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INTERACTIONS BETWEEN HYDRALAZINE, PROPILDAZINE AND PURINES ON ARTERIAL SMOOTH MUSCLE

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1 The interaction of hydralazine (Hyd) and propildazine (Pyd) with purine compounds was studied in the isolated tail artery from normotensive Wistar (NW) rats.

2 Exogenously added purines inhibit non competitively the antispasmogenic response to Hyd in denervated NW segments. The order of potency is 2-Cl-adenosine > adenosine > adenosine 5′-triphosphate (ATP) > inosine. Pyd action is modified only by the most active purine 2-Cl-adenosine, which displaces the dose-response curves to the right. Hyd and Pyd seem to act on the same site, since their maximal effects are not additive.

3 Theophylline (Theo) 50 μM induces the appearance of the antispasmogenic effect of Hyd in the usually poorly responsive innervated proximal NW arterial segments. The potentiating action of Theo is identical to the enhancement of the Hyd response observed after 6-hydroxydopamine denervation. This result suggests that the release of endogenous purines from sympathetic nerves is sufficient to block the smooth muscle responses to Hyd, under our experimental conditions. A similar potentiating effect is obtained with propranolol (5 μM).

4 The spontaneous release of ^{3}H, after loading with [^{3}H]-noradrenaline, was considered as an indirect indication of purine leakage from nerve terminals. There is an inverse relationship between the rate of ^{3}H release, under these conditions, and the magnitude of the relaxant response to Hyd, i.e., ^{3}H leakage is higher in proximal NW segments.

5 The most satisfactory explanation for the interaction of Hyd and Pyd with exogenous purines, and for the modulating actions of sympathetic nerve terminals, is that both antihypertensives act on a common receptor, sensitive to endogenous ATP and adenosine.

Introduction

In a previous paper, it was shown that hydralazine (Hyd) and a structural analogue, propildazine (Pyd), have an *in vitro* antispasmogenic action on arterial smooth muscle. In arteries from normotensive Wistar rats (NW) this action of Hyd, in contrast to that of Pyd, appears to be modulated by the sympathetic nerve terminals left in the tissue (Worcel, 1978). It seemed possible, from these experiments, that a molecule released from nerve terminals was involved in the modulation. Adenosine 5′-triphosphate (ATP) is a normal component of noradrenergic nerve storage vesicles (De Potter, 1971) and is released from sympathetic terminals during nerve activity (Su, 1975). ATP or adenosine might act as a co-transmitter, in association with noradrenaline, both at pre- and postsynaptic sites (Langer & Pinto, 1976; Verhaeghe, Vanhoutte & Shepherd, 1977). Furthermore, ATP at a concentration of 0.1 mM, is able to suppress completely the antispasmogenic effect of Hyd (Worcel, 1978). In the present work we have studied further the importance of sympathetic nerves on the modulation of the smooth muscle responses to Hyd and Pyd. The results obtained suggest strongly that both molecules act on a smooth muscle receptor, sensitive to exogenous and endogenous purines.

Methods

Pharmacological experiments

The rat tail artery was excised from either male normotensive Wistar (NW) or spontaneously hypertensive rats of the Okamoto strain (SHR) 20 weeks old, obtained from IFFA CREDO, France. The method used was described in detail in a previous publication (Worcel, 1978). In summary, the arteries were perfused at a constant flow of 75 μl/min. Variations in perfusion pressure were measured with a strain gauge transducer. The arteries were regularly contracted every 15 min by the addition of phenylephrine (Phe), 5 μM. All drugs were added to the bath. In some experiments a chemical sympathectomy by the application of 6-hydroxydopamine (6-

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OHDA) *in vitro*, was performed using the method described by Aprigliano & Hermsmeyer (1976) and Aprigliano, Rybarczyk, Hermsmeyer & Van Orden (1976). The effectiveness of the sympathectomy was assessed by the disappearance of the contractile response to field stimulation of the artery at a supramaximal voltage, with 1 ms square pulses, at 10 Hz frequency, for 30 s.

Measurement of noradrenaline (NA) concentration in arteries

The tail arteries were obtained from NW and SHR. After excision, the segments of the arteries were blotted lightly, and weighed. NA was extracted from the tissue, by use of a glass homogenizer, in 2 ml of 0.2 M perchloric acid containing 0.1% disodium edetate (EDTA) and 0.125% sodium sulphite. The homogenate was centrifuged at 1000 *g*. The NA concentration of 50 μl aliquots of the supernatant was measured by a radioenzymatic assay (Gauchy, Tassin, Glowinski & Cheramy, 1976). The method involves the 3 *O*-methylation of NA, in the presence of catechol-*O*-methyl transferase (COMT) and of the radioactive methyl donor, S [^{3}H-methyl]-adenosyl methionine. The normetadrenaline obtained was extracted into organic solvents, then converted to tritiated vanillin by mild periodic oxidation. Finally, vanillin was assayed by liquid scintillation counting. Quantitative results were obtained by comparison with the radioactivity of 250 and 500 pg of NA processed by the method used. Each measurement was made in triplicate and expressed as ng NA/mg fresh tissue.

Estimation of the spontaneous noradrenaline release from tail arteries

Proximal and distal segments of tail arteries from NW and SHR rats were incubated at 37°C for 5 min in 5 ml of physiological saline solution (PSS). Tritiated NA (10 μCi/ml) was added to the medium and the incubation continued for 1 h. After removing from the bath, the arteries were continuously superfused with PSS by the method of Chevillard, Mathieu, Saiag & Worcel (1980); the effluent was collected over 10 min periods. At the end of the experiment, the arteries were homogenized as described above. The tritium remaining in the tissue and released in each fraction was estimated by liquid scintillation counting of the aliquots. Spontaneous ^{3}H output was calculated as a percentage from the ratio between the cumulative ^{3}H released over a given period and the radioactivity present in the tissue at the beginning of the superfusion.

Drugs used

All substances were reagent grade: (−)-phenylephrine HCl, adenosine, inosine, the disodium salt of adenosine 5′-triphosphate, 2 chloro-adenosine, theophylline, glutathione (reduced form), 6-hydroxydopamine HCl and hydralazine were from Sigma Chemical Co, USA; (±)-propranolol was from ICI; propildazine was from Clin-Midy Laboratories, France (originally made by Ital Seber, Italy); (−)-noradrenaline HCl was from Calbiochem, USA; (±)-[7-^{3}H]-noradrenaline hydrochloride 10 Ci/mmol and 5-adenosyl-L-[methyl-^{3}H]-methionine, 15 Ci/mmol were from the Radiochemical Centre, Amersham.

Statistical analysis

The values are expressed as means ± s.e. mean. The statistical significance was calculated by Student's *t* test.

Results

Effect of exogenous purines on the arterial smooth muscle response to hydralazine and propildazine

There is a quantitative variation in the response to Hyd from different length sections of tail arteries from NW rats which disappears completely after *in vitro* denervation with 6-OHDA (Worcel, 1978). Consequently, all experiments described in this section were performed on either proximal or distal

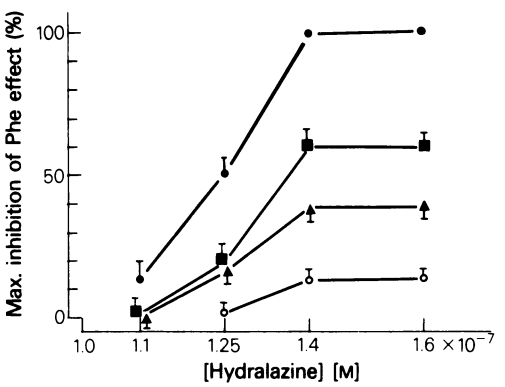

Figure 1 Antagonism of hydralazine (Hyd) action by ATP. Proximal or distal segments of the tail artery from NW rats were denervated with 6-hydroxydopamine. Dose-response curves to Hyd using (−)-phenylephrine (Phe) as a spasmogen were performed in the absence or presence of various concentrations of ATP: (■) 10^{-6} M; (▲) 10^{-5} M; (○) 5×10^{-5} M. The control curve (●) is the mean of 9 experiments. The bars represent s.e. mean. The ATP curves represent the mean of 3 experiments.

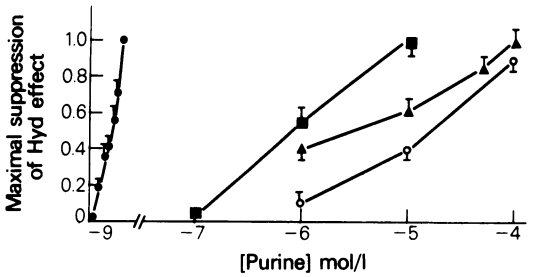

Figure 2 Effects of various purines on the maximal anti-spasmogenic action of hydralazine (Hyd) against phenylephrine on denervated arterial segments.

The compounds tested inhibited Hyd effects non-competitively. Ordinates represent the ratio of the reduction of the maximal response to Hyd. Each curve is the average of 3 experiments: (●) 2-Cl-adenosine; (■) adenosine; (▲) ATP and (○) inosine.

denervated segments of the tail artery, which under these conditions become identically responsive to Hyd. The previous observations that ATP (0.1 mM) completely suppressed the antispasmogenic responses to Hyd (Worcel, 1978) were extended to establish the complete dose-effect curve of the ATP-Hyd interaction. ATP inhibits in a non-competitive manner the antispasmogenic action of Hyd (Figure 1) so that the overall effect is a return of the contractile responses to Phe. Similarly, non-competitive effects were obtained with other purines and the most active

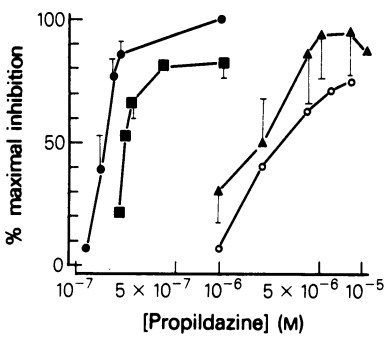

Figure 3 Antagonism of propildazine (Pyd) action by 2-Cl-adenosine. Denervated proximal and distal segments of the tail artery were obtained from NW rats. Dose-response curves to Pyd using (−)-phenylephrine (Phe) as a spasmogen were obtained in the presence or absence of 2-Cl-adenosine. The control curve is the mean of 5 to 7 experiments. The 2-Cl-adenosine curves represent 3 to 6 experiments. The s.e. mean is calculated and represented when $n > 4$. The control curve is normalized on the basis of the maximal anti-spasmogenic response. Subsequent curves (Pyd) are expressed as a percentage of the maximal control response. Control Pyd response (●); 2-Cl-adenosine 1.3 nM (■); 2-Cl-adenosine 1.6 nM (▲); 2-Cl-adenosine 2 nM (○).

compound in this respect is the non-metabolisable purine 2-Cl-adenosine, followed by adenosine > ATP > inosine (Figure 2). Confirming previous observations, the antispasmogenic response to Pyd is not affected by exposing the artery to concentrations of ATP as high as 0.1 mM. A similar absence of suppressive effects is observed in the presence of 0.1 mM adenosine. Only 2-Cl-adenosine is able to modify the action of Pyd on the rat tail artery. In contrast to a reduction of the maximal effect observed in the interaction between Hyd and the purines, the dose-response curve to Pyd appears to be displaced to the right by 2-Cl-adenosine without any suppression of the maximum (Figure 3). However, this interaction is not competitive since the slope of the Schild plot (4.3) is much higher than 1.

Interactions between hydralazine and propildazine

Such experiments show that the exogenous purines have different quantitiative actions but affect in a similar manner the responses to both Hyd and Pyd. This may indicate that both antihypertensive molecules act on the same receptor site. To investigate this possibility, experiments were performed by adding either drug at 1 μM once the maximal anti-spasmogenic effect for one had been attained. Neither of the antihypertensive drugs potentiated or modified the antispasmogenic action of the other, so that the contractile response to Phe 5 μM, already reduced to 40 to 60% of the control response, was not diminished further. Hyd and Pyd do not have mutually additive effects, suggesting that both drugs have the same site of action on the vascular smooth muscle.

Possible interaction between endogenous purines and hydralazine

The difference in the response to Hyd observed in proximal and distal tail artery segments obtained from NW rats might be due to a corresponding difference in the release of ATP (and NA) from the sympathetic nerve terminals (Worcel, 1978). The response to Hyd of the proximal segment from NW arteries might be due to a greater release of ATP from the proximal than from the distal sections of the artery. The inhibition by exogenous purines of the antispasmogenic effects of Hyd and Pyd, as well as their greater potency against Hyd compared to Pyd seems to fit with this proposition. Therefore, it is necessary to determine whether sufficient endogenous purines are released from the sympathetic nerve terminals under our experimental conditions. Two different pharmacological approaches have been used to examine this possibility. In all the following experiments the innervated proximal segments of the NW arteries were used, which are poorly responsive to Hyd.

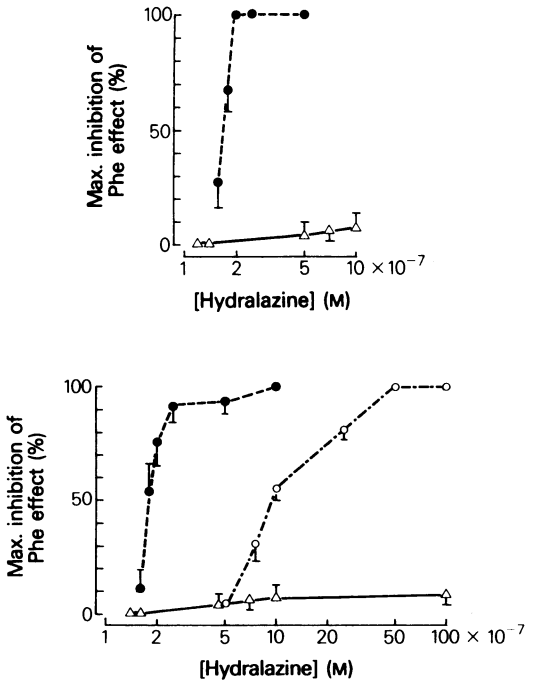

Figure 4 (a) Theophylline potentiation of hydralazine (Hyd) effects in innervated proximal tail artery segments of NW rats. The experiments were performed in the presence and in the absence of theophylline 50 μM. Theophylline curves represent the mean of 4 experiments (●). Control curves (△) are the mean of 12 experiments. (b) (±)-Propranolol potentiation of Hyd action on innervated proximal tail artery segments of NW rats. Propranolol curves represent the mean of 4 experiments: (●) propranolol 5×10^{-6} M; (○) 10^{-6} M. Control curves (△) is the mean of 12 experiments.

Effects of theophylline (Theo) It has been demonstrated that Theo at low concentrations may be a specific antagonist of purines on certain receptors (Burnstock, 1978; Fredholm, 1980). Theo at a 50 μM concentration, which does not relax the rat tail artery, potentiates the relaxant responses to Hyd (Figure 4). Under these conditions the proximal NW arterial segments, otherwise very poorly responsive to Hyd, become as responsive as the denervated segments (Figure 1).

Noradrenaline levels and release in tail arteries from NW and SHR rats In a previous paper it was found that the gradient in the response to Hyd, observed in NW arteries, does not exist in SHR arteries. The *in vitro* denervation of the vessels, obtained by treatment with 6-OHDA, eliminated the gradient of Hyd response in NW arteries, without having any effect on the Hyd responses of SHR arteries (Worcel, 1978). On the basis of this evidence, it was suggested that the

Table 1 Noradrenaline content in proximal and distal segments of NW rats and SHR rat tail arteries (ng/mg wet wt).

NW proximal	4.58 ± 0.38	
NW distal	2.71 ± 0.31	
		$n = 8$
SHR proximal	7.20 ± 0.34	
SHR distal	4.48 ± 0.33	

Values are expressed as mean ± s.e. mean. A significant difference ($P < 0.001$) was observed between proximal and distal segments of NW or SHR arteries. The difference between the mean values of proximal NW vs proximal SHR, as well as distal NW vs distal SHR, was significant at the $P < 0.001$ level.

poor relaxant response to Hyd observed in proximal segments of NW arteries may be due to a high spontaneous release of mediators (ATP + NA) from nerve terminals. Unfortunately it has not been technically possible to measure ATP content in nerve terminals separately from the total content of purines in the whole artery. Furthermore, the amount of ATP released during field stimulation, or spontaneously, is too low to be detectable by present techniques.

A possible way to estimate indirectly the output of ATP in nerve terminals is to study NA content and release. NA content appears to be higher in proximal than in distal segments from both NW and SHR, the levels of the amine being otherwise higher in the Okamoto rats (Table 1). The spontaneous release of tritium from arteries preloaded with [^{3}H]-NA was found to be 60% higher from proximal than from distal NW segments. In contrast, there is no difference in the spontaneous ^{3}H release between proximal and distal segments from SHR, which appears in both cases to be as low as in distal NW sections (Figure 5).

Effects of propranolol on the smooth muscle response to hydralazine In preliminary experiments, it was observed that propranolol 5 μM was able to suppress the contractile responses of proximal NW segments to field stimulation (1 min, 10 Hz, 1 ms square pulses, supramaximal voltage), perhaps by blocking completely the release of mediator NA, ATP and other vesicular contents. If propranolol exerted the same effect on non stimulated nerve terminals, it might potentiate Hyd relaxation in innervated tail arteries, by a reduction in the release of endogenous NA and ATP.

The action of Hyd was studied in the presence of two concentrations of propranolol using the innervated proximal segment of the rat tail artery from NW. The control relaxant response to Hyd was, as usual, very small but in the presence of propranolol the proximal segments became responsive to Hyd.

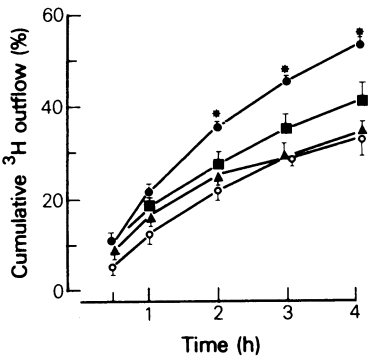

Figure 5 Spontaneous ^{3}H outflow from innervated arterial segments, preloaded with [^{3}H]-noradrenaline (see text): proximal NW (●); distal NW (○); proximal SHR (□); distal SHR (▲). The curves represent 9 experiments. The efflux from NW proximal segments is significantly different from all others, at 2 h and after: * $P < 0.01$.

Propranolol 5 μM, which depresses the contraction produced by field stimulation (see above), induced the appearance of a response to Hyd (Figure 4b), which was identical to that observed with proximal segments denervated by 6-OHDA (Worcel, 1978) or treated with Theo 50 μM (Figure 4a).

Discussion

The results presented in this paper substantiate our working hypothesis that the tail artery smooth muscle response to Hyd and Pyd is modulated by a substance released from the sympathetic nerve terminals.

The modulation appears to be the result of an interaction at the level of the specific receptor for Hyd and Pyd. The existence of this site, which we will call tentatively the 'Hyd receptor', is suggested by the following pharmacological data: (1) Hyd and Pyd act in a dose-dependent manner, the action of both drugs being limited by a maximal inhibitory response. Under these conditions there is no paralysis of the smooth muscle but merely a reduction of the spasmogenic effect. The relaxation induced by both Hyd and Pyd never surpasses 60% of the initial contractile response, suggesting that their effects are restricted by the availability of free receptor sites. (2) The absence of additivity between Hyd and Pyd suggests again that there is a receptor site, shared by both compounds. If the 'Hyd receptor' exists, which is its natural mediator? Both direct and indirect evidence presented in this paper indicate that either ATP or adenosine may be the transmitter involved in this interaction.

The experiments with exogenous purines give a partial answer to the question. The relaxant response to Hyd is either diminished or suppressed in a dose-dependent manner by ATP, adenosine and inosine, the more active exogenous purine being the non-metabolizable 2-Cl-adenosine. It must be stressed that the result of the interaction between Hyd or Pyd and purines, is the restoration of the contractile response to Phe. The effects of the purines cannot be explained by a direct action on the vascular smooth muscle. Indeed, it has not been possible to obtain any contractile or relaxant effect on the rat tail artery with ATP, adenosine or 2-Cl-adenosine. In consequence it seems difficult to interpret the effect of exogenous purines on Hyd and Pyd relaxation, other than by a modulation of the effects of the antihypertensive drugs at the level of a 'Hyd receptor' in smooth muscle cells (these experiments were performed on 6-OHDA denervated preparations). The affinity of the 'Hyd receptor' appears to be higher for Pyd than for Hyd itself. The antagonism between 2-Cl-adenosine and both antihypertensives may be different, since the Hyd maximal response is reduced but Pyd curves are displaced to the right. Nonetheless, the interaction between Pyd and 2-Cl-adenosine is not competitive, as revealed by the very high slope of the Schild plot. The different shape of the curves could be explained by a much higher efficiency (Stevenson, 1956) of Pyd action on the 'Hyd receptor'.

A different analysis of the experiments involving exogenous purines, Hyd and Pyd could be that the antihypertensive drugs bind *in vitro* to ATP, adenosine, inosine and 2-Cl-adenosine. This interpretation is not tenable since the antagonism should be 'competitive' and this is not the case for the interaction between Hyd and purines. Furthermore, the stochiometry of the suppressive action of purines on Hyd and Pyd relaxant effects precludes an *in vitro* interaction: 2-Cl-adenosine 2 nM completely block the action of Hyd 10 μM; each molecule of 2-Cl-adenosine would have to bind 5000 molecules of Hyd!

Are there sufficient endogenous purines released from the nerve terminals to explain a modulation of Hyd (and Pyd) effects on the smooth muscle 'Hyd receptor'? The best way to answer this question would have been to correlate the patterns of response to Hyd with measurements of ATP content of the nerve terminals, as well as with the purine release from nerves in the tail artery. Unfortunately, it is impossible to explore either parameter at the moment because of technical difficulties and indirect approaches had to be used.

It is generally accepted that NA is stored in the nerve vesicles bound to ATP (De Potter, 1971). The NA : ATP molar ratio measured in synaptosomes by different authors varies considerably (from 4 to 20), depending on the organ studied and the purity of the preparation (Blashke, 1979). Nonetheless, in any given preparation, whatever the ratio is, the NA content may reflect that ATP vesicular content. It has

been suggested (Worcel, 1978) that the existence of a gradient of response to Hyd in tail arteries from NW, could result from a concomitant gradient of content and/or release of purines. Conversely the absence of a gradient in response to Hyd in arteries from SHR, could reflect a lower content and/or release of purines and NA. Consequently, the NA content and the spontaneous ^3H release, after [^3H]-NA loading, have been used as indirect markers of sympathetic purine turnover.

The results obtained show that the magnitude of the Hyd response of the different segments from NW and SHR tail arteries is correlated with the relative size of the spontaneous ^3H release, after [^3H]-NA loading. If the magnitude of spontaneous ^3H efflux associated with the release of NA and its radioactive metabolites is a sign of purine leakage, our results would indicate that the size of the Hyd response may be modulated by endogenous purine release of nerve origin. However, the existence of a simultaneous release of NA and ATP, either spontaneous or stimulus induced, is not universally accepted. Luchelli-Fortis, Fredholm & Langer (1979) have shown that the radioactivity released by field stimulation of cat nictitating membrane, after tritiated adenine loading, comes mainly from the smooth muscle, the amount leaked from the nerve terminals being non measurable. Blashke (1979) has similarly shown that in the same preparation, there is no leak of ATP associated with the stimulus-induced release of NA. Nevertheless, Su (1975), and Westfall, Stitzel & Rose (1979) have obtained different results in blood vessels: they were able to show that ^3H is released from nerve terminals during field stimulation, following ^3H adenosine loading. Similarly, we have observed (unpublished results) a field stimulation-induced outflow of ^3H, after [^3H]-adenosine loading of NW innervated proximal segments. The radioactivity appears to come out from nerve terminals, since the contractions induced by Phe are not associated with an increased outflow. This finding indicates that, under our experimental conditions, there may be a joint efflux of catecholamines and purines during field stimulation. Further indirect experimental evidence for the modulating role of endogenous purines is provided by the experiments performed with Theo which at low concentrations acts as an antagonist of adenosine (Fredholm, 1980). This drug potentiates the relaxant response to Hyd in proximal segments from NW, otherwise poorly responsive. This result suggests again that under our experimental conditions, there may be enough ATP or adenosine released from the nerve terminals, to inhibit the smooth muscle response to the antihypertensive drug. It is interesting to analyse the interactions between Hyd and exogenous purines, as well as the potentiating action of Theo on the basis of a possible classification of purine receptors. According to Burnstock (1978), it could be possible to distinguish two types of purine receptors: (1) the P_1-receptor, preferentially stimulated by adenosine, blocked by Theo and associated with adenylate cyclase activation; and (2) the P_2-receptor, more sensitive to ATP than to adenosine and non specifically blocked by quinidine and 2,2'pyridilisatogen. The purine receptor involved in Hyd effects may be a P_1-receptor, since it is more sensitive to adenosine than to ATP, and blocked by Theo. It should be mentioned that in another preparation, the beef mesenteric artery, Hyd stimulates adenylate cyclase (Andersson, 1973).

The possible modulating role of the endogenous release of purines from nerves is stressed by the propranolol experiments. The effect of propranolol does not appear to be related to its β-blocking action, since atenolol, another β-blocker devoid of anaesthetic properties, does not enhance the Hyd effect on proximal innervated NW tail arteries (unpublished results). Whatever the mechanism of action of propranolol, it is important to stress the fact that the concentration of the blocker having a suppressant effect on field stimulation-induced contraction, has a potentiating effect similar to 6-OHDA denervation, or Theo pretreatment.

The most satisfactory single explanation for the modulation of Hyd effects by the sympathetic nerve terminals in NW rats; the 6-OHDA-, Theo- and propranolol-induced potentiation; the suppressive action of exogenous purines; the responsiveness of SHR arteries, and the effects of Pyd, is to suggest that Hyd and Pyd act on a smooth muscle receptor, sensitive to purines. This postjunctional action seems to be different from the prejunctional effects of Hyd, demonstrated in the same preparation (Chevillard *et al.*, 1980).

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References

ANDERSSON, R. (1973). Cyclic AMP as a mediator of the relaxing action of papaverine, nitroglycerine, diazoxide and hydralazine in intestinal and vascular smooth muscle. *Archs Pharmac. Tox.*, **32**, 321–336.

APRIGLIANO, O. & HERMSMEYER, K. (1976). In vitro denervation of the portal vein and caudal artery of the rat. *J. Pharmac. exp. Ther.*, **198**, 568–577.

APRIGLIANO, O., RYBARCZYK, K.E., HERMSMEYER, K. & VAN ORDEN, L.S. (1976). Adrenergic neuronal degeneration induced in portal vein and caudal artery by 6-hydroxydopamine in vitro. *J. Pharmac. exp. Ther.*, **198**, 578–588.

BLASHKE, E. (1979). Studies on sulphomucopolysaccharides and ATP in adrenergic neurons. *Acta physiol. scand.*, suppl. **466**, 1–42.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*, ed. Straub R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

CHEVILLARD, C., MATHIEU, M.N., SAIAG, B. & WORCEL, M. (1980). Hydralazine: effects on the outflow of noradrenaline and mechanical responses evoked by sympathetic nerve stimulation of the rat tail artery. *Br. J. Pharmac.*, **69**, 415–420.

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

FREDHOLM, B.B. (1980). Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends Pharmac. Sci.*, **1**, 129–132.

GAUCHY, C., TASSIN, J.P., GLOWINSKI, J. & CHERAMY, A. (1976). Isolation and radioenzymatic estimation of picogram quantities of dopamine and norepinephrine in biological samples. *J. Neurochem.*, **26**, 471–477.

LANGER, S.Z. & PINTO, J.E.B. (1976). Possible involvement of a transmitter different from norepinephrine in the residual responses to nerve stimulation of the cat nictitating membrane after pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **196**, 697–713.

LUCHELLI-FORTIS, M.A., FREDHOLM, B.B. & LANGER, S.Z. (1979). Release of radioactive purines from cat nictitating membrane labelled with ^{3}H adenine. *Eur. J. Pharmac.*, **58**, 389–397.

STEVENSON, R.P. (1956). A modification of receptor theory. *Br. J. Pharmac. Chemother.*, **11**, 379–393.

SU, C. (1975). Neurogenic release of purine compounds in blood vessels. *J. Pharmac. exp. Ther.*, **195**, 159–166.

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.

WESTFALL, D.P., STITZEL, R.E. & ROSE, J.N. (1979). The postjunctional effect and neural release of purine compounds in the guinea pig vas deferens. *Eur. J. Pharmac.*, **50**, 27–38.

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.

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