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Attenuation of Agonist-induced Desensitization of the Rat Substance P Receptor by Microinjection of Inositol Pentakis- and Hexakisphosphates in *Xenopus laevis* Oocytes

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SUMMARY

Recently, inositol hexakisphosphate (phytic acid) was shown to bind to photoreceptor arrestin and block its interaction with rhodopsin. Such an interaction might predict that inositol polyphosphates could alter G protein-coupled receptor desensitization. To investigate the possible roles of higher inositol polyphosphates on receptor desensitization, we have expressed the rat substance P receptor in *Xenopus laevis* oocytes. The functional expression of substance P receptor was monitored by voltage-clamp recording of substance P-induced Ca^{2+}-dependent Cl^- currents. When control oocytes were stimulated with substance P (30 nM), after 10 min of washing the second responses to substance P were approximately 15% of the first responses. Cytosolic injection of inositol pentakisphosphate (100 μM) or inositol hexakisphosphate (100 μM) inhibited the reduction of the second substance P-induced current responses, maintaining the second responses to 57–58% of the initial responses. The protective effects of inositol pentakisphosphate and inositol hexakisphosphate against agonist-induced desensitization were concentration and time dependent and structurally specific, in that inositol hexasulfate and inositol tris- and tetrakisphosphate isomers were inactive. Microinjection of inositol hexakisphosphate did not (a) change the potency of substance P or the sensitivity of the expressed substance P receptor to substance P, (b) inhibit 12-*O*-tetradecanoylphorbol-13-acetate-induced loss of substance P-induced current responses, or (c) alter the currents elicited by microinjection of inositol-1,4,5-trisphosphate. These results suggest that inositol pentakisphosphate and inositol hexakisphosphate have specific inhibitory effects on the agonist-induced loss of responsiveness of the rat substance P receptor. Moreover, these protective effects of inositol hexakisphosphate against desensitization were also observed with the endogenous lysophosphatidic acid/phosphatidic acid receptor, indicating that this mechanism is not specific to ectopic receptors. These results suggest that inositol pentakisphosphate and inositol hexakisphosphate may be novel pharmacological tools for the study of agonist-induced desensitization.

The desensitization of G protein-coupled receptors is now appreciated to be a complex phenomenon involving multiple kinases and specialized regulatory proteins, such as the β-arrestins (1, 2). Most of the current understanding of this adaptive process has come from a limited number of examples, including the β-adrenergic receptor and the visual photopigment rhodopsin. Thus, it is crucial to develop pharmacological approaches to modulate receptor sensitivity to identify general and cell-specific characteristics of receptor tachyphylaxis. Very few such tools have been identified, but recently $InsP_6$ (phytic acid) was shown to bind directly to visual arrestin (3) and to interfere with the binding of arrestin to phosphorylated rhodopsin, thereby potentiating light-induced responses under whole-cell voltage-clamp conditions (4). It was suggested that this result might be predictive of similar actions on the desensitization of other G protein-coupled receptors.

The SPR is a phospholipase C-coupled receptor that has been shown to be phosphorylated directly by β-adrenergic receptor kinases 1 and 2 (5) and is therefore an ideal model to test the generality and significance of $InsP_6$ action in protecting against desensitization. Accordingly, we have expressed the rat SPR in *Xenopus laevis* oocytes by nuclear microinjection and

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ABBREVIATIONS: $InsP_6$, inositol hexakisphosphate; SPR, substance P receptor; SP, substance P; $Ins1,4,5P_3$, inositol-1,4,5-trisphosphate; $Ins1,3,4P_3$, inositol-1,3,4-trisphosphate; $Ins2,4,5P_3$, inositol-2,4,5-trisphosphate; $Ins1,3,4,5P_4$, inositol-1,3,4,5-tetrakisphosphate; $Ins1,2,5,6P_4$, inositol-1,2,5,6-tetrakisphosphate; $Ins1,3,4,6P_4$, inositol-1,3,4,6-tetrakisphosphate; $Ins3,4,5,6P_4$, inositol-3,4,5,6-tetrakisphosphate; $InsP_5$, inositol pentakisphosphate; $InsS_6$, inositol hexasulfate; TPEN, *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; SEAP, secreted alkaline phosphatase; LPA, lysophosphatidic acid; $Ins1,3,4,5,6P_5$, inositol-1,3,4,5,6-pentakisphosphate; $Ins2,3,4,5,6P_5$, inositol-2,3,4,5,6-pentakisphosphate; $Ins1,2,4,5,6P_5$, inositol-1,2,4,5,6-pentakisphosphate; $Ins1,2,3,5,6P_5$, inositol-1,2,3,5,6-pentakisphosphate; $Ins1,2,3,4,5P_5$, inositol-1,2,3,4,5-pentakisphosphate; $Ins1,2,3,4,6P_5$, inositol-1,2,3,4,6-pentakisphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

investigated the ability of $InsP_6$ and related inositol compounds to modify agonist-induced desensitization of current responses.

Materials and Methods

Expression vector. The SPR cDNA open reading frame was removed from the prTKR2 plasmid (6) by digestion with *Eco*RI and *Pst*I restriction enzymes (2-kilobase fragment) and was subcloned into a *Xenopus* oocyte nuclear expression vector, pOEV, using the *Eco*RI and *Pst*I unique restriction sites (7).

Preparation of oocytes. *X. laevis* were injected with pregnant mares serum (50 IU) 24–48 hr before ovarectomy. The ovaries were washed well with OR-2 solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1.0 mM $CaCl_2$, 2.5 mM $NaHCO_3$, 5 mM HEPES, pH 7.4). Oocytes were isolated by treatment of the ovaries with collagenase type I (2 mg/ml) in Ca^{2+}-free OR-2 solution for 100–110 min. The stage VI oocytes were selected and then rolled on plastic culture dishes to ensure complete removal of the follicle cells. The defolliculated oocytes were cultured for 16–18 hr at 18° in modified Barth's solution [88 mM NaCl, 1.0 mM KCl, 0.82 mM $MgSO_4$, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 2.42 mM $NaHCO_3$, 10 mM HEPES, 300 μg/ml sodium pyruvate, 10 μg/ml penicillin, 10 μg/ml streptomycin, 10 μg/ml gentamycin, 0.5% chicken ovalbumin, pH 7.6].

Microinjection of pOEV-SPR cDNA and pMT2-SEAP cDNA. The oocytes were placed on a bed of 35% Ficoll with OR-2 solution in 50-ml plastic conical tubes and were centrifuged at 750 × *g* for 10 min. In each cell the nucleus was then visible as a white spot. The oocytes were transferred to an injection dish with a nylon mesh grid (1.5 mm) in the bottom to hold the oocytes. The oocytes were microinjected by pressure using a picospritzer (General Valve Corp, Fairfield, NJ). The amount injected was determined by injecting a droplet in the air and measuring the volume of the droplet. Five nanoliters of plasmid in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) were injected into the nucleus. To monitor successful microinjection of the nucleus, pMT2-SEAP cDNA (100 pg/oocyte) was always coinjected with variable amounts of pOEV-SPR cDNA (1–100 pg/oocyte), as described previously (8, 9). The oocytes were cultured for 4–5 hr in modified Barth's solution at 18°. The oocytes were then transferred individually to 96-well plates containing 200 μl of modified Barth's solution and were cultured for 48 hr. SEAP activity in the medium was assayed colorimetrically as described previously (8, 9).

Microinjection of inositol compounds. Alkaline phosphatase-secreting oocytes were selected and transferred for microinjection of inositol polyphosphates as described above. The inositol trisphosphate, inositol tetrakisphosphate, or $InsP_5$ isomers, $InsP_6$, or $InsS_6$ (10 nl, in water) for control experiments or $Ins1,4,5P_3$ (5 nl, in water) was injected into the cytosol. To calculate approximate cytosolic concentrations, the cytoplasmic volume of the oocytes was estimated to be 1 μl (10).

Electrophysiology. The oocytes were voltage-clamped at −60 mV, with a two-electrode voltage clamp (model TEV-200; Dagan, Minneapolis, MN) (11), 24–120 hr after microinjection of plasmids (time course experiments) or 48 hr after microinjection (all other experiments). The current was measured through a virtual-ground circuit. The electrodes were filled with 3 M KCl and selected for resistances of approximately 1.0 MΩ. The oocytes were continually perfused (flow rate, 3.4–3.6 ml/min) with OR-2 solution in the recording chamber (volume, 150 μl). SP was applied to the cell chamber from another reservoir. The oocytes were exposed to SP for 18.2 ± 0.5 sec (n = 24). The value was estimated visually by perfusion of a phenol red marker solution through the reservoir and chamber. The electrophysiology experiments were performed at 23–26°. The data were collected with Axotape software (Axon Instruments, Foster City, CA) and plotted with Axoplot software (Axon Instruments).

Materials. The following chemicals were obtained from the companies indicated: SP from Peninsula Laboratories (Belmont, CA); $Ins1,3,4P_3$, $Ins1,3,4,5P_4$, $InsP_6$, and TPEN from Calbiochem (La Jolla, CA); and $Ins1,4,5P_3$, $Ins2,4,5P_3$, $Ins1,2,5,6P_4$, $Ins1,3,4,6P_4$, $Ins3,4,5,6P_4$, $InsS_6$, TPA, LPA, collagenase type I, and Ficoll from Sigma Chemical Co. (St. Louis, MO). Defined $InsP_5$ isomers were prepared and characterized as described previously (12) and were kindly provided by Dr. P. T. Hawkins (Agricultural and Food Research Council, Babraham Institute, Cambridge, UK). The cDNA clone for the rat SPR (prTKR2) was a gift from Dr. Shigetada Nakanishi (Kyoto University, Kyoto, Japan). The pOEV plasmid was a gift from Dr. William L. Taylor (Vanderbilt University, Nashville, TN). The pMT2 plasmid containing SEAP cDNA was kindly provided by the Genetics Institute (Cambridge, MA).

Results

Functional expression of SPR in *Xenopus* oocytes. Expression of SPR using nuclear injection has been described elsewhere.[1] Briefly, the magnitude of current responses was sensitive to both the amount of cDNA injected (1–100 pg/oocyte) into the nucleus and the time of incubation. The SP (30 nM)-induced current was detectable at 24 hr after microinjection, reached a maximum level at 48 hr, and was sustained for at least 72 hr (data not shown).

Rapid loss of SP-induced Ca^{2+}-dependent Cl^- current responses. Effects of repeated application of SP on SPR-mediated currents were examined in oocytes injected with different amounts of SPR cDNA (Fig. 1). Oocytes (injected with 1 pg, 10 pg, or 100 pg of SPR cDNA/oocyte) were first stimulated with SP (30 nM), washed with OR-2 solution for 10 min, and then restimulated with SP (30 nM). The second current responses for each SPR cDNA amount used were clearly diminished and the magnitudes of the currents were 6 ± 7%, 17 ± 4%, and 35 ± 14%, respectively, of the first responses to SP (Fig. 1). In this work, a standardized protocol using 10 pg of SPR cDNA/oocyte for microinjection and a 48-hr incubation was adopted.

Effects of $InsP_5$ and $InsP_6$ on the rapid loss of SP-

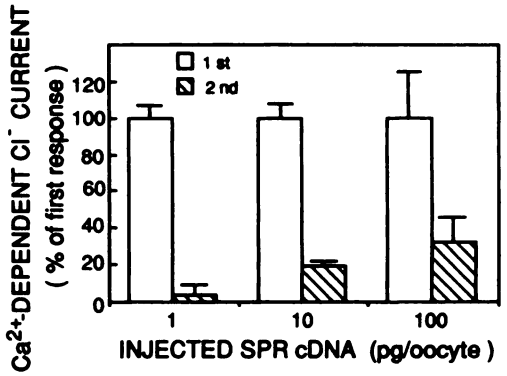

Fig. 1. SPR-mediated Ca^{2+}-dependent Cl^- current responses in oocytes injected with different concentrations of pOEV-SPR cDNA. The oocytes were microinjected with SPR cDNA (1, 10, or 100 pg/oocyte) and cultured for 48 hr. The oocytes were stimulated with SP (30 nM) for 18 sec (*1 st*). After the first stimulation, the oocytes were washed with OR-2 solution for 10 min and were restimulated with SP (30 nM) (*2 nd*). The results shown are percentages of the first response for four to eight determinations in each group from the same batch of oocytes. The absolute values of the first responses of each group (1, 10, and 100 pg/oocyte) were 1190 ± 250, 2250 ± 510, and 6410 ± 1720 nA, respectively. Typical data obtained from at least two different oocyte preparations are presented.

[1] J. E. Ferguson, N. Sasakawa, and M. R. Hanley. Functional characterization of coexpressed rat tachykinin receptors in *Xenopus* oocytes by nuclear cDNA injection. Submitted for publication.

induced current responses. Fig. 2 shows typical current traces from water (control)- and $InsP_6$ (100 μM intracellular concentration)-injected oocytes. $InsP_6$ had no significant effect on the first response to SP. When control oocytes were restimulated with SP (30 nM, 10-min wash), the peak current responses were nearly abolished (about 10% of the first response). However, microinjection of $InsP_6$ into cytosol largely prevented the loss of SP-induced current response. The second response was preserved to about 56% of the first response, roughly 5 times greater than in comparable control oocytes (Fig. 2). This phenomenon of resistance to desensitization was long lasting, even in response to a third application of SP (Fig. 2). The loss of peak responses to SP with repetitive application was also reflected in the latencies of responses, in that as the peak current response to SP diminished the latency of response became greater. In $InsP_6$-injected oocytes the response latency upon each addition of SP was invariably shorter than that in uninjected controls (Figs. 2 and 3).

Fig. 4A shows concentration-response curves for inhibition by $InsP_5$ and $InsP_6$ of the loss of SP-induced current responses. Both $InsP_5$ and $InsP_6$ inhibited the loss of current responses in a concentration-dependent manner and exerted a maximum effect (preserving second responses to about 60% of first responses) at 100 μM. These inhibitory effects on desensitization were not simply due to anionic charge, because the closely related compound $InsS_6$ had no effect. Fig. 4B shows the time course of onset of the inhibitory effect of $InsP_6$ on agonist-induced desensitization. The inhibitory effect reached a maximum level at 20 min after microinjection, and the effect was sustained for at least 6 hr. To investigate possible isomeric selectivity of this effect, defined $InsP_5$ isomers were tested for the loss of SP-induced current responses (Fig. 4C). The Ins1,3,4,5,6P_5, Ins2,3,4,5,6P_5/Ins1,2,4,5,6P_5, and Ins1,2,3,5,6P_5/Ins1,2,3,4,5P_5 isomers had similar inhibitory effects on the loss of current responses, but Ins1,2,3,4,6P_5 was invariably less effective than the other $InsP_5$ isomers at the concentrations tested. The results suggest that there is isomeric selectivity in the $InsP_5$ inhibition of the loss of current responses, which we investigated further using several defined inositol tris- and tetrakisphosphate isomers. No inhibitory effects on densensitization were observed for any of these isomers (Table 1). This result calls attention to special properties of $InsP_5$ and $InsP_6$. One documented possibility is that these isomers may act through selective ion chelation (13). This was evaluated using the heavy metal chelator TPEN (50 μM). It had no effect on densensitization.

Fig. 5 shows the concentration-response curves for SP in water (control)- and $InsP_6$-injected oocytes. Concentration-response curves for SP in $InsP_6$-injected oocytes coincided well with those in control oocytes (Fig. 5). The results suggest that microinjection of $InsP_6$ does not affect either the potency of SP or the sensitivity of SPR to SP.

Effects of $InsP_6$ on microinjected Ins1,4,5P_3-induced current responses. To exclude the possibility that microinjected $InsP_6$ exerted the inhibitory effects by interacting directly with Ca^{2+}-dependent Cl^- channels or affecting processes distal to Ins1,4,5P_3 formation, we examined the effects of $InsP_6$ on microinjected Ins1,4,5P_3-induced inward Cl^- currents. The first microinjection of Ins1,4,5P_3 (0.5 μM) induced inward Cl^- currents (91 ± 7 nA, $n = 6$) and the second microinjection after 10 min of washing with perfusion medium induced smaller currents (18 ± 4 nA, $n = 6$) in control oocytes. Microinjection of $InsP_6$ (100 μM) had no effects on the two inward currents induced by Ins1,4,5P_3 (first response, 87 ± 11 nA; second response, 13 ± 5; $n = 7$). Microinjection of $InsP_6$ itself did not induced significant currents (data not shown).

Effects of $InsP_6$ on TPA-caused loss of SP-induced current responses. To test for the selectivity of the $InsP_6$

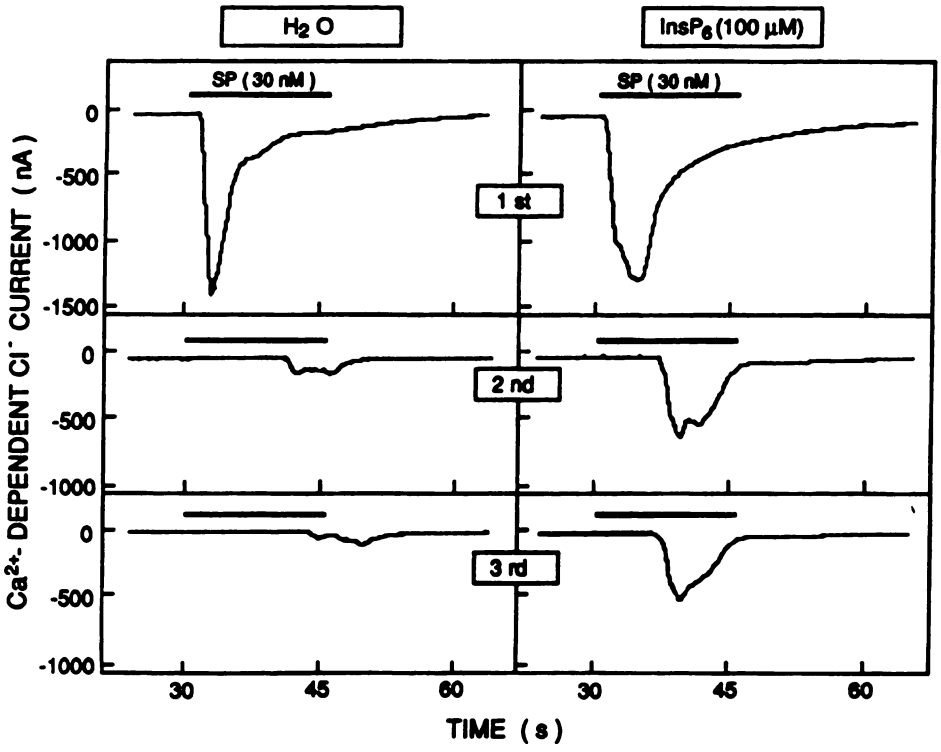

Fig. 2. Representative Ca^{2+}-dependent Cl^- current responses induced by SP in control and $InsP_6$-injected oocytes. The oocytes were microinjected in the cytosol with 10 nl of water (control) or $InsP_6$ solution in water and were incubated for 20 min in OR-2 solution. The cytosolic concentration of $InsP_6$ (100 μM) was determined as described in Materials and Methods. The oocytes were stimulated with SP (30 nM) (*1 st*). The oocytes were then washed for 10 min and restimulated with SP (*2 nd* and *3 rd*). The absolute values for current response and latency of response for water (control)- and $InsP_6$-injected oocytes were as follows: first, 1390 nA, 1.4 sec; second, 142 nA, 12.2 sec; third, 112 nA, 15.4 sec; and first, 1230 nA, 1.2 sec; second, 622 nA, 7.6 sec; third, 528 nA, 7.8 sec, respectively. There was no significant difference between noninjected oocytes and water-injected oocytes. Typical data obtained from six to 28 different oocyte preparations are presented.

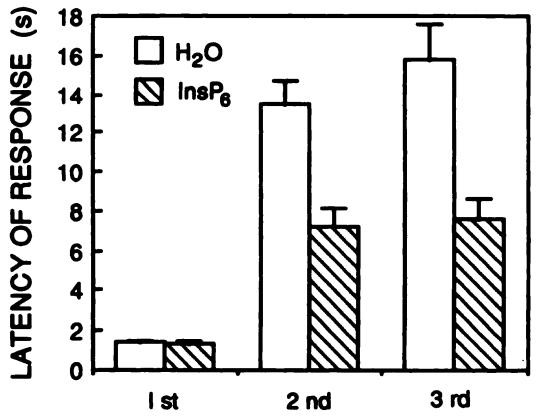

Fig. 3. Effects of $InsP_6$ on latency of SP-induced Ca^{2+}-dependent Cl^- current responses. The oocytes were microinjected with water or $InsP_6$ (100 μM) and were incubated for 20 min in OR-2 solution. The oocytes were stimulated with SP (30 nM) (*1 st*). The oocytes were then washed for 10 min and restimulated with SP (*2 nd* and *3 rd*). The latencies (in sec) for the initiation of a current response from the time of drug exposure are shown (mean ± standard error of three to six determinations from the same batch of oocytes).

effect on agonist-induced versus pharmacological receptor tachyphylaxis, we tested the influence of $InsP_6$ on protein kinase C-mediated attenuation of SPR current responses. It is widely recognized that activation of protein kinase C by treatment with TPA causes loss of phospholipase C-coupled receptor signaling (14, 15). Therefore, the possible effect of $InsP_6$ on TPA-caused loss of SP-induced current responses was examined. About 80% of SP-induced peak current was inhibited by TPA pretreatment (30 nM, 20 min) (control, 1820 ± 200 nA; TPA treated, 360 ± 41 nA; n = 8), but microinjection of $InsP_6$ (100 μM) had no effect on TPA-induced loss of current responses (control, 1740 ± 130 nA; TPA treated, 330 ± 67 nA; n = 8). The results suggest that the inhibitory effect of $InsP_6$ on the loss of current responses may be selective for agonist-induced homologous desensitization.

Effects of $InsP_6$ on LPA-induced current responses. To investigate whether the protective action of intracellularly injected $InsP_6$ was specific to an ectopic receptor, we examined responses of the intrinsic LPA receptor (11). As with the SPR, the intrinsic LPA receptor desensitization was attenuated by microinjection of $InsP_6$ (Fig. 6), so that responses to a second administration of LPA were maintained at 77% of the first response.

Discussion

The loss of responses to cell surface receptor activation during persistent stimulation with agonist has been demonstrated in many hormone and neurotransmitter receptor systems, including those that activate different G proteins and effector systems (1, 2, 16–18). This loss of receptor responsiveness has been classically called "desensitization." The process of the loss of receptor responsiveness is now more precisely divided into two major types of mechanisms, (a) desensitization and (b) down-regulation. The molecular mechanisms of desen-

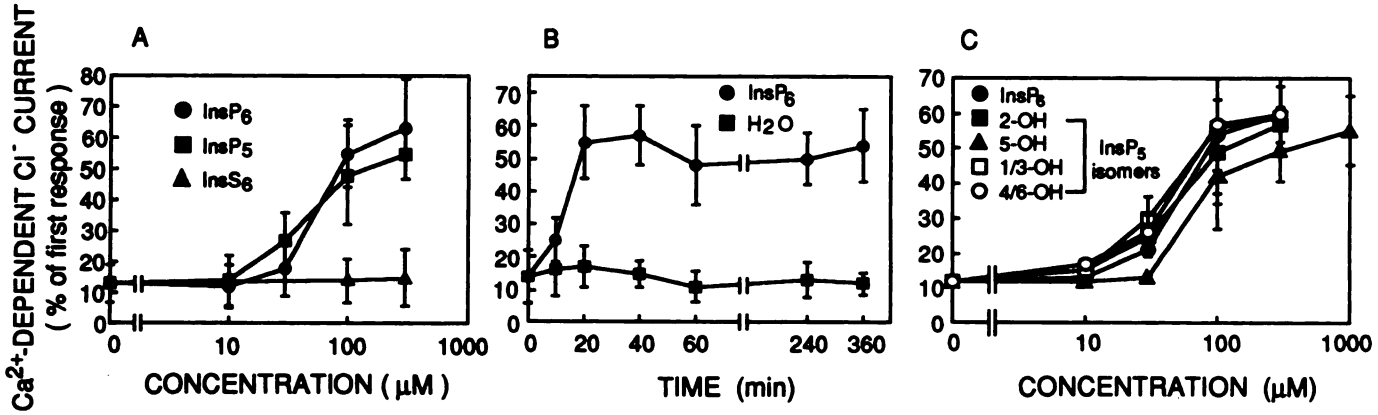

Fig. 4. A, $InsP_5$ and $InsP_6$ concentration dependence of the loss of SP-induced Ca^{2+}-dependent Cl^- current responses. The oocytes were microinjected in the cytosol with different concentrations of Ins1,3,4,5,6P_5, $InsP_6$, or $InsS_6$ and incubated for 20 min in OR-2 solution. The oocytes were stimulated with SP (30 nM), washed for 10 min, and then restimulated with SP (30 nM). The results shown are percentages of the second current response, relative to the first current response, of three to five determinations from the same batch of oocytes. The absolute values of the first responses of control, $InsP_5$ (100 μM)-injected, $InsP_6$ (100 μM)-injected, and $InsS_6$ (100 μM)-injected oocytes were 1850 ± 230, 1900 ± 260, 2080 ± 210, and 1780 ± 290, respectively. There was no significant difference in the peak values of the first responses between different concentrations (10–300 μM). Typical data obtained from at least two different oocyte preparations are presented. B, Time course of effects of $InsP_6$ on loss of SP-induced Ca^{2+}-dependent Cl^- current responses. The oocytes were microinjected in the cytosol with water or $InsP_6$ (100 μM) and incubated for different time periods in OR-2 solution. The oocytes were stimulated with SP (30 nM), washed for 10 min, and then restimulated with SP (30 nM). The results shown are percentages of the second current response, relative to the first current response, of three or four determinations from the same batch of oocytes. The absolute values of the first responses of control and $InsP_6$-injected oocytes after a 20-min incubation were 1680 ± 190 and 1840 ± 220 nA, respectively. There was no significant difference in the absolute values of first responses between different incubation time periods (10–360 min). Typical data obtained from at least two different oocyte preparations are presented. C, Defined $InsP_5$ isomer concentration dependence of the loss of SP-induced Ca^{2+}-dependent Cl^- current responses. The oocytes were microinjected with water, $InsP_5$ isomers, or $InsP_6$ and incubated for 20 min in OR-2 solution. The oocytes were stimulated with SP (30 nM), washed for 10 min, and then restimulated with SP (30 nM). The results shown are percentages of the second current responses, relative to the first current response, of four to 18 determinations. The absolute values of the first responses of control, $InsP_6$-injected, Ins1,3,4,5,6P_5-injected, Ins1,2,3,4,6P_5-injected, Ins2,3,4,5,6P_5/Ins1,2,4,5,6P_5-injected, and Ins1,2,3,5,6P_5/Ins1,2,3,4,5P_5-injected (100 μM) oocytes after a 20-min incubation were 1850 ± 220, 1800 ± 210, 1730 ± 170, 1690 ± 220, 1770 ± 220, and 1940 ± 280 nA, respectively. There was no significant difference in the peak values of the first responses between different concentrations (10–1000 μM). Typical data obtained from two different oocyte preparations are presented. *2-OH*, Ins1,3,4,5,6P_5; *5-OH*, Ins1,2,3,4,6P_5; *1/3-OH*, Ins2,3,4,5,6P_5/Ins1,2,4,5,6P_5; *4/6-OH*, Ins1,2,3,5,6P_5/Ins1,2,3,4,5P_5.

TABLE 1

Effects of inositol tris- and tetrakisphosphate isomers on loss of SP-induced Ca^{2+}-dependent Cl^- current responses

The oocytes were microinjected in the cytosol with water or inositol polyphosphates (30 or 100 μM) and incubated for 20 min in OR-2 solution. The oocytes were stimulated with SP (30 nM) (1st response), washed for 10 min, and then restimulated with SP (30 nM) (2nd response). Peak currents are shown. The results shown are mean ± standard error of three to eight determinations from the same batch of oocytes. Typical data obtained from two different oocyte preparations are presented.

Injected inositol polyphosphate	SP-induced peak current	
	1st response	2nd response
	nA	
Control (H_2O)	1980 ± 260	180 ± 48
Ins1,3,4P_3 (100 μM)	1880 ± 210	150 ± 72
Ins1,4,5P_3 (30 μM)	NA[a]	NA
Ins2,4,5P_3 (100 μM)	NA	NA
Ins1,2,5,6P_4 (100 μM)	1850 ± 190	120 ± 46
Ins1,3,4,5P_4 (30 μM)	NA	NA
Ins1,3,4,6P_4 (30 μM)	NA	NA
Ins3,4,5,6P_4 (100 μM)	2010 ± 320	140 ± 88
Ins1,3,4,5,6P_5 (100 μM)	1800 ± 360	890 ± 130
InsP_6 (100 μM)	1910 ± 390	1021 ± 220

[a] NA, not applicable. These isomers had direct effects on the induction of Cl^- current and therefore were not applicable to the examination of receptor desensitization.

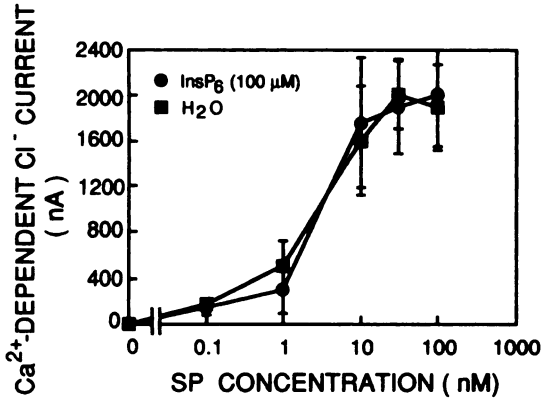

Fig. 5. SP concentration-response curves for Ca^{2+}-dependent Cl^- current responses in control and InsP_6-injected oocytes. The oocytes were microinjected in the cytosol with water or InsP_6 (100 μM) and incubated for 20 min in OR-2 solution. The oocytes were stimulated with different concentration of SP. Maximum values of the currents were plotted. The results shown are mean ± standard error of three to five determinations from the same batch of oocytes. Typical data obtained from two different oocyte preparations are presented.

sitization have been largely described for the β-adrenergic receptor, which is coupled to adenylyl cyclase (1, 19). The β-adrenergic receptor appears to undergo desensitization through a complex multistep process initiated by phosphorylation of specific serines and threonines in the carboxyl-terminal tail and the third intracellular loop. Once phosphorylated, the receptor is prevented from further interaction with G proteins by the binding of β-arrestins (1, 2, 19). Although the precise mechanisms for desensitization of receptors coupled to the phosphatidylinositol pathway are not known, desensitization of such receptors, such as SPR, is likely to be very similar (5).

As predicted from earlier work on rhodopsin (4), InsP_6 has been shown to attenuate desensitization of another type of G protein-linked receptor, the phospholipase C-linked SPR, as it did for rhodopsin. In addition, we have shown that InsP_5, but not inositol tetrakisphosphate or inositol trisphosphate, isomers also have this novel activity. In the earlier work, InsP_6

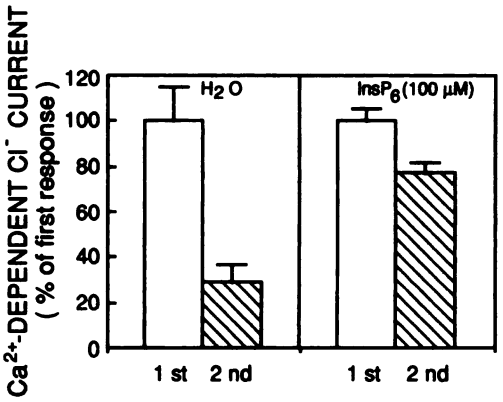

Fig. 6. LPA-induced Ca^{2+}-dependent Cl^- current responses in control and InsP_6-injected oocytes. The oocytes were microinjected with water or InsP_6 (100 μM) and incubated for 20 min in OR-2 solution. The oocytes were stimulated with LPA (50 nM) (*1 st*), washed for 10 min, and then restimulated with LPA (50 nM) (*2 nd*). The results shown are the percentages of the second current responses, relative to the first current response, of five to seven determinations. The absolute values of the first responses of control and InsP_6-injected oocytes after a 20-min incubation were 4970 ± 960 and 6210 ± 630 nA, respectively. Typical data obtained from two different oocyte preparations are presented.

was found to bind directly to visual arrestin with micromolar affinity (3), and it was speculated that this interaction was the basis for the blockade by InsP_6 of stimulus-induced rhodopsin desensitization (4). This speculation is consistent with the findings reported here, in that agonist-induced, but not phorbol diester-induced, desensitization was selectively attenuated by InsP_6 treatment. It is pertinent to note that immunoblotting of *Xenopus* oocyte extracts with antibodies to arrestins has revealed a single immoreactive band, indicating that the oocytes are likely to have a functional receptor regulatory pathway of this type,[2] although more work is clearly needed.

These results call attention to the possibility of InsP_5 and InsP_6 as pharmacological leads in the design of more potent drugs that selectively attenuate agonist-induced desensitization to enhance receptor responsiveness. However, because InsP_5 and InsP_6 are widespread, possibly ubiquitous, endogenous compounds, it is an appealing possibility that such higher inositol polyphosphates may contribute normally to the complex balance of biochemical events that mediate negative adaptation of stimulated receptors. Indeed, because InsP_5 and InsP_6 occur at substantial concentrations (up to 1–20 μM) in many cell types (20–22), the IC_{50} values (30–50 μM, estimated from Fig. 4A) for this pharmacological action are potentially physiologically significant. According to this perspective, the dynamics of stimulated changes in InsP_5 and InsP_6 levels (23–26) may contribute to resetting receptor sensitivity to agonist-induced desensitization. Intriguingly, InsP_6 also binds, with high affinity, to another protein participating in receptor regulation, the clathrin assembly protein AP-2 (27, 28). Thus, endogenous inositol polyphosphates may contribute to setting the level of both rapid adaptation of receptors through desensitization and longer term adaptation through down-regulation. This raises the question of to what extent the magnitude of agonist-induced desensitization may be influenced by resting levels of InsP_5 and InsP_6. *Xenopus* oocytes have been shown to have resting pools of endogenous InsP_5 and InsP_6 that are

[2] J. E. Ferguson and M. R. Hanley, unpublished observations.

turned over slowly and are relatively insensitive to various types of cell stimulants, although they are increased upon maturation (29). However, the resting concentrations of defined inositol phosphate isomers in oocytes are unknown, and it is unclear whether the inositol polyphosphates are cytosolic.

In summary, inositol polyphospates ($InsP_5$ and $InsP_6$) have been found to exhibit a novel effect in blocking agonist-induced desensitization of two phospholipase C-coupled receptors. The generality and physiological importance of these observations remains to be tested, but these compounds have immediate value as reagents to perturb desensitization processes. Few such reagents have been identified.

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