STUDIES INVOLVING INTERACTION BETWEEN ALPHAMETHYLDOPA AND COWPEA (VIGNA SINENSIS) USING BOTH NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS.

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THESIS
Submitted to
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D E C L A R A T I O N

This is to certify that I carried out the work reported in this thesis in the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University under the joint supervision of DRS. L. Sambo-Donga and C.N. Wambebe. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

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D E D I C A T I O N

TO GOD

and

In memory of my mother Mrs. Ibiyonu Kolawole.

PREFACE

The author entered the University of Ibadan Pre-degree Course in 1972, and later the Department of Pharmacy and Pharmacology, Ahmadu Bello University, Zaria in 1973. He qualified as a Pharmacist in 1976. After his Internship training and the National Youth Service, he joined the Department of Pharmacology, Ahmadu Bello University in October 1978 as a post-graduate student. He is now a member of staff in the same department as an Assistant Lecturer from October, 1979.

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RESULTS OBTAINED FROM COWPEA ANALYSIS

Samples	Ash weight(g)	Cowpea dry weight(g)
1	0.1863	10
2	0.1985	10
3	0.1658	10
4	0.1864	10
5	0.1614	10
6	0.1920	10

ESTIMATION OF Na^+, K^+, Ca^{++} in Ash Sample.

Sample 1,2,3,4,5,6 of known weights, were individually dissolved in 0.5ml of concentrated hydrochloric acid made up to 100mls with distilled water.

Na^+ estimation:- 15ml of each solution of samples was used in estimating sodium concentration. Result is as stated below:-

Sample 1	26 mmol/L
Sample 2	20 mmol/L
Sample 3	20 mmol/L
Sample 4	20 mmol/L
Sample 5	22 mmol/L
Sample 6	22 mmol/L

SUMMARY

i)) The effect of cowpea and its possible interactions with alphamethyldopa on the b.p. and heart rate of normotensive and spontaneously hypertensive rats have been studied.

ii) Cowpea (60%^{w}/w of Pfizer feed) increased the b.p. of normotensive rats (NTR), but had no such effect on spontaneously hypertensive rats (SHR). In addition the heart rate of SHR was significantly elevated while that of NTR was not affected. The sympathomimetic agents further elevated the b.p. in the NTR than in the controls, but the b.p. elevation in the SHR was much lower than in the controls. The heart rate of both NTR and SHR were not consistently affected.

lii) Cowpea (20%^{w}/w of Pfizer feed) did not alter the b.p. of NTR, but increased the heart rate. On the other hand, the b.p. of SHR was reduced, while the heart rate was apparently not affected. Noradrenaline and tyramine elevated the b.p. of this group lower than in controls, particularly with the SHR.

The results obtained with other sympathomimetics on both NTR and SHR were not consistent.

iv) Alphamethyldopa did not alter the b.p. of the NTR but lowered that of SHR. The heart rate uf both NTR and SHR was significantly elevated by alpha-methyldopa. The b.p. elevating effect of the sympathomimetics were not significantly more than those obtained in the controls.

V) Coadministration of alphamethyldopa and cowpea (60% and 20°/o^{w}/w) had no effect on the b.p. of NTR, but reduced that of SHR, Interaction of alphamethyl-dopa with cowpea (20%^{w}/w) increased the heart rate of both NTR and SHR. Similarly, cowpea (60%w/w) elevated the heart rate of SHR, but had no such effect on NTR. The sympathomimetic effects followed the pattern of responses obtained when administered to rats (NTR and SHR) treated with only cowpea (20% and 60%^{w}/w) . The treatment apparently had no influence on the effects of the sympathomimetics,

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Appendix I

MY PUBLICATION

SAMBO-DONGA, L., WAMBEBE, C. AND KOLAWOLE, S. O. (In Press): Effect of cowpea and its possible interaction with alphamethyldopa on the blood pressure and heart rate of normotensive and spontaneously hypertensive rats. West African J. Pharmacol. Drug Res.

GLOSSARY OF ABBREVIATIONS

AMD	Alphamethyldopa
B.P.	Blood Pressure
C.O.	Cardiac Output
DA	Dopamine
g	Gramme
H.R.	Heart Rate
IV	Intravenous
Kg	Kilogramme
mg	Milligramme
NA	Noradrenaline
NTR	Normotensive Rat
P.O.	Per Oral
P.R.	Peripheral Resistance
SHR	Spontaneously Hypertensive Rat.
µg	Microgramme.
sem	Standard error of mean

CHAPTER 1

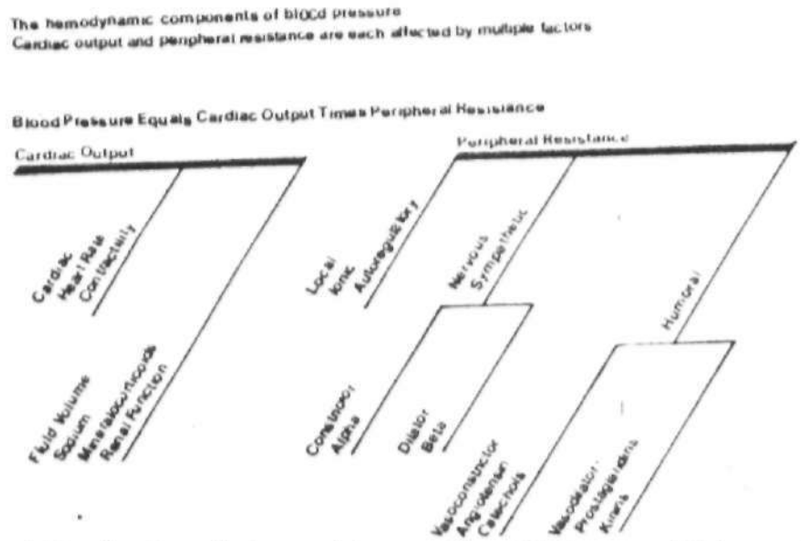
INTRODUCTION

The Pathogenesis of Hypertension

Hypertension is a condition characterised by high systemic blood pressure (b.p.) resulting from an abnormally high systolic b.p. in excess of 160 mmHg and diastolic b.p. exceeding 95 mmHg. The elevated arterial b.p. is symptomatic of some fundamental disturbances and is not itself a specific disease entity. Diastolic pressure correlates well with both the morbidity and mortality of untreated cases of hypertension (Pickering, 1971). However, the current findings indicate that the systolic b.p. may be a major contributing factor to cardiovascular catastrophies resulting from untreated hypertension (Kannel, Gordon and Schwartz, 1971).

In about 80 - 90% of hypertensive cases, the causes of b.p. elevation cannot be clearly defined. Such cases of unknown aetiology are called essential or idiopathic hypertension. It is for the treatment of essential hypertension that antihypertensive drugs have been found very useful. Haemodynamically, arterial pressure is determined by the cardiac output (c.o.) and peripheral resistance (p.r.), that is b.p. = c.o. x p.r. The p.r. is determined by the

Fig. A



Adapted from Maxwell, M.H. Aldomet® [methyldopa MSD]. In the managment of Hypertension. 1978 pp. 3.

viscosity of the blood and more importantly, by the caliber of the resistance vessels (see fig. A).

Although the aetiology of high b.p. is infrequently unknown, those b.p. disorders for which an understanding cause of cardiovascular, renal, neurogenic or endocrine origin may be determined are referred to as secondary hypertension. Amelioration of elevated b.p. in this form of hypertension is accomplished by therapy directed at the specific defect. The physiological mechanisms by which b.p. is determined are extremely complicated. All of these are interwoven in an almost inextricable web of feed-back mechanisms which should normally provide a large safety factor ensuring that the b.p. remains within acceptable limits. However, inspite of the in-built safety of such a system, the b.p. of a significant percentage of the population rises to levels which are associated with cerebrovascular, cardiac and renal complications (Dustan, Terazi and Bravo, 1972; Guyton, Coleman, Cowley, Scheel, Manning and Norman, 1972).

There are a large number of factors that can raise b.p. and it is widely thought that temporary rise in b.p. may in course of time become sustained. The barareceptor mechanism becomes 'reset', so that the b.p. is maintained at the elevated point (Sleight, Gribbin and Pickering, 1971).

The spasm of the arterioles leads to hypertrophy of their musculature and there is probably some degree of organic narrowing of the vessels. At this stage even a normal rate of autonomic discharge is associated with an elevated pressure (Ledingham, 1971; Tobian, 1972). Hypertensive subjects differ in the balance of factors which affect their b.p. and consequently, essential hypertension cannot be looked upon as a single entity, it is multifactoral.

The strongest evidence for this is the large difference which is being found in plasma renin values. Plasma renin has of course to be assessed in relation to salt intake; low salt intake will cause high plasma renin and high salt intake will cause low plasma renin (Brown, Davies, Lever and Robertson, 1963). Provided that this is taken into account, it can be inferred that hypertension in a subject with inappropriately high plasma renin may be at least partly due to renin activity as was reported by Brunner and co-workers (1972). They claimed that patients with high or normal levels of plasma renin in relation to sodium excretion had a higher incidence of stroke or myocardial infarction than those with low levels. Thus plasma renin activity could be regarded as a potential risk factor in hypertension (Laragh, Bear, Brunner, Buhler, Sealey and Vaughan, 1972;

Brunner, Laragh, Baer, Newton, Goodwin, Krakoff, Bard and Buhler, 1972). However, hypertension in a subject with low renin activity must have occured in spite of body's apparent ability to 'turn off' the production of renin.

It is in this field that the greatest progress has been made in determining the pathophysiology of hypertension and the findings have some relevance to therapy (Laragh et al, 1972).

High plasma renin levels are found in cases of malignant hypertension, primary and pseudo-primary aldosteronism, renovascular hypertension and hypertension due to oral contraceptives which cause increase in renin substrate (Laragh et al, 1972).

As stated earlier, cardiac output and peripheral resistance contribute to increase in the arterial b.p. However, it could be one or both of these factors that must be deranged, for the b.p. to remain high in hypertensive animals (Coleman, Granger and Guyton, 1971) and in man (Lund-Johansen, 1977). Some authorities suggested a primary increase in myocardial contractility and heart rate resulting from increased sympathetic and decreased parasympathetic nervous drive (Julius, Esler and Randall, 1975). It is now generally accepted that a high peripheral resistance is not the primary abnormality in most cases of hypertension.

Rather, the primary abnormality may be an increase either in adrenergic drive or expanded blood volume. The latter could be due to renin-angiotensin-aldosterone-sodium system as enumerated earlier, while the former could be due to a variety of causes.

Plasma levels of noradrenaline (NA) are widely used as an index of sympathetic activity and increased levels noted by some observers in a proportion of hypertensive subjects are interpreted as indicating increased sympathetic activity as a cause of high b.p. These correlations however are still not clean (Engelman, Portonoy and Sjoerdsma, 1970: Louis, Doyle and Anavekar, 1973).

Neurogenic components also play a role in the development of hypertension. Doyle and Smirk (1955) suggested that an alteration in the neurogenically maintained fraction of the b.p. may be the principal mechanism by which b.p. is regulated.

Genetic factors also play an important role in the development of essential hypertension in man. This is evident by diagnosis of the occurrence of essential hypertension in identical twins with hypertensive patients (Rose, Miller, Grim and Christian, 1979) and the studies of adopted versus natural children (Annest,

Sing, Biron and Mongeau, 1978). In addition, salt insensitive and salt sensitive strains of rats have been bread by Dahl (1972). The spontaneously hypertensive rats (SHR) developed by Okamoto and Aoki (1963) is also an evidence of genetic involvement in the development of hypertension.

The involvement of the kidney in the pathogenesis of hypertension cannot also be over-emphasised. The one-and two-kidney partial occlusion model of renovascular hypertension developed by Goldblatt, Linch, Henzal and Summerville, 1934) is evident of the involvement of the kidneys in hypertension.

Moreover, the experiment developed by De Champlain and Van Amerigan (1972) by the administration of deoxycorticosterone (DOCA) and 1% saline in the drinking water of uninephrectomized rats produced hypertension. This hypertension is associated with increased activity of peripheral sympathetic nerves and the adrenal medulla.

It is also possible for environmental factors to initiate hypertension. These are illustrated by various procedures, for example rats fed on excessive salt over a long period of time developed hypertension (Meneely, 1959).

Mice which have been progressively and continuously crowded developed hypertension with significant mortality due to uremia (Henry, Stephens and Santisteben, 1975). A caged male monkey observes his mate in the next cage copulating with another male monkey and then developed persistent hypertension (Lapin and Cherkovich, 1971). Excessive noise have also been reported to cause hypertension in animals (Medoff and Bongiovanni, 1945).

In the pathogenesis of essential hypertension, epidemiological factors should be considered. If we eat less food, take less salt and live a peaceful and unhurried life, then, the incidence of hypertension may be lowered profoundly.

Obesity is certainly enough to raise b.p. in many subjects. The stress of modern living may raise adrenergic drives sufficiently to set off a cycle of high cardiac output and high peripheral resistance in subjects who in other circumstances will remain normotensive.

ii Management of Hypertension

Essential hypertension is a disease with usually no well defined symptoms, and yet it increases the risk of many serious disorders (Van Zwieten, 1980). In only about 10% of all patients can the high b.p. be shown to have an

identifiable cause. In most of these, it is secondary to various kidney and adrenal diseases such as renal artery stenosis, parenchymal renal disease, cushing syndrome, primary aldosteronism and phaeochromocytoma (Laragh, 1972).

Essential hypertension requires management, since cure is not possible. General management of essential hypertension includes education, weight control, sodium restriction, exercise, relaxation and life style. These guidline may adequately control mild hypertension.

Education:- patient must be taught that hypertension is a condition that requires continuing observation and management. He must be made to understand that hypertension is a chronic and significant health problem. Hypertensive patients should also be motivated to follow a treatment programme that is life long.

Diet-Reduction of weight is important in obese persons. The avoidance of salt is advisable since a low salt diet will often result in a significant reduction in b.p. Atherosclerotic plague formation is a major cause of myocardial infarction and cerebrovascular disease. This formation is low-but has definite correlation with hypertensive vascular disease. Thus, a diet low in cholesterol and saturated fats may diminish the incidence of coronary artery disease (Page, 1972).

Physical Activity - Some form of exercise, suitable for one's age and general well-being, is part of the natural life

of a man and should be continued unless heart failure, angina or a stroke prevents it. The patient should be encouraged to lead as active a life as he possibly can without discomfort.

Socio-Cultural Management:- A disturbed, frustrated, unhappy life, psychic and emotional reactions to environmental factors do not help the patient. Tobacco smoking may aggravate coronary artery disease and should be avoided. Women with high blood pressure who are taking contraceptive pills should stop for six months, if hypertension improves then it may be due to oral contraception.

In the management of hypertensive subjects, a minimum work-up should include, when possible, pressure readings made on at least three visits before treatment is commenced. It should also include several electrolyte measurements, one standard multianalysis blood profile, a urinalysis and a chest reentgenogram and electrocardiogram, (Laragh, 1972).

It is clear that antihypertensive therapy if applied rapidly and effectively can yield brillant and often life-saving results. The patients who should receive treatment are those with family history of hypertensive cardiovascular diseases and evidence of persistent hypertension. Those with secondary hypertension not amenable to surgery should also be treated. In general, where the diastolic b.p. is persistently about 95 mmHg or higher should be treated.

In hypertensive therapy, an attempt is made to reduce sodium intake to approximately 2 to 3 grams per day. If this fails to reduce b.p., drug therapy is indicated. There is the need to use the least number of drugs possible to produce the greatest benefit with less side effects. No drug needs to be taken more frequently than twice daily. Frusemide should be reserved for patients with renal failure or sensitivity to thiazide (Ryan, 1979). Avoid beta-blockers when treating patients with congestive heart failure, heart block, chronic air-way obstruction and diabetes mellitus.

iii Pharmacology of Alphamethyldopa

Alphamethyldopa (L-isomer of alphamethyldopa) is a potent antihypertensive in animals and man. The mechanisms whereby it reduces human blood pressure are not completely established. The decrease in blood pressure produced by alphamethyldopa is not accompanied by significant changes in glomerular filtration rate or renal blood flow, but renal vascular resistance decreases and the renal fraction of cardiac output is increased (Onesti, 1976; Sannerstedt and Conway, 1962).

It was hypothesised that alphamethylnoradrenaline derived from alphamethyldopa entered noradrenaline storage sites within adrenergic neurons and was released as a false neurotransmitter upon nerve stimulation (Carlsson and Lindqvist, 1962; Day and Rand, 1963; 1964). The release of the false

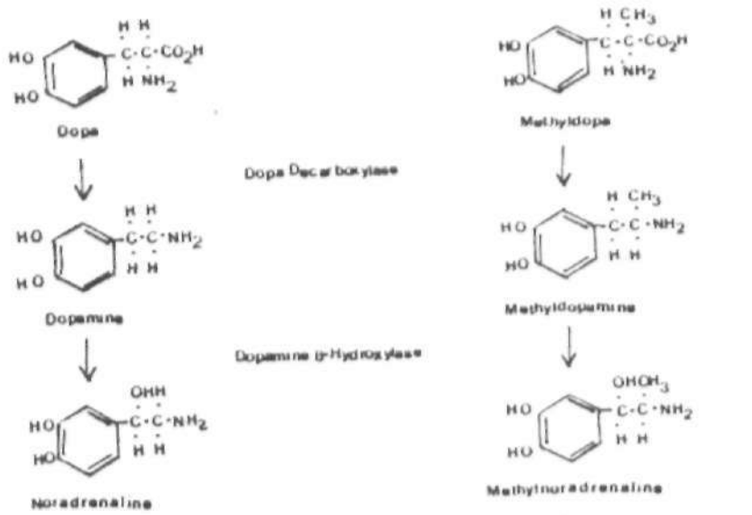
transmitter possessing less pressor effect compared to noradrenaline was said to be responsible for the hypotensive effect of alphamethyldopa.

Ayitey-Smith and Verma (1970) also reported effective blood pressure lowering by alphamethyldopa in immunosympathectomized rats, indicating some direct peripheral action. There is however no current persuasive evidence that alphamethyldopa acts by direct relaxation of vascular smooth muscles.

Alphamethyldopa was also found to lower plasma renin activity. It was also found that alphamethylnoradrenaline is only one-third as potent as noradrenaline in directly releasing renin (Mohammed, Fasola, Privitera, Lipicky, Martz and Gaffney, 1969; Weidmann, Hirsch, Maxwell, Okun and Scroth, 1974).

The decarboxylation of alphamethyldopa is a prerequisite for the antihypertensive effect of the drug (see fig. B). The drug is converted in the central nervous system (CNS) to the active metabolites: alphamethyldopamine and alphamethylnoradrenaline. It was previously assumed that alphamethylnoradrenaline played the major antihypertensive action of the drug (Van Zwieten, 1973; Finch and Haeusler, 1973). Recently however, it was shown that hypothalamic levels of alphamethyldopamine and not of alphamethylnoradrenaline in

Figure - 8
Synthesis of noradrenaline and methylnoradrenaline



Adapted from Reid, J. L., Dean, C. R., and Jones, D. H. Central actions of antihypertensive drugs, Cardiovasc. Med. 2:1186, December 1977

rats were closely related to the hypotensive effect of alphamethyldopa (Freed, Murphy and Quintero, 1978). There is also a good correlation between either central alphamethylnoradrenaline (decreased) or alphamethyldopamine (increased) levels and the decrease in b.p. (Van Zwieten, 1973; Finch and Haeusler, 1973; Freed, Murphy and Quintero, 1978). In human subjects the amine metabolites of alphamethyldopa (alphamethyldopamine and alphamethylnoradrenaline) showed lesser potency than noradrenaline. The pressor potency of alphamethyldopamine is about 1/100th that of noradrenaline and that of alphamethylnoradrenaline is about $\frac{1}{3}$rd as potent as noradrenaline in increasing the systolic blood pressure in man (Pettinger, Horwitz, Spector and Sjoerdsma, 1963).

Alphamethyldopa readily crosses the blood-brain barrier and enters adrenergic neurous, where it is transformed into the active metabolite-alphamethylnoradrenaline as in fig. B. In the nucleus tractus solitarius, alphamethylnoradrenaline is believed to act as a potent transmitter which stimulates alpha-adrenoceptors, thus inhibiting sympathetic outflow with an accompanying decrease in b.p. (DeJong and Nijkamp, 1976; Oates, 1980).

Alphamethyldopa treatment showed a slight but significant

mean increase in cerebral blood flow and a decrease in cerebrovascular resistance (Mayer, Sawada and Toyoda, 1968). Myocardial blood flow is consistently increased in hypertensive subjects following intravenous injection of alphamethyldopa (Cohen, Maxman, Ragheb, Baluron, Zaleski and Bing 1976).

The b.p. declines more in the hypertensive than in the normotensive subjects on alphamethyldopa. The hypotensive effect is often greater in the upright than in the supine position and also hypotension during exercise is rare (Nickerson and Ruedy, 1975). Alphamethyldopa has also been shown to cause a net reduction in the tissue concentration of serotonin, dopamine, noradrenaline and adrenaline due to its depleting effect on these amines. It also inhibits the decarboxylation of l-dopa to dopamine (Kopin, Fischer, Musacchio, Horst and Weise, 1965).

Studies on the mechanisms of action of alphamethyldopa in both man and laboratory animals have so far yielded conflicting results. However, recent findings favour a central mechanism rather than a peripheral site of action. It is apparent that alphamethyldopa exerts a major antihypertensive effect through a variety of mechanisms.

iv Drug-Diet Interaction

The relationships between drugs and diet is a subject fraught with much speculations and uncertainty with minor

exceptions. Foods are known to contain a variety of pharmacologically active substances, some of which are capable of producing severe toxicity or even death (Pierpaoli, 1972).

There are two major areas of concern with regards to drug effects and food. Some drugs are capable of delaying the absorption and utilization of nutrients. This is examplified by the effects of alcohol on nutritional status which are well documented with malabsorption of folic acid, vitamine B12 and increased excretion of magnesium (Faloon, 1970; Heaton and Pyroh, 1957). Certain foods or pattern of dietary consumption may alter drug absorption and response. There are several ways by which diet can influence any co-administered drug.

Place and Benson (1971) reviewed the effect of food on drug absorption. These workers reported that food can have variable effect on drug absorption, thus the absorption of some drugs may be significantly inhibited by food. On the other hand, the absorption of many drugs is slowed, but this effect may be clinically insignificant (Grower and Dash, 1969; Jaffe, Colazzi and Barry, 1971).

Dietary constituents may alter drug metabolism either through induction or inhibition of enzyme. Such effect may

principally involve food contaminants. Foods may also alter the rate of excretion of certain drugs possibly as a result of excessive acidification or alkalinization of the urine.

Pharmacologically active substances present in foods may alter the response of a coadministered drug or even produce pharmacological effects on their own. This type of interaction has been illustrated by the coadministration of monoamine oxidase inhibitors (e.g. paragyline) and the ingestion of foods containing large amounts of tyramine (e.g. cheese) and other biologically active amines (Horwitz, Lovenberg, Engelman and Sjoerdma, 1964; Blackwell and Mabbitt, 1965). It has been reported that broad beans (Vicia faba leguminosea) contain amine precursors (Hodge, Nye and Emerson, 1964). In general, combination therapy with monoamine oxidase inhibitors may be dangerous, where the levels of catecholamines are raised significantly thereby producing profound potentiation of their pressor effects.

However, very little is known about some of our indigenous foods like the cowpea(vigna sinensis leguminosea). This is widely eaten in this country. Literature search also indicated that it contains amino acids which are precursors of amines (Onayemi and Potter, 1976). This is prepared in various forms as cakes, moimoin, soup (Gbegiri) boiled alone or with rice or together with yam or maize.

v. Effect of anaesthesia (urethane) on b.p. and Heart Rate.

Response to cardiovascular agents may differ quantitatively when tested under different anaesthetic agents (Brezenoff, 1973; Miller and Wiegman, 1977).

Urethane has been used as a hypnotic/narcotic agent since 1885 (Altra and Weingberg, 1979). It is regarded as an anaesthetic of choice in many physiological and pharmacological experiments. Though widely used, it is known to lower b.p. (Hillerbrand, Van Der Meer and Ariens, 1971; Bunag and Mulenix, 1972; Brezenoff, 1973). It can also inhibit pressor responses to noradrenaline. (Bunag and Mullenix, 1972; Brezenoff, 1973).

There are several mechanisms put forward to explain this inhibitory action of urethane on pressor changes. Miller and Wiegman (1977) suggested that the decreased sensitivity of both artery and vein in urethane anaesthetized animals to noradrenaline could be via a vascular inhibitory effect of urethane. On the other hand, Pettinger, Tanaka, Keeton, Campbell and Brooks (1975) proposed that it may involve high levels of renin. However, altered calcium metabolism has also been implicated (Peng, Cooper and Munson, 1972).

Urethane has a well known b.p. lowering effect which may be due to either a loss of blood plasma into the peritoneal cavity (Vander Meer, Versluys-Broers, Tuynman and Burr, 1975) or a direct cardio- depressant effect (Giles, Quiroz and Burch,1969)

Brezenoff (1973) also suggested that the reflex bradycardia in rats was either reduced or unchanged by urethane.

Urethane was chosen as anaesthetic of choice in this project because it produces the minimal changes in respiration when compared to chloralose or pentobarbital in anaesthetic doses (Wang and Mims, 1948). It is known that alteration in respiration may reflexly affect responses to b.p. changes.

In this project the control as well as the experimental animals were subjected to the same anaesthetic treatment. Thus whatever influences urethane might possess on cardiodynamics would be common to both test and control animals.

vi. Objective of Project

Pharmacologically active substances present in some foods have been shown to alter the responses of a coadministered drug. This type of food-drug interaction has been observed with concomitant administration of monoamine oxidase inhibitors and the ingestion of foods containing large amounts of tyramine and other biogenic amines and their precursors. Several deaths were initially recorded from this interaction as a result of exaggerated pressor responses and subsequent hypertensive crisis with intracranial bleeding (Sapieka, 1969). Monoamine oxidase inhibition appears to modify and intensify the effects of endogenous biogenic

Estimation of K^+:- The original solution was diluted 1:200 by withdrawal of 0.1ml of sample solution plus 19.9mls of distilled water. Result is as stated below:-

Sample	1	9.0 x 2	=	18.00 mmol/L
Sample	2	9.3 x 2	=	18.60 mmol/L
Sample	3	7.8 x 2	=	15.60 mmol/L
Sample	4	9.1 x 2	=	18.20 mmol/L
Sample	5	7.6 x 2	=	15.20 mmol/L
Sample	6	8.8 x 2	=	17.60 mmol/L

Estimation of Ca^{2+}:- Volume of sample solution used = 0.05ml. Result is as stated below.

Sample	1	2.19 mmol/L
Sample	2	2.27 mmol/L
Sample	3	2.11 mmol/L
Sample	4	2.11 mmol/L
Sample	5	2.27 mmol/L
Sample	6	2.42 mmol/L

amines as well as their precursors. Tyramine containing foods and beverages have been the major offenders in this interaction.

Broad bean have also been implicated in this food-drug interaction phenomenon. The active substance appears to be the natural amino acid, L-3, 4-dihydroxyphenylalanine (L-dopa; Hodge, Nye and Emerson, 1964).

The above observations were made in a review of the influence of diet on drug therapy. It was therefore my intention to study the effect of cowpea alone on the heart rate and b.p. of normotensive and spontaneously hypertensive rats. In addition, the influence of coadministration of cowpea and alphamethyldopa on heart rate and b.p. would be investigated. It was also intended to study responses of animals treated as above to exogenously administered catecholamines.

CHAPTER II

MATERIALS AND METHODS

Test Animals

A colony of male and female spontaneously hypertensive rats (SHR - Okamoto-Aoki strain) and normotensive rats (NTR - Wistar strain obtained from Federal Veterinary Research Centre Vom, Nigeria) were used. The animals were about ten to thirteen months old and weighted between 200 and 400 g. However, the weight range within a particular group of animals was ± 50 g.

Drug Solutions.

Dopamine hydrochloride (DA), noradrenaline hydrochloride (NA), adrenaline hydrochloride and tyramine hydrochloride, obtained from Sigma Chemical Company, were used in this project. The solutions of DA, adrenaline, and NA were prepared by dissolving the drugs in normal saline (0.9%^{W}/v sodium chloride using recently boiled and cooled distilled water). In order to retard hydrolysis, 0.1%^{W}/w sodium metabisulphite was added to each solution. Tyramine hydrochloride solution was prepared using fresh normal saline. Alphamethyldopa tablet (Aldomet, Merck Sharp and Dohme Ltd) containing 250 mg anhydrous methyldopa base per

tablet was suspended in 1%^{W}/w compound tragacanth. Samples of the tablets used were analysed at the Ahmadu Bello University Quality Control Unit and confirmed as to British Pharmacopoea quality standard.

Due to the instability of some of the drugs used in this project, fresh drug solutions were prepared for each day's experiment. The solutions were kept in air-tight amber-coloured containers and stored in the refridgerator until ready for use.

Experimental Procedures.

Administration of alphamethyldopa.

The mixture of alphamethyldopa was administered orally using and adapted tube (19-gauge Terumo hypodermic needle with the sharp end smoothly cut off and the shaft covered with polyethylene tubing (PE 120 Clay Adams). This procedure allowed the dropping of the required amount of drug per unit time into the lower oesophageal area of the rat. The dose administered was 200 mg/kg body weight per day (Salmon and Iverson, 1970).

Analysis of Cowpea for Na^{+}, K^{+} and Ca^{2+} ion contents.

Ten grammes of dry cowpea was weighed into a porcelain container and burnt to ash at 800°C in a furnace (Carbolite$^{(R)}$

Banford, Sheffield, 972-ESF3-PD-IND) for 36 hours. The ash was weighed and dissolved in 0.5 ml concentrated hydrochloric acid (British Drug Houses Ltd, 11.456M, 1.18 S.G. Analar grade) and made up to 100 mls with deionised water. This solution was used for the analysis of sodium and potassium contents using the Galenkamp flame photometer with suitable filters. The analysis of calcium content was performed using the same solution as above and the colour developed was read utilizing the Galenkamp Colorimeter.

Method of feeding cowpea to rats.

Cowpea was powdered and mixed in the proportion of the required percentages with the powdered granules of Pfizer feed. The mixture was remoulded into bolus and dried at oven temperature of 60°C for 36 hours. The boli were prepared to allow easy removal of feed from the cage by the rats.

The rats were housed in batches of five per cage and were treated for six, twelve and eighteen weeks before blood pressure and heart rate recordings were made. The schedule of feeding the six groups of rats was as follows:

Group I - Rats fed on Pfizer feed only.

Group II - Rats fed on Pfizer feed and alphamethyldopa

(AMD) 200 mg/kg body weight daily.

Group III - Rats fed on Pfizer feed and 20% ^{w}/w cowpea

Group IV - Rats fed on Pfizer feed and 60%^{w}/w cowpea

Group V - Rats fed on Pfizer feed, 20%^{w}/w cowpea and AMD 200 mg/kg body weight daily.

Group VI - Rats fed on Pfizer feed, 60%^{w}/w cowpea and AMD 200 mg/kg body weight daily.

Surgical Procedure.

The rats were anaesthetised with Urethane (Ethyl Carbamate, Sigma Chemical Co.) 1.3 g/kg body weight given intraperitoneally (i.p.). Tranheotomies were performed and the trachea cannulated with polyethylene tubing (P.E. 240 Clay Adams) of sufficient diameter to provide adequate ventilation. Vascular access was provided at two sites. The left jugular vein was dissected free and cannulated with a polyethylene tubing (P.E. 50 Clay Adams) through which dopamine (100 ug/kg.);adrenaline (2 ug/kg.);noradrenaline (3 ug/kg.) and tyramine (100 ug/kg.)were infused into the rats. The carotid artery was dissected free and cannulated with polyethylene tubing (P.E. 50 Clay Adams) through which blood

pressure was recorded. The drug solutions were infused at intervals of 15 minutes. The Grass polygraph was calibrated before each day's experiment using an external pressure gauge cuff connected to the transducer by a three way polyethylene connector. This initial calibration was rechecked at the end of each experiment. After the cannulation procedure, a stability period of 15 - 20 minutes was allowed. The heart rate was recorded using intradermal electrodes connected to the Grass polygraph EKG recording channel.

The rats were pinned onto a regulated temperature board (Narco - Bio - system, INC, Huston, Texas, U.S.A. SER 1350) at 30°C to 32°C throughout the period of experiment.

Blood Pressure Measurement.

Blood pressure was recorded through the cannulated carotid artery using Gould Statham P 23Db transducer connected to Grass polygraph model 79D.

Readings were recorded utilizing four to five rats per group per time of treatment.

CHAPTER III

RESULTS

The following are results obtained from various treatments (as indicated on the graphs) on the b.p. and heart rate of both NTR and SHR. The data also include the influence of some sympathomimetics on the parameters mentioned above. All the results were with reference to controls fed on Pfizer rat pellets (group I) only.

i) The effect of cowpea (20% and 60%$^{w}/_{w}$) on b.p. and heart rate of NTR and SHR.

The group III NTR showed a marked decrease in heart rate at the twelveth week with a slight increase in b.p. However, there was a significant decrease in b.p. at the sixth week in the group III SHR with no corresponding change in the heart rate (fig. 1). The group IV NTR showed a significant b.p. increase at the sixth and the twelveth weeks with no corresponding alteration in the heart rate. However, there was a substantial increase in the heart rate of the group IV SHR at the eighteenth week (fig. 2).

Influence of noradrenaline on the b.p. and heart rate of groups III and IV NTR and SHR.

The group III NTR showed less response in heart

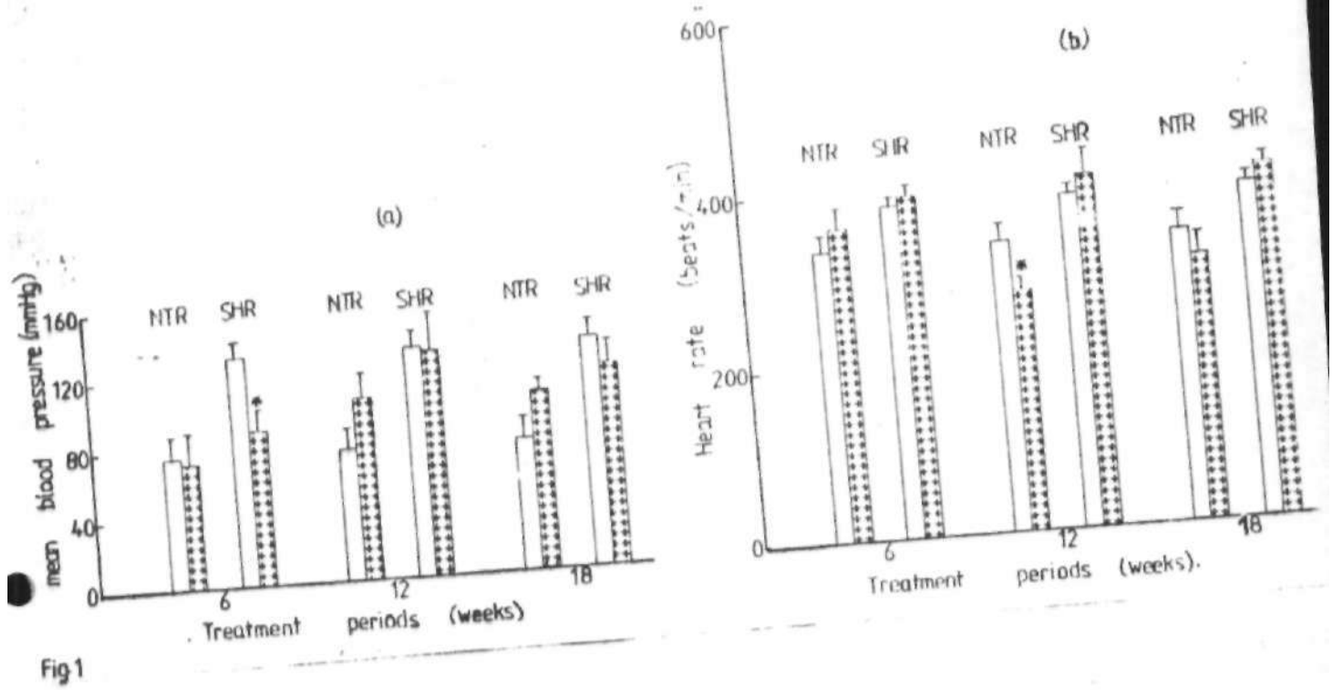


Fig 1

Changes in b.p. (a) and heart rate (b) of group III rats (20%^{w}/w cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4-5 observations ± sem.
* = $P<0.05$. Student's t - test.

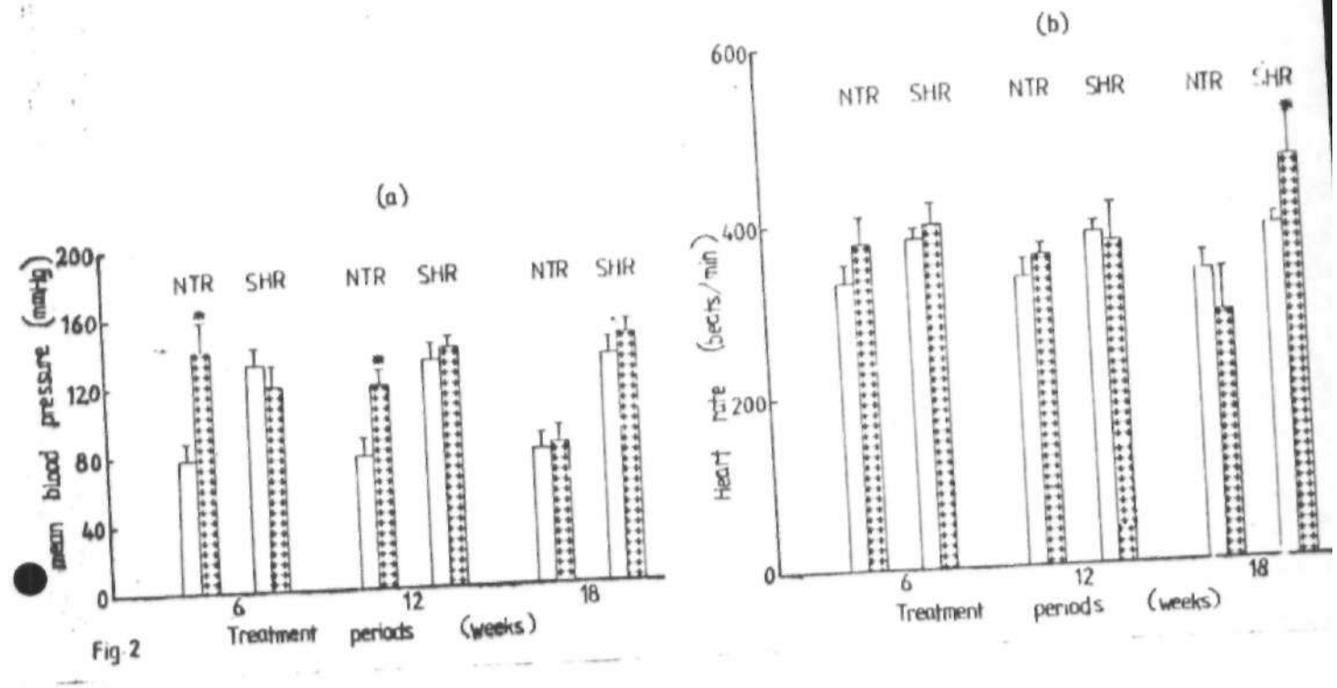


Fig 2

Changes in b.p. (a) and heart rate (b) of group IV rats (60%^{w}/w cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4-5 observations ± sem.
* = $P<0.025$. Student's t - test.

rate to the effect of noradrenaline when compared to control. However, noradrenaline increased the b.p. to a lesser extent in the group III SHR at the sixth and eighteenth weeks when compared to the group I SHR. The heart rate was not similarly affected (fig. 3). Noradrenaline in the group IV NTR, elevated the b.p. beyond the responses obtained in the group I NTR at the sixth and twelveth weeks. There was no similar effect on the heart rate. However, the group IV SHR showed greater response in heart rate at the eighteenth week (fig. 4).

Influence of adrenaline on the b.p. and heart rate of groups III and IV NTR and SHR.

The group III NTR showed a lesser positive chronotropic effect with no similar alteration in b.p. However, adrenaline elevated the b.p. to a lesser extent in the group III SHR at the sixth week than the group I rats. There was no similar effect on the heart rate (fig. 5). The magnitude of b.p. elevation induced by adrenaline was lower in group IV NTR than in group I rats at the sixth week. The heart rate was not similarly affected (fig. 6).

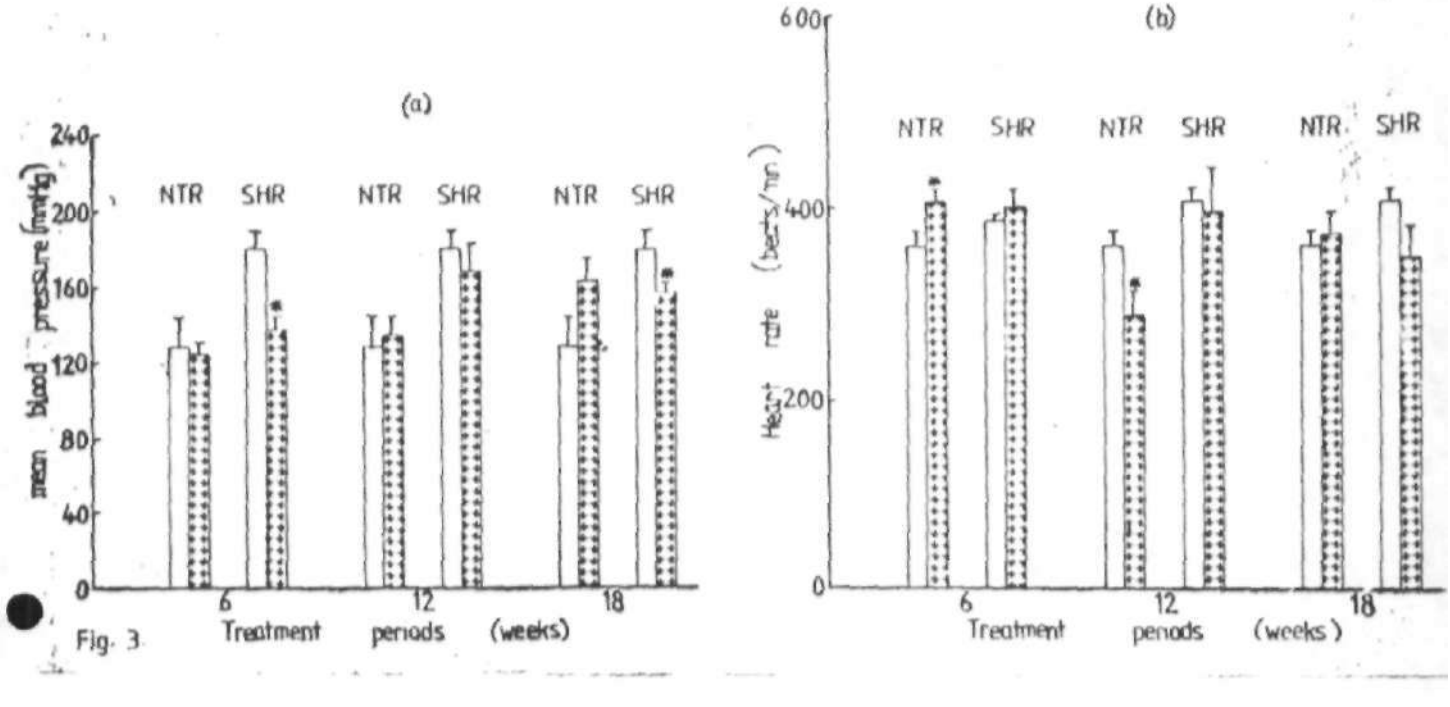


Fig. 3

Effect of noradrenaline (3 ug/kg i.v.) on b.p. (a) and heart rate (b) of group III rats (20%^{w}/w cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4-5 observations ± sem. * = $P<0.05$, Student's t - test.

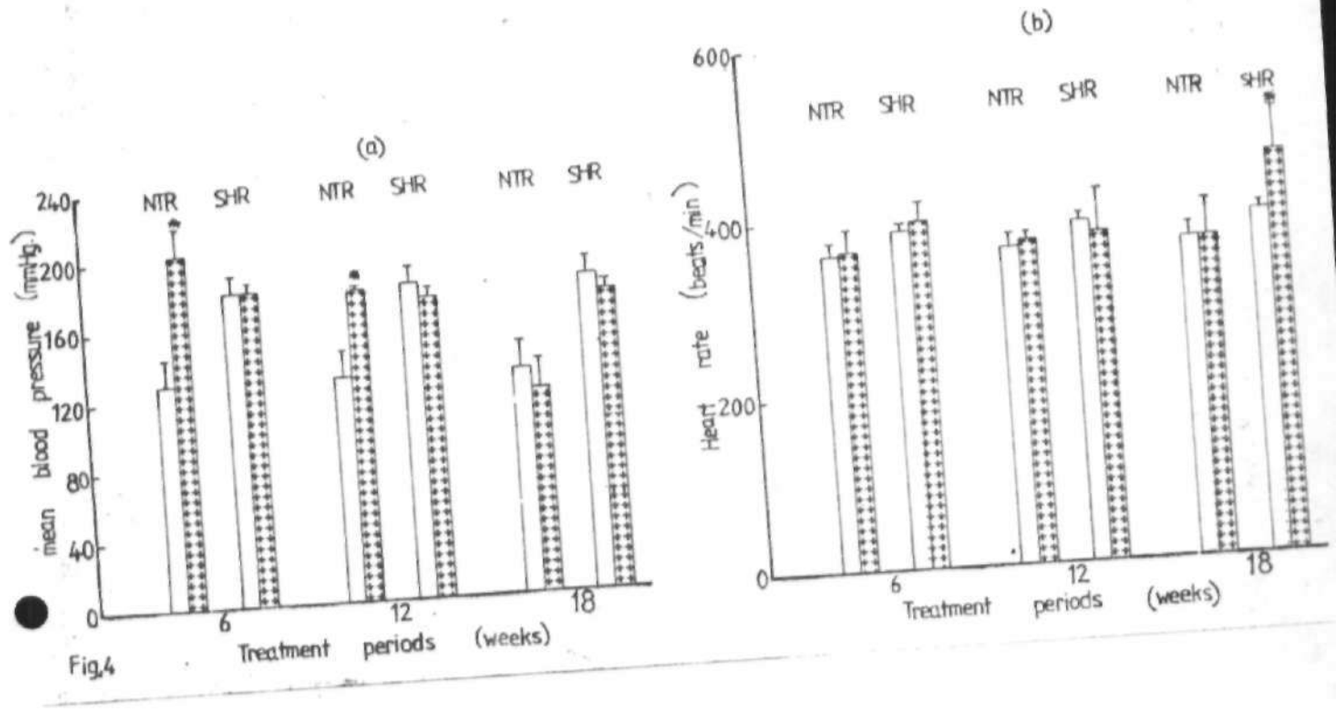


Fig.4

Effect of noradrenaline (3 ug/kg i.v) on b.p. (a) and heart rate (b) of group VI rats (60%^{W}/w cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4-5 observations ± sem. * = $P < 0.05$, Student's t-test.

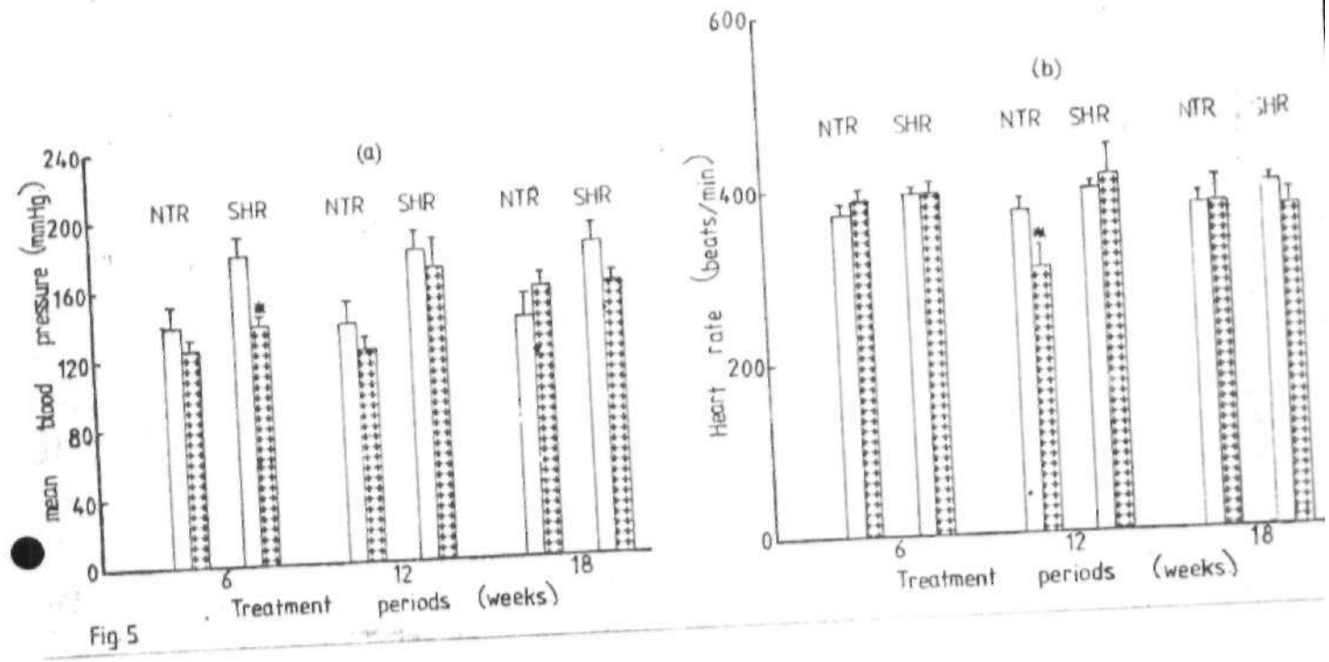


Fig 5

Effect of adrenaline (2ug/kg i.v.) on b.p. (a) and heart rate (b) of group iii rats (20% w/w cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P < 0.025$ Student's t - test.

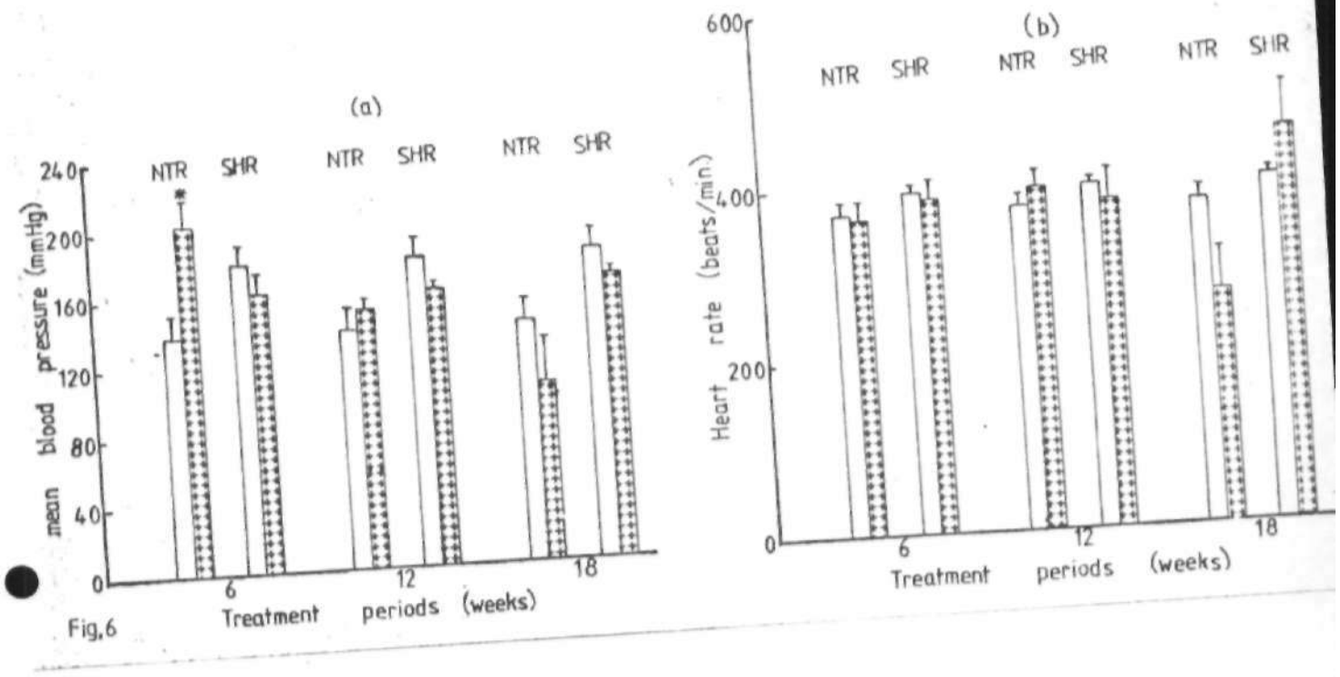


Fig.6

Effect of adrenaline (2ug/kg i.v.) on b.p. (a) and heart rate (b) of group iv rats (60% $^{w}/_{w}$ cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P < 0.01$. Student's t - test.

Influence of dopamine on the b.p. and heart rate of groups III and IV NTR and SHR.

The group III NTR and SHR did not show any marked alteration in b.p. than the group I rats. However, the group III NTR and SHR showed less increase in heart rate to dopamine at the twelveth and eighteenth weeks respectively (fig. 7). The results obtained with dopamine on group IV NTR (Fig. 8) were similar to those of noradrenaline (fig. 4).

Influence of tyramine on the b.p. and heart rate of groups III and IV NTR and SHR.

The positive chronotropic effect induced by tyramine was less at the twelveth week in the group III NTR compared to the group I rats. The elevation of b.p. induced by tyramine in group III SHR at the sixth week was less compared to group I rats. There was no similar change in heart rate. However, the heart rate response was more in the group III NTR and SHR at the sixth and twelveth weeks respectively than the corresponding group I rats (fig. 9). The results obtained with tyramine on group IV (fig. 10) were similar to those of noradrenaline (fig. 4).

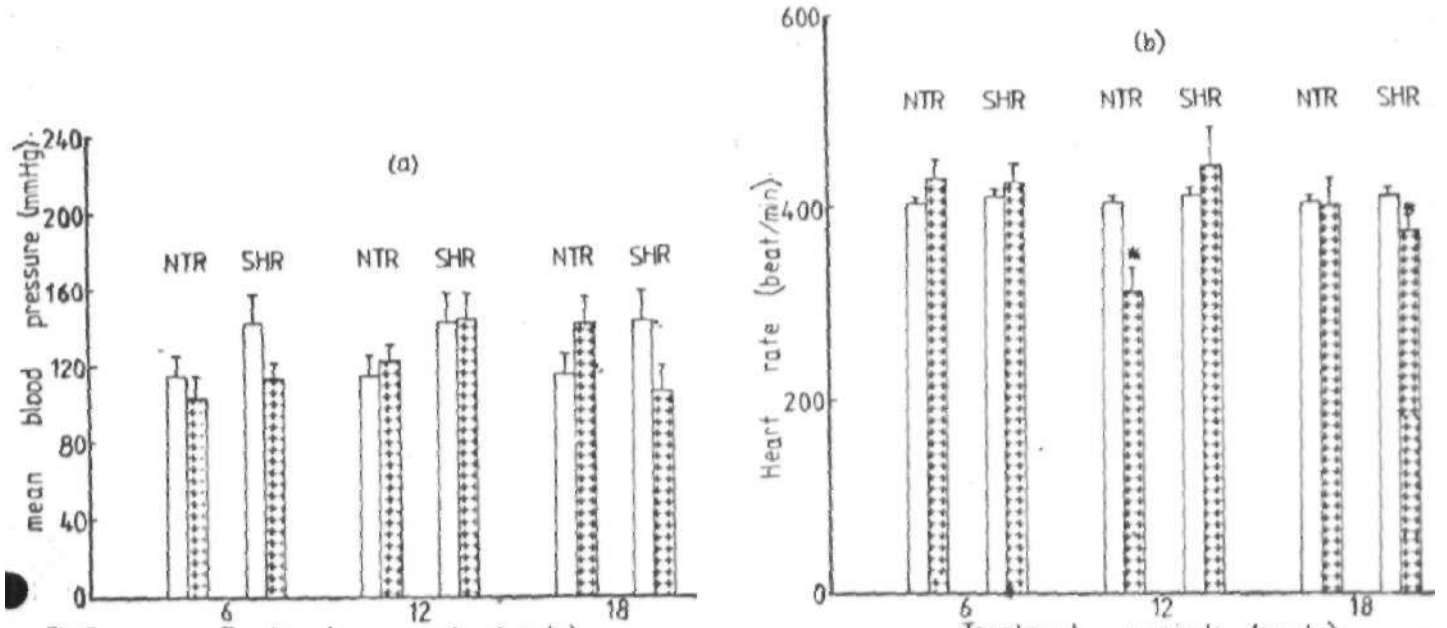


Fig.7

Effect of dopamine (100ug/kg i.v.) on b.p. (a) and heart rate of group iii rats (20% $^{w}/_{w}$ cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations $\pm$ sem. * = $P < 0.025$. Student's t - test.

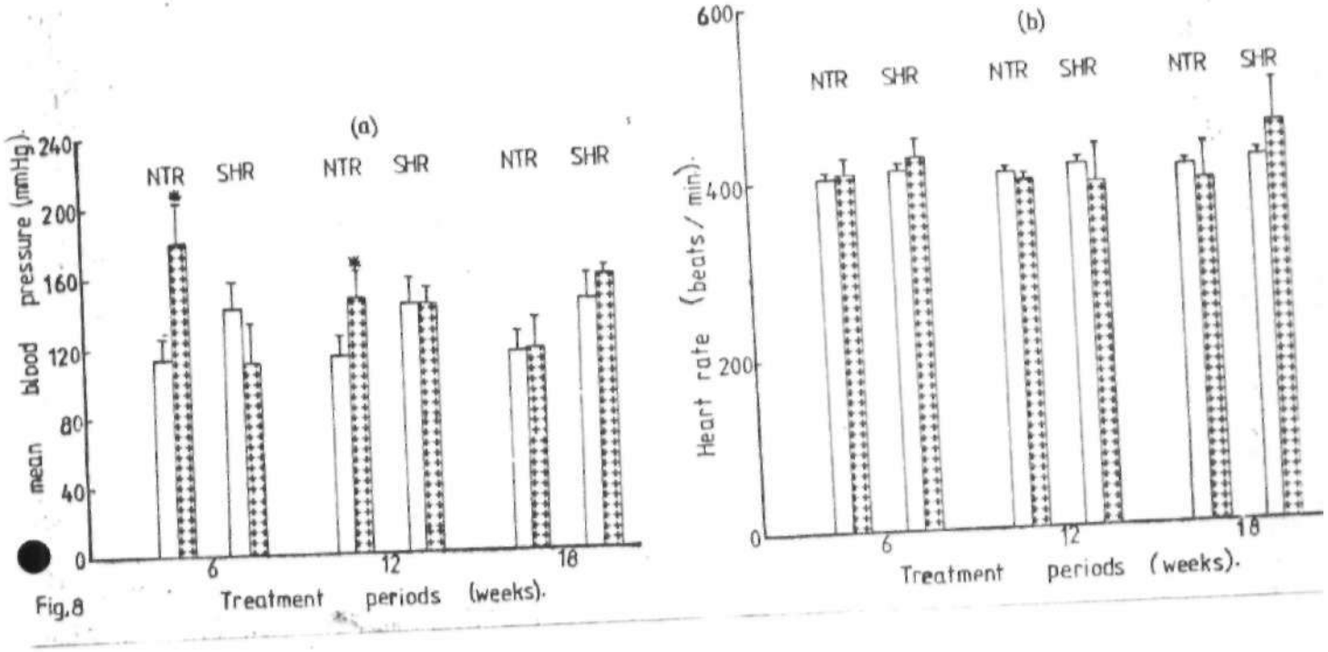


Fig.8

Effect of dopamine (100ug/kg i.v.) on b.p. (a) and heart rate (b) of group iv rats (60% $^{w}/_{w}$ cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P < 0.025$. Student's t - test.

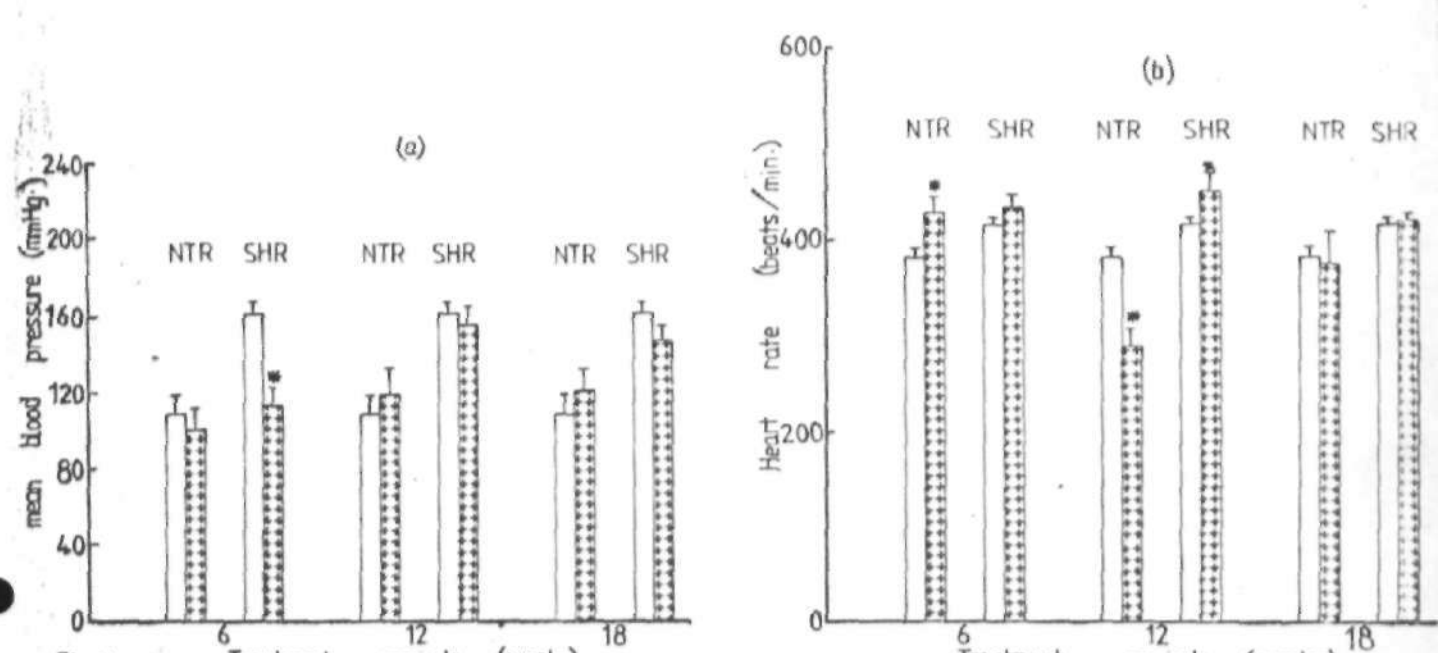


Fig 9

Effect of tyramine (100ug/kg i.v.) on b.p. (a) and heart rate (b) of group iii rats (20% $^{w}/_{w}$ cowpea in Pfizer feed, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem * = $P<0.05$. Student's t - test.

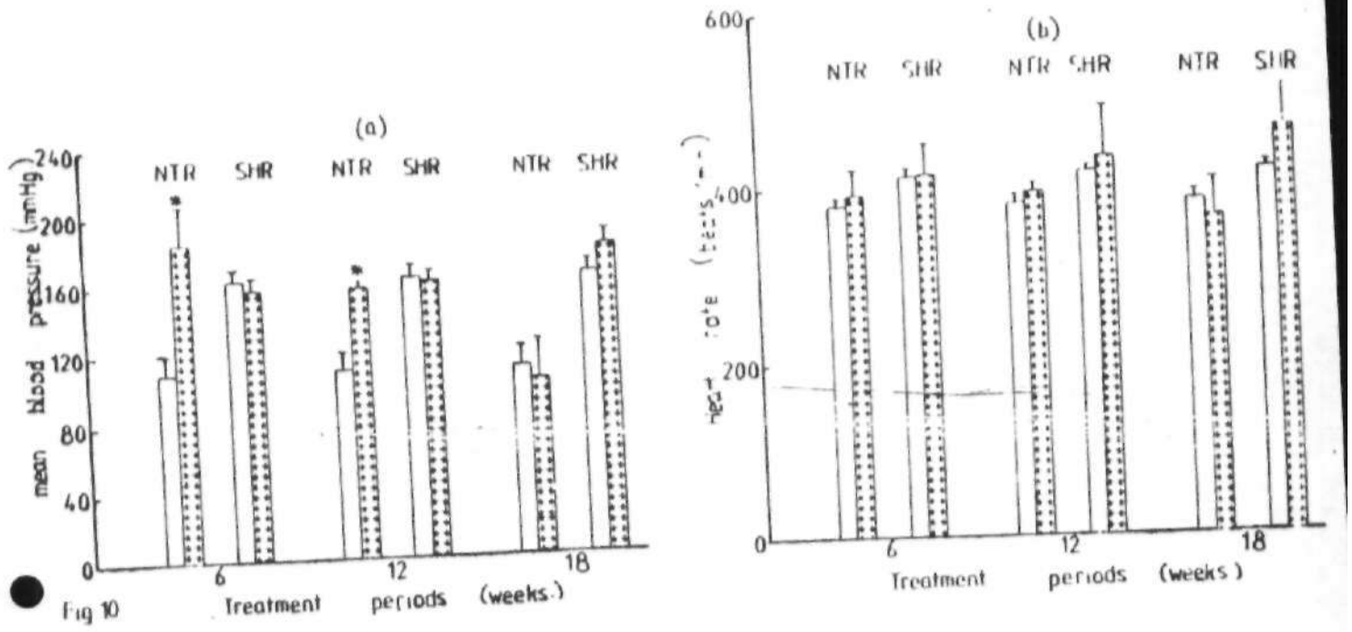


Fig 10

Effect of tyramine (100 ug/kg.i.v.) on b.p. (a) and heart rate (b) of group IV rats (60% w/w cowpea in Pfizer Feed, ▨) and group I rats (Pfizer Feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. *=$p < 0.0125$. Student's t - test.

ii) <u>The effect of alphamethyldopa (AMD 200mg/kg orally daily) on the b.p. and heart rate of NTR and SHR.</u>

The group II SHR showed a significant decrease in b.p. at the twelveth week with a corresponding increase in heart rate at the sixth and twelveth weeks (fig. 11).

<u>Influence of noradrenaline on the b.p. and heart rate rate of group II NTR and SHR.</u>

The b.p. of the group II NTR and SHR were not significantly altered from the controls. However, the heart rate was further increased by noradrenaline in the group II NTR at the sixth week, but it was decreased at the twelveth week. The group II SHR showed a higher increase in heart rate to noradrenaline at the twelveth and eighteenth weeks when compared to the group I SHR (fig. 12).

<u>Influence of adrenaline on the b.p. and heart rate of group II NTR and SHR</u>

The b.p. elevation induced by adrenaline in the group II NTR was lower than in control rats at the eighteenth week. There was a similar change in heart rate. However, group II SHR showed a higher increase in heart rate at the sixth week than the control rats (fig. 13).

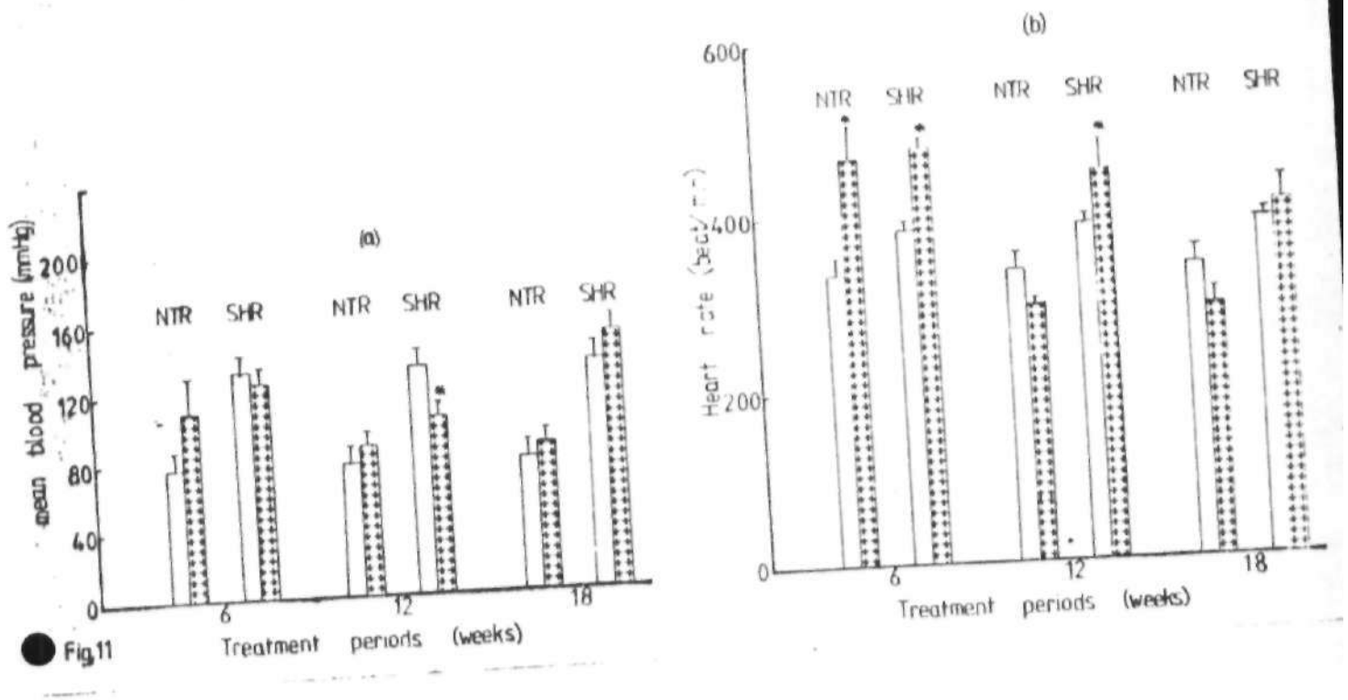


Fig.11

Changes in b.p. (a) and heart rate (b) of group II rats (Pfizer Feed and AMD 200 mg/kg orally, ▨) and group I rats (Pfizer Feed only, □) Each bar represents a mean of 4 - 5 observations ± sem. *=$p < 0.05$. Student's t - test.

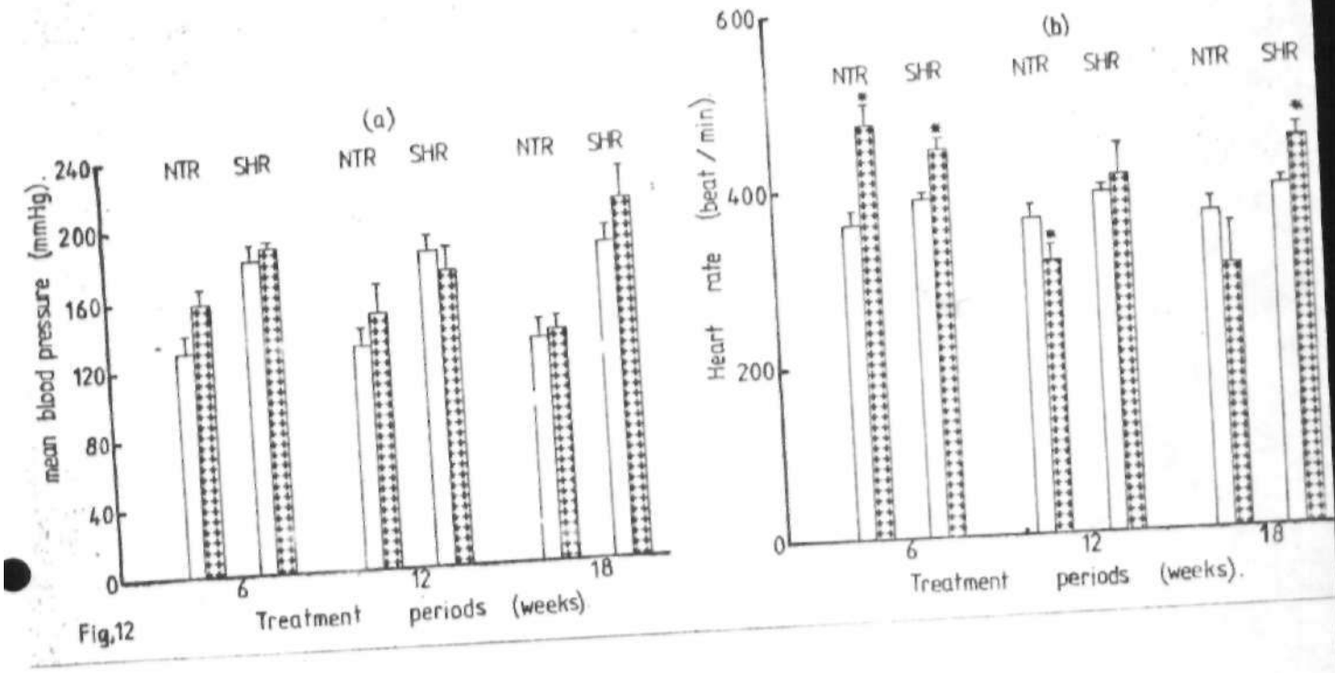
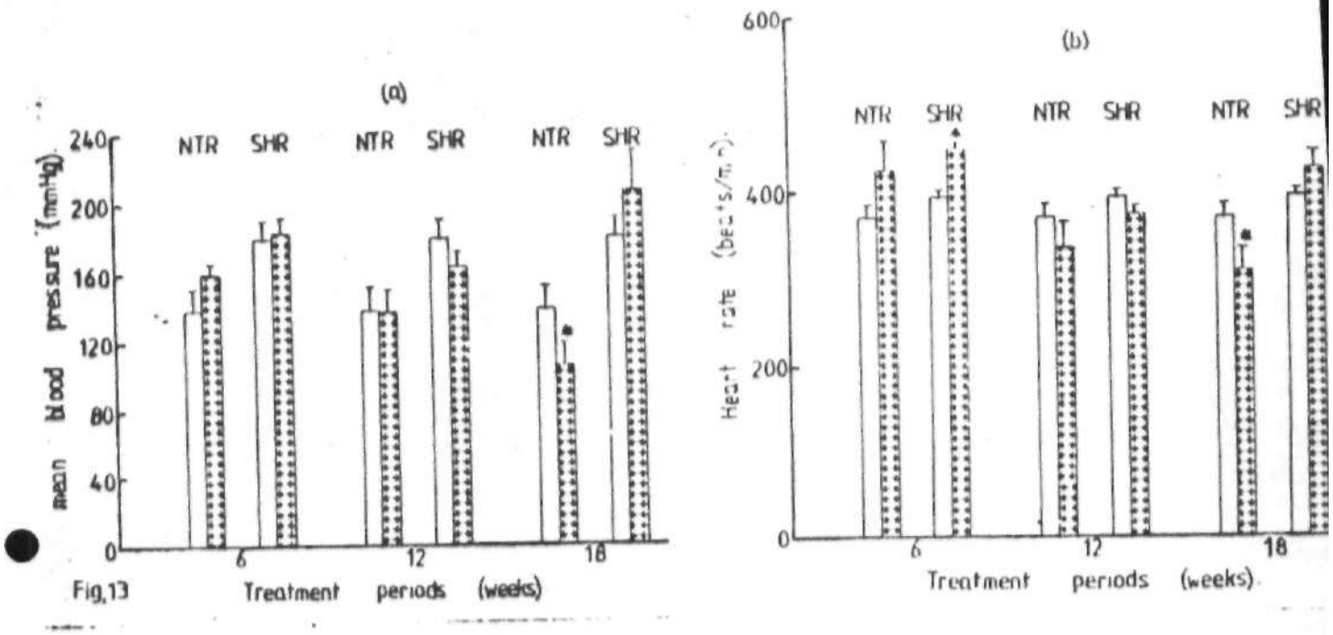


Fig.12

Effect of noradrenaline (3 ug/kg.i.v.) on b.p. (a) and heart rate (b) of group II rats (Pfizer Feed and AMD 200 mg/kg, orally ▨) and group I rats (Pfizer Feed only, ▱). Each bar represents a mean of 4-5 observations ± sem. *=$p < 0.05$, Student's t-test.



Fig,13

Effect of adrenaline (2ug/kg i.v.) on b.p. (a) and heart rate (b) of group II rats (Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. *=P< 0.05. Student's t - test.

Influence of dopamine on the b.p. and heart rate of group II NTR and SHR.

The group II NTR showed a significant b.p. change compared to the controls. The heart rate increase was more at the sixth week but it was less at the eighteenth week compared to the controls. However, the elevated b.p. response induced by dopamine was higher in the group II SHR than in the group I SHR at the eighteenth week. There was a higher heart rate response at the sixth week (fig. 14).

Influence of tyramine on the b.p. and heart rate of group II NTR and SHR.

The group II NTR showed a higher positive chronotropic effect at the sixth week compared to the control rats. Tyramine induced b.p. elevation was less in group II SHR at the twelveth week compared to control. However, the test rats responded more to tyramine at the eighteenth week. There was no similar changes in heart rate. The group II SHR manifested a higher response in heart rate at the sixth week (fig. 15).

iii) The effect of cowpea (20% and 60% $^{w}/_{w}$) and alphamethyldopa (200mg/kg orally daily) on the b.p. and heart rate of NTR and SHR.

The group V NTR did not show any significant

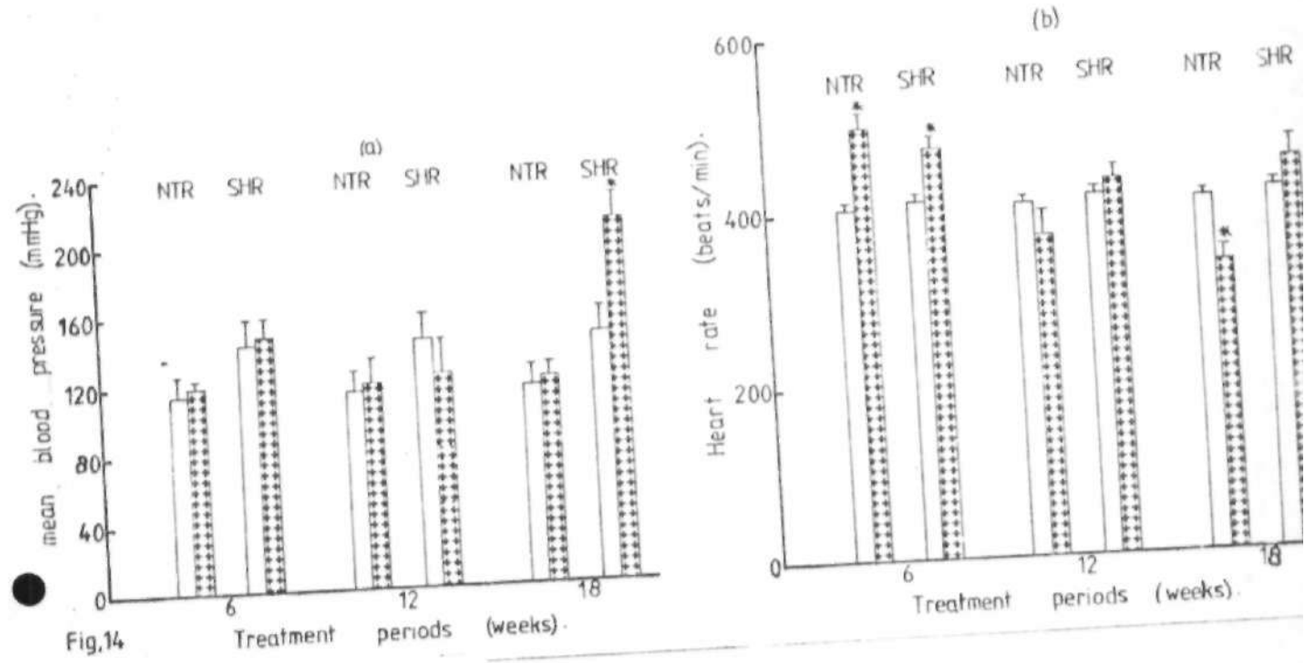


Fig.14

Effect of dopamine (100 ug/kg. i.v.) on b.p. (a) and heart rate (b) of group II rats (Pfizer Feed and AMD 200 mg/kg orally, ▨), and group I rats (Pfizer Feed only ▱). Each bar represents a mean of 4 - 5 observations ± sem. *= $p < 0.025$. Student's t - test.

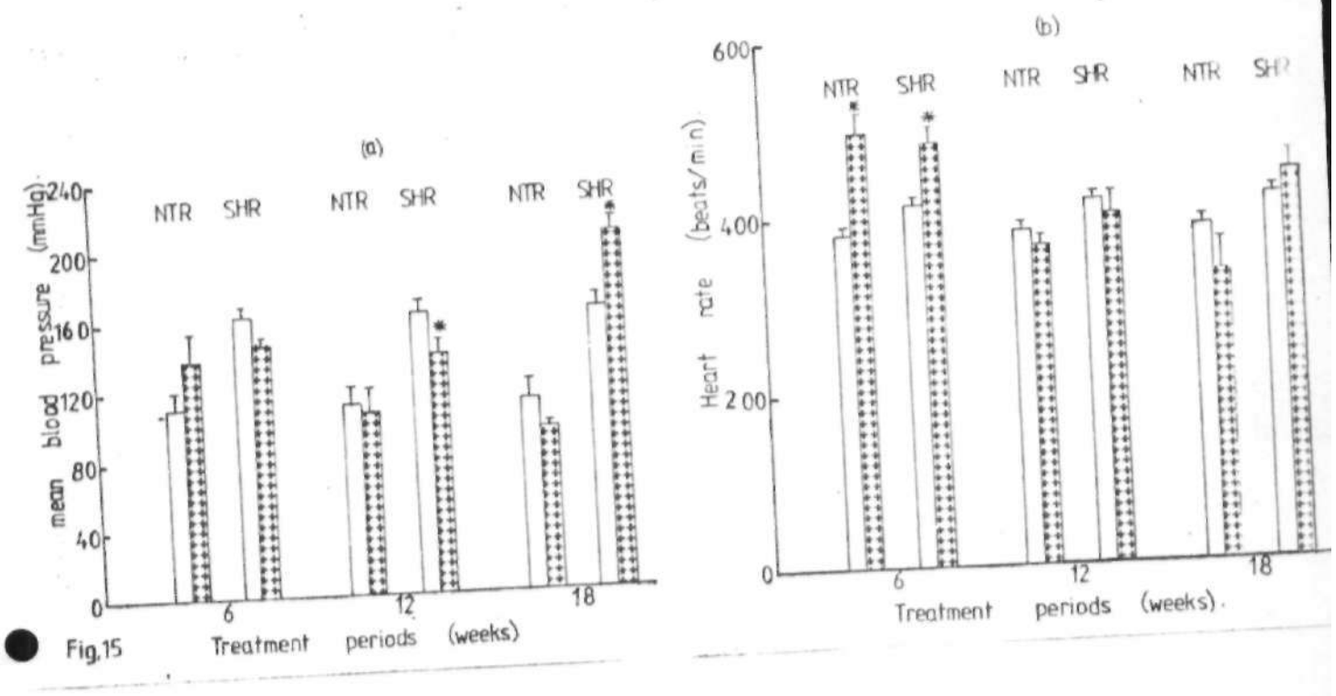


Fig.15

Effect of tyramine (100 ug/kg.i.v.) on b.p. (a) and heart rate (b) of group II rats (Pfizer Feed and AMD 200 mg/kg orally ▨) and group I rats (Pfizer Feed only ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $p < 0.05$. Student's t - test.

decrease in b.p. but showed an increase in heart rate at the sixth week. However, group V SHR showed a significant decrease in b.p. at the sixth week with a corresponding increase in heart rate. There was also an increase in heart rate of the group V SHR at the eighteenth week (fig. 16). Group VI SHR showed a significant decrease in b.p. at the eighteenth week with no corresponding change in heart rate. However, there was a significant increase in heart rate at the sixth and twelveth weeks. The group VI NTR did not show any significant change in both b.p. and heart rate (fig. 17).

Influence of noradrenaline on the b.p. and heart rate of groups V and VI NTR and SHR.

The magnitude of b.p. elevation induced by noradrenaline was higher in group V NTR at the twelveth and eighteenth weeks than in group I rats. The heart rate was increased more in test rats at the sixth week than in the controls. Noradrenaline, however increased the b.p. to a lesser extent in group V SHR at the twelveth week when compared to the controls. There was an increased positive chronotropic effect at the sixth and

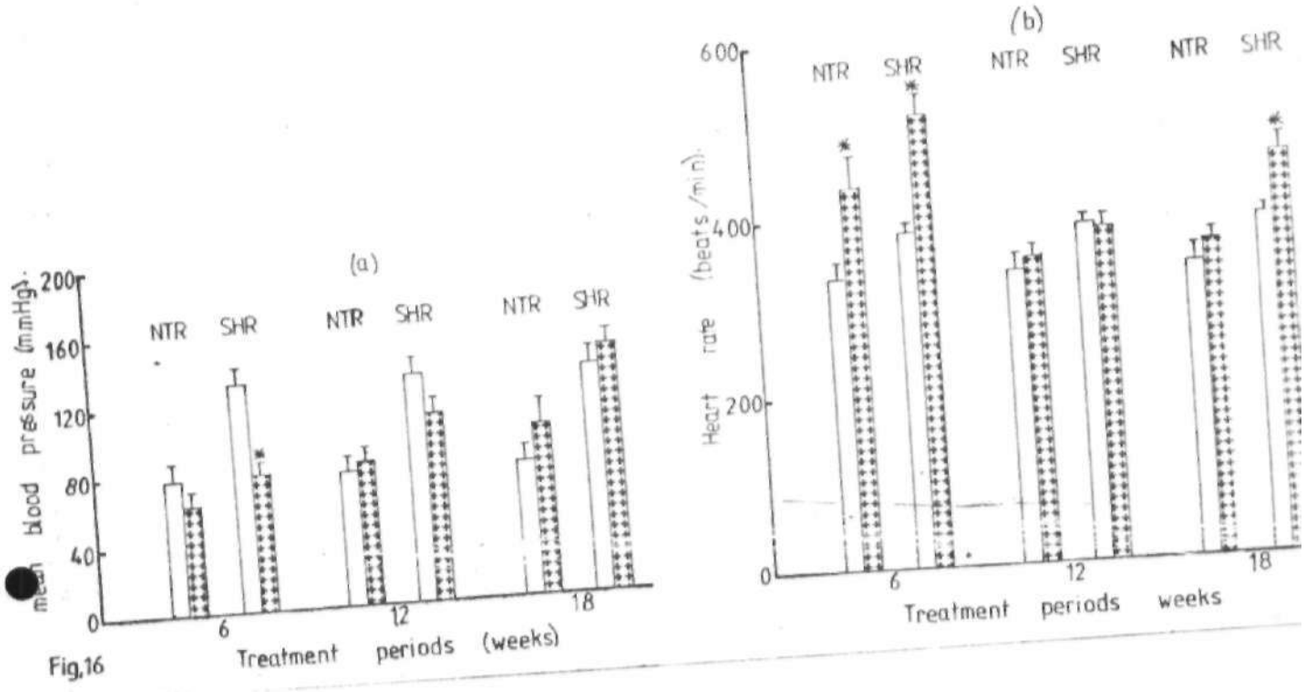


Fig.16

Changes in b.p. (a) and heart rate (b) of group V (20% w/w cowpea in (Pfizer Feed and AMD 200 mg/kg orally, ▨) and group I rats (Pfizer Feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $p < 0.025$. Student's t - test.

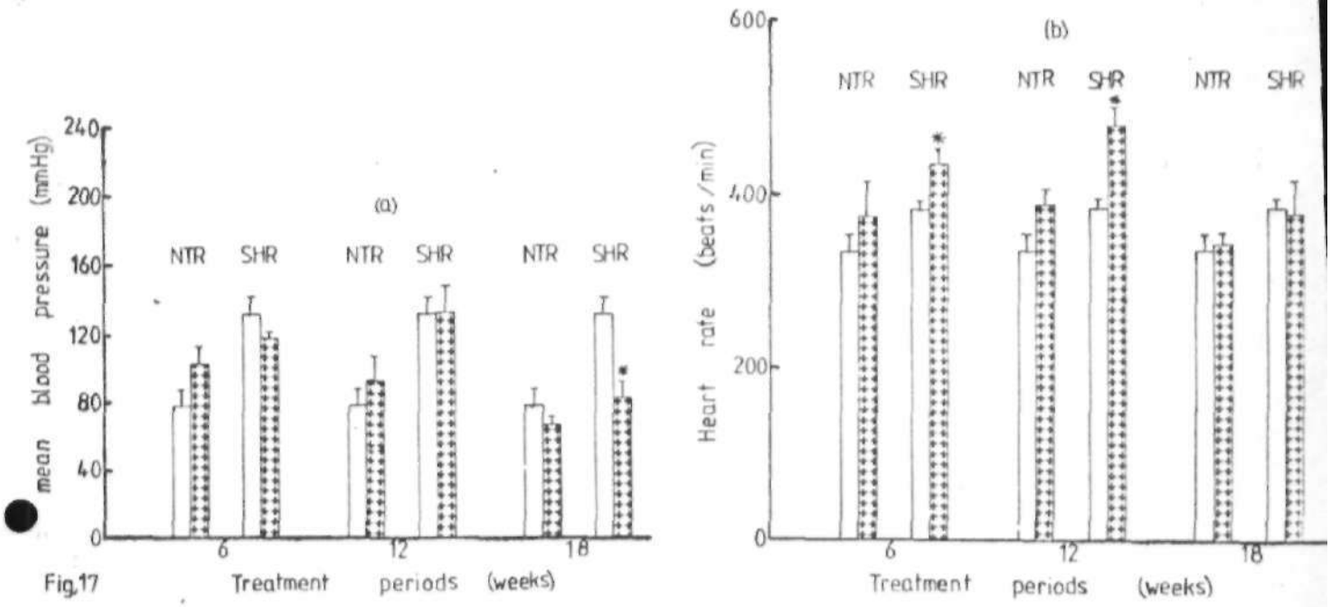


Fig.17

Changes in b.p. (a) and heart rate (b) of group VI rats (60% w/w cowpea in Pfizer Feed and AMD 200 mg/kg orally, ▨) and group I rats (Pfizer Feed only, ▱). Each bar represents a mean of 4 -5 observations ± sem. * = $p < 0.01$. Student's t - test.

twelveth weeks (fig. 18). The b.p. elevating effect of noradrenaline was more in the group VI NTR at the sixth week than in the controls. There was no similar change in heart rate. However, noradrenaline increased the b.p. to a lesser extent in the group VI SHR at the eighteenth week when compared with the group I rats. There was no such effect on the heart rate. On the other hand, the test rats showed an increased magnitude in heart rate at the twelveth week compared to the controls (fig. 19).

Influence of adrenaline on b.p. and heart rate of groups V and VI NTR and SHR.

The b.p. elevation induced by adrenaline was less at the sixth week, but more at the eighteenth week in group V NTR compared to control rats. The positive chronotropic effect induced by adrenaline showed a higher magnitude at the sixth week and lower magnitude at the twelveth and eighteenth weeks in the group V NTR. Adrenaline elevated the b.p. to a lesser extent in the group V SHR at the twelveth week when compared to group I SHR. The magnitude of the increase in heart rate of the group V SHR at the sixth week was higher

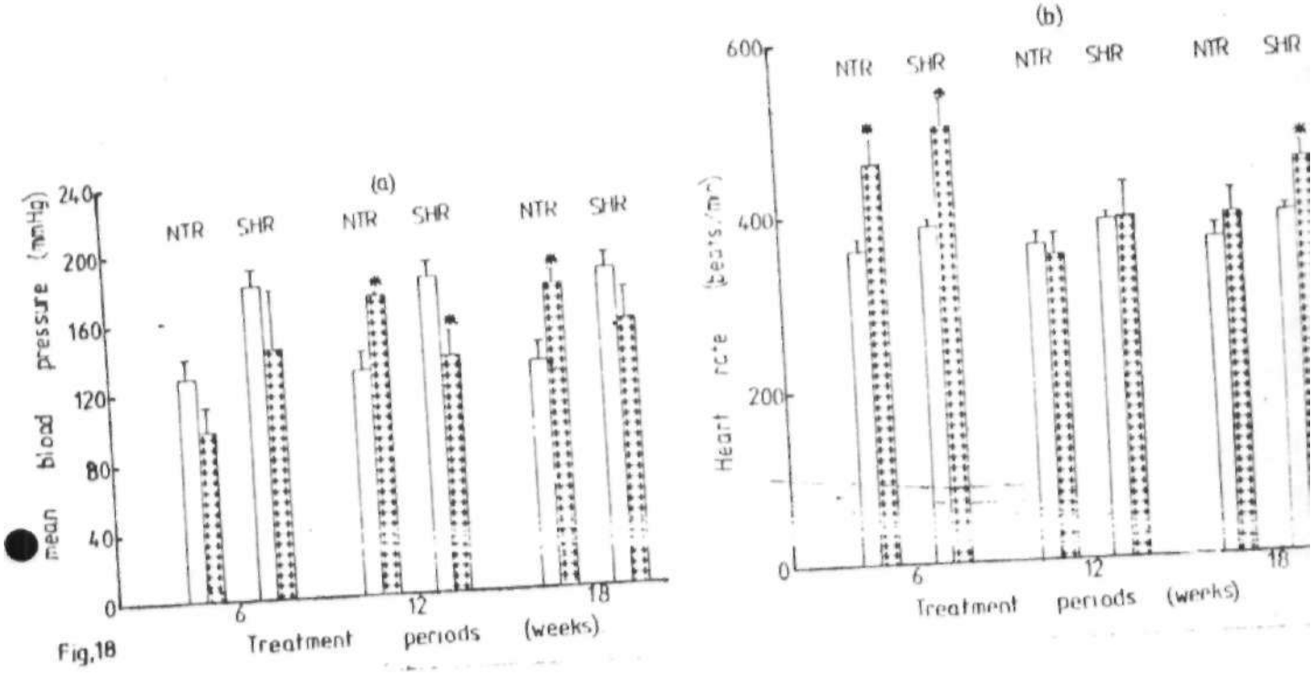


Fig.18

Effect of noradrenaline (3ug/kg i.v.) on b.p. (a) and heart rate (b) of group v rats (20% $^{w}/_{w}$ cowpea in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P<0.05$. Student's t - test.

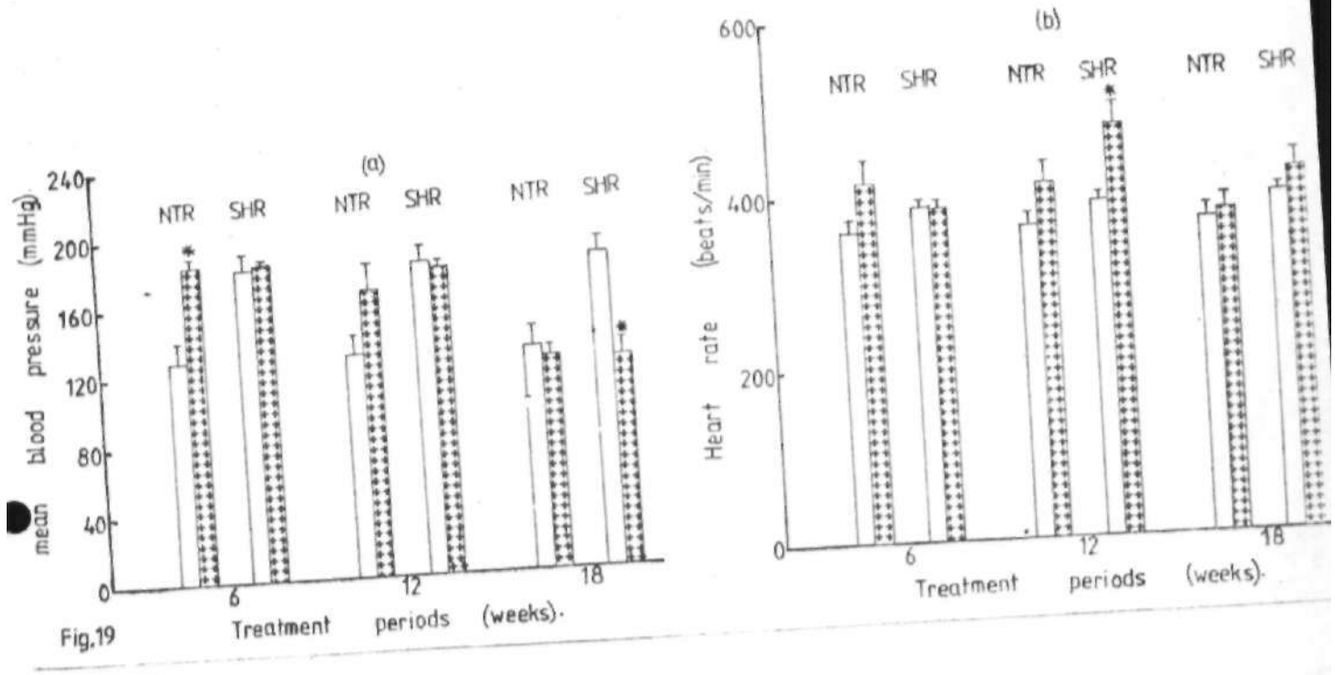


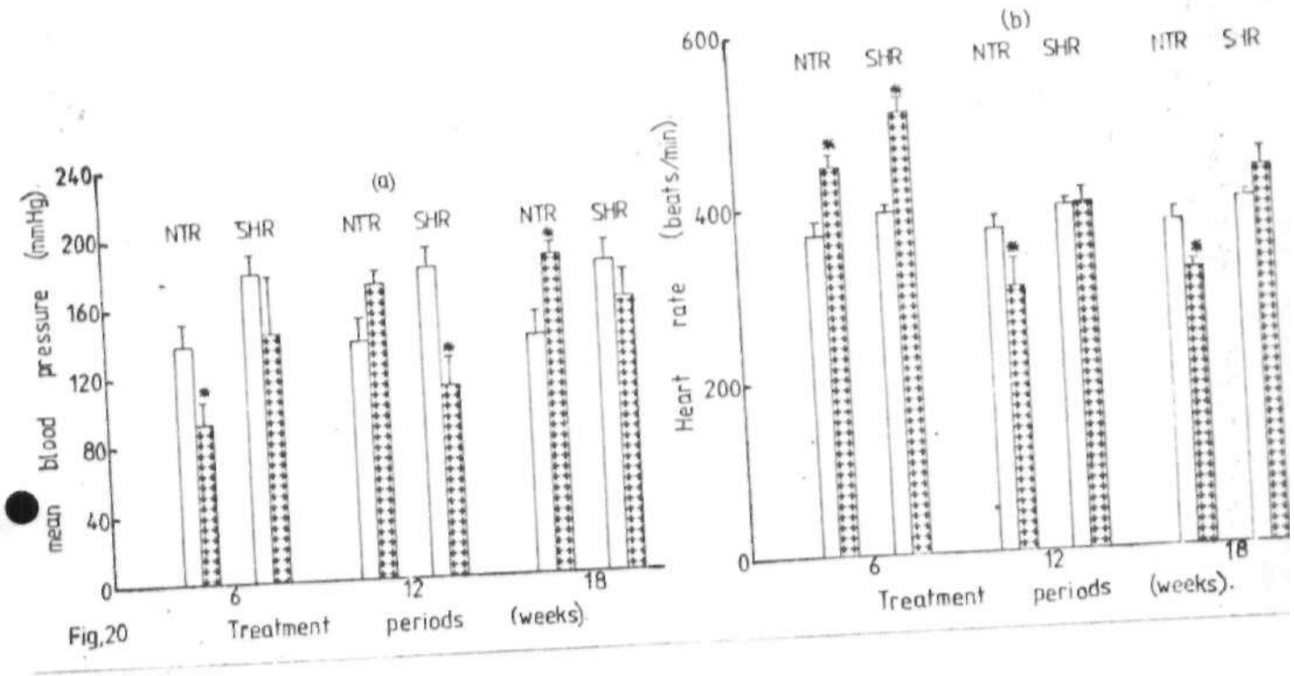
Fig.19

Effects of noradrenaline (3ug/kg i.v.) on b.p. (a) and heart rate (b) of group vi rats (60% w/w in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± - $P \leq 0.025$. Student's t - test.

compared to the controls (fig. 20). The group VI SHR showed lesser b.p. increase in response to adrenaline at the sixth week than the group I rats. There was no similar effect on the heart rate. However, the test rats showed a lower magnitude in heart rate at the twelveth week, but a higher magnitude at the eighteenth week when compared to control rats. The b.p. of the group VI NTR responded more to adrenaline effect at the sixth week than the group I NTR. There was a similar effect on the heart rate (fig. 21).

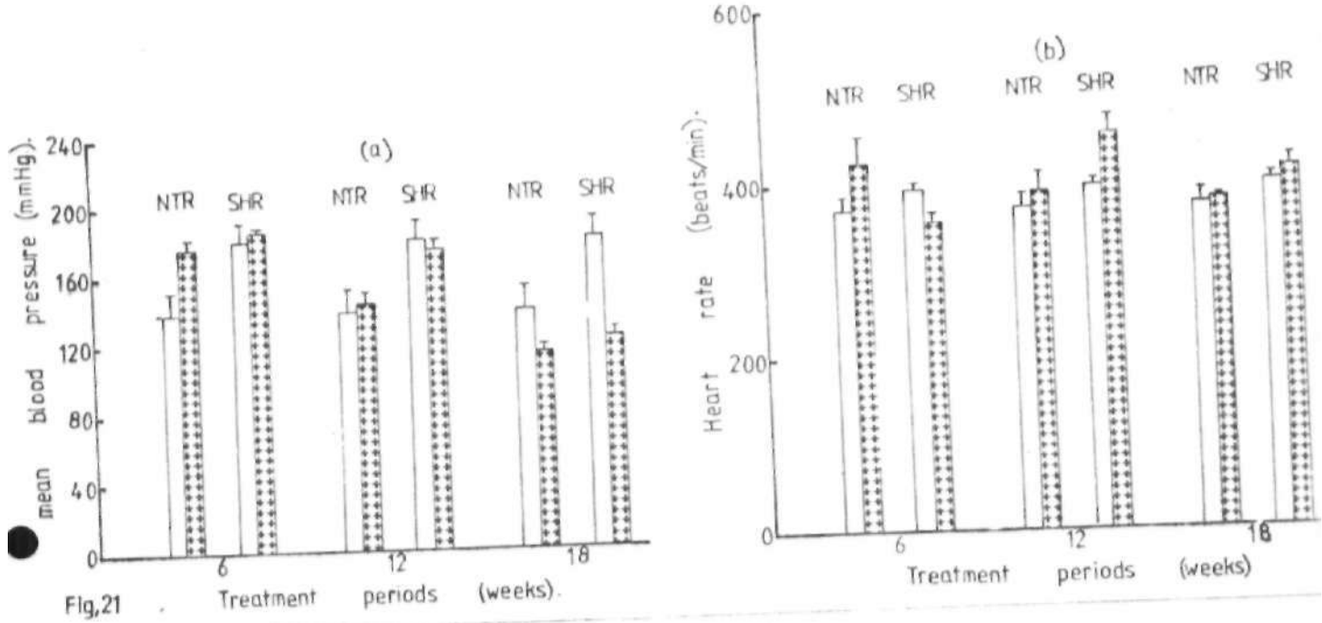
Influence of dopamine on the b.p. and heart rate of groups V and VI NTR and SHR.

Group V NTR showed a lesser b.p. elevation to dopamine at the sixth week than the control rats. There was a similar change in heart rate. The b.p. elevation induced by dopamine in the group V SHR at the twelveth week was less than that observed in the group I SHR. There was no similar change in heart rate. However, the magnitude of the positive chronotropic effect in the group V SHR at the sixth and eighteenth weeks was higher than in the group I rats (fig. 22). The group VI NTR and SHR did not show any marked alteration in



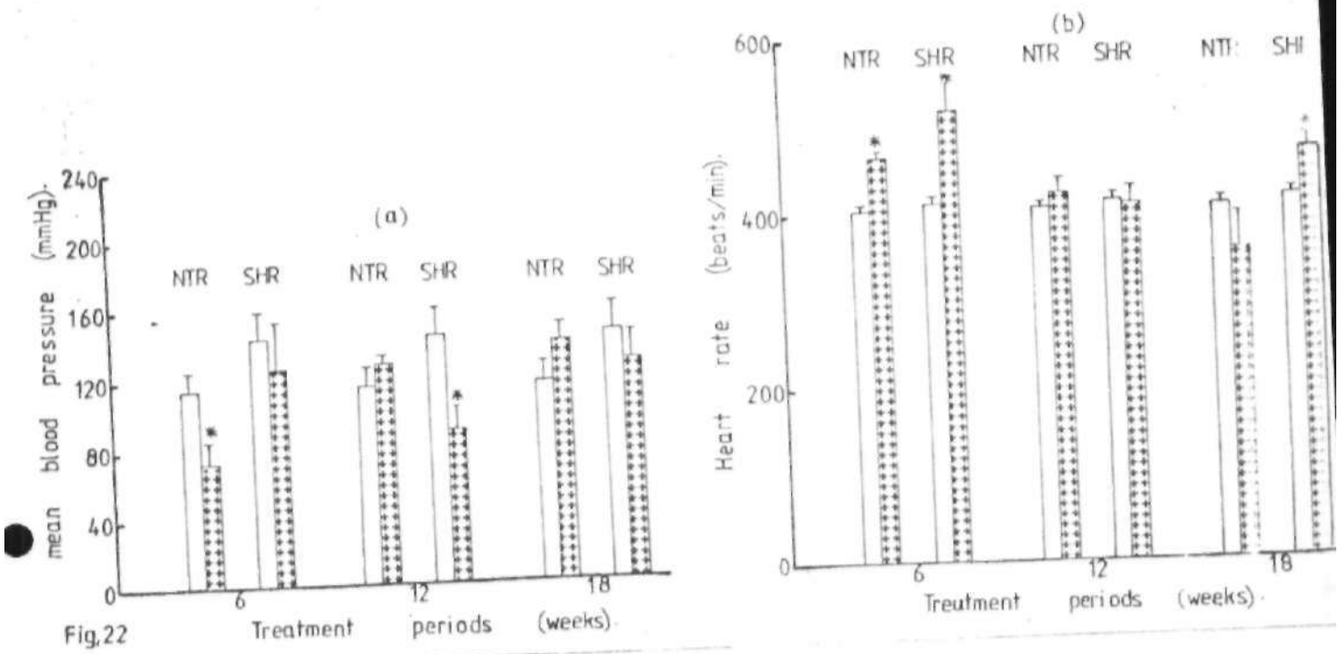
Fig,20

Effect of adrenaline (2ug/kg i.v.) on b.p. (a) and heart rate (b) of group v rats (20% $^{w}/_{w}$ cowpea in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * $P < 0.05$. Student's t - test.



Fig,21 .

Effect of adrenaline (2ug/kg i.v) on b.p. (a) and heart rate of group vi rats (60% cowpea in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P < 0.05$. Student's t - test.



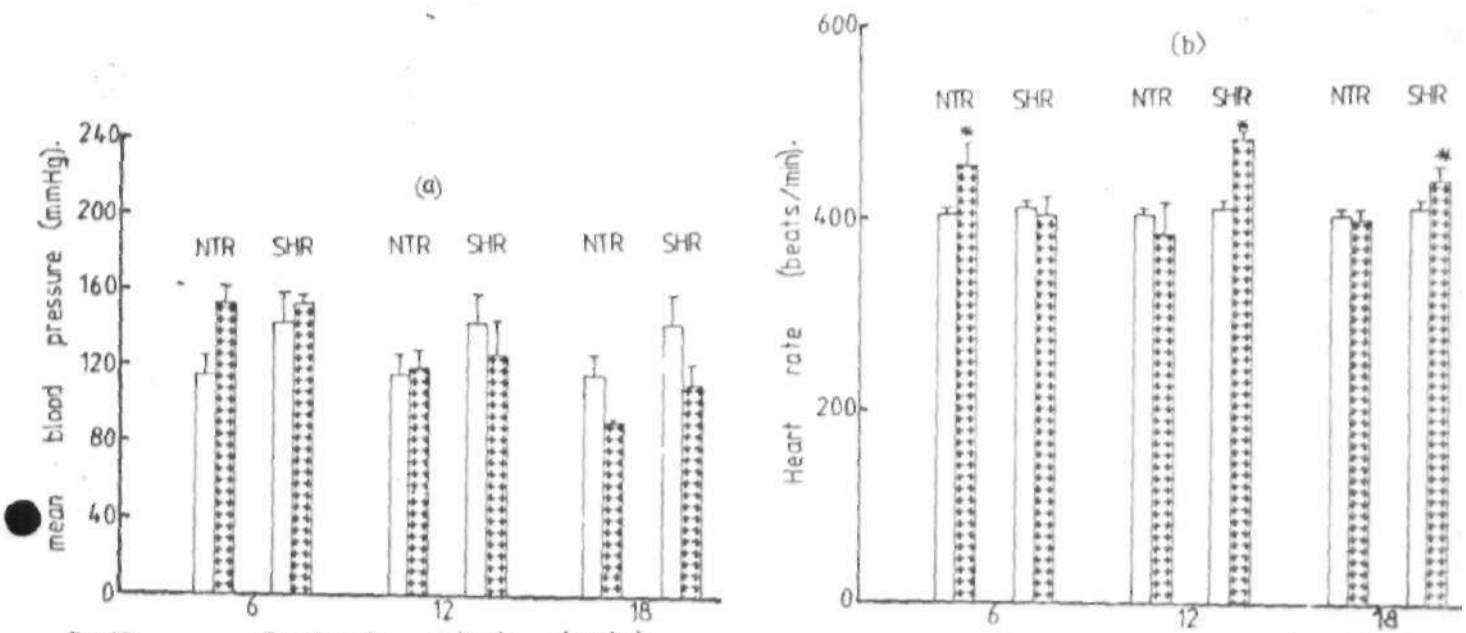
Fig, 22

Effect of dopamine (100ug/kg i.v.) on b.p. (a) and heart rate (b) of group v rats (20% $^{w}/_{w}$ cowpea in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P < 0.05$). Student's t - test.

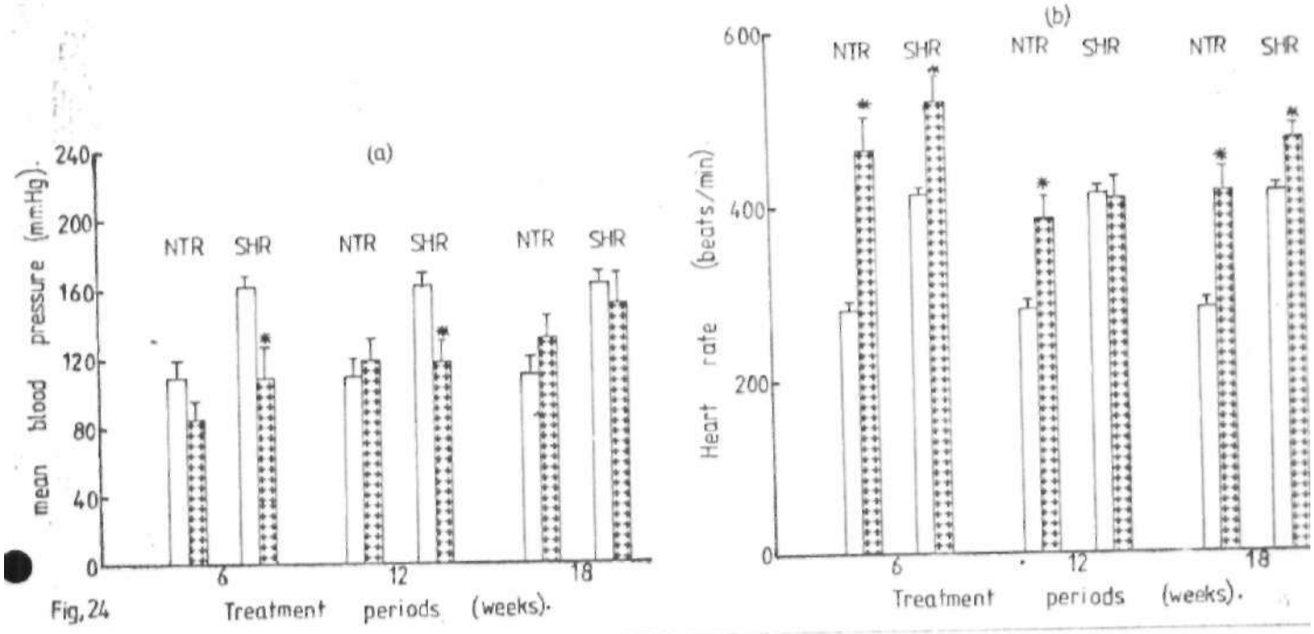
b.p. when compared to the group I rats. However, the group VI SHR showed more increase in heart rate to dopamine at the twelveth and eighteenth weeks than the group I rats. The group VI NTR showed a similar change at the sixth week (fig. 23).

Influence of tyramine on the b.p. and heart rate of groups V and VI NTR and SHR.

The group V NTR did not show any marked alteration in b.p. However, the magnitude of the increased heart rate was higher in the group V NTR at the sixth, twelveth and eighteenth weeks when compared to the controls. Tyramine increased the b.p. to a lesser extent in the group V SHR at the sixth and twelveth weeks than in the controls. The positive chronotropic effect induced by tyramine was of higher magnitude at the sixth and eighteenth weeks in the group V SHR than in the group I rats (fig. 24). The results obtained with tyramine on group VI NTR and SHR (fig. 25) were similar to those of noradrenaline (fig. 19).



Fig,23 Effect of dopamine (100ug/kg i.v.) on b.p. (a) and heart rate (b) of group vi rats (60% $^{w}/_{w}$ cowpea in Pfizer feed and AMD 200mg/kg orally, ▨ and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem.
* = $P < 0.05$. Student's t - test.



Fig, 24

Effect of tyramine (100ug/kg i.v.) on b.p. (a) and heart rate (b) of group v rats (20% $^{w}/_{w}$ cowpea in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observations ± sem. * = $P < 0.0125$. Student's t - test.

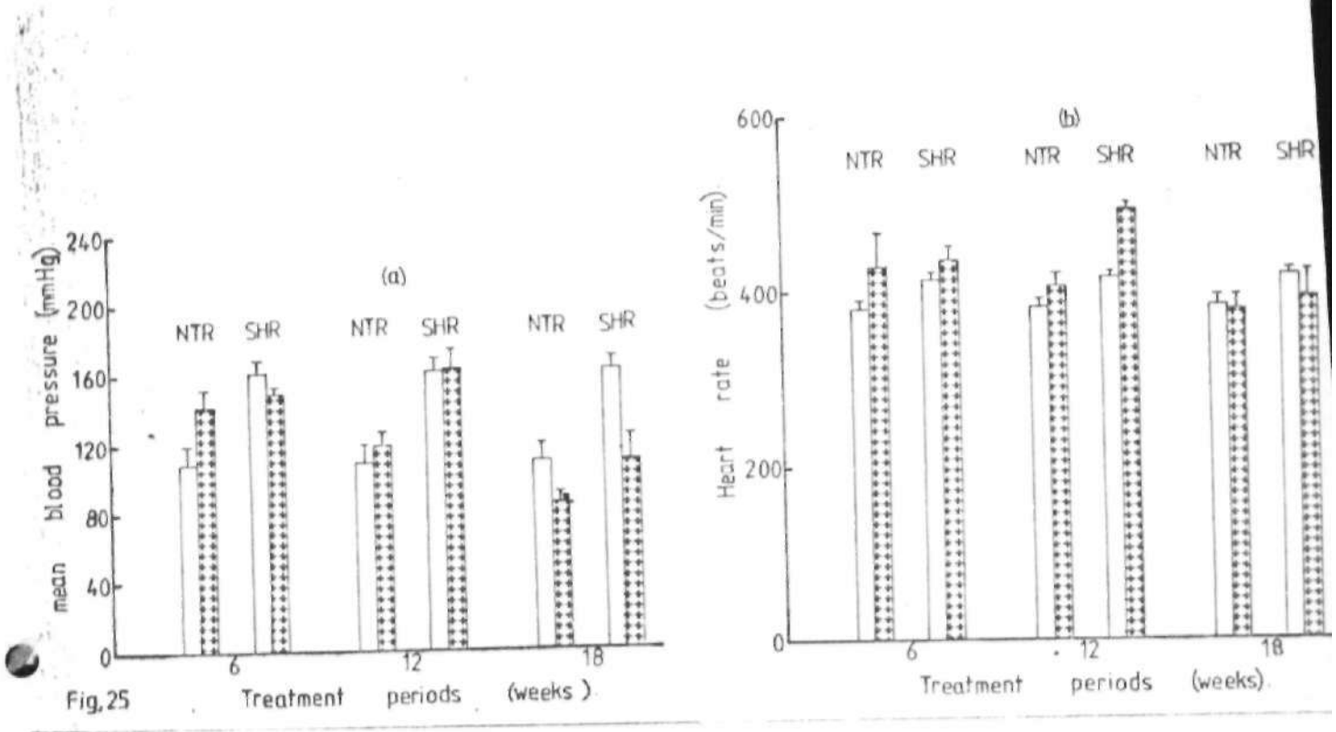


Fig. 25

Effect of tyramine (100ug/kg i.v.) on b.p. (a) and heart rate (b) of group vi rats (60% $^{w}/_{w}$ cowpea in Pfizer feed and AMD 200mg/kg orally, ▨) and group I rats (Pfizer feed only, ▱). Each bar represents a mean of 4 - 5 observation ± sem. * = $P < 0.05$. Student's t - test.

CHAPTER IV

DISCUSSION

i) The effect of cowpea (20% and 60% w/w) on the b.p. and heart rate of NTR and SHR.

The cowpea (vigna sinensis leguminosea) exhibited a pronounced pressor effect in group IV NTR at the sixth and twelveth weeks, without much change in the heart rate. There has been several reports on the presence of amino acids, such as l-tyrosine, l-phenylalamine and l-tryptophan in the cowpea (Graulier, 1968; Onayemi and Potter, 1976). Recently, unconfirmed preliminary studies in our laboratory indicated the presence of 3,4-dihydroxyphenylalanine (l-dopa) in the cowpea. This has also been claimed to be present in the broad bean (Hodge, Nye and Emberson, 1964; Hortwitz, Lovenberg, Engelman and Sjoerdsma, 1964). All these compounds are precursors of endogenous vasoconstrictor amines. Thus the pressor increase seen in group IV NTR might be due to the high plasma concentration of vasoconstrictor amines. This effect was not observed with the group IV SHR possibly due to receptor desensitization. The receptor desensitization in SHR might be attributed to persistent high plasma catecholamine levels. This suggestion is supported

by the work of DeChamplain (1977) as well as preliminary findings in our laboratory which showed that plasma catecholamine levels were higher in the SHR than in NTR.

The group III SHR showed a decrease in b.p. at the sixth week with no effect on the heart rate. It is possible that the major active metabolite of the cowpea content in this group of rats might be dopamine. According to Clark (1977 and 1980) dopamine in low doses induced hypotension in dogs, this however might be true for the rat. Thus, the decrease in b.p. in this project might be due to dopamine. The group III NTR showed a marked decrease in heart rate with a slight increase in b.p., an observation requiring further investigations.

Influence of the sympathomimetics on the b.p. and heart rate of groups III and IV NTR.

Noradrenaline increased the b.p. to a lesser extent in group III SHR when compared to the group I rats. From previous observations with group III SHR, it was thought that the decreased b.p. was due to the likely action of dopamine in low doses. Dopamine might be a possible major metabolite of the cowpea content. As a result of this physiological antagonism between dopamine

(hypotensive effect) and noradrenaline (pressure elevating effect) is a possibility. The response obtained with the group III NTR on heart rate might be a reflex compensation to the effect of the cowpea content on the b.p.

The b.p. elevating effect observed with noradrenaline in group IV NTR at the sixth and twelveth weeks might be due, (at least in part) to the additive effect of the cowpea content in the group IV NTR. Conversely, the haemodynamic adjustment of the animals to the cowpea contents might explain the lesser responses observed with the group IV SHR.

Group III NTR showed a lesser b.p. response to adrenaline when compared to group I rats. However, the group IV NTR showed an increased b.p. response. These effects observed with adrenaline are similar to those obtained with noradrenaline. It is therefore possible that similar mechanism might underly this response. In addition, the beta-adrenoceptor effect of adrenaline (i.e. vasodilatation) might have contributed to the effect obtained in group III NTR. The insignificant pressor increase observed in the group IV NTR at the twelveth and

eighteenth weeks, might have been due to haemodynamic adjustment of the rats to cowpea contents.

Group IV NTR showed increased b.p. in response to dopamine when compared to group I rats. This response might partly be due to the additive effect of the metabolites of cowpea contents and dopamine, Clark (1977) reported that high doses of dopamine increased the b.p. in dogs. Thus the high cowpea level in the group IV NTR could lead to an increase in cowpea metabolites, one of which could be dopamine. Such a proposal might explain dopamine induced b.p. elevation in the group IV NTR. This effect was not seen with the group IV SHR possibly due to haemodynamic adjustment of the rats to cowpea contents. Group III SHR showed a lesser b.p. response to tyramine than the group I SHR. However, the group IV NTR showed a greater b.p. response to tyramine at the sixth and twelveth weeks when compared to group I NTR. These results are similar to those obtained with noradrenaline. Tyramine however, acts through the release of endogenously stored noradrenaline (Pettinger et al, 1963; McCurdy et al, 1964). Thus the explanation advanced for noradrenaline effect is tenable.

ii) The effect of alphamethyldopa on the b.p. and heart rate of NTR and SHR.

The data showed that alphamethyldopa significantly lowered the b.p. in group II SHR, but there was no similar effect in the NTR. This result is compatible with that of Nickerson and Ruedy (1975) who used human subjects. This effect might be due to the higher peripheral resistance in the SHR compared to the NTR. There have been several reports indicating that alphamethyldopa might produce hypotensive effect principally via a lowering of the peripheral vascular resistance (Sandnerstedt et al, 1962). On the other hand, the effect of alphamethyldopa on cardiac output which has been found to be inconsistent, might be elicited indirectly (Onesti, 1976). It was also observed that the heart rate of group II NTR and SHR were significantly elevated by alphamethyldopa. This observation might be due to reflex adjustment to the fall in b.p. induced by the drug. The hypotensive effect of urethane (Hillebrand, Van Der Meer and Ariens, 1971), which is usually more pronounced in the hypertensive than in the normotensive (Couture and Regoli, 1980), might also contribute to the reflex adjustment.

Influence of sympathomimetics on the b.p. and heart rate of group II NTR and SHR.

Alphamethyldopa depletes tissue levels of noradrenaline, adrenaline and dopamine (Nickerson, 1975). This type of depletion may however lead to increase tissue sensitivity to an exogeneously administered amine (Pettinger et al, 1963). The positive chronotropic effect induced by noradrenaline (fig. 12) tends to agree with this explanation. The insignificant results obtained at the twelveth and eighteenth week might be related to the haemodynamic adjustment of the animals to the effect of alphamethyldopa. Group II NTR showed a decrease b.p. response to adrenaline compared to the group I NTR at the eighteenth week. This observation might have been due to sensitization of peripheral beta-adrenergic receptors to adrenaline and decrease in cardiac output by alphamethyldopa. The heart rate response was similar to that obtained with noradrenaline. Therefore the explanation tendered above for noradrenaline is applicable.

Group II SHR showed an increase b.p. and heart rate to dopamine when compared to group I SHR. According to Goldberg (1972) dopamine can act directly on adrenergic

receptors, and this might explain the result obtained with dopamine. Desensitization of adrenergic receptors by alphamethyldopa might contribute to the observed effect with dopamine. Group II SHR also showed increased b.p. response to tyramine infusion at the eighteenth week. This effect is also in agreement with those obtained by Pettinger and coworkers (1963). However, the same workers claimed that this effect could not be attributed to the depleting action of alphamethyldopa, since tyramine is believed to act through the release of endogeneously stored noradrenaline (Burn and Rand, 1958). It has also been suggested that alphamethylmonoamines formed from alphamethyldopa are not substrates to monoamine oxidases (Pettinger et al, 1963). This means that tyramine could release the accumulated alphamethylmonoamines to induce its pressor effect.

iii) <u>The effect of cowpea (20% and 60% w/w) and alphamethyldopa on the b.p. and heart rate of groups V and VI NTR and SHR.</u>

The lack of effect of alphamethyldopa in the group V NTR might be related to an interaction between cowpea contents and the drug. The profound hypotensive effect of alphamethyldopa in group V SHR might be due to the relatively high b.p. of the animal prior to drug administration.

The observed tachycardia in group V NTR and SHR might be due to reflex adjustments to the hypotensive effect of the drug.

Figure 17 shows that alphamethyldopa significantly lowered the b.p. at the eighteenth week with no obvious effect at the sixth and twelveth weeks. Haemodynamic adjustments to the b.p. elevating effect of cowpea might explain the delayed effect of alphamethyldopa. This suggestion is supported by our observation that the b.p. elevating effect of cowpea was only evident at the sixth and twelveth weeks (fig. 2). Alphamethyldopa increased the heart rate of group VI SHR at the sixth and twelveth weeks. These observations conflicted with the b.p. effects of the drug at the same periods and therefore require further work.

Influence of sympathomimetics on the b.p. and heart rate of groups V and VI NTR and SHR.

Noradrenaline increased the b.p. to a lesser extent in group V SHR when compared to group I rats. A possible antagonism between noradrenaline and alphamethyldopa might underly the observed effect. However, the insignificant effect of alphamethyldopa in NTR might explain the results

obtained with group V NTR at the sixth and twelveth weeks (Fig. 18). Noradrenaline induced tarchycardia in group V NTR and SHR might be due to adrenoceptor sensitization by alphamethyldopa.

The b.p. elevation resulting from noradrenaline in group VI NTR at the sixth week might be due to the additive effect of cowpea contents with the drug. On the other hand, the haemodynamic adjustment of the animals to the cowpea contents might explain the lesser pressor effect of noradrenaline in group VI SHR at the eighteenth week compared to the controls. The observation that alphamethyldopa lowered the b.p. of group VI SHR at the eighteenth week lends further support to the suggestion above. Adrenaline produced similar effects on both b.p. and heart rate as noradrenaline and thus the explanation given above for noradrenaline is tenable.

The lesser b.p. elevating effect of dopamine in group V NTR and SHR at the sixth and twelveth weeks respectively might be due to the hypotensive effect of alphamethyldopa. A possible interaction between cowpea contents and alphamethyldopa might explain the lesser b.p. effect of dopamine (Fig. 22). On the other hand, dopamine-induced

tarchycardia in group V NTR and SHR might be due to reflex response to the b.p. effect of the drug.

The observed dopamine induced heart rate increase might be due to cardiostimulating effect of dopamine resulting from sensitization of adrenoceptors following catecholamine depletion by alphamethyldopa (fig. 23). Figure 24 shows that tyramine had lesser effect in group V SHR than group I rats. This observation can be attributed more to the metabolites of cowpea contents rather than to the effect of alphamethyldopa on amine levels. The results here are more in agreement with those of figure 9 than with figure 15. Thus, the lesser b.p. increase with tyramine seen in fig. 24 might likely be due to effect of low levels of dopamine which had earlier been suggested to be one of the possible metabolites of the cowpea content. In addition, the b.p. lowering effect of alphamethyldopa might be a contributing factor. The induced tachycardia might be due to a reflex mechanism.

The greater pressor elevating effect of tyramine in group VI NTR at the sixth week compared to group I rats might be due to a positive interaction between some metabolites of cowpea contents and tyramine.

However, at the eighteenth week tyramine induced a lesser pressor effect (particularly with the group VI SHR) compared to the group I rats. This result might be related to haemodynamic adjustment of the animals to the pressor effect of the cowpea contents. The b.p. lowering effect of alphamethyldopa which is more in the SHR than the NTR might have contributed to the SHR results. The increased magnitude of heart rate seen in group VI SHR might be attributed to a reflex adjustment resulting from the lesser b.p. increase in this group compared to the controls.

CONCLUSION

Cowpea contents cause the precipitation of increased arterial blood pressure in the normotensive rats within a short period of twelve weeks. The blood pressure increase was observable only at the 60%^{w}/w used concentration.

The effects produced by the cowpea contents are potentiated by intravenous administration of amines like noradrenaline, adrenaline, dopamine and tyramine, while the blood pressure elevation due to the cowpea contents can be reversed or decreased by a co-administered alpha-methyldopa.

The spontaneously hypertensive rats were not much affected by the cowpea except for a slight alteration in the heart rate of those fed on the higher concentration of cowpea.

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