Br. J. Pharmac. (1980), **69**, 415–420

HYDRALAZINE: EFFECT ON THE OUTFLOW OF NORADRENALINE AND MECHANICAL RESPONSES EVOKED BY SYMPATHETIC NERVE STIMULATION OF THE RAT TAIL ARTERY

C. CHEVILLARD, M.N. MATHIEU, B. SAIAG* & M. WORCEL*[1]

INSERM FRA. 37 (Med. D), Hôpital St Charles, 34059 Montpellier, and INSERM U.7*, Hôpital Necker 75015, Paris, France

1 The effects of hydralazine on the vasoconstrictor responses to field stimulation of sympathetic nerves were studied in the isolated proximal segments of the rat tail artery. Vasoconstrictor responses to transmural stimulation were depressed by superfusion of hydralazine (0.3, 3 and 30 μM) in a concentration-dependent manner. The inhibition appeared slowly and was not easily reversed by washing.

2 Hydralazine (30 nM, 0.3 and 3 μM) reduced the stimulation-induced overflow of tritium from proximal and distal segments of the tail artery labelled with [^{3}H]-noradrenaline in a concentration-dependent manner. This phenomenon appeared rapidly and was easily reversed by washing.

3 Theophylline (0.5 mM) did not affect the inhibitory effect of hydralazine on the stimulation-induced tritium efflux from the distal segment of the rat tail artery.

4 The present results indicate that hydralazine has, in addition to its action on vascular smooth muscle, a very marked effect on sympathetic nerve terminals. The mechanism of this presynaptic inhibition appears to be different from the postsynaptic effect, in view of the much shorter delay, the shape of the dose-effect curve, and the lack of interaction with theophylline.

Introduction

Hydralazine (1-hydrazino-phtalazine: Hyd) has long been used as an antihypertensive drug, but its mechanism(s) of action remain(s) obscure (see review by Gross, 1977). In a previous paper (Worcel, 1978), we demonstrated that the effect of Hyd *in vitro* was modified by the state of the sympathetic innervation: in fact Hyd inhibited reversibly the contractions induced by phenylephrine in the distal segment of the rat tail artery, whereas the proximal segment, which was normally unresponsive, was relaxed by Hyd only after *in vitro* sympathectomy with 6-hydroxydopamine. Moreover, it was previously shown that adenosine triphosphate (ATP) completely blocked the response to Hyd (Worcel, 1978). ATP is associated with noradrenaline (NA) as a normal component of vesicular material in sympathetic nerve terminals (De Potter, 1971) being released together with the catecholamines following stimulation of the sympathetic nerves (Ribeiro, 1978). ATP and related substances may modify adrenergic transmission, inhibiting NA release in adrenergically innervated organs (Verhaeghe, Vanhoutte & Shepherd, 1976; Enero & Saïdman, 1977; Su, 1978) by acting on presynaptic inhibitory purine receptors. On the other hand, it has been shown previously that the effects of Hyd on arterial smooth muscle are modulated by the release of ATP and/or other purine compounds from sympathetic nerve terminals (Worcel, 1978; Worcel, Saiag & Chevillard, 1980).

In view of this, it appeared interesting to explore the possibility of the existence of a similar interaction between Hyd and endogenous purines at a presynaptic site. In this study, we have examined the effects of Hyd on the stimulation-induced (S-I) increase in perfusion pressure in the proximal part of the rat tail artery. We also studied the effects of Hyd on the tritium efflux evoked by transmural stimulation of rat tail arteries prelabelled with tritiated NA, in absence or in presence of theophylline (Theo).

Methods

Male Wistar rats (300 to 350 g) were anaesthetized with pentobarbitone (75 mg/kg; i.p.). After excision, the tail artery was divided in three parts measuring

[1] Present address: Centre de Recherches ROUSSEL-UCLAF, 111, Route de Noisy, BP 9, 93230 Romainville, France.

0007-1188/80/070415-06$01.00

5 cm each i.e.: the segment next to the body (proximal segment), the intermediate one which was discarded and the distal segment.

Vasoconstrictor responses to sympathetic nerve stimulations: effect of hydralazine

The proximal part of the caudal artery was excised after ligation of all collateral vessels, and cannulated at each end. Each segment was perfused with a physiological salt solution (PSS). The flow rate was maintained at 75 µl/min and the perfusion pressure monitored with a strain gauge. The artery was mounted between bipolar circular Pt electrodes, then continuously superfused with PSS (1 ml/min). The intramural sympathetic nerves were stimulated at supramaximal voltage for 40 s with pulses of 2 ms duration at 10 Hz. The intervals between stimulations were of 15 min. Hyd was infused by means of a Braun-Melsungen injection apparatus in the superfusion stream to achieve final concentrations of 30 nM, 0.3 µM, 3 µM and 30 µM; in these concentrations, Hyd had no effect on resting perfusion pressure. Results were expressed as % of the control (without Hyd) contractile responses evoked by electrical stimulation.

Radiolabelling with tritiated noradrenaline and determination of stimulation-induced tritium efflux

Transmitter stores of both proximal and distal segments were labelled with [^{3}H]-NA for 1 h in PSS containing 10 µCi/ml of [^{3}H]-NA (10 Ci/mmol). After 1 h rinsing, the arterial segments were mounted between Pt electrodes, superfused with PSS and stimulated as described above. Samples of the superfusate were collected for 5 min periods before, during and after each stimulation for measurement of the efflux of radioactivity. The S-I efflux of tritium was calculated as fractional release per shock: i.e. total radioactivity released per shock divided by radioactivity remaining in the tissue at the onset of stimulation (Langer & Enero, 1974). At the end of the experiment, arterial segments were homogenized in 2 ml of 0.2 M perchloric acid containing 0.1% disodium edetate and 0.125% sodium sulphite, then centrifuged. Radioactivity of 1 ml aliquots of superfusate and 0.1 ml of supernatant was estimated in 10 ml Instagel (Packard) with a liquid scintillation counter.

Effects of hydralazine on stimulation-induced tritium efflux

In each experiment, the S-I efflux in the second and subsequent periods of stimulation was calculated as ratios of the corresponding efflux during the first stimulation period (SX/S1).

The effect of Hyd was investigated by infusion of the drug in the superfusate 5 min before the second period of stimulation. In order to determine the delay of action, in a first series of experiments, Hyd (30 nM, 0.3 µM and 3 µM) was infused, each concentration being maintained during three periods of stimulation. In a second series of experiments, cumulative dose-response curves were obtained by increasing the concentration of Hyd in the superfusate (3 nM, 30 nM, 0.3 µM and 3 µM) every 15 min. The concentrations of Hyd were changed 5 min before each stimulation. In both cases, to evaluate the return to basal release, the Hyd infusion was terminated 5 min before the last stimulation.

Interaction of theophylline with hydralazine

To investigate the possible interaction of Hyd with endogenous purines at the presynaptic site, cumulative dose-response curves were obtained in the presence of 0.5 mM Theo. This drug was added to the superfusate from the beginning of the experiment.

Solutions used

Hyd was dissolved in a solution having the following composition (mg/ml H_2O): NaCl 7, NaH_2PO_4 3.85 and Na_2HPO_4 0.2; the pH was 5 ± 1.

The composition of the PSS used for radiolabelling, rinsing, perfusion and superfusion was (mmol/l): NaCl 137, KCl 2.5, $CaCl_2$ 1.4, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.36, glucose 5.6, ascorbic acid 0.1 and Dextran T 70 0.31; this solution was gassed with a 95% O_2: 5% CO_2 mixture and maintained at 37°C.

Drugs used

Hyd was obtained from Sigma Chemical Co., St Louis, Mo. U.S.A., Theo from Calbiochem, La Jolla, Ca. U.S.A. [^{3}H]-NA ((±)-[7-^{3}H]-noradrenaline hydrochloride) 10 Ci/mmol was obtained from the Radiochemical Centre, Amersham.

Statistical analysis of results

Student's *t* test was used to test for significant differences in results. Figures given represent means ± s.e. means.

Results

Effects of hydralazine on vasoconstrictor responses to transmural stimulation (Figure 1)

To discover whether Hyd modifies the vasoconstrictor responses induced by field stimulation by a pre-

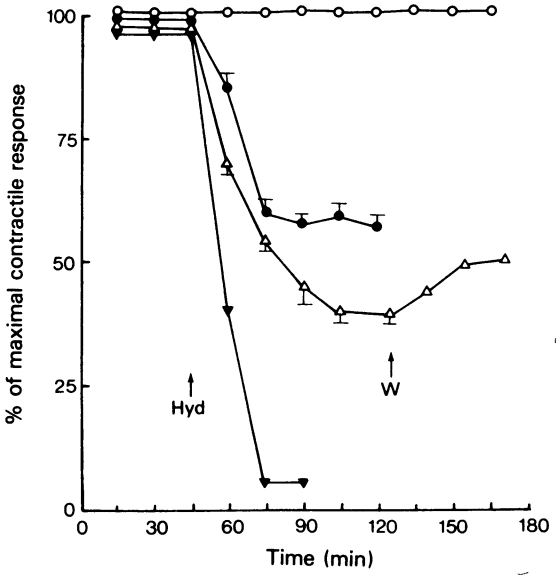

Figure 1 Effect of hydralazine on contractile responses induced by electrical transmural stimulation (duration: 2 ms, frequency: 10 Hz, supra-maximal voltage, for 40 s at 15 min intervals) in the isolated proximal segment of the rat tail artery. Hydralazine: (○) 30 nM; (●) 0.3 μM; (△) 3 μM and (▲) 30 μM. Responses were obtained from 3 to 8 preparations and calculated as a mean % inhibition for each point. Vertical lines show s.e. means. Hydralazine infusion was started at 45 min (Hyd); the drug was washed out (W) at 120 min for the concentration of 3 μM hydralazine.

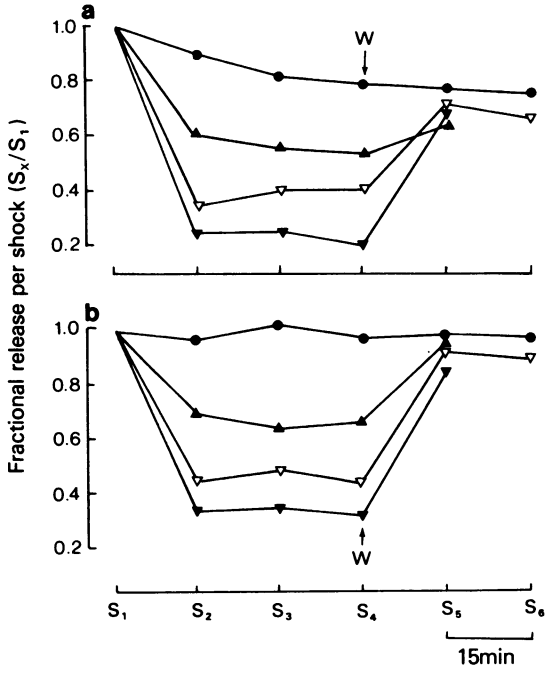

Figure 2 Effects of adding hydralazine to hydralazine-free physiological salt solution on tritium efflux induced by electrical stimulation (10 Hz, 2 ms during 40 s with supramaximal voltage at 15 min intervals) of proximal (a) and distal (b) segments of rat tail artery prelabelled with tritiated noradrenaline. SX/S1 indicates the ratio between the fractional release of ^3H per shock during a given period of stimulation (SX) and the first period (S1). Hydralazine was added to the medium 5 min before S2 then washed out 5 min before S6 (W). Note that hydralazine induced a decrease of ^3H release which appeared within 15 min, was maintained at the same level as long as the hydralazine infusion was maintained and was reversible by washing. (●) Hydralazine-free medium; hydralazine: (▲) 30 nM; (▽) 0.3 μM and (▼) 3 μM.

synaptic effect, we chose to study the action of this drug on the proximal segments of the tail artery. Indeed we had observed (Worcel, 1978) that the contractile responses induced by phenylephrine, 5-hydroxytryptamine or vasopressin in proximal segments of innervated arteries from normotensive Wistar rats was almost unaltered by concentrations of Hyd as high as 1 μM. However, the contractions induced by endogenous NA released during field stimulation were reduced in size by concentrations of Hyd of 0.3 and 3 μM (Figure 1). The maximal inhibition of the contractile response occurred after 30 to 45 min of Hyd exposure. After washout of the drug, the inhibition of the contractile responses was slowly reversed (see the effect of 3 μM Hyd). It is important to note that with the higher concentration of Hyd (30 μM), the inhibition of the contractile response induced by field stimulation was practically complete. This result differs from the inhibition by Hyd of the contractile effects of exogenous agonists which were reduced by 40%. The present result seems to indicate that Hyd may exert part of its action at a presynaptic site. Nevertheless the interpretation of these results is obscured by the possibility of a change in the postsynaptic response to Hyd due to the simultaneous reduction of NA and ATP release that could be expected under these circumstances.

Effect of hydralazine on stimulation-induced tritium efflux

In order to confirm the existence of a presynaptic effect of Hyd, we studied the actions of the drug on tritium released during field stimulation of arteries prelabelled with [^3H]-NA. The actual experiments were started (S1) at a time in which the fall in the

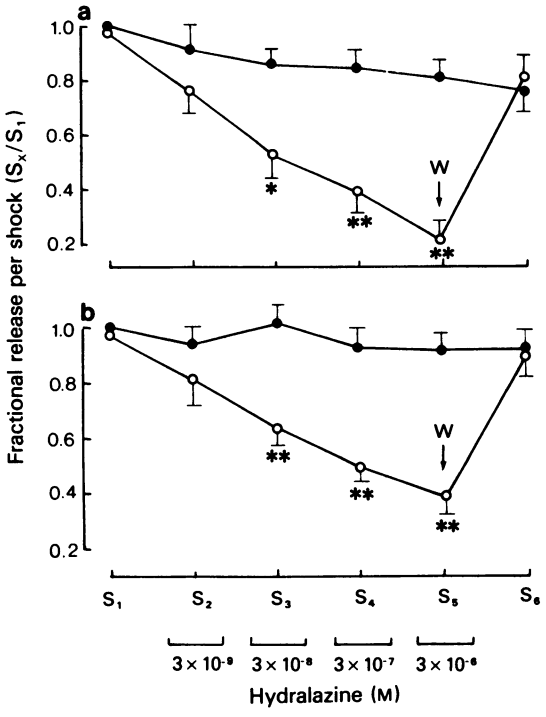

Figure 3 Cumulative dose-response curves of the effect of hydralazine on tritium release elicited by transmural stimulation of proximal (a) and distal (b) segments of the rat tail artery prelabelled with tritiated noradrenaline. S1 to S6 indicate the periods of electrical stimulation (10 Hz, 2 ms, during 40 s with supramaximal voltage at 15 min intervals). SX/S1 indicates the ratio between the fractional release of ^3H per shock during a given period of stimulation (SX) and the first period (S1). Hydralazine was added to the perfusion medium 5 min before S2 at the concentration indicated; the concentration of the drug was increased 5 min before each subsequent period of stimulation. Note that hydralazine induced a concentration-dependent decrease in tritium release, which was reversible by washing (W). (●) Controls; (○) hydralazine. Mean values of 6 or 7 experiments are shown. Vertical lines show s.e. means. * $P < 0.01$; ** $P < 0.001$.

fractional release per shock was either stabilized (distal segments) or considerably reduced (proximal segments).

In control experiments with the distal segment of the tail artery, SX/S1 was sustained from S2 to S6, whereas this value decreased progressively in the proximal segment (Figures 2 and 3). Hyd caused an inhibition of the S-I tritium efflux, which occurred rapidly, reaching a maximal value for a given concentration of the drug after only 5 min of Hyd superfusion, and was sustained as long as the Hyd infusion was maintained (Figure 2). It can be seen that the reduction of fractional release induced by Hyd is dose-dependent. This effect has been confirmed during the cumulative addition of the drug (Figure 3). In both cases, after washout of Hyd, SX/S1 returned within 15 min to control responses.

Theophylline and hydralazine interactions on stimulation-induced tritium efflux

It has been shown previously that in the presence of Theo 0.5 mM, phenylephrine-induced responses of innervated proximal segments of the tail artery obtained from normotensive Wistar (NW) rats are inhibited by Hyd. This potentiation of the postjunctional response to Hyd, observed in the presence of Theo contrasts with the poor response usually obtained using NW proximal segments (Worcel, 1978; Worcel *et al.*, 1980). Conversely, Theo appears to be ineffective presynaptically since 0.5 mM did not alter the inhibitory effect of Hyd on the S-I tritium efflux from the rat tail artery (Figure 4).

Discussion

The present investigation shows that Hyd causes a decrease in the S-I release of NA from sympathetic nerve endings of the rat tail artery. This assumption is substantiated by two pieces of evidence: (1) Hyd decreased the S-I tritium efflux from the proximal and distal segments of tail arteries prelabelled with tritiated NA; (2) Hyd produced depression of vasoconstrictor responses to transmural stimulation of the tail artery excised from normotensive rats. These innervated proximal segments were practically unresponsive to Hyd when contracted by phenylephrine (Worcel, 1978).

Nevertheless, there are numerous qualitative and quantitative differences between the results of both series of experiments: (a) Hyd 30 nM, which produced a significant inhibition of the S-I tritium efflux, was without effect on contractile response due to transmural stimulation. It seems that the decrease of NA overflow was possibly insufficient to depress the mechanical response. A similar threshold difference was observed for the presynaptic inhibitory action of dopamine in the rabbit ear artery (Hope, Law, McCulloch, Rand & Story, 1976) and the rabbit heart (Fuder & Muscholl, 1978). (b) On the other hand, the maximal inhibition by Hyd of the S-I tritium efflux occurred rapidly (within 5 min) whereas more than 30 min were necessary to achieve maximal inhibition of the mechanical response. (c) The Hyd-induced inhibition of [^3H]-NA efflux was rapidly reversed by washout of the drug, whilst the inhibition of the contractile

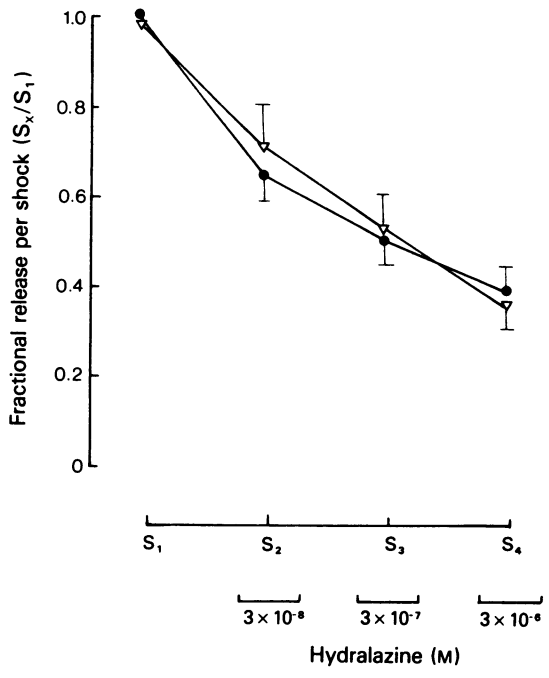

Figure 4 Cumulative dose-response curves to hydralazine in presence of theophylline 0.5 mM. S1 to S4 indicate the periods of electrical stimulation (10 Hz, 2 ms during 40 s with supramaximal voltage at 15 min intervals) of the rat tail artery prelabelled with tritiated noradrenaline. Hydralazine was added to the perfusion medium 5 min before S2 at the concentration indicated; the concentration of the drug was increased 5 min before each subsequent period of stimulation. Theophylline 0.5 mM was added to the perfusion medium at the start of the experiment. SX/S1 indicates the ratio between the fractional release per shock during a given period of stimulation (SX) and the first period (S1). (●) Cumulative dose-response curve to hydralazine; (▽) cumulative dose-response curve to hydralazine in the presence of theophylline. Mean values of 5 to 7 experiments are shown. Vertical lines show s.e. means.

response was not easily reversed. This fact is not surprising since the inhibition by Hyd of the S-I muscular contraction is a mixed phenomenon, in which a presynaptic inhibition occurs (the present work) as well as a postsynaptic inhibition, which was previously shown to be very difficult to reverse following the washout of the drug (Worcel, 1978).

The present results are unexpected considering our working hypothesis: in fact, since ATP and some other purines antagonized the postsynaptic action of Hyd (Worcel, 1978; Worcel *et al.*, 1980) and since ATP was shown to inhibit the S-I release of NA from sympathetic nerves (Verhaeghe *et al.*, 1976; Enero & Saïdman, 1977; Su, 1978), it could be expected that Hyd might be able to enhance the release of the neurotransmitter. Nevertheless, our results give evidence for an inhibitory effect of Hyd on the liberation of NA evoked by sympathetic stimulation.

Hyd does not appear to interact presynaptically with a purine receptor site, since the inhibition of NA release induced by Hyd was not altered by Theo, suggesting that at this prejunctional site, Hyd does not interfere with the presynaptic inhibitory purinergic feed-back of NA release. Furthermore, this result seems to exclude the possibility that Hyd might interfere with the reuptake of purines in nerve terminals, as dipyridamole does (Stafford, 1966).

Data available concerning the effects of Hyd on neural cardiovascular centres and sympathetic efferent pathways is scanty. A possible central mechanism of action seems unlikely since central administration of the drug caused a similar hypotensive response to an intravenous injection (Reis & Van Zwieten, 1967). Hyd was observed to produce ganglionic blockade by acting at a non-muscarinic site (Gomer & Hilton, 1978). At the level of the postganglionic sympathetic nerves, Hyd caused an early depletion of catecholamine concentration in several organs (Bydgeman & Stjarne, 1959; 1960; Linet, Van Zwieten & Hertting, 1969) which had been attributed to a reflexly induced overactivity of the sympathetic nerves due to the fall in blood pressure. On the other hand, Hyd did not induce any alteration of tyrosine-hydroxylase activity in vessels (Kohler, Berkowitz & Spector, 1975) but caused an inhibition of dopa-decarboxylase (Werle, Schauer & Hartung, 1955; Sano, Taniguchi, Gamo, Takesada & Kakimoto, 1960) and dopamine β-hydroxylase activity (Liu, Shen & Loken, 1974). Nevertheless, the prejunctional negative effect of Hyd inhibiting NA release which is suggested by the present work is probably independent of the inhibition of NA synthesis, since this phenomenon occurs rapidly and was easily reversed.

In conclusion, the present results indicate that Hyd has, in addition to its action on vascular smooth muscle, a very marked effect on sympathetic nerve terminals. The mechanism of this presynaptic inhibition appears to be different from the postsynaptic effect, in view of the much shorter delay, the shape of the dose-effect curve and the lack of interaction with Theo. More work is necessary to reveal the nature of this prejunctional action and its significance for the overall antihypertensive effect of Hyd.

This investigation was supported by grants from the INSERM, DGRST and CLIN-MIDY Laboratories, France. The authors would like to thank Ms Francesca Da Ponte for expert technical assistance.

References

BYDGEMAN, M. & STJÄRNE, L. (1959). Effects of 1,4-dihydralazinophtalazine in organ content and adrenal medullary secretion of catecholamines. *Nature, Lond.*, **184**, 1646–1647.

BYDGEMAN, M. & STJÄRNE, L. (1960) Effects of 1,4-dihydrazinophtalazine on the catecholamines. *Nature, Lond.*, **186**, 82–83.

DE POTTER, W.P. (1971). Noradrenaline storage particles in the splenic nerve. *Phil. Trans. R. Soc.*, B **261**, 313–317.

ENERO, M.A. & SAÏDMAN, B.Q. (1977). Possible feed-back inhibition of noradrenaline release by purine compounds. *Naunyn-Schmiedebergs Arch. Pharmac.*, **297**, 39–46.

FUDER, H. & MUSCHOLL, E. (1978). The effect of dopamine on the overflow of endogenous noradrenaline from the perfused rabbit heart evoked by sympathetic nerve stimulation. *Naunyn-Schmiedebergs Arch. Pharmac.*, **305**, 109–115.

GOMER, S.K. & HILTON, J.G. (1978). Effects of hydralazine on canine muscarinic ganglion stimulation. *Eur. J. Pharmac.*, **48**, 143–150.

GROSS, F. (1977). Drugs acting on arteriolar smooth muscle (vasodilator drugs), II. Hydralazines. In *Anti-hypertensive Agents*. ed. Gross, F. pp. 399–418. Berlin Heidelberg New York: Springer-Verlag.

HOPE, W., LAW, M., MCCULLOCH, M.W., RAND, M.J. & STORY, D.F. (1976). Effects of some catecholamines on noradrenergic transmission in rabbit ear arteries. *Clin. exp. Pharmac. Physiol.*, **3**, 15–28.

KOHLER, C., BERKOWITZ, B.A. & SPECTOR, S. (1975). Antihypertensive drugs and catecholamine metabolism: Effects of reserpine and hydralazine on tyrosine hydroxylase activity and norepinephrine concentrations in the spontaneously hypertensive rat. *J. Pharmac. exp. Ther.*, **193**, 443–451.

LINET, O., VAN ZWIETEN, P.A. & HERTTING, G. (1969) Effect of hydrazinophtalazines on catecholamines in rats. *Eur. J. Pharmac.*, **6**, 121–124.

LIU, T.Z., SHEN, J.T. & LOKEN, H.F. (1974). Inhibition of dopamine-β-hydroxylase by hydralazine. *Proc. Soc. exp. Biol.*, **145**, 294–297.

REIS, M.E. & VAN ZWIETEN, P.A. (1967). Hypotensive effect of hydralazine injected into the vertebral artery of the cat. *Archs int. Pharmacodyn* **169**, 494–499.

RIBEIRO, J.A. (1978). ATP; related nucleotides and adenosine on neurotransmission. *Life Sci., Oxford*, **22**, 1373–1380.

SANO, J., TANIGUCHI, K., GAMO, T., TAKESADA, M. & KAKIMOTO, Y. (1960). Die Katechinamine im Zentralnervensystem. *Klin. Vschr.*, **38**, 57–62.

SU, C. (1978). Purinergic inhibition of adrenergic transmission in rabbit blood vessels. *J. Pharmac. exp. Ther.*, **204**, 351–361.

STAFFORD, A. (1966). Potentiation of adenosine and the adenine nucleotides by dipyridamole. *Br. J. Pharmac. Chemother.*, **28**, 218–227.

VERHAEGHE, R.H., VANHOUTTE, P.M. & SHEPHERD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circulation Res.*, **40**, 208–215.

WERLE, E., SCHAUER, A. & HARTUNG, G. (1955). Einfluß von Hydrazin- und Guanylhydrazinderivaten auf die Aktivität der Monaminoxydase, Diaminoxydase, Dopadecarboxylase und Histidindecarboxylase. *Klin. Wschr.*, **33**, 562–567.

WORCEL, M. (1978). Relationship between the direct inhibitory effects of hydralazine and propildazine on arterial smooth muscle contractility and sympathetic innervation. *J. Pharmac. exp. Ther.*, **207**, 320–330.

WORCEL, M., SAIAG, B. & CHEVILLARD, C. (1980). An unexpected mode of action for hydralazine. *Trends in Pharmac. Sci.*, **1**, 136–139.

(Received May 15, 1979.
Revised September 21, 1979.)