EFFECTS OF AMINOGLYCOSIDE ANTIBIOTICS ON THE CONTRACTILE RESPONSE OF GUINEA-PIG URINARY BLADDER

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The present study was undertaken to investigate the effects of kanamycin (KM), aminodeoxy kanamycin (AKM), and ribostamycin (RSM) that belong to the aminoglycoside antibiotics on the contractile response of guinea-pig urinary bladder.

KM (5 x 10^-3 g/ml-1 x 10^-3 g/ml), AKM (5 x 10^-3 g/ml-1 x 10^-3 g/ml), and RSM (1 x 10^-4 g/ml-1 x 10^-3 g/ml) dose-dependently reduced the contractile responses induced by transmural electrical stimulation, respectively. In particular, these drugs in a concentration of 1 x 10^-3 g/ml remarkably reduced them, namely to 48±22% (n=10), 21±13% (n=10), and 53±15% (n=10) of the control, respectively. On the other hand, both KM (1 x 10^-8 g/ml) and AKM (1 x 10^-3 g/ml) slightly reduced the exogenous acetylcholine (1 x 10^-3 g/ml and 1 x 10^-3 g/ml)-induced contractile responses, while RSM (1 x 10^-8 g/ml) had almost no effect on them. These drugs in that concentration had almost no effect on the amplitude and frequency of spontaneous contractile response, while they slightly reduced the tonus level of the tissue. These phenomena which were observed as their effects on the spontaneous contractile response were also observed in the presence of tetrodotoxin (1 x 10^-7 g/ml). From these findings, it was concluded that KM, AKM, and RSM may affect on the intramural excitatory nerves but also on the muscle in the urinary bladder wall. It was also suggested that the effects of these agents are greater on the former than they are on the latter.

INTRODUCTION

Very few report is known concerning the effects of aminoglycoside antibiotics on the organs which have smooth muscle, while it has been reported that these antibiotics directly act on the muscle and reduce its contractile response\(^{1-4}\). In general, aminoglycoside antibiotics are not metabolized in the body and excreted intact in the urine\(^5\). In the present study, we investigated the effects of several aminoglycoside antibiotics (kanamycin, aminodeoxy kanamycin, and ribostamycin) on the contractile response of guinea-pig urinary bladder which were produced by transmural electrical stimulation and exogenous acetylcholine, which were induced spontaneously.

I. MATERIALS AND METHODS

Guinea-pig weighing 250~450 g stunned and bled to death. The urinary bladder was quickly removed and divided into two approximately equal parts, longitudinally, namely from the top to the fundus of urinary bladder. One of these pieces was used in an experiment. The preparation was mounted in an organ bath. As described with regard to the gall bladder by YOSHIDA and ISHIURA (1981)\(^6\), the fundus of the urinary bladder was fixed with pins and the top end was connected with a silk thread to a force-displacement transducer. The mechanical response of the urinary bladder were recorded isometrically under a load of 1 g. The preparation was bathed in the Krebs solution which has the following composition (mM): 133.5 NaCl, 4.7
KCl, 2.5 CaCl$_2$, 1.4 Na$_2$HPO$_4$, 16.3 NaHCO$_3$, and 7.8 glucose. This specimen was perfused with 95% O$_2$ and 5% CO$_2$, and pH was adjusted to 7.4. Both contractile response to transmural electrical stimulation and exogenous acetylcholine were observed in 23°C Krebs solution in order to prevent the appearance of spontaneous contractile response of the tissue. Observation relating to the spontaneous contractile response of the tissue was performed in 37°C Krebs solution. Two 5 mm x 5 mm silver plates coated with silver chloride were used as stimulus electrodes. As mentioned by YOSHIDA and ISHIURA (1981), the electrodes were placed, one on the serosal side and the other on the mucosal side of the tissue and connected to a stimulator. Tissue was stimulated transmurally with rectangular pulses (40 volt, 0.5 msec, 20 Hz) for a period of 5 sec at intervals of 5 minutes. The used drugs were: kanamycin sulfate (KM), $1 \times 10^{-8}$ g/ml-1 x $10^{-5}$ g/ml; aminodeoxy kanamycin sulfate (AKM), $1 \times 10^{-5}$ g/ml-1 x $10^{-3}$ g/ml; ribostamycin sulfate (RSM), $5 \times 10^{-3}$ g/ml-1 x $10^{-2}$ g/ml; acetylcholine chloride, $1 \times 10^{-4}$ g/ml and $1 \times 10^{-6}$ g/ml; tetrodotoxin (TTX), $1 \times 10^{-7}$ g/ml. The concentration indicate the final values in the organ bath. The concentration of KM, AKM, and RSM indicate the values which were converted to the potencies of antibiotics. The potencies of KM, AKM, and RSM used in this experiment are 673 µg/mg, 714 µg/mg, and 686 µg/mg, respectively.

II. RESULTS

Effects of KM, AKM, and RSM on the contractile response induced by transmural electrical stimulation. A monophasic contractile response was evoked by a transmural electrical stimulation. KM ($1 \times 10^{-8}$ g/ml) did not affect the monophasic contractile responses, while KM ($5 \times 10^{-5}$ g/ml-1 x $10^{-3}$ g/ml) dose-dependently reduced them (Fig.1). These reducing effects of KM ($5 \times 10^{-3}$ g/ml-5 x $10^{-4}$ g/ml) were immediately restored by washing with normal Krebs solution, while the reducing effect of KM ($1 \times 10^{-4}$ g/ml) was not well-restored by washing with normal Krebs solution (Fig.1).

AKM ($1 \times 10^{-5}$ g/ml) did not affect the transmural electrical stimulation-induced contractile responses, while AKM ($5 \times 10^{-5}$ g/ml-1 x $10^{-4}$ g/ml) dose-dependently reduced them (Fig.2). These reducing effects of AKM ($5 \times 10^{-3}$ g/ml-1 x $10^{-4}$ g/ml) were immediately restored by washing with normal Krebs solution, while those of AKM ($5 \times 10^{-4}$ g/ml and $1 \times 10^{-8}$ g/ml) were not well-restored by washing with normal Krebs solution (Fig.2).

RSM ($5 \times 10^{-4}$ g/ml) did not affect the transmural electrical stimulation-induced contractile responses, while RSM ($1 \times 10^{-4}$ g/ml-1 x $10^{-2}$ g/ml) dose-dependently reduced them (Fig.3). These reducing effects were immediately restored by washing with normal Krebs solution (Fig.3).

These reducing effects of KM, AKM, and RSM approximately reached a steady state in 14 to 29 minutes after addition of these drugs (Fig.1,2 and 3). When, therefore, each amplitude of transmural electrical stimulation-induced contractile response before treatment of KM, AKM, and RSM...
Fig.2 Effects of AKM on the contractile response induced by transmural electrical stimulation.

In all tracings, transmural electrical stimulation was given at dots beneath each tracing. 1-5: effects of AKM (1 x 10^{-4} g/ml-1 x 10^{-8} g/ml). Each right side tracing of 2-5 shows the phenomenon after washing with normal Krebs solution.

Fig.3 Effects of RSM on the contractile response induced by transmural electrical stimulation.

In all tracings, transmural electrical stimulation was given at dots beneath each tracing. 1-4: effects of RSM (5 x 10^{-1} g/ml-1 x 10^{-8} g/ml). Each right side tracing of 2-4 shows the phenomenon after washing with normal Krebs solution.

In each concentration, namely the control, was taken as 100%, its amplitude after 29 minutes treatment of each drug in each concentration were taken as a relative contractile response of 100%. These results were observed as shown in Table 1. In particular, KM (1 x 10^{-3} g/ml), AKM (1 x 10^{-3} g/ml), and RSM (1 x 10^{-3} g/ml) remarkably reduced the transmural electrical stimulation-induced contractile responses as shown in Fig.1, 2, 3 and Table 1. That is to say, these agents reduced them to 48±22% (n=10), 21±13% (n=10), and 53±15% (n=10) of the controls, respectively (Table 1). Thus the reducing effects of these agents on the transmural electrical stimulation-induced contractile response were in the following order: AKM>RSM.

Effects of KM, AKM, and RSM on the contractile response induced by exogenous acetylcholine. Since the effects of KM, AKM, and RSM on the exogenous acetylcholine-induced contractile response may be influenced by the concentration of exogenous acetylcholine, acetylcholines in both concentrations of 1 x 10^{-4} g/ml and 1 x 10^{-3} g/ml were used in this experiment. In this experiment, acetylcholine was added in an organ bath after 29 min treatment of each agent, because the reducing effects of each agent on the transmural electrical stimulation-induced contractile response approximately reached a steady state in 14 to 29 minutes after treatment of each agent. Thus the effects of KM, AKM, and RSM on the contractile response induced by exogenous acetylcholine were observed as shown in Fig.4 and Table 2. KM (1 x 10^{-3} g/ml) slightly reduced the contractile response induced by exogenous acetylcholine (1 x 10^{-4}
### Table 1
Effects of KM, AKM, and RSM on the contractile response induced by tranamural electrical stimulation.

Each amplitude of tranamural electrical stimulation-induced contractile response in the absence of KM, AKM, and RSM in each concentration, namely control, was taken as 100%. Each amplitude of tranamural electrical stimulation-induced contractile response at 29 min after addition of each agent in each concentration was registered as a relative contractile response of 100%. Each value represents a mean with S.D. of 10 experiments. *, **, ***: Significantly different from control at P<0.05, P<0.01 and P<0.001, respectively.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration (g/ml)</th>
<th>Amplitude of contractile response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM</td>
<td>1 × 10⁻⁵</td>
<td>100 ± 3</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁵</td>
<td>91 ± 5*</td>
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<tr>
<td></td>
<td>1 × 10⁻⁴</td>
<td>84 ± 6**</td>
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<td></td>
<td>5 × 10⁻⁴</td>
<td>66 ± 14***</td>
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<tr>
<td></td>
<td>1 × 10⁻³</td>
<td>48 ± 22***</td>
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<tr>
<td>AKM</td>
<td>1 × 10⁻⁵</td>
<td>100 ± 3</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁵</td>
<td>90 ± 2*</td>
</tr>
<tr>
<td></td>
<td>1 × 10⁻⁴</td>
<td>74 ± 12***</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁴</td>
<td>33 ± 14***</td>
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<tr>
<td></td>
<td>1 × 10⁻³</td>
<td>21 ± 13***</td>
</tr>
<tr>
<td>RSM</td>
<td>5 × 10⁻⁵</td>
<td>100 ± 3</td>
</tr>
<tr>
<td></td>
<td>1 × 10⁻⁴</td>
<td>92 ± 5*</td>
</tr>
<tr>
<td></td>
<td>5 × 10⁻⁴</td>
<td>77 ± 11***</td>
</tr>
<tr>
<td></td>
<td>1 × 10⁻³</td>
<td>53 ± 15***</td>
</tr>
</tbody>
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### Table 2
Effects of KM, AKM, and RSM on the contractile response induced by exogenous acetylcholine.

A: effect of each agent (1 × 10⁻³ g/ml) on the exogenous acetylcholine (1 × 10⁻⁴ g/ml)-induced contractile response. B: effect of each agent (1 × 10⁻³ g/ml) on the exogenous acetylcholine (1 × 10⁻⁵ g/ml)-induced contractile response. Each amplitude of exogenous acetylcholine-induced contractile response in the absence of each agent (1 × 10⁻³ g/ml), namely control, was taken as 100%. Each amplitude of exogenous acetylcholine-induced contractile response at 29 min after addition of each agent (1 × 10⁻³ g/ml) was registered as a relative contractile response of 100%. Each value represents a mean with S.D. of 10 experiments. *, **, ***: Significantly different from control at P<0.05, P<0.01 and P<0.001, respectively.

#### A: Exogenous acetylcholine (1 × 10⁻⁴ g/ml)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration (g/ml)</th>
<th>Amplitude of contractile response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM</td>
<td>1 × 10⁻³</td>
<td>93 ± 6*</td>
</tr>
<tr>
<td>AKM</td>
<td>1 × 10⁻³</td>
<td>90 ± 6***</td>
</tr>
<tr>
<td>RSM</td>
<td>1 × 10⁻³</td>
<td>100 ± 6</td>
</tr>
</tbody>
</table>

#### B: Exogenous acetylcholine (1 × 10⁻⁵ g/ml)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration (g/ml)</th>
<th>Amplitude of contractile response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KM</td>
<td>1 × 10⁻³</td>
<td>94 ± 6*</td>
</tr>
<tr>
<td>AKM</td>
<td>1 × 10⁻³</td>
<td>88 ± 7**</td>
</tr>
<tr>
<td>RSM</td>
<td>1 × 10⁻³</td>
<td>103 ± 4</td>
</tr>
</tbody>
</table>
Fig. 4 Effects of KM, AKM, and RSM on the contractile response induced by exogenous acetylcholine.

A: effects of KM, AKM, and RSM in the concentration of $1 \times 10^{-8}$ g/ml on the contractile response induced by exogenous acetylcholine ($1 \times 10^{-4}$ g/ml). B: effects of KM, AKM, and RSM in the concentration of $1 \times 10^{-8}$ g/ml on the contractile response induced by exogenous acetylcholine ($1 \times 10^{-4}$ g/ml).

In all tracings, acetylcholine was given at a black triangle beneath each tracing. KM, AKM, and RSM were given 29 min before addition of acetylcholine, respectively. Each left side tracing of 1, 2, and 3 in both A and B shows the contractile response in the absence of each agent, namely control. Each middle tracing of 1, 2, and right side tracing of S in both A and B shows the contractile response in the presence of each agent. Each right side tracing of 1 and 2 in both A and B shows the contractile response which was observed at 20 min after washing with normal Krebs solution.

$g/\text{ml}$, that is, to 93±6% (n=10) of the control (Fig. 4-1 and Table 2). AKM ($1 \times 10^{-5}$ g/ml) slightly reduced it, that is, to 90±5% (n=10) of the control (Fig. 4-2 and Table 2). These reducing effects were immediately restored by washing with normal Krebs solution (Fig. 4). As shown in Fig. 4, RSM ($1 \times 10^{-3}$ g/ml) did not affect the contractile response induced by exogenous acetylcholine ($1 \times 10^{-4}$ g/ml). That is to say, the amplitude of contractile response induced by exogenous acetylcholine ($1 \times 10^{-4}$ g/ml) after treatment of RSM ($1 \times 10^{-3}$ g/ml) was 100±6% (n=10) (Table 2). This value almost equal to the value of control (100%). In addition, effect of each agent in concentration of $1 \times 10^{-4}$ g/ml on the exogenous acetylcholine ($1 \times 10^{-4}$ g/ml-induced contractile response was almost similar to that observed as the effect of each agent in that concentration on the exogenous acetylcholine ($1 \times 10^{-4}$ g/ml)-induced contractile response (Fig. 4 and Table 2).

Effects of KM, AKM, and RSM on the spontaneous contractile response. KM ($1 \times 10^{-8}$ g/ml), AKM ($1 \times 10^{-5}$ g/ml), and RSM ($1 \times 10^{-8}$ g/ml) had almost no effect on the amplitude and frequency of spontaneous contractile response, while these agents slightly reduced the tonus level of the tissue (Fig. 5). As shown in Fig. 5, these phenomena were also observed when each agent was added in the presence of tetrodotoxin ($1 \times 10^{-7}$ g/ml).

III. DISCUSSION

In general, aminoglycoside antibiotics are not metabolized in the body and excreted intact in the urine. When KM, AKM, and RSM that belong to aminoglycoside antibiotics were intramuscularly administrated to man at a clinical dosage, the concentration of each drug in the blood and urine was observed by several workers. That is to say,
CHEMOTHERAPY

Fig. 5 Effects of KM, AKM, and RSM on the spontaneous contractile response.

1, 3, 5: effect of each agent (1x10^{-3} g/ml).
2, 4, 6: effect of each agent (1x10^{-4} g/ml) in the presence of tetrodotoxin (1x10^{-7} g/ml).

Tetrodotoxin (TTX) which was indicated with an arrow was given 15 min before addition of each agent.

The authors, again, have previously suggested that the exogenous acetylcholine-induced contractile response is induced by the action of acetylcholine to the muscle in the urinary bladder regardless of intramural nerves. In the present study, effects of KM, AKM, and RSM on a property mentioned above were observed.

KM (5x10^{-4} g/ml-1x10^{-6} g/ml), AKM (5x10^{-4} g/ml-1x10^{-4} g/ml) dose-dependently reduced the transmural electrical stimulation-induced contractile response (Fig. 1, 2, 3 and Table 1). In particular, KM and AKM in concentration of 1x10^{-4} g/ml remarkably reduced them, that is, to 48±22% (n=10), 21±13% (n=10), and 53±15% (n=10) of control, respectively (Fig. 1, 2, 3 and Table 1). The reducing effects of RSM were immediately restored by washing with normal Krebs solution while those of KM and AKM were not well-stored (Fig. 1, 2, 3). On the other hand, KM (1x10^{-3} g/ml) and AKM (1x10^{-3} g/ml) slightly reduced the exogenous acetylcholine (1x10^{-4} g/ml)-induced contractile response (Fig. 4 and Table 2). That is to say, KM (1x10^{-4} g/ml) reduced it to 93±6% (n=10) of the control (Table 2). Also, AKM (1x10^{-4} g/ml) reduced it to 90±1% (n=10) of the control (Table 2). These reducing effects were immediately restored by washing with normal Krebs solution (Fig. 4). And RSM (1x10^{-3} g/ml) did not affect the exogenous acetylcholine (1x10^{-4} g/ml)-induced contractile response (Fig. 4). That is to say, the amplitude of exogenous acetylcholine (1x10^{-4} g/ml)-induced contractile response after treatment of RSM (1x10^{-3} g/ml) was 100±6% (n=10) (Table 2). This value was almost equal to the value of the control (100%).

In addition, effect of each agent in concentration 1x10^{-3} g/ml on the exogenous acetylcholine (1x10^{-4} g/ml)-induced contractile response was almost equal to that observed as the effect of each agent in that concentration on the exogenous acetylcholine (1x10^{-4} g/ml)-induced contractile response (Fig. 4 and Table 2). From these results, it can be suggested that both KM and AKM affect the intramural excitatory nerves but also on muscle in the urinary bladder wall, while RSM effect on the intramural excitatory nerves. It should be considered, moreover, that the effects of these agents are greater on the intramural excitatory nerves than they are on the muscle. And

The highest concentration of these drugs in the blood was in the range of about 19 µg/ml to 80 µg/ml [14]. That in the urine, however, was in the range of about 750 µg/ml to 1,450 µg/ml [12-14]. It is thus demonstrated that the excretion of these drugs is carried out in a good condition. We can not yet, however, see the reports concerning the effects of these drugs on the motility of urinary bladder. The present study was, thus, undertaken to investigate the effects of these drugs on the contractile response of the urinary bladder.

The authors have previously shown that the most part of the transmural electrical stimulation-induced contractile response may be induced by release of acetylcholine from ending of intramural cholinergic excitatory nerves, and the intramural non-cholinergic excitatory nerves may produce only a slight contractile response to transmural electrical stimulation, namely it is about 25% of the contractile response to transmural electrical stimulation [10].
effects of these agents on the muscle may be very weak. And also, RSM seems to have no effect on the muscle, while it remarkably affects the intramural excitatory nerves. As mentioned above previously, intramural cholinergic excitatory nerves may produce the most part of the contractile response to transmural electrical stimulation, and intramural non-cholinergic excitatory nerves may produce only a slight contractile response to it. Therefore, KM, AKM, and RSM may mainly act on the intramural cholinergic excitatory nerves. It can be supposed that these agents may also act on the intramural non-cholinergic excitatory nerves. This possibility, however, is not revealed by the present study alone. In addition, as shown in Table 1, Table 2 and above mentioned results, the reducing effects of KM, AKM, and RSM on both transmural electrical stimulation and exogenous acetylcholine-induced contractile responses were in the following order: AKM > KM > RSM. Thus the effects of these agents on the intramural excitatory nerves and the muscle in the urinary bladder wall may be in stronger order of AKM > KM > RSM. KM (1×10^-3 g/ml), AKM (1×10^-3 g/ml), and RSM (1×10^-3 g/ml) had almost no effect on the amplitude and frequency of spontaneous contractile response, while these agents slightly reduced the tonus level of the tissue (Fig.5). As shown in Fig.5, these phenomena were also observed when each agent was added in the presence of tetrodotoxin (1×10^-7 g/ml). It is, therefore, possible that this reducing effect of each agent on the tonus level of the tissue is mediated by the direct action of each agent on the muscle in the urinary bladder wall. In 23°C Krebs solution, however, such a change of the tonus level of the tissue was not observed after addition of these agents (Fig.1,2,3). Thus the effects of these agents on the tonus level of the tissue may depend on the temperature. In addition, the authors previously mentioned that RSM did not affect the muscle, while the above mentioned results indicate that RSM also may act on the muscle. Moreover, these results which were observed as the effects of KM, AKM, and RSM on the spontaneous contractile response may be another evidence to support that the effects of these agents on the muscle are very weak. There are reports concerning the effects of KM^{10}, AKM^{11} and RSM^{12-14}. These reports suggest that these agents directly act on the smooth muscle and reduce its contractile response. The same view was observed in the present study. But it was also suggested that these agents act on the intramural excitatory nerves in the urinary bladder wall and reduce the contractile response of the tissue mediated by release of transmitter from the ending of intramural excitatory nerves.

From all findings, it may be concluded that KM, AKM, and RSM affect not only on the intramural excitatory nerves but also on the muscle in the urinary bladder wall. It also seems possible that the effects of these agents are greater on the former than they are on the latter.

References


モルモット膀胱の収縮反応に及ぼす aminoglycoside antibiotics の影響

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小枝武美
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モルモット膀胱の収縮反応に及ぼす kanamycin (KM), aminodeoxy kanamycin (AKM) および ribostamycin (RSM) の影響について検討した。

KM (5x10⁻⁴g/ml-1x10⁻⁴g/ml), AKM (5x10⁻⁴g/ml-1x10⁻³g/ml) および RSM (1x10⁻⁴g/ml-1x10⁻³g/ml) は、各々、濃度依存的に、経摂刺激により誘発される収縮反応を減弱させ、特に 1x10⁻³g/ml の濃度において、その減弱作用が著しかった。また、その作用は AKM>KM>RSM の順であった。1x10⁻⁴g/ml の濃度の KM および AKM は、各々、1x10⁻⁴g/ml あるいは 1x10⁻³g/ml の外来性 acetylcholine により誘発される収縮反応をわずかに減弱させたが、1x10⁻³g/ml の濃度の RSM は、それらに対して、ほとんど影響を示さなかった。また、これら 3 薬物 (1x10⁻³g/ml) は、各々、自動収縮の頻度や振幅に対して、ほとんど認めべき影響を示さなかったが、いずれも、組織の tonus をわずかに低下させた。この tonus の低下は tetrodotoxin (1x10⁻⁷g/ml) 存在下でも同様に観察された。

以上より、KM, AKM および RSM は、膀胱の壁内神経に対して作用を及ぼすと共に、筋に対しても、わずかに作用を及ぼすものと思われる。