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Transient Resensitization Interrupting the Development of Carbachol-induced Desensitization in Smooth Muscle of Guinea-pig Taenia Caeci: Ca^{2+}-dependent Termination of Resensitization

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It is important to clarify developmental mechanisms of desensitization because of their great significance in regulation of cellular responsiveness. We have found that carbachol-induced desensitization to carbachol develops in three successive phases in the presence of extracellular Ca^{2+} in the smooth muscle of guinea pig taenia caeci: fast desensitization within 15 s, transient resensitization reaching a peak at 1 min and the subsequent re-development of desensitization to terminate resensitization for up to 30 min. In contrast, in the absence of extracellular Ca^{2+}, desensitization develops without resensitization. To further clarify the roles of Ca^{2+} in the formation of the transient resensitization phase, we examined the developmental process of carbachol-induced desensitization in the absence of extracellular Ca^{2+}, following the induction of desensitization by a 15-s treatment with carbachol in the presence of extracellular Ca^{2+}. Desensitization to carbachol occurred due to pretreatment with 10^{-4} M carbachol for 15 s in normal physiological solution, and continued pretreatment with carbachol in Ca^{2+}-free solution containing 0.2 mM EGTA induced resensitization followed by the obscure progress of re-desensitization for up to 30 min resulting in a long-lasting phase of resensitization. These results suggest that resensitization is promptly terminated by the Ca^{2+}-dependent development of subsequent desensitization for further regulation of cellular responsiveness via G_q protein-coupled Ca^{2+}-mobilizing receptors against sustained stimuli.

Key words——desensitization; resensitization; G protein-coupled receptor; muscarinic M_3 receptor; Ca^{2+}; smooth muscle

INTRODUCTION

Cells or tissues undergo a process of desensitization upon stimulation with a receptor agonist for cellular adaptation or homeostasis.[1–10] Resensitization is also a crucial process for cells or tissues to regain their normal responsiveness.[4,5] It is important to clarify mechanisms of desensitization and resensitization because of their great significance in regulation of cellular responsiveness. It has been thought that desensitization develops with an exponential reduction in cellular responsiveness depending on the time period of agonist exposure and that resensitization becomes obvious only after removal of the agonist. However, we have found that pretreatment with carbachol develops desensitization in contractile responses to carbachol in three successive phases in the smooth muscle of guinea pig taenia caeci: fast desensitization within 15 s followed by transient interruption by resensitization, which reaches a peak at 1 min, and the subsequent re-development of desensitization to terminate resensitization up to 30 min with concomitant changes at muscarinic receptor/G protein levels.[11–13] The resensitization process, which is novel in that it occurs even in the continued presence of a desensitizing agonist, appear to function as a further modulatory mechanism against the initial development of desensitization. In contrast, carbachol pretreatment in the absence of extracellular Ca^{2+} induces the simple and slow development of desensitization without resensitization, suggesting that resensitization is caused by Ca^{2+}-dependent mechanisms.[12,13] To further characterize this Ca^{2+}-dependent resensitization process, we examined how the transient resensitization phase might be modulated by the deprivation of extracellular Ca^{2+} during carbachol pretreatment after induction of the initial desensitization by 15-s treatment with carbachol in the presence of extracellular Ca^{2+}. We herein present an elaborate regulatory mechanism that resensitization is promptly terminated by the Ca^{2+}-dependent re-development of desensitization so as to refuse prolonged stimulation.

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MATERIALS AND METHODS

Measurement of Control Responses The experimental protocols of the present study were approved by the Institutional Review Board, Meiji Pharmaceutical University. Strips of taenia caeci were prepared from guinea pigs of either sex, weighing 250–400 g, as described previously.[11] Differences between the sexes did not significantly affect the results obtained. The muscle strips were maintained in normal Locke-Ringer solution (normal solution) with the following composition (mM): NaCl 154; KCl 5.6; $CaCl_2$ 2.2; $MgCl_2$ 2.1; $NaHCO_3$ 6; glucose 5.6. Contractions were induced with carbachol (10^{-9} to 10^{-4} M) at 30°C.

Induction of Desensitization in the Presence and Absence of Extracellular Ca^{2+} One hour after the control curve was completed, muscle strips were pretreated with 10^{-4} M carbachol-containing normal solution for 15 s and then with 10^{-4} M carbachol-containing Ca^{2+}-free Locke-Ringer solution (Ca^{2+}-free solution) for the indicated time periods. Ca^{2+}-free solution was prepared by adding EGTA to normal solution at a final concentration of 0.2 mM, instead of adding $CaCl_2$. For comparison, the muscle strips were treated with 10^{-4} M carbachol-containing normal solution for the indicated time periods.

Measurement of Desensitized Responses After carbachol pretreatment, the muscle strips were washed with normal or Ca^{2+}-free solution, and a further 10 min later, concentration-response curves were recorded in normal solution. Concentration–response curves, without carbachol pretreatment, were also measured after the control responses were first recorded. This was done to statistically test the differences between responses with and without carbachol pretreatment (shown as "0 min" treatment in Fig. 2). Concentration-response curves for carbachol were expressed as percentages of the maximal contraction of the control response, taking 0% as the baseline in the resting state measured just before the addition of carbachol for the control response.

Statistical Analyses Results are presented as the mean±SEM. Statistical significance was evaluated by Student's t test with $p<0.05$ taken to indicate significance.

Materials We purchased carbachol and EGTA from Wako Pure Chemicals Industries (Osaka, Japan).

RESULTS

Carbachol-induced Desensitization to Carbachol Contractile responses to carbachol were measured in normal solution before and after pretreatment with 10^{-4} M carbachol for 15 s in normal solution followed by continued pretreatment with 10^{-4} M carbachol in normal or Ca^{2+}-free solution up to 30 min (Fig. 1). Carbachol pretreatment resulted in desensitization in contractile responses, in particular, to 10^{-7} M carbachol, with increases in the EC_{50} values for carbachol, but no changes were observed in maximal contraction to 10^{-4} M carbachol.

Developmental Process of Carbachol-induced Desensitization to Carbachol The developmental process of desensitization to carbachol was then evaluated by measuring the reduction in contractile responses to 10^{-7} M carbachol or the increase in the EC_{50} values for carbachol induced by pretreatment with 10^{-4} M carbachol for 15 s in normal solution followed by continued pretreatment with 10^{-4} M carbachol in normal or Ca^{2+}-free solution for the indicated time periods (Fig. 2). Three successive phases were observed in the desensitizing process to carbachol in normal solution, *i.e.* fast desensitization within 15 s, transient resensitization reaching a peak at 1 min, and the subsequent re-development of desensitization up to 30 min. By carbachol treatment in Ca^{2+}-

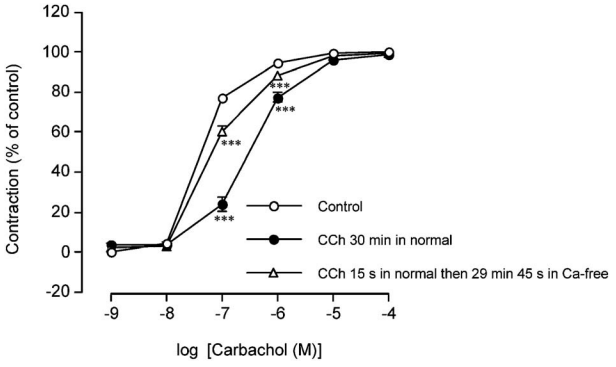

Fig. 1. Carbachol-induced desensitization to carbachol. Concentration-response curves for carbachol in normal solution were measured before (control; open circle) and after treatment with 10^{-4} M carbachol-containing normal solution for 30 min (closed circle) or after treatment with 10^{-4} M carbachol-containing normal solution for 15 s followed by 10^{-4} M carbachol-containing Ca^{2+}-free solution for 29 min 45 s (open triangle). Values represent the mean±SEM of 8–10 experiments, expressed as percentages of the maximal response to 10^{-4} M carbachol in the control response. The error bar lies within the dimension of the symbol where error bars are not shown. ***$p<0.001$, compared with control.

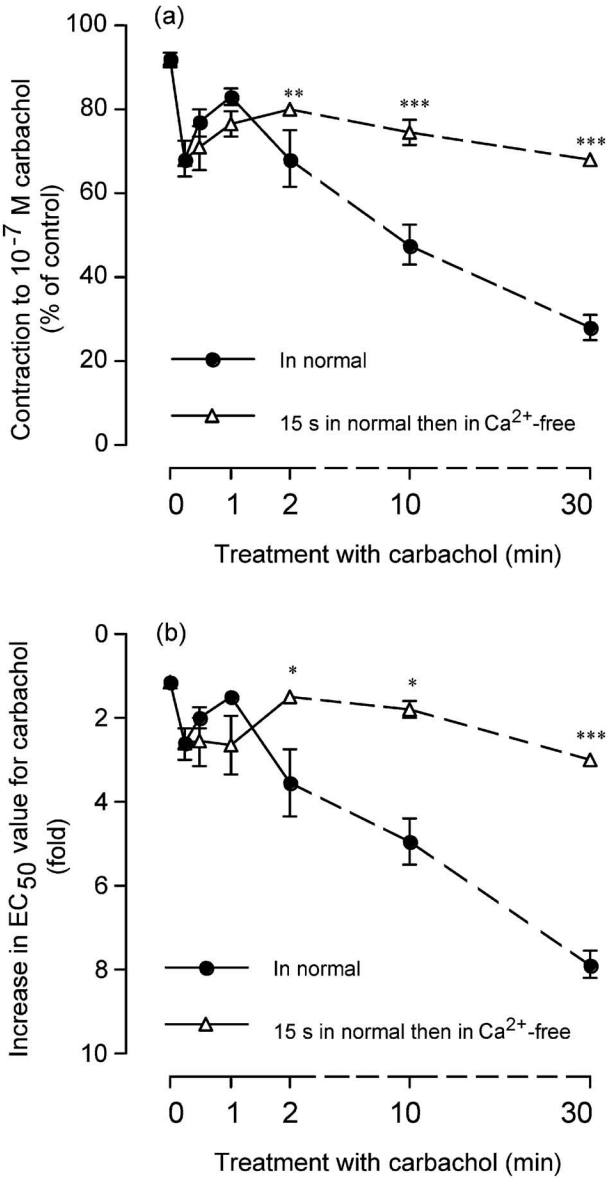

Fig. 2. The developmental process of carbachol-induced desensitization to carbachol. Contractions to carbachol in normal solution were measured after treatment with 10^{-4} M carbachol-containing normal solution for 0 s (no treatment) to 30 min (closed circle) or after treatment with 10^{-4} M carbachol-containing normal solution for 15 s followed by 10^{-4} M carbachol-containing Ca^{2+}-free solution for up to 29 min 45 s (open triangle). Values represent the means ± SEM of 8–10 experiments, expressed as percentages of the control response to 10^{-7} M carbachol (a) or ratios of EC_{50} values for carbachol before and after carbachol treatment (b). The error bar lies within the dimension of the symbol where error bars are not shown. All points are significantly different from the value at 0 s (no carbachol treatment). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, compared with the value for carbachol treatment in normal solution.

free solution after 15-s pretreatment with carbachol in normal solution, resensitization was induced to reach a peak at 2 min followed by the slow development of re-desensitization to result in a long-lasting phase of resensitization. It is noted that development of re-desensitization showed a time course similar to that induced in Ca^{2+}-free solution throughout carbachol treatment.[12]

DISCUSSION

Although resensitization has been thought to occur in a Ca^{2+}-dependent manner,[12,13] resensitization occurred even in the absence of extracellular Ca^{2+} in this study. It is most likely that resensitization occurred due to the initial increase in intracellular Ca^{2+} concentration caused by carbachol pretreatment for 15 s in the presence of extracellular Ca^{2+} followed by a relatively slow decrease in intracellular Ca^{2+} concentration after deprivation of extracellular Ca^{2+}. In contrast, the subsequent development of desensitization was delayed and became obscure by deprivation of extracellular Ca^{2+}, resulting in a long-lasting phase of resensitization. This is the first observation that resensitization was induced without marked development of subsequent desensitization. These results suggest that Ca^{2+}-dependent resensitization, which interrupts the early progress of carbachol-induced desensitization, is promptly terminated by the Ca^{2+}-dependent re-development of desensitization. These Ca^{2+}-dependent processes appear to occur as far as an increase in intracellular Ca^{2+} concentration is maintained even by stimulation with a lower concentration of agonist, since 10^{-6} M carbachol induced a similar process of desensitization/resensitization.[14] This is consistent with our hypotheses that the early process of desensitization and resensitization of G_q protein-coupled Ca^{2+}-mobilizing receptors is determined via Ca^{2+}-dependent mechanisms, *i.e.* Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) and protein phosphatase 2B (calcineurin: PP2B), respectively.[15]

In G_s protein-coupled cAMP-generating receptors such as beta-adrenergic receptors, desensitization may involve second messenger-dependent and -independent mechanisms mediated *via* protein kinase A and G protein-coupled receptor kinases (GRKs), respectively,[1–10] and resensitization may involve protein phosphatase 2A (PP2A) in endosomes.[4,5] In G_q protein-coupled Ca^{2+}-mobilizing receptors such as muscarinic M_3 receptors and histamine H_1 receptors, we estimate that desensitization and resensitization are dually regulated by second messenger-dependent

activation of CaM kinase II/PP2B in their early processes as well as second messenger-independent activation of GRKs/PP2A in their later processes, respectively.[11–16] Further research, however, is required for clarifying precise mechanisms for time-dependent desensitization and resensitization that determine cellular responsiveness.

In conclusion, we emphasize that Ca^{2+} plays a crucial role in the prompt and thorough regulation of cellular responsiveness upon stimulation with an agonist by the radical formation of three successive processes of fast desensitization, resensitization and subsequent desensitization that terminates resensitization. In particular, Ca^{2+}-dependent re-development of desensitization provides an important way for cellular homeostasis by functioning as a long-lasting safety apparatus against prolonged stimulation. Furthermore, if cells do not normally respond to receptor agonist with an impaired increase in intracellular Ca^{2+} concentration, desensitization will develop much more slowly so as not to reduce cellular responsiveness further. Such Ca^{2+}-dependent elaborate regulation of desensitization progress may be well reflected in its physiological, pathophysiological and pharmacological significance in the regulation of smooth muscle function *via* G_q protein-coupled Ca^{2+}-mobilizing receptors *in vivo*.

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