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IRREVERSIBLE INHIBITORY EFFECT OF ATROPINE ON THE CONTRACTILE RESPONSES TO DRUGS IN ISOLATED RAT RECTUM, PARTICULARLY IN RELATION TO Ca

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Indexing Words
rat rectum; acetylcholine chloride; potassium chloride; irreversible inhibitory effect of atropine; release of Ca; influx of Ca

Kouhei OGINO, Juei-Tang CHENG and Hiromasa ARAKI. *Irreversible Inhibitory Effect of Atropine on the Contractile Responses to Drugs in Isolated rat Rectum, Particulatrly in Relation to Ca.* Kobe J. Med. Sci. 22, 239-246, December 1976——Effect of atropine sulfate (atropine) on the contractile responses to acetylcholine chloride (ACh) and potassium chloride (K) was investigated in isolated rat rectum. The tension of the strips suspended in the bath medium (Locke solution, 30° C) was isotonically recorded. The K (ED50)-induced contraction was not affected by atropine at 1.5×10^{-3} mM, which reversibly abolished the ACh (ED100)-contraction. After washout of 6.0×10^{-3} mM atropine, the phasic, but not the tonic, component of ACh (ED50)- and K (ED50)-contractions were to some extent inhibited irreversibly. However, such irreversible inhibition of the phassic component was not produced by the atropine methylbromide even in the high concentration of 3.0×10^{-2} mM. The irreversible inhibition by atropine of 6.0×10^{-3} mM on the phasic component of K-contraction was protected by pretreatment with a high concentration of ACh or K. In addition, these irreversible inhibition by atropine was potentiated by the absence of Ca and was abolished by the increase of Ca content in bath media. In preparations in which the irreversible inhibitory effect of atropine is obtained, K-contraction at about 3 min after the removal of Ca from bath medium was significantly reduced. However, contraction by exogenous Ca in high K-depolarizing Ca-free bath media was affected neither by the combined treatment nor by the incubation with atropine. These results suggest that the irreversible inhibition of the contractile responses to drugs by atropine in high concentrations may be due to the interference with the release of Ca in the deep layer of the membrane, rather than by a blockade of muscarinic receptor sites.

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INTRODUCTION

It is well known that atropine is a highly selective antagonist at the muscarinic receptor site in smooth muscle and some organs. And several evidences have indicated that atropine in high concentrations more than that needed to block the action of acetylcholine may also reduce the responses to receptor stimulating drugs, histamine, serotonin and norepinephrine.[4]

Effect of atropine is generally believed to be reversible after washout, but the irreversible inhibitory effect of atropine on the drug-induced contractions has been found out in our laboratory and this effect of atropine is presumed to be produced by the interference with Ca mobilization rather than by the blockade of receptor sites.[1] Otherwise, the finding that the contraction induced by the direct stimulation to muscle through the electrical stimulation was inhibited by the atropine has been reported,[4] although the mechanism of this inhibition by atropine has not been reported.

The aim of the present study was to determine whether or not atropine produces an irreversible inhibitory effect on the contractions by acetylcholine chloride and potassium chloride in isolated rat rectum. If the irreversible inhibition by atropine was obtained, the effect of atropine was further investigated in relation to the movement of Ca which is responsible for the muscle contractions.

MATERIALS AND METHODS

Both sexes rats (approximately 250 g) were sacrified by a blow on the neck and then the rectum (about 1.5 cm long) was dissected. The muscle strips were fixed vertically between hooks in a 30 ml organ bath containing (mM): NaCl 154.0, KCl 5.6, $CaCl_2$ 2.2, glucose 5.6 and $NaHCO_3$ 4.8 (pH 7.4). The medium was maintained at 30±1°C and continuously bubbled with a mixture of 95% O_2—5% CO_2. Tension of the strips was isotonically recorded on a smoked drum by a lever adjusted to give an approximately 10-fold amplification. The Ca-free bath medium was prepared in the same way except that $CaCl_2$ was omitted and osmotic adjustment was not made. All preparation were allowed to equilibrate in the bath media for one hour before measurements were taken. During the eqilibration period the bath media were replaced every 30 min. Drug solutions were applied by dropping directly into the bath media and final concentrations were expressed in mM. Atropine was treated for 10 min in bath medium.

When contractile responses to drugs became constant between 90-240 min after washout of atropine with normal medium, irreversible inhibition was determined if the contractile responses could be initiated for about 5 min at 30 min intervals.

Drugs employed were acetylcholine chloride (Tokyo Kasei), potassium chloride (Wako Pure Chemicals), calcium chloride (Yoneyama Chemicals), atropine sulfate (Merck, Germany), atropine methylbromide (Tokyo Kasei).

RESULTS

1) Effect of Atropine on the Contractile Responses to Drugs.

In this experiment, 5.5×10^{-4} mM acetylcholine (ACh) and 26 mM potassium chloride (K) were used as approximately ED50.

In this preparation, ACh (ED100)-induced contraction was completely abolished in the presence but reversed after washout of 1.5×10^{-3} mM atropine sulfate (atropine), whereas the contractile response to 26 mM K remained unaffectedly by atropine in the same dosage. Otherwise, although contractile response to K is not modified in the presence of 6.0×10^{-3} mM atropine, the phasic component, but not the tonic component, of K-induced contraction was gradually decreased after washout of this concentrating of atropine and finally remained at the constant magnitude about 90 min later. The phasic component of ACh-contraction which was abolished in the presence of 6.0×10^{-3} mM atropine was to some extent reversed gradually after washout of the antagonist and also remained at the constant magnitude about 90 min later. That is, the irreversible inhibitory effect of atropine was obtained on the phasic components of ACh and K. However, irreversible inhibition could not be observed on the tonic component of contractions by K and ACh. Exampples of these experiment are illustrated in Fig. 1 and irreversible inhibitory effect of atropine in the dose of 6.0×10^{-3} and 3.0×10^{-2} mM on the drug-induced phasic contractions are

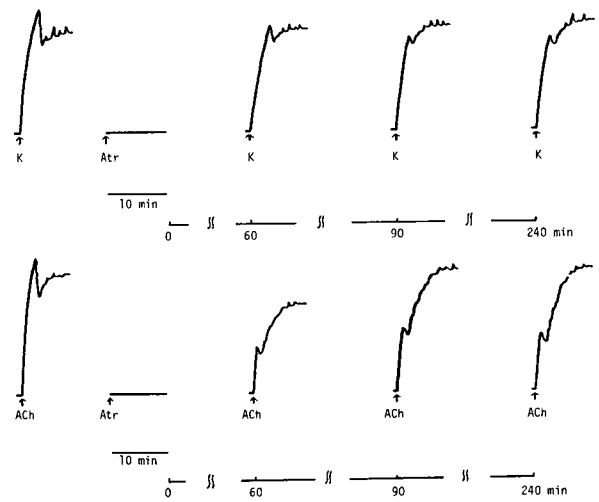

Fig. 1 Influence of incubation with atropine on the contractions by K and ACh. Magnitude of the response to 26 mM K (upper) or 5.5×10^{-4} mM ACh (below) on the left side was taken as control. Time schedule below the contraction showed that responses are initiated at 60, 90 and 240 min after the incubation with 6.0×10^{-3} mM atropine (Atr) (10 min and then washout), respectively. Note the recovery of tonic component and the some extent inhibition of phasic component on these contractions after the washout of atropine.

shown in Table 1. However, such inhibitions were not obtained with atropine methylbromide, a quaternary ammonium compound, 6.0×10^{-3} and 3.0×10^{-2} mM (Table 1).

Table 1 Influence of incubation with atropine on the contractile responses to acetylcholine and potassium.

	ACh-contraction		K-contraction	
	PC	TC	PC	TC
Control (no treatment with atropine)				
	100	100	100	100
After incubation with atropine sulfate				
5.0×10^{-7}	98.0±1.4 (6)	98.7±1.0 (6)	99.2±1.1 (6)	98.3±1.2 (6)
2.0×10^{-6}	46.1±2.4*(7)	98.0±1.8 (7)	83.8±1.6*(14)	99.3±1.0 (14)
10^{-5}	20.2±2.9*(7)	97.2±2.1 (7)	57.8±2.7*(9)	101.2±1.2 (9)
After incubation with atropine methylbromide				
2.0×10^{-6}	97.8±1.6 (6)	98.2±1.3 (6)	99.6±1.5 (7)	99.8±1.0 (7)
10^{-5}	98.7±1.9 (6)	98.4±1.1 (6)	97.8±1.0 (7)	99.1±2.0 (7)

PC: phasic component. TC: tonic component
Value (means±S.E.), the heights of contraction are expressed as percentages.
100% is the height of contraction for a given agonist at its approximate ED50.
Figures in parentheses indicate number of experiments.
*: value significantly different from control ($p<0.05$)

2) Influence of the Change of Ca Content in Bath Medium on the Irreversible Inhibitory Effect of Atropine.

As stated above, the phasic comonent of K-induced contraction was irreversibly reduced by 6.0×10^{-3} mM atropine to 83.8±1.6% (N=14). However, in preparations in which 11 mM Ca was added previous to the application of 6.0×10^{-3} mM atropine, the phasic component of K-contraction did not decrease (99.4±0.3%, N=9). On the other hand, when 6.0×10^{-3} mM atropine was added to the Ca-free bath media and then washed out with normal media, the phasic component of K-contraction was further reduced irreversibly to 43.9±2.4% (N=6). Difference in the effect of atropine in the presence and the absence of Ca was statistically significant ($p<0.05$).

3) Protection Experiments Against the Irreversible Inhibitory Effect of Atropine.

As mentioned above, the irreversible inhibitory effect of atropine on the phasic component of ACh- and K-contractions was observed with the concentration of 6.0×10^{-3} mM. However, this irreversible inhibitory effect of atropine could not be obtained in the preparation that had been incubated with a high concentration of 5.5×10^{-1} mM ACh or 104 mM K previous to the application with atropine.

The self-protection and cross-protection between ACh and K were evident. The result of this experiment is shown in Table 2.

Table 2 Influence of incubation with atropine under application of acetylcholine or potassium at high concentration on phasic component of acetylcholine- and potassium-contractions.

	ACh-contraction	K-contraction
Control (no treatment with atropine)	100	100
After incubation with atropine sulfate (2.0×10^{-6})		
under application of ACh	98.4±1.2 (7)	98.6±2.0 (7)
under application of K	98.2±1.6 (8)	97.9±1.9 (8)

Values (means±S.E.), the heights of contraction are expressed as percentages. 100% is the height of contraction for a given agonist at its approximate ED50. Figures in parentheses indicate the number of experiments.

4) Effect of Atropine on the Contraction by Exogenous Ca and Ba in Ca-free Medium.

In preparations in which the contractile response to K was completely disappeared in Ca-free bath medium, contraction by exogenous Ca (2.2 mM) under high K-depolarization was not modified by the incubation with 6.0×10^{-3} mM atropine (for 10 min and then washout) in normal medium (98.1±1.2%, N=6) (Fig. 2), as compared with that (100%) before incubation with atropine, and by the combined treatment with 6.0×10^{-3} mM atropine in Ca-free medium (97.7±2.3%, N=6) (Fig. 3).

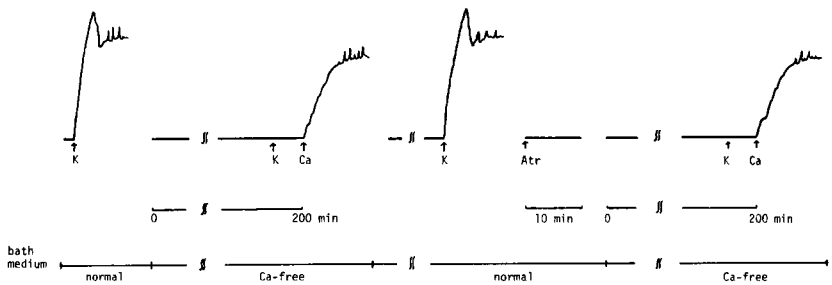

Fig. 2 Influence of incubation with atropine in normal bath medium on the contraction induced by exogenous Ca in Ca-free medium. Response to exogenous Ca is initiated by the addition of 2.2 mM Ca into K (26 mM)-depolarizing Ca-free medium at 200 min after the removal of Ca from bath medium. Contractile response to K (26 mM) in normal medium is employed to indicate the recovery of response to drugs in this preparation. Atropine (Atr) (6.0×10^{-3} mM) is incubated in normal bath medium for 10 min and then washed out. Procedure of this experiment is carried out in the same preparations. Note the no difference of the contraction by exogenous Ca between after and before the incubation with atropine.

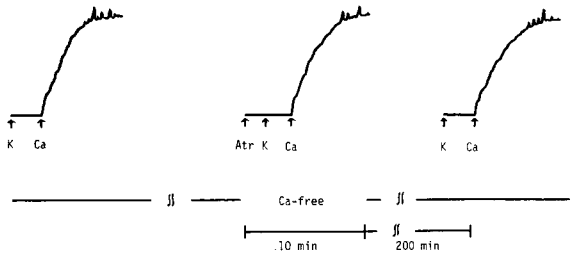

Fig. 3 Influence of atropine on the contraction induced by exogenous Ca in high K-depolarizing Ca-free medium. Response to 2.2 mM exogenous Ca on the left side is taken as control. Atropine (Atr) (6.0×10^{-3} mM) is treated before the addition of exogenous Ca (middle). Response to exogenous Ca at 200 min after the washout of atropine is also shown on the right side. Procedure of this experiment is carried out in the same preparations.

In the preparation which is exposed in Ca-free bath medium for a long time, contraction by Ba finally became constant by the repeated treatment for 3 min at 30 min intervals. That is, the residual contraction by Ba obtained herein and it is taken as 100%. The residual contraction by Ba was not modified by the incubation ($98.6\pm1.0\%$, $N=6$) or the combined treatment ($98.2\pm1.0\%$, $N=6$) with 6.0×10^{-3} mM atropine.

5) Effect of Atropine on the Contractile Response to K in Ca-free Bath Media.

In this experiment, the height of phasic component of K-contraction in normal bath medium was taken as 100%. Contractile response to K decreased to $65.6\pm2.6\%$ ($N=6$) at about 3 min after the removal of Ca from bath medium. After the recovery

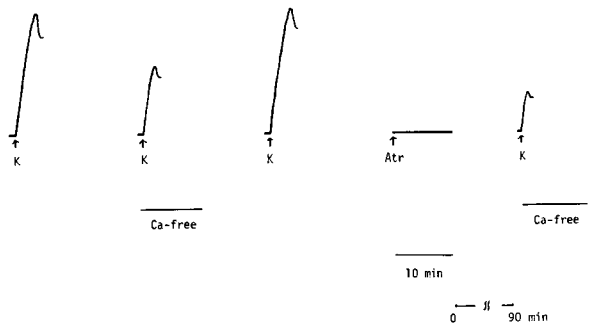

Fig. 4 Influence of incubation with atropine on the phasic component of K-contraction at 3 min after the removal of Ca from bath medium. Response to 26 mM K in Ca-free medium is taken as control. Response to 26 mM K is employed to indicate the recovery of response to drug in this preparation after the re-exposed in normal bath medium. Atropine (6.0×10^{-3} mM) is incubated in normal bath medium for 10 min and then washed out. Note the difference of K-induced contraction in Ca-free bath medium between after and before the incubation with atropine.

of K-contraction in the normal bath medium, the same preparations were treated with 6.0×10^{-3} mM atropine for 10 min. At 90 min after the treatment with 6.0×10^{-3} mM atropine, the contractile response to K which was applied at about 3 min after the removal of Ca from bath medium decreased to $31.3\pm2.7\%$ (N=6) (Fig. 4). Difference about the phasic component of K-contraction in Ca-free bath medium between before and after the treatment with atropine is statistically significant ($p<0.05$).

DISCUSSION

We found that atropine in concentrations higher than required to completely block the cholinergic receptor sites produced a partial irreversible inhibition on the phasic component of the contractions by ACh and K after the washout of atropine. However, absence of the effect of 1.5×10^{-3} mM atropine which completely abolished the contraction by ACh (ED100) on K-induced contraction show that participation of the neurogenic effect through the release of acetylcholine in the contractile response to K can be ruled out. Thus, that the irreversible inhibitory effect of atropine on the phasic component of contraction by two drugs is not likely mediated through the blocking of the cholinergic receptor sites. The irreversible inhibitory effect of atropine on the drug-induced contractions may be due to the interference with the excitation-contraction coupling of smooth muscle. The only datum concerning the interaction between atropine and some physiological ions on the excitation of cell membrane is that atropine prevents the activation of the sodium "carrier" in cell membrane.[2)]

We also found that this irreversible inhibition was produced by atropine sulfate but not by atropine methylbromide. Because atropine methylbromide, a quaternary ammonium compound, unlike atropine sulfate, hardly penetrates the cell membrane, it is indicated that the action of atropine in the deep layer, and not that on the surface of membrane, is responsible for the irreversible inhibitory effect.

The irreversible inhibitory effect of atropine was potentiated by the removal of Ca and was prevented by the increase of Ca in the bath media. These findings indicate that atropine in a high concentration may irreversibly inhibit the mobilization of Ca in this preparation. Thus, it can be considered that the mechanism of irreversible inhibition by atropine may indeed be mediated through the interference with the mobilization of Ca rather than by the blockade of the cholinergic receptor sites. Protection from this irreversible inhibitory effect of atropine by the high concentration of K lends further support to our view, as Ca ions may be powerfully moved by K in high concentrations. This view is consistent with the previous study in isolated rabbit ileum.[1)]

It is also demonstrated that the contraction by K which is applied at about 3 min after the removal of Ca from bath media was significantly reduced by the incubation with atropine. Since contraction in Ca-free bath medium by drug is presumed to be initiated through the release of Ca from storage sites, it is assumed that atropine in high dosages may irreversibly inhibit the release of Ca.

Otherwise, contraction by 2.2 mM exogenous Ca in high K-depolarizing Ca-free

bath medium was influenced neither by the combined treatment nor by the incubation with atropine in normal bath medium. Thus, effect of atropine on the influx of Ca in this preparations can be ruled out. And since the residual contraction by Ba initiated through direct stimulation to muscle contractile element[5] was not affected by atropine, the effect of atropine on the muscle contractile element can be ruled out.

In agreement with the previous study in isolated rabbit ileum,[1] our present data suggested that attention should be paid on the direct inhibitory effect of atropine in this preparation when this agent is employed in high concentrations.

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