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THE LACK OF EFFECT OF OXYTETRACYCLINE ON RESPONSES TO SYMPATHETIC NERVE STIMULATION AND CATECHOLAMINES IN VASCULAR TISSUE

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1 The effects of oxytetracycline, an inhibitor of amine binding in connective tissue, on the responses of perfused rabbit ear arteries to sympathetic nerve stimulation and to intraluminally administered noradrenaline were examined. The contractions of aortic strips to catecholamines in the presence of oxytetracycline were also examined.

2 Oxytetracycline (0.1 mM) had no discernable effect on the magnitude of constrictions, measured as reductions in flow, produced by either nerve stimulation (0.5–10 Hz) or noradrenaline (0.5–50 ng) in the ear artery. In addition, the time taken for vessels to recover towards control flow values after endogenously released or exogenously applied noradrenaline had acted was not increased by oxytetracycline.

3 Oxytetracycline (0.1 mM) did not alter the position or shape of the concentration-response curve to noradrenaline nor did it enhance the amplitude of individual responses to catecholamines in aortic strips.

4 It is concluded, contrary to the observations of Powis (1973), that oxytetracycline does not increase the magnitude or duration of responses to sympathetic nerve activation or to catecholamines and that binding to connective tissue is of no material consequence in terminating their action in vascular tissue.

Introduction

The recent report that responses of isolated blood vessels to sympathetic nerve stimulation and to pulses of injected noradrenaline are considerably enhanced and prolonged by oxytetracycline (Powis, 1973), presumably by blockade of catecholamine binding to collagen and elastin in the vascular wall, needs further investigation. If this finding is verifiable it severely complicates the analysis of termination of action of catecholamine-induced responses in vascular tissue, since it is not easily reconciled with the bulk of available evidence on agonist disposition. For this reason, experiments were undertaken to re-examine the effects of oxytetracycline on responses to nerve stimulation and to catecholamines in the central artery of the rabbit ear and to include, as well, observations on rabbit aortic tissue, which has an even greater amount of connective tissue than does the relatively muscular ear vessel.

Methods

The central artery was removed from each ear in rabbits under urethane anaesthesia (7 ml/kg of a 25% solution, intraperitoneally) after cannulation at both ends, essentially as described by De La Lande & Rand (1965) and De La Lande, Frewin & Waterson (1967). The arteries were suspended in individual 30 ml muscle chambers containing Krebs-Henseleit (Krebs) solution of the following composition (mM): NaCl 115.3, KCl 4.6, $CaCl_2$ 2.3, $MgSO_4$ 1.1, $NaHCO_3$ 22.1, KH_2PO_4 1.1 and glucose 7.8, to which the disodium salt of ethylene diamine tetra-acetic acid was added (0.03 mM), to retard oxidation of catecholamines catalyzed by traces of heavy metals. The preparations were maintained at 37°C and continuously oxygenated with 95% O_2 and 5% CO_2.

A gravity-feed aparatus was used to perfuse the vessels at a constant pressure of 85 cm of water with warmed (37°C) and oxygenated Krebs solution. A record of the rate of flow of Krebs solution through each artery was provided by an Andrews (1952) outflow recorder connected to a piston recorder writing on a slowly revolving smoked kymograph drum. The height of each vertical stroke of the writing lever is proportional to the volume of air displaced from the outflow recorder over an 8 s period and thus to the volume of Krebs solution flowing through the

vessel lumen and into the recorder during that time interval. Other details and a schematic diagram of the perfusion apparatus are presented elsewhere (Kalsner, 1972).

The procedure used for stimulation of the periarterial sympathetic nerves was that used by De La Lande & Rand (1965). Platinum electrodes were arranged around the proximal end of each artery over the area where the perfusion cannula lay within the artery. The nerves were stimulated at supramaximal voltage with biphasic pulses of 1 ms pulse width delivered by a Grass model S5 stimulator. Supramaximal voltage was determined for each preparation by stimulating initially at 5 Hz for 1 min at each test voltage (beginning with 10 V and increasing in 5 V increments) and recording the vasoconstriction produced. A voltage which was about 25% above that which produced the most vasoconstriction was routinely used in all experiments.

Strips of rabbit aorta were prepared and suspended under 2 g of tension in muscle chambers of 15 ml capacity (Kalsner & Nickerson, 1968). The strips were immersed in Krebs solution as described above, maintained at 37°C, and constantly bubbled with 95% O_2 and 5% CO_2. Isotonic contractions were recorded, after a 90 min equilibration period, by means of frontal-writing levers on a slowly moving kymograph drum (about 2.0 mm/min) with a lever magnification of 6.8-fold.

The drugs used were (−)-noradrenaline and (−)-adrenaline bitartrates and oxytetracycline dihydrate. The catecholamines are expressed as the weight of the base in g/ml and oxytetracycline is in terms of molarity. For intraluminal injections of noradrenaline in the ear arteries the desired absolute amount in ng was always delivered in a total volume of 0.2 ml, by injection into the tubing immediately above the vessel. The administration of 0.2 ml of Krebs solution, as a routine control measure, had no effect on flow through the artery. Oxytetracycline was dissolved directly into a reservoir of Krebs solution at a concentration of 0.1 mM shortly before use and the flow of drug-treated or control Krebs solution through the vessel was determined by means of a two-way stopcock located above the proximal end of the artery. In addition, Krebs solution containing oxytetracycline (0.1 mM) was added to the muscle chamber so as to bathe the extraluminal surface of the vessel. Preparations were exposed to the tetracycline compound, for 20 min before testing and for the entire test period. With respect to aortic strips, the oxytetracycline was either dissolved directly in the Krebs solution at 0.1 mM and the muscle chambers filled with the treated Krebs for 20 min, followed without washout, by agonist; or the tetracycline was made up in 0.9% w/v NaCl solution at a concentration of 2 mM, adjusted to pH 8.0 with 1 N NaOH, and the appropriate volume added to the muscle chambers after responses to the agonist had attained plateau values.

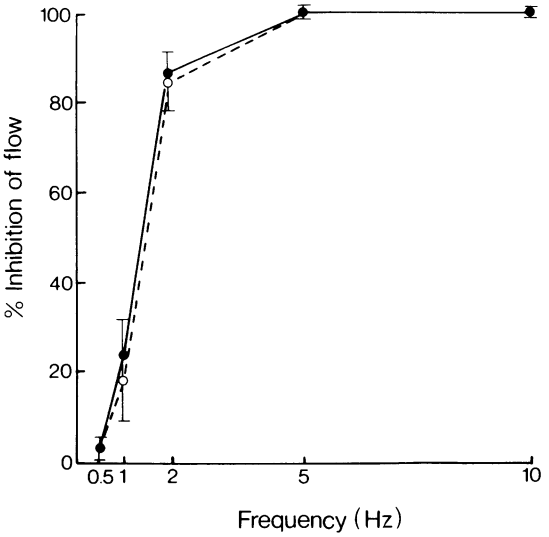

Figure 1 Effect of oxytetracycline on the frequency-response curve to periarterial stimulation in the rabbit ear artery. Frequency-response curves in 7 preparations are shown, first in the absence (●) and then in the presence (○) of oxytetracycline (0.1 mM). Stimulation was for 2 min at each test frequency. Mean values are shown; vertical lines show s.e. mean. Details of test conditions are provided in the Methods section.

Response sensitivities in aortic strips at the mean effective dose (ED_{50}) are expressed as geometric mean values (Fleming, Westfall, De La Lande & Jellett, 1972). Each ED_{50} was converted to its log and the mean for each group was recorded. The antilog of each mean log is presented as the geometric mean. In addition, mean values of all data are presented with their standard errors and were compared, where appropriate, by Student's *t* test.

Results

Responses to nerve stimulation and to noradrenaline in the ear artery

The central artery responds to periarterial stimulation of its sympathetic innervation with constriction, decreased lumen diameter and a consequent reduction in intraluminal flow. Detectable inhibition of flow is most often seen at 0.5–1.0 Hz and obliteration of lumen diameter and cessation of flow at 5 Hz. For the present experiments, two sets of frequency-response curves (0.5–10 Hz) were obtained for each vessel, one in the absence and the other in the presence of oxytetracycline (0.1 mM), so as to minimize variability.

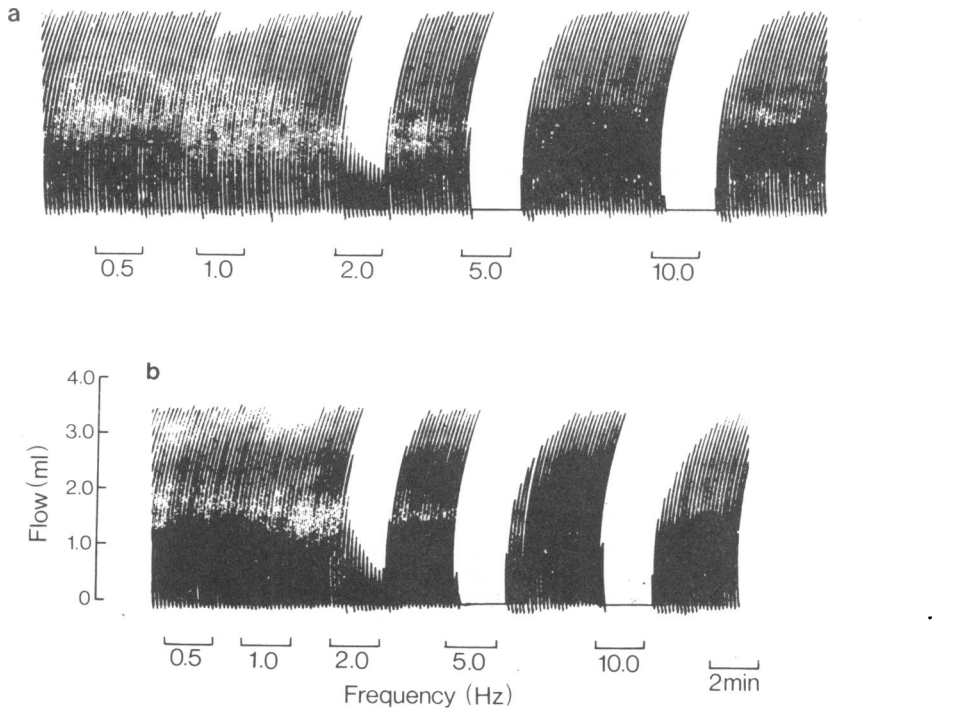

Figure 2 Effect of oxytetracycline on responses of an ear artery to periarterial nerve stimulation. Responses of a typical vessel are shown (a) in the absence and (b) in the presence of oxytetracycline (0.1 mM). The duration of stimulation at each frequency was 2 min and is indicated by enclosed horizontal bars. Vasoconstrictor responses are recorded as reductions in outflow.

The cumulative results are shown in Figure 1 and the record of one such experiment is seen in Figure 2. No detectable effect of the tetracycline compound on the magnitude of flow inhibition at any of the tested frequencies was observed.

Additionally, analysis of response duration, measured as the time taken after the cessation of stimulation at 5 and 10 Hz, for flow to return to 50% of pre-stimulation values failed to reveal any statistically significant effect of the inhibitor in prolonging duration of action. The mean half-time for recovery in 7 control and 7 treated preparations was 33.4 ± 8.0 and 37.9 ± 8.0 s respectively at 5 Hz; and it was 39.0 ± 9.4 and 39.4 ± 9.0 s at 10 Hz. It was confirmed in matching experiments that, in the absence of oxytetracycline, first and second frequency-response curves do not differ significantly from each other either in the size or in the duration of the response.

Further, oxytetracycline (0.1 mM) had no enhancing effect on the constrictions elicited by intraluminal injections of noradrenaline (0.5–50 ng); a method of agonist administration similar to that which was used by Powis (1973). The dose-response curves obtained in the absence or in the presence of the inhibitor of amine binding did not differ significantly (Figure 3). Further, analysis of response duration again established the lack of interference by oxytetracycline with termination of action. The mean half-time for recovery from peak effects in 5 control and 5 treated preparations was 13.6 ± 1.7 and 15.2 ± 1.3 s respectively after 20 ng; and it was 46.0 ± 6.3 and 58.0 ± 10.9 s after 50 ng of noradrenaline.

Responses to noradrenaline and adrenaline in aortic strips

Experiments were done on aortic strips using two different protocols. In one case, strips were contracted with low concentrations of noradrenaline (3 or 10 ng/ml) or adrenaline (10 ng/ml), chosen from the steep portions of the concentration-response curves, to maximize the size of any enhancing effect. After responses had reached a stable plateau value, oxytetracycline was added to the bathing fluid, either cumulatively (10, 30, 100 μM), or in one single addition (0.1 mM) (Figure 4). In a total of 8 strips

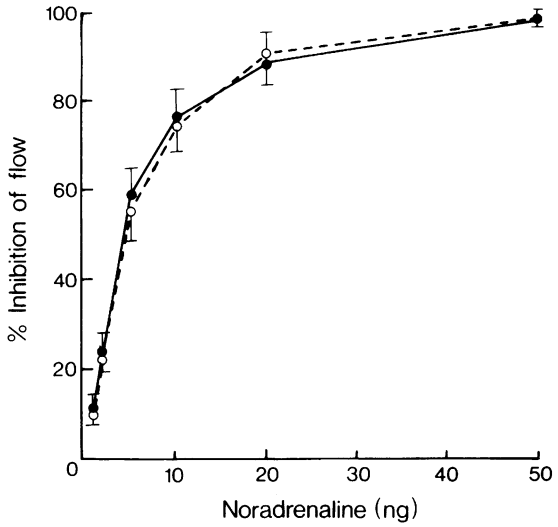

Figure 3 Effect of oxytetracycline on responses of the ear artery to intraluminal injections of noradrenaline. Dose-response curves in 7 preparations, first in the absence (●) and then in the presence (○) of oxytetracycline (0.1 mM) are shown. Mean values are shown; vertical lines show s.e. mean. Details of agonist administration are given in the Methods section.

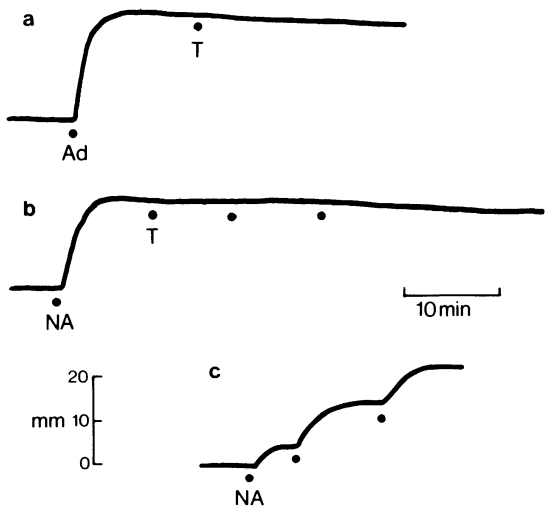

Figure 4 Effect of oxytetracycline on responses of aortic strips to catecholamines. (a) Strip contracted by adrenaline (Ad, 10 ng) and exposed to oxytetracycline (T, 0.1 mM); (b) strip contracted by noradrenaline (NA, 3 ng) and exposed to oxytetracycline (T, 10, 30 and 100 μM at dots) (c), strip contracted cumulatively by noradrenaline (1, 3 and 10 ng) to show typical increments in amplitude obtained with approximately 3-fold increments in agonist concentration.

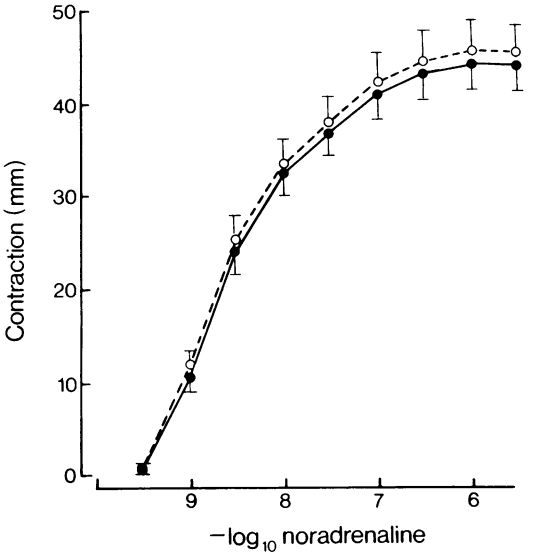

Figure 5 Effect of oxytetracycline on the concentration-response curve to noradrenaline in aortic strips. Responses of 10 preparations in the absence (●) and 11 preparations in the presence (○) of oxytetracycline are shown. Mean values are shown; vertical lines show s.e. mean. Details are given in text.

examined no increment in response amplitude due to the oxytetracycline was detectable.

In the other case, strips were matched initially for responsiveness to noradrenaline by exposure to low concentrations (0.3, 1 and 3 ng/ml) and then after washout of agonist and recovery of basal tone half of the strips were exposed to oxytetracycline (0.1 mM) for 20 min and the remainder served as controls. Complete concentration-response curves to noradrenaline were then obtained in all strips, and this was done without washout of the oxytetracycline in the treated ones. The results are shown in Figure 5. Again, the postulated inhibitor of amine binding had no material effect on the magnitude of responses to noradrenaline, at any point along the curve. Also, geometric mean ED_{50} values of the control and treated groups (2.8 and 2.6 ng/ml respectively), did not differ significantly ($P > 0.5$).

Discussion

The observations of Powis (1973) on response potentiation by oxytetracycline could in no way be confirmed in the present experiments. Oxytetracycline, an inhibitor of amine binding to collagen and elastic tissue, neither sensitized the central ear artery to intraluminal injections of noradrenaline or to

sympathetic nerve stimulation nor prolonged the duration of its responses. Similarly, aortic strips of the rabbit, rich in connective tissue, did not give increased contractions to noradrenaline in the presence of oxytetracycline; regardless of whether it was added before the catecholamine or after individual responses had reached stable equilibrium values.

Although the present findings cannot account for the enhanced contractions observed by Powis in the presence of oxytetracycline (in the order of 10- and 6-fold for responses to noradrenaline and nerve stimulation), certain apparent inconsistencies in his analysis should be mentioned. He concluded that in tissues with a high content of collagen and elastin 'binding to extracellular sites is the major mechanism for terminating the response to noradrenaline or to adrenergic nerve stimulation'. However, his data reveal that blockade of neuronal and effector cell uptake, with cocaine and 17β-oestradiol, produces a sensitization perhaps only slightly less than that caused by oxytetracycline (his Figure 4); but the addition of oxytetracycline to vessels already treated with cocaine and 17β-oestradiol does not produce any material additional enhancement. This is contrary to what would be expected if independent mechanisms of inactivation were inhibited together.

The suggestion that overlapping mechanisms of inactivation are involved such that binding to connective tissue is a prelude to definitive inactivation by neuronal or extraneuronal (effector cell) uptake is also not sustained by his data. For if, as he states, 'extracellular tissue binding is sufficient for the immediate inactivation of all the noradrenaline presented in a pulse injection, as employed in the present study, or liberated by nerve stimulation over a short period' then no demonstrable enhancing effect of 17β-oestradiol and cocaine, the inhibitors of effector cell and neuronal uptake respectively, should be observed under these conditions. And, enhancement was observed.

The explanation for the potentiating effect observed by Powis, which could not be duplicated here, is at present obscure. In this context, it should be noted that Guimarães, Azevedo, Cardoso & Oliveira (1975) did not detect enhancement by oxytetracycline of the inhibitory dose-response curves to isoproterenol in saphenous vein preparations of the dog.

A good deal of evidence obtained from various laboratories and by the use of several different techniques points to an important role for effector cell uptake, followed by enzymatic inactivation, in termination of action in most preparations of vascular tissue examined (Kalsner, 1966; Kalsner & Nickerson, 1969; Gillespie, Hamilton & Hosie, 1970; Berkowitz, Tarver & Spector, 1971; Bevan & Su, 1973; Kalsner, 1975a; Kalsner, Frew, Smith, 1975); although the role for neuronal uptake is still controversial (De La Lande, 1975; Kalsner, 1975b). Thus, although binding of catecholamine to connective tissue may occur, it is improbable that it could be an additional independent factor of any significance in termination of action, particularly with moderate concentrations of amine; unless our concept of biophase kinetics (Furchgott, 1955; Kalsner, 1976) is to be extensively revised. The available evidence does not warrant such a revision.

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