EFFECTS OF CALCIUM AND GUANETHIDINE ON NORADRENAline OUTPUT INDUCED BY SODIUM REDUCTION IN RABBIT ATRIA

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Kubo and Misu (1) have shown that guanethidine-induced blockade of adrenergic transmission in rabbit hearts is attenuated when nerves are stimulated during subsequent perfusion with a low sodium solution and is accentuated with a high sodium solution and proposed that the drug increases the permeability of adrenergic nerve endings to sodium ions, thereby leading to blockade. Guanethidine $4 \times 10^{-4}$ M increased the maximum upstroke velocity of action potentials without hyperpolarization for the first 30 min after the addition, after which the value decreased concomitantly with a gradual decrease in resting potentials until 2 hr, and the changes of action and resting potentials were antagonized by tetrodotoxin in rabbit atria (2). In the present experiments, effects of calcium and guanethidine on an increase in the output and a decrease in the endogenous contents of noradrenaline induced by sodium reduction were investigated in rabbit atria.

Atria were incubated for 2 hr in 10 ml Krebs solution of the following composition expressed in mM: NaCl 143.4, KCl 5.9, CaCl$_2$ 2.5, MgCl$_2$ 1.18, glucose 11.1, tris buffer 2.0 and EDTA 0.03. The solution was bubbled with 5% CO$_2$ in oxygen and maintained at 37°C. Sodium deficient media were prepared by replacement of NaCl with an equiosmotic amount of sucrose or choline chloride. Calcium free solution was made by elimination of CaCl$_2$ and addition of EGTA 0.5 mM. Excess calcium solution was prepared by addition of CaCl$_2$ without compensating for changes in tonicity. The final pH of solutions was 7.1 to 7.4. Incubation medium was collected at intervals of 30 or 60 min and noradrenaline output into media and final endogenous noradrenaline contents after 2 hr incubation periods were assayed fluorometrically (3).

As demonstrated in Fig. 1, when NaCl was reduced from 143 to 72, 36, 18 or 0 mM with sucrose, noradrenaline output at each incubation interval increased (upper columns) and
FIG. 1. Noradrenaline output induced by sodium reduction in rabbit atria. Upper columns demonstrate output of noradrenaline (abscissa) induced by sodium reduction with sucrose into incubation media at time intervals shown in ordinate, which is common to Fig. 2-A and B. Lower columns show endogenous contents of noradrenaline remaining after an incubation period for 2 hr. Sodium concentrations in media are shown in the ordinate, shaded columns show control, horizontal bars standard errors and parentheses numbers of estimations, which are common to Fig. 2-A and B.

As shown in Fig. 2-A, the output of noradrenaline induced by sodium deprivation with sucrose was not affected by simultaneous omission of external CaCl₂ and addition of EGTA 0.5 mM or raising calcium concentrations to 10 mM. Decreases in final endogenous noradrenaline contents were also not affected by these procedures. The result is consistent with data presented by Garcia and Kirpekar (6) in the cat spleen. On the other hand, it has been reported that in the rat heart, increases in the release and decreases in the stores of ³H-noradrenaline induced by sodium deprivation especially when replaced with choline,
FIG. 2. Effects of calcium (A) and guanethidine (B) on noradrenaline output induced by sodium reduction in rabbit atria. Simultaneously with sodium reduction, calcium was deprived or added in A and guanethidine was applied in B, the concentrations being shown in ordinate. [Ca]out 0 mM solution contained EGTA 0.5 mM. NaCl was replaced with an equiosmotic amount of sucrose. *: P<0.05.

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>[Ca]out (mM)</th>
<th>[Na]out (mM)</th>
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</thead>
<tbody>
<tr>
<td>0 - 30</td>
<td>0 (+EGTA)</td>
<td>2.5 0</td>
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<tr>
<td>10.0</td>
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<tr>
<th>Incubation Guanethidine time (min)</th>
<th>[Na]out (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 30</td>
<td>0</td>
</tr>
<tr>
<td>2 x 10^{-6}</td>
<td>2 x 10^{-5}</td>
</tr>
<tr>
<td>4 x 10^{-6}</td>
<td>36</td>
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</tbody>
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Fig. 2. Effects of calcium (A) and guanethidine (B) on noradrenaline output induced by sodium reduction in rabbit atria. Simultaneously with sodium reduction, calcium was deprived or added in A and guanethidine was applied in B, the concentrations being shown in ordinate. [Ca]out 0 mM solution contained EGTA 0.5 mM. NaCl was replaced with an equiosmotic amount of sucrose. *: P<0.05.

depend upon external calcium concentrations (4, 5). However, in rabbit atria, noradrenaline output by sodium deprivation with choline also was not significantly inhibited by calcium omission and EGTA addition 30 min prior to sodium deprivation. These results indicate that in rabbit atria, noradrenaline output induced by sodium reduction is qualitatively different from that by sympathetic nerve stimulation, which evidently depends on external calcium concentrations (7-9).

As shown in Fig. 2-B, simultaneous addition of guanethidine 4 x 10^{-6} M inhibited increases in noradrenaline output by 36 mM sodium solution at 60 to 120 min, while 2 x 10^{-5} produced no inhibition: the drug in doses higher than 2 x 10^{-5} alone increased noradrenaline output. Final endogenous noradrenaline contents were not modified by the drug in doses
of $2 \times 10^{-6}$ to $2 \times 10^{-3}$. On the other hand, guanethidine $4 \times 10^{-5}$ significantly ($P<0.05$) inhibited ($0.83 \pm 0.08 \mu g/g, n=5$) the decrease in final endogenous noradrenaline contents induced by 18 mM sodium solution ($0.59 \pm 0.07 \mu g/g$, shown in lower columns of Fig. 1), in spite of the decrease in the parameter induced by the drug $4 \times 10^{-5}$ alone ($1.21 \pm 0.11 \mu g/g, n=3$). The site of inhibitory action of guanethidine on increase in the output and decrease in endogenous contents of noradrenaline induced by sodium reduction does not appear to be an excitation-secretion coupling of adrenergic nerve endings, and the present findings are consistent with the hypothesis that guanethidine increases the permeability of the terminals to sodium ions, thereby leading to blockade (1). Both factors of possible decreases in the uptake of guanethidine in the condition of sodium deficient media (10, 11) and of increases in the output of noradrenaline by the drug alone appear to mask a clear-cut inhibitory action of the drug on the output of noradrenaline by sodium reduction. Further detailed studies concerning effects of incubation with guanethidine and modified concentrations of calcium prior to sodium reduction on the noradrenaline output are in progress in rabbit ventricular slices.

REFERENCES


INHIBITORY EFFECT OF TRICHLORMETHIAZIDE ON DEVELOPMENT OF DOC HYPERTENSION IN RATS

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Despite the popularity as an antihypertensive drug of first choice (1), the action mechanism of benzothiadiazine derivatives remains controversial (2–4). Trichlormethiazide (TCM) is a benzothiadiazine derivative with potent diuretic activity (5, 6). Although its antihypertensive effect is well documented clinically (7–10), there is apparently no documentation of its effect in experimental animals. We carried out studies on the antihypertensive effect of TCM in hypertensive rats. Diuretic activity of TCM was also observed in the course of this study.