DUAL ACTION OF HYDRALAZINE ON RAT HEART IN VIVO

Prasert Songkittiguna and Giatbungon Jindagul

Department of Pharmacology, Faculty of Dentistry,
Chulalongkorn University, Bangkok 11330, Thailand.

SUMMARY

1. Hydralazine 2, 4, 6, 12, 16, mg/kg IV produced a dose-dependent bradycardic and tachycardic effects on the heart rate of anaesthetized and pithed rats in situ.

2. The change in the rate of beating of isolated rat atria produced by high dose of hydralazine (2.0 mM) was enhanced by either theophylline or ICI 63 197.

INTRODUCTION

Not many information on the direct action of hydralazine on the heart is currently available. Only the tachycardia produced by hydralazine during the course of treatment has been documented which is generally attributed to reflex stimulation of cardiac sympathetic nerves (Gross, 1977; Gerber and Nies, 1991). Although a dose-dependent negative chronotropic effect due to the inhibition of spontaneous release of noradrenaline was reported to produced by low concentrations of hydralazine in isolated rat atria, and a positive chronotropic effect due to release of noradrenaline by high concentration of hydralazine (Songkittiguna and Rand, 1982). In addition, hydralazine was reported to inhibit noradrenaline synthesis (Songkittiguna et al., 1980; Houchi et al. 1986). Therefore, the experiments on the effect of hydralazine in different doses in intact heart of anaesthetized, pithed rat and the effects of indomethacin and ICI 63 197 on the bradycardic and tachycardic effects of spontaneously-beating isolated rat atria, produced by high dose of hydralazine were investigated.

METHODS

Anaesthetized normotensive rat

Wistar rats of either sex weighing 250-300 g were anaesthetized with urethane. Urethane (25%) in normal saline solution was injected intraperito neally. The trachea was cannulated to maintain the animals on positive ventilation in order to avoid any complicating effects of respiration. The rectal temperature was also maintained throughout the course of the experiments by irradiation with infrared light. The blood pressure was recorded directly form carotid artery on a Grass Polygraph Model 79 (FT03). The heart rate was recorded from tachometer. The freshly-prepared hydralazine including other drugs under investigation in NSS were injected into the femoral vein cannula.

Pithed rat

Normotensive wistar rats of either sex weighing 250-300 g were anaesthetized with diethyl ether and immediately cannulated with tracheal cannula. The brain and spinal cord were then destroyed by passing a steel pithing rod through the orbit and artificial respiration was started immediately by connecting the tracheal cannula to a small animal respiratory pump. The rate of respiration was set at 70 strokes per minute. Both anesthetized and pithed rats blood pressures were measured from the carotid artery through a small bore polyethylene cannula (No 260) and recorded on a Grass recorder (as above). The heart rate was recorded by triggering a Grass tachometer with the pulse wave derived from the blood pressure channel.

Spontaneously beating atria

Wister rats of either sex weighting 250-300 g were killed by cervical dislocation and

Table 1. The percentage change of heart rate produced by hydralazine 2, 4, 6, 12, 16 mg/kg in anesthetized rats.

	Dose of hydralazine (mg/kg)	2	4	6	12	16	N
Anaesthetized rats.	% change of Bradycardia	6.0 ± 0.3%	10.3 ± 0.6%	12.3 ± 0.6%	21.5 ± 1.1%	21.7 ± 1.1%	
	% change of Tachycardia	6.6 ± 1.2%	9.8 ± 1.3%	13.6 ± 1.0%	25.2 ± 2.6%	26.8 ± 2.1%	

Table 2. The dual action of hydralazine 2, 4, 6, 12, 16 mg/kg in pithed rats.

	Doses of hydralazine (mg/kg)	2	4	6	12	16	N
Pithed rats	% change of Bradycardia	4.2 ± 0.5%	7.7 ± 0.4%	11.5 ± 0.6%	20.9 ± 1.9%	29.2 ± 2.5%	10
	% change of Tachycardia	4.9 ± 0.7	7.3 ± 1.2	10.3 ± 0.9	30.9 ± 4.5%	51.3 ± 4.9%	

Talbe 3 Pattern of the changing of atrial rate produced by hydralazine (0.1, 1 and 2 mM) in spontaneously-beating isolated atria of rat pretreated with theophylline (0.5 mM) or ICI 63 197 (0.1 mM).

	ATRIAL RATE (BEATS/MIN)							
	Before hydralazine	After hydralazine						
		0.1 mM		1.0 mM		2.0 mM		
	phase 1	phase 2	phase 1	phase 2	phase 1	phase 2	phase 1	phase 2
Theophylline (0.55 mM)	403.8 ± 13.3 (n = 8)	–	381.7 ± 15.8 (n = 6)	–	325.8 ± 16.8 (n = 6)	–	302.5 ± 16.1 (n = 6)	325.0 ± 17.1 (n = 6)
ICI 63 197 (01. mM)	306.3 ± 7.8 (n = 6)	–	278.1 ± 6.2 (n = 6)	–	232.5 ± 4.9 (n = 6)	254.0 ± 6.2 (n = 5)	222.5 ± 5.5 (n = 6)	306.7 ± 13.5 (n = 6)

Phase 1 = An initial decrease in atrial rate
Phase 2 = An secondary increase in atrial rate
= No phase 2 is seen

exsanguination. The chest was opened, the heart was rapidly taken out and transferred to Krebs-Henseleit solution at room temperature, and the atria were dissected free. The atria were set up in a 10 ml jacketed organ bath containing Krebs-Henseleit solution of the following composition (mmol/l) : NaCl, 118; KCl, 417; $NaHCO_3$, 25; $MgSO_4$, 0.45; KH_2PO_4, 1.03; $CaCl_2$, 2.5; D-(+)-glucose, 11.1; disodium edetate, 0.067; and ascorbic acid, 0.14. The solutions in the organ bath and in the reservoir supplying the organ bath were gassed with 5% CO_2 in O_2 and maintained at a temperature of 37 ± 0.5 °C. The rate and force of contractions of the atria were measured with a Grass force displacement transducer (FT03) coupled to a Grass tachograph (model 7p 433) and displayed on a Grass recorder. The initial resting diastolic tension was 10 mN (approx. 1 gramforce). The effects of hydralazine were studied after a 1 h stabilization peroid, during which the bathing solution was changed several times.

Statistical analysis of results

One-way analysis of variance was performed on grouped data followed by the paired or unpaired Student's t-test (where appropriate).

Drugs

Hydralazine hydrochloride (Ciba-Geigy); Theophylline (Sigma); ICI 63 197' (Sigma); urethane (May & Baker).

RESULTS

The bradycardia and tachycardia produced by intravenously-given hydralazine in anaesthetized rat.

In a group of fifty rats divided into 10 rats in five sub-groups. In each sub-group of 10 rats, hydralazine in doses of 2, 4, 6, 12, and 16 mg/kg was administered through a venous cannula implanted into the femoral vein. The bradycardic responses were measured between the predrug value and the maximal effect value, whereas the tachycardic responses were measured from the maximal bradycardic response to the maximal tachycardia.

Hydralazine (2 mg/kg IV) produced the percentage change of bradycardia in a value of 6.0 ± 0.3; 4 mg/kg, it was 10.3 ± 0.6; 6 mg/kg. it was 12.3 ± 0.6; 12 mg/kg, it was 21.5 ± 1.1; 16 mg/kg, it was 21.7 ± 1.1, whereas the percent change of tachycardia, it was 6.6 ± 1.2; 9.8 ± 1.3; 13.6 ± 1.0; 25.2 ± 2.6 and 26.8 ± 2.1 respectively. The typical tracings showed the cardiovascular responses produced by the increasing doses of hydralazine (2, 4, 6, 12 and 16 mg/kg) were show on the Fig. 1, 2 and 3). The percentage change of the heart rate produced by hydralazine (2, 4, 6, 12 and 16 mg/kg) in anesthetized rats is summarized in the table 1, whereas in the pithed rats, the results are summerized in the table 2 and the typical tracings of the cardiovascular effects of hydralazine (2, 4, 6, 12 and 16 mg/kg) are shown in Fig. 4, 5 and 6 respectively.

The effects of theophylline, ICI 63 197 on the cardiac responses produced by hydralazine in a high concentration (2.0 mM) on the spontaneously beating rat isolated atria.

A control group of 10 experiments, hydralazine (2 mM) in bathing solution caused a sudden decrease in a rate of beating of rat atria from 297.5 beats/min (s.e.m. = 9.8) to 192.5 beats/min (s.e.m. = 8.3) and then rose from 192.5 beats/min (s.e.m. = 8.3) to 289.5 beats/min (s.e.m. = 10.8). Theophylline (0.55 mM) alone caused an increase in the rate of beating from 272.5 beats/min (s.e.m. = 7.2) to 403.7 beats/min (s.e.m. = 13.3), whereas ICI 63 197 (0.1 mM) alone; it was risen from 278.7 beats/min (s.e.m. = 6.8) to 306.2 beats/min (s.e.m. = 7.8). After maximal response, hydralazine (2 mM) was added into the bathing fluid that previously contained either theophylline or ICI 63 197 at the above concentrations under separated experiment. In eight isolated atrin pretreated with theophylline (0.55 mM), the beating rate fell immediately from a mean of 408.3 beats/min (s.e.m. =

13.3) to 302.5 beats/min (s.e.m. = 16.1) and then rose to 325.0 beats/min (s.e.m. = 17.1) In six atria pretreated with ICI 63 197 (0.1 mM) the rate of beating fell from a mean of 306.3 beats/min (s.e.m. = 7.8) to 222.5 beats/min (s.e.m. = 5.5) and then rose rapidly to 306.7 beats/min (s.e.m. = 13.5). The patterns of the changes in atrial rate produced by hydralazine (0.1 mM, 1.0 mM and 2.0 mM) after pretreatment with theophylline (0.5 mM) and ICI 63 197 (0.1 mM) are shown in Table. 2

DISCUSSION

Hydralazine has been extensively studied and widely used to treat hypertension that occurs during pregnancy. Parenteral administration of hydralazine has been also used fro the treatment of hypertensive emergencies (Gerber and Nies, 1991). The tachycardia experienced by the patient during the causes of hydralazine therapy has been generally attributed to the reflex. A few information on the direct effect of hydralazine on heart has been documented we have reported a direct action of hydralazine on isolated rat atria (Songkittiguna and Rand, 1982) and the inhibition of the conversion of dopamine to noradrenaline both in vitro and in vivo (Songkittiguna, Majewski and Rand, 1980). The results of the present study show that hydralazine produces a dose-dependent primary (initial) decrease (bradycardia) and secondary (subsequent) increase in the heart rate (tachycardia) in both anesthetized and pithed rats. Our previous report has shown dual action of hydralazine in rat isolated atria and reported that the decrease in the rate of beating of rat isolated atria was associated with the decrease in tritiated noradrenaline efflux in spontaneously-beating rat atria, whereas the increase in the rate of beating was associated with the increase in the radioactive labelled noradrenaline. The decrease and subsequent increase in the rate of beating of rat isolated atria was coined dual action of hydralazine by us. The present study showed that the dual action of hydralazine was seen in vivo, both anaesthetized and pith rats which would tell the direct action of hydralazine in the intact animal and would exclude the reflex involvement of the heart rate. Since, in the pithed rat both brain and spinal cord were destroyed; the reflex pathways were cut off. In addition the dual action of hydralazine, in the present result, was concentration dependent and potentiated in the presence of theophylline and ICI 63 197 (the known phosphodiesterase inhibiters) in sponstaneously beating rat isolated atria. In the presence of theophylline (0.5 mM) in the bathing fluid, hydralazine 0.1 and 1.0 and 2.0 mM decreased the rate of atrial beating in a concentration-dependent manner (Fig. 8a). But 2.0 mM of hydralazine produced a subsequent tachycardia (Fig. 8b). The same results were observed in the atria pretreated with ICI 63 197 (0.1 mM), except the subsequent increased in the atrial rate was seen in the 1.0 mM concentration of hydralazine (Fig. 8.b). Hydralazine, 1.0 mM and 2.0 mM, produced an increase in the atrial rate in the presence of ICI 63 197 in a concentration-dependent manner. Only 2.0 mM hydralazine produced a subsequent increase of the atrial rate in the presence of theophylline (0.5 mM). The primary decrease and secondary increase in heart rate of both anesthetized and pithed rats including isolated atria may explained by the finding of the author and coworkers mentioned earlier (Songkittiguna and Rang, 1982). The increase of atrial rate produced by theophylline is believed to be mediated through cyclic nucleotide phosphodiesterase, which causes an increase in the intracellular concentration of cAMP (Sutherland, et al 1968). It is interesting on the results obtained from ICI 63 197 pretreatment in that hydralazine (1.0 mM) cause a secondary increase in atrial rate instead of hydralazine in 2.0 mM concentration as seen in the previous report (Songkittiguna and Rand, 1982). Furthermore hydralazine at a 2.0 mM concentration caused the same effect in the

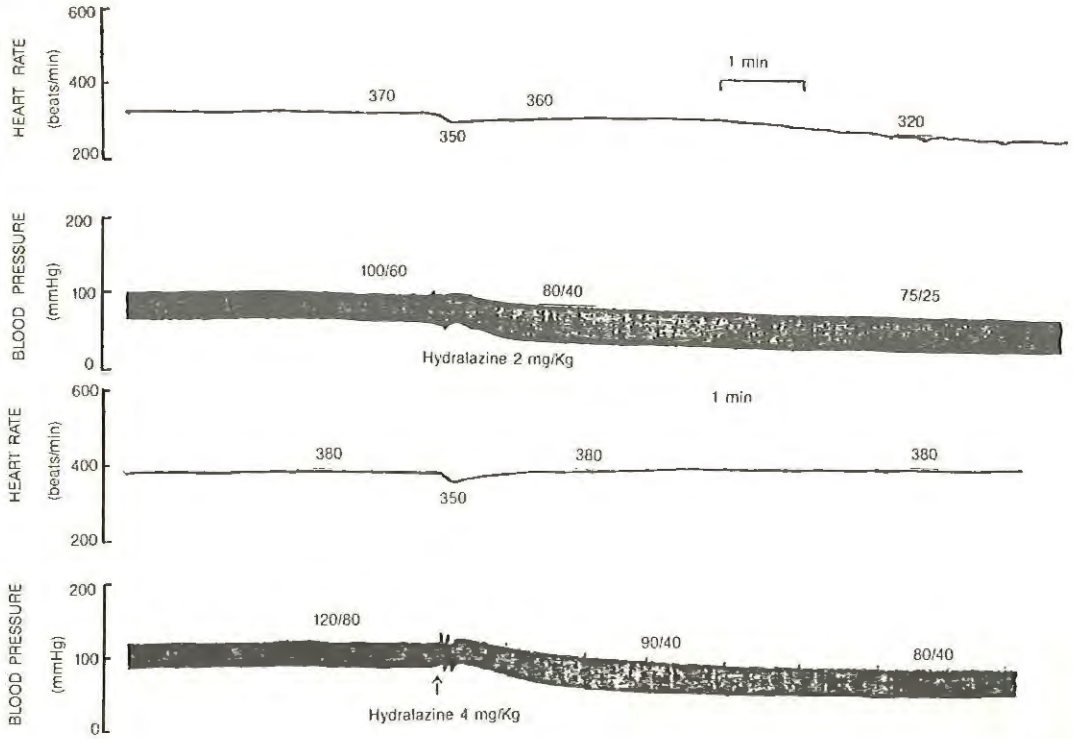

Fig. 1. The effect of hydralazine 2 and 4 mg/kg on blood pressure and heart rate in anaesthetized rats.

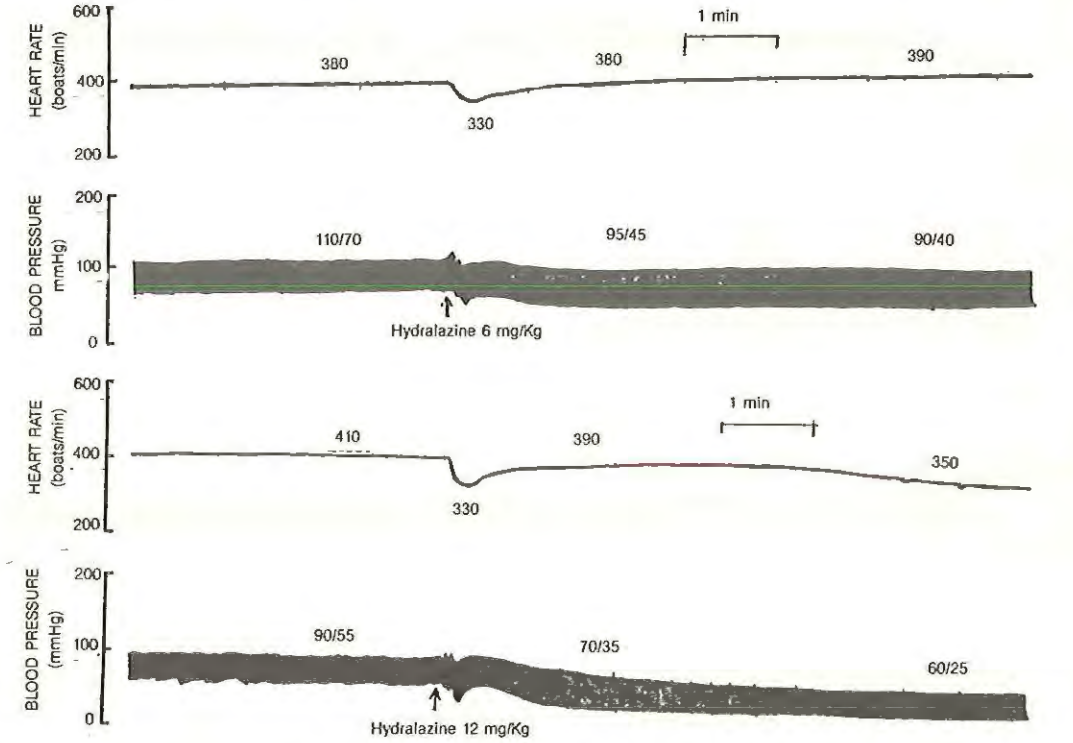

Fig. 2. The effect of hydralazine 6 and 12 mg/kg on blood pressure and heart rate in anaesthetized rats.

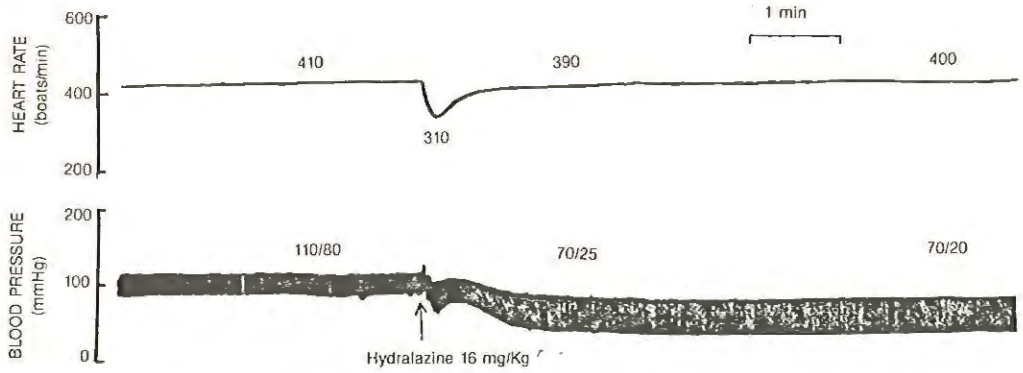

Fig. 3. The effect of hydralazine 16 mg/kg on blood pressure and heart rate in anaesthetized rats.

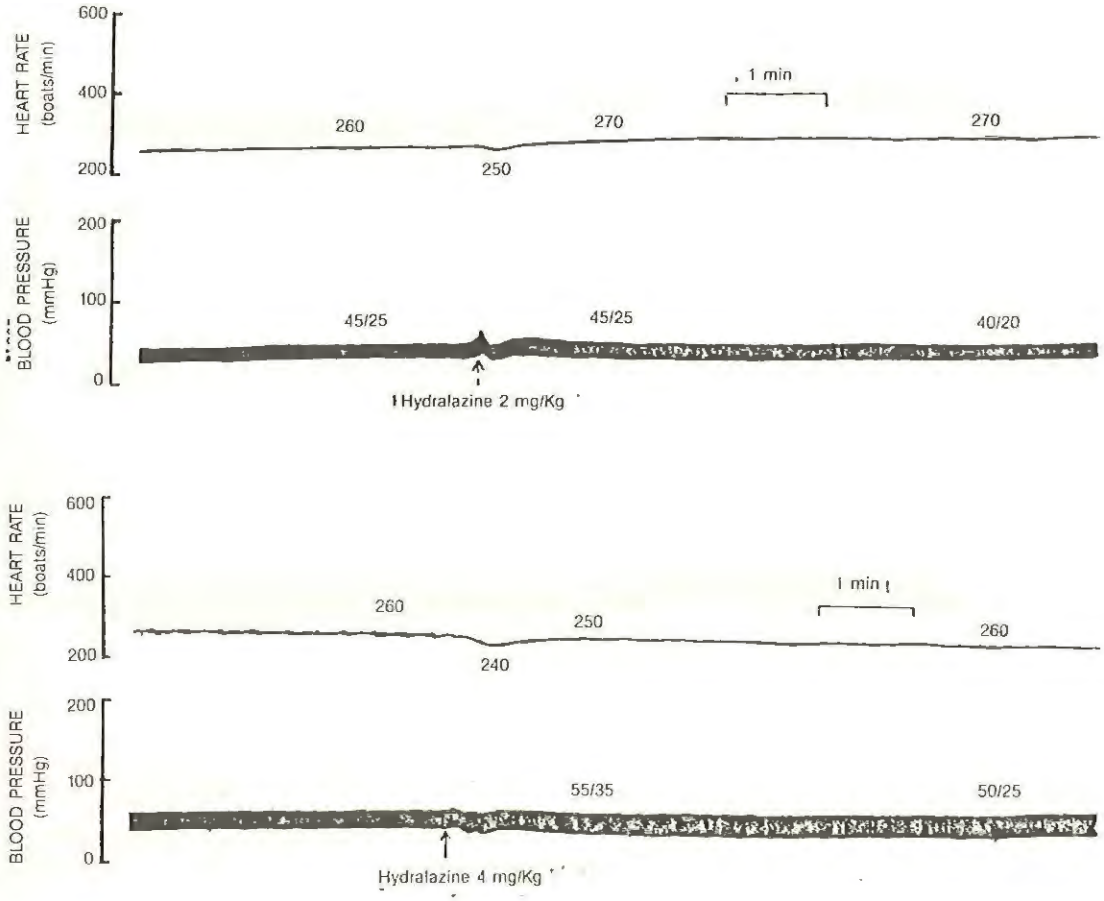

Fig. 4. The effect of hydralazine 2 and 4 mg/kg on blood pressure and heart rate in pithed rats.

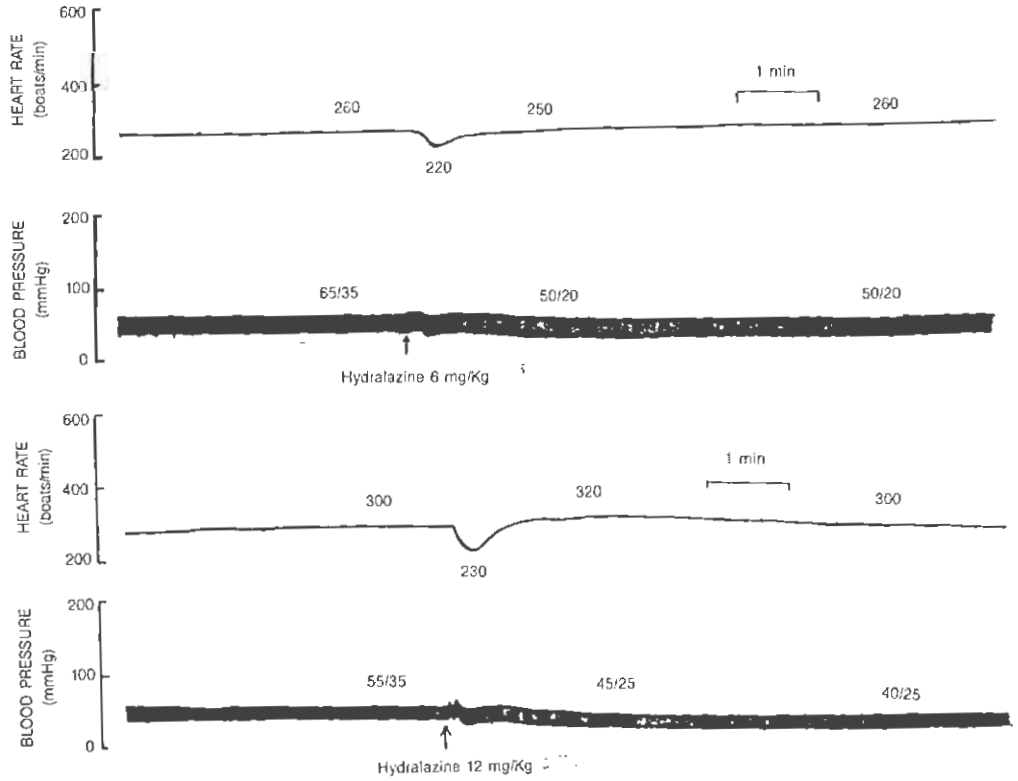

Fig. 5. The effect of hydralazine 6 and 12 mg/kg on blood pressure and heart rate in pithed rats.

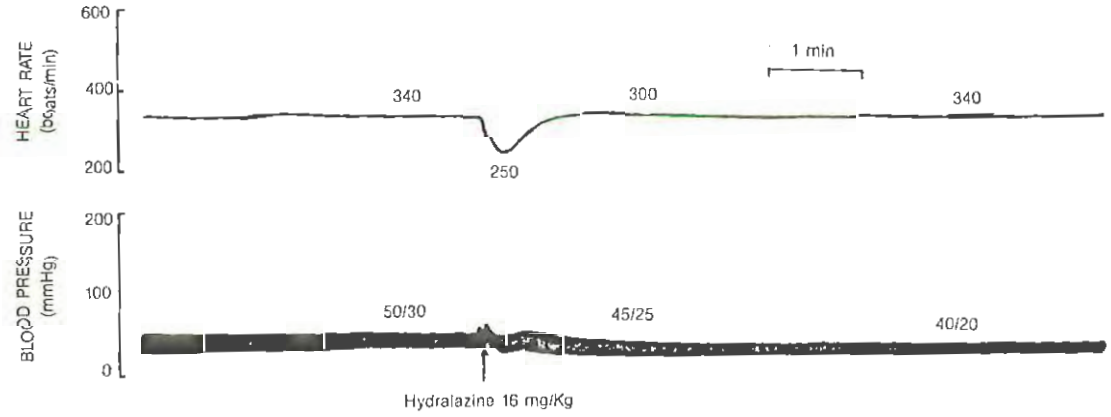

Fig. 6. The effect of hydralazine 16 mg/kg on blood pressure and heart rate in pithed rats.

presence of theophylline in the present study (Fig. 8b). This may be due to the potentiation of ICI 63 197 on the hydralazine induced an increase in the rate of beating of rat isolated atria.

It may be suggested, therefore, that in addition to the vasodilator-induced a change in heart rate, the direct action of hydralazine on the important part of the heart, the atrium with pace-maker, should be accounted for those side effects of the patients' hears.

Refferences

1. Gerber, J.G., and Nies, A.S. (1991) Antihypertensive agents and the drug therapy of hypertension. In : Goodman and Gilman's The Pharmacological Basis of Therapeutics. pp. 784-813. Edited by A.G. Gilman et al. Pergamon Press, Inc. Singapore.
2. Gross, F. (1977) Drugs acting on arteriolar smooth muscle (vasodilator drugs). In "Antihypertensive Agents", pp. 397-476. Ed. Gross, F. Springer-Verlag, Berlin.
3. Houchi, H., Morita, K., Minakuchi, K. and Oka, M. (1986) Inhibition by hydralazine of catecholamine biosynthesis in cultured bovine adrenal medullary cells. Biochemical Pharmacology, 35 (12), 2047-2049.
4. Songkittiguna, P., Majewski, H. and Rand M.J. (1980) Inhibition by hydralazine of the conversion of dopamine to noradrenaline in rat atria in vitro and in vivo. Clinical and Experimental Pharmacology & Physiology, 7, 509-514.
5. Songkittiguna, P. and Rand, M.J. (1982) The mechanism of the inotropic and chronotropic actions of hydralazine on rat isolated atria. Arch. Int. Pharmacodyn. 255, 269-280.
6. Sutherland, E.W., Robinson, G.A., and Butcher, R.W. (1968) Some aspects of the biological role of adenosine 3', 5'-monophosphate (cyclic AMP). Circulation, 37, 279-306.