PROTECTIVE ACTION OF GUANETHIDINE ON ATRIA ISOLATED FROM RESERPINIZED RABBITS DURING EXPOSURE TO SODIUM-DEFICIENT MEDIA

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Kubo and Misu (1) have done studies on the adrenergic neuron blockade induced by guanethidine which is restored by washing out the drug with the solution containing low sodium and accentuated with the solution containing high sodium in the perfused heart of rabbits. The hypothesis has been presented that guanethidine increases the permeability of the membrane of nerve endings to sodium ions, thereby leading to adrenergic neuron blockade. Whether or not guanethidine could protect atria from a standstill of spontaneous beating and from a decrease in the maximum rate of rise of the action potential induced by exposure to sodium-deficient media has been investigated herein.

About 15 rabbits of both sexes weighing 1.4 to 1.9 kg were used. Reserpine (3 mg/kg) was injected i.p. to eliminate sympathomimetic actions of high concentrations of guanethidine, as noradrenaline increases the upstroke velocity of the action potential (2, 3) and increases the heart rate. The atrium was isolated 18 to 24 hr after reserpinization. For recording of transmembrane potentials, the left atrium was mounted by means of stainless-steel pins on a cork plate in a shallow open plastic chamber filled with modified Tyrode's solution maintained at 30°C and bubbled with 95% O₂ and 5% CO₂. The composition of the solution was as follows (mM): NaCl 147.2, KCl 2.7, CaCl₂ 2 H₂O 1.4, MgCl₂ 6 H₂O 0.25, NaH₂PO₄ 2 H₂O 1.3, Na₂HPO₄ 12 H₂O 4.5 and glucose 5.6. The preparation was stimulated at 1 cps rectangular pulses of 1 msec and a 3 times threshold voltage generated by an electronic stimulator MSE-3R (Nihon Kohden). The glass microelectrodes filled with 3 M KCl had a resistance of 10 to 25 megohms. Intracellular action potentials were displayed on one beam of a dual-beam oscilloscope VC-7 (Nihon Kohden). Zero level and the position of an upper line of rectangular pulses applied for calibration were always adjusted to a horizontal line on the scale of the oscilloscope. The maximum rate of rise of the action potential was obtained by an electronic differentiation circuit (time constant 10 μsec) and displayed on the other beam. A solution containing 12.7 or 78.8 mM sodium was prepared by isoosmotic replacement of sodium chloride by sucrose. Student's t-test was used to evaluate data.

As demonstrated in Fig. 1, immediately after exposure to Locke-Ringer's solution containing 12.7 mM sodium, the contraction force markedly increased. On the other
FIG. 1. Typical protective effect of guanethidine on standstill time of spontaneous rhythm induced by exposure to sodium-deficient medium in atria isolated from reserpinized rabbits. At upward arrows, Locke-Ringer's solution (Na 158.9 mM) was changed by overflowing with solution containing 12.7 mM sodium. At downward arrows, guanethidine $4 \times 10^{-4}$ M was added 10 min before and immediately after overflowing. Numbers over the trace demonstrate atrial beats/min. Numbers under the trace demonstrate standstill time of atrial spontaneous beating.

TABLE 1. Protective effect of guanethidine on decreases in the maximum rate of rise and height of action potential induced by exposure to a sodium-deficient medium in electrically driven left atria isolated from reserpinized rabbits.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of potentials</th>
<th>Resting potential (mV)</th>
<th>Action potential</th>
<th>Maximum rate of rise (V/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(% change)</td>
<td>(μV) % change</td>
<td>(% change)</td>
</tr>
<tr>
<td>Before</td>
<td>28</td>
<td>$83.3 \pm 2.3^*$</td>
<td>$101.0 \pm 3.9$</td>
<td>$145.2 \pm 6.7$</td>
</tr>
<tr>
<td>78.8 mM Na-Tyrode's solution (control)</td>
<td>26</td>
<td>$82.4 \pm 1.5$</td>
<td>$87.4 \pm 1.4$</td>
<td>$153.2 \pm 6.6$</td>
</tr>
<tr>
<td>Guanethidine ($4 \times 10^{-4}$ M), 78.8 mM Na-Tyrode's solution</td>
<td>18</td>
<td>$81.2 \pm 1.9$</td>
<td>$96.8 \pm 1.7^*$</td>
<td>$162.2 \pm 8.8$</td>
</tr>
</tbody>
</table>

Solution was changed by overflowing. Exposure to Tyrode's solution containing 78.8 mM sodium was done twice in the same preparation. Guanethidine $4 \times 10^{-4}$ M was added 10 min before and immediately after the 2nd exposure. Intracellular action potentials were recorded before the 1st exposure, 10 to 25 min after the 1st (control) and the 2nd (lowest line) exposure.

* Mean ± standard error.

+ Significant difference from control at $P<0.01$.

hand, the atrial spontaneous rate decreased gradually, sometimes resulting in arrhythmia and ceased completely 11 to 13 min after exposure. Pretreatment with guanethidine $4 \times 10^{-4}$ M delayed decreases in atrial rate and prolonged a standstill time of atrial rhythm to 59 min. The standstill time thus induced by the 1st exposure to the sodium deficient medium was $13'02'' \pm 0'08''$ (min', sec'', $n=10$). It was not modified by the 2nd exposure ($14'55'' \pm 1'20'', n=3$). Guanethidine $4 \times 10^{-5}$ prolonged it in 2 out of 3 preparations (>25', >20' and 13'00''). The drug $4 \times 10^{-4}$ clearly prolonged it in every preparation tested (22'45'', >30', >30' and 59'00''). Results of electrophysiological studies obtained in 3 left atria are summarized in Table 1. After exposure to Tyrode's solution containing
78.8 mM sodium, the maximum rate of rise of the action potential markedly decreased, compared with that before exposure. Action potential amplitude slightly decreased. Resting potential and total duration at a 90° repolarized level showed no modifications. The maximum rate of rise and height of the action potential recovered almost completely 30 min after washing with a fresh solution. These results are consistent with the findings presented by other investigators (4, 5). Guanethidine 4 x 10^{-4} appeared to produce no visible modifications in action and resting potentials for at least 10 min after application. Pretreatment with the drug significantly (P<0.01) blocked decreases in the maximum rate of rise and height of the action potential induced by exposure to the sodium-deficient medium. Resting potential is one of several factors which influence the maximum rate of rise of the action potential (4, 6). In the present experiment, however, guanethidine produced no hyperpolarization.

Upstroke of the action potentials is considered to be chiefly due to a rapid influx of sodium ions (4), although part of the positive inward current noted during the upstroke may reflect calcium ion movement (7). Present observation is concerned with the effectiveness of guanethidine in protecting atria from the standstill of spontaneous beating and the decreases in the maximum rate of rise and height of action potential induced by exposure to sodium-deficient media. Results herein suggest that the drug has, at least partially, a direct positive action in increasing the permeability of the atrial membrane to sodium ions during depolarization. Further detailed investigations on the actions of guanethidine on the membrane of the atrial fibers are in progress.

REFERENCES

NARCOTIC ANTAGONIST ACTIVITY OF THE 3-PHENYLPIPERIDINES WITH N-ANTAGONISTIC SUBSTITUTION

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Since the narcotic antagonist, N-allylnorcodeine, for counteracting morphine overdose was synthetized in 1914, a number of other potentially useful known narcotic antagonists have been reported (1-4). Recently, the use of potent narcotic antagonists for treatment of narcotic abuse is being investigated in the U.S.A. Naloxone is a favorable and avail-