Involvement of Different Ca^{2+} Sources in Changes of Responsiveness of Guinea-Pig Trachea to Repeated Administration of Histamine and Acetylcholine

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Abstract. The role of Ca_i and Ca_o in changes of responsiveness of guinea pig tracheal smooth muscle strips to repeated applications of histamine and acetylcholine was investigated.

Homologous desensitization to histamine developed when the airways were exposed to concentrations higher than 10^{-5} mol/l, while sensitization to acetylcholine was recorded even when its highest concentration did not exceed 10^{-5} mol/l. The maximum of the concentration response curves (CRC) was reduced upon repeated histamine, and enhanced upon repeated acetylcholine administration. There was, however, no significant difference in EC_{50} values for repeated CRCs of the stimulants.

In Ca^{2+} free, EGTA (10^{-4} mol/l) containing solution the second contraction elicited by single (10^{-3} mol/l) or cumulative (10^{-9}–10^{-3} mol/l) histamine application was significantly smaller, while that elicited by acetylcholine did not differ significantly from the first one.

In Ca^{2+}-free, caffeine (10^{-2} mol/l) and EGTA containing solution the contractile responses to repeated additions of Ca^{2+} (2.7 mmol/l) in histamine and acetylcholine (10^{-3} mol/l) treated tracheae was decreased and unchanged, respectively. Addition of nifedipine (10^{-6} mol/l) to this solution fully prevented Ca^{2+} in inducing contraction in histamine treated tracheae, while Ca^{2+} still induced contraction in acetylcholine treated tracheae. TMB-8 (10^{-5} mol/l) was ineffective in blocking the remaining acetylcholine induced contractions.

The present data suggest that contractions of the guinea pig trachea elicited by histamine and acetylcholine are due to release of intracellular Ca^{2+} from a caffeine sensitive store and to influx of Ca^{2+} from the extracellular space via voltage operated channels (VOC). Moreover, acetylcholine activated Ca^{2+} entry into guinea pig tracheal smooth muscle cells via the nifedipine insensitive mechanism, probably receptor operated channels (ROC). It is concluded that desensitization to histamine in the guinea pig trachea is most probably due to alterations in intracellular Ca^{2+}

mobilization and Ca^{2+} influx via VOC. In contrast, sensitization to acetylcholine involved primarily enhanced Ca^{2+} influx via VOC and Ca^{2+} induced Ca^{2+} release.

Key words: Guinea pig trachea — Histamine — Acetylcholine — Intra- and extracellular Ca^{2+}

Introduction

Attenuation of the receptor mediated responses of smooth muscles, i.e. tachyphylaxis or desensitization, is a frequently observed phenomenon upon repeated exposure of tissues to certain agents, and can be generally classified as homologous or heterologous.

Repeated applications of histamine in different animal species result in desensitization of H_1-receptors on both the central and the peripheral airways. The development of this desensitization depends on the highest histamine concentration administered and on the time lag between the first and the consecutive CRC (Bradley and Russell 1983; Shore and Martin 1985; Antol et al. 1988; Cortijo et al. 1989). There are however some observations which do not support the notion of receptor desensitization to histamine (Drazen and Schneider 1978). Anderson et al. (1979a) could not find a significant difference between the first and the second concentration response curve (CRC) elicited in dogs also by two other stimulants of the airways, acetylcholine and serotonin.

The molecular mechanism of desensitization may involve reduction in the number and in affinity of receptors to the corresponding agonist, changes in the membrane potential, in coupling mechanisms such as G-proteins, in ion gradients, in Ca^{2+} influx or release, in depletion or synthesis of second and third messengers (e.g. eicosanoids, cyclic nucleotides) etc. Since the contractile agonists utilize extracellular and intracellular Ca^{2+} sources, the aim of the present study was to analyze their involvement in the altered responsiveness of the guinea pig trachea to histamine and acetylcholine.

Materials and Methods

Animals and tissue preparation

Male guinea pigs (250–350 g) were sacrified by cervical dislocation. The tracheae were quickly removed and dissected free from adhering tissues and cut spirally along their longitudinal axis (Todorov 1977).

Tracheal spirals were suspended in a 20 ml organ bath filled with Krebs solution (mmol/l): NaCl 123, KCl 6.2, $MgCl_2$ 1.2, $CaCl_2$ 2.7, $NaHCO_3$ 15.4, Na_2HPO_4 1.2, and glucose 11.5. The temperature of the bath solution was 37 °C and it was gassed with 5% CO_2 and 95% O_2. The tracheal experiments started after an equilibration period of 20

min under an initial tension of 40 mN and additional 30 min under the experimental basal tension of 20 mN. Contractions of the trachea were recorded isometrically by means of a strain gauge system.

Repeated cumulative concentration – response curves (CRC)

The CRCs for histamine (10^{-9}–10^{-3} mol/l) and acetylcholine (10^{-9}–10^{-3} mol/l) were constructed according to Van Rossum (1963). Increasing concentrations of the agonists were applied at intervals allowing full development of the effect of each concentration tested. The response elicited by the highest concentration of the agonist used in each individual experiment was taken as 100%. Three washings at 15 min intervals were needed to restore the basal tension. In a portion of the experiments in which the cumulative CRCs were constructed the maximum concentrations of acetylcholine or histamine did not exceed 10 μmol/l. In experiments designed to elucidate homologous or heterologous sensitization or desensitization, 25 minutes wash-out intervals were needed.

Study of the role of different Ca^{2+} sources

In order to study the role of extra- and intracellular calcium, two approaches were used:

a) The tissues were equilibrated in Ca^{2+}-free, caffeine (10 mmol/l) containing Krebs solution (mmol/l): NaCl 123, KCl 6.2, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, Na_2HPO_4 1.2, glucose 11.5, and EGTA 0.1. During equilibration which lasted 45 min, the solution was exchanged every 15 min. In experiments with nifedipine (10^{-6} mol/l) and TMB-8 (10^{-5} mol/l), the agents were added to the organ bath 15 min before the application of the corresponding stimulant.

b) The tissues were equilibrated in Krebs solution for 45 min. Fresh solution was added every 15 min. At the end of this period the bathing fluid was changed to Ca^{2+}-free, EGTA containing Krebs solution. The stimulants were applied 5 min after the introduction of this Ca^{2+}-free media. The same procedure preceded each application of the stimulants.

Chemicals

The following drugs were used: histamine hydrochloride (Merck, Darmstadt, FRG), acetylcholine hydrochloride (Germed, Berlin, FRG), caffeine and sodium benzoate (Spofa, Prague, Czechoslovakia), TMB-8 (8- /N,N-dietylamino/ octyl-3,4-trimetoxybenzoate; Sigma, Deisenhofen, FRG), nifedipine (Bayer AG, Leverkusen, FRG). Stock solutions were prepared with distilled water with the exception of nifedipine which was dissolved in propylene glycol. All the drugs were added by injection in a volume of 0.1–0.2 ml into a 20 ml organ bath. Nifedipine was added to an organ bath shielded from light.

Statistics

The results are expressed as arithmetic means ± S.E.M. Differences were tested by Student's t-test for paired observations. Changes were considered significant at $p < 0.05$. pD_2 values (–log of concentrations of the agonists producing 50% maximum contraction; EC_{50}) were calculated by linear regression (Delaunois 1973).

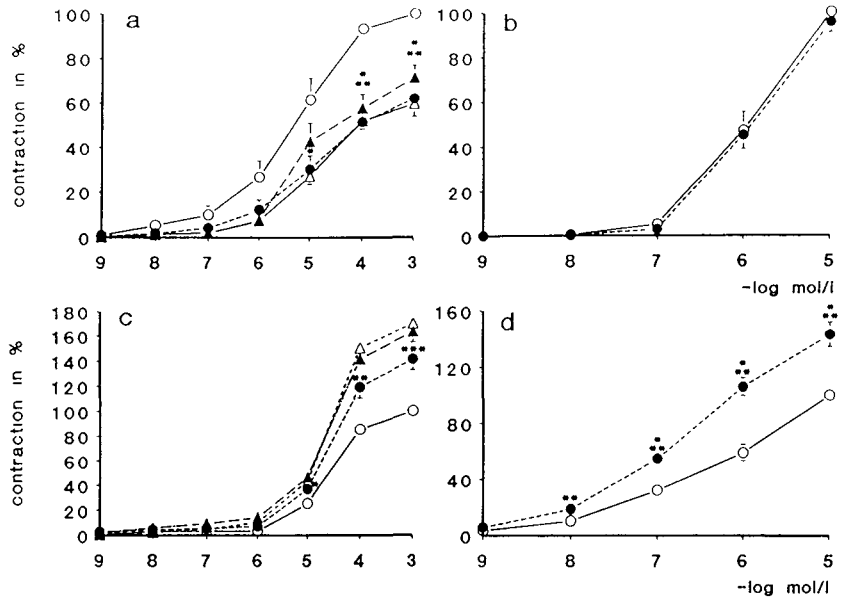

Figure 1. Concentration response curves (CRCs) elicited by repetitive cumulative applications of histamine (**a** $n = 10$, **b** $n = 8$) and acetylcholine (**c** $n = 12$, **d** $n = 8$) in guinea pig trachea. o – 1st, ● – 2nd, △ – 3rd, and ▲ – 4th CRC, * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.005$.

Results

Repeated cumulative concentration-response curves

The second cumulative application of histamine (concentrations graded from 10^{-9} to 10^{-3} mol/l) yielded CRCs with a significantly lower ($p < 0.005$) maximum (by about 35–45%) and a flatter slope than the CRC for the first application. On the first application, the maximum was 15.8±0.17 mN ($n = 10$). The repeated (second, third, fourth) CRCs did not differ significantly from each other (Fig. 1*a*). When the maximal responses of CRCs were taken as 100%, there was no significant difference among their pD_2 values: 5.6 ± 0.26 (1st), 5.3 ± 0.31 (2nd), 5.27 ± 0.36 (3rd), and 5.2 ± 0.38 (4th) ($n = 10$). In order to analyze the role of the highest histamine concentration used, CRCs were also constructed for repeated applications when the highest concentration did not exceed 10^{-5} mol/l. Under such a condition the second histamine CRC did not differ from the first one (Fig. 1*b*; $n = 8$).

In contrast, the second, third and fourth application of acetylcholine (10^{-9}–10^{-3} mol/l) yielded CRCs with 40–60% higher ($p < 0.005$) maximal values than the first application. On the first application the maximum was 18.9±2.1 mN ($n = 12$).

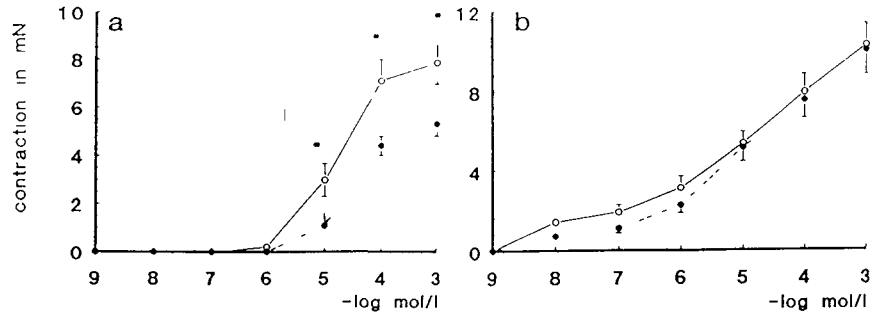

Figure 2. Contractions of guinea pig trachea elicited by histamine (**a** $n = 8$) and acetylcholine (**b** $n = 8$) treated preparations in the absence of extracellular Ca and in the presence of EGTA (10^{-4} mol/l) ○ – 1st and ● – 2nd CRC, $*$ – $P < 0.05$.

There was no significant difference between the CRCs for the second, third and fourth acetylcholine dosis (Fig. 1*c*). The pD_2 values did not differ significantly from the first dosis, being 4.9 ± 0.56 (1st), 4.9 ± 0.55 (2nd), 5.0 ± 0.51 (3rd), and 5.1 ± 0.53 (4th) ($n = 12$). The enhancement of the tissue sensitivity to the second application of acetylcholine (Fig. 2*b*) remained unchanged even at concentrations not exceeding 10^{-5} mol/l ($n = 8$).

To measure the possible modification of the action of one stimulant by a previous injection of the other, the tissues were also exposed to alternative doses of histamine and acetylcholine. Addition of histamine (10^{-3} mol/l) between two consecutive applications of acetylcholine did not modify the sensitizati of the trachea to acetylcholine ($n = 6$). In contrast, when acetylcholine (10^{-3} mol/l) was added between two applications of histamine (10^{-3} mol/l), the second response to histamine did not significantly differ from the first one ($n = 6$).

Repeated applications of KCl (40 mmol/l), which contracts smooth muscle without the involvement of membrane receptors, elicited smooth muscle contraction with a phasic and a tonic component (Fig. 5*a*).

Participation of different Ca^{2+} sources in response to repeated applications of agonists

To analyze the participation of intracellular Ca^{2+} in the responses elicited by repeated applications of histamine and acetylcholine, the agonists were cumulatively added to the organ bath under Ca^{2+}- free condition in the presence of EGTA (10^{-4} mol/l).

The maximal tracheal contraction induced by the second cumulative (10^{-9}–10^{-3} mol/l) histamine application under this condition was significantly ($p < 0.05$) smaller (by about 30%; Fig. 2*a*) than the first one. If, however histamine was added

in concentrations of 10^{-9}–10^{-5} mol/l, there was no significant difference between the first and the second CRC ($n = 8$).

The CRC for repeated doses of acetylcholine in Ca^{2+}-free, EGTA (10^{-4} mol/l) containing solution did not differ from the control CRC, either when acetylcholine was added in concentrations from 10^{-9} to 10^{-3} mol/l (Fig. 2*b*) or when the concentrations ranged from 10^{-9} to only 10^{-5} mol/l ($n = 8$).

The role of transmembrane Ca^{2+} influx was analyzed in tissues after Ca^{2+} release from the caffeine (10^{-2} mol/l) sensitive intracellular Ca^{2+} pool in Ca^{2+}-free EGTA (10^{-4} mol/l) containing solution. Addition of histamine (10^{-3} mol/l) did not alter the basal tension of the muscle under these conditions. Addition of Ca^{2+} (2.7 mmol/l) to tissues under such conditions in the presence of histamine (10^{-3} mol/l, Fig. 3*a* and 10^{-5} mol/l) resulted in smooth muscle contraction. On repeated Ca^{2+} application there was no difference in the Ca^{2+}-induced contraction amplitude when the concentration of histamine was low (10^{-5} mol/l; $n = 8$). Yet, when a high concentration was used (10^{-3} mol/l) the second response to Ca^{2+} was smaller (by about 40%; Fig. 3*a*).

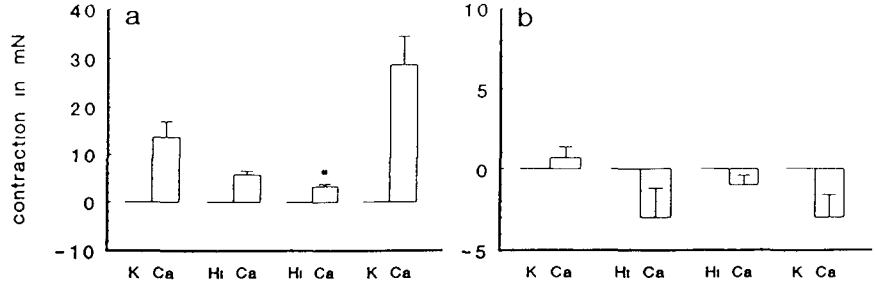

Figure 3. Contractions of guinea pig trachea elicited by $CaCl_2$ (2.7 mmol/l Ca) in potassium chloride (40 mmol/l K) or histamine (10^{-3} mol/l) treated preparations: **a** – in the absence of extracellular Ca^{2+} and in the presence of EGTA (10^{-4} mol/l) and caffeine (10^{-2} mol/l) ($n = 8$). In **b**, nifedipine (10^{-6} mol/l) was also present in the bathing fluid ($n = 8$). $*$ – $P < 0.05$.

Under the same conditions (Ca^{2+}-free solution containing EGTA and caffeine) acetylcholine (10^{-3} mol/l) was also ineffective, and the second response to Ca^{2+} in the presence of acetylcholine (10^{-3} mol/l) in the bathing fluid did not differ significantly from the first one (Fig. 4*a*; $n = 8$).

In the absence of extracellular Ca^{2+} and in the presence of EGTA (10^{-4} mol/l) and caffeine (10^{-2} mol/l), KCl did not change significantly the basal tension of the guinea pig trachea. Administration of Ca^{2+} in the presence of KCl (40 mmol/l)

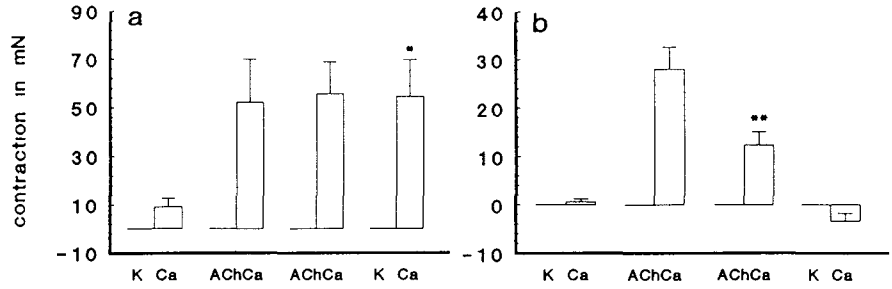

Figure 4. Contractions of guinea pig trachea elicited by $CaCl_2$ (2.7 mmol/l Ca) in potassium chloride (40 mmol/l K) or acetylcholine (10^{-3} mol/l) treated preparations: **a** – in the absence of extracellular Ca^{2+} and in the presence of EGTA (10^{-4} mol/l) and caffeine (10^{-2} mol/l) ($n = 8$). **b**, nifedipine (10^{-2} mol/l) was also present in the bathing fluid ($n = 8$). * – $P < 0.05$, ** – $P < 0.01$.

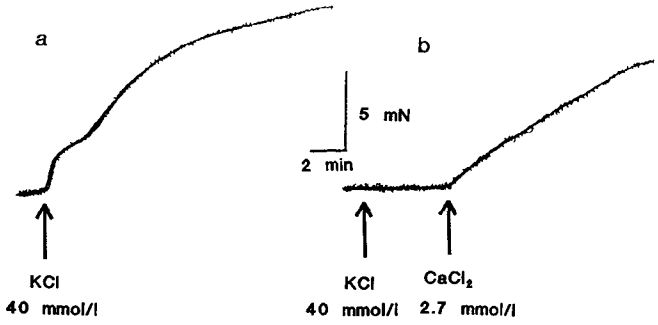

Figure 5. Original records of the contractions of guinea-pig trachea evoked by KCl in the presence of extracellular Ca^{2+}(**a**) and by Ca^{2+} in potassium- depolarized trachea under Ca^{2+}-free condition (in the presence of EGTA 10^{-6} mol/l and caffeine 10^{-2} mol/l; **b**).

caused only a slowly developing contraction of the smooth muscle (Fig. 5*b*). Repeated addition of Ca^{2+} to potassium (40 mmol/l)-depolarized tissues at 45 min intervals resulted in an enhancement of the amplitude of the second and third Ca^{2+}-induced contractions (14.7 ± 1.9; 18.8 ± 3.2 and 19.7 ± 2.1 mN, $n = 7$). The differences between the first, the second or the third amplitude were significant ($p < 0.05$). Moreover, when two doses of Ca^{2+} were between the two KCl treatments in the presence of histamine or acetylcholine, the contraction elicited by the second addition of Ca^{2+} to KCl (40 mmol/l)-depolarized tissues was enhanced even more, by about 100% ($p < 0.1$; Fig. 3*a*) with histamine and by up to 500% ($p < 0.001$; Fig. 4*a*) with acetylcholine.

To differentiate between the influx of Ca^{2+} via voltage operated channels (VOC) and receptor operated channels (ROC), nifedipine (10^{-6} mol/l) was applied to Ca^{2+}-free, EGTA and caffeine containing bathing solution for 15 min before the application of the corresponding stimulant. This procedure prevented Ca^{2+} from inducing significant elevation in muscle tension in the presence of both KCl and histamine (Fig. 3*b*; $n = 8$) but not in the presence of acetylcholine (Fig. 4*b*; $n = 8$). The second contraction induced by Ca^{2+} in the presence of acetylcholine was however significantly smaller ($p < 0.01$), by about 50%, than the first one (Fig. 4*b*). This action of acetylcholine remained unaffected by TMB-8 (10^{-5} mol/l) treatment between the two consecutive applications of acetylcholine ($n = 8$).

Discussion

Whether airways contract or relax in response to different stimulants, including histamine, depends on the level of basal tone (Mansour and Daniel 1986) and on the relative proportions of excitatory and inhibitory (e.g. H_1 and H_2) receptor subtypes present (Bradley and Russel 1983).

The results presented herein suggest that histamine contracts guinea pig trachea due to the influx of Ca^{2+} from extracellular space as well as to Ca^{2+} release from its intracellular stores. Since nifedipine fully blocked the effect of extracellular Ca^{2+} in the presence of histamine this influx must have been realized via nifedipine sensitive (voltage operated) Ca^{2+} channels. It has been suggested that receptor activation results in phospholipase C-coupled phosphoinositol hydrolysis and the IP_3 formed releases Ca^{2+} from the sarcoplasmic reticulum. The intracellular store from which histamine releases Ca^{2+} in the guinea pig trachea is assumed to be a caffeine sensitive store because in the absence of extracellular Ca^{2+} and in the presence of caffeine histamine failed to contract this tissue.

As with the canine trachea (Bradley and Russel 1983), repeated applications of histamine to the guinea pig trachea resulted in contractions of the same amplitude both in the presence and in the absence of intracellular or extracellular Ca^{2+} when the highest single or cumulatively applied histamine concentrations did not exceed 10^{-5} mol/l. In agreement with the observations of Anderson et al. (1979a,b), Shore and Martin (1985), and in contrast to those of Drazen and Schnieder (1978), Cortijo et al. (1989), and Anderson et al. (1983), the presented results showed that the second application of histamine in concentrations exceeding 10^{-5} mol/l (regardless of whether a single concentration or reached cumulatively) resulted in a significant reduction in the contraction amplitude of the guinea pig trachea. This desensitization remained constant on further repetition of histamine application. The desensitization was found to be homologous because histamine failed to affect the responses to repeated acetylcholine applications. Yet, the intrinsic activity of

histamine was significantly reduced, there was no significant change in the affinity of the tracheal smooth muscle to histamine on its repeated cumulative applications, even in high concentrations. Thus, the described reduction of the amplitude of the histamine induced contractions probably does not involve alterations in histamine receptors themselves.

After the application of high histamine concentrations, desensitization developed in both the presence and absence of extracellular Ca^{2+} and also when Ca^{2+} was applied in the course of histamine induced depolarization of the caffeine treated tissues in Ca^{2+}-free EGTA-containing bathing solution. Thus the development of desensitization to histamine was due to alterations in Ca^{2+} influx via nifedipine-sensitive voltage-operated Ca^{2+} channels, and to the release of Ca^{2+} from its intracellular caffeine-sensitive store.

Besides the above described histamine-activated mechanims, the contraction elicited by acetylcholine involved utilization of extracellular Ca^{2+} also via nifedipine insensitive influx (probably due to activation of receptor-operated Ca^{2+} channels, Bolton 1979). In contrast to histamine, the contractions elicited by repeated acetylcholine applications in the presence of extracellular Ca^{2+} were augmented. Yet, the intrinsic activity of acetylcholine was significantly enhanced, there was no significant change in the affinity of the tracheal smooth muscle to acetylcholine on its repeated cumulative applications, even in high concentrations. Thus the described augmentation of the amplitude of the acetylcholine-induced contractions probably does not involve alterations in acetylcholine receptors themselves. The contraction amplitude did not increase either in tissues with only intracellular Ca^{2+} present (in Ca^{2+}-free EGTA-containing solution) or in tissues whith the Ca^{2+} influx being responsible for the contraction due to the application of Ca^{2+} into Ca^{2+}-free caffeine and acetylcholine containing solution. Under the latter conditions, when voltage operated Ca^{2+} channels were blocked by nifedipine, desensizitation developed. This could only be via nifedipine insensitive Ca^{2+} influx. Thus, two membrane permeability changes of opposite direction and roughly of the same amplitude were involved when acetylcholine was repeatedly applied. While the influx of Ca^{2+} via receptor operated channels (ROC) was reduced the influx via voltage operated channels (VOC) was enhanced. The sensitization induced by repeated acetylcholine applications required primarily the presence of extracellular Ca^{2+} and of its nifedipine sensitive influx. The absence of sensitization of the Ca^{2+}-induced contraction in the presence of ACh in Ca^{2+}-free, caffeine and EGTA containing Krebs solution, and its presence with untreated tissues suggest moreover that for the development of sensitization a facilitated Ca^{2+}-induced Ca^{2+} release or enhancement of the releasable intracellular Ca^{2+} store is also essential. Otherwise, an enhanced influx via VOC would be counteracted by a reduced influx via ROC. The latter assumption is supported by the marked difference between the development of K^+-induced contraction in the presence of Ca^{2+} and of Ca^{2+}-

induced contraction in K^+ depolarized Ca^{2+} depleted tissue when acetylcholine or histamine were applied between two K^+ applications.

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