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

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The present study was undertaken to investigate the effects of cephalothin (CET), cephalexin (CEX), and 7-aminocephalosporanic acid (7-ACA) on the contractile response of guinea-pig urinary bladder.

CET, CEX, and 7-ACA in the concentration of 1×10^{-6} g/ml-1×10^{-4} g/ml had no effect on the transmural electrical stimulation-induced contractile responses. These agents in the concentration of 1×10^{-3} g/ml slightly reduced them as 97±3% (n=12), 97 ±2% (n=10), and 97±3% (n=14) of the controls, respectively. These agents in its concentration, however, had no effect on the contractile responses whether induced by exogenous acetylcholine (1×10^{-4} g/ml) and KCl (40 mM) or spontaneously induced. From these results, it may be concluded that these agents very slightly affect the intramural excitatory nerves of urinary bladder, while they don't affect the muscle in the urinary bladder wall. It is also considered that the effects of these agents on the contractile responses of urinary bladder are extremely weak.

Introduction

Experimental data[1~10] show that cephalosporins, in general, have no or reducing effect for the contractile response of smooth muscle, while some of them have enhancing effect for it. These different effects seem to depend on the difference of the kind of animals and/or organs. In general, cephalosporins may be roughly classified in two groups, as follows[11] : (1) Agents are not metabolized in the body and excreted intact in the urine. (2) Agents are metabolized in the body and excreted as a desacetylated product in the urine. It appears that the excretion of cephalosporins into urine is in any event carried out in a good order[11]. We have not yet seen any reports concerning the effects of these agents on the contractile response of smooth muscle of the urinary bladder. In the present study, we investigated the effects of cephalosporins(cephalothin and cephalexin) on the contractile responses whether induced by transmural electrical stimulation, exogenous acetylcholine and KCl or spontaneously induced. The effects of 7-aminocephalosporanic acid, the basic structure of semisynthetic cephalosporins, on the contractile responses were also investigated.

I. MATERIALS AND METHODS

This experiment was carried out by the same method described by YOSHIDA and ISHIURA[12], and YOSHIDA and KOEDA[13]. That is to say, a guinea-pig weighing 250-450 g was stunned and put to death by bleeding. The urinary bladder was quickly removed and devided into two approximately equal parts, longitudinally, namely from top to fundus of the urinary bladder. One of these pieces was used in an experiment. The preparation was mounted in an organ bath filled with Krebs solution which was perfused with 95% O_2+5% CO_2. The fundus of the preparation was fixed with pins and the top end was connected with silk thread to a forcedisplacement transducer. The mechanical response of the preparation was recorded isometrically under a load of 1 g. Two 5 mm×5

Fig. 1 Effects of CET, CEX, and 7-ACA on the contractile response induced by transmural electrical stimulation.

In all tracings, transmural electrical stimulation was given at dots beneath each tracing. 1 : effect of CET (1×10^{-4} g/ml), 2 : effect of CEX (1×10^{-4} g/ml), 3 : effect of 7-ACA (1×10^{-4} g/ml).

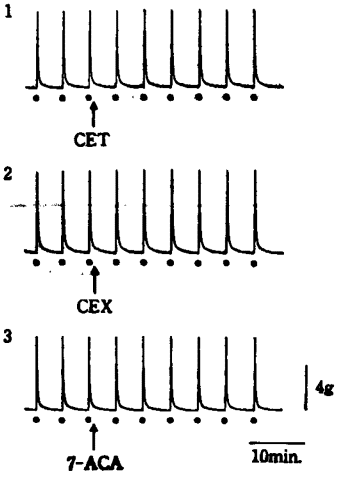
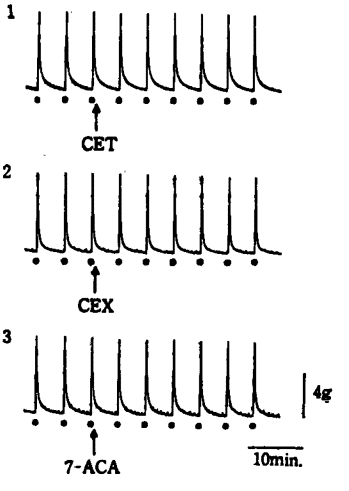


Fig. 2 Effects of CET, CEX, and 7-ACA on the contractile response induced by transmural electrical stimulation.

In all tracings, transmural electrical stimulation was given at dots beneath each tracing. 1 : effect of CET (1×10^{-3} g/ml), 2 : effect of CEX (1×10^{-3} g/ml), 3 : effect of 7-ACA (1×10^{-3} g/ml).



mm silver plate electrodes coated with silver chloride were used for electrical stimulation. The 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 contractile response of the tissue to transmural electrical stimulation, exogenous acetylcholine and KCl were observed in 23°C Krebs solution. Observation related to the spontaneous contractile response of the tissue was performed in 37°C Krebs solution. The used drugs were : cephalothin Na salt (CET), 1×10^{-6} g/ml-1×10^{-3} g/ml ; cephalexin (CEX), 1×10^{-6} g/ml-1×10^{-3} g/ml ; 7-aminocephalosporanic acid (7-ACA), 1×10^{-6} g/ml-1×10^{-3} g/ml ; acetylcholine chloride, 1×10^{-4} g/ml ; KCl, 40 mM. The concentration indicates the final value in the organ bath. Each concentration of CET and CEX indicates the value which was converted to the potency of antibiotic substances. The potencies of CET and CEX used in this experiment are 950 μg/mg and 934 μg/mg, respectively.

II. RESULTS

Effects of CET, CEX, and 7-ACA on the contractile response induced by transmural electrical stimulation. A monophasic response was evoked by a transmural electrical stimulation. CET, CEX, and 7-ACA in the concentration of 1×10^{-6} g/ml and 1×10^{-5} g/ml did not affect the monophasic contractile responses, respectively (data not shown). CET, CEX, and 7-ACA in the concentration of 1×10^{-4} g/ml were in a similar manner as above, respectively (Fig. 1). CET (1×10^{-3} g/ml) had no effect on the monophasic contractile responses in five cases out of twelve experiments and showed only a very slight reducing effect on them in the other seven cases (Fig. 2-1). CEX (1×10^{-3} g/ml) had no effect on them in three cases out of ten experiments and showed only a very slight reducing effect on them in the other seven cases (Fig. 2-2). And also, 7-ACA (1×10^{-3} g/ml) had no effect on them in four cases out of fourteen experiments and showed a similar manner as above in the other ten cases (Fig. 2-3).

These reducing effects of CET, CEX, and 7-ACA reached a steady state in approximately 4 to 19 minutes after addition of these drugs, respectively (Fig. 2). When, therefore, each amplitude of transmural electrical stimulation-induced contractile response before the treatment of CET, CEX, and 7-ACA in the concentration of 1×10^{-3} g/ml, namely the control, was taken as 100%, its amplitude after 19 minutes treatment of each drug in

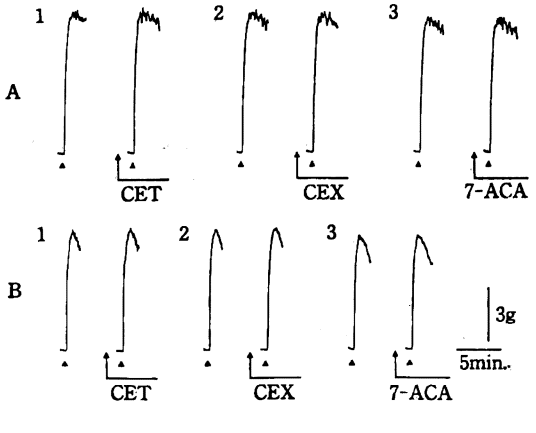
Table 1 Effects of CET, CEX, and 7-ACA on the contractile response induced by transmural electrical stimulation.

Each amplitude of transmural electrical stimulation-induced contractile response in the absence of CET, CEX, and 7-ACA in the concentration of 1×10^{-3}g/ml, namely the control, was taken as 100%. Each amplitude of transmural electrical stimulation-induced contractile response at 19 min. after addition of each agent in its concentration was registered as a relative contractile response of 100%. Each value represents the mean±S.D. of 10 to 14 experiments.

Agent	No. of experiment	Concentration (g/ml)	Amplitude of contractile response (%)
CET	12	1×10^{-3}	97± 3
CEX	10	1×10^{-3}	97± 2
7-ACA	14	1×10^{-3}	97± 3

Fig. 3 Effects of CET, CEX, and 7-ACA on the contractile response induced by exogenous acetylcholine and KCl.

A : effects of CET, CEX, and 7-ACA 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 CET, CEX, and 7-ACA in the concentration of 1×10^{-3} g/ml on the contractile response induced by KCl (40 mM). In all tracings, acetylcholine and KCl are given at black triangle beneath each tracing, respectively. CET, CEX, and 7-ACA were given 19 min before addition of acetylcholine or KCl, 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 the control. Each right side tracing of 1, 2, and 3 in both A and B shows the contractile response in the presence of each agent.

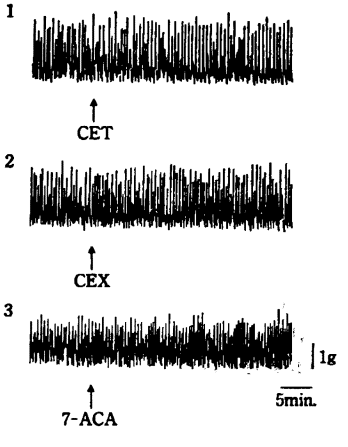


the concentration of 1×10^{-3}g/ml was taken as a relative contractile response of 100%. These results were observed as shown in Table 1. That is to say, CET, CEX, and 7-ACA in its concentration reduced the transmural electrical stimulation-induced contractile response to 97±3% (n=12), 97±2% (n=10), and 97±3% (n=14) of the controls, respectively (Table 1).

Effects of CET, CEX, and 7-ACA on the contractile response induced by exogenous acetylcholine and KCl. In this experiment, acetylcholine (1×10^{-4} g/ml), or KCl (40 mM) was added in an organ bath after 19 min treatment of each agent in the concentration of 1×10^{-3}g/ml, because

Fig. 4 Effects of CET, CEX, and 7-ACA on the spontaneous contractile response.

1 : effect of CET (1×10^{-3} g/ml), 2 : effect of CEX (1×10^{-3} g/ml), 3: effect of 7-ACA (1×10^{-3} g/ml).



the reducing effects of each agent on the transmural electrical stimulation-induced contractile response reached a steady state in approximately 4 to 19 min after the treatment of each agent. CET, CEX, and 7-ACA in the concentration of 1×10^{-3} g/ml had no effect on the exogenous acetylcholine-induced contractile response, respectively (Fig. 3-A). Effects of these agents on the KCl-induced contractile response were also similar to that described above (Fig. 3-B).

Effects of CET, CEX, and 7-ACA on the spontaneous contractile response. It appeared that CET, CEX, and 7-ACA in the concentration of 1×10^{-3} g/ml had almost no significant effect on the amplitude and frequency of spontaneous contractile responses and the tonus level of the tissue, respectively (Fig. 4).

III. DISCUSSION

Most of CET is not metabolized in the body and is excreted intact in the urine, while a part of it is metabolized in the body and is excreted in the urine as o-desacetyl compound[14,15]. CEX is not metabolized in the body and excreted intact in the urine[11]. When CET was intramuscularly administrated to man at a clinical dosage (500 mg), its concentration in the blood and urine was observed by several workers[16~18]. When CEX orally administrated to man at a clinical dosage (500 mg), its concentration in the blood and urine was also observed by several workers[19~24]. That is to say, the highest concentrations of CET and CEX in the blood were in the range of about 8-16 μg/ml[16~18] and 7-20 μg/ml[19~24], respectively. Those in the urine, however, were in the range of about 1,340 μg/ml-1,580 μg/ml[16] and 1,300 μg/ml-9,000 μg/ml[19,21,22,24], respectively. It is thus likely that the excretion of these drugs in the urine are carried out in a good order. However, we have not yet seen any reports concerning the effects of these drugs and 7-ACA which is the basic structure of semisynthetic cephalosporins on the motility of the 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 transmural electrical stimulation-induced contractile response may be induced by release of transmitters from endings of both intramural cholinergic and noncholinergic excitatory nerves[25]. The authors, again, have previously suggested that the exogenous acetylcholine-induced contractile response is induced by the action of acetylcholine on the muscle in the urinary bladder wall, regardless of intramural nerves[26]. While very few report is known concerning the effects of antibiotics on the former property, the reports by KUBOTA et al.[10], and YOSHIDA and KOEDA[18,25,26] are notable. In the present study, effects of CET, CEX, and 7-ACA on each property mentioned above were observed. Not only those, but effects of them on the contractile responses which are induced by KCl or spontaneously induced were also observed in the present study.

CET, CEX, and 7-ACA in the concentration of 1×10^{-6} g/ml-1×10^{-4} g/ml did not affect the transmural electrical stimulation-induced contractile responses. CET (1×10^{-3} g/ml) had no effect on the transmural electrical stimulation-induced contractile responses in three cases out of twelve experiments and showed only a very slight reducing effect on them in the other seven cases, while this agent reduced them to 97±3%(n=12) of the control (Fig. 2 & Table 1). CEX (1×10^{-3} g/ml) had no effect on them in three cases out of ten experiments and showed only a very slight reducing effect on them in the other seven cases, while this agent reduced them to 97±2% (n=10) of the control (Fig. 2 & Table 1). 7-ACA (1×10^{-3} g/ml), moreover, had no effect on them in four cases out of fourteen experiments and showed only a very slight reducing effect on them in the other ten cases, while this agent reduced them to 97±3% (n=14) of the control (Fig. 2 & Table 1). On the other hand, CET, CEX, and 7-ACA in the concentration of 1×10^{-3} g/ml had almost no effect not only on the exogenous acetylcholine-induced contractile responses, but on the KCl-induced contractile responses, respectively (Fig. 3). Each agent in the concentration of 1×10^{-3} g/ml, moreover, had almost no significant effect on the amplitude and frequency of spontaneous contractile responses and the tonus level of the tissue, respectively (Fig. 4). It may be, therefore, considered that each agent in the concentration of 1×10^{-3} g/ml does not affect the muscle and very slightly affect the intramural excitatory nerves.

The previous experimental data[1~10] show that cephalosporins, in general, have no or reducing effect for the contractile responses of the intestinal, tracheal and uterine muscles of several kinds of

animal, but some cephalosporins have also enhancing effect for them. These different effects seem to depend on the difference of the kind of animals and/or organs. In the present study, the enhancing effects of CET, CEX, and 7-ACA on the contractile response of the tissue were not observed. Very few report is known concerning the mode of action of cephalosporins, while it has been reported that three cephalosporins, namely cephaloridine, cephapirin, and ceftazidime, directly act on the muscle and affect the contractile response of organs which have smooth muscle[1, 8, 10]. This finding is not compatible with that obtained by cephalosporins used in the present study. When CET and CEX were respectively administrated to man at a clinical dosage as mentioned previously, each highest concentration of them in the blood did not reach the level of 10^{-3} g/ml[16~24]. Each of them in the urine, however, reached the same level[16, 19, 21, 22, 24]. In the present study, CET, CEX, and 7-ACA in the concentration of 1×10^{-3} g/ml very slightly reduced the transmural electrical stimulation-induced contractile response alone as mentioned above, respectively.

From all these findings, it may be concluded that CET, CEX, and 7-ACA very slightly affect the intramural excitatory nerves of urinary bladder, while they don't affect the muscle in the urinary bladder wall. It is also considered that the effects of these agents on the contractile responses of the urinary bladder are extremely weak.

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

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モルモット膀胱の収縮反応に及ぼす cephalothin (CET), cephalexin (CEX) および 7-aminocephalosporanic acid (7-ACA) の影響について検討した。

1×10^{-6} g/ml～1×10^{-4} g/ml の濃度の CET, CEX および 7-ACA は，経壁電気刺激により誘発される収縮反応に対して，影響を示さなかった。1×10^{-3} g/ml の濃度の CET, CEX および 7-ACA は，各々，その収縮反応を対照の 97±3% (n=12), 97±2% (n=10) および 97±3% (n=14) のように軽度に減弱せしめた。しかし，1×10^{-3} g/ml の濃度のこれら薬物は，外来性 acetylcholine (1×10^{-4} g/ml) や KCl (40mM) により，各々誘発される収縮反応並びに自動収縮反応に対しては，影響を示さなかった。以上より，CET, CEX および 7-ACA は，膀胱の壁内神経に対して極めて弱い作用を及ぼすが，筋に対しては，作用を及ぼさないものと思われる。また，これらの薬物の膀胱の収縮反応に及ぼす影響は，極めて弱いものと考えられる。