PHARMACOLOGICAL STUDIES OF NITROHARMIDINE NITRATE ON CARDIOVASCULAR SYSTEM

O.U. Khawaja and S.M. Yusuf

The pharmacological actions of nitroharmidine nitrate, a modified alkaloid of Peganum harmala, have been studied on the cardiovascular system in rats, cats and rabbits. The drug was administered to intact rats and cats in doses ranging between 100 and 1,600 ug/kg B.W. whereas a total dose ranging between 10 and 160 ug was injected into the perfusion fluid in isolated rabbit heart preparations. In rats the drug produced a marked hypertensive effect abolished by phenoxybenzamine premedication. This effect of the drug was not observed in pithed rats and in rat hind quarter preparation. In cats, on the other hand, the drug produced hypotensive effect prevented by atropine premedication. This hypotensive effect could not be produced in spinal cats. The isolated rabbit heart studies showed myocardial depression. The actions produced on the blood vessels were probably central whereas those on the myocardium direct one.

Nitroharmidine nitrate, a modified alkaloid of Peganum harmala, was studied in our laboratories for its pharmacological actions on the central nervous system (Khawaja and Yusuf, 1977). It was considered that the studies on cardiovascular system may also be undertaken as the drug in previous studies was expected to produce its effect mainly due to MAO inhibitory properties.

Experimental

Material.

1. Cats of either sex weighing 2-3.5 kg were employed. These were kept under observation for one week and fed on meat, bread and milk. Watcr was allowed adlibitum.

2. Rabbits of either sex weighing between 1.5-2.5 kg fed on lucerne, carrots and water were used.

3. Albino rats, Sprague Dawly strain, of either sex, weighing 250-300 G and 3-4 months age fed on mouse feed and water, were employed.

4. Nutrient solutions used in the isolated organ studies included Locke's solution and Ringer's solution.

Department of Pharmacology and Therapeutics, Jinnah Postgraduate Medical Centre, Karachi.

5. Pentobarbitone sodium, phenoxybenzamine, heparin, atropine sulphate and nitroharmidine nitrate wer used in these studies.

Heparin from Weddel Pharmaceutical Ltd., London was diluted so that 1 ml of the solution contained 50 units of the drug.

Nitroharmidine nitrate 12.35 mg contained 10 mg of the base. The drug was dissolved in distilled water heated upto 60°C to make a stock solution. Further dilutions were made in normal saline. Phenoxybenzamine (Dibenzyline) was supplied by Smith Kline and French Labs. Ltd., Welwyn Garden City, Herts, England.

Atropine sulphate from C.H. Boehringer, Ingelheim, Germany was employed.

6. Grass polygraph model 5D, and Langendorff's isolated heart perfusion apparatus were used in these studies.

Methods

A. Rat Blood Pressure Recording

Rat blood pressure preparation was made after Crawford and Outchorn (Akhtar 1971).

Rat weighing 250-300 G were anaesthetized with pentobarbitone sodium (40 mg/kg B.W. I/P) and tied down in supine position to wooden board. A midline incision was made on the aspect of the neck of the animal. The trachea was exposed and cannulated with a polythene tubing to prevent choking of respiratory passages (Chai and Wang, 1966). The common carotid artery on one side was then exposed and separated from vagus nerve in the entire length of the neck.

The artery was ligated at cephalic end and cannulated with a polythene cannula filled with heparinized saline. The other end of the tubing was connected to Statham's pressure strain guage transducer that was also filled with heparinized fluid medium. The jugular vein on the other side was cannulated with polythene tubing which was connected to a 10 ml burette containing normal saline through a rubber tubing. The solutions of drugs were injected into jugular vein through this rubber tubing and 0.5 ml saline solution run to ensure entry of drug into circulation.

The polygraph was so calibrated that one cm deflection of the writing pen was equal to 100 mm of Hg pressure.

Systolic and diastolic pressures were recorded and the mean and the pulse pressure calculated before and after the injection of the drug. A dose response with doses ranging from 100 ug to 1,600 ug per kg B.W. was recorded.

B. Effect on B.P. of Anaesthetized Rat Premedicated with Phenoxybenzamine

In a preparation noted above, a dose of 500 ug/kg B.W. of nitroharmidine nitrate was injected intravenously and response recorded till B.P. turned to normal level. An injection of 10 mg/kg B.W. of phenoxybenzamine was given I.V. and response recorded. After 10 minutes the same dose of nitroharmidine nitrate (500 ug/kg B.W.) was repeated and again response recorded. Any difference between the two records was noted.

C. Effect on Pithed Rat Blood Pressure Preparation

Pithed rats were prepared by modifying the technique employed by Burn (1952) in spinal cat preparations.

The animal was prepared as above but both carotid arteries were ligated and normal blood pressure tracings recorded. Then the vertebral column of the rat was exposed by a mid line incision at the back of the neck, the muscles retracted and a sharp probe passed into the brain through the foramen magnum and continuity between the brain and spinal cord severed. Before this procedure the animal was connected to respirator and artifical respiration switched on after severing the spinal cord connection. The foramen magnum was then stuffed with plasticin tightly to check any bleeding.

When the animal had rested for 15 minutes after this operative procedure, blood pressure was recorded before and after medication with nitroharmidine nitrate (500 ug/kg B.W.).

D. Effect on Rat Hind Quarter Preparation

A rat weighing 250-300 G was killed with a blow on the head and then cutting the throat. The abdomen was opened by a mid line incision. The rectum was cut between ligations and abdominal viscera were removed. The vertebral column was bisected below the level of the origin of renal arteries and the lower half of the body of the rat was completely isolated. It was pinned down on a slanting wooden board fixed on a stand and the aorta was cannulated with a polythene cannula connected through a rubber tubing with a bottle containing Ringer's solution to be used as perfusion fluid. The level of the perfusion fluid in the bottle was maintained at a constant height throughout the experiment.

The perfusion fluid entering through the aorta circulated through the blood vessels of the lower extremeties and returned via the inferior vena cava. The returned fluid trickled down the slanting wooden board. When the out flow of perfusion fluid became constant it was collected in a graduated cylinder. A collection of 10 minutes was recorded and mean of three recordings taken. A dose of 500 ug/kg B.W. of ntact animal of nitroharmidine nitrate dis-olved in 0.5 ml of normal saline was injected very lowly through the rubber tubing as close to the orta as possible and collection of fluid as before ecorded. The difference in two recordings was oted. The temperature of the perfusion fluid vas kept as 27-28°C.

E. Effect on Anaesthetized Cat Blood Pressure

The cats were anaesthetized with intraperitoneal injection of pentobarbitone sodium 30 mg/kg B.W. and subsequently tied on an operating table in supine position. The common carotid artery, jugular vein and trachea were exposed and cannulated as was done in rat blood pressure preparation. The polygraph had already been so calibrated that 1 cm deflection of writing pen was equal to 100 mm of Hg pressure. The drug solutions were administered in 1 ml volume through the tubing into the jugular vein and flushed with 3 ml of normal saline. The animal was left for 15 minutes to recover operative manipulation and stabilize. A dose response curve with doses ranging between 100 ug and 1,600 ug/kg B.W. was recorded.

Systolic and diastolic pressures were recorded and the mean and pulse pressure calculated before and after the administration of each dose.

The experiment was repeated after premedication with 2 mg/kg B.W. dose of atropine sulphate.

F. Effect on Spinal Cat Blood Pressure

Spinal cats were prepared following the method of Burn (1952) .The animal was allowed to rest for half an hour after surgical operation.

The blood pressure tracings before and after medication with nitroharmidine were then recorded.

G. Effect on Isolated Perfused Rabbit Heart Preparation

The animal was killed with a blow on the head. The chest was opened, sternum retracted and the heart isolated by cutting its connections with a pair of scissors. The heart was at once transferred to a dish containing properly oxygenated and heparinized Locke solution kept at 37°C and was gently squeezed to remove residual blood from the cardiac chambers. Any extra tissue still sticking to the heart was also excised. The heart was cannulated through aorta with the cannula connected to perfusion apparatus. It was perfused by Langendorff technique (Burn 1952) using oxygenated Locke solution kept at 37°C. A hook was attached to the apex of the heart and cardiac contractions recorded on a smoked drum of kymograph through Starling's heart lever.

After recording normal contractions graded doses of nitro-harmidine nitrate ranging 10-160 ug were administered through the rubber tubing. The force of contraction of heart was calculated from the amplitude of the tracings and difference in normal and those recorded after medication was noted.

Results

A. Effect of Nitroharmidine Nitrate on Rat Blood Pressure

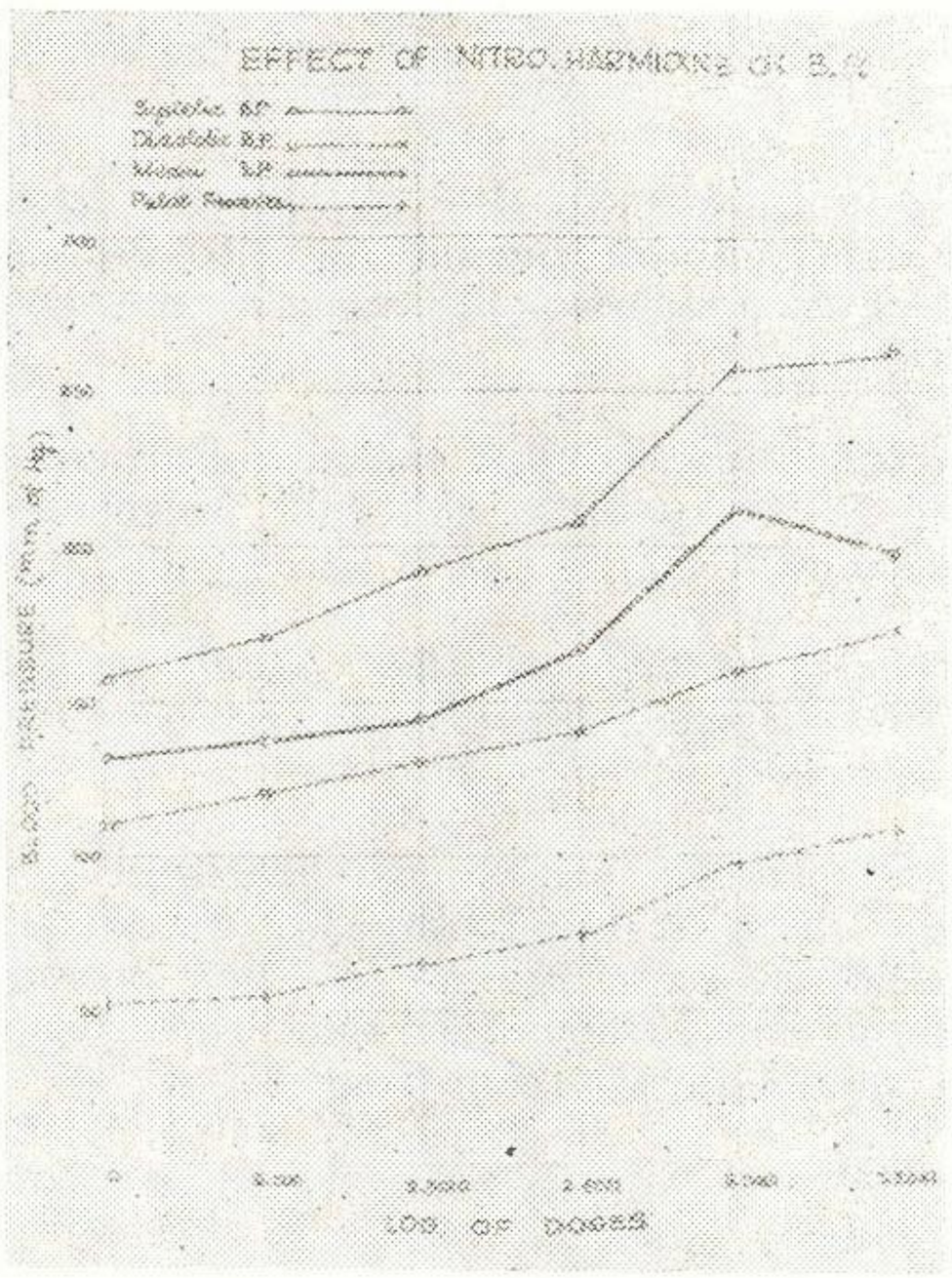

Fig. 1: Graph showing the effect of different doses of nitro-harmidine nitrate on various components of anaesthetized rat blood pressure.

Nitroharmidine nitrate produced hypertensive effect on anaesthetized rat blood pressure with all the doses tried. The results are presented in Figure 1.

The increase in blood pressure following 100 ug/kg B.W. dose was statistically insignificant whereas with other doses it was significant.

B. Effect of Nitroharmidine Nitrate on Rat Blood Pressure After Phenoxybenzamine Pretreatment

Pretreatment with phenoxybenzamine in a dose of 10 mg/kg B.W. intravenously successfully checked the expected rise in blood pressure following nitroharmidine in these experiments. The results are shown in Table I.

C. Effect of Nitroharmidine Nitrate on Blood Pressure in Pithed Rats

The doses of nitroharmidine nitrate which produced statistically significant rise in blood pressure in normal anaesthetized rats could not produce the same effect on blood pressure in pithed rats. On the contrary there was a slight statistically insignificant fall in blood pressure. The results are tabulated in Table II.

D. Effect of Nitroharmidine Nitrate On Rat Hind-quarter Preparation

Nitroharmidine nitrate produced slight statistically insignificant decrease in perfused fluid volume collections showing some vasoconstrictor effect. The results are given in Table III.

Table I: Effect of Nitroharmidine Nitrate on Blood Pressure in Anaesthetized Rats after Pretreatment with Phenoxybenzamine (10 mg/kg B.W.)

Before Phenoxybenzamine Premedication					*After Pretreatment with Phenoxybenzamine*				
Parameter	*Blood Pressure in mm Hg ± S.E.**		*% rise*	*P value*	*Parameter*	*Blood Pressure in mm Hg ± S.E.**		*% rise*	*P value*
	Control	*After Treatment*				*Control*	*After Treatment*		
Systolic	152.8 ± 12.82	214.0 ± 13.7	40.0	⩽0.01	Systolic	138.0 ± 8.18	143.0 ± 9.8	3.6	N.S.
Diastolic	95.8 ± 9.37	130.0 ± 7.75	35.9	⩽0.05	Diastolic	71.7 ± 7.6	70.0 ± 8.16	2.3	N.S.
Mean	116.1 ± 10.02	158.3 ± 9.51	18.3	⩽0.02	Mean	9.29 ± 7.71	94.4 ± 8.23	1.6	N.S.
Pulse Pressure	55.3 ± 4.45	± 84.1 7.56	52.0	⩽0.01	Pulse Pressure	63.8 ± 6.14	7.33 ± 6.28	14.8	N.S.

*Standard error.

Table II: Comparison of the Effect of Nitroharmidine Nitrate on Blood Pressure on Intact and Pithed Rats (500 ug/kg B.W. I/V)

Before pithing					*After pithing*				
Parameter	*Blood Pressure in mm Hg ± S.E.**		*% rise*	*P value*	*Parameter*	*Blood Pressure in mm Hg ± S.E.**		*% rise*	*P value*
	Control	*After Treatment*				*Control*	*After Treatment*		
Systolic	182.0 ± 9.3	232.0 ± 7.75	27.4	⩽0.01	Systolic	146.0 ± 15.11	135.0 ± 17.89	7.5	N.S.
Diastolic	130.0 ± 8.95	154.0 ± 7.79	18.4	⩽0.05	Diastolic	88.4 ± 19.16	79.0 ± 18.89	10.6	N.S.
Mean	147.3 ± 8.84	180.0 ± 7.08	22.1	⩽0.02	Mean	109.6 ± 13.96	59.0 ± 19.31	9.6	N.S.
Pulse Pressure	52.0 ± 3.39	78.0 ± 6.63	50.0	⩽0.01	Pulse Pressure	57.6 ± 4.72	56.0 ± 5.1	2.7	N.S.

*Standard error.

Table IV: Effect of Nitroharmidine Nitrate on Blood Pressure of Anaesthetized Cats Treated with Atropine (2 mg/kg B.W.)

Before atropine premedication					*After pretreatment with atropine sulphate*				
Parameter	*Blood Pressure in mm Hg ± S.E.**		*% fall*	*P value*	*Parameter*	*Blood Pressure in mm Hg ± S.E.**		*% fall*	*P value*
	Control	*After Treatment*				*Control*	*After Treatment*		
Systolic	148.0 ±11.71	112.2 ± 6.1	24.2	⩽0.05	Systolic	139.3 ±12.74	129.3 ±10.29	7.2	N.S.
Diastolic	98.7 ±12.32	59.5 ± 3.68	39.7	⩽0.05	Diastolic	88.8 ±13.39	78.8 ±13.45	11.3	N.S.
Mean	115.2 ±11.89	75.6 ± 3.19	34.8	⩽0.02	Mean	105.6 ±12.9	95.5 ±12.25	9.6	N.S.
Pulse Pressure	49.2 ± 5.36	48.3 ± 2.46	1.8	N.S.	Pulse Pressure	50.5 ± 4.4	50.5 ± 5.41	—	N.S.

*Standard error.

Table III: Volume of Fluid Collected in 10 Minutes Duration in Rat Hind Quarter Preparation at Various Intervals

*Total volume of fluid collected in 10 minutes (in ml) ± S.E.**		
Before the administration of nitroharmidine 500 ug/kg B.W. of the intact animal.	After the administration of nitroharmidine nitrate 500 ug/kg B.W. of the intact animal.	P value
55.0± 3.32	48.0 ±4.82	P ⩽0.2

*Standard error.

E. *Effect of Nitroharmidine Nitrate on Anaesthetized Cat Blood Pressure (Normal and Atropine Treated)*

Nitroharmidine nitrate, contrary to its effect on rat blood pressure, produced fall in blood pressure in this study. The fall in blood pressure was statistically insignificant after 200 ug/kg B.W. doses, but was significant following the larger doses. The pretreatment of animal with 2 mg/kg B.W. doses of atropine sulphate intravenously abolished the hypotensive effect of nitroharmidine nitrate. The results are shown in Figure 2 and Table IV respectively.

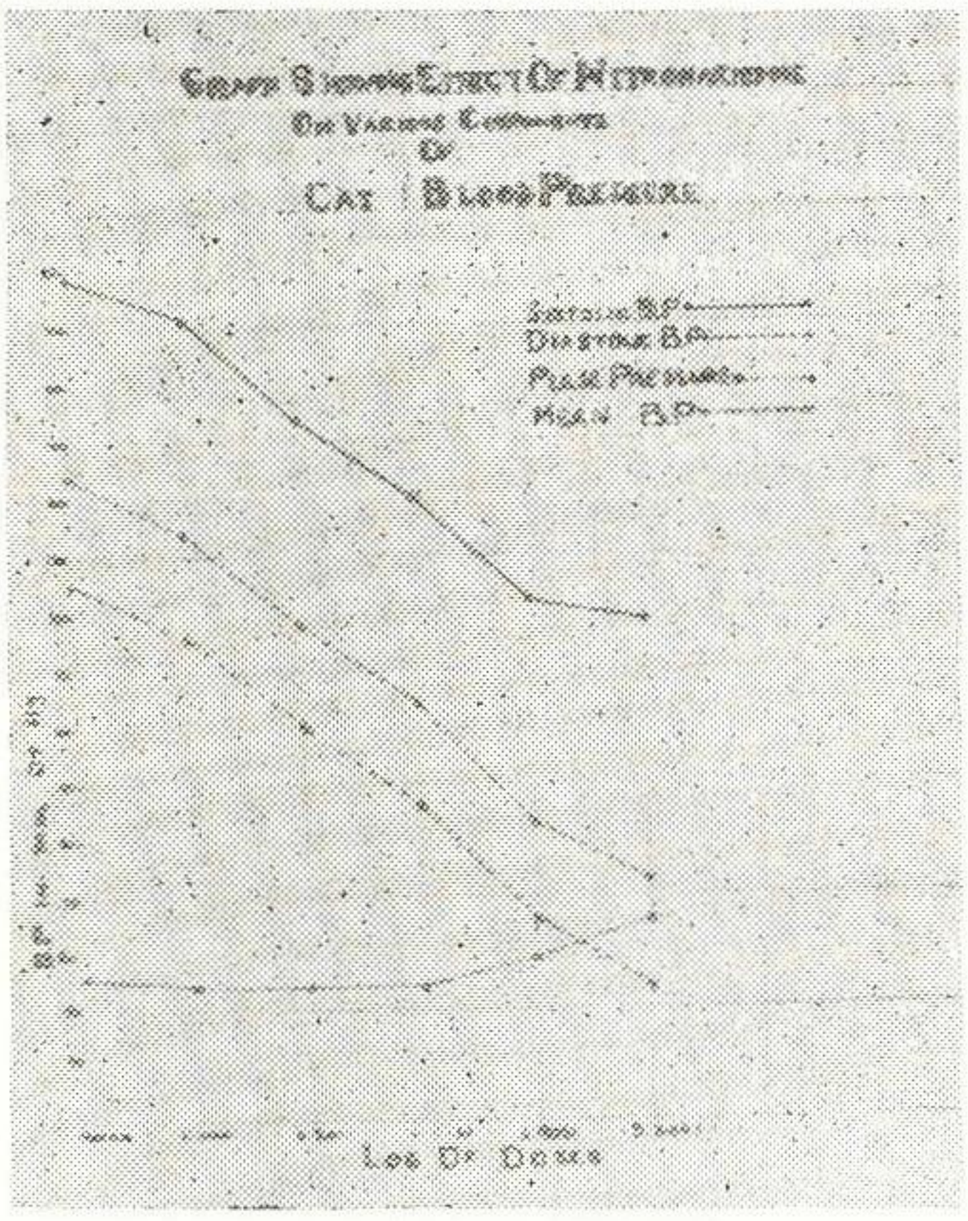

Fig. 2: Graph showing the effect of different doses of nitroharmidine nitrate on various components of anaesthetized cat blood pressure.

F. Effect of Nitroharmidine Nitrate on Spinal Cat Blood Pressure

The hypotensive effect of nitroharmidine nitrate produced in normal anaesthetized cats was not reproduced when same doses of drug were tried in spinal cats. However, a slight rise in blood pressure though statistically insignificant was observed. Table V presents the results of this study.

Table V: Effect of Nitroharmidine Nitrate on Spinal Cat Blood Pressure
(Dose 500 ug/kg B.W.)

Parameter	*Blood pressure in mm Hg ± S.E.**		*Percentage rise or fall*	*P value*
	Control	*After treatment*		
Systolic	113.7 ±12.82	118.3 ±10.54	4.0 rise	N.S.
Diastolic	54.2 ± 8.51	46.6 ± 8.03	14.0 fall	N.S.
Mean	73.8 ± 9.75	70.55 ± 8.43	3.0 fall	N.S.
Pulse Pressure	59.5 ± 6.02	70.5 ± 6.54	18.4 rise	N.S.

*Standard error.

G. Effect of Nitroharmidine Nitrate on Force of Contraction of Isolated Rabbit Heart

Nitroharmidine nitrate significantly depressed the force of contraction of isolated perfused heart of rabbit. The degree of depression was determined in terms of decrease in the amplitude of contractions measures in centimeters. The decrease was observed with all doses tried but statistically significant effect was produced with a dose of 40 ug of the drug. The successive dose showed further decrease in force of contraction and with a dose of 160 ug the heart stopped beating. This cardiac arrest was, however, transient and the heart restarted beating within 5 minutes as the drug was washed off the organ. The results have been tabulated in Table VI.

Table VI: Effect of Nitroharmidine Nitrate on Force of Contraction of Isolated Rabbit Heart

S. No	*Dose*	*Force of contra;tion in cm ± S.E.**		*Percentage decrease*	*P value*
		Control	*After treatment*		
i	10 ug	5.0±0.84	4.1±0.58	18.0	N.S.
ii	20 ug	4.9±0.81	3.5±0.62	28.6	N.S.
iii	40 ug	5.0±0.82	2.9±0.49	42.0	P ⩽0.05
iv	80 ug	4.7±0.75	1.6±0.51	65.9	P ⩽0.01
v	160 ug	4.5±0.64	0.2±0.06	95.5	P ⩽0.001

*Standard error.

Discussion

Harmidine, an alkaloid from Peganum harmala, in our laboratories, was shown to possess MAO inhibitory properties. Nitroharmidine is a modification of the structure of harmidine and is presumed to possess the properties of harmidine; many of its actions on central nervous system in our earlier studies have been tried to be explained due to these properties (Khawaja and Yusuf, 1977). It was considered worthwhile to study the actions of the drug on cardiovascular system in the hope of finding out some useful effects.

Nitroharmidine for its cardiovascular actions showed species differences. It produced marked rise in blood pressure in anaesthetized rats and on the contrary a significant fall in anaesthetized cats.

A. Effects on Anaesthetized Rat Blood Pressure

Nitroharmidine nitrate produced a rise in blood pressure in rats. It has been shown by Schmit and Schmitt (1964) that harmala alkaloids in addition to their being MAO inhibitors, can also cause a direct release of catecholamines from the stores and hence bring about a rise in blood pressure by their direct central action like amphetamine. A similar mechanism may be responsible for rise in blood pressure in this study. Being itself an MAO inhibitor like other alkaloids of Peganum harmala, nitroharmidine nitrate escapes the oxidative deamination by MAO like tyramine and may cause release of norepinephrine from its stores by its direct action like amphetamine.

B. Effect of Phynoxybenzamine Premedication

The pressor effect produced by nitroharmidine nitrate was successfully prevented by pretreatment with 10 mg/kg B.W. of phenoxybenzamine. This may explain the mechanism of pressor effect to be through adrenergic transmitter at the alpha receptors in blood vessels.

C. Effect of Nitroharmidine on Blood Pressure in Pithed Rats

In order to elucidate the central nature of action of nitroharmidine, the drug was tried on pithed rats. The drug in doses which produced marked rise in blood pressure in normal rats failed to produce any positive effect in pithed rats. This shows that the drug produced its action by some mechanism involving the higher centres.

D. Effect of Rat Hind Quarter Preparation

A rat hind quarter preparation study was made to find out if nitroharmidine nitrate was producing the pressor effect by its direct action on the blood vessels. A decrease or increase in the flow of perfusion fluid indicated the constriction or dilatation of the blood vessels. The drug

in present study did not produce any change in the volume of perfusate collected. This showed that the drug had no direct effect on the blood vessels. Hence the effect produced was either through the adrenergic innervation or the result of central effect.

E. Effect of Nitroharmidine in Cat Blood Pressure

The drug, contrary to its pressor action or rat blood pressure, produced depressive effect in cat resulting in fall in blood pressure. Probably the drug could not release norepinephrine by its direct action on the higher centres of cat.

The hypotensive effect observed with MAO inhibitors has been suggested by Gessa et al. (1963) to be due to prevention of sympathetic nerve impulses to release norepinephrine at synapses. Brodie and Shore (1957) suggested that 5 HT in the brain may act as humoral transmitter and produce effects either by stimulating parasympathetic centres or inhibiting the sympathetic ones. The action of 5 HT on blood pressure are complex with marked species differences. In cat, dog and rabbit 5 HT causes a brief fall, preceding a short lived rise that is followed finally by a prolonged fall in the blood pressure. The hypotension and bradycardia thus produced can be abolished by vagotomy. Nitroharmidine nitrate may thus pro duce hypotensive effect in cat by any of the above noted mechanisms.

Moreover, nitroharmidine has been found to be a direct depressant of the myocardium. This has been observed in experiments on isolated perfused heart preparation. This direct depressant action of myocardium may also partly account for hypotensive effect of the drug. It is supported by the observation that fall in blood pressure following a dose of 600 ug/kg B.W. of nitroharmidine nitrate produced hypotension that could not be completely abolished by pretreatment with atropine.

The myocardial depression produced by nitroharmidine nitrate may be a direct action like bretylium, especially with large doses where atropine could not completely prevent the hypotensive effect of the drug. The bradycardia produced with nitroharmidine may also be partly the result of decrease in release of adrenergic transmitter as has been observed with nialamide, a mono amine oxidase inhibitor (Davey et al., 1963). The direct depression of the myocardium may be presumed to play a more pronounced role in the production of hypotension when the larger doses are employed as they elicited a significant prolongation of hypotensive effect of the drug.

The central locus of action of nitroharmidine has been further supported by the observations made in spinal cat blood pressure preparation where the drug failed to produce hypotensive action.

There is possibility that nitroharmidine nitrate might have raised the concentrations of, free 5 HT in the higher centres through MAO inhibition and this resulted in parasympathetic stimulation.

The bradycardia seen after tne administration of various doses of nitroharmidine nitrate points towards a vagal stimulation by the drug. This presumption can further be supported by the observation that the usual hypotension and bradycardia following nitroharmidine nitrate were absent in the cats pretreated with atropine sulphate.

Acknowledgements

The authors would like to thank Dr. Saleemuzzaman Siddiqui, Director Postgraduate Institute of Chemistry, Karachi University, for a liberal supply of nitroharmidine nitrate, and to M/S. Smith Kline & French Laboratories Limited, Welwyn Garden City, Herts, England, for a gift of dibenzyline.

References

Akhtar, M.S. (1971) Study of Norepinephrine and 5 HT content of hypothalami of rats treated with harmidine hydrochloride. University of Karachi.

Brodie, B.B. and Shore, P.A. (1957) A concept for a role of serotonin and norepinephrine as chemical mediators in brain. Ann. N.Y. Acad. Sci., 66:631.

Burn, J.H. Practical Pharmacology, Oxford, Black-well p. 68.

Chai, C.Y. and Wang, S.C. (1966). Cardiovascular actions of diazepam in the cat. J. Pharmacol. Exp. Therap., 154:271.

Davey, M.J., Farmer, J.B. and Reinert, H. (1963). The effects of nialamide on adrenergic functions. Brit. J. Pharmacol., 20: 121.

Gessa, G.L., Cuenca, E and Costa, E. (1963). On the mechanism of hypotensics effect of MAO Inhibitors. Ann. N.Y. Acad. Sci., 107:935.

Khawaja, O.U. and Yusuf, S.M. (1977) Under publication.

Schmitt, H. and Schmitt, H. (1964). Interrelationship between amphetamine and harmidine alkaloids. Nature, 203:877.