TABLE 1.

<table>
<thead>
<tr>
<th></th>
<th>Colonic temperature (°C)</th>
<th>Plasma NEFA (mEq/l)</th>
<th>Plasma glucose (mg/dl)</th>
<th>Plasma corticosterone (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>35.6 ± 0.317</td>
<td>0.159 ± 0.0176</td>
<td>125.6 ± 6.20</td>
<td>0.192 ± 0.0170</td>
</tr>
<tr>
<td>2. Cold-exposure</td>
<td>33.5 ± 0.273</td>
<td>0.876 ± 0.0770*</td>
<td>146.6 ± 13.12*</td>
<td>0.863 ± 0.0355</td>
</tr>
<tr>
<td>3. DMP treated</td>
<td>35.4 ± 0.305</td>
<td>0.174 ± 0.0260</td>
<td>119.0 ± 7.50</td>
<td>0.496 ± 0.0193</td>
</tr>
<tr>
<td>4. DMP treated plus cold-exposure</td>
<td>33.6 ± 0.371</td>
<td>0.496 ± 0.0152*</td>
<td>189.7 ± 12.72*</td>
<td>0.818 ± 0.0266</td>
</tr>
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</table>

Each value is the mean ± S.E. of five to six animals.

*: Significant difference: Control group (1→2) vs Experimental group (3→4)

After cold-exposure, the significant fall in colonic temperature was observed in both the intact and DMP treated rats, but there was no significant difference between them. These findings suggested that in acute cold-exposure both plasma glucose and NEFA were needed for maintaining the body temperature and therefore the significant compensatory increase in plasma glucose occurred as a consequence of the significant decrease in plasma NEFA in the DMP treated rats.

REFERENCES


THE ACUTE PARTIAL FAILURE OF NEUROMUSCULAR TRANSMISSION BY RESERPINE IN THE ISOLATED PERFUSED RABBIT'S HEART

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It has been well established that the application of reserpine in vivo leads to a prolonged decrease in endogenous noradrenaline (NA) content and reduces the responses of various kinds of sympathetically innervated organs to the nerve stimulation. However, there are few available reports about the action of reserpine in acute phase. In the present experiment, it was investigated in the isolated perfused rabbit's heart.

Langendorff's preparations with bilateral stellate ganglia were perfused with Krebs bicarbonate solution (sodium (Na) 143 mm) as described previously (1). Procedures of the electrical nerve stimulation and perfusion of reserpine (Serpasil® CIBA) solution (10⁻⁶ g/ml) are explained in Fig. 1. About one-third of the preparations was suspended by means of the perfusion apparatus (Natsumec KN-206) for recording isotonic changes in spontaneous contractile responses to the nerve stimulation or exogenously apply...
plied NA of 0.1 to 0.3 ml of $10^{-5}$, the concentration of which was selected to produce almost the same responses to those to the nerve stimulation. In the other preparations, total NA output released in venous effluent (2) by the electrical nerve stimulation and by perfusion for 2 minutes of nicotine solution ($6.6 \times 10^{-6}$) or perfusion for 4 minutes of KCl solution (100 and 300 mm) instead of electrical stimuli and endogenous NA content (3) was measured fluorometrically. Calculations of the results were performed as explained in Fig. 1.

As shown in Fig. 1-A, changes in heart rate produced by the 2nd period of the nerve stimulation decreased scarcely in control preparations. Responses in contraction force showed the same tendency to those in heart rate. On the other hand, by the 2nd nerve stimulation total NA output decreased to $37.7 \pm 5.0\%$ (Fig. 1-B) compared to that by the 1st procedure and this control value was almost the same to that described

Fig. 1. Effects of reserpine on responses of the isolated perfused rabbit's heart to the sympathetic nerve stimulation. Bilateral stellate ganglia were stimulated 6 times, each for 30 seconds at intervals of 30 seconds. The stimulation was repeated 2 times at an interval of 30 minutes. Reserpine solution ($10^{-6}$) was perfused for 20 minutes between the 1st and 2nd periods of the nerve stimulation and washed out by Krebs solution 4 to 5 minutes before the 2nd procedure to rule out a direct action to the muscular cell membrane as much as possible. Numbers of experiments are shown in parenthesis. Vertical bars show standard error.

A. On changes in heart rate.

Abscissa shows each number of the 1st and 2nd periods of the nerve stimulation. In ordinate, responses of the heart to each number of the 2nd period of the nerve stimulation and to the 2nd application of exogenous NA are expressed as a percentage of those to the 1st procedures. Broken line connecting closed circles shows control responses. Solid line connecting closed circles shows effect of reserpine, which was washed out by normal Krebs solution. Single open circle in the upper right-hand corner shows a ratio of responses to exogenously applied NA before and after perfusion of reserpine in normal Krebs solution. Chain line connecting open circles shows effect of reserpine, which was washed out by $2 \times Na$ Krebs solution. The procedure recovered significantly effect of reserpine.

B. On total NA output in perfusion medium.

In ordinate, NA output released by the 2nd period of the nerve stimulation is expressed as a percentage of that by the 1st procedure (See Text).
Previously (1). A clear parallel relationship between contractile responses to and NA output released by the nerve stimulation as described by Huković and Muscholl (4) was not found in the present experiment.

Perfusion of reserpine solution reduced significantly spontaneous beats/minute. After perfusion of reserpine, responses in heart rate to the later phase, the 5th and 6th numbers, of the 2nd period of the nerve stimulation decreased significantly compared to those in the control hearts (Fig. 1-A), and responses in contraction force decreased more clearly than those in heart rate. On the other hand, in a corresponding later phase, the sensitivity of the heart to exogenously applied NA decreased scarcely compared to control before perfusion of the drug solution (Fig. 1-A). About the action of reserpine in acute phase, von Euler (5) observed a block of neuromuscular transmission in the guinea pig's vas deferens after the application in the low concentration of the drug, suggesting that the block was functional and dependent on local deficiency of transmitter immediately available for release. However, in the isolated perfused rabbit's heart, even the higher concentration of reserpine $10^{-6}$ did not produce this kind of the complete block, but a partial failure of neuromuscular transmission. As shown in Fig. 1-B, the same perfusion of reserpine reduced significantly NA output released by the nerve stimulation by about 50% compared to the corresponding output in control hearts. The present results give a direct evidence that the major mechanism of the partial failure by reserpine derives from the decrease in NA output released by the nerve stimulation. In another series of the experiment, as shown in Table 1, endogenous NA content was reduced in whole parts of the heart, especially, significantly, in the atria by the same perfusion of reserpine in vitro.

<table>
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<tr>
<th>Procedures</th>
<th>Noradrenaline content ($\mu$g/g)</th>
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<tbody>
<tr>
<td></td>
<td>Atrium</td>
</tr>
<tr>
<td>None</td>
<td>1.73±0.17*</td>
</tr>
<tr>
<td>Control Krebs solution</td>
<td>0.85±0.04</td>
</tr>
<tr>
<td>Reserpine solution ($10^{-6}$) for 20 min</td>
<td>0.66±0.07**</td>
</tr>
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* : Mean±standard error.
** : Value differs from control at p=0.05.
Numbers of experiments are shown in parenthesis.

In next one step of the experiment, it was investigated whether the decrease in NA output released by the nerve stimulation after perfusion of reserpine might be derived from an action of the drug like as local anesthetics or membrane stabilizing agents on the neural membrane or not. If so, the partial failure by the drug might be recovered by perfusion of extracellular high Na Krebs solution, as suggested in the giant axon of the squid by Hodgkin and Katz (6). Responses in heart rate were ascertained to be used for the purpose, because external high osmotic pressure did not produce so marked depression on the parameter, compared to a very marked nonspecific depression on responses in contraction force. Perfusion of external $2 \times$ Na Krebs (286 mm) solution 4 to 5 minutes before the 2nd period of the nerve stimulation recovered significantly, in value of the 6th number of the stimulation, the partial failure by reserpine, as shown in Fig. 1-A, despite of no recovery by perfusion of Krebs solution of isoosmotic amount of sucrose (n=3). Which is a major possibility, presynaptic or postsynaptic membrane, about the result? It was confirmed as follows: the decrease in NA output released by nicotine instead of electrical stimuli, after perfusion of reserpine, could be recovered by perfusion of $2 \times$ Na Krebs solution. The 1st perfusion for 2 minutes of nicotine solution ($6.6 \times 10^{-6}$) released totally 1059.6 ng of NA on an average of 7 cases. The ratio of NA output released by the 1st and 2nd ap-
Applications of nicotine in control hearts was 95.3±14.1% (n=7), the control value of which made it easy to analyze the problem compared to that less than 1/2 by the electrical nerve stimulation. It was reduced to 17.9±3.5% (n=3) by perfusion of reserpine in normal Krebs solution (2.5×10^-6) and recovered significantly (P<0.05) to 48.1±2.3% (n=4), after perfusion of the same dose of reserpine in 2×Na Krebs solution for 20 minutes, then by perfusion of 2×Na Krebs solution without the drug 4 to 5 minutes before the 2nd application of nicotine, despite of no recovery by perfusion of Krebs solution of isoosmotic amount of glucose (300 mm) (n=2).

Furthermore, the ratio of NA output released by the 1st and 2nd applications of 100 and 300 mm KCl solutions in untreated hearts was 124.1±7.2% (n=3) and 109.9±9.0% (n=3), respectively. The ratio by 100 mm KCl was reduced markedly to 32.9±9.3% (n=3) by perfusion of reserpine solution (10^-6). The ratio by 300 mm KCl, however, was reduced scarcely (80.7±23.8%, n=3) by the same procedure. Reserpine produced a ceiling effect on the decrease in NA output released by the higher concentration of KCl.

Judging from the present results, it was concluded that the major mechanism of the acute partial failure of neuromuscular transmission by reserpine in the rabbit’s heart was the decrease in NA output released by the sympathetic nerve stimulation, which derived mainly from a membrane stabilizing action of the drug on the neural membrane. It should be investigated in future study how the action of reserpine in the acute phase is correlated with that of the application of the drug in vivo or not.

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REFERENCES

TERATOGENIC ACTION OF ADENINE* ON MOUSE EMBRYOS

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Several purine analogues are reported to show a teratogenic action in mammalian embryos when administered at calculated dosage [reviewed by Giroud and Tuchmann-Duplessis (1), Cahn (2) and Nishimura (3, 4); Kury et al. (5)].

We established that some methylated xanthines such as theophylline, theobromine and caffeine induce skeletal defects and subcutaneous hematoma in mice (6). Hypoxanthine also caused skeletal malformations and hematomas as well as the resorption of embryos (7).

As methylated xanthines are also purine derivatives, it is of interest to study which, xanthine nucleus in

* Adenine was manufactured by Sigma Chemical Company (St. Louis, U.S.A.) and obtained through Katayama Chemical Company (Osaka, Japan).