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Short-term desensitization of guinea-pig taenia caecum induced by carbachol occurs at intracellular Ca stores and that by histamine at H_1-receptors

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1 In Ca-free solution, the contractile response of guinea-pig taenia caecum to 10^{-4} M carbachol was mediated through muscarinic receptors and was reduced time-dependently by desensitization with 10^{-4} M carbachol, but not 10^{-4} M histamine. On the other hand, the response to 10^{-4} M histamine was shown to be mediated through H_1-receptors and to be reduced time-dependently by desensitization with either 10^{-4} M histamine or 10^{-4} M carbachol.

2 The maximal K^+ contraction was not changed by desensitization with carbachol or histamine. Thus, contractile proteins and voltage-dependent Ca channels maintain their normal functions.

3 To study the coupling of Ca channel activity in cell surface membrane to receptor activation, the contractile responses elicited by carbachol and histamine added simultaneously with Ca to Ca-free solution were measured. The response elicited by carbachol plus Ca was not changed by desensitization with carbachol, while that elicited by histamine plus Ca was reduced by desensitization with histamine. These results show that desensitization by carbachol occurs at a post-receptor site, whereas that induced by histamine occurs at H_1-receptors.

4 After desensitization with carbachol, but not histamine, the contractile response to 5×10^{-2} M caffeine in Ca-free solution was significantly reduced.

5 These results show that short-term desensitization of guinea-pig taenia caecum by carbachol is heterologous and occurs at intracellular Ca stores, while that induced by histamine is homologous and occurs at histamine H_1-receptors.

Introduction

Desensitization, the phenomenon of diminished cellular responses after prolonged or repeated treatment with an agonist, is very important in some disorders and in drug therapy. Desensitization can be classified into two types: homologous, i.e. specific for a given agonist, and heterologous, i.e. not specific for the agonist. The first example of homologous desensitization, histamine-induced desensitization, was described by Barsoum & Gaddum (1935), and the first example of heterologous desensitization, acetylcholine-induced desensitization, was demonstrated by Cantoni & Eastman (1946). Homologous desensitization has been thought to be due to changes in the affinity or number of receptors for an agonist, whereas heterologous desensitization is assumed to be due to the change of a transducer, such as guanosine 5'-triphosphate (GTP)-binding protein, or an effector, such as adenylate cyclase in the adenylate cyclase system. In the contractile system in smooth muscle, a change in some common processes such as Ca channels, Ca stores, or contractile proteins may lead to heterologous desensitization.

Short-term desensitization has not been investigated extensively because the short duration of the desensitized state makes its biochemical characterization difficult. We have described the effects of local anaesthetics on short-term desensitization of contraction induced by histamine under normal physiological conditions (Hishinuma & Uchida, 1987). In this study, we investigated the short-term desensitizations to carbachol and histamine of intracellular Ca-dependent contractile responses, measured by a slight modification of the method of Casteels & Raeymaekers (1979).

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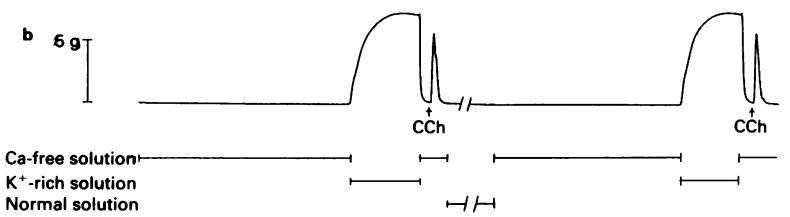
Methods

Guinea-pigs of either sex, weighing 250–400 g were killed by a blow on the neck and exsanguinated. Strips of taenia caecum were suspended in 10 ml organ baths bubbled with air at 30°C. The bathing solution was normal Locke-Ringer solution and was of the following composition (mM): NaCl 154, KCl 5.63, $CaCl_2$ 2.16, $MgCl_2$ 2.10, $NaHCO_3$ 5.95 and glucose 5.55. $CaCl_2$ was omitted and 2 mM EGTA was added to Ca-free solution. The 42 mM K^+-rich solution had the same composition as normal solution but with 117.63 mM NaCl and 42 mM KCl. Contractile responses were recorded isometrically. The muscles were equilibrated in normal solution for 1 h with a resting tension of 0.5 g. As shown in Figure 1, after the muscles had been incubated in Ca-free solution for 1 h with a resting tension of 0.05 g, they were soaked in K^+-rich solution and K^+ contractions were elicited for 10 min to load Ca into intracellular Ca stores. Then the muscles were placed in Ca-free solution, and exactly 2 min later, carbachol, histamine or caffeine was added for about 2 min during the transient tension development (at final concentrations of 10^{-4} M carbachol and histamine, and 5×10^{-2} M caffeine) and this tension development

A a

Cycle	Tension (g) K^+ contraction	Tension (g) CRCF	Tension (% of control) K^+ contraction	Tension (% of control) CRCF
1st	5.54 ± 0.52	4.94 ± 0.13	90.1 ± 1.5	90.3 ± 10.8
2nd	6.14 ± 0.54	5.16 ± 0.24	100.0 ± 0.0	100.0 ± 0.0
3rd	6.17 ± 0.50	5.45 ± 0.26	100.8 ± 1.6	100.7 ± 9.5
4th	5.85 ± 0.34	5.20 ± 0.32	96.3 ± 4.6	96.1 ± 10.0



B a

Cycle	Tension (g) K^+ contraction	Tension (g) CRCF	Tension (% of control) K^+ contraction	Tension (% of control) CRCF
1st	5.77 ± 0.27	2.95 ± 0.29	95.7 ± 2.8	107.1 ± 6.1
2nd	6.04 ± 0.22	2.80 ± 0.29	100.0 ± 0.0	100.0 ± 0.0
3rd	5.96 ± 0.25	2.90 ± 0.27	98.6 ± 0.8	104.8 ± 2.7
4th	5.75 ± 0.29	2.73 ± 0.29	95.0 ± 1.2	98.2 ± 4.3
5th	5.58 ± 0.28	2.43 ± 0.25	92.1 ± 1.6	87.6 ± 3.9

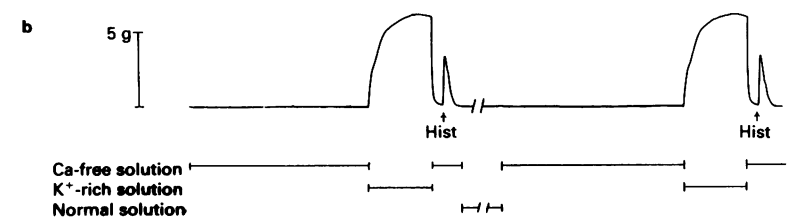


Figure 1 K^+-induced contraction and contractile responses in Ca-free solution elicited by carbachol and histamine. For conditions see Methods. (A, a) Tensions generated by 42 mM K^+-rich solution containing 2.16 mM Ca^{2+} (K^+ contraction) and by 10^{-4} M carbachol in Ca-free solution (CRCF) are shown in g on the left and as percentages of the K^+ contraction and the CRCF in the second cycle (control) on the right. Values are means ± s.e. of four experiments. (A, b) Traces of the second (control) and third cycles. One cycle was as follows: the muscle was suspended in Ca-free solution for 1 h, contracted by 42 mM K^+-rich solution for 10 min, and resuspended in Ca-free solution. Then the CRCF was elicited by carbachol (CCh) exactly 2 min later. The carbachol was present for about 2 min. The chart speed for about 15 min during the contractile responses was 2.5 times faster than that during preincubation in Ca-free solution for 1 h. The height indicates the tension in g. (B) Conditions were as in (A), except that 10^{-4} M histamine was used instead of 10^{-4} M carbachol. Histamine (Hist) was added at the indicated times for about 2 min during these transient responses. Values shown are means ± s.e. of seven experiments.

was recorded as the contractile response in Ca-free solution (CRCF). The muscle strips were resuspended in normal solution for about 45 min before the next cycle. Similarly, muscle strips were suspended in Ca-free solution for 1 h, induced to contract by suspension in 42 mM K^+-rich solution for 10 min, and resuspended in Ca-free solution. Then exactly 2 min later, their responses to histamine, carbachol and caffeine were recorded. This cycle was performed 4 or 5 times and the responses in the second cycle were taken as controls. In the third cycle, the strips were treated with histamine or carbachol (desensitizing incubation) before inducing the K^+ contraction in Ca-free solution and then after washing out the histamine or carbachol, K^+ contraction and the test responses were recorded (desensitization). In some experiments, carbachol or histamine were added simultaneously with $CaCl_2$ (2.16 mM Ca ion, final concentration). K^+ contractions and the contractile responses in Ca-free solution were expressed as percentages of the control values. Statistical significance was evaluated by Student's paired t test, and $P = 0.05$ was taken as the upper limit of significance.

Drugs used: histamine dihydrochloride and carbamylcholine chloride were obtained from Sigma, and caffeine was from Wako Pure Chemical Industries, Ltd.

Results

Contractile responses in Ca-free solution elicited by 10^{-4} M carbachol or 10^{-4} M histamine

Figure 1 shows the tension of the contraction induced by K^+ and the response in Ca-free solution in each cycle, and the traces of the second and third cycles when 10^{-4} M carbachol or 10^{-4} M histamine were used as agonists. These values tended to decrease in successive cycles but the changes were not statistically significant. The carbachol and histamine responses were mediated through muscarinic cholinoceptors and histamine H_1-receptors, since

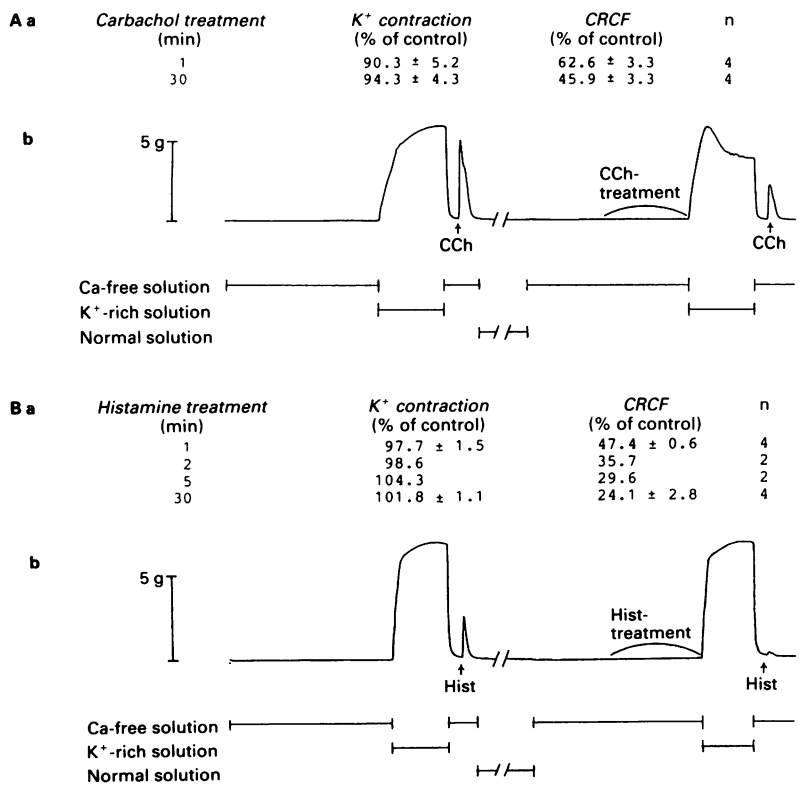


Figure 2 K^+-induced contraction and contractile responses in Ca-free solution elicited by carbachol (CCh) and histamine (Hist) in the desensitized muscle. Conditions were as for Figure 1, except that a desensitizing incubation with 10^{-4} M carbachol (A) or 10^{-4} M histamine (B) was performed before K^+ contraction in the third cycle. n = number of experiments.

they were inhibited by 10^{-6} M atropine and 10^{-5} M mepyramine, respectively (data not shown). To facilitate comparison of the tensions generated by muscles in different experiments, we expressed K^+ contractions and the agonist responses as percentages of the values in the second, control, cycle.

Desensitization of the contractile response in Ca-free solution to histamine or carbachol

Figure 2Ab shows a trace elicited after desensitizing treatment with 10^{-4} M carbachol for 30 min. After this treatment, the carbachol-elicited response in Ca-free solution was significantly smaller than the control value (Figure 2Aa). After carbachol-induced desensitization, the maximal tension of the K^+-induced contraction did not change, but the contraction was more rapid and tonic tension was not maintained. Figure 2Bb shows the histamine-elicited response in Ca-free solution after desensitizing incubation with 10^{-4} M histamine for 30 min. This response was significantly smaller than the control value, but the K^+-induced contraction did not change. The histamine-elicited responses after various periods of desensitizing incubation with histamine are shown in Figure 2Ba. Desensitization to carbachol and histamine increased as the period of desensitizing incubation increased. In intestinal muscles such as guinea-pig taenia caecum, intracellular Ca ion is known to be depleted rapidly in the absence of external Ca ion (Ohashi *et al.*, 1974; Casteels & Raeymaekers, 1979). Thus, since the medium had been changed to Ca-free solution more than 30 min before the start of the desensitizing incubation, no tension was generated during the desensitizing incubation with histamine, although a slight tonic tension of about 0.03 g was observed during the desensitization with carbachol.

Cross-desensitization between histamine and carbachol of the contractile response in Ca-free solution

The carbachol-elicited responses after the desensitizing incubation with 10^{-4} M histamine for 1 or 30 min were not significantly smaller than the control value; that is, histamine-induced desensitization was of the homologous type and did not affect the carbachol-elicited responses (Figure 3B). In

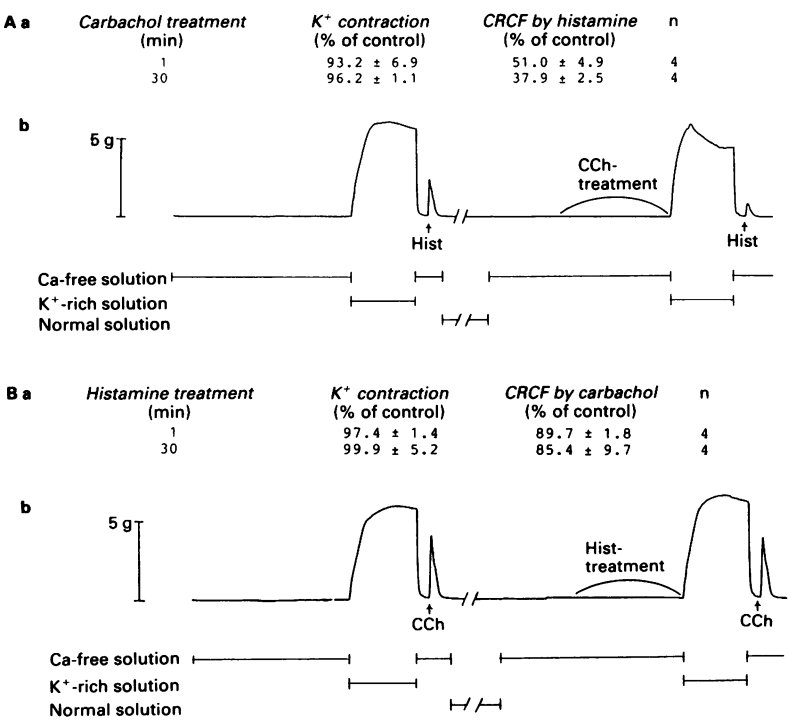


Figure 3 Cross-desensitization between carbachol and histamine. Conditions were as for Figure 2, except that the responses were elicited by 10^{-4} M histamine (Hist) (A) and 10^{-4} M carbachol (CCh) (B) after a desensitizing incubation with 10^{-4} M carbachol and 10^{-4} M histamine for 30 min, respectively, before K^+ contraction in the third cycle. n = number of experiments.

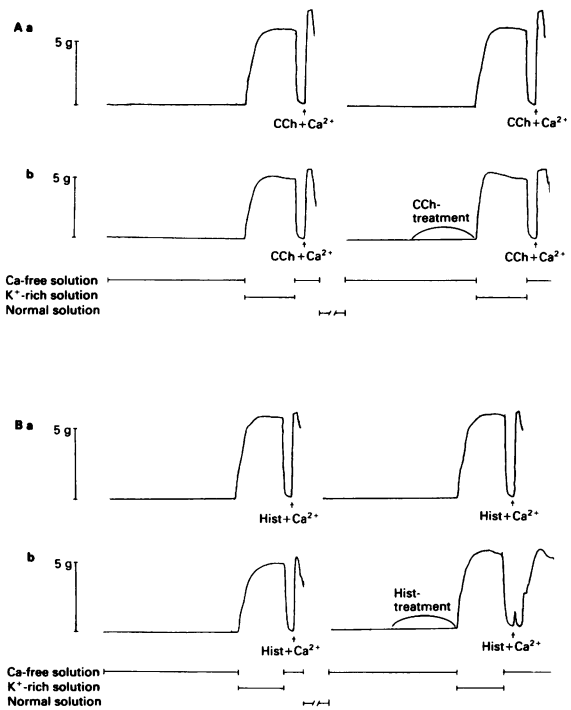


Figure 4 Response to carbachol (CCh) and histamine (Hist) added simultaneously with Ca to Ca-free solution after desensitizing treatment. (A) Conditions were as for Figure 1, except that carbachol was added simultaneously with Ca. The final concentrations added were 10^{-4} M carbachol and 2.16 mM Ca. (a) Trace of the response without desensitizing treatment with carbachol; (b) trace of the responses before and after desensitizing treatment with 10^{-4} M carbachol for 30 min. (B) Conditions were as for (A), except that 10^{-4} M histamine was used instead of 10^{-4} M carbachol.

contrast, the histamine-elicited responses in Ca-free solution after the desensitizing incubation with 10^{-4} M carbachol for 1 or 30 min were significantly smaller than the control value; that is, carbachol-induced desensitization was of the heterologous type and affected histamine-elicited responses (Figure 3A). Therefore, desensitization induced by carbachol may occur at a post-receptor site, wihle that induced by histamine probably occurs at the receptor level.

Desensitization of the contractile response elicited by carbachol or histamine added simultaneously with Ca

To investigate signal transduction from receptors to Ca channels in the desensitized state, we examined the contractile responses of muscles induced by 10^{-4} M carbachol added simultaneously with Ca (2.16 mM, final concentration) after desensitizing treatment. As shown in Figure 4A, no change was observed in the contractile response elicited by 10^{-4} M carbachol added simultaneously with Ca after desensitizing treatment with carbachol (97.8 ± 1.0% of control, $n = 4$). These results suggest that carbachol-induced desensitization does not affect these responses, and that the signals from muscarinic receptors to Ca channels may induce enough influx of Ca ion to elicit a contraction. Figure 4B shows the contractile response elicited by 10^{-4} M histamine added simultaneously with Ca. The response was reduced after desensitizing treatment with histamine (30.4 ± 8.3% of control, $n = 5$). The gradual generation of tension after the first peak of the contractile response elicited by histamine and Ca seemed not to be specific for histamine, since it was also observed with Ca alone, in the absence of histamine (data not shown). These results suggest that the signals from H_1-receptors to Ca channels to elicit the contractile responses dependent on extracellular Ca ion were also desensitized by histamine treatment.

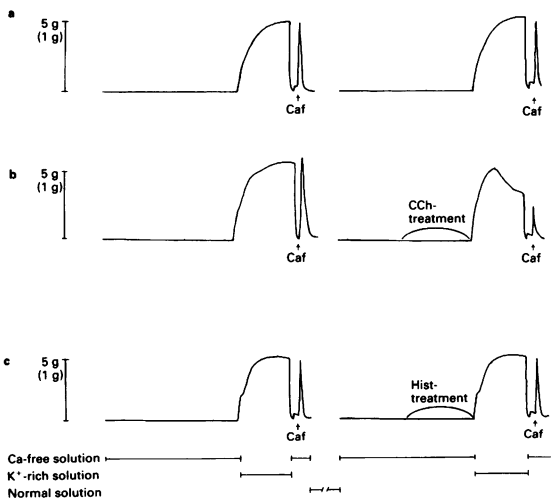


Figure 5 Contractile response in Ca-free solution elicited by caffeine in desensitized muscle. Conditions were as for Figure 1, except that contractile responses in Ca-free solution were elicited by 5×10^{-2} M caffeine (Caf) at the times indicated instead of by 10^{-4} M carbachol or 10^{-4} M histamine. The tension calibration in parentheses (i.e. 1 g) is for the caffeine-induced contraction only. Traces show the response before desensitizing treatment (a) and responses before and after desensitizing treatment with 10^{-4} M carbachol (CCh) for 30 min (b) or 10^{-4} M histamine (Hist) for 30 min (c).

Desensitization of the contractile response in Ca-free solution elicited by 5×10^{-2} M caffeine

Caffeine has been shown to release Ca from intracellular Ca stores through a Ca-induced Ca release mechanism in guinea-pig taenia caecum (Iino, 1987). Therefore, we measured caffeine-induced contraction after desensitizing treatment with carbachol or histamine. Caffeine-induced contractions were reproducible throughout the experiment. As shown in Figure 5, the caffeine-induced contraction was significantly reduced by desensitization with carbachol ($60.1 \pm 9.1\%$ of control, $n = 6$), but not histamine ($100.2 \pm 5.6\%$, $n = 8$).

Discussion

The Ca stores of enteric smooth muscles such as guinea-pig taenia caecum are in equilibrium with extracellular Ca and easily depleted in the absence of extracellular Ca. The method of Casteels & Raeymaekers (1979) is useful for replenishing the intracellular Ca stores after their depletion in Ca-free solution. Using this procedure, we found that short-term desensitization of guinea-pig taenia caecum to carbachol or histamine still occurred in the ability of these drugs to release Ca from the intracellular Ca store (Figure 2), and that carbachol-induced desensitization was heterologous (Figure 3A), whereas histamine-induced desensitization was homologous (Figure 3B). Even in the desensitized state induced by carbachol or histamine, the functions of contractile proteins and voltage-dependent Ca channels seemed to be normal and muscle contraction could be elicited when the Ca concentration in the cytosol was sufficiently elevated in some way, such as by soaking the muscles in K^+-rich solution containing Ca (Figure 2).

Carbachol must desensitize the intracellular Ca stores. Since signals from muscarinic cholinoceptors to Ca channels in the carbachol-induced desensitized state appears to be enough to lead to the same contractile responses being elicited as in the non-desensitized state (Figure 4A). Hence, carbachol-induced desensitization probably does not occur at the receptor but at a post-receptor site. This post-receptor site may be the intracellular Ca stores because, in guinea-pig taenia caecum, intracellular Ca is thought to be released from the same stores by carbachol, histamine and caffeine (Casteels & Raeymaekers, 1979) and desensitization by carbachol also caused desensitization of the histamine- and caffeine-induced contraction (Figures 3A and 5B). With regard to the signal transduction mechanism of Ca-mobilizing hormones or transmitters, it has been shown that inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and diacylglycerol (DG) are produced by the hydrolysis of phosphatidylinosotol 4,5-bisphosphate (PIP_2) and that the former releases Ca from the endoplasmic reticulum (Streb *et al.*, 1983), while DG activates C-kinase (Takai *et al.*, 1979). In guinea-pig taenia caecum, this mechanism seems to operate on stimulation of muscarinic cholinoceptors and histamine receptors, as observed in enteric smooth muscle (Sekar & Roufogalis, 1984; Donaldson & Hill, 1985). $Ins(1,4,5)P_3$ receptors in insulinoma microsomes were shown to be desensitized rapidly by treatment with $Ins(1,4,5)P_3$ (Prentki *et al.*, 1984), and this is probably one cause of heterologous desensitization. Reduced function of Ca channels in Ca stores may also reduce Ca release from the stores. Another possible cause of heterologous desensitization, such as that induced by carbachol, may be depletion of intracellular Ca stores of Ca. The latter possibility is particularly likely, since caffeine-induced contraction was also desensitized by carbachol-treatment and caffeine-sensitive intracellular Ca stores of guinea-pig taenia caecum are also thought to be $Ins(1,4,5)P_3$-sensitive (Iino, 1987). This depletion of intracellular Ca may result from reduced Ca-loading into the intracellular Ca stores due to a lowered function of the Ca pump in the stores, enhanced leakiness of the store membrane, or increased Ca efflux from the cytosol to the extracellular space before repletion of the stores with Ca. Inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$), produced by the phosphorylation of $Ins(1,4,5)P_3$, may be involved in the opening of Ca channels in the cell surface membrane of sea urchin eggs (Irvine & Moor, 1986). If this signal transduction of $Ins(1,4,5)P_3$ and $Ins(1,3,4,5)P_4$ functions normally and Ca in the intracellular Ca stores is depleted, the $Ins(1,4,5)P_3$-elicited response would be reduced without a reduction of the $Ins(1,3,4,5)P_4$-elicited response dependent on extracellular Ca. Although, release of Ca from intracellular stores by $Ins(1,4,5)P_3$ is thought to be necessary for $Ins(1,3,4,5)P_4$-induced influx of extracellular Ca (Irvine & Moor, 1986). This hypothesis is plausible because, after carbachol-induced desensitization, the responses in Ca-free solution were reduced without a reduction of the contractile responses dependent on external Ca and the Ca-free responses elicited by carbachol in the desensitized state were about 50% of the control value, indicating that some stored Ca was released. Another possible cause for heterologous desensitization is lowered function of GTP-binding proteins. It has been suggested that GTP-binding proteins mediate agonist-promoted phosphatidylinositol turnover (Gomperts, 1983). But lowered function of GTP-binding protein involving the signal transduction system of Ca has not been implicated in heterologous desensitization, even

though lowered function of the GTP-binding protein involving the signal transduction system of cyclic AMP was proposed to cause heterologous desensitization of β-adrenoceptors in turkey erythrocytes (Pike & Lefkowitz, 1980; Briggs *et al.*, 1983). We could not detect any effect of the islet activating protein (IAP), which inactivates the inhibitory GTP-binding protein, on the contractile responses to carbachol and histamine, although we cannot rule out problems of penetration, or any GTPase activity in membrane preparations of guinea-pig taenia caecum stimulated by these agonists (data not shown).

Histamine must desensitize H_1-receptors, since histamine-induced desensitization was homologous (Figure 3B) and histamine desensitized the signals from H_1-receptors to Ca channels (Figure 4B) and to intracellular Ca stores (Figure 2B) but not voltage-dependent Ca channels (i.e. K^+ contraction, Figure 2B). The Ca content of the intracellular Ca stores did not seem to be depleted in the histamine-induced desensitized state, because the carbachol-elicited and caffeine-elicited contractions in Ca-free solution were not changed after the desensitizing incubation with histamine (Figures 3B and 5C). Homologous desensitization, such as that induced by histamine, may involve modulating factors (desensitizing factors) specific for the agonist-occupied form of the receptors. An attractive hypothesis described in our previous study (Hishinuma & Uchida, 1987) is that histamine H_1-receptors may be regulated by a desensitizing factor that modifies the agonist-occupied form of receptors, such as β-adrenoceptor kinase (Benovic *et al.*, 1986; Strasser *et al.*, 1986).

In conclusion, the mechanisms involved in the increase in intracellular Ca concentration, induced by activation of histamine H_1-receptors and muscarinic cholinoceptors, may not function well during histamine-induced desensitization of H_1-receptors and carbachol-induced desensitization of intracellular Ca stores. To obtain information on the biochemical basis of desensitization, we are now investigating phosphatidylinositol turnover elicited by these agonists in the desensitized state.

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