Desensitization to ANG II in guinea pig ileum depends on membrane repolarization: role of maxi-K⁺ channel

Bagnólnia A. Silva, Viviane L. A. Nouailhetas, and Jeannine Aboulafia

Department of Biophysics, Universidade Federal de São Paulo, Escola Paulista de Medicina, 04023-062 São Paulo, Brazil

Silva, Bagnólnia A., Viviane L. A. Nouailhetas, and Jeannine Aboulafia. Desensitization to ANG II in guinea pig ileum depends on membrane repolarization: role of maxi-K⁺ channel. Am. J. Physiol. 277 (Cell Physiol. 46): C739–C745, 1999.—Desensitization of ANG II tonic contractile response of the guinea pig ileum is related to membrane repolarization determined by Ca²⁺-activated K⁺ (maxi-K⁺) channel opening. ANG II-stimulated depolarized myocytes presented sustained activation of maxi-K⁺ channels, characterized by reduction from 415 to 12 ms of the closed time constant. ANG II desensitization was prevented by 100 nM iberiotoxin, being reversible within 30 min. Depolarization by KCl, higher than 4 mM, impaired desensitization, suggesting that the membrane potential must attain a threshold to counteract the repolarization induced by maxi-K⁺ channel opening. Once this value is attained, there is no time dependency because the desensitization process was shut off by addition of KCl along the time course of the tonic response. In contrast, the sustained ACh tonic component was not altered by these maneuvers. We conclude that desensitization of the ANG II tonic component is foremost due to the opening of maxi-K⁺ channels, leading to membrane repolarization, thus closing the voltage-dependent Ca²⁺ channels responsible for the Ca²⁺ influx that sustains the tonic component in this muscle.

Isometric contraction; patch-clamp technique; acetylcholine; maxi-K⁺ channel; iberiotoxin

The contractile response of the guinea pig ileum to stimulants, such as ANG II or ACh, depends on membrane depolarization, which leads to an increase of the intracellular free Ca²⁺ concentration. This increase is due to an enhanced Ca²⁺ influx, mainly through nonselective cation channels (NSCC) and/or voltage-dependent Ca²⁺ channels rather than the release of Ca²⁺ from intracellular pools. ANG II, in addition to its potent vasoconstrictor action and other physiological effects, has a direct action on the guinea pig ileum. Its contractile response presents two components: a fast and transient increase of the tonus, the phasic component, followed by a partial relaxation of the tissue to a sustained tonus, the tonic component. Prolonged treatment of this tissue with ANG II promotes a gradual decay of this component to near basal levels usually within 15 min. This response, named desensitization, does not occur when the guinea pig ileum is stimulated with ACh, since the tonic component remains sustained.

The molecular mechanisms underlying desensitization of AT₁ receptor-mediated responses in vascular smooth muscles appear to involve receptor phosphorylation, downregulation, and internalization. In guinea pig ileum, it has been proposed that desensitization of the contractile response to ANG II may be due to a negative-feedback mechanism mediated by protein kinase C (PKC) affecting a step in the stimulus-response chain after phospholipase C activation. On the other hand, over the last years, the reports converge to the concept that K⁺ channels may be an important cell strategy for controlling smooth muscle function. The relationship between increased K⁺ channel activity and smooth muscle relaxation was explored in many tissues and K⁺ channel types, such as ATP-dependent K⁺ channels in intestinal smooth muscles, small-conductance Ca²⁺-dependent K⁺ channels in vascular smooth muscle, and high-conductance Ca²⁺-dependent K⁺ (maxi-K⁺) channels in vascular and intestinal smooth muscles.

Maxi-K⁺ channels are ubiquitously distributed among tissues, and it has been suggested that they contribute to the resting potential and the repolarization of the action potential. In addition to its sensitivity to intracellular Ca²⁺ concentration and membrane potential, it has been reported that it is modulated by a wide variety of agents, including hormones, lipids, cyclic nucleotides, neurotransmitters, and PKC. In a previous paper, we demonstrated that the pharmacomechanical coupling of ANG II to the AT₁ receptor is well preserved in high-K⁺ depolarized longitudinal myocytes of the guinea pig ileum by indirect and persistent activation of maxi-K⁺ channels. This long-lasting ANG II effect contrasts with the suppression of Ca²⁺-dependent K⁺ currents by ACh in other tissues. So, that it raises a major possibility that there is a close relationship between ANG II desensitization and maxi-K⁺ channel activity.

In this study, we explored the role of maxi-K⁺ channel on the desensitization mechanism of the guinea pig ileum to ANG II. We compared the maxi-K⁺ channel activity resulting from prolonged treatment of the cells with ANG II and ACh and studied the contractile response to these agonists when the membrane potential was altered, by either blocking maxi-K⁺ channel activity or adding KCl in the physiological solution. We conclude that desensitization of the tonic component of the contractile response to ANG II is largely due to the sustained opening of maxi-K⁺ channel population, leading to repolarization of the membrane potential, thus closing the voltage-dependent Ca²⁺ channels respon-
sible for the Ca\(^{2+}\) influx that sustains the tonic component in this smooth muscle.

**METHODS**

**Animals.** Either male or female albino guinea pigs, weighing between 200 and 250 g, were used in this study.

**Cell preparation.** Guinea pig ileum smooth muscle cells were isolated according to the method described by Romero et al. (23). Briefly, 2.5-cm segments of the longitudinal muscle layer of the guinea pig ileum were washed in 5 ml of Ca\(^{2+}\)-free solution and exposed to Ca\(^{2+}\)-free solution containing 0.5 mg/ml collagenase, 0.3 mg/ml pronase (from Streptomyces griseus), and 2.0 mg/ml BSA for 7 min at room temperature. The enzymatic digestion was interrupted by washing the tissue fragments in high-Ca\(^{2+}\) solution containing 20 mg/ml BSA and 0.1% trypsin inhibitor. The digested fragments were rinsed in Ca\(^{2+}\)-free solution, and the cells were released by successively drowning the tissue fragments in and out of a blunt glass pipette. Cells were collected by centrifugation at 700 g for 30 s, and the cell pellet was resuspended in Ca\(^{2+}\)-free solution, seeded on circular coverslips, and kept at 4°C for 1 h. At the time of the experiment, one coverslip was washed with the appropriate saline solution and transferred to the stage of a microscope for electrophysiological measurements.

**Recording techniques.** Single-channel currents were recorded using either the inside-out or cell-attached modes of the patch-clamp technique (10). Patch electrodes were made of borosilicate glass (Garner Glass, Claremont, CA) through a two-stage puller (model PP-83; Narishige, Japán) and fire-polished (model MF-83 forge; Narishige) to a final pipette tip resistance of 5–10 MΩ. A 1 M KCl-agar bridge connecting the Ag-AgCl reference electrode was used to ground the bath solution. The cells and the electrode were visualized with an inverted microscope (model Diaphot, Nikon, Japán). Single-channel currents were captured and amplified through a patch-clamp amplifier (EPC7; List Electronics, Darmstadt, Germany) and were stored on videotape (model PVC-6000; Philco-Hitachi, São Paulo, Brazil) through an analog-to-digital converter (model DR-384; Neuro-Corder, Neuro Data Instruments, New York, NY). Data were displayed on-line or from the videotape to a physiograph (model RS 3200; Gould, Cleveland, OH) and to an oscilloscope (model MO 1221; Minipa, São Paulo, Brazil) via a low-pass filter (8-pole Bessel filter; Frequency Devices, Haverhill, MA) at 3 kHz. All experiments were done at room temperature and at −40 mV membrane potential.

**Data acquisition and analysis.** Currents were acquired through a 16-bit analog-to-digital converter (TL-1 DMA interface; Axon Instruments, Foster City, CA) controlled by the Fetchex software (pClamp 5.1, Axon). Records were analyzed using the computer program Transit (version 1.0, kindly offered by R. Latorre, Universidad de Chile). The duration and amplitude of each current level were determined using idealized records from the original data, constructed through the recognition of the transitions between distinct levels. Transitions were detected any time \(\text{dI/dt}\) (where \(I\) is current amplitude) was higher than the slope threshold criterion, usually set at ±3\(\sigma\) of the mean baseline noise. \(\sigma\) values, where \(N\) is the number of channels in the patch available to open and \(P_o\) is the open probability of the channel, were calculated by the ratio of the mean current to the unitary single-channel current. The mean current was obtained from the amplitude current distribution histogram, using the following expression: \(I_{\text{mean}} = A_1 f_1 + A_2 f_2 + \ldots + A_n f_n\), where \(A_1\), \(A_2\), and \(A_n\) represent the area under the Gaussian curve for each current level \((f_1, f_2, f_n)\) present in the patch.

**Tension measurements.** Guinea pig ileum longitudinal smooth muscle strips were prepared as previously described (22). Segments of the longitudinal muscle ileum (3–3.5 cm) were suspended in a 5-ml chamber containing Tyrode solution at 37°C and bubbled with air. The isometric tension was recorded through a Narco Bio-System model F-60 force transducer connected to an EOB model 102-B potentiometric recorder. The agonist concentrations used were maximal, the time contact of the tissues with the agonist was 15 min, and a control response was obtained before any experimental protocol and between two successive agonist challenges after a 30-min resting period to fully recover the initial contractile response (21).

**Solutions and drugs.** The following solutions were used for isolation of the cells. Ca\(^{2+}\)-free solution contained (in mM) 132.4 NaCl, 5.9 KCl, 1.2 MgCl\(_2\), 6H\(_2\)O, 11.5 glucose, and 10 HEPES, pH 7.4 (1 M NaOH), in the presence of 100 U/ml penicillin and 100 µg/ml streptomycin. For high-Ca\(^{2+}\) solution plus albumin, 2.0 mg/ml BSA and 2.5 mM CaCl\(_2\)-2H\(_2\)O were added to Ca\(^{2+}\)-free solution. For patch-clamp experiments, the composition of both the bath and pipette solutions was (in mM) 150 KCl, 1 MgCl\(_2\), 6H\(_2\)O, and 10 HEPES, pH 7.4 (1 M KOH), referred to as high-K\(^{+}\) solution. In cell-attached experiments, all chemicals were added to the cells by perfusion of homogeneous solutions, and data were obtained after 3 min, so that the complete substitution of the volume of the chamber was guaranteed. The composition of the Tyrode solution was (in mM) 137 NaCl, 2.68 KCl, 1.36 CaCl\(_2\):2H\(_2\)O, 0.49 MgCl\(_2\):6H\(_2\)O, 12 NaHCO\(_3\), 0.36 NaH\(_2\)PO\(_4\), and 5.5 d-glucose.

**Chemicals.** All chemicals were of analytical grade. Penicillin, streptomycin, HEPES, trypsin inhibitor, BSA, iberotoxin, and ACh were purchased from Sigma (St. Louis, MO). Collagenase I (217 U/ml) was from Worthington Biochemical (Freehold, NJ), and pronase was from Boehringer (Mannheim, Germany). ANG II and [2-lysine]ANG II (Lys\(^2\)-ANG II) were purified peptides routinely synthesized in our laboratory. Stock solutions (1 mg/ml) were prepared in water and kept at 0°C, and an appropriate dilution was made at the moment of the experiment. Other salts and d-glucose were from Merck (Darmstadt, Germany).

**RESULTS**

**Indirect effect of ANG II and ACh on maxi-K\(^+\) channel activity.** To test the possibility that desensitization of the contractile response might be related to repolarization of the membrane potential through activation of K\(^+\) channels, we compared the effect of prolonged exposure of the guinea pig intestinal myocytes, bathed in high-K\(^{+}\) solution, to maximal concentration of 10\(^{-7}\) M ANG II and 10\(^{-6}\) M ACh on maxi-K\(^{+}\) currents recorded through cell-attached configuration of the patch-clamp technique at −40 mV membrane potential. ANG II invariably enhanced maxi-K\(^+\) channel activity by keeping \(P_o\) values elevated as long as the peptide was maintained in the bath solution, as already reported by Romero et al. (23). It usually caused simultaneous channel openings as illustrated by the multiple current levels in Fig. 1. Contrasting with ANG II, prolonged exposure to maximal ACh concentration caused quite variable effects on maxi-K\(^+\) channel activity: a transient activation returning to the control activity within 12 min even in the presence of ACh in the bath solution (Fig. 1; in 4 of 8 experiments), no effect at all (data not shown; in 2 of 8 experiments), or a
sustained increase, which was reversible upon washout (data not shown; in 2 of 8 experiments). In this latter group, the ACh activation was lower than that caused by ANG II challenged at the same patches. The observed sustained \( P_0 \) induced by ANG II strongly suggests that relaxation of the whole tissue might be related to the repolarization of the cell.

In a unique experiment, out of 100 trials, containing one channel in the patch, the kinetic parameters were determined (Fig. 2 and Table 1). In the control condition, the channel presented two open and two closed states. The open time constants were 0.8 and 5.7 ms, which yielded a mean open time value of 3.7 ms. With regard to the closed state, these values were 0.6 and 415.3 ms, resulting in a mean closed time value of 159.4 ms. Upon addition of ANG II, there was mainly a reduction of one order of magnitude for the longest time constant value of the closed state after a 2-min stimulation of the cells with the peptide (Fig. 2 and Table 1). This behavior, evaluated at the 5- and 10-min stimulation periods, was basically maintained, presenting minor differences for the time constants or component distributions (Table 1). Therefore, in this one patch, it appears that the sustained elevated \( P_0 \) values are due to shorter mean closed time, leading to higher frequency of channel opening.

Desensitization and membrane potential. If a sustained activation of maxi-K\(^+\) channels by ANG II would repolarize the cell, leading to tissue relaxation, it would be expected that maxi-K\(^+\) channel blockers would interfere with the desensitization of its contractile response. Thus we investigated the effects of iberiotoxin, a specific maxi-K\(^+\) channel blocker (5), on the contractile response of the muscle tissue to ANG II, its synthetic analog Lys\(^2\)-ANG II, or ACh. Because there were no previous reports that the maxi-K\(^+\) channels present in our experimental model are sensitive to iberiotoxin, we first tested its effect at single-channel level, in inside-out patches of longitudinal layer myocytes bathed in symmetrical high-K\(^+\) solutions. Iberiotoxin (100 nM) in

---

Table 1. Kinetic parameters of closed and open states of the maxi-K\(^+\) channel in control and ANG II-stimulated myocytes

<table>
<thead>
<tr>
<th>Condition</th>
<th>MCT (ms)</th>
<th>( \tau_1 ) (ms)</th>
<th>( \tau_2 ) (ms)</th>
<th>MOT (ms)</th>
<th>( \tau_1 ) (ms)</th>
<th>( \tau_2 ) (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>159.4</td>
<td>0.6 (63)</td>
<td>415.3 (37)</td>
<td>3.7</td>
<td>0.8 (42)</td>
<td>5.7 (58)</td>
</tr>
<tr>
<td>2 min</td>
<td>1.0</td>
<td>0.4 (89)</td>
<td>11.9 (11)</td>
<td>4.4</td>
<td>2.6 (35)</td>
<td>5.2 (65)</td>
</tr>
<tr>
<td>5 min</td>
<td>3.6</td>
<td>0.4 (90)</td>
<td>27.0 (10)</td>
<td>3.9</td>
<td>1.3 (17)</td>
<td>0.8 (37)</td>
</tr>
<tr>
<td>10 min</td>
<td>4.5</td>
<td>0.7 (75)</td>
<td>15.0 (25)</td>
<td>3.3</td>
<td>0.8 (37)</td>
<td>4.7 (63)</td>
</tr>
</tbody>
</table>

Mean closed time (MCT) and mean open time (MOT) values as well as time constants (\( \tau \)) are expressed in ms. Numbers in parentheses represent percentage of contribution of each component to mean dwell-time values. Values were measured at \(-40\) mV.
the delayed onset for dissipation of the Lys-ANG II tonic component and slower increase of the tonus for ACh (Fig. 3).

To further characterize the relationship between desensitization and repolarization of the membrane potential in intestinal smooth muscle, we investigated the effect of distinct levels of membrane depolarization on the contractile responses to ANG II, Lys-ANG II, and ACh. Addition of 1 or 2 mM KCl, 2 min after exposing the strips to either 10^{-7} M ANG II (Fig. 4) or 10^{-6} M ACh (data not shown), did not modify the usual contractile pattern to both agonists. However, for KCl concentrations higher than 4 mM, desensitization to ANG II (or Lys-ANG II) was prevented, as illustrated in Fig. 4 for 8 and 16 mM KCl. On the other hand, the effect of KCl added to ACh-stimulated tissues was dependent on the control contractile response. The most usual waveform of ACh contractile response is the presence of a sustained tonic component at its onset (Fig. 5), but in 20% of the isolated guinea pig preparations, the tonic component undergoes a partial relaxation before a steady level is attained (Fig. 5, inset). Then, addition of 8 mM KCl did not affect the ACh time course response or prevented the partial relaxation of the ACh tonic component (Fig. 5). Similar results concerning the time course of the tonic component (data not shown) were observed when KCl additions were done 5 min before the exposure of the muscle to ANG II, Lys-ANG II, or ACh, suggesting that the phasic component is not modulated by maxi-K^{+} channel activity. Because desensitization is a long-term process, it was interesting to verify the time dependency of this maneuver along the dissipation of the tonic component, using a KCl concentration that did not cause any sustained contraction per se. Figure 6 shows that depolarization of the membrane potential with 8 mM KCl, at any time along the tonic response induced by Lys-ANG II,
the AT1 and M3 muscarinic receptors, are coupled to the voltage-sensitive channel (17). Both receptors, namely, desensitized by iberiotoxin and elevation of extracellular K+ concentration. The sustained increase of the maxi-K+ channel activity that would lead to the repolarization of the membrane potential and the dissipation of the ANG II tonic contractile response (Fig. 3). ACh was used as a counterproof in the electrophysiological studies as well as in the whole tissue contraction experiments. In contrast to the homogeneity of the sustained increase of maxi-K+ channel activity in long-term ANG II-stimulated myocytes (Fig. 1), ACh caused variable responses. Most of the cells tested presented transient (50% of the cells) or no activation (25%) of maxi-K+ channels, and just in 25% of the ACh-stimulated cells there was a sustained maxi-K+ channel activation, although lower than the ANG II effect. Similarly, ACh-induced contractile response of the guinea pig ileum also presented some variability concerning the amplitude of the steady tonic component, which however never attains basal level in the presence of this agonist. The most usual ACh waveform presents a tonic component already sustained at its onset (Fig. 5), and in a few cases, there is a partial relaxation before a steady level is attained (Fig. 5, inset). This parallelism between ACh-induced maxi-K+ channel activity and ACh contraction suggests that the major contribution to the most usual ACh contractile response results from myocytes that presented transient or no activation of maxi-K+ channels. This would lead to a sustained depolarization of the cell membrane, favoring Ca2+ currents through L channels, and thus contraction. On the other hand, the observations that iberiotoxin increased the fluctuation of basal and stimulated tension in ACh contraction (Fig. 3) and the partial relaxation of the tonic component in 25% of the cases (Fig. 5, inset) indicate an active involvement of maxi-K+ channels throughout the contraction induced by this agent. However, the eventual repolarization of the membrane potential, as a consequence of maxi-K+ channel activity, would not be enough to overcome the initial depolarization induced by ACh, which involves activation of Ca2+-sensitive NSCC (12) sustaining the depolarization level of the membrane. In the case of ANG II, on the contrary, the cell balance does converge to the repolarization of the cell membrane and thus relaxation of the tissue. Indeed, repolarization due to sustained maxi-K+ channel activation would exert a negative-feedback modulation on L-type Ca2+ channels. The non-desensitization in the presence of iberiotoxin (Fig. 3) reinforces the close dependency of maxi-K+ channel activity and modulation of the ANG II tonic component. When this channel population is blocked, the contractile response to ANG II is similar to the ACh contraction (Fig. 3), as sustained ANG II depolarization should occur, probably by maintaining elevated the P0 of voltage-dependent channels, mainly NSCC and L channels, thus keeping the tissue contracted. If this interpretation is correct, then any maneuver causing membrane depolarization should prevent this phenomenon. Indeed, exposure of the tissue to KCI concentrations, which did not cause a sustained response per se, prevented the fade of the ANG II tonic component.}

![Fig. 6. Effect of same membrane depolarization level on Lys2-ANG II tonic component. Shown are isometric contractile responses of muscle strips to 10^-6 M Lys2-ANG II (control) and in presence of 8 mM KCl added in external medium at 7, 4, and 0.5 min of tonic contraction time course. Muscle tissues were bathed in Tyrode solution, at 37°C, and allowed to rest for 30 min between preparation washout and next challenge with peptide. Individual traces were from different experiments and are representative of 5 experiments.](http://ajpcell.physiology.org/)

caused the maintenance of the tonus at the same level as it was before the KCl addition, thus counteracting the desensitization process.

**DISCUSSION**

In this study we provide evidence that desensitization of the contractile response induced by ANG II in the longitudinal layer of the guinea pig ileum is intimately related to the membrane potential. This conclusion was supported by the fact that ANG II activated maxi-K+ channels in cell-attached patches and the desensitization of the contractile response was abolished by iberiotoxin and elevation of extracellular K+ concentration.

In intestinal smooth muscles, ACh and ANG II share some common pathways of the transduction signaling. Both activate a NSCC population (12, 17) promoting Ca2+ and/or Na+ influx, depolarization, activation of L-type Ca2+ channels, rise in intracellular Ca2+ concentration, and thus contraction. In guinea pig ileum, the NSCC channels activated by ACh and ANG II present different characteristics. Although both are permeable to Ca2+, the first is very sensitive to intracellular Ca2+ (12), whereas the second is not, being a Na+- and voltage-sensitive channel (17). Both receptors, namely, the AT1 and M3 muscarinic receptors, are coupled to the Gq/G11 protein class (16, 30). However, the effector response differs in the maintenance of the tonic component during prolonged contact of the tissue with ANG II or ACh. ANG II invariably promotes dissipation of the tonic component to basal level (desensitization), whereas ACh does not (1; Fig. 5).

In cell-attached membrane patches, prolonged treatment of the myocytes with ANG II peptides promoted a sustained increase of the maxi-K+ channel activity (23, Fig. 1). Because the pharmacomechanical coupling of ANG II to its receptor was preserved in this preparation (23), it was attractive to verify whether there is a relationship between the enhanced maxi-K+ channel activity that would lead to the repolarization of the membrane potential and the dissipation of the ANG II tonic contractile response (Fig. 3). ACh was used as a counterproof in the electrophysiological studies as well as in the whole tissue contraction experiments. In contrast to the homogeneity of the sustained increase of maxi-K+ channel activity in long-term ANG II-stimulated myocytes (Fig. 1), ACh caused variable responses. Most of the cells tested presented transient (50% of the cells) or no activation (25%) of maxi-K+ channels, and just in 25% of the ACh-stimulated cells there was a sustained maxi-K+ channel activation, although lower than the ANG II effect. Similarly, ACh-induced contractile response of the guinea pig ileum also presented some variability concerning the amplitude of the steady tonic component, which however never attains basal level in the presence of this agonist. The most usual ACh waveform presents a tonic component already sustained at its onset (Fig. 5), and in a few cases, there is a partial relaxation before a steady level is attained (Fig. 5, inset). This parallelism between ACh-induced maxi-K+ channel activity and ACh contraction suggests that the major contribution to the most usual ACh contractile response results from myocytes that presented transient or no activation of maxi-K+ channels. This would lead to a sustained depolarization of the cell membrane, favoring Ca2+ currents through L channels, and thus contraction. On the other hand, the observations that iberiotoxin increased the fluctuation of basal and stimulated tension in ACh contraction (Fig. 3) and the partial relaxation of the tonic component in 25% of the cases (Fig. 5, inset) indicate an active involvement of maxi-K+ channels throughout the contraction induced by this agent. However, the eventual repolarization of the membrane potential, as a consequence of maxi-K+ channel activity, would not be enough to overcome the initial depolarization induced by ACh, which involves activation of Ca2+-sensitive NSCC (12) sustaining the depolarization level of the membrane. In the case of ANG II, on the contrary, the cell balance does converge to the repolarization of the cell membrane and thus relaxation of the tissue. Indeed, repolarization due to sustained maxi-K+ channel activation would exert a negative-feedback modulation on L-type Ca2+ channels. The non-desensitization in the presence of iberiotoxin (Fig. 3) reinforces the close dependency of maxi-K+ channel activity and modulation of the ANG II tonic component. When this channel population is blocked, the contractile response to ANG II is similar to the ACh contraction (Fig. 3), as sustained ANG II depolarization should occur, probably by maintaining elevated the P0 of voltage-dependent channels, mainly NSCC and L channels, thus keeping the tissue contracted. If this interpretation is correct, then any maneuver causing membrane depolarization should prevent this phenomenon. Indeed, exposure of the tissue to KCI concentrations, which did not cause a sustained response per se, prevented the fade of the ANG II tonic component.
(Fig. 4) and did not alter the time course of the most usual ACh response (Fig. 5). Moreover, it appears that the membrane potential must attain a threshold to counteract the repolarization induced by maxi-K⁺ opening (Fig. 4), but once it is attained, there is no time dependency along the tonic component because the desensitization process was shut off by addition of KCl at any moment of the tonic response time course (Fig. 6). This prompt response suggests that the balance of the voltage-dependent channels recruited by ANG II (maxi-K⁺, NSCC, and L channels) is a very effective regulatory mechanism for the contractility of the guinea pig ileum. However, our data also suggest that this is probably not the only mechanism underlying desensitization, since it is not clear how ACh and ANG II, which initiate their response through the same G protein (16, 30), may present differential channel type coupling. Our data convey the notion that the two receptors must differentially activate distinct G proteins and hence balance the types of channels activated and the responses achieved. Nevertheless, some caution is required for this straightforward conclusion because no data are yet available focusing this intriguing point, and alternatively, the signaling transduction of one or both agonists may involve other pathways. It has been shown that tyrosine kinase activity is of great functional importance in regulating muscarinic NSCC in guinea pig ileum (for a review, see Ref. 14) or that muscarinic activation enhanced production of arachidonic acid, thereby increasing Ca²⁺ influx into the cell (14). Another point liable to speculation concerns the molecular mechanism for the gating properties of the channels recruited by ACh and ANG II. It has been proposed that the concentration of active G protein α-subunits might critically determine the state of voltage-dependence of the channel (14). Furthermore, one must also consider that different β,γ-subunits can also regulate a number of cellular effectors, including ionic channels (14). Finally, we cannot rule out the involvement of PKC or some other unknown intracellular mechanisms that transduce cell-surface signals to the channel proteins (14), which might be different according to the agonist used. Indeed, it has been proposed that desensitization to ANG II in guinea pig ileum may be due to a negative-feedback mechanism exerted by PKC on a step of the stimulus-response chain after phospholipase C activation (24).

In conclusion, ANG II desensitization in guinea pig ileum is likely to rely on membrane repolarization, assigning a relevant role to maxi-K⁺ channel population to the modulation of this phenomenon, although the precise type and contribution of G protein other than Gα/G₁₁ remains unclear.

We are grateful to Nelson A. Mora, Chandler Tahan, Andréa Simonato, and Alexandre L. F. Pascotto for technical assistance.

B. A. Silva was on a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Estado de São Paulo.

Present address of B. A. Silva: Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, Paraíba, PB, Brazil.

Address for reprint requests and other correspondence: J. Aboulafia, Departamento de Biofísica, Universidade Federal de São Paulo, Escola Paulista de Medicina, Rua Botucatu 862, 7° andar. CEP 04023–062 São Paulo, Brazil (E-mail: jan@biofis.epm.br).

Received 16 March 1999; accepted in final form 6 July 1999.

REFERENCES


