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THE INFLUENCE OF AN EXTRANEURONAL COMPARTMENT ON THE RELAXATION OF THE CAT NICTITATING MEMBRANE *in vivo*

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1 Contractions of the cat nictitating membrane were elicited on stimulation of the internal carotid nerve, and the effects were studied of desipramine and two inhibitors of catechol-*O*-methyltransferase, U-0521 and pyrogallol, on the subsequent relaxation of the muscle.

2 The relaxation of the nictitating membrane occurred in at least two phases. The late phase of relaxation was prolonged after increase in the period of nerve stimulation and the duration of this phase was further prolonged after treatment with pyrogallol.

3 After inhibition of neuronal uptake of noradrenaline with desipramine both the early and late phases of relaxation were increased in duration, and subsequent administration of pyrogallol or U-0521 caused a further increase in the duration of the late phase of relaxation.

4 The results suggest that the late phase of relaxation of the nictitating membrane is influenced by efflux of noradrenaline from an extraneuronal pool.

Introduction

Responses of the rabbit ear artery (Avakian & Gillespie, 1968) and the cat nictitating membrane (Draskoczy & Trendelenburg, 1970) to exogenous noradrenaline have been investigated *in vitro*. These authors have proposed that, following return of the tissue to an amine-free medium, responses may be prolonged by efflux of unchanged amine from an extraneuronal pool.

A possible mechanism for this extraneuronal accumulation of noradrenaline and its subsequent release is the transport mechanism described by Iversen (1965). A number of reports have indicated that this transport is carrier-mediated and is capable of transferring amines in either direction (Gillespie & Hamilton, 1967; Kalsner, 1975; Gillespie, 1976).

In vivo the relaxation of the cat nictitating membrane, after a period of nerve stimulation, has been reported to occur in two or three phases following inhibition of the neuronal uptake process; this pattern of relaxation was attributed to the mechanism described above (Eccles & MacLean, 1977).

The experiments described here were undertaken to establish whether the relaxation of the nictitating membrane *in vivo* may be affected by release of noradrenaline from an extraneuronal pool, and to investigate the site of this extraneuronal compartment.

Methods

Cats of either sex and with body weights between 1.5 and 3 kg were anaesthetized with pentobarbitone sodium in an initial dose of 40 mg/kg intraperitoneally and maintained by infusion of pentobarbitone into the femoral vein at a rate of approximately 8 mg kg^{-1} h^{-1}. The trachea was cannulated and the cats breathed spontaneously. The cats were maintained at a constant temperature (38°C).

Isometric contractions of the nictitating membrane above a resting tension of 3 to 5 g were recorded on a rectilinear pen recorder (Physiograph Six). Femoral blood pressure and respiratory movements were also monitored. Contractions of the membrane were elicited by electrical stimulation of the internal carotid nerve with pulses of 0.5 ms duration at a supramaximal stimulus voltage.

Drugs were injected via a cannula in the cephalic vein and a period of 20 min was allowed before further responses were recorded. Desipramine hydrochloride (Geigy Pharmaceuticals) and pyrogallol were each given in 1 ml of 0.9% w/v NaCl solution (saline). U-0521 (Upjohn Ltd.) was injected in 1 ml of 30% ethanol.

Results

The relaxation of the nictitating membrane following short periods of high frequency nerve stimulation (5–20 s, $\geqslant$ 20 Hz) occurs in two or more phases. The initial rate of relaxation is quite rapid, while the late phase of relaxation is much slower. In three experiments responses to nerve stimulation at 20 Hz were

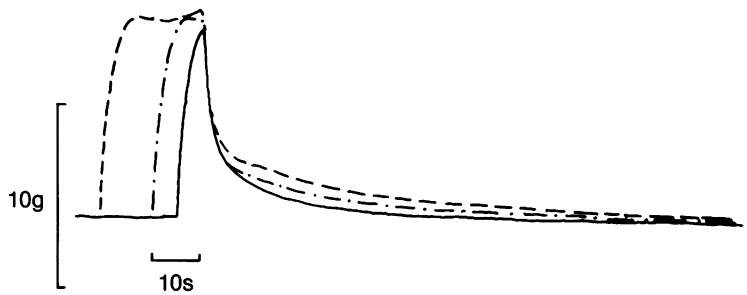

Figure 1 A typical experiment showing responses of the cat nictitating membrane *in vivo* to electrical stimulation of the internal carotid nerve at a frequency of 20 Hz for periods of 5, 10 and 20 seconds. Traces are superimposed for comparison. The longer the period of stimulation the more prolonged was the second phase of relaxation.

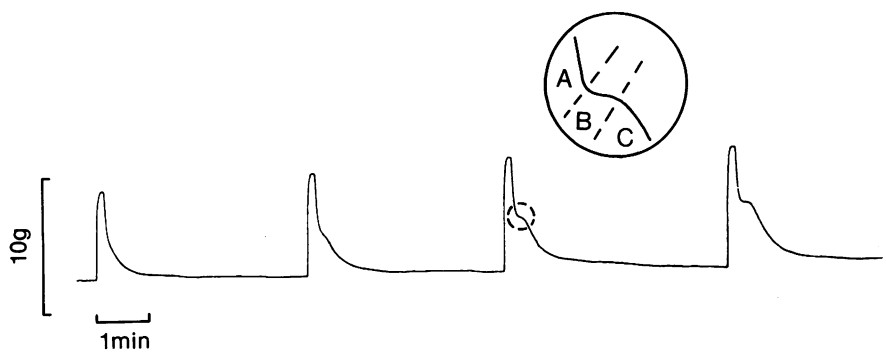

Figure 2 Responses of the cat nictitating membrane *in vivo* to electrical stimulation of the internal carotid nerve for 10 s at 5, 10, 15 and 20 Hz after the administration of desipramine (1.2 mg/kg i.v.). Inset, the three phases of relaxation are labelled, (A) initial phase, (B) middle phase, (C) late phase.

maintained for 5, 10 and 20 seconds. Comparison of these responses revealed that the initial phase of the relaxation was unaffected by the period of stimulation. The late phase of the relaxation response, however, progressively increased in duration with the period of stimulation (Figure 1).

In three experiments desipramine (0.2 mg/kg i.v.) slowed the initial phase of the relaxation, a result which we have already reported (Eccles & MacLean, 1977). Under these conditions the transition between the early and late phases of relaxation was more abrupt and occurred at an amplitude which increased with the period of stimulation. This change was even more marked following a higher dose of desipramine (1.2 mg/kg) when the relaxation of the muscle after stimulation at frequencies $\geqslant 10$ Hz was triphasic, as is illustrated in Figure 2. In each of a series of twelve experiments, in which a 10 s period of stimulation was used, the amplitude and duration of the middle phase of the relaxation was progressively increased with increasing impulse frequency (Figure 2).

In six of these experiments subsequent injection of the catechol-*O*-methyltransferase (COMT) inhibitor U-0521 (25 mg/kg i.v.) further prolonged the relaxation of the membrane, and in every case there was a transient increase in tension during the middle phase of the relaxation which followed contractions to stimulation frequencies between 5 and 20 Hz (Figure 3). In two experiments pyrogallol (50 mg/kg), when given after desipramine (1.2 mg/kg), had an effect similar to that of U-0521. In two experiments responses were recorded to stimulation at 20 Hz for 20 s before and after the administration of pyrogallol alone (50 mg/kg i.v.). The late phase of relaxation of the membrane was prolonged following pyrogallol, although the early phase of relaxation was unaffected. In four experiments, U-0521 alone (25 mg/kg) did not prolong the relaxation after nerve stimulation at 20 Hz for 10 seconds.

Discussion

In an earlier paper it was concluded that the rate of relaxation of the nictitating membrane, after nerve

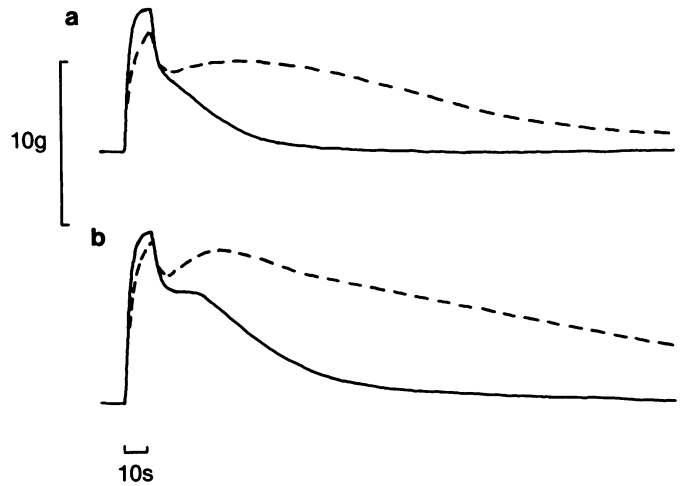

Figure 3 Responses of the cat nictitating membrane *in vivo* to electrical stimulation of the internal carotid nerve at (a) 5 Hz and (b) 15 Hz after the intravenous administration of 1 mg/kg desipramine (solid line) and subsequently 25 mg/kg U-0521 (dashed line). Traces are superimposed for comparison.

stimulation *in vivo*, is directly related to the removal of noradrenaline from the neuro-effector gap (Eccles & MacLean, 1977). The present results indicate that the relaxation of the nictitating membrane following high frequency stimulation occurs in at least two distinct phases and that the secondary phase is lengthened after administration of the COMT inhibitors pyrogallol and U-0521, particularly when they are given after desipramine.

The noradrenaline released during nerve stimulation, as well as acting on adrenoceptors, might be expected to accumulate in the extracellular space upon collagen and within the smooth muscle (Avakian & Gillespie, 1968; Gillespie, 1976), the amount being dependent on the extracellular concentration and the time for which it is maintained. Extraneuronal uptake of noradrenaline is thought to be carrier-mediated and this mechanism may allow movement of substrate in either direction (Gillespie & Hamilton, 1967; Kalsner, 1975). Since the extraneuronal uptake process in the isolated nictitating membrane appears to be able to generate only a 3 to 5 fold concentration difference across the cell membrane (Trendelenburg, Hohn, Graefe & Pluchino, 1971), it is possible that an efflux of noradrenaline could occur once the extracellular concentration has been reduced to less than a quarter of the intracellular concentration.

The present results indicate that the duration of nerve stimulation, i.e. the time for which a high concentration of noradrenaline is maintained in the extracellular space, selectively affects the late phase of relaxation.

Desipramine dose-dependently reduces the initial rate of relaxation of nictitating membrane responses (Eccles & MacLean, 1977), presumably through its inhibitory action on the neuronal uptake process (Titus & Spiegel, 1962). This inhibition may explain why the relaxation of the nictitating membrane after high frequency stimulation occurs in three distinct phases following the administration of desipramine 1.2 mg/kg: 1) during the initial phase the extracellular concentration of noradrenaline drops rapidly until it reaches a level where the removal of noradrenaline by neuronal uptake is exactly balanced by a net efflux from extraneuronal stores; 2) during the middle or 'holding' phase this efflux from the extraneuronal stores continues until the concentration of noradrenaline in these stores is so reduced that the rate of efflux no longer compensates for the activity of the neuronal uptake process; 3) the late phase of relaxation represents the period during which the extracellular concentration of noradrenaline is once more declining although more slowly than during the initial phase as there is still some slow efflux from the extraneuronal stores.

The progressive development of the middle or 'holding' phase of relaxation with increasing frequency of stimulation, which is seen after desipramine, is quite consistent with this hypothesis. During stimulation at higher frequencies, the extracellular noradrenaline concentration is raised, and consequently the concentration in the extraneuronal pool should also be higher. Efflux from this pool might then occur at higher extracellular concentrations of

noradrenaline, and this efflux could be expected to last longer.

Efflux of noradrenaline from a neuronal pool has been stated to be responsible for the late phase of relaxation of the nictitating membrane when studied *in vitro* (Trendelenburg, 1971). However, the present results indicate that, *in vivo* efflux from an extraneuronal pool is responsible for the late phase of relaxation and that neuronal uptake of noradrenaline is a site of loss of transmitter from the extracellular space. The difference between the *in vitro* and *in vivo* results may be explained by the fact that in the present experiments the transmitter was released on nerve stimulation whereas in the *in vitro* experiments the nictitating membrane was pretreated with reserpine and pargyline and the relaxation was studied after the tissue had been incubated with noradrenaline.

The administration of the COMT inhibitors pyrogallol and U-0521 (Wylie, Archer & Arnold, 1961; Giles & Miller, 1967) after desipramine caused a marked prolongation of the late phase of relaxation. These results are consistent with the hypothesis that efflux of noradrenaline from an intracellular pool prolongs the late phase of the relaxation, since in the nictitating membrane COMT activity is located primarily within smooth muscle cells (Jarrot & Langer, 1971). Neither the duration of stimulation nor inhibition of COMT affected the initial component of relaxation, possibly because this component reflects the rapid reduction of the extracellular concentration of noradrenaline by neuronal uptake until a level is reached which permits net efflux of noradrenaline from the extraneuronal compartment. These results are consistent with previously published work demonstrating the significance of COMT in terminating the action of noradrenaline after inhibition of neuronal uptake of noradrenaline (Trendelenburg *et al.*, 1971). Inhibition of COMT in the absence of desipramine did not have such marked effects on the relaxation of the muscle, pyrogallol having a slight effect and U-0521 no significant effect. This may be because in the absence of desipramine the majority of noradrenaline is taken up into the nerve terminals and very little enters the smooth muscle.

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