Hydralazine: mode of action at the neuroarterial junction

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Summary

1. Hydralazine relaxes the rat tail artery by a direct action on vascular smooth muscle cells, which appears to be modulated by the action of sympathetic nerve terminals.
2. There is a gradient of response to hydralazine in arteries from normotensive Wistar rats, the proximal segments being poorly responsive. This gradient disappears after denervation with 6-hydroxydopamine in vitro.
3. Exogenously added purines inhibit non-competitively the vasodilator response to hydralazine in denervated segments from normotensive Wistar rats. Their order of potency is 2-C1-adenosine > adenosine > ATP > inosine.
4. The effect of hydralazine in innervated, poorly responsive segments is greatly potentiated by theophylline (50 μmol/l) and propranolol (5 μmol/l). These results, together with the effect of denervation, suggest that there are endogenous purines leaking from the nerve terminals under our experimental conditions.
5. Hydralazine produces a marked inhibition of stimulus-induced contraction and [3H]release after [3H]noradrenaline loading. The mechanism of this prejunctional action appears to be different from the mechanism of the postjunctural effect.

Key words: arterial smooth muscle, hydralazine, 'hydrallazine receptors', postjunctional effects, prejunctional effects, purines.

Abbreviations: HYD, hydralazine; 6-OHDA, 6-hydroxydopamine.

Introduction

Hydralazine (HYD) relaxes the rat tail artery by a direct action on vascular smooth muscle cells (Worcel, 1978). Not all segments of the artery from normotensive male Wistar rats (NW rats) are equally sensitive to the drug. Indeed, there is a gradient of response to HYD in these arteries of NW rats, characterized by a very slight (or absent) relaxation in the proximal third of the artery, the effect of the drug being more marked towards the distal end of the vessel. After sympathectomy in vitro with 6-hydroxydopamine (6-OHDA), the proximal segments from NW rats become responsive to HYD. The gradient of response to the antihypertensive drug seems to be determined by the sympathetic nerve terminals remaining in the vessel after dissection. Under our experimental conditions it seemed possible to consider that this modulating effect could be exerted by a substance released from the nerves. Having supposed that either ATP or adenosine could be involved in this interaction, we have studied the effect of purines on the relaxant response to HYD.

Methods

Rat tail arteries were excised from NW rats and spontaneously hypertensive rats from the Okamoto strain purchased from IFFA CREDO, France.

The methods used for the study of the post- and pre-junctional effects have been published by Worcel (1978) and Chevillard, Mathieu, Saiag & Worcel (1980) respectively.

The noradrenaline concentration in arteries was measured as follows: after excision, the arterial segments were blotted lightly and weighed. Noradrenaline was extracted by homogenization of the tissue, in a glass Potter apparatus, in 2 ml of perchloric acid (0-2 mol/l) containing 0.1% disodium edetate and 0.125% sodium sulphite. The homogenate was centrifuged at 1000 g and the noradrenaline concentration was measured (in 50 μl aliquots of the supernatant) by a radioenzymatic assay (Gauchy, Tassin, Glowinski & Cheramy, 1976).
The spontaneous noradrenaline release was estimated by studying $^3$H outflow after loading of arterial segments with $[^3]$Hnoradrenaline. Segments of the tail artery were incubated during 1 h in the physiological salt solution containing 10 $\mu$Ci of $[^3]$Hnoradrenaline/ml (10 Ci/mmol).

Results

Postjunctional effects of HYD

HYD had a dose-dependent action on 6-OHDA-denervated proximal and distal segments from NW rats. The concentration–relaxation curve obtained was steep, with a threshold response at 0.15 $\mu$mol/l and a maximum at 1 $\mu$mol/l. Four different exogenous purines inhibited the relaxant effect of HYD, their order of potency being 2-CI-adenosine > adenosine > ATP > inosine.

Theophylline, a purine receptor antagonist (Fredholm, 1980), at a concentration of 50 $\mu$mol/l (a non-relaxant dose), greatly potentiated the effects of HYD on the usually poorly responsive, innervated proximal NW rat arteries. A similar potentiating effect was obtained with propranolol at 5 $\mu$mol/l. These results suggest that the amount of endogenous purines released in our experimental conditions from the sympathetic nerve endings may be enough to inhibit the HYD relaxant effect.

Unfortunately it has not been technically possible to measure directly this purine output. The spontaneous release of $^3$H, after loading with $[^3]$Hnoradrenaline, was considered as an indirect marker of purine leakage from nerve terminals (Worcel, Saiag & Chevillard, 1980). There was an inverse relationship between $^3$H release, under these conditions, and the magnitude of the relaxant response to HYD. Namely, $^3$H leakage was higher in proximal segments from NW rats.

On the contrary, there was no difference in the ratio of $^3$H outflow from proximal and distal arteries of spontaneously hypertensive rats, which appeared to be as low as in distal segments of NW rats. It must be recalled that arteries of spontaneously hypertensive rats do not show any gradient in the relaxant effect to HYD, all segments being equally responsive (Worcel, 1978). Furthermore, the noradrenaline content is higher in the proximal NW rat artery (unresponsive to HYD: 3.6 ± 0.3 ng/mg) than in the

![Fig. 1. Effects of hydrallazine on the neurovascular junction.](image-url)
distal NW rat segments: 2.3 ± 0.2 ng/mg; n = 8, P < 0.01.

Prejunctional effects of HYD

The effects of HYD on vasoconstrictor responses to field stimulation of sympathetic nerves have been studied in the innervated proximal segments of the rat tail artery. Vasoconstrictor responses to transmural stimulation were depressed in a concentration-dependent manner by 0.3, 3 and 30 μmol/l, the responses of phenylephrine-contracted proximal segments of NW rats being poorly relaxed by HYD. The same concentrations of HYD reduced the stimulus induced overflow of ^3^H from proximal and distal segments of the tail artery labelled with [^3^H]noradrenaline in a concentration-dependent manner with a threshold of about 30 nmol/l but a shallow dose-inhibition curve. Theophylline (0.5 mmol/l) did not affect the inhibitory action of HYD on the stimulation-induced ^3^H efflux of distal segments of tail arteries labelled with [^3^H]noradrenaline.

Discussion

We present in this paper a series of experimental results indicating that HYD has, at least in vitro, both pre- and post-junctional effects.

It has been suggested that HYD may act on a smooth muscle receptor, sensitive to endogenous purines released from the sympathetic nerve terminals remaining in the artery under our experimental conditions.

This seems to be the best unifying explanation for the influence of, (1) the sympathetic nerve terminals, (2) the potentiating action of 6-OHDA, theophylline and propranolol, (3) the suppressing action of exogenous purines, and (4) the behaviour of arteries of spontaneously hypertensive rats, as well as the characteristics of noradrenaline content and ^3^H release after [^3^H]noradrenaline loading.

The mechanism of the very marked presynaptic inhibitory action of HYD appears to be different from the smooth muscle effect, given the much shorter delay, the shape of the dose-effect curve (Fig. 1) and the lack of action of theophylline.

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References


