IRREVERSIBLE INHIBITORY EFFECT OF ATROPINE ON CONTRACTILE RESPONSES TO DRUGS IN ISOLATED RABBIT ILEUM

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Abstract—Effect of atropine sulfate (atropine) on the contractile responses to acetylcholine chloride (ACh), potassium chloride (K) and barium chloride (Ba) was investigated in isolated rabbit ileum. Tension of the strips suspended in the bath medium (Locke solution, 30°C) was isotonically recorded. The K(ED50) and Ba(ED50)-induced contractions were not affected by atropine at $3.0 \times 10^{-4}$ mM, which reversibly abolished the ACh(ED100)-contraction. After washout of $6.0 \times 10^{-4}$ mM atropine, the phasic, but not the tonic, component of ACh(ED50)-, K(ED50)- and Ba(ED50)-contractions was to some extent inhibited irreversibly. On the contrary, such irreversible inhibition of the phasic component was not produced by atropine methylbromide even in the high concentration of $3.0 \times 10^{-3}$ mM. The irreversible inhibitions by atropine of $6.0 \times 10^{-4}$ mM on the phasic component of ACh- and K-contractions were protected by pretreatment with a high concentration of ACh or K. Further, these irreversible inhibitions by atropine were potentiated by the absence of Ca and were abolished by the increase of Ca content in bath media. These results suggest that the irreversible inhibition of the contractile responses to drugs by atropine in high concentration may be due to the interference with the mobilization of Ca in the deep layer of the membrane, rather than by a blockade of muscarinic receptor sites.

It is well known that atropine is a highly selective antagonist at the muscarinic receptor site in smooth muscle and some other organs. In addition, several lines of evidence have indicated that atropine in higher concentrations than are needed to block the action of acetylcholine may reduce the responses to receptor stimulating drugs, histamine, serotonin and norepinephrine (1). These inhibitory effects of atropine to agonists are generally believed to be reversible after washout, and there is no report about the irreversible inhibitory effect of atropine on the drug-induced contractions in smooth muscle organs. On the other hand, it has been accepted that atropine also shows a local anesthetic action such as is seen with cocaine and procaine. If the anesthetic action of local anesthetics is indeed due to the competition between the drugs and calcium (2, 3), atropine ought to inhibit the mobilization of Ca ions. Actually, the finding that the contraction induced by direct stimulation to muscle through the electrical stimulation was inhibited by atropine has been reported by Munro (4), although the mechanism of this inhibition by atropine has not been reported.

The aims of the present study were to determine whether or not atropine produces an

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irreversible inhibitory effect on the contractions by acetylcholine chloride, potassium chloride and barium chloride, and whether or not there is an interaction between atropine and Ca ion in isolated rabbit ileum.

**MATERIALS AND METHODS**

The ileum from mature exsanguinated rabbits (2.3–3.4 kg) was cut into strips of approx. 1.5 cm and fixed vertically between hooks in a 30 ml organ bath containing (mM): NaCl 154, KCl 5.6, CaCl2 2.2, glucose 5.6 and NaHCO3 4.8 (pH 7.4). The medium was maintained at 30±1 °C and continuously bubbled with a mixture of 95% O2-5% CO2. Tension of the strips was isotonicly recorded on a smoked drum by a lever adjusted to give an approx. 8-fold amplification. The Ca-free bath medium was prepared in the same way except that CaCl2 was omitted and osmotic adjustment was not made. All preparations were allowed to equilibrate in the bath media for one hour before measurements were taken. During the equilibration period the bath media were replaced every 30 min. Drug solutions were applied by dropping directly into the bath media and final concentrations are expressed in mM. Transmural stimulation (50 Hz, 1 msec, 100 V) by the use of an electronic square-pulse generator (Nihon Kohden, MSE-3R) was applied through parallel bipolar platinum electrodes.

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

Drugs used were acetylcholine chloride (Tokyo Kasei), potassium chloride (Wako Pure Chemicals), barium chloride (Yoneyama Chemicals), calcium chloride (Yoneyama Chemicals), atropine sulfate (Merck, Germany), atropine methylbromide (Tokyo Kasei) and tetrodotoxin (Sankyo).

**RESULTS**

*Effect of atropine on the contractile responses to drugs*

In this experiment, the approx. ED50's of acetylcholine (ACh) (5.5×10^-4 mM), potassium chloride (K) (13.0 mM) and barium chloride (Ba) (2.0 mM) were used.

In this preparation, 5.5×10^-2 mM ACh (ED100)-induced contraction was completely abolished in the presence but reversed after washout of 3.0×10^-4 mM atropine sulfate (atropine), although the contractions induced by K and Ba in ED50 remained unaffected by atropine of this concentration. Furthermore, the presence of atropine 6.0×10^-4 mM did not modify the contractions by K and Ba, but after washout of this concentration of atropine, only the phasic components of K- and Ba-contractions gradually decreased and finally remained at the constant magnitude about 90 min later. The phasic component of ACh-contraction which was abolished in the presence of atropine 6.0×10^-4 mM was to some extent reversed gradually after washout of the antagonist and 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, K and Ba. On the other hand, irreversible
inhibition was not observed against tonic component of the contractions by the three agonists. Examples of these experiments are illustrated in Fig. 1. The irreversible inhibitions of phasic component by atropine $6.0 \times 10^{-4}$ and $3.0 \times 10^{-3}$ mM are shown in Table 1.

**Fig. 1.** Typical recordings of the contractile responses to ACh at $5.5 \times 10^{-4}$ mM (upper) and K at 13.0 mM (below). a): control responses to ACh and K, b): responses to ACh and K under treatment with atropine at $6.0 \times 10^{-4}$ mM, c), d), e) and f): responses to ACh and K at 30, 60, 90 and 240 min after the washout of atropine, respectively.

**Fig. 2.** Typical recordings of the contractile responses to transmural stimulation (TS) (50 Hz, 1 msec, 100 V) and K at 13.0 mM. Left side: control responses to TS and K. Right side: under treatment with atropine at $3.0 \times 10^{-4}$ mM (upper) and tetrodotoxin (TTX) at $10^{-4}$ mM (below).
| Control (no treatment with atropine) | ACh-contraction | K-contraction | Ba-contraction |
|-------------------------------------|----------------|--------------|---------------|
|                                    | PC  | TC  | PC  | TC  | PC  | TC  |
| 100       | 100 | 100 | 100 | 100 | 100 | 100 |

After incubation with atropine sulfate

| 3.0 × 10⁻⁴ mM | 99.0±1.4 (7) | 98.7±1.9 (7) | 98.4±1.9 (7) | 99.6±1.1 (7) | 99.8±1.3 (6) | 99.5±1.2 (6) |
| 6.0 × 10⁻⁴ mM | 79.2±1.1* (9) | 98.0±1.8 (9) | 82.9±1.8* (9) | 99.2±2.0 (9) | 88.3±6.9* (6) | 99.1±1.5 (6) |
| 3.0 × 10⁻³ mM | 67.0±2.9* (7) | 98.1±2.2 (7) | 70.6±2.6* (8) | 98.8±2.1 (8) | 76.5±4.2* (6) | 99.0±1.6 (6) |

After incubation with atropine methylbromide

| 6.0 × 10⁻⁴ mM | 100.2±1.6 (8) | 100.4±2.0 (8) | 99.6±1.3 (7) | 99.8±1.7 (7) | 99.5±1.8 (6) | 99.7±2.0 (6) |
| 3.0 × 10⁻³ mM | 99.7±1.9 (7) | 100.0±1.1 (7) | 99.8±2.0 (7) | 100.1±1.5 (7) | 100.0±1.1 (6) | 99.8±0.9 (6) |

PC: phasic component,  TC: tonic component.
Values (means ±S.E.), the heights of contraction are expressed as percentages.
100% is the height of contraction for a given agonist at its approx. ED50.
Figures in parentheses indicate number of experiments.
*: value significantly different from control (p<0.01)
Such inhibitions were not obtained with atropine methylbromide, a quaternary ammonium compound, $6.0 \times 10^{-4}$ and $3.0 \times 10^{-3}$ mM (Table 1).

Contractile responses to K and Ba were not modified by the treatment with $10^{-4}$ mM tetrodotoxin or $3.0 \times 10^{-4}$ mM atropine both of which completely abolished the contractile response to transmural stimulation. Examples of these experiments concerning the K-contraction are illustrated in Fig. 2.

The effect of atropine was not observed on the residual contraction by Ba in Ca-free bath media (5).

Influence of the change of Ca content in bath medium on the irreversible inhibitory effect of atropine

As stated above, the phasic component of K-induced contraction was irreversibly reduced by $6.0 \times 10^{-4}$ mM atropine to $82.9 \pm 1.8\%$ ($N=9$). However, in preparations in which $6.6$ mM Ca was added previous to the application of $6.0 \times 10^{-4}$ mM atropine, the phasic component of K-contraction did not decrease ($99.5 \pm 0.6\%$, $N=8$). On the other hand, when $6.0 \times 10^{-4}$ 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 $74.7 \pm 3.7\%$ ($N=11$). Difference in the effect of atropine in the presence and absence of Ca was statistically significant ($p<0.05$).

Protection experiments against the irreversible inhibitory effect of atropine

Protection experiments were carried out concerning the contractile responses to ACh and K.

As mentioned above, the irreversible inhibitory effect of atropine on the phasic component of ACh- and K-contractions was observed with the concentration of $6.0 \times 10^{-4}$ mM. However, this irreversible inhibitory effect of atropine could not be obtained in the preparation that had been incubated with a high concentration of ACh or 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 ($3.0 \times 10^{-4}$ mM) |                 |              |
| under application of ACh | 99.0 ± 1.5 (7)  | 98.6 ± 2.0 (7) |
| under application of K   | 98.7 ± 1.8 (7)  | 98.2 ± 2.4 (7) |

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

**DISCUSSION**

We found that atropine in concentrations higher than are required to completely block
the cholinergic receptor sites produced a partial irreversible inhibition on the phasic component of the contractions by ACh, K and Ba after the washout of atropine. However, absence of the effect of $3.0 \times 10^{-4}$ mM atropine which completely abolished the contraction by $5.5 \times 10^{-2}$ mM ACh (ED100) and that of $10^{-4}$ mM tetrodotoxin on the K- and Ba-induced contractions show that participation of the neurogenic effect through the release of acetylcholine in the contractile responses to K and Ba can be ruled out. Thus, the irreversible inhibitory effect of atropine on the phasic component of contractions by ACh, K and Ba 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 ion on the excitation of cell membrane is that atropine prevents the activation of the sodium "carrier" in cell membrane (6).

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, hardly penetrates the cell membrane, it is presumed that the action of atropine in the deep layer, and not that on the surface of the membrane, is responsible for the irreversible inhibitory effect. Furthermore, since the residual contraction by Ba initiated through direct stimulation to muscle contractile elements (5) was not affected by atropine, the effect of atropine on the muscle contractile element can be ruled out.

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, we are left with the conclusion 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 the mobilization of extracellular Ca ions into the cell induced by K may result in the increase of cellular Ca levels. Finally, since the phasic component of ACh-, K- and Ba-contractions in this preparation is initiated by the release of Ca from storage sites (5), it is assumed that atropine inhibits the release of Ca induced by agonists.

Our present data also suggested that attention should be paid to the direct inhibitory effect of atropine in this preparation when this agent is employed in high concentrations.

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