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Pharmacological properties of isoidide dinitrate

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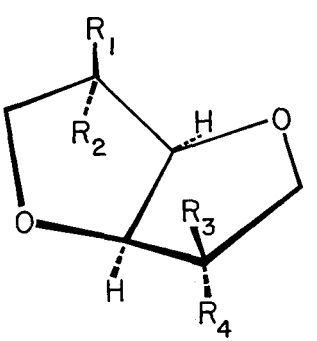
Isoidide dinitrate is a more active vasodepressor agent in anaesthetised animals than either of its geometric isomers, isosorbide dinitrate and isomannide dinitrate. Isoidide dinitrate also has the greatest vasodilator action of the three isomers on the vascular system of the dog hind leg and is a more potent hypotensive agent than isosorbide dinitrate in hypertensive rats. The greater activity of isoidide dinitrate may be attributable to its higher oil : water partition coefficient or enhanced drug-receptor interaction resulting from the spatial positions of the nitrate groups or both factors. No evidence was found for involvement of adrenergic receptors in responses of vascular or intestinal smooth muscle to isoidide dinitrate.

MANY nitrate esters have been examined for vasodilator activity including 1,4:3,6-dianhydro-D-mannitol 2,5-dinitrate (isomannide dinitrate; IMDN) and its geometric isomer 1,4:3,6-dianhydro-D-sorbitol 2,5-dinitrate (isosorbide dinitrate; ISDN; Carvasin; Isordil) (Krantz, Carr, Forman & Ellis, 1939a & 1939b). These two esters were the subjects of a comparative study by Goldberg (1948) who found that isosorbide dinitrate was a more active vasodepressor and coronary vasodilator than isomannide dinitrate. He also reported the former to be effective in lowering the blood pressure in hypertensive patients and of value in angina pectoris and intermittent claudication.

More recently Jackson & Hayward (1960) have prepared 1,4:3,6-dianhydro-L-iditol 2,5-dinitrate (isoidide dinitrate; IIDN) thus making three known isomeric dianhydrohexitol dinitrates. These three esters have their nitrate groups attached to the same carbon atoms and differ only in the spatial arrangement of these groups. The configurational formula for the 1,4:3,6-dianhydrohexitol (isohexide) molecule with the various positions of the nitrate groups has been represented as shown by I.



I

The molecule possesses two fused five-membered rings having a *cis* arrangement of the ring junction. The rings, being nearly planar, are inclined to one another at an angle of about 120°. Substituents on the

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carbon atoms at positions 2 and 5 can either be *exo* (R_2R_4) or *endo* (R_1R_3). The conformation of the ring system is rigid (Jackson & Hayward, 1959).

We have compared the pharmacological properties of isoidide dinitrate with its two geometric isomers and investigated possible mechanisms of action for the compound.

Experimental

BLOOD PRESSURE IN ANAESTHETISED ANIMALS

Arterial blood pressure was recorded in 6 cats and 5 dogs anaesthetised with pentobarbitone sodium and in 4 rabbits anaesthetised with allobarbitone and urethane. Respiration was recorded simultaneously in some animals. The nitrates, as a 1% solution in 20% ethanol, were administered intravenously in doses ranging from 0·1 to 0·5 mg/kg depending on the responsiveness of the animal. In the 5 dogs the nitrates were administered intraduodenally as a warmed aqueous suspension containing 5 mg of the nitrate per ml with acacia as a suspending agent. Administration was preceded and followed by 5 ml of warm water by the same route. The order in which the nitrates were given was changed from animal to animal each nitrate being administered at least on one occasion, first, second and last in order.

HYPERTENSIVE RATS

Male Wistar rats, 250–300 g weight, were made hypertensive by the method of Grollman (1944). Within 4 to 6 weeks most of the rats had blood pressures ranging from 170 to 230 mm of mercury (Photoelectric Tensometer, Metro Scientific, Inc.). Before taking blood-pressure readings the rats were fasted for 16 hr then given 50 mg/kg of phenobarbitone sodium intraperitoneally. Control blood-pressure levels were determined 1½ to 2 hr after administration of phenobarbitone and then isosorbide dinitrate or isoidide dinitrate, 10 mg/kg, was given by stomach tube as a 0·1% solution in 10% ethanol. Blood-pressure readings were continued at 5 to 10 min intervals until control levels were restored. A cross-over comparison was made by testing both nitrates in 14 hypertensive rats; at least 3 days were allowed between the administration of drugs to the same rat. Following the same procedure, the effect of the solvent alone was then determined in 8 of the hypertensive rats.

PERIPHERAL BLOOD FLOW

The effect of the three nitrates on the blood flow in the hind limb was determined in 4 dogs using a bubble flowmeter to intercept the flow through the femoral artery. The drugs were injected into the return tube and each nitrate, 0·1 mg in 0·1 ml of 20% ethanol, was tested at least twice in each dog. Glyceryl trinitrate, in the same solvent, was sometimes used for comparison. The dogs were anaesthetised with pentobarbitone sodium and given 3 mg/kg of heparin before making measurements. Supplementary heparin, 1 mg/kg, was given at hourly intervals through a cannula in the external jugular vein. Arterial blood pressure was recorded from the opposite femoral artery.

PHARMACOLOGICAL PROPERTIES OF ISOIDIDE DINITRATE

WATER SOLUBILITY AND OIL : WATER PARTITION COEFFICIENT

The water solubility of the nitrates was determined at 20°. The oil : water partition coefficient for each nitrate was found by dissolving 10 mg in 50 ml of maize oil and shaking with an equal volume of water for 90 min at 20°. After separation the nitrate content of the aqueous layer was determined by the method of Whalen (1930).

MECHANISM OF ACTION

Male Wistar rats, 300–500 g, were anaesthetised with pentobarbitone sodium or urethane and prepared for recording arterial blood pressure by a technique similar to that of Dekanski (1952). Heparin, 1·5 mg/100 g, was injected intravenously before cannulation of the carotid artery with polythene tubing (PE50). Pentolinium tartrate, 0·125 mg/rat was injected initially and the animal left for about 1 hr to allow blood-pressure levels to stabilise. This procedure, recommended by Gillis & Nash (1961), minimised fluctuations in blood pressure during the experiment. Rectal temperatures were maintained at 33°. Isoidide dinitrate was administered intravenously in doses of 0·05 mg/rat. Some rats were given 2 mg/kg of reserpine the day before the experiment to deplete tissue catecholamines.

Segments of rabbit duodenum were suspended in a 25-ml bath containing Tyrode solution at 38° and gassed with oxygen 95% and carbon dioxide 5%. The lever was lightly weighted so that the longitudinal muscle maintained a high level of tone and exhibited good responses to relaxant drugs. To deplete catecholamines, reserpine 0·5 mg/kg was administered subcutaneously to some rabbits 18 hr before the experiment.

DRUGS

Samples of crystalline isomannide dinitrate, isosorbide dinitrate and isoidide dinitrate were provided by Dr. L. D. Hayward and a commercial sample of powdered isosorbide dinitrate with 75% lactose was also used. Other drugs used were glyceryl trinitrate (hypodermic tablets Parke-Davis), adrenaline hydrochloride, isoprenaline hydrochloride, phenylephrine hydrochloride, tyramine hydrochloride, dichloroisoprenaline hydrochloride (DCI), pronethalol hydrochloride, phenoxybenzamine hydrochloride, guanethidine, papaverine, histamine diphosphate and diphenhydramine hydrochloride. Concentrations of the salts are expressed as such. Reserpine (Serpasil) was used as the parenteral solution.

Results

BLOOD PRESSURE IN ANAESTHETISED ANIMALS

Intravenous administration of small doses of the three nitrates produced a transient fall in blood pressure : in rabbits and dogs, 0·1 mg/kg was usually sufficient; in cats, doses of 0·2 mg/kg or more were required. The response to isoidide dinitrate was always greater and persisted longer than the responses to either of its isomers. The responses to isosorbide dinitrate were sometimes slightly greater than those to isomannide dinitrate

but in most instances were similar (Fig. 1). In terms of average fall in blood pressure the potency exhibited by isoidide dinitrate was 1·7 to 2·1 times that of isomannide dinitrate and 1·4 to 2·1 times that of isosorbide dinitrate.

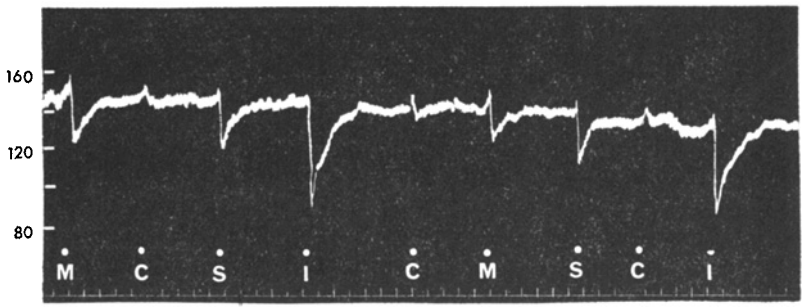

FIG. 1. Effects of isohexide dinitrates on carotid arterial pressure of an anaesthetised cat. Intravenous injection of 0·2 mg/kg of isomannide dinitrate (M), isosorbide dinitrate (S) and isoidide dinitrate (I). Control injections of solvent at C. Time, 1 min.

Introduction of the nitrates into the duodenum of dogs was followed by a depressor response indicating that absorption readily occurred. The fall in blood pressure after isoidide dinitrate was always greater than that after either of its isomers, regardless of the order of administration (Fig. 2).

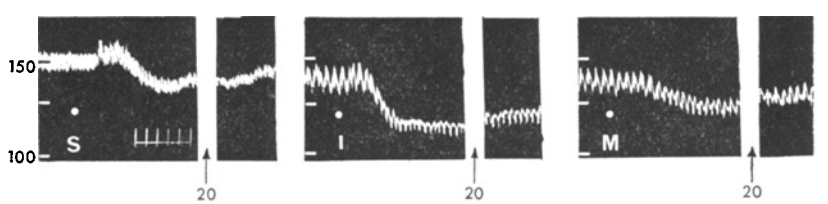

FIG. 2. Effect of isohexide dinitrates on carotid artery blood pressure of anaesthetised. dog. Intraduodenal administration of 4 mg/kg of isosorbide dinitrate (S), isoidide dinitrate (I) and isomannide dinitrate (M) in order shown. Intervals between giving drugs 2½ hr. Interval between consecutive traces 20 min. Time, 1 min.

HYPERTENSIVE RATS

Oral administration of 10 mg/kg of isosorbide dinitrate or isoidide dinitrate caused a decline in blood pressure which became maximal at 20 to 25 min. No hypotensive effect was produced in the rats which received the solvent alone. The mean fall % in blood pressure produced by isoidide dinitrate was significantly greater than that produced by isosorbide dinitrate. The activities of the isomers did not differ significantly in onset or duration of action. The results are summarised in Table 1.

PERIPHERAL BLOOD FLOW

Injection of 0·1 mg of any of the isomers into the femoral artery of the dog caused a transient increase in rate of blood flow through the hind limb.

TABLE 1. EFFECT OF ISOHEXIDE DINITRATES ON BLOOD PRESSURE OF HYPERTENSIVE RATS. ORAL ADMINISTRATION OF 10MG/KG

Ester	Mean fall in blood pressure % ± s.e.	Time to maximum effect (min) ± s.e.	Duration* (min) ± s.e.
Isosorbide dinitrate ..	14·0 ± 1·5	21·7 ± 0·97	34·8 ± 3·47
Isoidide dinitrate ..	20·9 ± 1·98	23·5 ± 0·94	47·4 ± 5·56
	P < 0·01	P > 0·2	P > 0·05

* Time until return to control levels

No difference was demonstrated between the effects of isomannide dinitrate and isosorbide dinitrate on blood flow. The mean response to isoidide dinitrate was significantly greater than the response to either of the other isomers, but much less than the response to glyceryl trinitrate. (Table 2). Intra-arterial injection of 0·1 mg of any of the isomers had no effect on systematic blood pressure.

TABLE 2. EFFECT OF ISOHEXIDE DINITRATES ON BLOOD FLOW IN THE DOG HIND LEG. EXPRESSED AS INCREASE % IN FLOW AFTER INTRA-ARTERIAL INJECTION OF 0·1 MG

Dog No.	Isomannide dinitrate	Isosorbide dinitrate	Isoidide dinitrate	Glyceryl trinitrate
1	61·0	65·8	89·3	
	87·5	85·0	131·0	209·0
2	34·9	37·4	66·7	
	45·8	78·2	138·8	
	47·0	59·4	90·0	
3	59·2	50·0	89·0	299·0
	39·0	36·5	95·0	204·0
4	80·2	64·3	112·5	
	80·2	88·7	143·3	240·0
Average ± s.e.	59·4 ±6·46	62·8 ±6·36	109·2* ±7·68	238·0 ±22·8

* Significantly different from the mean values for isosorbide and isomannide dinitrates (P < 0·001).

WATER SOLUBILITY AND OIL : WATER PARTITION COEFFICIENTS

The results, each based on 5 determinations, are shown in Table 3. The water-solubility values found for isomannide dinitrate and isosorbide dinitrate correspond closely to those reported (1·7 mg/ml for isomannide dinitrate, Krantz, Carr, Forman & Ellis, 1939b; 1·08 mg/ml for isosorbide dinitrate, Sherber & Gelb, 1961).

TABLE 3. WATER SOLUBILITY AND OIL: WATER PARTITION COEFFICIENTS OF ISOHEXIDE DINITRATES

Ester	Water solubility* mg/ml	Oil/water coefficient*
Isomannide dinitrate	1·67 ± 0·3	81·42 ± 2·15
Isosorbide dinitrate	1·08 ± 0·2	112·0 ± 8·80
Isoidide dinitrate	0·845 ± 0·02	158·4 ± 7·50

* average of 5 values ± s.d.

STUDIES ON MECHANISM OF ACTION

The tone of isolated rabbit intestine was reduced when any of the isomers was added to the bath at a concentration of 2 mg%. The relaxant

effect of isoidide dinitrate was greater than that of either of the other isomers. The effect produced by isoidide dinitrate was not prevented by dichloroisoprenaline, pronethalol, phenoxybenzamine or a combination of pronethalol and phenoxybenzamine. Exposure of the gut for 90 min to guanethidine, or pre-treatment of the rabbit with reserpine did not prevent the response to isoidide dinitrate.

When addition of any one of the nitrates was repeated several times, tachyphylaxis developed to all of the isomers, but the gut still responded to isoprenaline, adrenaline or papaverine. The vasodepressor effect of isoidide dinitrate in the rat was not reduced by previous administration of pronethalol, and in rats pretreated with reserpine responses were unaltered. The depressor effect produced by isoidide dinitrate was not prevented by diphenhydramine.

Discussion

The three isomeric dinitrate esters are structurally identical, except for the spatial orientation of their nitrate groups. Alteration in the position of these groups results in different oil: water partition coefficients and different pharmacological potencies. The isomer most active pharmacologically, isoidide dinitrate, also has the highest oil: water coefficient. For some series of nitrate esters an increase in oil: water partition coefficient seems to be responsible for increased pharmacological activity (Krantz, Carr, Forman & Cone, 1940). But it does not seem likely that this physical property has a direct influence in determining the potency of the isohexide dinitrates, since isosorbide dinitrate, which has a considerably higher oil: water coefficient than isomannide dinitrate, did not differ from it in potency. The position of the nitrate groups probably has a more direct effect on the activity of these compounds. Both of the nitrate groups of isoidide dinitrate are in the *exo*-position (see 1) and may thus combine most readily with tissue receptors. The nitrate groups of isomannide dinitrate, both being in the *endo*-position, may approach receptors the least readily because of hindrance by the isohexide skeleton. The arrangement of only one of the nitrate groups in the *exo*-position, as in isosorbide dinitrate, does not significantly increase potency and this seems to indicate that combination of both groups with tissue receptors is necessary for activity.

Relaxation of smooth muscle, the most characteristic effect of nitrate esters, is associated physiologically with adrenergic stimulation, particularly of β-receptors. The present availability of a variety of pharmacological tools for studying adrenergic mechanism prompted us to see if any evidence could be found for the involvement of adrenergic receptors in responses to isoidide dinitrate. However, though the responses of the preparations to this drug resembled those resulting from stimulation of β-receptors by agents such as isoprenaline, or with the gut, from the results of stimulation of both types of receptors by adrenaline, there was no indication that either an action on adrenergic receptors or a release of noradrenaline contributed to its action.

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