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FURTHER EVIDENCE FOR THE PREVENTION BY GUANETHIDINE OF NORADRENALINE EFFLUX FROM RABBIT VENTRICULAR SLICES INDUCED BY A LOW EXTERNAL SODIUM CONCENTRATION

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When guanethidine was applied to rabbit ventricular slices at 37°C for an initial 30 min incubation period, doses of 4×10^{-5} and 8×10^{-5} M irreversibly inhibited increases in efflux of noradrenaline induced by the subsequent reduction of external sodium concentrations to 18 mM. This inhibition was prevented if the temperature was reduced to 0°C during the drug application period. Guanethidine transported into adrenergic nerve endings appears to increase permeability to Na^+, thereby leading to the reduction in efflux.

Introduction. We have presented the hypothesis that guanethidine increases the permeability of adrenergic nerve endings to sodium ions, thereby leading to adrenergic neurone blockade (Kubo & Misu, 1974; Misu & Nishio, 1978; Hosotani & Misu, 1978). If such is indeed the case, guanethidine could prevent other phenomena in the terminals which occur when the concentration of external sodium is reduced. Hosotani & Misu (1978) demonstrated that, in rabbit ventricular slices, guanethidine (4×10^{-6} and 4×10^{-5} M) inhibited calcium-independent increases in the efflux of noradrenaline induced by low sodium and that this inhibition was prevented by tetracaine, while the accumulation of guanethidine was not prevented by tetracaine. However, whether or not this inhibition by guanethidine occurred consistently with the time course of the adrenergic neurone blocking action was not clear and attempts have been made to clarify it in the present experiments.

Methods. Methods were the same as those described previously (Hosotani & Misu, 1978), except that the application period of guanethidine was limited to 30 min. Rabbit ventricles were sliced transversely into sections approximately 400 mg in weight and 0.5 mm in thickness. These slices were incubated in 10 ml Tris buffer Krebs solution, bubbled with 95% O_2 and 5% CO_2 and usually kept at 37°C. The composition of the solution was as follows (mM): NaCl 143.4, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.18, glucose 11.1, Tris buffer 2.0 and disodium edetate 0.03. A 30 min stabilization period was allowed before exposure of these slices to the test solutions. A sodium-deficient medium was prepared by replacement of NaCl with an equiosmotic amount of sucrose. The final pH of solutions was 7.1 to 7.4. The incubation medium was collected at intervals of 30 min and its noradrenaline content was measured by the method of Anton & Sayre (1962).

Results. When the external sodium concentration was reduced from 143 to 18 mM, the efflux of noradrenaline during each successive incubation period markedly increased (Figure 1a). Even when guanethidine was present for only the first 30 min incubation period, concentrations of 4×10^{-5} and 8×10^{-5} M irreversibly inhibited increases in the efflux of noradrenaline induced by the subsequent sodium reduction, despite small increases during the drug contact period. However, no significant changes were seen if a lower concentration (4×10^{-6} M) was used. The concentrations required to produce this inhibition are somewhat higher than those used in previous experiments (Hosotani & Misu, 1978), in which guanethidine was present throughout. The present results demonstrate that a 30 min contact with guanethidine is sufficient for prolonged inhibition.

When slices were initially incubated with guanethidine at a temperature of 0°C there was no longer a modification in either the resting output nor in the efflux of noradrenaline induced by low sodium (Figure 1b). Exposure to a low temperature in the absence of guanethidine during the initial period did not modify the efflux ($n = 6$).

Discussion. Our results demonstrate that the time course of guanethidine-induced inhibition of increases in the efflux of noradrenaline by low sodium parallels its adrenergic neurone blocking action (Boura &

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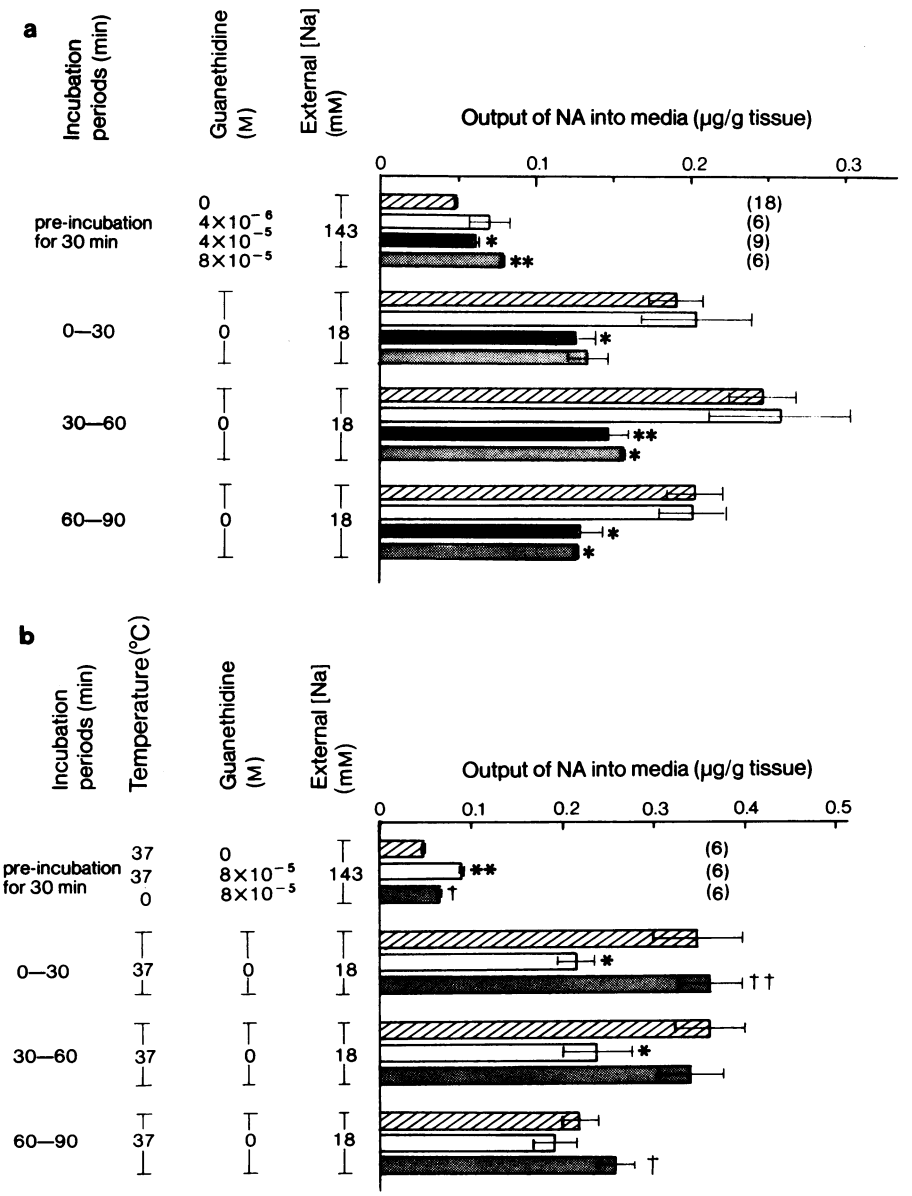

Figure 1 Effects of guanethidine on the efflux of noradrenaline induced by low sodium in rabbit ventricular slices. (a) At 37°C after an initial incubation period for 30 min in 143 mM sodium solution, slices were incubated in 18 mM sodium solution with sucrose for 3 further periods of 30 min. Guanethidine was added only during the initial incubation period at concentrations of 4×10^{-6}, 4×10^{-5} or 8×10^{-5} M denoted by open, filled and stippled columns respectively. Abscissa scale indicates output of noradrenaline and hatched columns show control values. Horizontal bars indicate standard errors and in parentheses are the number of estimations. Statistically significant differences: $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with control. (b) At 0°C. The experiment was identical to (a) except that the initial incubation only was conducted at 0°C, the temperature being reduced 10 min before the addition of guanethidine (8×10^{-5} M, stippled columns). For comparison, the results are shown of controls (hatched columns) and guanethidine 8×10^{-5} M (open columns) when initial incubation was carried out at 37°C. Statistically significant differences: $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with controls; $\dagger P < 0.05$ and $\dagger\dagger P < 0.01$ compared with guanethidine at 37°C.

Green, 1965; Misu, Nishio, Hosotani & Hamano, 1976). Furthermore, the inhibitory action was prevented by incubation with guanethidine at 0°C, which may be explained by a failure of uptake of guanethidine into the terminals, since low temperatures greatly reduce accumulation into the slices which are abundantly innervated by adrenergic nerves (Hosotani & Misu, 1978). The result also suggest that such accumulation of guanethidine is a necessary process for this inhibition. This idea is also consistent with the fact that the accumulation within nerves of adrenergic neurone blocking agents such as guanethidine (Gulati & Jaykar, 1971) and bretylium (Hosotani & Misu, 1977) is essential for the development of the transmission failure.

In conclusion, guanethidine transported into the adrenergic nerve endings appears to act inside the membrane to increase the permeability to ionized sodium, thereby leading to the decreased efflux of noradrenaline described here.

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