ENHANCEMENT OF Ca-CONTRACTIONS BY CATECHOLAMINES AND TEMPERATURE DEPENDENCY IN THE DEPOLARIZED TEANIA COLI OF THE GUINEA-PIG

Hidenori OHASHI*, Akira OHGA and Koji SAITO
Department of Pharmacology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan
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Abstract—Guinea-pig taenia coli suspended in a depolarizing solution without Ca responded to Ca by a contraction. The effects of adrenaline, noradrenaline and isoprenaline on the contractile response were investigated at 20°C and at 37°C. Contractile response to Ca was not significantly affected by adrenaline and noradrenaline, but was reduced by isoprenaline at 37°C. At 20°C, all the three catecholamines inhibited Ca-contraction. The potency in producing the inhibition was isoprenaline, adrenaline, noradrenaline, the same sequence of potency as related to beta-receptor mediated responses. Treatment with a beta-receptor blocking agent markedly reduced or abolished the inhibitory effects, and consequently the Ca-contraction was enhanced by adrenaline and noradrenaline at 37°C. Treatment with an alpha-receptor blocking agent increased the inhibitory effects so that adrenaline and noradrenaline reduced the Ca-contraction at 37°C as well as at 20°C. The order of potency in producing the inhibition after alpha-receptor blockade was again isoprenaline, adrenaline, noradrenaline. It was concluded that the stimulatory effect is mediated by alpha-receptor activation and the inhibitory effect by beta-receptor activation. After beta-receptor blockade and at 37°C, adrenaline and noradrenaline applied after Ca caused further development of tension. The contractile response to adrenaline resembles the slow component of the ACh-induced contraction, regarding time course and susceptibility to removal of Ca from the extracellular medium. Adrenaline enhanced the Ca-contraction to the same extent before and after frequent exposures to ACh to deplete cell-bound Ca. These findings suggest that the enhancement of the Ca-contraction by adrenaline and noradrenaline is dependent on increased permeation of Ca ions across the cell membrane.

It is well known that catecholamines such as adrenaline, noradrenaline and isoprenaline cause relaxation of intestinal smooth muscle and that the relaxing action is mediated by both adrenergic alpha- and beta-receptor mechanisms (1-4). The initial effect is suppression of the spontaneous discharge of the action potentials (3-6), usually with hypopolarization of the muscle membrane associated with an increase of the membrane conductance mainly due to potassium and to chloride (3, 7). It has been established that Ca ions are essential for the action of the catecholamines (8, 9). The effects of raising the external concentration of Ca on the membrane potential and membrane resistance resemble those of catecholamines (10, 11). These observations have led to the view that the catecholamines may alter the process of Ca-binding in and Ca-removal from the mem-

* Present address: Department of Pharmacology, Faculty of Agriculture, Gifu University, Gifu, Japan
brane, resulting in a change in distribution of Ca ions in the membrane, and thereby changing the permeability (9, 10).

It is generally accepted that Ca ions are the final activator of the contractile system of smooth muscle as in skeletal muscles and it is assumed that smooth muscle membrane may play a role in controlling the concentration of intracellular free Ca ions as in the sarcoplasmic reticulum of skeletal muscle, since the cellular Ca exchanges rather quickly with the extracellular Ca (12), the sarcoplasmic reticulum is very poorly developed (13, 14) and due to the small size of the cells (surface/volume ratio approaching 1:1) the possibility has been considered by Peachey & Porter (14) that the contractile elements may be activated sufficiently fast by diffusion of Ca across the cell membrane or by liberation of Ca from the membrane structure.

Thus, if the view presented by Bülbbring and Tomita (9, 10) that the amines change the process of Ca-binding in and Ca-removal from the membrane is correct, one can expect that the drugs may also affect directly contractile responses to Ca of smooth muscle depolarized in a high K medium.

The aim of the present work was to investigate the effects of catecholamines on the responses to Ca of depolarized taenia coli of the guinea-pig at different temperatures and to draw inferences about the movements of Ca from the mechanical changes produced by the catecholamines. It was found that the catecholamines enhanced the Ca-responses by alpha-receptor activation and this effect could be due mainly to increased permeation of Ca ions across the cell membrane.

A preliminary account of some of these observations has been given (15).

MATERIALS AND METHODS

The experiments were carried out with isolated taenia coli of the guinea-pig. Strips approx. 20 mm long were mounted in Tyrode solution of the following composition: NaCl, 137 mM; KCl, 1.5 mM; NaH₂PO₄, 0.4 mM; CaCl₂, 1.8 mM; MgCl₂, 0.4 mM; glucose, 5 mM; NaHCO₃, 12 mM and bubbled with air.

The experiments were performed at 20 ± 1°C and at 37 ± 1°C. After equilibration with the Tyrode solution for approx. 10 min at 20°C and 60 min at 37°C, the preparations were then transferred to Ca-free and K-rich Tyrode solution in which Ca contamination is as low as 0.04 mM (referred to as K-Tyrode in this paper): the NaCl was replaced with equimolecular KCl or K₂SO₄, the CaCl₂, NaH₂PO₄ and NaHCO₃ were omitted and Tris-maleate buffer (pH 7.4), 5 mM was added. In most of the experiments, KCl-Tyrode was used as the depolarizing solution. Some experiments were performed with K₂SO₄ instead of KCl as the depolarizing solution. The results obtained under these different conditions were not qualitatively different.

The volume of the bathing fluid was 3.0 ml. Calcium chloride and drugs were added to the bathing fluid from the concentrated solutions by rapid injection of a small volume (0.3 ml at 20°C and 0.1 ml at 37°C) to give the final concentration required. All concentrations in this paper refer to the final concentration in the bathing fluid.
Tension responses were isometrically measured with a mechano-electronic transducer (Toshiba 5734 A) and records were obtained on a pen-recorder (Nihon Kohden, WI-260 or Hitachi, 056).

Drugs used were ( )-adrenaline (Burroughs Wellcome), (—)-noradrenaline bitartrate (Koch-Light), ( )-isoprenaline sulphate (Burroughs Wellcome), dibenamine hydrochloride (Tokyo Kasei), phenoxybenzamine hydrochloride (Tokyo Kasei), pronethalol (‘Alderlin’, Sumitomo Kagaku), propranolol (‘Inderal’, ICI) and acetylcholine chloride (‘Ovisot’, Daiichi).

RESULTS

Responses to Ca at 20°C and 37°C

The guinea-pig taenia coli suspended in depolarizing and Ca-free Tyrode solution (K-Tyrode solution) responded by a contraction to a small amount of Ca added to the organ bath. The sensitivity to Ca varied in the course of depolarization; the amplitude of successive contractions evoked by Ca at a constant concentration increased to a stable level which was maintained over a period of 2 to 3 hr. Fig. 1 shows typical records of isometric tension obtained from the same strip at 20°C and 37°C. The final Ca concentrations were 0.5 mM and 2.0 mM. The tension of the contractile responses rose slowly and was sustained. The tension developed within 3 min increased as the amount of Ca added increased. The maximum rate of rise in tension also increased but less consistently. Frequently, the tracing of tension development at 37°C showed a curve with a hump as illustrated in Fig. 1 b. The hump made it difficult to measure the rate of rise in tension. It can also be seen in Fig. 1 that the tension evoked by either dose of Ca rose to a higher level and removal of the Ca by washing with K-Tyrode solution led to a faster relaxation, at 37°C. Time taken for relaxation to half amplitude of the peak tension was 72 sec in one muscle strip and 36 sec in another at 20°C, and after raising the temp. of the bathing solution to 37°C it was shortened to 55 and 21 sec respectively.

Enhancement of the Ca-contractions by catecholamines

The concentration of Ca employed was 2.5 mM at 20°C and 2.0 mM at 37°C. The
dose-response curve in the preliminary experiments predicts that a small change of Ca concentration in the medium around 2.0 or 2.5 mM would give rise to a great change in tension development. Thus if catecholamines modify the process of Ca-binding in and Ca-removal from the membrane \(9, 10\), leading to changes in the intracellular Ca ions available for the contractile elements, one can expect that the drugs would affect responses to these Ca concentrations to a great extent. Each exposure of the strips to Ca was limited to 3 min at 20°C and to 2 min at 37°C, although sometimes contractions did not reach a steady level. The tension developed within these periods, however, was found to be highly reproducible provided that 15 min (at 20°C) or 10 min (at 37°C) intervals between applications were allowed.

In each experiment, after the sensitivity of the muscle strip to Ca was stabilized by several applications of Ca, two Ca-contractions were further obtained and then a catecholamine was added to the bathing solution 3 min before the following Ca-contraction. Changes in the magnitude of Ca-contractions produced by the catecholamines were expressed as percentages of the control Ca-contractions (the second Ca-contraction of the two in the absence of catecholamines). The first Ca-contractions were also presented as percentages of the same control to show variation in Ca-contractions before application of each catecholamine.

At 37°C responses to Ca were apparently enhanced by adrenaline and noradrenaline. An example of the effect of adrenaline, \(5 \times 10^{-7} \text{ M}\), is shown in Fig. 2 (upper row). The results are summarized in Table 1. Differences between the mean values in a and b in

![Fig. 2](image)

**Fig. 2.** Effect of adrenaline on Ca-contractions at 37°C and influence of alpha-receptor blocking agents on the enhancing effect.

Ca was added to the bath for 2 min periods at intervals of 10 min. (a) Control; (b) in the presence of adrenaline \(5 \times 10^{-7} \text{M}\) applied 3 min before Ca; (c) after removal of adrenaline. Upper row: control effect of adrenaline; lower row: in the presence of phenoxybenzamine \(2 \times 10^{-6} \text{M}\). Horizontal bar shows the scale for 1 min and vertical bar for 1 g.
**α-RECEPTOR IN DEPOLARIZED SMOOTH MUSCLE**

Table 1. Comparison of tensions evoked by 2.0 mM Ca++ in the presence and absence of each catecholamine at 37°C.

| Muscle preparations | Adrenaline (mean±S.E. %) | Noradrenaline (mean±S.E. %) | Isoprenaline (mean±S.E. %) |
|---------------------|---------------------------|------------------------------|-----------------------------|
| Normal              |                           |                              |                             |
| a                   | 104.8±5.7 (n=7)           | 103.3±4.4 (n=6)              | 101.4±2.4 (n=12)            |
| b                   | 107.6±7.9                 | 108.7±5.6                   | 71.8±5.0                   |
| Treated with an α-receptor blocking agent | 101.1±4.7 (n=9) | 100.0±3.0 (n=8)              | 101.0±4.7 (n=9)             |
| Treated with a β-receptor blocking agent | 70.1±2.8                  | 87.6±4.4                    | 62.6±7.3                   |
| e                   | 99.0±5.9 (n=9)            | 102.0±2.8 (n=8)             | 105.2±3.4 (n=9)            |
| f                   | 125.4±8.2                 | 121.1±4.7                   | 103.7±7.7                  |

1) Figures in b, d and f show the mean tensions±S.E. in the presence of catecholamines relative to the second control values in the absence of the amines.
2) Figures in a, c and e show the mean tensions±S.E. in the first Ca-contractions before application of the amines relative to the second control as in b, d or f.
3) Figures in parentheses indicate the number of measurements. See text for further details.

Table 1 were not however statistically significant. On the other hand, responses were significantly reduced by isoprenaline.

It is well known that adrenaline and noradrenaline activate both alpha- and beta-receptors whereas isoprenaline almost solely activates beta-receptors, and that the inhibitory effect in the depolarized taenia coli is mediated by beta-receptor activation (16). Therefore, it is possible that the insignificant effects of adrenaline and noradrenaline may be due to resultant antagonism between an enhancement of Ca-contractions through activation of the alpha-receptors and a reduction of these through activation of the beta-receptors. The possibility was examined by comparing the effects of the catecholamines in the presence of either an alpha-receptor blocking agent or a beta-receptor blocking agent with those in the absence of the blocker. The blockers were allowed to remain in contact with each muscle strip for more than 30 min, because of the well known slow onset of action of alpha-receptor blockers. Adrenaline and noradrenaline enhanced significantly Ca-contractions after treatment with a beta-receptor blocking drug, propranolol or pronethalol (2 or 4×10⁻⁶ M) (Table 1-f). As shown in Fig. 2, the drugs reduced Ca-contractions after treatment with an alpha-receptor blocking agent, phenoxybenzamine or dibenamine (1.5 or 3.0×10⁻⁶ M) (Table 1-d); the order of potency in producing the inhibition was isoprenaline, adrenaline, noradrenaline, which was the same sequence as that related to beta-responses (17). Both adrenergic-receptor blocking drugs, individually had a weak inhibitory effect on Ca-contractions so that the contractions were sometimes reduced when used in higher concentrations.

Thus the most likely interpretation for the results at higher temperatures is that the catecholamines tested enhance the Ca-contractions through activation of the alpha-receptors, and the stimulatory effects are usually masked or made obscure by their inhibitory effects through simultaneous activation of the beta-receptors.

At 20°C, responses to Ca were reduced by all the three catecholamines, as observed
by Jenkinson and Morton (16). Typical effects are illustrated in Fig. 3. The evoked tension by Ca was less in the presence of the catecholamines. All average changes in the tension which developed within 3 min are summarized in Table 2. The order of potency

![Graphs illustrating effects of catecholamines on Ca-contractions at 20°C.](image)

**Table 2. Comparison of tensions evoked by 2.5 mM Ca++ in the presence and absence of each catecholamine at 20°C.**

| Muscle preparations                  | Adrenaline (mean ± S.E. %) | Noradrenaline (mean ± S.E. %) | Isoprenaline (mean ± S.E. %) |
|--------------------------------------|-----------------------------|--------------------------------|-------------------------------|
| Normal                               |                             |                                |                               |
| a                                    | 101.3 ± 2.3 (n=7)           | 104.3 ± 2.6 (n=10)             | 105.4 ± 6 (n=14)              |
| b                                    | 70.6 ± 3.3                  | 81.7 ± 6.9                     | 49.8 ± 4.7                   |
| Treated with an α-receptor blocking  |                             |                                |                               |
| agent                                | 100.2 ± 3.3 (n=5)           | 102.0 ± 4.8 (n=5)              | 101.4 ± 2.7 (n=5)            |
| d                                    | 55.8 ± 4.2                  | 66.4 ± 6.1                     | 30.0 ± 4.1                   |
| Treated with a β-receptor blocking    |                             |                                |                               |
| agent                                | 98.0 ± 2.0 (n=4)            | 105.5 ± 6.0 (n=6)              | 99.4 ± 5.0 (n=6)             |
| e                                    | 99.3 ± 4.8                  | 101.2 ± 8.9                    | 87.3 ± 5.0                   |

1) Figures in b, d and f show the mean tensions ± S.E. in the presence of catecholamines relative to the second control values in the absence of the amines.
2) Figures in a, c and e show the mean tensions ± S.E. in the first Ca-contractions before application of the amines relative to the second control as in b, d or f.
3) Figures in parentheses indicate the number of measurements. See text for further details.
in producing the inhibition was again isoprenaline, adrenaline, noradrenaline. The inhibitory effect of the catecholamines was markedly reduced or abolished in the presence of pronethalol or propranolol (2 or $4 \times 10^{-4}$ M) (Table 2-f). In contrast, responses to Ca were more inhibited by each catecholamine in the presence of an alpha-receptor blocking drug (Table 2-d). This also suggests that all catecholamines may have a stimulatory effect on the Ca-contraction, which may be mediated by alpha-receptor activation in addition to their well known inhibitory effect.

Comparison of contractile responses to adrenaline and to ACh

At 37°C, in some muscle strips treated with a beta-receptor blocking drug, adrenaline or noradrenaline produced an increase of tension in Ca-contractions when introduced at the stage of a tonic phase of the contractions as presented in Fig. 4-a. During prolonged exposure to the drugs the increased tension was not maintained but gradually passed off with time. The contractile response to adrenaline was compared with a contractile response to ACh in the same condition. It may be seen that the ACh response consists of a fast (transient) and a slow component, whereas the adrenaline response has a single component which is very similar in time course to the slow component of the ACh-induced contraction. The magnitude of each component of the responses to both drugs was decreased by a decrease in Ca concentration in the medium, but the fast component persisted for a long period even in K-Tyrode solution without Ca ions as shown in Fig. 4-b. Here, the response to 1.0 mM Ca was observed first and then the added Ca was removed by washing three times with the K-Tyrode solution (at Wash). Adrenaline, $5 \times 10^{-7}$ M, was added 2 min after removal of the Ca and in turn ACh, $5 \times 10^{-8}$ M, 3 min after

![Fig. 4. Comparison of contractile effect of adrenaline $5 \times 10^{-7}$ M and ACh $5 \times 10^{-9}$M, applied during Ca-contraction and during relaxation after removal of Ca, in the presence of pronethalol $2 \times 10^{-6}$M at 37°C. (a) Contractile responses to adrenaline applied 5 min after 1.0 mM Ca followed by ACh 4 min after the adrenaline; (b) responses to adrenaline applied 2 min after removal of 1.0 mM Ca (at Wash) followed by ACh 3 min after the adrenaline. Horizontal bar shows the scale for 1 cm and vertical bar for 1 g.](image-url)
the adrenaline. In keeping with the concept that Ca ions which activate smooth muscle contraction can be supplied from more than one site (18-20), the present finding may be interpreted as reflecting two sources of Ca ions which are differentially affected by changing the extracellular concentration of Ca. One source responsible for the fast contraction may be a cellular site, and another responsible for the slow contraction may be the extracellular space. Assuming this to be true, adrenaline appears to act through increasing Ca entry from the extracellular medium into the cells, whereas ACh appears to act through increasing the mobilization of Ca ions from both Ca pools.

Adrenaline, like ACh, may also release Ca ions from the cellular site but it may do so with a slower time course and to a lesser degree thereby not reaching the threshold Ca concentration sufficient to develop isometric tension. Thus adrenaline would lead to an increase of background free Ca concentration available for activation of contraction, resulting in enhancement of a contraction produced by the addition of Ca to the bathing solution. Should this be the case, it can be expected that the stimulatory effect of adrenaline would be modified by changing the amount of Ca in the cellular pool. The following experiments were carried out to determine in what way depletion of the cellular Ca depot with ACh would alter the potentiation effect of adrenaline on Ca-contractions, since it has been observed that ACh releases a quantity of cell-bound Ca in smooth muscle tissues (20-22). The decline of ACh responses in a Ca-free environment was accelerated by frequent stimulation with this drug. Abolition of the ACh response was taken as an indication of the depletion of Ca from the storage site. Fig. 5 shows one of this series of experiments. ACh, 5 x 10^{-6} M, was introduced to the bath at each dot (2 min interval). Everytime the ACh remained for 20 sec and was then removed by washing with the fresh K-Tyrode solution, the ACh responses declined progressively and finally were completely abolished. At this stage the contractile response to 2 mM Ca was ob-

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**Fig. 5.** Potentiating effects of adrenaline on Ca-contractions after possible depletion of the cellular Ca depot by frequent stimulation with ACh in the presence of propranolol, 2 x 10^{-6} M at 37°C. ACh 5 x 10^{-6} M was added at each dot to the bath for 20 sec periods at intervals of 2 min. Between two successive additions of ACh bathing solution was replaced with fresh Ca-free K-Tyrode solution. (a) Ca-contraction after abolition of ACh response; (b) Ca-contraction at the same stage as (a) in the presence of adrenaline 5 x 10^{-6} M applied 3 min before Ca. Horizontal bar shows the scale for 1 min and vertical bar for 1 g.
served (Fig. 5-a). The same muscle strip was then subjected to a second series of ACh exposures to deplete Ca in the same manner as described above. After this, adrenaline, $5 \times 10^{-7}$ M was introduced to the bath and then the response to the same dose of Ca was obtained (Fig. 5-b). In eight experiments, the average response in the presence of adrenaline was 125.0% of the reference contraction (ranging from 106 to 144%). The average value is comparable to that in the strips unexposed to ACh (125.4%, see Table 1-f), which means that the stimulatory effect of adrenaline is not reduced after a possible depletion of the cellular Ca-store. Therefore, the possibility that the enhanced contractile response to Ca in the presence of adrenaline may be due to a release of Ca ions from a cellular depot is remote.

**DISCUSSION**

The results presented in this paper confirmed the observations made by previous authors that catecholamines caused inhibition or a fall in tension of the K-contracture and of Ca-contractions of the guinea-pig taenia coli through beta-receptor activation (16, 23). In addition, the present results clearly indicate that adrenaline and noradrenaline can also cause enhancement of the Ca-contraction through alpha-receptor activation. One of the possible reasons why the stimulatory effect has not been observed hitherto may be that an investigation of the action of catecholamines on the Ca-contraction has not been done at a higher temp. (37°C). In fact, the stimulatory effect was very temp. dependent to the point where it was difficult to demonstrate the alpha-effect at a lower temp. (20°C). The result could be explained by a reduction of the alpha-effect or augmentation of the beta-effect at low temp. If the alpha-effect is mediated by accelerated Ca entry due to increased permeation of this ion across the cell membrane as suggested in the present paper, it could be effectively reduced when the Ca concentration gradient between the bathing solution and the tissue is lowered. Bauer, Goodford and Huter (24) observed that the tissue Ca content of the guinea-pig taenia coli increased after exposure to room temp., and also they suggested that the elevated Ca content was due to inhibition of a Ca extrusion mechanism which requires energy supply. This may be the case also in the depolarized taenia coli and, consequently, the alpha-effect may be reduced. It has been suggested that some metabolic process is involved in the beta-effect, which supplies energy for the process of removing Ca ions from the environment of contractile elements (9). Therefore, by assuming that the metabolic process related to the beta-effect is saturable and that it can be saturated to a less extent at a low temp., an augmentation of the beta-effect could be expected.

Two possible reasons for the difficulty in demonstrating the alpha-effect at a lower temp. have been mentioned. There may still be however, additional underlying causes. To fully comprehend the difference of the effects of catecholamines on Ca-contractions at different temp., detailed information concerning genesis of the Ca-contraction and dependency of each step of the genesis on temp. is required as well as knowledge of the mechanism of the action of catecholamines.
In similar experiments on rabbit uterus and rat seminal vesicle, contractile responses to adrenaline were obtained in polarized as well as in depolarized muscle preparations (25). The taenia coli of the guinea-pig is exceptional in that adrenaline produces the opposite effect through alpha-receptor activation before and after the membrane depolarization.

In view of the importance of Ca inside the cell to regulate contraction, it is logical to assume that the stimulatory effect of adrenaline and noradrenaline may be due to an increase in concentration of intracellular Ca ions which activate the smooth muscle contraction. The catecholamines may possibly be responsible by liberation of Ca ions from a cellular store and/or by an increase in permeation of Ca ions across the cell membrane. It has been suggested that ACh causes a liberation of cell-bound Ca (20-22) as well as an increase in the membrane permeability to the ions (26, 27). The present findings are that (1) the ACh-induced contraction is composed of two components, a fast and a slow one, (2) the fast component of the ACh-contraction has a much faster rate of rise than a Ca-contraction of about the same magnitude and the tension is lost within less than 15 sec, (3) the fast component is very resistant to removal of Ca from the extracellular medium, while the slow component is very susceptible, and (4) the abolition of the fast ACh response in a Ca-free environment is much slower than the loss of Ca by diffusion from the extracellular space (28). It is therefore reasonable to assume that Ca liberation from a cellular depot can account for the fast component of the ACh contraction and increased Ca entry due to increase in the membrane permeability for the slow component of the contraction.

The similarity of the adrenaline-induced contraction, regarding dependency on the extracellular Ca concentration, the time course of tension development and its decay, to the slow component of the ACh-contraction suggests that the adrenaline contraction may be mediated by the same mechanism as that of the slow component of the ACh contraction, i.e. an increase in the membrane permeability to Ca ions. This assumption is indirectly supported by the fact that enhancement by adrenaline of the Ca-contractions was not reduced even after a possible depletion of a cellular Ca-store with ACh.

If the present inference made about the movements of Ca from mechanical changes produced by the catecholamines is correct, changes in fluxes of Ca would be observed in association with the increased or decreased contractile response to Ca. There are however no studies of Ca fluxes under these conditions available. Briggs and Melvin (29), observed that in the rabbit aorta, contractions by adrenaline were accompanied by increased \(^{45}\)Ca influx and Grossman and Furchgott (30), in the guinea-pig atria, showed that adrenaline increased exchange of \(^{45}\)Ca in association with the increased force of contraction.

Many available reports suggest that intracellular Ca is an important regulator of membrane permeability to cations as well as of contractile elements. Recently, it was found that high internal Ca caused an increase in the potassium permeability of human red cell membrane (31, 32). In polarized smooth muscle, catecholamines may also allow more Ca to enter the cells via the alpha-receptor mechanism as in depolarized smooth muscle.
and thereby increase Ca ions in the intracellular fluid. The increment of intracellular Ca ions would be expected to cause an increase in the membrane permeability to potassium in the way suggested by Bülbring and Tomita (9, 10). Thus hyperpolarization of the membrane and reduction of the membrane resistance would result (3, 6, 7, 10).

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