermostatically controlled table. Arterial blood pressure, expressed as the mean, was taken from a cannulated common carotid artery and was recorded via a Statham P 23 Db pressure transducer. The arterial pulse wave triggered a heart ratemeter. Both arterial pressure and heart rate were recorded continuously on a Physiograph MK III. A femoral vein was cannulated for the administration of drugs.

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Pithed rats

General procedures. Male Wistar rats, 300–350 g, were lightly anaesthetized with ether. After cannulation of the trachea, the animals were pithed by inserting a steel rod into the spinal canal via the right orbit. They were then immediately ventilated with room air by a Harvard Ventilator (10 ml kg^{-1}, 50 strokes min^{-1}). Rectal temperature was kept at about 37 °C. Blood pressure, expressed as mean arterial pressure, and heart rate were recorded continuously as described for the intact rats. All animals were vagotomized and given atropine (1 mg kg^{-1} i.v.) before the start of each experiment. This was to eliminate parasympathetic interference and block muscarinic receptors. Drugs were injected into a femoral vein.

Tachycardia induced by electrical stimulation of the cardiac accelertor nerves from the spinal cord. The pithing rod was coated with enamel except for a 1 cm length 6 cm from the tip. The unprotected part of the rod was located in the region where the nervi accelerantes leave the cord (C_7-Th_1). An indifferent electrode was placed subcutaneously in the dorsum and the animals were given (+)-tubocurarine (1 mg kg^{-1} i.v.). The preganglionic nerves to the heart were electrically stimulated between the pithing rod and the indifferent electrodes with monophasic square wave pulses (11·5 V, 1 ms, 0·5, 1, 3 and 6 Hz) delivered for 30 s and applied at 2 min intervals. The parameter measured was the maximal tachycardia elicited by electrical stimulation at each frequency. Frequency–response curves were obtained before and 5 min after i.v. injection of the test drug (one dose per animal).

To demonstrate the antagonism between dihydroergotoxine and the α_2-adrenoceptor blocking agent yohimbine, the following experiment was carried out. After determination of a frequency–response curve for each animal, the rats were given 1 mg kg^{-1} i.v. yohimbine and 5 min later a new frequency–response curve was determined. After a 5 min rest, the rats were injected with dihydroergotoxine, and 5 min later a further series of electrical stimulations was given.

To determine the potency of dihydroergotoxine, the maximal inhibition (as percentage of the control) of the electrically evoked (0·5 Hz) tachycardia was plotted against the log of the dose of dihydroergotoxine. This log dose–response curve was used to determine the dose which caused a 50% reduction in the electrically induced tachycardia (ID50).

Hypertension induced by (−)-phenylephrine. The pressor effects (measured at the peak of the response) of phenylephrine, regardless of the presence of the dihydroergotoxine were studied by administering no more than three different doses of the agonist per rat, in random order. The log dose–response curves were established by plotting the log dose of the drug as a function of the increase (mmHg) in blood pressure. Each point on the curve represents the mean of 5 observations, and nine or ten rats were used for each curve. In other pithed rats, dihydroergotoxine (one dose per animal) was injected i.v. 10 min before the first agonist dose and the testing procedure was identical to that described above for untreated animals. The doses of phenylephrine which increased blood presure by 50 mmHg both in the absence and in the presence of different doses of dihydroergotoxine were estimated from the log dose–response curves.

The pressor action of phenylephrine was evaluated by considering only the pressure increases due to phenylephrine above the level induced by dihydroergotoxine. In addition we checked that after dihydroergotoxine, the increased doses of phenylephrine induced the same maximal increases in blood pressure as those seen in control rats.

Drugs used

(−)-Noradrenaline bitartrate (Sigma), (+)-isoprenaline hydrochloride (Fluka), (+)-propranolol hydrochloride (ICI Pharma) pentobarbitone sodium (Nembutal, Abbott), urethane (Rhône-Poulenc), (−)-phenylephrine hydrochloride (Koch-Light), yohimbine hydrochloride (Sigma), atropine sulphate (Rhône-Poulenc), (+)-tubocurarine chloride (Abbott), B-HT 933 (2-amino-6-ethyl-5,6,7,8-tetrahydro-4-H-oxazolo (4–5d) azepine dihydrochloride) (Boehringer Ingelheim), methysergide bimaleate (Sandoz), prazosin hydrochloride (Pfizer), dihydroergotoxine methane sulphonate (Fabre, Sandoz), nifedipine (Bayer). Nifedipine was dissolved in a 5% glucose solution containing 1% v/v polysorbate 80 and 10% v/v ethanol. Prazosin was made up in a 5% w/v glucose solution and the other drugs in 0·9% NaCl (saline). Appropriate controls were performed with the vehicle used to dissolve nifedipine. All doses mentioned refer to the free base. Nifedipine was injected into the left carotid artery, all other drugs were given i.v. in a volume of 0·5 ml kg^{-1}. When the drugs were administered in cumulative doses, subsequent doses were applied when the pressor response to the previous dose had reached a plateau.

Statistical analysis

Student's *t*-test at a 95% level of confidence was used for the statistical comparisons.

RESULTS

General cardiovascular effects of dihydroergotoxine in anaesthetized normotensive rats

In pentobarbitone anaesthetized rats, dihydroergotoxine had no significant pressor action at low doses (0·01–0·4 mg kg^{-1}) although it had a slight but significant effect at high doses (1–4 mg kg^{-1}) (Table 1) not exceeding 18 mmHg and lasting 5–10 min. In urethane treated rats dihydroergotoxine (0·1–4 mg kg^{-1}) always induced a significant ($P < 0·05$) and long-lasting drop in blood pressure (Table 1), which was not proportional to the dose. The magnitude of the response to a given dose was directly related to the resting pressure level. At the doses used, dihydroergotoxine had no influence on the positive chronotropic effects of noradrenaline and isoprenaline (0·5 and 1 μg kg^{-1} i.v.) which are antagonized by propranolol (1 mg kg^{-1} i.v.). It reduced the hypertensive effect of noradrenaline, but not the hypotensive response to isoprenaline.

Table 1. Effect of dihydroergotoxine on blood pressure in anaesthetized, normotensive rats.

Dose mg kg^{-1} i.v.	n	Blood pressure (mmHg) Pentobarbitone Control	Pentobarbitone Treated	Urethane Control	Urethane Treated
Saline 0·5 ml kg^{-1}	25	123 ± 4	121 ± 6	126 ± 5	125 ± 5
0·01	5	124 ± 9	120 ± 7		
0·1	5	133 ± 12	132 ± 11	130 ± 10	82 ± 9*
0·4	5	128 ± 8	136 ± 8	126 ± 8	90 ± 5*
1	5	110 ± 11	128 ± 9*	140 ± 3	88 ± 4*
2	5	128 ± 7	146 ± 6*	111 ± 7	82 ± 6*
4	5	112 ± 4	124 ± 6*	122 ± 10	77 ± 3*

* Significantly different from corresponding controls. $P < 0·05$.

In urethane or pentobarbitone anaesthetized rats the intravenous injection of dihydroergotoxine in a dose range from 0·01 to 2 mg kg^{-1} consistently reduced the heart rate.

Effect of yohimbine and prazosin on the pressor action of dihydroergotoxine in pithed rats

Before drug administration, the mean arterial pressure of the pithed rat was 37 ± 3 (mmHg) (mean ± s.e.m., n = 30). Injected intravenously in cumulative doses, dihydroergotoxine increased blood pressure in a dose-dependent manner (Fig. 1). Yohimbine (0·5 and 1 mg kg^{-1} i.v.) administered 5 min previously caused a parallel shift to the right of the log dose–hypertensive response curves for dihydroergotoxine, without affecting the maximal response (Fig. 1). Under the same conditions prazosin (0·1, 0·5 and 1 mg kg^{-1} i.v.) also displaced the log dose–response curves to the right (Fig. 1) but to a lesser extent and in a non dose-dependent way. A combination of both α-blockers (yohimbine 0·5 mg kg^{-1} + prazosin 0·5 mg kg^{-1}) shifted the dose–response curve to the right to the same extent as yohimbine (0·5 mg kg^{-1}) alone. The doses of dihydroergotoxine which increased blood pressure by 50 mmHg (DE50) in the absence and in the presence of yohimbine or prazosin were estimated from the log dose–response curves. These DE50 values were respectively 24 ± 5 μg kg^{-1} (n = 25) for dihydroergotoxine alone, 130 ± 19, 255 ± 25 μg kg^{-1} (n = 5) in the presence of yohimbine (0·5, 1 mg kg^{-1}) and 60 ± 10, 62 ± 13, 58 ± 14 μg kg^{-1} (n = 5) in the presence of prazosin (0·1, 0·5, 1 mg kg^{-1}).

Influence of nifedipine and methysergide on the pressor effect of dihydroergotoxine

Nifedipine (0·3 and 1 mg kg^{-1}) a calcium antagonist, shifted the dose–response curves of dihydroergotoxine to the right, but in a non-parallel manner (Fig. 2).

Methysergide (0·005 and 0·05 mg kg^{-1} i.v.) a 5-HT blocking agent (Fanchamps et al 1960) did not alter the pressor response induced by dihydroergotoxine.

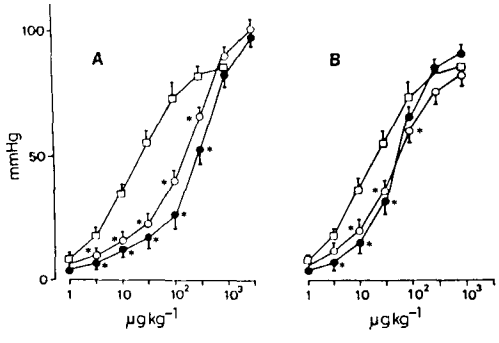

FIG. 1. Log dose–response curves with respect to the increase in mean arterial pressure of pithed rats induced by i.v. injection of dihydroergotoxine 5 min after i.v. A—saline (□), yohimbine (0·5 mg kg^{-1} ○–1 mg kg^{-1} ●); B—saline (□), prazosin (0·5 mg kg^{-1} ○–1 mg kg^{-1} ●). The data are given as the mean ± s.e.m. (n = 5). The significance of the difference from the control is represented by an asterisk (*$P < 0·05$). Ordinate = increase in blood pressure (mmHg). Abscissa = dose of dihydroergotoxine in μg kg^{-1}.

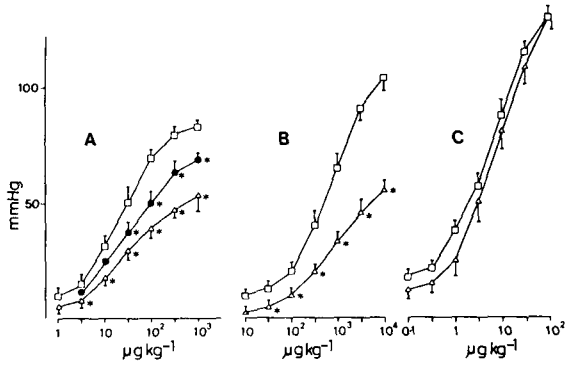

FIG. 2. Log dose–response curves with respect to the increase in mean arterial pressure of pithed rats induced by i.v. injection of dihydroergotoxine (A), B-HT 933 (B) and phenylephrine (C) 10 min after i.v. vehicle (□) and various amounts of nifedipine administered intra-arterially (0·3 mg kg^{-1} ●, 1 mg kg^{-1} △). Data are presented as mean values ± s.e.m. (n = 6). The significance of the difference from the control is represented by an asterisk (*$P < 0.05$). Ordinate = increase in blood pressure (mmHg). Abscissa = dose of agonist in μg kg^{-1}.

Effect of dihydroergotoxine on the tachycardia induced by electrical stimulation of the cardiac accelerator nerves

Dihydroergotoxine (50, 100, 200 μg kg^{-1} i.v.) had no significant effect on the spontaneous heart rate (324 ± 25 beats min^{-1}, n = 15) of the vagotomized, atropine treated, pithed rat. At the doses used, it reduced the tachycardia evoked by stimulation at the low (0·5, 1 Hz) but not at the high frequency (3, 6 Hz) (Fig. 3). The dose of dihydroergotoxine which determined graphically reduced the electrically (0·5 Hz) induced tachycardia by 50%, was found to be 30 μg kg^{-1} i.v. The reduction of the stimulation induced tachycardia by dihydroergotoxine could be antagonized in rats with the α_2-adrenoceptor blocking agent yohimbine (1 mg kg^{-1} i.v.) administered either before (Fig. 4) or after the dihydroergotoxine.

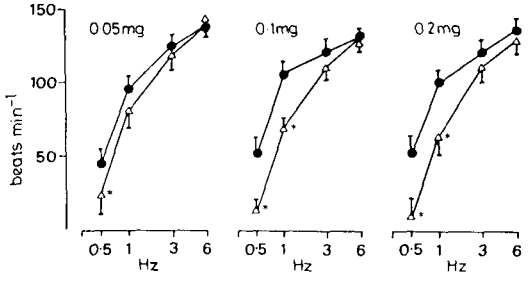

FIG. 3. Effects of i.v. dihydroergotoxine on the tachycardia produced by stimulation of the cardiac accelerator nerve at increasing frequencies in pithed rats; (●), control before injection of test drug; (△), dihydroergotoxine. The data are presented as mean values ± s.e.m. (n = 5). The significance of the difference from the control is represented by an asterisk (*$P < 0.05$). Ordinate = increase in heart rate (beats min^{-1}). Abscissa = frequency of stimulation (Hz).

Effect of dihydroergotoxine on the hypertensive response to (−)-phenylephrine

Intravenous administration of (−)-phenylephrine induced a dose-dependent increase in arterial pressure in pithed rats. Dihydroergotoxine at 100, 200 and 400 μg kg^{-1} injected 10 min previously, reduced the effects of (−)-phenylephrine, producing dose-dependent and approximately parallel shifts of the log dose–response curve to the right (Fig. 5). The agonist doses which increased mean arterial pressure by 50 mmHg in the absence and presence of different doses of dihydroergotoxine were interpolated from the dose–response curves; these were 2·1 ± 0·4 μg kg^{-1} in the absence, and 4 ± 0·5, 12·8 ± 2·6 and 35·8 ± 10 μg kg^{-1} in the presence of 100, 200, 400 μg kg^{-1} respectively of dihydroergotoxine. The differences from the corresponding controls were significant ($P < 0.05$). Under the same conditions in the presence of prazosin (0·01 mg kg^{-1} i.v.) or yohimbine (1 mg kg^{-1} i.v.) the ED50 values of phenylephrine were 28 ± 2 μg kg^{-1} and 5·9 ± 1 μg kg^{-1} respectively.

DISCUSSION

In pentobarbitone anaesthetized normotensive rats, dihydroergotoxine at small doses (0·01–0·4 mg kg^{-1}) did not change blood pressure significantly, but at the higher doses (1–4 mg kg^{-1}) a significant elevation was seen. It seems that the inability of small

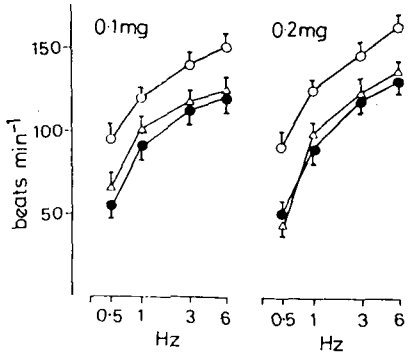

FIG. 4. Effect of yohimbine on the reduction by dihydroergotoxine of the tachycardia elicited by stimulation of the cardiac accelerator nerve at increasing frequencies in pithed rats; (●), control; (○), yohimbine 1 mg kg^{-1} i.v.; (△), dihydroergotoxine 23 min after yohimbine. The data are presented as mean values ± s.e.m. (n = 5). The differences between control and dihydroergotoxine treated rats are not statistically significant. Ordinate = increase in heart rate (beats min^{-1}). Abscissa = frequency of stimulation (Hz).

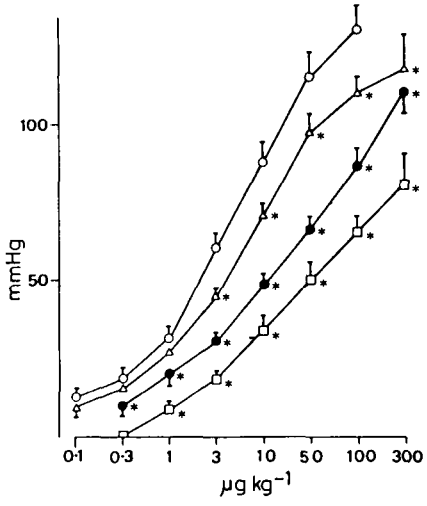

FIG. 5. Log dose–response curves with respect to the increase in mean arterial pressure of pithed rats induced by i.v. injection of (−)-phenylephrine, 10 min after i.v. saline (○) and dihydroergotoxine (100 μg kg^{-1} △, 200 μg kg^{-1} ● and 400 μg kg^{-1} □). The data are presented as mean values ± s.e.m. (n = 5). The significance of the difference from the control is represented by an asterisk (*$P < 0.05$). Ordinate = increase in blood pressure (mmHg). Abscissa = dose of phenylephrine in μg kg^{-1}.

doses of dihydroergotoxine to produce a pressor effect can be partly explained by the drop in heart rate. In urethane anaesthetized rats, under the same conditions, dihydroergotoxine always induced a long-lasting depressor effect, the magnitude of which largely depended on the pre-existing blood pressure. The differences observed in the pressor responses between rats anaesthetized with pentobarbitone and or urethane, are due to the nature of anaesthetic used. Armstrong (1981) has shown that urethane in the dose often used to anaesthetize rats, depresses pressor responses produced by a variety of α-agonists. The most inhibited responses were those produced by α_2-adrenoceptor agonists. Thus the use of urethane as an anaesthetic agent should be avoided for studies of agents acting at post-synaptic α_2-adrenoceptors.

In pithed rats, dihydroergotoxine administered in cumulative doses always induced a dose-dependent increase in mean arterial pressure. The increase in blood pressure induced by dihydroergotoxine in pentobarbitone anaesthetized animals is of peripheral origin, since it is observed after destruction of the CNS. In pithed rats the pressor responses were antagonized by the relatively selective α_2-adrenolytic drug yohimbine (0·5 and 1 mg kg^{-1}) (Weitzell et al 1979) which shifted the log dose–response curves to the right approximately in parallel. It did not change the maximal effect, suggesting a competitive type of antagonism. Prazosin (0·1, 0·5 and 1 mg kg^{-1}), a selective α_1-adrenoceptor antagonist (Cambridge et al 1977), also antagonized the hypertensive effect of dihydroergotoxine but to a smaller extent and in a non dose-dependent manner. The combination of both α-blockers did not enhance the antagonist effect due to yohimbine alone, suggesting that α_1-adrenoceptors are not involved in the pressor action of dihydroergotoxine. Methysergide (0·005 and 0·5 mg kg^{-1}), a blocking agent of 5-HT receptors (Fanchamps et al 1960), did not alter the pressor response induced by dihydroergotoxine. This indicated that its vasopressor action was not mediated by vascular 5-HT receptors. This experiment showed that most of the pressor activity of dihydroergotoxine in rats is mediated via vascular α_2-adrenoceptors. Nifedipine, a calcium antagonist, depressed the vasoconstriction elicited by dihydroergotoxine. Calcium antagonists, have been shown to interfere selectively with the vasoconstriction mediated by α_2-adrenoceptor stimulation, in-vivo, leaving the α_1-adrenoceptors hypertensive effects relatively unaffected (Van Meel et al 1981). This finding supports the idea of an essential involvement of vascular post-synaptic α_2-adrenoceptors in the pressor action of dihydroergotoxine.

The α_2-agonist activity of dihydroergotoxine is confirmed by the fact that the reduction of the tachycardia, due to electrical stimulation of cardioaccelerator nerves, induced by this drug, is more pronounced for low frequency stimulation (0·5 Hz compared with 6 Hz), and is antagonized by the α_2-adrenoceptor blocking agent yohimbine (Starke et al 1974). Similar results have been obtained in experiments on dog isolated saphenous veins by Müller-Schweinitzer (1982).

(−)-Phenylephrine is considered to be a potent α_1-adrenoceptor stimulant (Starke et al 1975). Dihydroergotoxine shifted log dose–response curves of phenylephrine to the right approximately in parallel, in a dose-dependent way, without affecting the maximal effect. This suggests competitive antagonism at α_1-adrenoceptors.

From these results it can be suggested that, in rats, the vasoconstrictor action exerted by dihydroergotoxine, on the peripheral vascular system, is predominantly mediated by post-junctional α_2-adrenoceptors. The peripheral action of dihydroergotoxine seems to be that of an agonist of α_2-adrenoceptors and an antagonist of α_1-adrenoceptors.

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