Br. J. Pharmac. Chemother. (1967), **30**, 123–133.

INFLUENCE OF ADRENERGIC BETA-RECEPTOR BLOCKADE ON THE ACUTE CARDIOVASCULAR EFFECTS OF HYDRALAZINE*

BY

H. BRUNNER, P. R. HEDWALL AND M. MEIER

From the Biological Department, CIBA Limited, Basle, Switzerland

(*Received November* 30, 1966)

Hydralazine lowers peripheral resistance and, therefore, the blood pressure, mainly by a direct action on vascular smooth muscle (Bein, Gross, Tripod & Meier, 1953; Åblad, 1963). In the human (Stein & Hecht, 1955; Åblad, 1963) and in the conscious dog (Grimson, 1952) the fall in blood pressure is accompanied by tachycardia. In anaesthetized dogs an increase in myocardial contractility has been found (Barrett, Povalski & Rutledge, 1965). Furthermore, in the dog, both the tachycardia (Brunner, Hedwall & Meier, 1965a) and the increase in myocardial contractility (Barrett *et al.*, 1965) could be inhibited by adrenergic beta-receptor blockade. Thus it seemed possible that the action of hydralazine might involve direct stimulation of adrenergic beta-receptors. We therefore studied the influence of two adrenergic beta-receptor blocking agents, propranolol and CIBA 39,089-Ba [1-(o-allyloxyphenoxy)-3-isopropylamino-2-propanol hydrochloride] (Brunner, Hedwall & Meier, to be published), on the acute cardiovascular effects of hydralazine in the dog and rat.

METHODS

Arterial blood pressure was measured in the conscious rat using the method of Weeks & Jones (1960). Three to five days before the experiment a polyethylene cannula was implanted into the abdominal aorta. During blood pressure measurement the animals were placed in plexiglass restraining cages. The cannulae were filled with heparin solution; and pressure was recorded with a Statham strain gauge. Heart rate was derived from the pulse wave. Test substances were injected into a tail vein.

In a parallel group of rats anaesthetized with pentobarbitone sodium (Nembutal,® Abbott) (25 mg/kg intraperitoneally+50 mg/kg subcutaneously) blood pressure was measured with a Statham strain gauge attached to a cannula in the carotid artery. Test substances were injected into a jugular vein.

In trained, unanaesthetized, mongrel dogs a needle was inserted in a femoral artery and blood pressure was measured with a Statham transducer or a mercury manometer. Heart rate was derived from an electrocardiogram tracing. Test substances were injected into a catheter inserted from a superficial leg vein and lying in the inferior vena cava.

Blood pressure and heart rate were measured in a group of dogs anaesthetized with pentobarbitone sodium (initial injection of 20–30 mg/kg intravenously+additional amounts as needed during the experiment) using the same technique.

* A preliminary report of these results was presented at the 7th Meeting of the Deutsche Pharmakologische Gesellschaft, Mainz, 1966.

Cardiac output was determined in trained, unanaesthetized dogs and in pentobarbitone-anaesthetized dogs by the dye dilution method. Indocyanine green was injected through a catheter inserted from a superficial vein in the hind limb into the inferior vena cava. Blood was withdrawn from a femoral artery and the dye concentration determined continuously by a photometric method. Total peripheral resistance was calculated from mean arterial blood pressure and cardiac output. Test substances were injected into the catheter lying in the inferior vena cava.

Substances and doses used were: hydralazine (Apresoline®, CIBA) 1 mg/kg intravenously, propranolol (Inderal,® ICI) 1 mg/kg intravenously and CIBA 39,089-Ba (Trasicor®) 1 mg/kg intravenously. The adrenergic beta-receptor blockade produced by this dose of propranolol or CIBA 39,089-Ba inhibits the vasodepressor and positive chronotropic effects of 0.1–0.5 γ/kg isoprenaline intravenously (Aleudrin®, C. H. Boehringer, Ingelheim) in the dog and the vasodepressor effect of 1 γ/kg isoprenaline intravenously in the rat for longer than 1 hr.

In parallel groups of conscious and anaesthetized dogs and rats, the effects of hydralazine, propranolol, CIBA 39,089-Ba, and hydralazine after pretreatment with propranolol or CIBA 39,089-Ba were determined. In the dog hydralazine was injected 10 min after pretreatment; in the rat 30 min after pretreatment.

The statistical methods of Mather (1943), Lord (1947) and Hogben (1964) were used.

RESULTS

Effects of hydralazine, propranolol and CIBA 39,089-Ba on blood pressure and heart rate in the conscious and anaesthetized rat

In the conscious rat 1 mg/kg hydralazine intravenously decreased aortic blood pressure and increased heart rate (Fig. 1a). In the pentobarbitone-anaesthetized rat, on the other hand, hydralazine (1 mg/kg) produced a more pronounced fall in blood pressure, accompanied by a decrease in heart rate (Fig. 1b). In both the anaesthetized and the conscious rat, the effect of hydralazine reached a maximum within 10 min and remained unchanged for over 30 min.

Propranolol (1 mg/kg) and CIBA 39,089-Ba (1 mg/kg) had no significant effect on blood pressure or heart rate in the conscious rat. In the anaesthetized rat propranolol (1 mg/kg) and CIBA 39,089-Ba (1 mg/kg) reduced blood pressure slightly, while heart rate remained unchanged (Table 1).

Effects of hydralazine on blood pressure and heart rate of the rat after pretreatment with propranolol or CIBA 39,089-Ba

In the conscious rat the fall in blood pressure produced by hydralazine was less pronounced after pretreatment with propranolol 1 mg/kg ($P<0.01$) or CIBA 39,089-Ba 1 mg/kg ($P<0.05$) (Fig. 1c). After pretreatment with CIBA 39,089-Ba the increase in heart rate was slighter than in control animals ($P<0.05$). After pretreatment with propranolol, however, heart rate was increased to the same extent in pretreated and in control animals (Fig. 1c).

The effect of hydralazine on the blood pressure of the anaesthetized rat was reduced slightly by propranolol pretreatment ($P<0.05$), but was not influenced by pretreatment with CIBA 39,089-Ba (Fig. 1d). In the anaesthetized rat after pretreatment with propranolol, hydralazine did not reduce heart rate significantly. The decrease in heart rate due to hydralazine in animals pretreated with CIBA 39,089-Ba was in the same range as in control rats.

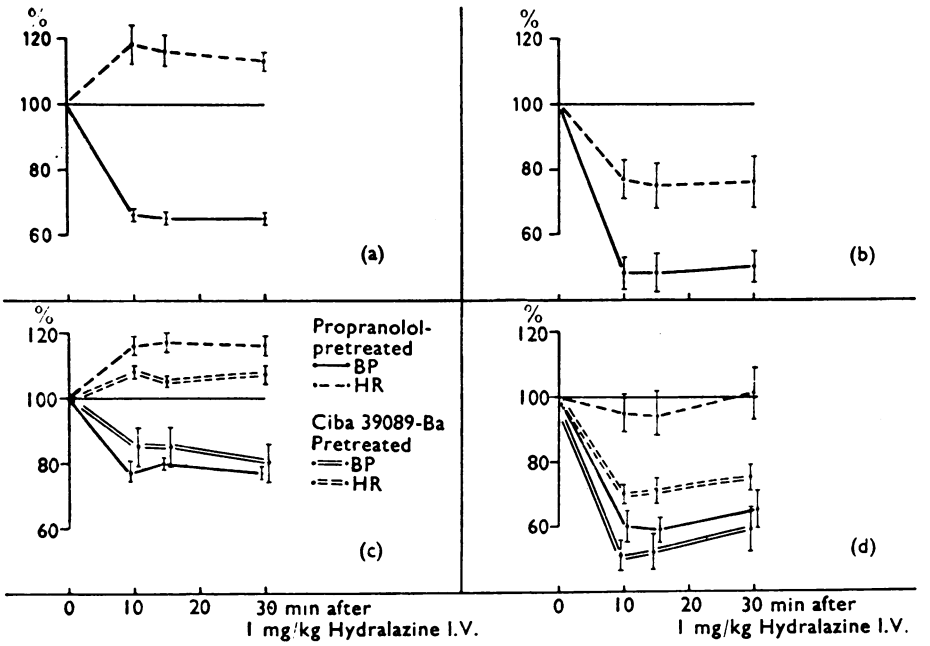

Fig. 1. Effects of 1 mg/kg hydralazine intravenously on blood pressure and heart rate in the rat. (a) Conscious control animals. Initial values: blood pressure (BP) 127±5 mm Hg, heart rate (HR) 453±16 beats/min, number of experiments (n=8). (b) Pentobarbitone-sodium-anaesthetized control animals. Initial values: BP 128±7 mm Hg, HR 383±28 beats/min, n=8. (c) Conscious animals, pretreated with 1 mg/kg propranolol I.V. or 1 mg/kg CIBA 39,089-Ba I.V. Initial values: propranolol-pretreated animals: BP 121±3 mm Hg, HR 368±12 beats/min, n=8. CIBA 39,089-Ba-pretreated animals: BP 116±4 mm Hg, HR 399±17 beats/min, n=6. (d) Pentobarbitone-sodium-anaesthetized animals, pretreated with propranolol or CIBA 39,089-Ba. Initial values: propranolol-pretreated animals: BP 116±6 mm Hg, HR 329±18 beats/min, n=8. CIBA 39,089-Ba-pretreated animals: BP 116±5 mm Hg, HR 393±19 beats/min, n=6. The ordinates represent the change in blood pressure and heart rate expressed as % of pre-injection values; the abscissae the time elapsing after injection of hydralazine. All values are means; the cross bars represent standard errors.

TABLE 1

EFFECTS OF INTRAVENOUS INJECTION OF 1 mg/kg PROPRANOLOL OR CIBA 39,089-Ba ON BLOOD PRESSURE AND HEART RATE IN THE RAT

The values given are the means and standard errors. The changes in blood pressure and heart rate 30 min after injection are expressed as % of pre-injection values. n = Number of experiments. * = Significant at $P<0.05$

	Initial values		30 min after injection	
	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (Δ%)	Heart rate (Δ%)
Propranolol				
Conscious rat n = 6	114±6	390±24	−1±3	−9±4
Pentobarbitone-anaesthetized rat n = 7	133±4	384±17	−14±5*	−12±6
CIBA 39,089-Ba				
Conscious rat n = 7	118±4	429±12	−2±2	−7±3
Pentobarbitone-anaesthetized rat n = 6	135±3	412±14	−14±5*	−5±4

Effects of hydralazine, propranolol and CIBA 39,809-Ba on blood pressure, heart rate, cardiac output and total peripheral resistance in the dog

In the conscious, trained dog hydralazine produced only a slight decrease in femoral arterial blood pressure, but a marked increase in heart rate (Fig. 2a). The fall in blood pressure in the pentobarbitone-anaesthetized dog (Fig. 2b) was more rapid and more pronounced ($P<0.05$). Heart rate was increased only slightly.

Owing to the higher initial heart rate in the anaesthetized dog (188 ± 12 beats/min compared with 81 ± 10 beats/min in the conscious dog) the ability to increase heart rate

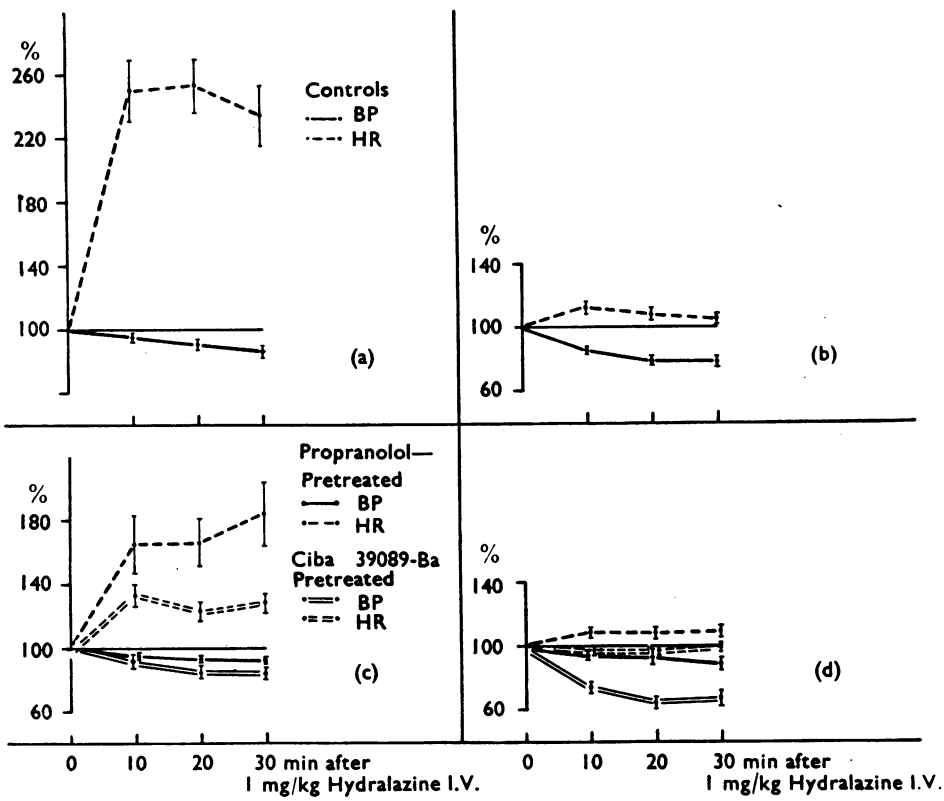

Fig. 2. Effects of 1 mg/kg hydralazine intravenously on blood pressure and heart rate in the dog. (a) Conscious control animals. Initial values: blood pressure (BP) 125 ± 8 mm Hg, heart rate (HR) 81 ± 10 beats/min, number of experiments (n=7). (b) Pentobarbitone-sodium anaesthetized control animals. Initial values: BP 155 ± 6 mm Hg, HR 188 ± 12 beats/min, n=7. (c) Conscious animals pretreated with 1 mg/kg propranolol I.V. or 1 mg/kg CIBA 39,089-Ba I.V. Initial values: propranonol-pretreated animals: BP 127 ± 7 mm Hg, HR 73 ± 9 beats/min, n=6. CIBA 39,089-Ba-pretreated animals: BP 116 ± 6 mm Hg, HR 104 ± 8 beats/min, n=8. (d) Pentobarbitone-sodium-anaesthetized animals, pretreated with propranolol or CIBA 39,089-Ba. Initial values: propranolol-pretreated animals: BP 152 ± 9 mm Hg, HR 129 ± 7 beats/min, n=7. CIBA 39,089-Ba-pretreated animals: BP 116 ± 8 mm Hg, HR 148 ± 6 beats/min, n=9. The ordinates represent the change in blood pressure and heart rate expressed as % of pre-injection values; the abscissae the time elapsing after injection of hydralazine. All values are means; the cross bars represent standard errors.

may have been limited. In two anaesthetized dogs with lower initial heart rates (average 150 beats/min), however, heart rate was not increased by hydralazine (average maximum increase = 8 beats/min).

The suppression of tachycardia after hydralazine seemed to be correlated with the depth of anaesthesia. In one dog in extremely superficial pentobarbitone-sodium anaesthesia hydralazine increased heart rate from 165 beats/min to 240 beats/min. After injection of additional anaesthetic the rate fell to 170 beats/min within 10 min.

In the conscious dog propranolol did not influence blood pressure or heart rate significantly. In the anaesthetized dog blood pressure remained unchanged while heart rate decreased slightly (Table 2).

TABLE 2

EFFECTS OF INTRAVENOUS INJECTION OF 1 mg/kg PROPRANOLOL OR CIBA 39,089-Ba IN THE DOG

The values given are the means and standard errors. The changes in blood pressure and heart rate 10 min after injection are expressed as % of pre-injection values. n = number of experiments. * = $P<0{\cdot}05$. † = $P<0{\cdot}01$

	Initial values		10 min after injection	
	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (Δ%)	Heart rate (Δ%)
Propranolol				
Conscious dog n = 6	123±8	82±17	+4±6	−5±8
Pentobarbitone-anaesthetized dog n = 7	155±8	157±13	−2±2	−16±3†
CIBA 39,089-Ba				
Conscious dog n = 8	118±7	84±11	−1±3	+35±15*
Pentobarbitone-anaesthetized dog n = 9	131±8	164±7	−12±3†	−10±2†

In the conscious dog CIBA 39,089-Ba did not influence blood pressure, but increased heart rate moderately. In the anaesthetized dog blood pressure and heart rate were decreased slightly (Table 2).

In smaller groups of conscious and anaesthetized dogs, blood pressure, heart rate and cardiac output were measured simultaneously and total peripheral resistance was calculated.

In the experiments described above it was determined that the effects of hydralazine in the dog reached a virtually constant level after 10 min. Therefore, in the following experiments, the values reached 10 min after injections of hydralazine are given.

In the conscious dog hydralazine increased cardiac output but had little or no effect on arterial blood pressure, from these observations it was calculated that hydralazine reduced total peripheral resistance markedly (Fig. 3).

In the anaesthetized dog hydralazine decreased peripheral resistance to approximately the same extent as in the conscious dog. The increase in cardiac output, however, was much less pronounced than in the conscious dog ($P<0.05$), although the initial control values were in the same range in both groups of animals (Fig. 4).

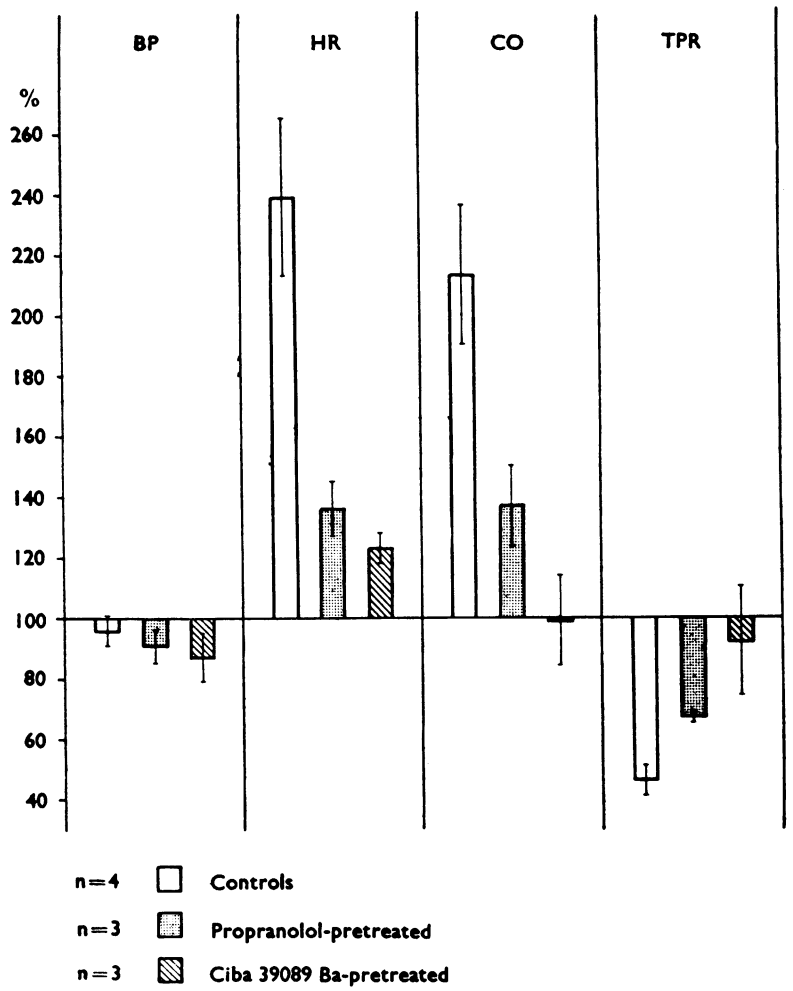

Fig. 3. Effects of 1 mg/kg hydralazine intravenously on blood pressure, heart rate, cardiac output and total peripheral resistance in the conscious dog 10 min after injection. The ordinate represents blood pressure (BP), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR) expressed as % of pre-injection values. The values are means, the cross bars represent standard errors, n = number of experiments.

Initial values:	BP (mm Hg)	HR (beats/min)	CO (l./min)	TPR dyn. sec. cm^{-5}
Control animals	124±15	89±16	2·39±0·41	4,260±360
Propranolol-pretreated animals (1 mg/kg I.V.)	153± 4	83± 9	2·18±0·17	5,680±480
CIBA 39,089-Ba-pretreated animals (1 mg/kg I.V.)	127± 7	100± 7	3·07±0·46	3,413±430

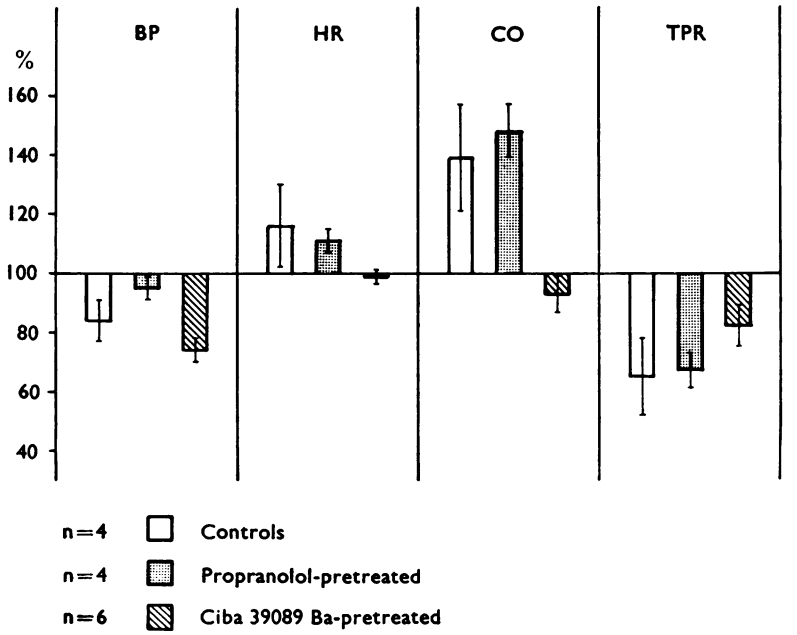

Fig. 4. Effects of 1 mg/kg hydralazine intravenously on blood pressure, heart rate, cardiac output and total peripheral resistance in the pentobarbitone-sodium-anaesthetized dog 10 min after injection. The ordinate represents blood pressure (BP), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR) expressed as % of pre-injection values. The values are means, the cross bars represent standard errors, n = number of experiments.

Initial values:	BP (mm Hg)	HR (beats/min)	CO (l./min)	TPR dyn. sec. cm^{-5}
Control animals	137± 9	164±11	1·59±0·14	7,110±1,200
Propranolol-pretreated animals (1 mg/kg I.V.)	160± 7	124± 7	1·54±0·24	8,720±1,200
CIBA 39,089-Ba-pretreated animals (1 mg/kg I.V.)	118±12	138± 4	2·21±0·35	4,720±640

Propranolol and CIBA 39,809-Ba had no significant effect on total peripheral resistance or cardiac output in the conscious or anaesthetized dog (Table 3).

The effects of hydralazine, propranolol and CIBA 39,809-Ba on blood pressure and heart rate corresponded roughly to those seen previously.

Effects of hydralazine on blood pressure, heart rate, cardiac output and total peripheral resistance after pretreatment with propranolol or CIBA 39,089-Ba

The fall in blood pressure produced by hydralazine in the conscious dog was not influenced by pretreatment with propranolol or CIBA 39,089-Ba (Fig. 2c). The increase in heart rate was, however, less in animals pretreated with propranolol ($P<0.01$) or CIBA 39,089-Ba ($P<0.001$). In the anaesthetized dog pretreated with propranolol the

TABLE 3

EFFECTS OF INTRAVENOUS INJECTION OF 1 mg/kg PROPRANOLOL OR CIBA 39,089-Ba ON BLOOD PRESSURE (BP), HEART RATE (HR), CARDIAC OUTPUT (CO) AND TOTAL PERIPHERAL RESISTANCE (TPR) IN THE DOG

The values given are the means and standard errors. The changes 10 min after injection are expressed as % of pre-injection values. n = Number of experiments. * = $P<0.05$. † $P<0.01$

	Initial values				10 min after injection			
	BP (mm Hg)	HR (beats/min)	CO (l./min)	TPR dyn. sec. cm^{-5}	BP (Δ%)	HR (Δ%)	CO (Δ%)	TPR (Δ%)
Propranolol								
Conscious dog n = 3	145±3	95±14	2·54±0·40	4,720±652	+4±2	−14±2*	−11±16	+23±24
Pentobarbitone-anaesthetized dog n = 4	161±11	146±15	1·80±0·23	7,520±1,128	0±3	−13±10	−14±7	+18±13
CIBA 39,089-Ba								
Conscious dog n = 3	136±9	88±22	2·21±0·32	5,067±628	−6±8	+28±40	+45±35	−30±17
Pentobarbitone-anaesthetized dog n = 6	130±12	153±4	2·24±0·37	5,147±632	−10±2†	−10±3*	+1±13	−7±10

fall in blood pressure produced by hydralazine was not as marked as in control animals (Fig. 2d, $P<0.05$), while after pretreatment with CIBA 39,089-Ba the decrease in blood pressure was more pronounced than in control animals ($P<0.05$). After pretreatment with propranolol, as in control animals, heart rate was increased slightly by hydralazine, whereas after pretreatment with CIBA 39,089-Ba, hydralazine did not influence heart rate.

Hydralazine decreased peripheral resistance to a lesser extent in the conscious dog pretreated with propranolol or CIBA 39,089-Ba ($P<0.05$); and the increase in cardiac output was less pronounced than in the control animals ($P<0.05$ and $P<0.01$ respectively). In the anaesthetized dog pretreated with propranolol or CIBA 39,089-Ba the decrease in peripheral resistance produced by hydralazine did not differ significantly from the decrease seen in the control animals. Cardiac output increased slightly in control animals and in propranolol pretreated animals, but not after pretreatment with CIBA 39,089-Ba. The changes in blood pressure and heart rate corresponded to those seen previously (Fig. 2).

DISCUSSION

Marked differences were found in the effects of hydralazine in the conscious and the anaesthetized animal. In the conscious animal the fall in arterial blood pressure was accompanied by pronounced tachycardia and an increase in cardiac output. These effects could be inhibited by pretreatment with propranolol or CIBA 39,089-Ba. In the anaesthetized animal the fall in blood pressure was more pronounced; there was little or no change in heart rate or cardiac output in the anaesthetized dog, while bradycardia was seen in the rat. The differences in the effects of hydralazine in conscious and anaesthetized animals suggest that hydralazine does not stimulate the heart directly. Furthermore, no increase in heart rate was seen in the isolated rabbit or cat heart (Bein *et al.*, 1953), or in the canine heart–lung preparation (Craver & Yonkman, 1950). Likewise, section of the cardiac nerves (Grimson, 1952) or pretreatment with hexamethonium (Moyer, Huggins & Handley, 1953) suppressed the tachycardia after application of hydralazine.

The cardiac stimulation seen after administration of hydralazine in the conscious animal may be the result of reflex adjustment to the fall in blood pressure, as suggested by Åblad (1963). Reflex cardiovascular adjustment initiated by a fall in blood pressure consists of increased sympathetic discharge rather than a decrease in vagus activity as shown by Glick & Braunwald (1965) in the dog. An increase in sympathetic activity has also been found to result after administration of hydralazine in man (Åblad, 1963). An increase in sympathetic outflow should result in constriction of the peripheral blood vessels by a predominant stimulation of adrenergic alpha-receptors and increased cardiac activity by adrenergic beta-receptor stimulation. The vasoconstrictor component of this reflex adjustment is, however, masked by hydralazine (Åblad, 1963). The effects of beta-receptor stimulation, tachycardia and an increase in cardiac output, were evident in the conscious animal.

The cardiac stimulant effects of hydralazine in unanaesthetized animals could be inhibited by beta-blockade. At the dose level used (1 mg/kg) CIBA 39,089-Ba seemed to be more effective than propranolol. In the dog after pretreatment with CIBA 39,089-Ba,

the heart rate was higher than after pretreatment with propranolol ($P<0.05$). Thus the relative increase in heart rate produced by hydralazine differs in the two groups, while the absolute values reached were in the same range. In the conscious rat, however, only CIBA 39,089-Ba inhibited the tachycardia produced by hydralazine.

The slight tachycardia and increase in cardiac output produced by hydralazine in the anaesthetized dog were abolished by CIBA 39,089-Ba, but not by propranolol. In the anaesthetized rat hydralazine did not increase heart rate, so that it is not possible to draw any conclusion from this parameter. After blockade of adrenergic beta-receptors, for example, suppression of reflex cardiac stimulation, hydralazine should produce a more pronounced fall in blood pressure. This was seen only in one case. The relative decrease in blood pressure in the anaesthetized dog, pretreated with CIBA 39,089-Ba was slightly more pronounced than in control animals. The absolute decrease in blood pressure, however, did not differ significantly.

In the conscious dog inhibition of reflex cardiac stimulation, as produced by propranolol or CIBA 39,089-Ba, was not sufficient to potentiate the fall in blood pressure. In this case the reduction in peripheral resistance due to hydralazine was also less pronounced. After beta-blockade in the conscious rat the hypotensive effect of hydralazine was less marked than in control animals. Thus the decrease in peripheral resistance was probably also slighter. In an earlier investigation in renal hypertensive rats the hypotensive effect of hydralazine was antagonized by pronethalol (Brunner, Hedwall & Meier, 1965b) or propranolol (Bein & Brunner, 1966).

That portion of the decrease in peripheral resistance which can be inhibited by CIBA 39,089-Ba or propranolol may be the result of reflex stimulation of vascular beta-receptors. However, the possibility that inhibition of the cardiac component of reflex adjustment may intensify reflex vasoconstriction cannot be excluded.

SUMMARY

1. The influence of adrenergic beta-receptor blockade on the cardiovascular effects of hydralazine was studied in conscious and pentobarbitone-anaesthetized dogs and rats.

2. In the conscious dog hydralazine produced a marked reduction in total peripheral resistance; but, owing to a pronounced increase in cardiac output, blood pressure fell only slightly.

3. In the anaesthetized dog hydralazine decreased total peripheral resistance to the same extent as in the conscious dog. Blood pressure was, however, decreased more markedly since only slight cardiac stimulation occurred.

4. Cardiac stimulation and, to a certain extent, the decrease in total peripheral resistance could be inhibited by adrenergic beta-receptor blockade. After suppression of cardiac stimulation no potentiation of the hypotensive effect of hydralazine was seen.

5. In the conscious rat hydralazine produced a marked fall in blood pressure and tachycardia. Both effects could be inhibited by adrenergic beta-receptor blockade.

6. In the anaesthetized rat no definite influence of beta-receptor blockade on the effects of hydralazine was seen.

REFERENCES

ÅBLAD, B. (1963). A study of the mechanism of the hemodynamic effects of hydralazine in man. *Acta pharmac. tox.*, **20**, Suppl. No. 1, 1–53.

BARRETT, W. E., POVALSKI, H. & RUTLEDGE, R. (1965). A hypothesis concerning the mechanism of action of hydralazine HCl. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **24**, 712.

BEIN, H. J. & BRUNNER, H. (1966). Mode of action of antihypertensive drugs. In *Antihypertensive Therapy*, ed. GROSS, F., pp. 15–28. Springer, Berlin, Heidelberg, New York.

BEIN, H. J., GROSS, F., TRIPOD, J. & MEIER, R. (1953). Experimentelle Untersuchungen über die Kreislaufwirkung der blutdrucksenkenden Hydrazinophthalazinderivate Apresolin und Nepresol. *Schweiz. med. Wschr.*, **83**, 336–340.

BRUNNER, H., HEDWALL, P. R. & MEIER, M. (1965a). Inhibition of acute effects of hydralazine by an adrenergic beta-receptor blocking agent. *Experientia*, **21**, 136–137.

BRUNNER, H., HEDWALL, P. R. & MEIER, M. (1965b). Influence of an adrenergic beta-receptor blocking agent on the effect of various hypotensive agents in the hypertensive rat. *Experientia*, **21**, 231–232.

CRAVER, B. M. & YONKMAN, F. F. (1950). Some pharmacological properties of L-hydrazinophthalazine, a hypotensive agent. *Fedn Proc. Fedn Am. Socs exp. Biol.*, **9**, 265–266.

GLICK, G. & BRAUNWALD, E. (1965). Relative roles of the sympathetic and parasympathetic nervous system in the reflex control of the heart rate. *Circulation Res.*, **16**, 363–375.

GRIMSON, K. S. (1952). In *Apresoline® Conference*. New York City, N.Y. Cited by MOYER, J. H., HUGGINS, R. A. & HANDLEY, C. A. (1953). *J. Pharmac. exp. Ther.*, **109**, 175–182.

HOGBEN, C. A. M. (1964). A practical and simple equivalent for student's t test of statistical significance. *J. Lab. clin. Med.*, **64**, 815–819.

LORD, E. (1947). The use of range in place of standard deviation in the t-test. *Biometrika*, **34**, 41–67.

MATHER, K. (1943). *Statistical Analysis in Biology*. Methuen, London.

MOYER, J. H., HUGGINS, R. A. & HANDLEY, C. A. (1953). Further cardiovascular and renal hemodynamic studies following the administration of hydrallazine (1-hydrazinophthalazine) and the effect of ganglionic blockade with hexamethonium on these responses. *J. Pharmac. exp. Ther.*, **109**, 175–182.

STEIN, D. H. & HECHT, H. H. (1955). Cardiovascular and renal responses to the combination of hexamethonium and 1-hydrazinophthalazine (Apresoline) in hypertensive subjects. *J. clin. Invest.*, **34**, 867–874.

WEEKS, J. R. & JONES, J. A. (1960). Routine direct measurement of arterial pressure in unanesthetized rats. *Proc. Soc. exp. Biol. Med.*, **104**, 646–648.