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 (5×10^{-5} g/ml-1×10^{-3} g/ml), AKM (5×10^{-5} g/ml-1×10^{-3} g/ml), and RSM (1×10^{-4} g/ml-1×10^{-3} g/ml) dose-dependently reduced the contractile responses induced by transmural electrical stimulation, respectively. In particular, these drugs in a concentration of 1×10^{-3} g/ml remarkably reduced them, namely to $48 \pm 22\%$ (n=10), $21 \pm 13\%$ (n=10), and $53 \pm 15\%$ (n=10) of the control, respectively. On the other hand, both KM (1×10^{-3} g/ml) and AKM (1×10^{-3} g/ml) slightly reduced the exogenous acetylcholine (1×10^{-4} g/ml and 1×10^{-5} g/ml)-induced contractile responses, while RSM (1×10^{-3} 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 (1×10^{-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 CaCl₂, 1.4 NaH₂PO₄, 16.3 NaHCO₃, and
7.8 glucose. This specimen was perfused with
95% O₂ and 5% CO₂, and pH was adjusted to 7.4.
Both contractile response to transmural electrical
stimulation and exogenous acetylcholine were ob-
served in 23°C Krebs solution in order to prevent
the appearance of spontaneous contractile response
of the tissue. Observation relating to the spon-
taneous contractile response of the tissue was per-
formed in 37°C Krebs solution. Two 5 mm × 5 mm
silver plates coated with silver chloride were used
as stimulus electrodes. As mentioned by YOSHIDA
and ISHIURA (1981)[6], the electrodes were placed,
one on the serosal side and the other on the mu-
cosal side of the tissue and connected to a stimula-
tor. Tissue was stimulated transmurally with rec-
tangular 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
×10⁻⁵ g/ml–1×10⁻³ g/ml; aminodeoxy kanamycin
sulfate (AKM), 1×10⁻⁵ g/ml–1×10⁻³ g/ml; ribos-
tamycin sulfate (RSM), 5×10⁻⁵ g/ml–1×10⁻³ g/
ml; acetylcholine chloride, 1×10⁻⁴ g/ml and 1×
10⁻⁵ g/ml; tetrodotoxin (TTX), 1×10⁻⁷ 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 675
μg/mg, 714 μg/mg, and 686 μg/mg, respectively.

II. RESULTS

**Effects of KM, AKM, and RSM on the con-
tractile response induced by transmural elec-
trical stimulation.** A monophasic contractile
response was evoked by a transmural electrical
stimulation. KM (1×10⁻⁵ g/ml) did not affect the
monophasic contractile responses, while KM (5×
10⁻⁵ g/ml–1×10⁻³ g/ml) dose-dependently reduced
them (Fig. 1). These reducing effects of KM (5×
10⁻⁵ g/ml–5×10⁻⁴ g/ml) were immediately restored
by washing with normal Krebs solution, while
the reducing effect of KM (1×10⁻³ g/ml) was not
well-restored by washing with normal Krebs solu-
tion (Fig. 1).

AKM (1×10⁻⁵ g/ml) did not affect the transmural
electrical stimulation-induced contractile responses,
while AKM (5×10⁻⁵ g/ml–1×10⁻³ g/ml) dose-de-
pendently reduced them (Fig. 2). These reducing
effects of AKM (5×10⁻⁵ g/ml–1×10⁻⁴ g/ml) were
immediately restored by washing with normal

Fig. 1 Effects of KM on the contractile response
induced by transmural electrical stimula-
tion.

In all tracings, transmural electrical stimula-
tion was given at dots beneath each tracing.
1–5: effects of KM (1×10⁻⁵ g/ml–1×10⁻³ g/ml).
Each right side tracing of 2–5 shows the phe-
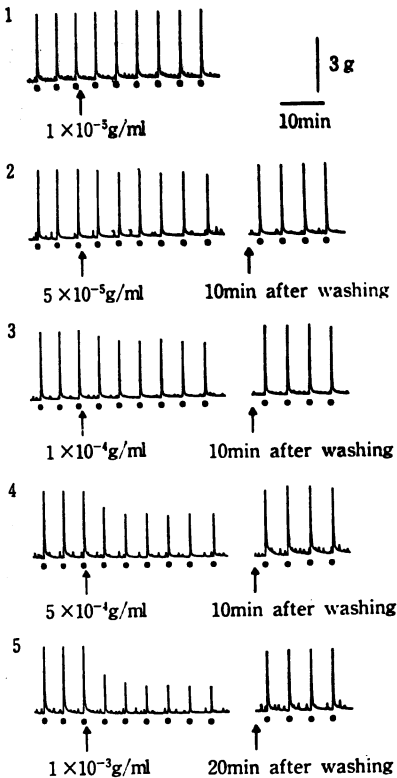nomenon after washing with normal Krebs
solution.
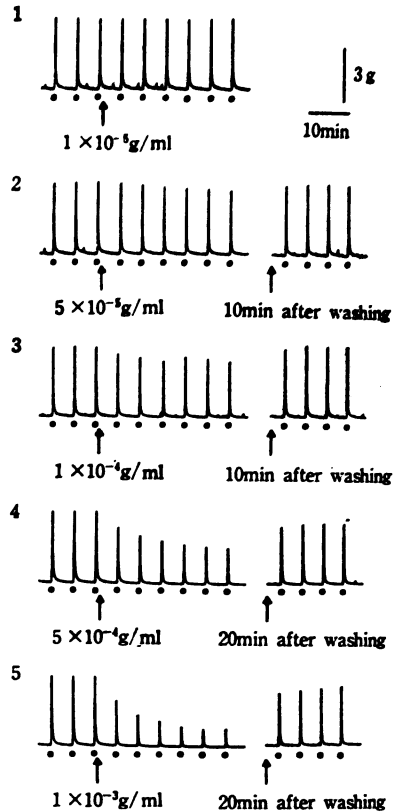
Krebs solution, while those of AKM (5×10⁻⁴ g/ml
and 1×10⁻³ g/ml) were not well-restored by wash-
ing with normal Krebs solution (Fig. 2).

RSM (5×10⁻⁵ g/ml) did not affect the transmural
electrical stimulation-induced contractile responses,
while RSM (1×10⁻⁴ g/ml–1×10⁻³ g/ml) dose-de-
pendently 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 20
minutes after addition of these drugs (Fig. 1, 2 and
3). When, therefore, each amplitude of trans-
mural electrical stimulation-induced contractile re-
sponse before treatment of KM, AKM, and RSM

Fig. 2 Effects of AKM on the contractile response induced by transmural electrical stimulation.
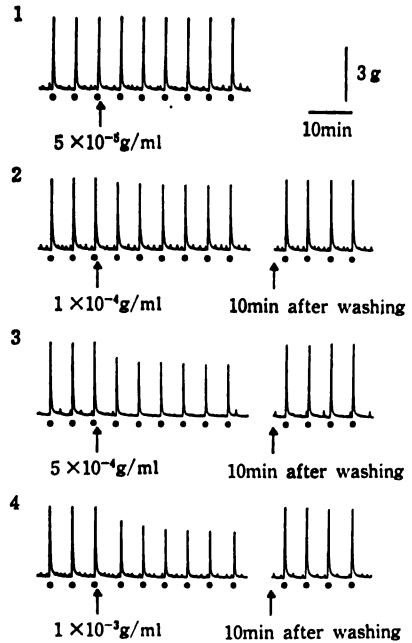
In all tracings, transmural electrical stimulation was given at dots beneath each tracing. 1–5: effects of AKM (1×10^{-5} g/ml–1×10^{-3} g/ml). Each right side tracing of 2–5 shows the phenomenon after washing with normal Krebs solution.

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 (5×10^{-5} g/ml–1×10^{-3} g/ml). Each right side tracing of 2–4 shows the phenomenon after washing with normal Krebs solution.

KM > 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 1×10^{-4} g/ml and 1×10^{-5} 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 (1×10^{-3} g/ml) slightly reduced the contractile response induced by exogenous acetylcholine (1×10^{-4}

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 (1×10^{-3} g/ml), AKM (1×10^{-3} g/ml), and RSM (1×10^{-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 $48 \pm 22\%$ (n=10), $21 \pm 13\%$ (n=10), and $53 \pm 15\%$ (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 >

Table 1   Effects of KM, AKM, and RSM on the contractile response induced by transmural electrical stimulation.

     Each amplitude of transmural 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 transmural 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.

Agent	Concentration (g/ml)	Amplitude of contractile response (%)
KM	1×10^{-5}	100 ± 3
	5×10^{-5}	91 ± 5*
	1×10^{-4}	84 ± 6**
	5×10^{-4}	66 ± 14***
	1×10^{-3}	48 ± 22***
AKM	1×10^{-5}	100 ± 3
	5×10^{-5}	90 ± 2*
	1×10^{-4}	74 ± 12***
	5×10^{-4}	33 ± 14***
	1×10^{-3}	21 ± 13***
RSM	5×10^{-5}	100 ± 3
	1×10^{-4}	92 ± 5*
	5×10^{-4}	77 ± 11***
	1×10^{-3}	53 ± 15***

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)

| Agent	Concentration (g/ml)	Amplitude of contractile response (%)
KM	1×10^{-3}	93 ± 6*
AKM	1×10^{-3}	90 ± 6***
RSM	1×10^{-3}	100 ± 6

B : Exogenous acetylcholine (1×10^{-5}g/ml)

Agent	Concentration (g/ml)	Amplitude of contractile response (%)
KM	1×10^{-3}	94 ± 6*
AKM	1×10^{-3}	88 ± 7**
RSM	1×10^{-3}	103 ± 4

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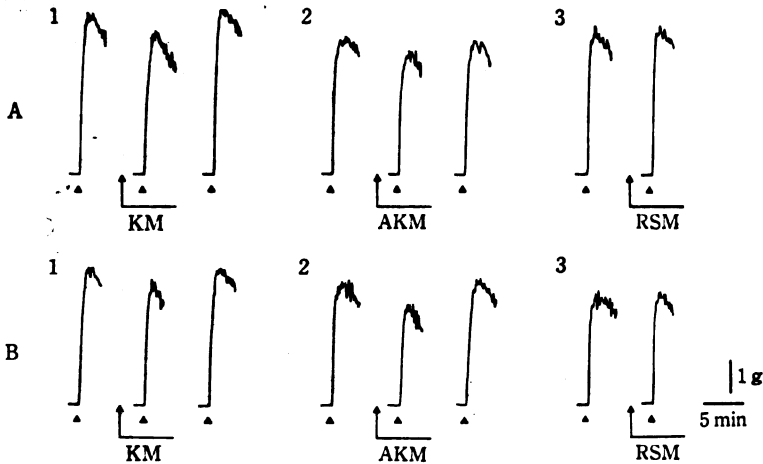
g/ml), that is, to $93 \pm 6\%$ (n=10) of the control (Fig. 4-1 and Table 2). AKM (1×10^{-3} g/ml) slightly reduced it, that is, to $90 \pm 6\%$ (n=10) of the control (Fig. 4-2 and Table 2). These reducing effects were immediately restored by washing with normal Krebs solution (Fig. 4). As shown in Fig.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

(Fig. 4 and Table 2).

Effects of KM, AKM, and RSM on the spontaneous contractile response. KM (1×10^{-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. 5-1, 3, 5). As shown in Fig. 5-2, 4, 6., these phenomena were also observed when each agent was added in the presence of tetrodotoxin (1×10^{-7} g/ml).

III. DISCUSSION

In general, aminoglycoside antibiotics are not metabolized in the body and excreted intact in the urine[5]. When KM, AKM, and RSM that belong to aminoglycoside antibiotics were intramuscularly administered to man at a clinical dosage, the concentration of each drug in the blood and urine was observed by several workers[7-15]. That is to say,

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

1, 3, 5 : effect of each agent (1×10^{-3} g/ml).

2, 4, 6 : effect of each agent (1×10^{-3} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml). Tetrodotoxin (TTX) which was indicated with an arrow was given 15 min before addition of each agent.
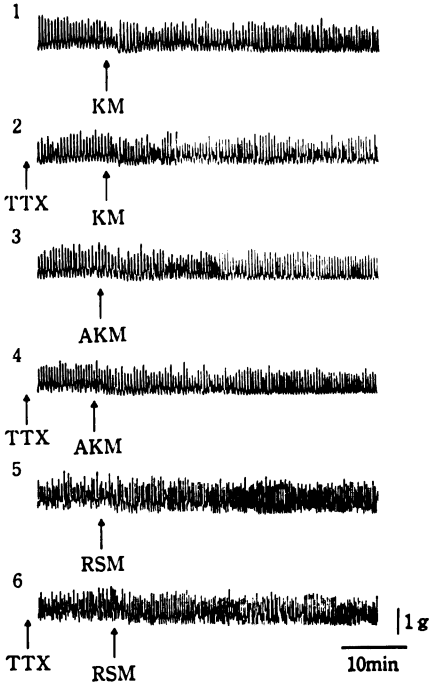
the highest concentration of these drugs in the blood was in the range of about 19 μg/ml to 80 μg/ml[7~14]. That in the urine, however, was in the range of about 750 μg/ml to 1,450 μg/ml[12~15]. It is thus demonstrated that the excretion of these drugs is carried out in a good condition. We can not yet, 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 23% of the contractile response to transmural electrical stimulation[16].

The authors, again, have previously sugges that the exogenous acetylcholine-induced contr tile response is induced by the action of acetylc line to the muscle in the urinary bladder w regardless of intramural nerves[16]. In the pres study, effects of KM, AKM, and RSM on e property mentioned above were observed.

KM (5×10^{-5} g/ml-1×10^{-3} g/ml), AKM (5×1 g/ml-1×10^{-3} g/ml), and RSM (1×10^{-4} g/ml- 10^{-3} g/ml) dose-dependently reduced the transmu electrical stimulation-induced contractile respon (Fig. 1, 2, 3 and Table 1). In particular, K AKM, and RSM in concentration of 1×10^{-3} g remarkably reduced them, that is, to 48±22% (10), 21±13% (n=10), and 53±15% (n=10) of control, respectively (Fig. 1, 2, 3 and Table The reducing effects of RSM were immediately stored by washing with normal Krebs soluti while those of KM and AKM were not well stored (Fig. 1, 2, 3). On the other hand, KM (10^{-3} g/ml) and AKM (1×10^{-3} g/ml) slightly duced the exogenous acetylcholine (1×10^{-4} g/m induced contractile response (Fig. 4 and Table That is to say, KM (1×10^{-3} g/ml) reduced it 93±6% (n=10) of the control (Table 2). A also, AKM (1×10^{-3} g/ml) reduced it to 90± (n=10) of the control (Table 2). These reduc effects were immediately restored by washing w normal Krebs solution (Fig. 4). And RSM (10^{-3} g/ml) did not affect the exogenous acetylchol (1×10^{-4} g/ml)-induced contractile response (F 4). That is to say, the amplitude of exogen acetylcholine (1×10^{-4} g/ml)-induced contractile sponse after treatment of RSM (1×10^{-3} g/ml) 100±6% (n=10) (Table 2). This value alm equal to the value of the control (100%). addition, effect of each agent in concentration 1×10^{-3} g/ml on the exogenous acetylcholine (10^{-5} g/ml)-induced contractile response was alm equal to that observed as the effect of each ag in that concentration on the exogenous acetylc line (1×10^{-4} g/ml)-induced contractile respo (Fig. 4 and Table 2). From these results, it be suggested that both KM and AKM effect the intramural excitatory nerves but also on muscle in the urinary bladder wall, while R effect on the intramural excitatory nerves. It be considered, moreover, that the effects of th agents are greater on the intramural excitat nerves than they are on the muscle. And

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[1],

AKM[2] and RSM[3~4]. 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.

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

　KM (5×10^{-5} g/ml-1×10^{-3} g/ml), AKM (5×10^{-5} g/ml-1×10^{-3} g/ml) および RSM (1×10^{-4} g/ml-1×10^{-3} g/ml) は，各々，濃度依存的に，経壁電気刺激により誘発される収縮反応を減弱せしめ，特に 1×10^{-3} g/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。1×10^{-3} g/ml の濃度の KM および AKM は，各々，1×10^{-4} g/ml あるいは 1×10^{-5} g/ml の外来性 acetylcholine により誘発される収縮反応を，わずかに減弱せしめたが，1×10^{-3} g/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 (1×10^{-3} g/ml) は，各々，自動収縮の頻度や振幅に対して，ほとんど認む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 (1×10^{-7} g/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，わずかながら作用を及ぼすものと思われる。