ACTION MECHANISMS OF THE CONTRACTING DRUGS, K, ACETYLCHOLINE AND Ba, AND OF THE RELAXANTS, ISOPROTERENOL AND PAPAVERINE IN THE RAT'S ISOLATED ILEUM, ESPECIALLY IN RELATION TO Ca

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ACTION MECHANISMS OF THE CONTRACTING DRUGS, K, ACETYLCHOLINE AND Ba, AND OF THE RELAXANTS, ISOPROTERENOL AND PAPAVERINE IN THE RAT'S ISOLATED ILEUM, ESPECIALLY IN RELATION TO Ca

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Indexing Words

rat ileum; K; acetylcholine; Ba; Isoproterenol; papaverine; Ca influx; Ca release; concentration-action curve; contraction curve

Kohtaro TANIYAMA. *Action Mechanisms of the Contracting Drugs, K, Acetylcholine and Ba, and of the Relaxants, Isoproterenol and Papaverine in the Rat's Isolated Ileum, especially in Relation to Ca.* Kobe J. Med. Sci. 19, 67-89, June, 1973——By the functional method of observation, it was attempted to clarify the action mechanisms of contracting and relaxing drugs in the rat's excised ileum, particularly in relation to Ca. Various methods of analysis were used in order to obtain informations as detailed as possible. The analysis by means of the shape of contraction curve and that by means of the concentration-action curve, which had not so far been widely utilized, proved considerably useful.

INTRODUCTION

It is generally accepted that Ca ion is the physiological link between excitation of the membrane and shortening of the contractile protein (actomyosin, AM) of the muscle cell,[4, 35] and that physiological relaxation of the muscle is caused by the dissociation of Ca ion from the AM-regulating protein. It is, therefore, an important problem of research to investigate the mechanism of drug-induced contraction or relaxation of the smooth muscle in relation to Ca.

Already it has been reported[5, 8, 21, 22, 24, 27, 45] that the contractions by various drugs were mediated through Ca mobilization (Ca influx or Ca release) in various isolated organs of smooth muscle, and the view[20] has also been advanced that the part of Ca store from which Ca was released to elicit contraction was different between some contracting drugs. However, it is not yet fully known as to what similarity or peculiarity of the mode of Ca mobilization may exist according to the kinds of contracting

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drugs or of smooth muscle organs. And further investigation in detail seems to be particularly required concerning the contraction mechanism of Ba on smooth muscle organs, because there are discrepancies between the opinions about this, that is, the indirect action through Ca mobilization[13, 27, 28, 33, 40], the direct action on AM,[36] or the other actions[8, 47] of Ba being reported.

On the other hand, concerning the relation between Ca and the drug-induced relaxation in the excised smooth muscle organs, the evidences hitherto obtained are remarkably less advanced as compared with those concerning the drug-induced contraction, although there are some reports[15, 16, 26, 37, 42, 43] ascribing the drug-induced relaxation to the inhibition against the contracting role of Ca. This seems to be due to the complexity of factors concerend with the relaxation and the difficulty in differentiating the participations of these factors, in the organ level. As to the relaxation factors, it is clear that, besides the inhibition of Ca influx or Ca release, the inhibition of the energy utilization required for the contraction of AM is also important.

In the present study, as an attempt to contribute to the elucidation of the problems as stated above, which are yet unsettled or lacking in knowledge, the action mechanisms of the contracting and relaxing drugs were examined in the rat's ileum strip, especially in relation to Ca, using the functional method of observing the modification in the tonus of this strip. In order to obtain findings as detailed as possible, various items of experiments were performed including the analysis of the contraction curve of drugs and the analysis with the concentration-action curve, which have so far been not widely used.

In the present paper, the word, "contractile system of muscle", is used in the broad sense, which includes not only the contractile element of muscle (AM) but also the energy metabolism required for its contraction.

MATERIALS AND METHODS

The strip of about 1 cm in length was obtained from the ileum (in the distance of 5 to 10 cm from the appendix) of mature rats killed by blow on the head and hung in the Locke's solution (30 ml) according to the Magnus' method, and the tension of the strip was isotonically recorded on a smoked kymograph by the lever adjusted to give about 10-fold amplification.

The preparation was left alone in the bath solution (abbreviated as "bath" in the following) for at least one and a half hour before initiation of the experiment.

The Locke's solution (bath) used consists of NaCl 9.0, KCl 0.42, $CaCl_2$ 0.24, glucose 1.0 and $NaHCO_3$ 0.4 (g/l). The bath was set at the temperature of 25°C and aeration was made of bubbled air.

In the following, "Ca-free bath" is the bath in which $CaCl_2$ was removed from the Locke's solution.

All drug solutions tested were applied by dropping into the bath.

The concentration-action curve of the contracting drug was constructed according to the cumulative dose method.

The details of the method of other experiments are described in the part of

the results.

The drugs used in this research are as follows:
acetylcholine choloride (Tokyokasei), barium chloride (Wakojunyaku), calcium chloride (Yoneyamayakuhin), potassium chloride (Yoneyamayakuhin), papaverine hydrochloride (Fujisawayakuhin), 1-isoproterenol hydrochloride (Proternol-L, Nikkenkagaku), phentolamine mesylate (Regitine, Ciba), tolazoline hydrochloride (Imidaline, Yamanouchiseiyaku), yohimbine hydrochloride (Onoyakuhin), propranolol hydrochloride (Inderal, Sumitomokagaku).

RESULTS

I. The Action of the Exogenous Ca

In the normal bath, the preparation showed the relaxation by exogenous Ca from the concentration of 2 mM and this relaxation was inhibited neither by α-blockers such as phentolamine (10^{-5}), tolazoline (10^{-5}) and yohimbine (10^{-5}) nor by propranolol (10^{-5}), a β-blocker. On the other hand, in the preparation, in which the contraction by K had disappeared in the Ca-free bath (this preparation is described as "Ca-depleted preparation" in the following), exogenous Ca always showed the prolonged contraction. This contraction increased with increased concentration of Ca, although the contraction was followed by the relaxation in the supramaximal contracting concentration of Ca.

II. The Action of Contracting Drugs (K, Acetylcholine and Ba)

1. The shape of the contraction curve of contracting drugs and the effect of anoxia and removal of bath Ca on it

In this experiment, the concentrations used of K (10^{-3}), acetylcholine (ACh) (10^{-7}) and Ba ($10^{-4}\sim5\times10^{-4}$) were those corresponding to ED_{50} (shown in parentheses).

a) The shape of contraction curve: In the normal bath, the shape of contraction curve of K consisted of the initial fast contraction (phasic contraction, PC), which was followed by fade, and the subsequent slow contraction (tonic contraction, TC), which was without fade. The shapes of the contraction curves of ACh and Ba also consisted of the fast component (PC) and the subsequent slow component (TC), although there was seemingly no clear-cut distinction between them because of the absence of fade in PC.

b) The effect of anoxia: In the following, anoxia is meant by the discontinuation of aeration to the bath. In the normal bath, anoxia showed the transient contraction, and then the tonus gradually fell to fix 15 - 30^{m} later at a slightly lower level than the original base line. The height of the TC by K began to decrease at about 60^{m} after anoxia. It decreased more with lapse of time and finally disappeared at about 2^{h} after anoxia. The height by PC of K did not change even at the time of disappearance of TC. The influence of anoxia on the contraction curve of ACh or Ba was

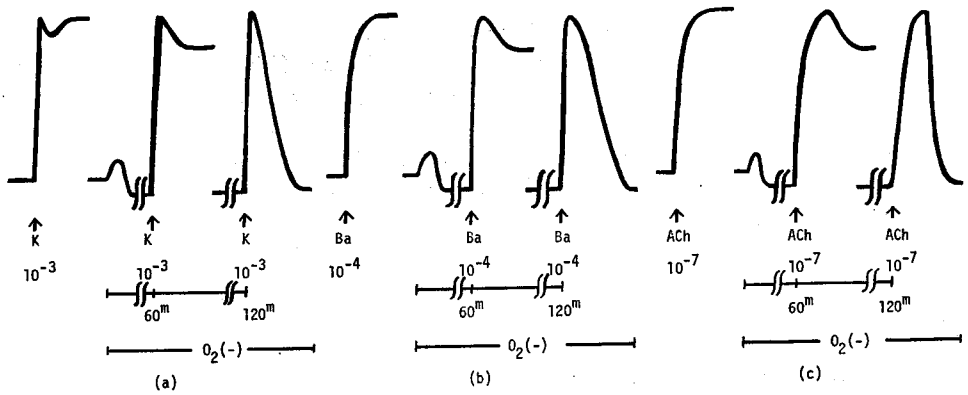

Fig. 1 Effect of anoxia (discontinuation of aeration to the bath solution) on the contraction curves of K (a), Ba (b) and ACh (c).

similar to that on the contraction curve of K as described above (Fig. 1). Re-aeration to the bath completely removed the effect of anoxia on the contraction curves of K, ACh and Ba in 15～20ᵐ.

c) *The effect of the removal of Ca from the bath:* About 15ᵐ later after the exchange of the bath for the one without Ca, the height of TC by K decreased without the modification in the height of PC by K. About 30ᵐ later after the exchange, the height of PC by K also decreased, but the decrease in the TC by K was much more. The changes similar to this were observed also for the contraction curves of ACh and Ba by the removal of bath Ca. When both the PC and the TC by K had disappeared at about 4ʰ after the removal of bath Ca, the TC by K was recovered more rapidly than the PC by K by the re-exchange of the bath to normal, complete recovery of both PC and TC being obtained about 20ᵐ later. Summing up the above results, it is considered that, for any of K, ACh and Ba, PC is caused by Ca release, which hardly depends on energy (aerobic energy), whereas TC is maintained by the active influx of Ca requiring energy. This is consistent with the view expressed by Urakawa et al.,[45] and Karaki et al.[29] concerning the contraction by K in the guinea pig's taenia coli.

2. *Time course of decrease in the action of contracting drugs in the Ca-free bath*

When the bath was exchanged for the one without Ca, the preparation immediately showed a transient contraction and the tonus gradually fell to fix about 150ᵐ later.

In a short time (about 2ᵐ) after the exchange of the bath from normal to Ca-free, the contraction levels of K (about 10^{-3}), ACh (about 10^{-7}) and Ba (10^{-4}～5×10^{-4}) of ED_{50} concentration did not decrease. But each contraction gradually decreased with the time. In about 4ʰ after the exchange, the contractions by K and

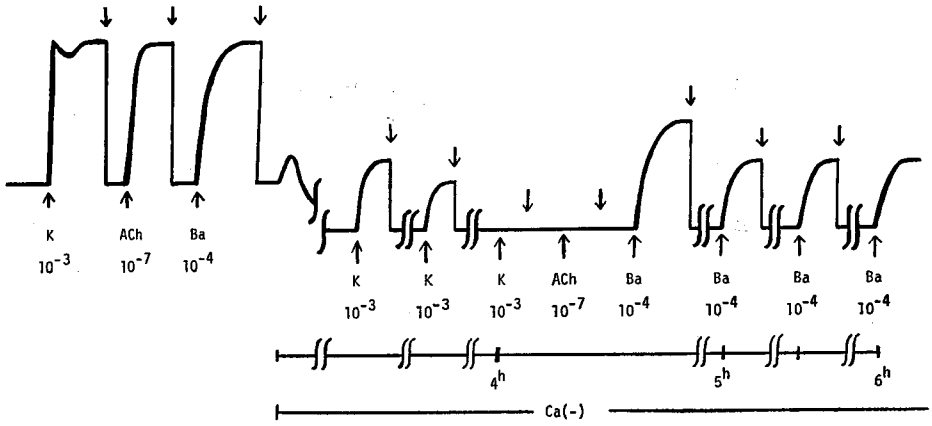

Fig. 2 Modification in the contractions by K, ACh and Ba with the lapse of time after the exchange of the bath solution from normal to Ca(−).

ACh disappeared almost simultaneously. At this time, the contraction by Ba was 40～60% of its initial height. The contraction by Ba further decreased with more lapse of time, but when the time exceeded about 5^h from the exchange, it decreased no more and remained constant at 20～40% of its initial height for a long time (more than 1^h). In the following this constant contraction remaining is named "the residual contraction by Ba" (Fig. 2).

The time required for disappearance of the contraction by K or ACh in the Ca-free bath was markedly shortened by the prior application of Ba contraction.

In about 20^m after the re-exchange of the bath to normal, the contractions by K, ACh and Ba were recovered.

Next, it was analysed with the concentration-action (C-A) curve how the action of each contracting drug was varied by the removal of Ca from the bath.

In the Ca-free bath, once the contraction by K or ACh of ED_{50} disappeared, no contraction occurred even at the higher concentration of K or ACh, and so the C-A curve of K or ACh could not be obtained. On the other hand, at this time, the log C-A curve of Ba showed a nearly parallel shift to the right, as compared with the one in the normal bath. Then, at the time when the contraction by ED_{50} of Ba remained constant, the log C-A curve of Ba showed the combined shift of a marked downward compression and a parallel shift to the right, as compared with the one in the normal bath. The log C-A curve of this residual contraction by Ba could be reproducible in the same shape several times for several hours after this (Fig. 3).

III. The Action of the Relaxants (Isoproterenol and Papaverine)

1. The effect of the removal of bath Ca on the action of relaxants

In the normal bath, 50% effective (ED_{50}) and maximal effective (ED_{100}) concentrations of isoproterenol (Iso) and papaverine (Pap) were approximately 10^{-7}, 2×10^{-6} and 10^{-5}, 10^{-4}, respectively.

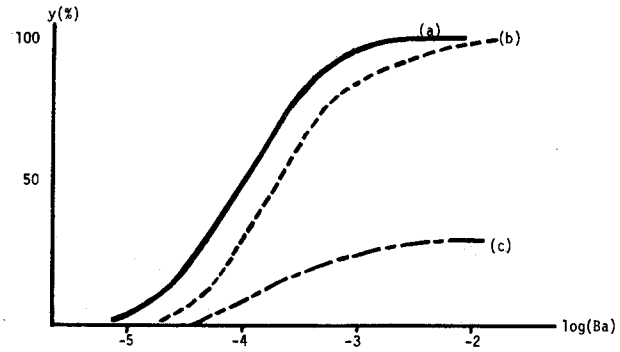

Fig. 3 Modification in the concentration-action curve of Ba with the lapse of time after the exchange of the bath solution from normal to Ca(−).
(a) in the normal bath, (b) at the time when the contraction by ED_{50} of K disappeared and (c) after the time when the contraction by ED_{50} of Ba remained constant.

After changing the normal bath for the one without Ca, at every time before the tonus reached the lowest, the relaxation levels induced by Iso and Pap of ED_{50} or ED_{100} were the same as those in the normal bath. After the tonus reached the lowest, this tonus was of the same level as that induced by ED_{100} of these relaxants in the normal bath, and so, the relaxations by them could not be observed. Also under the contraction by ACh (10^{-6}), the relaxation levels of these relaxants in the Ca-free bath were the same as those in the normal bath.

2. *The effect of the high potassium-depolarizing bath on the relaxations by isoproterenol and papaverine*

Adding K 6×10^{-3} in the normal bath elicited a depolarizing contraction. Under this condition (contraction), the relaxing action of Iso 10^{-8} (concen-

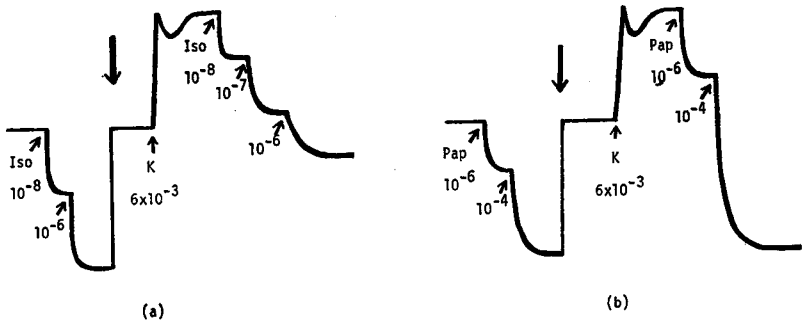

Fig. 4 Effect of the high K-depolarizing bath solution on the relaxing actions of isoproterenol (Iso) (a) and papaverine (Pap) (b).

tration below ED_{50}) was strongly suppressed as regards both the level and the magnitude of relaxation, and about 100-fold higher concentration was required in order that the relaxation level in the normal bath could be obtained. Further, in the high K-depolarizing bath, the maximal level of relaxation attained by high concentration of Iso was depressed, compared with the one in the normal bath (Fig. 4-a).

Under the high K-depolarizing bath, the relaxation level induced by Pap 10^{-6} (concentration below ED_{50}) was depressed, although the magnitude of relaxation by it was hardly decreased, and the relaxation level by Pap 10^{-4} (ED_{100}) did not change, the magnitude of relaxation by it being rather increased (Fig. 4-b).

3. *The comparison of the relaxing potency against the Ca-induced contraction and against the Ba-induced contraction in the Ca-free bath*

In the Ca-depleted preparation suspended in the Ca-free bath, in which the contraction by K had already disappeared as stated before, the contraction by exogenous Ca 2 mM and that by Ba $10^{-4}\sim5\times10^{-4}$ were similar in extent. Iso 10^{-7} strongly relaxed this Ca-contraction, but it relaxed this Ba-contraction weakly (Fig. 5). On the other hand, Pap $10^{-6}\sim5\times10^{-6}$ showed the relaxation of a similar extent against the contraction by Ca 2mM and against that by Ba $10^{-4}\sim5\times10^{-4}$.

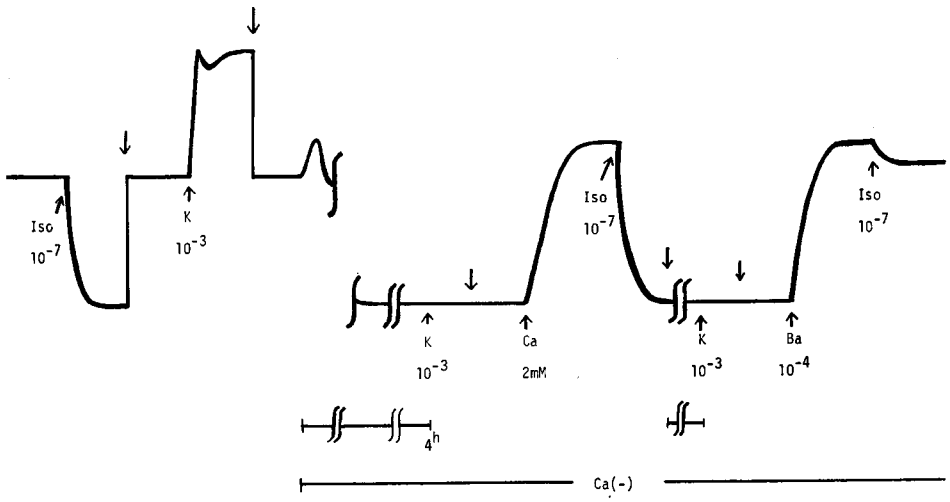

Fig. 5 Relaxing actions of isoproterenol (Iso) against the Ca contraction and the Ba contraction in the Ca(−) bath solution.

Then, the relaxing action against the residual contraction by Ba obtained in the strongly Ca-depleted preparation suspended in the Ca-free bath as stated before was compared with that against the Ba-contraction in the normal bath. The relaxing action of Iso (10^{-7}) against the former was very weaker, only a slight relaxation being observed, while Pap ($10^{-6}\sim5\times10^{-6}$) showed the relaxation level of a similar extent against the both contractions (Fig. 6).

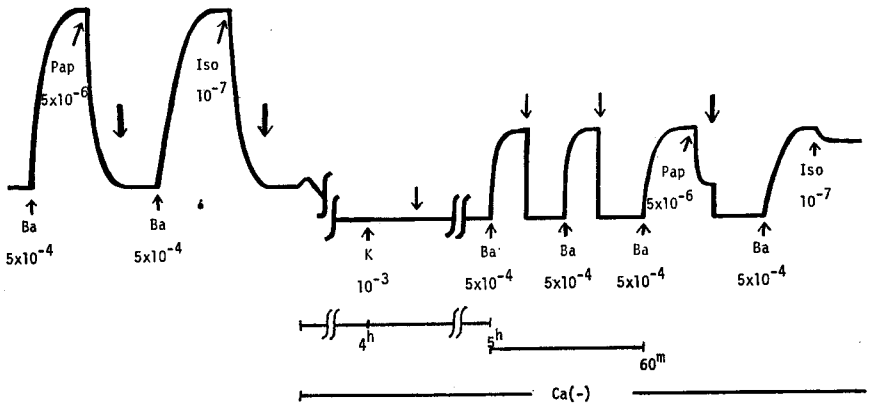

Fig. 6 Relaxing actions of isoproterenol (Iso) and papaverine (Pap) against the Ba contraction in the normal bath solution and against the residual contraction by Ba in the Ca(−) bath solution.

4. The action of the relaxants on the Ca uptake and the Ca release in the low Ca-bath

When the bath was exchanged from normal to low Ca (0.11 mM, 1/20 of the normal), ACh (10^{-8})-induced contraction gradually decreased to fix about 180^{m} later. At this time, adding Ca 2 mM (approximately corresponding to the content in the normal bath) for 15^{m}, recovered the decreased ACh-contraction to its initial height in the normal bath. This is considered to be due to that the added Ca was taken up into the Ca store of the pereparation. Then, added Ca was washed out with the low Ca-bath. By this procedure, ACh contraction gradually decreased again. This is considered to be due to that the Ca taken up into the Ca store by the above procedure was gradually released. Using the above two phenomena that mean Ca uptake and Ca release respectively, as the control, the effect of drugs on them was studied.

As shown in Fig. 7, the coexistence of Iso (5×10^{-8}) for 15^{m} (following 10^{m} pretreatment) during the process of Ca uptake, strongly inhibited the recovery of ACh contraction dependent on Ca uptake. But, the coexistence of Pap (10^{-6}) had no effect.

As shown in Fig. 8, the presence of Iso (5×10^{-8}) for 13^{m} in the initial stage of the process of Ca release made the ACh contraction higher than that of the control, after the time, when the ACh contraction-inhibiting effect of Iso probably remaining after its washout had disappeared, and prolonged the time course concerning the decrease of ACh contraction. However, the presence of Pap (10^{-6}) had no effect.

Out of the above mentioned, it is considered that Iso inhibits both Ca uptake and Ca release in this experimental method, but Pap had no effect on both.

5. The action of relaxants on the shape of the contraction curves of K and ACh

The concentrations used of K and ACh were 2×10^{-3} and 10^{-8}, respectively. The relaxants were applied by the pretreatment (10^{m}) method.

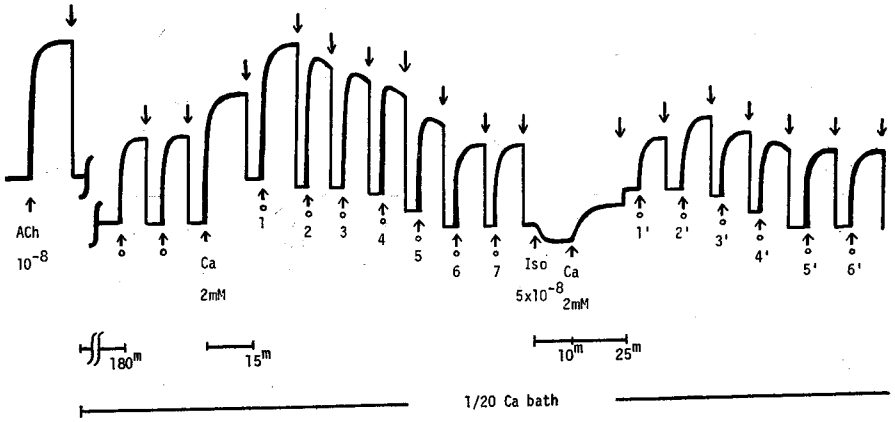

Fig. 7 Effect of isoproterenol (Iso) on the Ca uptake into the Ca store of the preparation in the 1/20 Ca-bath solution.
The mark ♂ indicates the application of acetylcholine (ACh) (10^{-8}).

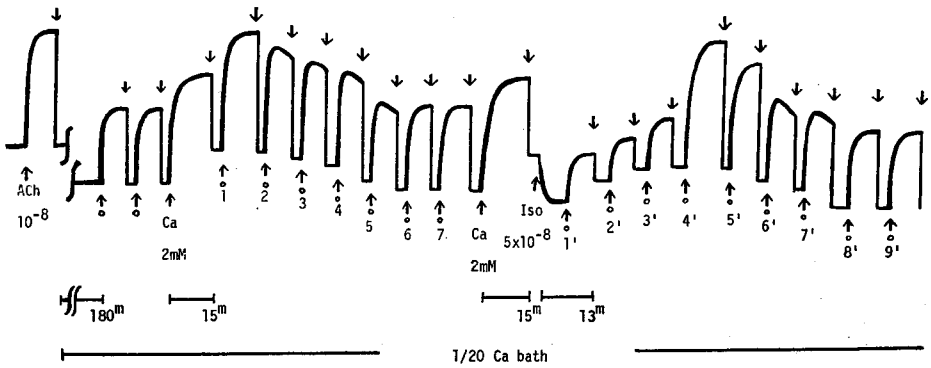

Fig. 8 Effect of isoproterenol (Iso) on the Ca release from the Ca store of the preparation in the 1/20 Ca-bath solution.
The mark ♂ indicates the application of acetylcholine (ACh) (10^{-8}).
In both of the two series of procedures, the control (left) and the test (right), the time interval between the end of the Ca incubation and the first ACh application (♂ attatched with the number 1 or 1') was 15^{m} and thereafter the time interval between each application of ACh was 13^{m} (3^{m} preceding plus 10^{m} following the washout of ACh).

Against the contraction curve of K, Iso in low concentration (10^{-8}) remarkably inhibited PC, without inhibiting TC. With the increased concenration of Iso (2×10^{-7}), TC was also inhibited, but less than the inhibition of PC. On the other hand, low concentration (10^{-6}) of Pap inhibited TC remarkably without inhibiting PC. Higher concentration (10^{-5}) of Pap inhibited PC also, but much less than the inhibition of TC (Fig. 9).

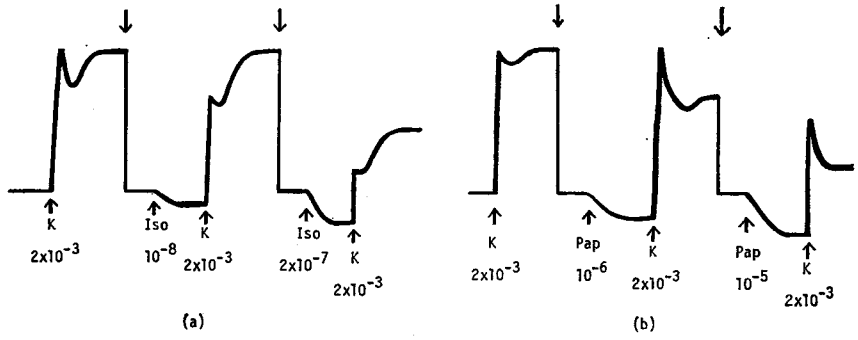

Fig. 9 Effect of isoproterenol (Iso) (a) and papaverine (Pap) (b) on the contraction curve of K in the normal bath solution.

Against the contraction curve of ACh, both Iso ($2\times10^{-8}\sim2\times10^{-7}$) and Pap ($10^{-6}$) in low concetration strongly inhibited TC without inhibiting PC. Both Iso (10^{-6}) and Pap (10^{-5}) in high concentration inhibited PC also, but they inhibited TC much more strongly and almost abolished TC (Fig. 10).

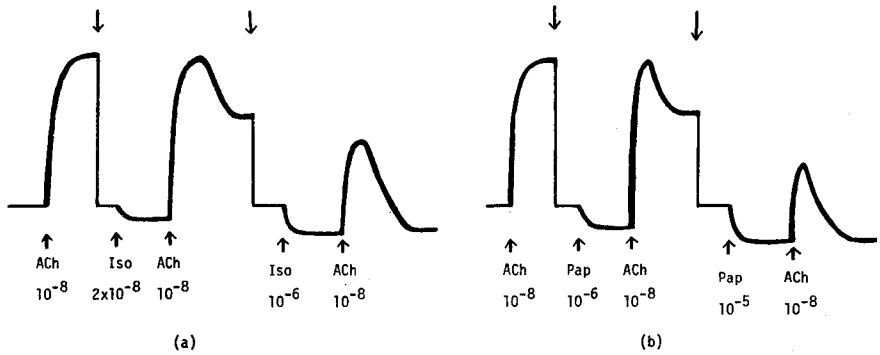

Fig. 10 Effect of isoproterenol (Iso) (a) and papaverine (Pap) (b) on the contraction curve of acetylcholine (ACh) in the normal bath solution.

6. *The types of inhibition of contraction by the relaxants viewed from the concentration-action curve*

As an indicator of the action of contracting drugs for constructing the concentration-action (C-A) curve, in the case of Ca, the contraction by exogenous Ca under the high-K depolarization in the normal bath or the Ca-free bath was used and in the cases of ACh and Ba, PC obtained in the normal bath was used. Besides, in the case of Ba, the residual contraction in the strongly Ca-depleted preparation suspended in the Ca-free bath as stated before was also used as an indicator of action.

a) The action of Iso : In both the normal bath and the Ca-free bath, the log C-A curve of exogenous Ca showed a parallel shift to the right under the influence of Iso 10^{-8}, the concentration of Ca producing maximal contraction being increased from about 10 mM to 20~30 mM. This shift is the same type as the competitive (type I) antagonism.[31, 32] This type of antagonism was also obtained with Iso 10^{-6}, the shift of the curve to the right becoming larger (Fig. 11). Iso also showed the type I antagonism against the log C-A curve of ACh in the whole range of concentrations used (up to 10^{-6}).

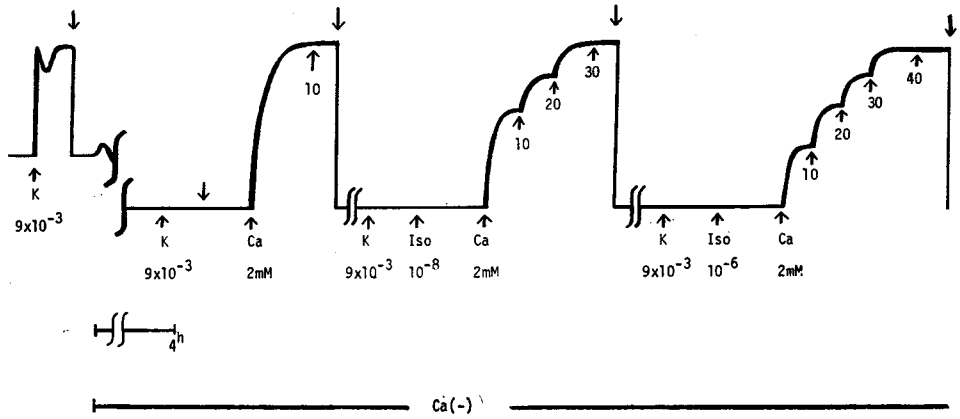

Fig. 11 Effect of isoproterenol (Iso) on the concentration-action curve of Ca in the high K-depolarizing Ca(−) bath solution.

On the other hand, Iso (5×10^{-7}) shifted the log C-A curve of Ba both to the right and downwards, the magnitude of the maximal contraction being decreased. This shift is the same type as the competitive and non-competitive (type III) antagonism (Fig. 12).[31, 32] Iso in high concentration (10^{-6}) also showed the type III antagonism against the log C-A curve of the residual contraction by Ba.

For all of the above contracting drugs, complete inhibition of the contraction by Iso did not occur in the concentrations used (up to 10^{-6}).

b) The action of Pap : Pap showed the type III antagonism against the log C-A curves of exogenous Ca (in the normal or Ca-free bath), ACh and Ba in the inhibitory concentrations tested (5×10^{-6}~10^{-4}). However, Pap (10^{-5}~5×10^{-5}) shifted the log C-A curve of the residual contraction by Ba downwards. This shift is the same type as the non-competitive (type II) antagonism. By the high concentration (10^{-4}) of Pap, the contractions by all of the above contracting drugs were abolished nearly or entirely (Fig. 13). The antagonistic potency of Pap against each of the above contractions was similar (Tabe 1).

The above results analysed by the C-A curve are summarized in Table 1.

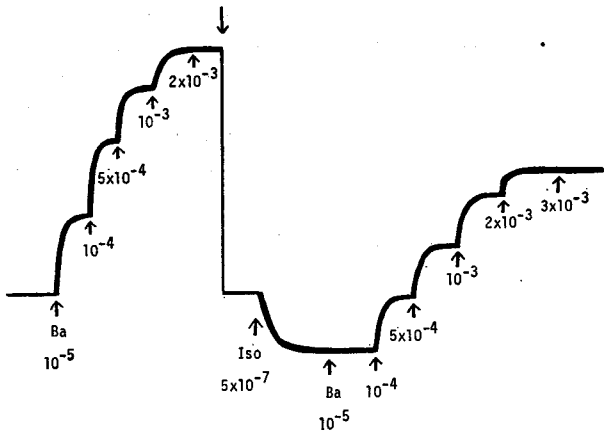

Fig. 12 Effect of isoproterenol (Iso) on the concentration-action curve of Ba in the normal bath solution.

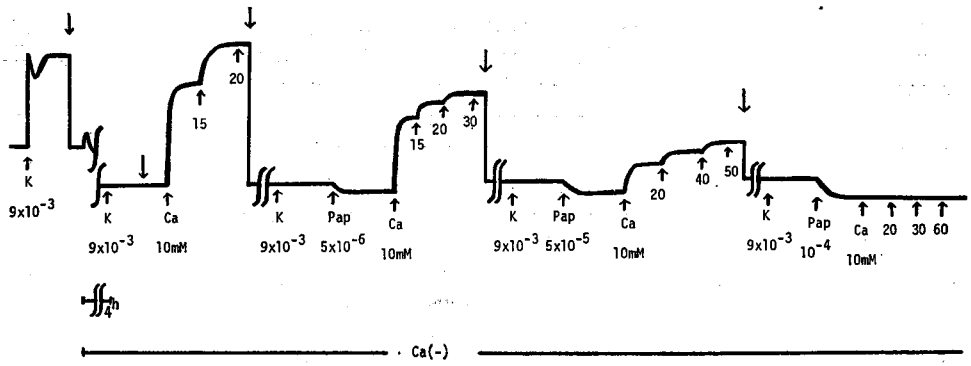

Fig. 13 Effect of papaverine (Pap) on the concentration-action curve of Ca in the high K-depolarizing Ca(−) bath solution.

Table 1 Patterns of the antagonism of the drugs tested (isoproterenol, papaverine) to the concentration-action curves of the spasmogens.

Bath solutions	Normal			Ca(−)	
Spasmogens / Drugs tested	Ca	Acetylcholine	Ba	Ca	Ba (residual contraction)
Isoproterenol	I (10^{-8}〜10^{-6})	I (10^{-8}〜10^{-6})	III (5×10^{-7})	I (10^{-8}〜10^{-6})	III (10^{-6})
Papaverine	III (5×10^{-6} 〜 5×10^{-5})	III (5×10^{-6} 〜 5×10^{-5})	III (5×10^{-6} ～ 5×10^{-5})	III (5×10^{-6} 〜 10^{-4})	II (10^{-5} 〜 5×10^{-5})

I : type I (competitive) antagonism.
II : type II (non-competitive) antagonism.
III : type III (competitive and non-competitive) antagonism.
Figures in the parentheses indicate the concentration of the drugs tested showing the respective patterns of antagonisms.

DISCUSSION

1. *The significance of bath Ca in the tonus and the action of exogenous Ca on the tonus*

After the exchange of the bath for the one without Ca, the preparation immediately showed a transient contraction and then its tonus gradually fell to fix about 150^{m} later.

This transient contraction is, in agreement with the explanation by Hurwitz et al.[21] of the similar phenomenon in the guinea pig's ileum, considered to be due to the following, that is, the Ca in the superficial layer of the muscle cell membrane leaves into the bath immediately after the exchange of the bath and so the release of Ca from the deep layer which has been inhibited by the membrane stabilizing action of Ca in the superficial layer arises into the cell to elicit contraction transiently. And it is considered that the relaxation following the transient contraction is due to the absence of Ca influx from the bath, but the gradual progress in relaxation is due to the gradual depletion of Ca store and so, the continuous release of Ca from the Ca store into the cell is serving to maintain the tonus in the Ca-free bath. The action of exogenous Ca was relaxation (this was proved to be non-adrenergic and is probably due to the membrane stabilizing action of Ca[17]) in the normal bath, but it changed to the sustained contraction in the Ca-depleted preparation, in which K contraction had disappeared, in the Ca-free bath. This contraction may be explained by the acceleration of Ca influx due to the decrease of the membrane stabilizing

action of Ca,[4] which has been elicited by the membrane depolarization[6, 10] and Ca depletion due to the removal of Ca from the bath, and the well-known fact that the increased Ca^{++} in the muscle cell produces contraction. With increase of the exogenous Ca, the contraction increased, but the supramaximal contraction was followed by relaxation. This relaxation probably depends on the re-establishment of the predominance for the membrane stabilizing action of Ca.[4]

2. The shape of the contraction curves of contracting drugs (K, ACh, Ba) and its significance

The contraction curves of K, ACh and Ba consisted of the initial fast contraction (phasic contraction, PC) and the following slow contraction (tonic contraction, TC). Against the contraction curves of any of these contracting drugs, the removal of Ca from the bath or metabolic inhibition (anoxia) preferentially inhibited TC before inhibiting PC. On the other hand, at the time when PC besides TC had been strongly inhibited after the removal of bath Ca, the re-exchange of the bath to normal recovered TC more rapidly than PC. Out of these findings, it is considered that for all of these contracting drugs, PC is induced by Ca release hardly depending on energy (aerobic energy), whereas TC is maintained by the active influx of Ca requiring energy.

The preferential inhibition of TC by the removal of bath Ca or the metabolic inhibition stated above is similar to the report by Urakawa et al.[46] concerning the K contraction of the guinea pig's taenia coli, and the above interpretation on the mechanisms of PC and TC is consistent with the view of Urakawa et al.[45] and Karaki et al.[29] regarding the K contraction on the guinea pig taenia coil, but was not consistent with the view of Imai and Takeda,[24] in which the mechanism of PC was ascribed to Ca influx and that of TC to Ca release for the K contraction on the guinea pig taenia coli.

3. The action of contracting drugs in relation to Ca in the bath

In short time (about 2^m) after the exchange of the bath from normal to Ca-free, the PC level of K, ACh or Ba (of ED_{50}) was the same as that in the normal bath. This also suggests that the mechanism of PC of these drugs mainly depend on Ca release.

With the time after the exchange to Ca-free bath, the contraction height of these drugs gradually decreased. The contractions by K and ACh disappeared about 4^h later almost simultaneously, while the small contraction of Ba remained constant (at 20~40% of the initial height) for a long time from about 5^h later. Further, it was ascertained that the disappearance of the contraction by K or ACh in the Ca-free bath was markedly accelerated by applying Ba contraction in advance.

Putting these findings and the above findings concerning contraction curves together, the following assumptions can be obtained: (1) In the muscle cell membrane, there are two kinds of Ca stores; one, the store where Ca is comparatively

easily released and the other, the store where Ca is not easily released, and the Ca release from the former store has to do with PC by K and ACh, while the Ca release from both the former and the latter stores is concerned with PC by Ba. (2) The TC by each contracting drug is maintained by the active influx of Ca. (3) The direct action on the contractile system of muscle (probably on AM), which is not mediated through the mobilization of Ca, is also concerned partly with the Ba contraction, as suggested by the residual contraction by Ba.

The analysis with the concentration-action(C-A) curve revealed the followings. In the Ca-free bath, when the contraction by K or ACh of ED_{50} disappeared, no contraction was induced by the higher concentraction of K or ACh and so the C-A curve of K or ACh could not be obtained. This suggests that the disappearance of the contraction by K or ACh is due to the depletion of the Ca store on which K or ACh acts. At this time, the log C-A curve of Ba showed a nearly parallel shift to the right as compared with that in the normal bath, indicating that the comparative amount of Ca was still remaining in the store of not easily releasable Ca on which Ba acts. Further, at the time when the contraction by Ba of ED_{50} finally remained fixed, the log C-A curve of Ba showed a shift to the right with a concomitant strong downward compression, comparing with the one in the normal bath. This log C-A curve of the residual Ba contraction could be reproducible in the same shape several times for a long time after this. This reproducibility gives a strong support to the view that the residual contraction by Ba mainly depends on the direct action on the contractile system of muscle (probably on AM), with little dependence on Ca release if any.

The above results concerning K and ACh are consistent with the literatures [2, 14, 19, 23] mentioning that the Ca was required for the smooth muscle contracting action of these drugs and also coincide with the report saying that K[5] and ACh[35] induce the contraction by releasing Ca, so far as the mechanism of PC is concerned.

There are discrepancies between the reports concerning the mechanism of contracting action of Ba on the smooth muscle organs, such as, (1) the indirect action mediated by Ca release,[13, 27, 28, 33, 40] (2) the direct action on the muscle contractile protein[36] and (3) the combined action of (1) and (2).[8] Moreover, there is a report mentioning that the existence of Ca in the bath rather inhibits[47] the Ba contraction. The conclusion obtained in the present study coincides, as stated above, with the view of (3).

Recently, it was clarified that the primary site of action of Ca^{++} in inducing the contraction of AM is troponin, a regulatory protein of muscle, that the binding of Ca^{++} to troponin releases (de-inhibits) the inhibitory function of troponin on the sliding of actin and myosin, and Ba^{++} and Sr^{++} also have the same action on troponin as $Ca,^{++}$[12] although weaker than $Ca.^{++}$ According to this, it is expected that if Ba could reach the muscle contractile system in sufficient amount, it would be able to contract AM directly (not via Ca release). It is considered, however, that according to the kinds of smooth muscle organs, the amount of Ba attainable to the contractile system would be different, and so discrepancies would occur in the contraction mechanism of Ba as stated above.

The presumption of the above-stated division in the Ca store, which was derived

from the difference between Ba and K or ACh concerning the Ca released to elicit contraction, is consistent with the similar view of Hudgins and Weiss[20] in the rabbit's aorta strip.

4. The action of the relaxants (isoproterenol and papaverine) in relation to Ca in the bath

After exchanging the bath for the one without Ca, at everytime before the tonus reached the lowest, the relaxation level induced by isoproterenol (Iso) or papaverine (Pap) was quite the same as the one in the normal bath. From this, it is suggested that these relaxants have the inhibitory action on the tonus-maintaining mechanism other than the Ca influx, that is, on the Ca release or its subsequent processes, although it cannot be judged whether these relaxants have the inhibitory action on Ca influx or not.

5. The effect of the high potassium-depolarizing bath on the relaxing action of the relaxants

Under the high K-depolarization (contraction), the relaxing action of Iso was strongly depressed as regards both the level and the magnitude of relaxation, and the maximal level of relaxation by Iso in the normal bath could not be obtained, however high the concentraction of Iso was increased. On the other hand, in the case of Pap, the relaxation level by its low concentraction was depressed, although the extent of relaxation by it was hardly decreased, while the maximal level of relation by high concentraction was not changed, the magnitude of relation by it being rather increased. From this, it is suggested that the inhibition of the membrane activity (the hyperpolarization or the stabilization of membrane) concerns less with the relaxing action of Pap as compared with that of Iso. This difference is similar to the report of Harigaya et al.[18] in the guinea pig intestine.

6. The comparison of the relaxing potency against the Ca contraction and against the Ba contraction

In the Ca-depleted preparation in the Ca-free bath, in which the K contraction had disappeared, the relaxing action of Iso was strong against the contraction by exogenous Ca and it was weak against the Ba contraction of a similar height, whereas Pap relaxed the both contractions to a similar extent. And, when the relaxing action against the residual contraction by Ba (chiefly dependent on the direct action on AM) in the strongly Ca-depleted preparation in the Ca-free bath was compared with that against the Ba contraction in the normal bath, the relaxing action of Iso against the former was extremely weaker, only a slight relaxation being observed, whereas Pap showed the similar extent of relaxation against the both contractions. Putting these findings and the above findings concerning the effect of depolarization together, it is considered that the relaxation by Iso mainly depends on the antagonism against Ca in the muscle cell membrane, and hardly depends on the inhibition of the muscle

contractile system, while for the relaxation by Pap the inhibition of the contractile system plays a major role in addition to the inhibition against Ca in the membrane.

7. The action of relaxants on the Ca uptake and the Ca release in the low Ca-bath

The effect of relaxants on the uptake of exogenous Ca into the Ca store and on the Ca release was examined in the low Ca-bath by the functional method of observation. Iso inhibited the both, but Pap did not affect the both. It is considered, however, that these results do not always reflect wholly the actions of relaxants on the Ca uptake and Ca release under the influence of contracting drugs. To clarify the latter actions, the next item of observations was performed.

8. The action of relaxants on the shape of the contraction curve of contracting drugs

As suggested before, the PC by K or ACh is induced by Ca release hardly depending on energy, whereas its TC is maintained by the active influx of Ca requiring energy. The low concentration of Pap selectively inhibited the TC by K or ACh without inhibiting the PC by K or ACh (and therefore, without inhibiting the contractile system of muscle). From this, it can be assumed that Pap has the inhibitory action on the active influx of Ca. On the other hand, the low concentration of Iso selectively inhibited the PC against K, while it selctively inhibited the TC against ACh, and so it can be assumed that Iso has the inhibitory action against both the Ca release and the active Ca influx. For the present, it is difficult to explain why Iso showed the different manner of action against the contractions by K and ACh.

The high concentration of Pap inhibited also the PC by K or Ach, and the high concentration of Iso also inhibited the TC by K and the PC by ACh. Concerning the mechanism of the additional appearance of the inhibition against TC or PC in high concentration of the relaxants, two explanations are possible, that is, firstly, the addition of the inhibition against the active Ca influx or the inhibition of Ca release, respectively, and secondly, the addition of the inhibition against the contractile system of muscle. It is not easy in general to differentiate the validity between these explanations. However, in the case of Pap, the second explanation is more plausible because it was suggested that the high concentration of Pap inhibits the muscle contractile system, as stated before, and in the case of Iso, the first explanation seems to be more plausible, because Iso in low concentration inhibits both PC and TC (as the sum of the action against K and ACh), and because, as stated before, the evidence is hardly present suggesting the inhibitory action of Iso on the contractile system.

The reports (Ferrari et al.,[15] Imai and Takeda[24]) mentioning that Pap inhibited TC preferentially than PC by K in the guinea pig's taenia coli are similar to the above results concerning Pap in the present study. The report concerning the action of Iso on the contraction curve is not available.

9. The contraction-inhibiting mechanism of the relaxants viewed from the concentration-action curve

Iso showed a parallel shift to the right, that is, the same shift as the competitive (type I) antagonism,[31, 32] against the log C-A curve of exogenous Ca inducing contraction (under the high K-depolarization in the normal or Ca-free bath). However, this Ca contraction is due to Ca influx and the primary site of action of Iso is the β-receptor, and therefore, it is considered that the meaning of this type I antagonism between Ca and Iso is not the true competition, but the functional antagonism showing pseudo-competition.[1, 41] The mechanism of this antagonism is not clear in detail, but as its possible explanation, it may be considered that Iso facilitates the Ca uptake into the Ca store (as assumed by Schild[36]) and that the inhibitory action on the membrane which Iso has inhibits the mobilization of Ca resulting in muscle contraction. Iso showed the type I antagonism, also against the PC by ACh (which is due to the Ca release, as stated before) in the normal bath. This type I antagonism is also considered to mean the functional antagonism. On the other hand, Iso showed the combined shift of a parallel shift to the right and a downward compression, that is, the same shift as the competiive and non-competitive (type III) antagonism,[31, 32] against PC by Ba in the normal bath as well as against the residual contraction by Ba in the Ca-free bath. Thus, in this case, besides the factor of type I (functional) antagonism, the factor of the non-competitive (type II) antagonism,[31,32] which was not seen against exogenous Ca or ACh, appeared in addition. It is considered that the mechanism of this type II antagonism is presumably due to the non-competitive inhibition of Ba influx by Iso, because, as suggested before, the site of action of Iso chiefly consists in the membrane, but not in the contractile system of muscle.

On the other hand, Pap showed the type III antagonism against the contraction by exogenous Ca (under the high K-depolarization in the normal or Ca-free bath), PC by ACh and PC by Ba (in the normal bath), while it showed type II antagonism against the residual contraction by Ba (in the Ca-free bath). In this case, the factor of type I antagonism, which is involved in the type III antagonism, probably means the true competition, and so it is considered that Pap possesses the competitive inhibitory action against the influx of exogenous Ca, and it also possesses the competitive inhibitory action against Ca mobilization within the cell (Ca release or the subsequent arrival of released Ca at the muscle contractile system), as suggested by the existence of the factor of type I antagonism against the PC by ACh and Ba. The factor of type II antagonism, which was observed towards all of the contracting drugs in the similar concentration of Pap, strongly suggests the non-competitive inhibition by Pap on the contractile system of muscle, which is the common pathway in the contraction by each contracting drug. The partial difference between the inhibition by Pap against the PC by Ba in the normal bath (type III antagonism) and that against the residual contraction by Ba in the Ca-free bath (type II antagonism) is interesting. This is probably explicable by the assumption that the factor of type I antagonism by Pap against PC by Ba is due to the competitive inhibition of Ca mobilization and that the residual contraction by Ba has little to do with the Ca mobilization.

According to the literature concerning the C-A curve, it has been already reported[1, 41] that the antagonisms of Iso and other adrenergic drugs against the contractions of intestine by ACh and the other cholinergic drugs were of the type I, which means the functional antagonism. The result on the antagonism between ACh and Iso in the present study is consitent with this report.

Concerning the type of antagonism against the contraction by exogenous Ca, it was reported that Pap showed the type I antagonism in the rabbit's ear blood vessels (Ferrari et al.[16]) and in the guinea pig's taenia coil (Simonis, Ariëns et al.,[37] Ferrari et al.[15]). In partial difference from these reports, in the present study, Pap showed the type II antagonism concomitantly with the type I antagonism. This difference is probably due to the difference in the organs employed or the concentrations of Pap used.

The factor of type I (competitive) antagonism which Pap possesses against contracting drugs is considered to occur most likely in the membrane of muscle cell. This view is consistent with the reports suggesting the action of Pap on the membrane, such as the hyperpolarizing action[25] on the muscle cell membrane, the inhibiting action on Ca influx[42, 43] and the inhibiting action on Ca release[42] in the guinea pig's taenia coli. On the other hand, the factor of type II (non-competitive) antagonism which Pap possesses is most easily explained by the inhibitory action of Pap on the oxydative phosphorylation,[34] which results in the inhibition of the generation of energy required for muscle contraction and consequently the inhibition of the muscle contractile system.

Recently, various actions based on various viewpoints have been reported concerning the relaxing mechanism of Pap on smooth muscles, that is, (1) the competitive inhibition against Ca,[15] (2) the hyperpolarization of membrane,[25] (3) the increase of cyclic AMP due to the inhibition of phosphodiesterase[30, 44] and (4) the inhibition of oxydative phosphorylation.[34] It is possible that the actions of (2) and (3) are ultimately concerned with the inhibition against the Ca mobilization for contraction.[7, 9] The above assumptions on the mechanism of action of Pap which were obtained in the present research is considered to be not contradictory with each of these opinions.

10. The relaxing mechanism of the relaxants on the normal tonus

Summing up the findings and discussions in all of the above items, the relaxing mechanism on the normal tonus is assumed as the following, that is, (1) the relaxing mechanism of Iso is mainly in the membrane and is due to the functional antagonism (pseudo-competition) against the Ca influx and the Ca release, and (2) the relaxation by Pap is induced both by the competitive inhibition against the Ca influx and the non-competitive inhibition against the contractile system of muscle.

The reason why the inhibition against Ca release by Pap was not included in the above is that such action could not be clearly demonstrated in the experiments concerning the inhibition against Ca release and against contraction curve, but not that it was definitely denied.

SUMMARY

Using the strips of rat's ileum, the action mechanisms of the contracting drugs, K, ACh and Ba and of the relaxants, isoproterenol (Iso) and papaverine (Pap) were investigated.

1. The action of the contracting drugs

(1) Any of the contraction curves of K, ACh and Ba consists of the phasic contraction (PC) and the following tonic contraction (TC). Evidences have been obtained suggesting that, for any of these contracting drugs, PC is induced by the Ca release hardly depending on energy and TC is maintained by the active influx of Ca requiring energy. And from the fact that only the small residual contraction by Ba remains constant in the Ca-free bath, it is assumed that the direct action on the musale contractile system without the mediation of Ca mobilization is also concerned partly with the contracting mechanism of Ba.

(2) It was suggested that the Ca stores in the muscle cell membrane are distinguished into the one where Ca is comparatively easily released and the one where Ca is not easily released, and that K and ACh release Ca from the former store, while Ba does so from both the former and the latter stores.

2. The action of the relaxants

(1) From the change of the relaxing action by the high K-depolarizing bath, it was assumed that the inhibition against membrane activity is more concerned with the relaxing mechanism of Iso than with that of Pap.

(2) The functional method of observing the effect on the Ca release from and the Ca uptake into the Ca store in the low Ca-bath, revealed that Iso inhibited the both, while Pap did not affect the both.

(3) In the Ca-free bath, Iso relaxed the Ca contraction strongly, the Ba contraction weakly and the residual contraction by Ba extremely slightly, whereas Pap relaxed these three contractions at the same degree. It is assumed from this that the relaxation by Iso is mainly due to the antagonism against Ca, whereas that by Pap is more due to the inhibition of the muscle contractile system (in a broad sense, including energy metabolism) than to the antagonism against Ca.

(4) Pap in low concentration selectively inhibited TC of the contraction curve of K or ACh, while Iso in low concetration selectively inhibited PC against K and seletively inhibited TC against ACh. From this, it can be assumed that Pap has the inhibitory action on the active Ca influx and Iso has the inhibitory action against both the Ca release and the active Ca influx.

(5) Using the concentration-action curve, the results of Table 1 were obtained concerning the antagonistic types of the relaxants against each contraction by exogenous Ca, ACh and Ba (including the residual contraction of Ba).

(6) Summing up the above findings, the relaxing mechanism on the normal tonus was assumed as the following. That is, (1) the relaxation mechanism of Iso

is mainly in the membrane and is due to the functional antagonism (pseudo-competition) against the Ca influx and the Ca release, and (2) the relaxation by Pap is induced both by the competitive inhibition against the Ca influx and the non-competitive inhibition against the muscle contractile system.

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