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THE EFFECT OF UPTAKE BY ADRENERGIC NERVE TERMINALS ON THE SENSITIVITY OF ARTERIAL VESSELS TO TOPICALLY APPLIED NORADRENALINE

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1 Direct observations were made of the rat mesenteric vasculature *in vivo*. They showed a gradual increase in the sensitivity to topically applied noradrenaline from the larger arteries to the precapillary arterioles which was paralleled by a gradual decrease in the density of the adrenergic innervation.

2 Both cocaine and chronic denervation increased the sensitivity to noradrenaline of the innervated arterial vessels, approximately in proportion to the density to their innervation. They did not affect the sensitivity of the non-innervated precapillary arterioles.

3 It is concluded that the sensitivity gradient to topically applied noradrenaline results mainly from the uptake of the applied catecholamine by the perivascular nerve terminals.

Introduction

Zweifach (1961), in an account of the behaviour of the microcirculation, stated that there is a gradual increase in the sensitivity to humoral agents along the arterial tree towards the capillary bed and a concomitant decrease in the influence of the sympathetic nervous system. The idea of a gradient of increasing sensitivity to humoral substances from the large to the small arteries is supported by many direct observational studies in which vasoactive agents have been applied topically to the microcirculation (Baez, 1961; Altura, 1967; Duling, Berne & Born, 1968; Gore, 1972; Furness & Marshall, 1974).

The reason for this differential sensitivity has not been fully determined. Gore (1972) showed that segmental differences in the responses of frog mesenteric vessels to noradrenaline could be explained in terms of the resting tangential stress in the vessel wall but it is not clear whether this explanation can be extended to other vascular beds. In a recent study of the rat mesentery, Furness & Marshall (1974) obtained results which seem to suggest that another factor might be involved. The density of the adrenergic innervation gradually decreased from the principal arteries of the mesenteric arcades to the terminal arterioles associated with a gradual increase in the sensitivity to topically applied noradrenaline. The precapillary arterioles, which arise from the terminal arterioles, had no sympathetic innervation at all and were by far the most sensitive to noradrenaline. In other words, there is a graduation in the density of adrenergic innervation along the arterial tree which is directly opposite to the gradient in the sensitivity to topically applied noradrenaline.

It is well known that the adrenergic nerve terminals of blood vessels have a considerable capacity to take up and retain exogenous noradrenaline (Haeusler, Haefely & Huerlimann, 1971). It, therefore, seems reasonable to propose that a gradual increase in the sensitivity to topically applied catecholamines towards the capillary bed might be a reflection, in part at least, of a gradual decrease in the amount of applied catecholamine which can be taken up by the nerve endings.

If this is the case, the sensitivity of innervated vessels, as compared with that of non-innervated vessels, should be substantially increased when neuronal uptake is prevented. This hypothesis was tested in the present study by comparing the responses of consecutive sections of the arterial tree to topically applied noradrenaline in the presence and absence of cocaine, and before and after chronic denervation.

Methods

The experiments were performed on female rats (100–200 g body weight) anaesthetized with urethane (250 mg/100 g body weight, subcutaneously). The mesentery was prepared for direct observation as described previously (Furness & Marshall, 1974). An area of vasculature was chosen for study which included vessels from the four main sections of the arterial tree, i.e. the principal arteries, small arteries, terminal arterioles and precapillary arterioles with internal diameters of 80–350 μm, 30–40 μm, 18–30 μm and 10–18 μm respectively. Control

internal diameters were measured at a selected site along each vessel with a calibrated micrometer eye piece.

In order to study the effect of noradrenaline, the level of Krebs solution in the bath was lowered so that it was well below the level of the tissue and 0.1 ml of the test solution applied from a syringe. The internal diameter of a single vessel was measured at 3 to 5 s intervals over a period of 60 s and the preparation was then flushed with fresh Krebs solution. This procedure was repeated for a range of noradrenaline concentrations on all the vessels selected. A period of at least 5 min was allowed between successive applications. In 7 experiments the solution bathing the preparation was replaced with one containing cocaine (1 μg/ml). The responses of the vessels to noradrenaline were then examined again.

Chronic denervation was performed aseptically on 5 rats, under ether anaesthesia, by mechanically pinching the principal vessels of a mesenteric arcade with fine forceps, to crush the paravascular nerve bundles. Small incisions were made in the mesentery on either side of these vessels for identification later. After 4 or 5 days the mesentery was prepared for observation and the responses of the vessels to noradrenaline were examined. In some of these experiments cocaine (1 μg/ml) was administered as above and the responses to noradrenalin were re-examined. The efficacy of the denervation procedure was established by the fluorescence histochemical method for monitoring monoamines (Falck, 1962) on air-dried stretched preparations of the mesentery (Furness & Malmfors, 1971).

The drugs used were (–)-noradrenaline bitartrate and cocaine hydrochloride. Concentrations of noradrenaline are expressed in terms of the base and concentrations of cocaine in terms of the salt.

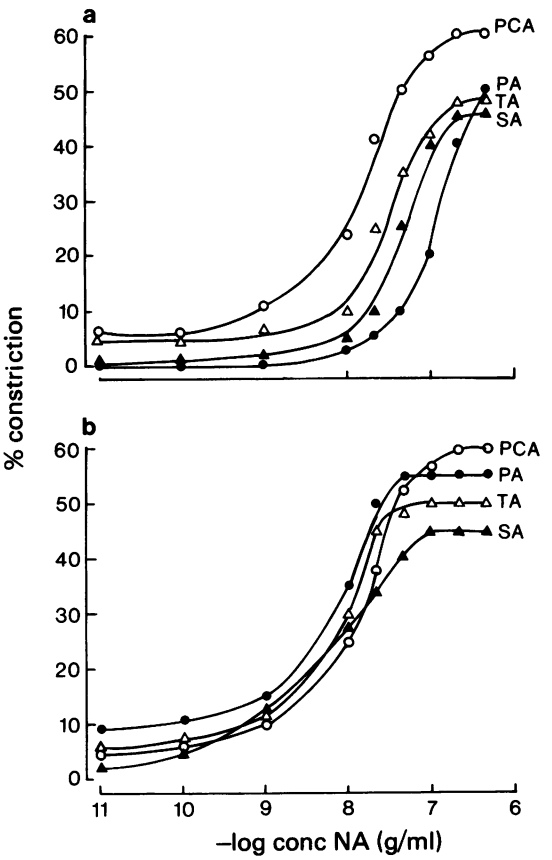

Figure 1 Responses of arterial vessels of a single preparation of rat mesentery to topically applied noradrenaline before (a) and after (b) addition of cocaine (1 μg/ml). Each point represents the mean of three measurements. (○) PCA, precapillary arteriole; (△) TA, terminal arteriole; (▲) SA, small artery; (●) PA, principal artery. Control internal diameters were 12, 24, 30 and 110 μm respectively.

Results

The present experiments confirmed the previous finding that there is a gradient of increasing sensitivity to topically applied noradrenaline from the principal arteries to the precapillary arterioles (Figure 1a). The threshold concentrations for constrictor responses ranged from 10 ng/ml for principal arteries, to 10 pg/ml for precapillary arterioles. The concentrations necessary to produce maximal constrictor responses were 0.6 μg/ml for principal arteries and 0.1 μg/ml for precapillary arterioles.

After inclusion of cocaine (1 μg/ml) in the bathing solution the dose-response curves of principal arteries, small arteries and terminal arterioles were shifted to the left whereas those of precapillary arterioles remained essentially unchanged (Figure 1b). In all preparations the degree of shift was such that the principal arteries had now become the most sensitive and the precapillary arterioles the least sensitive vessels. Chronic denervation produced similar effects to cocaine: in every case the principal arteries became the most sensitive vessels. The changes in sensitivity produced by cocaine and by denervation were such that the principal arteries responded to noradrenaline in concentrations as low as 10 pg/ml and reached maximum degrees of constriction when the concentration was raised to 60 ng/ml. In the experiments in which cocaine was administered to denervated preparations, this did not cause a potentiation of the responses to noradrenaline beyond that already induced by the denervation.

Fluorescence histochemical studies demonstrated that 4 days after performing the denervation there were no fluorescent fibres in the area of mesentery

served by the nerves that had been crushed. Control preparations taken from other mesenteric areas of the same animal, at the same time, revealed a normal distribution of fluorescent fibres.

Discussion

In a previous study of the mesenteric vasculature (Furness & Marshall, 1974) it was confirmed that there is a gradual increase in the sensitivity to topically applied noradrenaline from the larger arteries towards the capillary bed. Histochemical examination of these same vessels revealed that the density of the adrenergic innervation decreases gradually from the principal arteries to the terminal arterioles and that precapillary arterioles have no adrenergic innervation. In the present study, cocaine and chronic denervation increased the sensitivity shown by the innervated arterial vessels to topically applied noradrenaline in direct proportion to the density of their adrenergic innervation; dose-response curves of principal arteries were always shifted more than those of small arteries and terminal arterioles. In contrast, neither cocaine nor chronic denervation had any effect on the sensitivity of the non-innervated precapillary arterioles.

It has been suggested that cocaine, besides blocking the neuronal uptake mechanism, causes a direct increase in the sensitivity of vascular smooth muscle by an action on the post-junctional membrane (Bevan & Verity, 1967; Kalsner & Nickerson, 1969). However, as cocaine did not potentiate the responses of the non-innervated precapillary arterioles and since it had no effect on denervated vessels, there is no reason to suppose that this occurred in the present experiments. It is also unlikely that the post-junctional type of supersensitivity which follows sympathectomy contributed to the results because this takes at least 7 days to develop (Trendelenberg, 1966) and the present experiments were performed only 3 or 4 days after denervation. Thus, it seems reasonable to assume that all the changes in sensitivity produced in these experiments were due solely to inactivation or removal of the neuronal uptake mechanism. It is then apparent that the uptake mechanism is of considerable importance in determining the sensitivity exhibited by the innervated sections of the arterial tree to topically applied noradrenaline. Indeed, the fact that cocaine and chronic denervation essentially reversed the sensitivity profile along the arterial tree suggests that the gradient of increasing sensitivity to topically applied noradrenaline towards the capillary bed is entirely due to the different amounts of applied catecholamines taken up by the adrenergic nerve terminals of the vessel walls.

From a functional point of view, an important question is whether this uptake mechanism exerts a similar influence over the responsiveness to noradrenaline circulating in the blood stream. Since the adrenergic nerve terminals are only to be found on those smooth muscle cells furthest removed from the vessel lumen, the noradrenaline penetrating from the blood stream might be less affected by the uptake processes into nerve terminals than the noradrenaline applied to the outside of the vessels. This is supported by the work of de la Lande & Waterson (1967) who showed that innervated arteries are more sensitive to intraluminal than to extraluminal noradrenaline and that this difference could be substantially reduced or even abolished by cocaine or denervation. It appears therefore that when neuronal uptake has been blocked, arterial vessels exhibit a similar sensitivity to topically applied noradrenaline as they would to circulating noradrenaline. From the present results, noradrenaline in a concentration of 10 pg/ml in the blood stream might be expected to produce small constrictor responses in all arterial vessels of the mesentery while concentrations of 10 ng/ml would constrict each section of the arterial tree by up to 30% from the resting arterial diameter. Experiments on conscious rats have shown that resting levels of noradrenaline in the plasma are approximately 1 ng/ml, while after severe haemorrhage they may become as high as 6 ng/ml (Ridigas & Cession-Fossion, 1972). Thus, it appears that concentrations of noradrenaline in the blood stream are sufficient to produce significant constrictor responses in all arterial vessels of the mesentery even under resting conditions. Since precapillary arterioles have no sympathetic innervation, circulatory catecholamines may be of particular importance in maintaining the tone of these vessels.

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