Effects of Aminobenzyl penicillin (AB-PC), Methylchlorophenyl isoxazolyl penicillin (MCI-PC) and 6-Aminopenicillanic acid (6-APA) on the Contractile Response of Guinea-pig Urinary Bladder

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Abstract

Yoshida, M. and Koeda, T. *Effects of aminobenzyl penicillin (AB-PC), methylchlorophenyl isoxazolyl penicillin (MCI-PC) and 6-aminopenicillanic acid (6-APA) on the contractile response of guinea-pig urinary bladder.* Japanese Journal of Smooth Muscle Research, 1984, 20 (1), 1-12. — The present study was undertaken to investigate the effects of aminobenzyl penicillin (AB-PC), methylchlorophenyl isoxazolyl penicillin (MCI-PC), and 6-aminopenicillanic acid (6-APA) on the contractile response of guinea-pig urinary bladder. AB-PC, MCI-PC, and 6-APA, respectively, diminished the intramural electrical stimulation-induced contractile response. AB-PC had almost no effect on the contractile response to exogenous acetylcholine, while MCI-PC remarkably reduced it. The reducing effect of MCI-PC was greater on the intramural electrical stimulation-induced contractile response than it is on the exogenous acetylcholine-induced contractile response. 6-APA slightly reduced the exogenous acetylcholine-induced contractile response. The reducing effect of 6-APA was a little weaker on the exogenous acetylcholine-induced contractile response than it is on the intramural electrical stimulation-induced contractile response. By the addition of AB-PC, the amplitude of spontaneous contractile response of the tissue was reduced gradually with slight lowering of the tonus level of the tissue. By the addition of MCI-PC, the spontaneous contractile response of the tissue was almost prevented with a little lowering of the tonus level of the tissue. The effects of AB-PC and MCI-PC on the spontaneous contractile response of the tissue mentioned above were also

Accepted for publication, December 17, 1983.

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observed in the presence of tetrodotoxin. On the other hand, by the addition of 6-APA, the reducing effect on the amplitude of spontaneous contractile response of the tissue and increasing effect on its frequency were recognized with remarkable raise of the tonus level of the tissue. These phenomena which were induced by 6-APA were also observed in the presence of tetrodotoxin. From these results, the conclusion comes as follows: 1) AB-PC may affect not only on the muscle but also on the intramural cholinergic nerves. 2) MCI-PC may affect on the muscle, but its effect on the intramural nerves is also not likely to be negligible at this time. 3) 6-APA may affect mainly on the muscle.

Key words: aminobenzyl penicillin, methylchlorophenyl isoxazolyl penicillin, 6-aminopenicillanic acid, contractile response of urinary bladder.

Introduction

It is well known that both aminobenzyl penicillin (AB-PC) and methylchlorophenyl isoxazolyl penicillin (MCI-PC) represent the β -lactum group of penicillins. Aratani et al. (1963) reported that AB-PC reduced the spontaneous contractile response of guinea-pig intestine and the blood pressure of rabbit. Aratani et al. (1964) also reported MCI-PC diminished the spontaneous contractile response of rabbit intestine and the blood pressure of a rabbit. The effects of these penicillins and 6-aminopenicillanic acid (6-APA), basic structure of these penicillins, on the contractile response of the guinea-pig gallbladder were observed by Yoshida & Koeda (1984). We can not yet, however, see the reports concerning the effects of these drugs on the other organs which have smooth muscle.

It is demonstrated that the excretion of these penicillins into urine is carried out in good condition (Ichikawa, Itō & Terawaki, 1963; Ichikawa, Nijima & Terawaki, 1964). The present study, thus, undertaken to investigate the effects of both AB-PC and MCI-PC on the contractile response of guinea-pig urinary bladder which were induced by both transmural electrical stimulation and exogenous acetylcholine, and which were induced spontaneously. The effects of 6-APA, the basic structure of these penicillins (AB-PC and MCI-PC), on these contractile response were also investigated.

Materials and Methods

Guinea-pigs (250-300 g) were killed by a blow on the head and exsanguinated. The urinary bladder was removed and divided into two approximately equal parts, longitudinally, namely from the top to the fundus of the urinary bladder. One of these pieces was used in an experiment. The preparation was mounted in an organ bath which was filled with Krebs solution. As described with regard to the gallbladder by Yoshida & Ishiura (1981), the fundus of the urinary bladder was fixed with pins and the top end was connected with a silk thread to a force transducer. The mechanical response of the urinary bladder were recorded isometrically under a load of 1 g. Two 5 mm $\times$ 5 mm silver plates coated with silver chloride were used as stimulating eld the top end was connected with a silk thread to a force transducer. The mechanical response of the urinary bladder were recorcosal 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 4 minutes. 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. Observations relating to the spontaneous contractile response of the tissue was performed in 37°C Krebs solution. 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, perfused with 95% O₂+5% CO₂, pH 7.4.

The used drugs were: aminobenzyl penicillin Na salt (AB-PC), 1×10^{-3} g/ml– 1×10^{-2} g/ml; methylchlorophenyl isoxazolyl penicillin Na salt (MCI-PC), 1×10^{-4} g/ml– 1×10^{-2} g/ml; 6-aminopenicillanic acid (6-APA), 1×10^{-4} g/ml– 3×10^{-3} g/ml; guanethidine sulfate, 1×10^{-6} g/ml; atropine sulfate, 1×10^{-6} g/ml; tetrodotoxin, 1×10^{-7} g/ml; acetylcholine chloride, 1×10^{-4} g/ml. The concentrations indicate the final values in the organ bath. The concentrations of AB-PC and MCI-PC indicate the values which were converted to the potencies of antibiotic substances.

Results

Effects of AB-PC, MCI-PC, and 6-APA on the contractile response induced by transmural electrical stimulation.

Transmural electrical stimulation was not affected by guanethidine (1×10^{-6} g/ml), while it was inhibited to $23 \pm 3\%$ (n=8) of control by atropine (1×10^{-6} g/ml) (Fig. 1). And also it was

Fig. 1 Effects of guanethidine, atropine and tetrodotoxin on the transmural electrical stimulation-induced contractile response. In all tracings, transmural electrical stimulation was given at triangular dots beneath each tracing. 1: effect of guanethidine (Gua), 1×10^{-6} g/ml., 2: effect of atropine (Atr), 1×10^{-6} g/ml., 3: effect of tetrodotoxin (TTX), 1×10^{-7} g/ml. Each agent was applied at an arrow.

Fig. 2 Effects of aminobenzyl penicillin (AB-PC) on the transmural electrical stimulation-induced contractile response. 1-3: effect of AB-PC (1×10^{-3} g/ml, 3×10^{-3} g/ml and 1×10^{-2} g/ml). Each right side tracing of 2-3 shows the phenomenon after washing with normal Krebs solution.

almost completely prevented by tetrodotoxin (1×10^{-7} g/ml) (Fig. 1). Effects of AB-PC, MCI-PC, and 6-APA on the transmural electrical stimulation-induced contractile response showing such a property were observed as shown in Fig. 2, 3, 4 and 5.

AB-PC (1×10^{-3} g/ml) did not affect the contractile response to transmural electrical stimulation-induced contractile response (Fig. 2-1). AB-PC (3×10^{-3} g/ml) slightly diminished it, while AB-PC (1×10^{-2} g/ml) remarkably reduced it (Fig. 2-2, 3). These reducing effects of AB-PC were immediately restored by washing with normal Krebs solution (Fig. 2-2, 3).

MCI-PC (1×10^{-4} g/ml) did not affect the transmural electrical stimulation-induced contractile response (Fig. 3-1). MCI-PC (1×10^{-3} g/ml) slightly reduced it, while its reducing effect was restored by washing with normal Krebs solution for 10 minutes (Fig. 3-2). MCI-PC (3×10^{-3} g/ml) remarkably reduced it, but its reducing effect was not completely restored by washing with normal Krebs solution for 30 minutes (Fig. 3-3). As shown in Fig. 3-4, MCI-PC (1×10^{-2} g/ml) reduced still harder the contractile response to transmural electrical stimulation, and its reducing effect was not completely restored by washing with normal Krebs solution for 40 minutes.

6-APA (1×10^{-3} g/ml) did not affect the transmural electrical stimulation-induced contractile response (Fig. 4-1). As shown in Fig. 4-2, 6-APA (3×10^{-3} g/ml) reduced it, while its reducing effect was restored immediately by washing with normal Krebs solution. Moreover, the effect of 6-APA (1×10^{-2} g/ml) on the contractile response was not studied owing to the poor solubility of 6-APA.

When the amplitude of transmural electrical stimulation-induced contractile response before treatment of AB-PC, MCI-PC, and 6-APA in each concentration was taken as 100%, its amplitude after 15 min treatment of each drug in each concentration was registered as a relative contractile response of 100%. These results were observed as shown in Fig. 5. That is to say, the amplitude of transmural electrical stimulation-induced contractile response was $100 \pm 2\%$ ($n=6$) in the presence of AB-PC (1×10^{-3} g/ml), namely almost equal to the value in its absence. It was reduced to $92 \pm 2\%$ ($n=6$) in the presence of AB-PC (3×10^{-3} g/ml) and $52 \pm 7\%$ ($n=6$) in the presence of AB-PC (1×10^{-2} g/ml). And also the amplitude of transmural electrical stimulation-induced contractile response was $99 \pm 3\%$ ($n=6$) in the presence of MCI-PC (1×10^{-4} g/ml), namely almost equal to the value in its absence. It was $86 \pm 2\%$ ($n=6$) in the presence of MCI-PC (1×10^{-3} g/ml) and $64 \pm 10\%$ ($n=6$) in the presence of MCI-PC (3×10^{-3} g/ml). Then it was $3 \pm 2\%$ ($n=6$) in the presence of MCI-PC (1×10^{-2} g/ml). As shown in Fig. 5, moreover, the amplitude of the contractile response mediated by transmural electrical stimulation was $101 \pm 2\%$ ($n=6$) in the presence of 6-APA (1×10^{-3} g/ml), while it was $86 \pm 3\%$ ($n=6$) in the presence of 6-APA (3×10^{-3} g/ml).

Effects of AB-PC, MCI-PC, and 6-APA on the contractile response induced by exogenous acetylcholine (1×10^{-4} g/ml).

The exogenous acetylcholine (1×10^{-4} g/ml)-induced contractile response showed the following property. That is to say, the exogenous acetylcholine-induced contractile response was not affected by tetrodotoxin (1×10^{-7} g/ml), while it was inhibited by atropine (1×10^{-6} g/ml) (Fig. 6). Effects of AB-PC, MCI-PC, and 6-APA in each concentration on the exogenous

Fig. 3 Effects of methylchlorophenyl isoxazoly penicillin (MCI-PC) on the transmural electrical stimulation-induced contractile response.

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

Fig. 4 Effects of 6-aminopenicillanic acid (6-APA) on the transmural electrical stimulation-induced contractile response.

1-2: effect of 6-APA (1×10^{-3} g/ml and 3×10^{-3} g/ml). Right side tracing of 2 shows the phenomenon after washing with normal Krebs solution.

Fig. 5 Effects of aminobenzyl penicillin (AB-PC), methylchlorophenyl isoxazoly penicillin (MCI-PC), and 6-aminopenicillanic acid (6-APA) on the transmural electrical stimulation-induced contractile response. Each amplitude of transmural electrical stimulation-induced contractile response in the absence of AB-PC, MCI-PC, and 6-APA in each concentration was taken as 100% (Control). Ordinate shows each amplitude of transmural electrical stimulation-induced contractile response at 15 min after addition of each agent which is registered as a relative contractile response of 100%.

□: 1×10^{-4} g/ml, ▤: 1×10^{-3} g/ml, ▨: 3×10^{-3} g/ml, ■: 1×10^{-2} g/ml. Values are mean $\pm$ S.D. (n=6).

Fig. 6 Effects of tetrodotoxin and atropine on the exogenous acetylcholine (1×10^{-4} g/ml)-induced contractile response.

□: control (no-drugs), ▤: effect of tetrodotoxin (1×10^{-7} g/ml), ■: effect of atropine (1×10^{-6} g/ml). Values are mean $\pm$ S.D. (n=8).

Fig. 7 Effects of aminobenzyl penicillin (AB-PC) and 6-aminopenicillanic acid (6-APA) on the exogenous acetylcholine (1×10^{-4} g/ml)-induced contractile response. In all tracings, acetylcholine (1×10^{-4} g/ml) was given at a triangular dot beneath each tracing. AB-PC and 6-APA in each concentration were given 15 min before addition of acetylcholine. Each left side tracing of 1-4 shows the contractile response in the absence of each agent, namely control. Each right side tracing of 1-4 shows the contractile response in the presence of each agent.

Fig. 8 Effects of methylchlorophenyl isoxazolyl penicillin (MCI-PC) on the exogenous acetylcholine (1×10^{-4} g/ml)-induced contractile response. In all tracings, acetylcholine was given at a triangular dot beneath each tracing. MCI-PC in each concentration was given 15 min before addition of acetylcholine. 1: Left side tracing shows the contractile response in the absence of MCI-PC, namely control. Right side tracing shows the contractile response in the presence of MCI-PC. 2-3: Left side tracing shows the contractile response in the absence of MCI-PC, namely control. Middle tracing shows the contractile response in the presence of MCI-PC. Right side tracing shows the contractile response after washing (W) with normal Krebs solution.

acetylcholine (1×10^{-4} g/ml)-induced contractile response showing such a property were observed after 15 min treatment of each agent in each concentration as shown in Fig. 7, 8. In addition, when the amplitude of exogenous acetylcholine-induced contractile response before the treatment of these agents in each concentration was taken as 100%, its amplitude after 15 min treatment of each agent in each concentration was registered as a relative contractile

Fig. 9 Effects of aminobenzyl penicillin (AB-PC), methylchlorophenyl isoxazolyl penicillin (MCI-PC), and 6-aminopenicillanic acid (6-APA) on the exogenous acetylcholine (1×10^{-4} g/ml)-induced contractile response.

Each amplitude of exogenous acetylcholine-induced contractile response in the absence of AB-PC, MCI-PC, and 6-APA in each concentration was taken as 100% (Control). Ordinate shows each amplitude of exogenous acetylcholine-induced contractile response at 15 min after addition of each agent which is registered as a relative contractile response of 100%.

□: 1×10^{-4} g/ml, ▤: 1×10^{-3} g/ml, ▨: 3×10^{-3} g/ml, ■: 1×10^{-2} g/ml. Values are mean $\pm$ S.D. (n=10).

Fig. 10 Effects of aminobenzyl penicillin (AB-PC) on the spontaneous contractile response.

1-3: effects of AB-PC (1×10^{-3} g/ml – 1×10^{-2} g/ml). 4: effect of AB-PC (1×10^{-2} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml). Tetrodotoxin (TTX) which was indicated with an arrow was given 10 min before addition of AB-PC. In left side tracings, AB-PC was added an arrow beneath each tracing. Each right side tracing of 2-4 shows the phenomenon after washing with normal Krebs solution.

response of 100%. These results were observed as shown in Fig. 9.

AB-PC (1×10^{-3} g/ml, 3×10^{-3} g/ml and 1×10^{-2} g/ml) had almost no effect on the exogenous acetylcholine-induced contractile response (Fig. 7, 9).

MCI-PC (1×10^{-4} g/ml and 1×10^{-3} g/ml) had almost no effect on it, while MCI-PC (3×10^{-3} g/ml and 1×10^{-2} g/ml) remarkably reduced it (Fig. 8). That is to say, the exogenous acetylcholine-induced contractile response was reduced to $76 \pm 12\%$ (n=6) in the presence of MCI-PC (3×10^{-3} g/ml) and $17 \pm 3\%$ (n=6) in the presence of MCI-PC (1×10^{-2} g/ml). These reducing effects of MCI-PC were not completely restored by washing with normal Krebs solution for 20 minutes.

In addition, 6-APA (1×10^{-3} g/ml) had almost no effect on the contractile response induced by exogenous acetylcholine, while 6-APA (3×10^{-3} g/ml) slightly reduced it, namely it was reduced to $92 \pm 3\%$ (n=6) of the control (Fig. 7, 9).

Effects of AB-PC, MCI-PC, and 6-APA on the spontaneous contractile response.

AB-PC (1×10^{-4} g/ml) had almost no effect on the spontaneous contractile response of the tissue (Fig. 10-1). By the addition of AB-PC (3×10^{-3} g/ml), the amplitude of spontaneous contractile response of the tissue was slightly reduced, while its reducing effect was immediately restored by washing with normal Krebs solution (Fig. 10-2). By the addition of AB-PC (1×10^{-2} g/ml), it was remarkably reduced with slight lowering of the tonus level of the tissue, but it was liable to be recovered immediately by washing with normal Krebs solution (Fig. 10-3). As shown in Fig. 10-4, the same view was demonstrated by the addition of AB-PC (1×10^{-2} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml).

MCI-PC (1×10^{-4} g/ml) had almost no effect on the spontaneous contractile response of the tissue (Fig. 11-1). By the addition of MCI-PC (1×10^{-3} g/ml), the amplitude of spontaneous contractile response of the tissue was slightly inhibited, while its inhibitory effect was immediately restored by washing with normal Krebs solution (Fig. 11-2). By the addition of MCI-

Fig. 11 Effects of methylchlorophenyl isoxazolyl penicillin (MCI-PC) on the spontaneous contractile response. 1-4: effects of MCI-PC (1×10^{-4} g/ml – 1×10^{-2} g/ml). 5: effect of MCI-PC (1×10^{-2} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml). Tetrodotoxin (TTX) which was indicated with an arrow was given 10 min before addition of MCI-PC. In left side tracings, MCI-PC was added at an arrow beneath each tracing. Each right side tracing of 2-5 shows the phenomenon after washing with normal Krebs solution.

Fig. 12 Effects of 6-aminopenicillanic acid (6-APA) on the spontaneous contractile response. 1-4: effects of 6-APA (1×10^{-4} g/ml – 3×10^{-3} g/ml). 5: effect of 6-APA (3×10^{-3} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml). Tetrodotoxin (TTX) which was indicated with an arrow was given 10 min before addition of AB-PC. In left side tracings, 6-APA was added at an arrow beneath each tracing. Each right side tracing of 2-5 shows the phenomenon after washing with normal Krebs solution.

PC (3×10^{-3} g/ml), it was remarkably reduced, while its reducing effect was restored by washing with normal Krebs solution for about 20 minutes (Fig. 11-3). By the addition of MCI-PC (1×10^{-2} g/ml), the spontaneous contractile response of the tissue was almost prevented with slight lowering of the tonus level of the tissue, but this effect was inclined to be restored by washing with normal Krebs solution for about 40 minutes (Fig. 11-4). As shown in Fig. 11-5, a similar result was obtained by the addition of MCI-PC (1×10^{-2} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml).

6-APA (1×10^{-4} g/ml) had almost no effect on the spontaneous contractile response of the tissue (Fig. 12-1). By the addition of 6-APA (1×10^{-3} g/ml), the frequency of the spontaneous contractile response of the tissue was slightly increased with very slight raise of the tonus level of the tissue (Fig. 12-2). By the addition of 6-APA (3×10^{-3} g/ml), it was liable to be increased with slight raise of the tonus level of the tissue in four cases (Fig. 12-3) and was remarkably increased with remarkable raise of the tonus level of the tissue in six others (Fig. 12-4). The latter phenomenon was also observed by the addition of 6-APA (3×10^{-3} g/ml) in the presence of tetrodotoxin (1×10^{-7} g/ml). The effects of 6-APA on the spontaneous contractile response of the tissue as mentioned previously were liable to be restored immediately by washing with normal Krebs solution (Fig. 12).

Discussion

The contractile responses induced by transmural electrical stimulation and exogenous acetylcholine which were used in the present study showed the following property, respectively. That is to say, the transmural electrical stimulation-induced contractile response was almost completely inhibited by tetrodotoxin (1×10^{-7} g/ml), reduced to $23 \pm 3\%$ of control by atropine (1×10^{-6} g/ml), and was unaffected by guanethidine (1×10^{-6} g/ml) (Fig. 1). It was thus suggested that this contractile response was mediated by intramural excitatory nerves, including cholinergic and non-cholinergic excitatory nerves, that is, it may be induced by release of transmitter substances from the ending of intramural excitatory nerves. The same view was previously demonstrated by other workers (Ambache & Zar, 1970; Burnstock, Dumsday & Smyth, 1972; Groat & Saum, 1976; Johns & Paton, 1977; Burnstock et al, 1978). From the present study, moreover, it is suggested that the most part of the contractile response may be induced by release of acetylcholine from the 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 $23 \pm 3\%$ of the contractile response to transmural electrical stimulation. On the other hand, the exogenous acetylcholine-induced contractile response was unaffected by tetrodotoxin (1×10^{-7} g/ml), while it was inhibited by atropine (1×10^{-6} g/ml) (Fig. 6). It thus can be considered that this contractile response is induced by the action of acetylcholine to the muscle in the urinary bladder wall, regardless of intramural nerves. In the present study, effects of aminobenzyl penicillin (AB-PC), methylchlorophenyl isoxazolyl penicillin (MCI-PC), and 6-aminopenicillanic acid (6-APA) on each property mentioned above were observed.

AB-PC (1×10^{-3} g/ml) had almost no effect on the transmural electrical stimulation-induced contractile response (Fig. 2-1). AB-PC (3×10^{-3} g/ml) slightly reduced the transmural

electrical stimulation-induced contractile response, while AB-PC (1×10^{-2} g/ml) diminished it to $52 \pm 7\%$ of control (Fig. 2, 5). AB-PC (3×10^{-3} g/ml and 1×10^{-2} g/ml), however, had almost no effect on the exogenous acetylcholine-induced contractile response (Fig. 7, 9). Therefore, it is possible that the reducing effect of AB-PC on the transmural electrical stimulation-induced contractile response may be mediated by the action of AB-PC on the intramural cholinergic nerves. These results alone, however, are based on the failure to estimate whether AB-PC acts not only on the intramural cholinergic excitatory nerves but also on the intramural non-cholinergic excitatory nerves.

MCI-PC (1×10^{-4} g/ml) had almost no effect on the transmural electrical stimulation-induced contractile response, while MCI-PC (1×10^{-3} g/ml, 3×10^{-3} g/ml, and 1×10^{-2} g/ml) diminished it (Fig. 3). And MCI-PC (1×10^{-4} g/ml and 1×10^{-3} g/ml) had almost no effect on the exogenous acetylcholine-induced contractile response, while MCI-PC (3×10^{-3} g/ml and 1×10^{-2} g/ml) reduced it (Fig. 8, 9). In addition, the reducing effect of MCI-PC was greater on the intramural electrical stimulation-induced contractile response than it is on the exogenous acetylcholine-induced contractile response (Fig. 5, 9). Consequently, it is suggested that MCI-PC may mainly act on the muscle in the urinary bladder wall. It is, however, considered that its effect on the intramural nerves is not likely to be negligible at this time.

6-APA (1×10^{-3} g/ml) had almost no effect on both intramural electrical stimulation-induced contractile response and exogenous acetylcholine-induced contractile response (Fig. 4, 5, 7, 9). 6-APA (3×10^{-3} g/ml) reduced the intramural electrical stimulation-induced contractile response to $86 \pm 3\%$ of control and it reduced the exogenous acetylcholine-induced contractile response to $92 \pm 3\%$ of control (Fig. 4, 5, 7, 9). The reducing effect of 6-APA, thus, was slightly greater on the intramural electrical stimulation-induced contractile response than it is on the exogenous acetylcholine-induced contractile response. These results suggest that 6-APA acts on the muscle in the urinary bladder wall, but its effect on the intramural nerves may also be not neglected completely.

Further, AB-PC (1×10^{-3} g/ml), MCI-PC (1×10^{-4} g/ml), and 6-APA (1×10^{-4} g/ml) had almost no effect on the spontaneous contractile response of the tissue (Fig. 10, 11, 12). AB-PC (3×10^{-3} g/ml), however, slightly reduced the amplitude of spontaneous contractile response of the tissue (Fig. 3-2). AB-PC (1×10^{-2} g/ml) remarkably diminished it with slight lowering of the tonus level of the tissue (Fig. 10-3). MCI-PC (1×10^{-3} g/ml) slightly reduced the amplitude of the spontaneous contractile response and MCI-PC (3×10^{-3} g/ml) remarkably reduced it (Fig. 11-2, 3). And MCI-PC (1×10^{-2} g/ml) almost prevented the spontaneous contractile response of the tissue (Fig. 11-4). And then 6-APA (1×10^{-3} g/ml) slightly increased the frequency of spontaneous contractile response of the tissue with very slight raise of the tonus level of tissue, while 6-APA (3×10^{-3} g/ml) remarkably increased it with remarkable raise of the tonus level of the tissue (Fig. 12). In addition, each phenomenon mentioned above which was shown by AB-PC (1×10^{-2} g/ml), MCI-PC (1×10^{-2} g/ml), and 6-APA (3×10^{-3} g/ml) was also observed after the addition of each agent in the presence of tetrodotoxin (Fig. 10-4, 11-5, 12-5). Therefore, these results suggest that each phenomenon which was mentioned as the effects of AB-PC, MCI-PC, and 6-APA on the spontaneous contractile response of the tissue is mediated by the action of each agent on the muscle, regardless of its action on the intramural nerves.

Further, the effects of both AB-PC and 6-APA on the contractile response were restored immediately by washing with normal Krebs solution, while the effects of MCI-PC on them were not always restored immediately (Fig. 2, 3, 4, 11, 12, 13). When the spontaneous contractile response were observed, the lowering of the tonus level of the tissue was caused by the addition of AB-PC and MCI-PC, but the raise of the tonus level of the tissue was produced by the addition of 6-APA (Fig. 10, 11, 12). It thus seems likely that the mechanism of the action of AB-PC, MCI-PC, and 6-APA on the muscle differs from one another. Further studies may necessary to elucidate these differences. In 37°C Krebs solution, however, the change of the tonus level of the tissue was observed by the addition of AB-PC, MCI-PC, and 6-APA (Fig. 10, 11, 12). On the other hand, in 23°C Krebs solution, such a change of the tonus level of the tissue was not observed after the addition of these drugs (Fig. 2, 3, 4). Thus the effects of these drugs on the tonus level of the tissue may depend on the temperature.

When either of AB-PC or MCI-PC was given to man per oral administration at a dosage of 250 mg, namely at a clinical dosage, the concentration of each drug in the blood and urine was observed by Knudsen, Rolinson & Stevens (1961), Ichikawa, Itō & Terawaki (1963) and Ichikawa, Nijima & Terawaki (1964). That is to say, the highest concentration of AB-PC in the blood was 2.19×10^{-6} g/ml (Knudsen, Rolinson & Stevens, 1961) and 3.3×10^{-6} g/ml (Ichikawa, Itō & Terawaki, 1963). And also that of MCI-PC in the blood was 2.8×10^{-6} g/ml– 4.2×10^{-6} g/ml (Ichikawa, Nijima & Terawaki, 1964). The highest concentration of AB-PC in the urine, however, was 1.5×10^{-4} g/ml– 1×10^{-3} g/ml (Knudsen, Rolinson & Stevens, 1961) and 2.2×10^{-4} g/ml– 3.8×10^{-4} g/ml (Ichikawa, Ito & Terawaki, 1963). And that of MCI-PC in the urine was 3.8×10^{-5} g/ml– 5.9×10^{-5} g/ml (Ichikawa, Nijima & Terawaki, 1964). It is thus demonstrated that the excretion of these drugs into urine is carried out in good condition. As shown in our study, AB-PC, MCI-PC, and 6-APA at low concentration had almost no effect on the contractile response of the tissue, but these drugs at high concentration, although there were few exceptional cases, had effect on it remarkably. This result is compatible with the observations of Aratani et al. (1963, 1964). Aratani et al. (1963) suggested that AB-PC showed cholinergic action to the organ which has smooth muscle. Aratani et al. (1964), again, suggested that MCI-PC acted mainly paralytically on that organ, but its cholinergic action on that organ was also not likely to be negligible. In the present study, such a cholinergic action of these drugs was not observed.

The conclusion comes as follows: 1) AB-PC may affect not only on the muscle but also on the intramural cholinergic nerves. The present study alone, however, is based on the failure to estimate whether AB-PC acts not only on the intramural cholinergic nerves but also on the intramural non-cholinergic excitatory nerves. 2) MCI-PC may affect on the muscle, but its effect on the intramural nerves is also not likely to be negligible at this time. 3) 6-APA may affect mainly on the muscle, while its effect on the intramural nerves may be still not neglected completely at this time. This conclusion supports the suggestion concerning the effects of these drugs on the contractile response of the guinea-pig gallbladder which was reported by Yoshida & Koeda (1984).

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