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Dihydroergotoxine-induced Bradycardia in Rats

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Abstract — Dihydroergotoxine (0·01–0·3 mg kg^{-1} i.v.) decreased heart rate in pentobarbitone-anaesthetized rats. The bradycardia was reduced but not blocked by pre-treatment with guanethidine, yohimbine, propranolol or pithing. It was not prevented by bivagotomy, atropine, sulpiride or haloperidol. Dihydroergotoxine failed to affect, either the bradycardia produced by electrical stimulation of the vagus, or the cardioacceleration induced by i.v. isoprenaline. The increase in heart rate elicited in pithed rats by electrical stimulation of the spinal cord was reduced by dihydroergotoxine; this effect being inhibited by yohimbine but not by sulpiride. In conclusion, the main mechanism by which dihydroergotoxine (i.v.) induces bradycardia in the rat involves stimulation of α_2-adrenoceptors located predominantly at the cardiac sympathetic nerve endings.

Although bradycardia is almost invariably observed following administration of dihydroergotoxine in conscious and anaesthetized animals, the mechanism of its bradycardial action is not well understood (Clark et al 1978). However, the drug has been shown to reduce the positive chronotropic response of the heart to nerve stimulation, in the pithed cat, by activation of presynaptic receptors (Scholtysik 1975).

Dihydroergotoxine can interact with α-adrenoceptors, and dopamine receptors (Müller-Schweinitzer & Weidmann, 1978; Markstein 1983; Markstein et al 1983). In a previous investigation, we showed that dihydroergotoxine acts as an α_2-agonist and α_1-antagonist in the vascular bed of the rat (Roquebert & Demichel 1985). α_2-Agonists can cause bradycardia by activation of central and cardiac pre-synaptic α_2-adrenoceptors (Langer 1977; Schmitt 1977; Starke 1977; Armstrong et al 1980; Pichler et al 1980). In addition, α_1-antagonists may cause bradycardia by increasing the vagal outflow to the heart (Huchet et al 1981), and by reduction of the sympathetic tone of the heart (Fozard 1982). On the otherhand, it has been demonstrated in the rat that dopaminergic agonists have bradycardial effects via a central mechanism (Cavero et al 1981). Dihydroergotoxine may, therefore, lower heart rate via an interaction with adrenergic and/or dopaminergic systems.

The aim of the present investigation was to determine the mechanism responsible for the bradycardial effect of dihydroergotoxine in rats.

Materials and Methods

Anaesthetized, intact rats

Male, normotensive rats (Wistar), 250 g, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.) and artificially ventilated with room air via a tracheal cannula attached to a Harvard Ventilator (10 ml kg^{-1}, 60 strokes min^{-1}). Rectal temperature was kept at about 37°C by 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.

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The arterial pulse wave triggered a heart ratemeter. Both arterial pressure and heart rate were recorded continuously on a Physiograph MK IV. A femoral vein was cannulated for the administration of drugs.

Effects on the heart rate of injections of dihydroergotoxine (0·010 to 0·100 mg kg^{-1} i.v. one dose per animal) were studied initially in intact rats. Heart rate response to isoprenaline (0·5 μg kg^{-1} i.v.) were elicited before and 10 min after administration of dihydroergotoxine (0·100 mg kg^{-1} i.v.).

The effects of dihydroergotoxine (0·100 mg kg^{-1} i.v.) on heart rate were studied in rats pretreated with either i.v. saline (1 mL kg^{-1}-control), atropine (1 mg kg^{-1}), yohimbine (1 mg kg^{-1}), guanethidine (5 mg kg^{-1}), prazosin (0·1 mg kg^{-1}), propranolol (1·5 mg kg^{-1}), haloperidol (0·15 mg kg^{-1}), or sulpiride (0·300 mg kg^{-1}). For certain experimental procedures more than one of these pretreatments was employed. Dihydroergotoxine was always injected 10 min after the first treatment.

Studies were also carried out in rats with sectioned cervical vagosympathetic nerves.

Experiments in pithed rats

For pithing, the rats were anaesthetized with ether and after cannulation of the trachea a steel rod was inserted into the spinal cord via the right orbit. Immediately after operation they were artificially ventilated with room air (1 mL/100 g/60 strokes min^{-1}). Blood pressure and heart rate were recorded as described above. The femoral vein was cannulated for drug administration, and the vagus nerves were cut in the neck.

For electrical stimulation of the spinal cord an indifferent electrode was placed subcutaneously in the dorsum and then atropine and (+)-tubocurarine (1 mg kg^{-1} each i.v.) were administered. To stimulate the preganglionic cardioaccelerator nerves selectively, the metal rod inside the spinal canal was coated with enamel except for a length of 1 cm, 6 cm from the tip. The uncovered segment was situated at spinal cord level C_7-Th_1. The preganglionic nerves to the heart were stimulated continuously with monophasic square wave pulses (10 V; 0·5 ms; 0·5 Hz) applied between the pithing rod and the indifferent electrode. The position of the pithing rod

was adjusted so that heart rate was increased, but blood pressure remained constant. When the heart rate increase had reached a plateau, dihydroergotoxine (one dose per animal) was injected intravenously. Changes in heart rate (Δ HR beats min^{-1}) were used to judge the effect of the drug on receptors located at the cardiac sympathetic nerves terminals. For study of the antagonism between dihydroergotoxine and yohimbine or sulpiride, the antagonist was administered at the moment of maximal tachycardia 5 min before injection of dihydroergotoxine.

In pithed rats given propranolol (1·5 mg kg^{-1} i.v.), the caudal stump of the cervical vagus nerve was stimulated (10 V; 0·5 ms; 3, 5, 10 Hz for 15 s) before and after dihydroergotoxine (0·100 mg kg^{-1} i.v.), atropine (1 mg kg^{-1} i.v.) or neostigmine (0·25 mg kg^{-1} i.v.) administration.

Analysis of results

Results are given as mean ± SEM. Analysis of variance or *t*-tests were used for the statistical comparisons. A P value of less than 0·05 was considered to indicate a significant difference.

Drugs used

Atropine sulphate (Rhône-Poulenc), guanethidine (Ciba-Geigy), haloperidol (Haldol, injectable preparation, Janssen), prazosin hydrochloride (Pfizer), propranolol hydrochloride (ICI Pharma), sulpiride (Dogmatil, injectable preparation Delagrange), yohimbine hydrochloride (Sigma), (+)-tubocurarine chloride (Sigma), sodium pentobarbitone (Pentobarbital injectable Clin-Midy), neostigmine bromide (Prostigmine, injectable preparation, Roche), dihydroergotoxine mesylate (Sandoz), isoprenaline hydrochloride (Fluka). Prazosin was dissolved in DMSO and thereafter diluted with saline (end concentration of DMSO not more than 4%). In control experiments DMSO (4% 1 mL kg^{-1} i.v., 10 min before) did not affect the cardiovascular response to dihydroergotoxine. The other drugs were dissolved in saline. All doses mentioned refer to the free base. All drugs were given i.v. in a volume of 1 mL kg^{-1}.

Results

Effects of dihydroergotoxine in intact rats

The mean heart rate before drug administration was 385 ± 10 beats min^{-1} (n = 20). Dihydroergotoxine administered intravenously reduced heart rate without affecting arterial blood pressure. The mean ± s.e.m. (n = 5) reduction in heart rate by dihydroergotoxine (0·010; 0·030; 0·100; 0·300 mg kg^{-1}) was 46·5 ± 5, 66 ± 6, 93 ± 7 and 93 ± 6 beats min^{-1}, respectively. 0·100 mg kg^{-1} of dihydroergotoxine was found to induce maximal bradycardia. This dose was used in subsequent experiments.

The chronotropic response to isoprenaline was not affected by dihydroergotoxine. Isoprenaline (0·5 μg kg^{-1} i.v.) increased heart rate from 395 ± 10 (control value) to 475 ± 7 beats min^{-1} (n = 5). Rats treated with dihydroergotoxine (0·100 mg kg^{-1}) had a resting heart rate of 295 ± 8 beats min^{-1} which was raised by administration of isoprenaline (0·5 μg kg^{-1}) to 465 ± 6 beats min^{-1} (n = 5).

Effects of dihydroergotoxine in rats subjected to various pretreatments

The effects on heart rate, of various surgical or pharmacological pretreatments designed to elucidate the mechanism of action of dihydroergotoxine are showed in Table 1.

Fig. 1 displays the effect of dihydroergotoxine on the heart rate, in rats after various pretreatments. Dihydroergotoxine (0·100 mg kg^{-1} i.v.) reduced heart rate. This effect was significantly reduced but not abolished by pretreatment with yohimbine (1 mg kg^{-1} i.v.) or guanethidine (5 mg kg^{-1} i.v.). In contrast, prazosin pretreatment (0·100 mg kg^{-1} i.v.) did not affect the dihydroergotoxine response. Propranolol (1·5 mg kg^{-1} i.v.) reduced heart rate by approximately 25%. In propranolol pretreated rats, dihydroergotoxine was still able to lower heart rate significantly although the response was smaller than that observed after yohimbine or guanethidine. Dihydroergotoxine also decreased cardiac chronotropism in

Table 1. Effects on heart rate (HR) of surgical and/or pharmacological treatment in pentobarbitone anaesthetized rats.

Treatment	Dose mg kg^{-1} i.v.	n	H.R. beats min^{-1} Control	After T
Saline	1 mL × kg^{-1}	20	385 ± 10	388 ± 8
Atropine	1	5	374 ± 6	364 ± 7
Bivagotomy		6	370 ± 8	375 ± 6
Guanethidine	5	5	358 ± 10	296 ± 7*
Haloperidol	0·15	5	364 ± 11	355 ± 9
Pithed		10	382 ± 9	299 ± 8*
Propranolol	1·5	5	384 ± 9	283 ± 8*
Prazosin	0·1	8	385 ± 11	394 ± 6
Sulpiride	0·3	5	394 ± 6	390 ± 5
Yohimbine	1	9	374 ± 7	370 ± 7

*Value significantly different ($P < 0·005$, t-test) from that before treatment or saline treated animals.

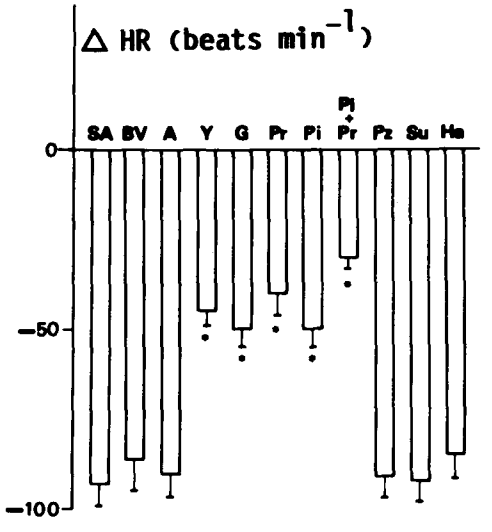

FIG. 1. Changes in heart rate (Δ HR beats min^{-1}) produced by dihydroergotoxine (0·100 mg kg^{-1} i.v.) in anaesthetized or pithed rats (n = 5–20/group) subjected to various pretreatments (see Table 1 for doses of antagonists and initial HR values) The HR changes shown are those measured 5 min after dihydroergotoxine injection. An asterisk indicates that the response after the treatment is significantly different ($P < 0·05$ analysis of variance) from that obtained in control (saline pretreated) rats. A: atropine; BV: bivagotomy; G: guanethidine; Ha: haloperidol; Pi: pithed; Pr: propranolol; Pz: prazosin; SA: saline; Su: sulpiride; Y: yohimbine.

the pithed rat, both in the presence and absence of propranolol (1·5 mg kg^{-1} i.v.). Section of the cervical vagosympathetic nerves or administration of atropine (1 mg kg^{-1} i.v.) 10 min before the ergot alkaloid had no effect on the response (Fig. 1) to dihydroergotoxine.

The fall in heart rate produced by dihydroergotoxine was not reduced by sulpiride (0·300 mg kg^{-1} i.v.) or haloperidol (0·15 mg kg^{-1} i.v.), two dopamine receptor antagonists, which in the doses used did not affect the basal heart rate (Table 1).

Effect of dihydroergotoxine on the tachycardia produced by electrical stimulation of the spinal cord

The effect of dihydroergotoxine on the synaptic mechanisms in the sympathetic neuroeffector junction in the heart was also studied in the pithed rat preparation.

Continuous stimulation (0·5 Hz) of the cervico-thoracic spinal cord, significantly increased heart rate (by 70 ± 5 beats min^{-1}) from the initial value of 296 ± 10 beats min.$^{-1}$ This effect was not significantly altered in the three groups pretreated with either saline (1 mL kg^{-1} i.v. n = 5), yohimbine (1 mg kg^{-1} i.v. n = 5) or sulpiride (0·300 mg kg^{-1} i.v. n = 5); the changes in heart rate were 70 ± 5; 63 ± 7, and 67 ± 9 beats min^{-1}, respectively. As shown in Fig. 2 in the saline or sulpiride pretreated rats dihydroergotoxine reduced heart rate, but in the yohimbine pretreated group the heart rate response to dihydroergotoxine was significantly less than that in the control (saline) group.

Effects of dihydroergotoxine on the bradycardia produced by vagal stimulation

In pithed rats given propranolol (1·5 mg kg^{-1} i.v.), electrical stimulation of the peripheral stump of the cervically sectioned right vagus, produced similar falls in heart rate, before or after i.v. injection of dihydroergotoxine. However, these responses were potentiated by neostigmine (0·25 mg kg^{-1} i.v.) and antagonized by atropine (1 mg kg^{-1} i.v.) (Fig. 3).

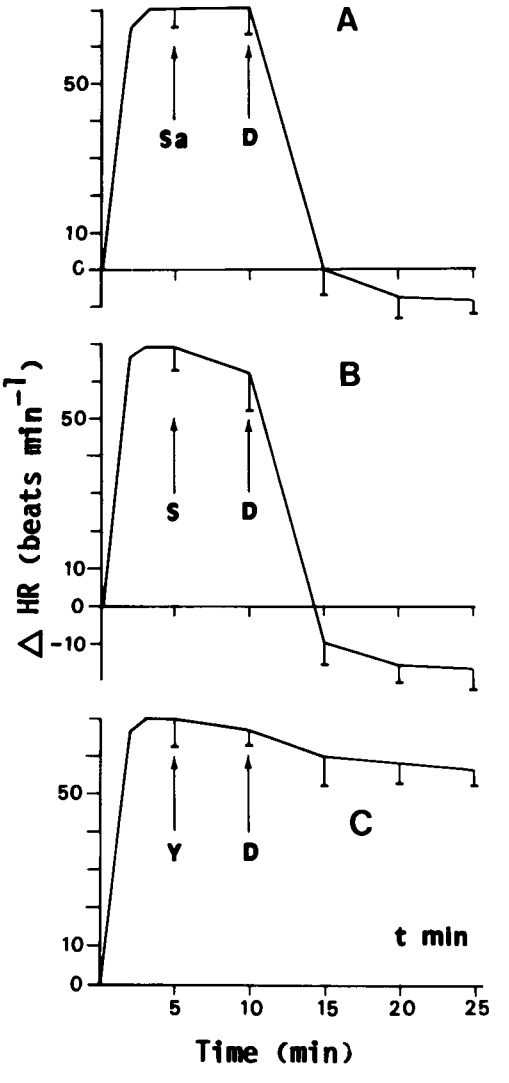

FIG. 2. Increase in heart rate (Δ HR beats min^{-1}) during continuous stimulation (10 V; 0·5 ms; 0·5 Hz) of the cardiac sympathetic nerve in pithed rats. (A) The rise in frequency is inhibited by dihydroergotoxine (D: 0·1 mg kg^{-1} i.v.) injected 5 min after saline (Sa: 1 mL kg^{-1}). (B) Sulpiride (S:0·300 mg kg^{-1} i.v.) applied 5 min before dihydroergotoxine is inactive. (C) Yohimbine (Y: 1 mg kg^{-1} i.v.) antagonizes the effect of dihydrogotoxine. The results are presented as mean values ± SEM for 5 separate experiments.

Discussion

Dihydroergotoxine reduces heart rate in pentobarbitone anaesthetized, intact and pithed rats. Various mechanisms can be invoked to explain this effect.

The dihydroergotoxine-induced bradycardia was not prevented by bivagotomy, nor by prior administration of atropine, sulpiride or haloperidol. In the pithed rat, dihydroergotoxine did not affect the bradycardia elicited by electrical stimulation of the peripheral end of the right vagus. These results indicate that the parasympathetic and dopaminergic systems do not play significant roles in this bradycardial effect.

The bradycardial effects of dihydroergotoxine were reduced but not abolished by the following sympathetic treatments: yohimbine, which blocks α_2-adrenoceptors, guanethidine, which leads to a functional sympathectomy by inhibiting noradrenaline release, propranolol, which is an antagonist of noradrenaline at cardiac β-adrenoceptors, or pithing, which eliminates sympathetic tone. Furthermore, in pithed rats dihydroergotoxine decreases the tachycardia induced by stimulation of the cardioaccelerator nerves. This latter effect was antagonized by yohimbine but not by sulpiride. In addition, this ergot alkaloid did not reduce the cardiac effects of isoprenaline. This indicates that dihydroergotoxine reduces cardiac sympathetic activity by a presynaptic action mediated predominantly through α_2-adrenoceptors on the nerve terminals. The involvement of presynaptic dopaminergic receptors in the action of dihydroergotoxine appears unlikely since sulpiride pretreatment did not reduce the effect. This is not unreasonable in view of the absence of presynaptic dopamine receptors in the sympathetic innervation of the heart (Cavero & Lefevre-Borg 1981; Clapham & Hamilton 1982).

Overall, these findings provide evidence that the dihydroergotoxine-induce bradycardia in the rat is mainly due to inhibition of cardiac sympathetic nerve activity. This reduction in sympathetic tone produced by dihydroergotoxine appears to be due to stimulation of α_2-adrenoceptors since it

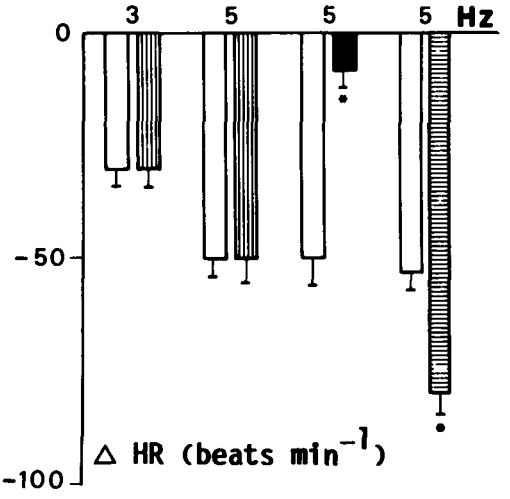

FIG. 3. Heart rate response (Δ HR beats min^{-1}) evoked by electrical stimulation of the peripheral end of the severed right vagus in propranolol (1·5 mg kg^{-1} i.v.) pretreated pithed rats (n = 5) before (□ control) and 10 min after intravenous administration of dihydroergotoxine (▥ 0·100 mg kg^{-1}), atropine (■ 1 mg kg^{-1}) or neostigmine (▤ 0·25 mg kg^{-1}). An asterisk indicates that the response was significantly altered ($P < 0·05$ paired t-test) by the treatment.

was reduced by yohimbine, an α_2-adrenoceptor antagonist (Weitzell et al 1979) but not by prazosin an α_1-blocker (Cambridge et al 1977). Dihydroergotoxine which acts as an α_2-agonist in the rat (Roquebert & Demichel 1985) may induce this effect via, an activation of predominantly peripheral presynaptic α_2-adrenoceptors.

Dihydroergotoxine may, also, reduce cardiac sympathetic tone via a central action, since significant bradycardia is observed after direct intracerebroventricular injection (Schmitt & Schmitt 1964). However, a central action was not considered likely to play a significant role in the cardiovascular effects after acute administration of this drug since: (1) slow intervertebral artery infusion of dihydroergotoxine (10 $\mu g\ kg^{-1}$) leads to a smaller fall in blood pressure than intravenous infusion of the same dose in the dog; the reverse is true for the centrally acting α_2-adrenoceptor stimulant guanfacine. (2) efferent splanchnic nerve activity in the cat, is not affected by doses up to 100 $\mu g\ kg^{-1}$; the dose of dihydroergotoxine depressing nerve activity by 50% is approximately 900 $\mu g\ kg^{-1}$; the dose of clonidine producing comparable inhibition is 3·4 $\mu g\ kg^{-1}$ i.v. (Clark et al 1985). (3) the effects of ergot alkaloids on sympathetic centres are observed only at high doses (Schmitt & Fenard 1970; Clark et al 1985).

After abolition of cardiac sympathetic tone in propranolol pretreated pithed rats, dihydroergotoxine still induces a low but significant fall in heart rate, suggesting a depressant action on the myocardium itself.

In summary, the main mechanism by which dihydroergotoxine (i.v.) leads to bradycardia in anaesthetized rats, involves stimulation of α_2-adrenoceptors predominantly located at the cardiac sympathetic nerve endings, and an additional direct depressant effect on the myocardium.

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