0026-895X/94/040731-06$03.00/0

MOLECULAR PHARMACOLOGY, 45:731-736

Differential Heterologous and Homologous Desensitization of Two Receptors for ATP (P_{2Y} Purinoceptors and Nucleotide Receptors) Coexisting on Endothelial Cells

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Received August 25, 1993; Accepted January 20, 1994

SUMMARY

Bovine aortic endothelial cells in culture contain two coexisting phosphoinositidase C-linked receptors for ATP, the P_{2Y}-purinoceptors [for which 2-methylthio-ATP (2MeSATP) is a selective agonist] and the nucleotide (or P_{2U}) receptors (for which UTP is a selective agonist). Here we have investigated the occurrence of homologous and heterologous desensitization of these two receptors and the involvement of protein kinase C-dependent mechanisms. Measuring total [^{3}H]inositol (poly)phosphate accumulation in the presence of lithium, we showed that with long (15-min) stimulations with UTP or 2MeSATP desensitization occurred to a maximum of 40% within several minutes of preexposure to either agonist, i.e., with this procedure there is no difference between the heterologous and the homologous experimental design. In the remainder of the experiments reported we measured inositol-1,4,5-trisphosphate mass levels, using a protocol of 5-min preincubation, 2-min wash, and 5-sec stimulation. We found that preincubation with either agonist led to desensitization of the response to the same agonist of about 40%. However, whereas preincubation with 2MeSATP did not affect the subsequent response to UTP, preincubation with UTP did attenuate the 2MeSATP response. These results demonstrate that homologous desensitization occurs with both P_{2Y} and nucleotide receptors but that heterologous desensitization follows only from activation of the nucleotide receptors. Preincubation with the protein kinase C inhibitor Ro 31–8220 enhanced the subsequent inositol-1,4,5-trisphosphate response to 2MeSATP but did not affect the desensitization of this response by preincubation with the same agonist. However, whereas the response to UTP was not enhanced by preincubation with the protein kinase C inhibitor, the desensitization caused by preincubation with UTP was partially inhibited by Ro 31–8220. These results show that multiple desensitizing events occur during the first few minutes of receptor activation and that these events are different for each of the receptors for ATP.

Desensitization of responses to cell surface receptors is a widespread phenomenom occurring upon continual or repeated exposure to agonists. The molecular events involved in desensitization of seven-transmembrane segment G protein-linked receptors have been described in some detail for the β-adrenergic receptors acting through adenylyl cyclase (1). In contrast, there have been relatively few studies on the desensitization of receptors linked to PIC and the consequent generation of Ins(1,4,5)P_3 and diacylglycerol. This is in part because in a number of reports continual exposure to agonists produces an apparently linear rate of inositol phosphlolipid hydrolysis over an extended period of time (2–5). However, other studies show that desensitization of PIC-linked receptors does occur, with a time course that varies from seconds to hours (e.g., see Refs. 5–11). These include reports on P_{2Y} purinergic receptors (7) and vascular endothelial cells (11), which are the subject of the present study. Several mechanisms are likely to be involved in desensitization of tissues to PIC-linked receptors, including receptor events (phosphorylation, uncoupling, and internalization), depletion of inositol phospholipids, accelerated Ins(1,4,5)P_3 metabolism, and adaptation of the intracellular receptor for Ins(1,4,5)P_3 (see Ref. 12 for review).

One consequence of activation of PIC-linked receptors is likely to be the stimulation of isoforms of PKC. It has been shown in several systems that stimulation of PKC with phorbol esters such as PMA can attenuate agonist-stimulated inositol phospholipid hydrolysis. Combined with an enhancement of PIC responses when PKC is down-regulated or inhibited (8, 13–16), this suggests the presence of a short (PKC-dependent) inhibitory feedback loop. The extent to which such feedback can be demonstrated varies considerably. For example, in studies on bradykinin- and histamine-stimulated chromaffin cells we have shown a minor role for such feedback (15), whereas with endothelin-stimulated neuroblastoma cells there is a massive inhibition by agonist-activated PKC (16). These experi-

This work was supported by The Wellcome Trust, The Medical Research Council, and Fisons plc.

ABBREVIATIONS: PIC, phosphoinositidase C; BAEC, bovine aortic endothelial cell; 2MeSATP, 2-methylthio-ATP; Ins(1,4,5)P_3, inositol-1,4,5-trisphosphate; PKC, protein kinase C; PMA, phorbol myristate acetate; BSS, balanced salt solution; ADPβS, adenosine-5′-*O*-(2-thio)diphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

ments used a mass assay of Ins(1,4,5)P_3 as an index of PIC activation within the first few seconds of stimulation. It has been reported by others that there is a role for PKC in the attenuation of response upon continual or repeated exposure to an agonist (e.g., Refs. 8 and 17). These approaches suggest a link between the short (PKC-dependent) feedback loop, with its inhibitory influence occurring with the onset of stimulation, and early desensitization events.

In the experiments reported here we have investigated the occurrence of homologous and heterologous desensitization of the receptors for ATP that are found on vascular endothelial cells. We and others have previously shown that on BAECs these comprise two coexisting populations of receptors, the P_{2Y} purinergic and the nucleotide (or P_{2U}) receptors (18, 19). The P_{2U} receptors respond to purines (such as ATP) but also to UTP, a pyrimidine, whereas the P_{2Y} receptors do not respond to UTP (18, 20). These two PIC-linked receptor populations interact to enable the regulation of endothelial cells by ATP and ADP. In recent studies we have provided evidence that activation of these receptors has different consequences, with respect to both the pattern of second messenger generation and the nature of the vasodilator response (21, 22). One of the differences we observed was that PIC stimulation by P_{2Y} receptors, but not by P_{2U} receptors, was subject to regulation by PKC (21). In the experiments reported here we set out to describe the desensitization characteristics of these coexisting receptors for ATP and to investigate the relationship between desensitization and short (PKC-dependent) feedback.

Materials and Methods

Cell culture. Fresh aortae were used to prepare BAECs by a modification of the method of Boysee *et al.* (23). Briefly, an aorta was trimmed free of fat and connective tissue, the collateral arteries were ligated, and the endothelial cells were removed by digestion with collagenase solution (0.5 mg/ml). BAECs were maintained in minimal essential medium with D-valine, 10% fetal calf serum, 10% newborn calf serum, 25 IU/ml penicillin, 25 μg/ml streptomycin, 250 μg/ml fungizone, 27 mg/ml glutamine, and 10 mg/ml gentamycin. Cells were seeded into 24-well multiplates for experimental use and were maintained at 37° in water-saturated 5% CO_2/95% air. Cells were used at confluence, when they formed a cobblestone pattern of homogeneous cells that were positive for factor VIII immunoreactivity.

[^{3}H]Inositol phosphate formation. Cells were incubated with 0.074 MBq/ml *myo*-[2-^{3}H]inositol (15 Ci/mmol) at 37° for 48 hr, in 0.5 ml of medium M199 supplemented with 25 IU/ml penicillin, 25 μg/ml streptomycin, and 250 μg/ml fungizone. Before use the loading medium was removed and the cells were washed with BSS (125 mM NaCl, 5.4 mM KCl, 16.2 mM $NaHCO_3$, 1 mM NaH_2PO_4, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5.5 mM glucose, 30 mM HEPES, buffered to pH 7.4 with NaOH and gassed with 95% O_2/5% CO_2). Cells were exposed to agonists in 0.5 ml of BSS for varying times and then washed three times with 1 ml of BSS supplemented with 10 mM LiCl. After an additional 5-min incubation, cells were stimulated for 15 min in the continued presence of lithium. The reaction was terminated by the addition of 0.5 ml of cold methanol and the cells were scraped and extracted into chloroform. Total [^{3}H]inositol phosphates were counted after purification on Dowex-1 (Cl^-).

Measurement of D-Ins(1,4,5)P_3 mass. Cells were washed twice with 1 ml of BSS and allowed to equilibrate for 10 min at 37°. The BSS was aspirated and replaced with 200 μl of BSS with preincubation drugs as required. After 5 min this solution was aspirated and the cells were washed twice with 200 μl of BSS. After an additional 2 min the cells were stimulated with BSS with or without 2MeSATP (30 μM) or UTP (300 μM), for the times indicated. The reaction was stopped by the addition of 100 μl of 1.5 μM ice-cold trichloroacetic acid. The acid was removed by three washes with 2 ml of water-saturated diethyl ether, and the mass of Ins(1,4,5)P_3 was determined by the protein-binding assay of Challiss *et al.* (24).

Where appropriate, statistical analysis was by Student's *t* test, with significance accepted at $p < 0.05$. Data are quoted as mean ± standard error. Curves were analyzed by the GraphPAD logistic curve-fitting program.

Materials. Tissue culture medium was from GIBCO (Paisley, Scotland, UK) and tissue culture flasks and multiplates were from Nunc Ltd. D-[^{3}H]Ins(1,4,5)P_3 was obtained from Amersham International UK. Ro 31–8220 (compound 3 in Ref. 25) was a kind gift of Dr. G. Lawton (Roche Products Ltd., Welwyn, Hets, UK). All other chemicals were obtained from either Sigma (Poole, UK) or Fisons (Loughborough, UK).

Results

Desensitization of [^{3}H]inositol phosphate responses. Preliminary studies were carried out on desensitization of the total [^{3}H]inositol phosphate response in the presence of lithium. These studies used long (15-min) stimulation times, with preincubation times varying from 5 to 60 min. We used as our P_{2Y} agonist in these long incubation experiments the ectonucleotidase-resistant agonist ADPβS, which is not active at the P_{2U} receptor in these cells (18). UTP was chosen as the agonist active at P_{2U} but not P_{2Y} receptors (18). The concentrations of agonists used were maximal for each, as determined in earlier experiments characterizing these responses (18). Fig. 1 shows the effect of various times of preincubation with ADPβS on the subsequent response to 15-min exposure to either ADPβS or UTP. By 20 min there was a loss of about 40% of the response to both agonists. The reduction in mean response apparently began during the first few minutes of preincubation, reaching significance by 10 min. The degree of desensitization reached by 20 min was the same for stimulation with either ADPβS or UTP, i.e., apparently homologous and heterologous desensitization was observed. Results of experiments on desensitization by UTP (data not shown) produced very similar results (reduction of response to either UTP or ADPβS to about 40% with 20-min preincubation with UTP). Our previous work has shown that UTP and ADPβS are selective with respect to their agonist

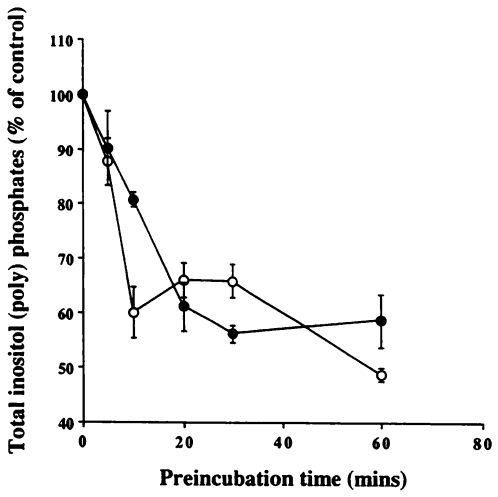

Fig. 1. Effect of preincubation with 30 μM ADPβS for the times shown on the subsequent stimulation of total [^{3}H]inositol (poly)phosphates by 15-min exposure to 30 μM ADPβS (○) or 300 μM UTP (●). LiCl was not present during the preincubation period but was included for the washes and stimulation period. There was no effect of preincubation on the basal (no agonist) condition. Data are mean ± standard error (three determinations) from one of two experiments with very similar results.

activities at the two receptors involved (18). However, to investigate the possibility that the apparent heterologous desensitization was due to some activity of these concentrations of ADPβS at P_{2U} receptors and of UTP at P_{2Y} receptors, we determined concentration-response curves for the preincubation agonists. We found that the concentrations required to generate desensitization to the same agonist were the same as those required for a different agonist (data not shown). This is consistent with the data from mass assay of Ins(1,4,5)P_3 shown in Fig. 4, indicating that the cross-desensitization was truly heterologous and not due to lack of selectivity of agonists.

These studies showed that the process of desensitization was initiated during the first few minutes of preincubation. A 5-min preincubation was therefore used in subsequent analyses using the Ins(1,4,5)P_3 mass assay.

Desensitization of Ins(1,4,5)P_3 responses. The mass Ins(1,4,5)P_3 experiments used short incubation times, so that breakdown of nucleotides was not significant. We therefore used the archetypal P_{2Y}-selective agonist 2MeSATP in these studies; we retained UTP as the P_{2U}-selective agonist. We used the concentrations of these agonists giving maximal responses with 5-sec incubation in the Ins(1,4,5)P_3 assay,[1] which were essentially the same as those established in our earlier report on the total [^{3}H]inositol phosphate assay (18).

Our preliminary experiments showed that changes of medium increased Ins(1,4,5)P_3, consistent with earlier reports of shear stress stimulation of PIC in these cells, so in the present study we deducted a BSS (i.e., no agonist added to the 5-sec stimulation) control for each condition. These control values showed small responses (in absolute amounts, compared with the agonist responses) and rather variable dependence on the preincubation conditions. For example, expressed as a percentage of the control value with BSS alone in the preincubation, 5-min preincubation with Ro 31–8220 (10 μM) followed by a 5-sec incubation with BSS gave 126.5 ± 15.2%, preincubation with UTP (300 μM) gave 70.4 ± 16.4%, and preincubation with both gave 79.4 ± 28.4% (data are mean ± standard error from seven separate experiments, each assayed in triplicate).

When maximal concentrations of 2MeSATP (30 μM) or UTP (300 μM) were applied to BAECs, there was a rapid rise in Ins(1,4,5)P_3 to a maximum at 5 sec, with a decline at each of the subsequent times measured (Fig. 2). In some experiments the time course was extended to 5 min, when no further responses to these two agonists were seen (data not shown).

Preincubation of BAECs for 5 min with either 2MeSATP or UTP, followed by a 2-min wash period, produced significant alterations in the profile of Ins(1,4,5)P_3 mass measured upon subsequent stimulation with the same agonist. Fig. 2 shows the changes observed with 2MeSATP and UTP. This homologous decrease in response was maximal at 5 sec for both agonists. The response to 2MeSATP was reduced by 45.2 ± 7.1% (four experiments), whereas that to UTP was reduced by 64.6 ± 5.6% (four experiments). When stimulations were performed with the alternative agonist, preincubation with 2MeSATP produced no corresponding decrease in responses to UTP at any of the times examined (Fig. 3A). However, preincubation with UTP reduced the stimulation induced by 2MeSATP by 44.7 ± 4.8% at 5 sec and by 38.7 ± 1.9% at 10 sec (four experiments) (Fig. 3B).

To determine whether the stimulations by 2MeSATP and UTP differed in their sensitivity to desensitization by UTP, experiments were performed with various concentrations of

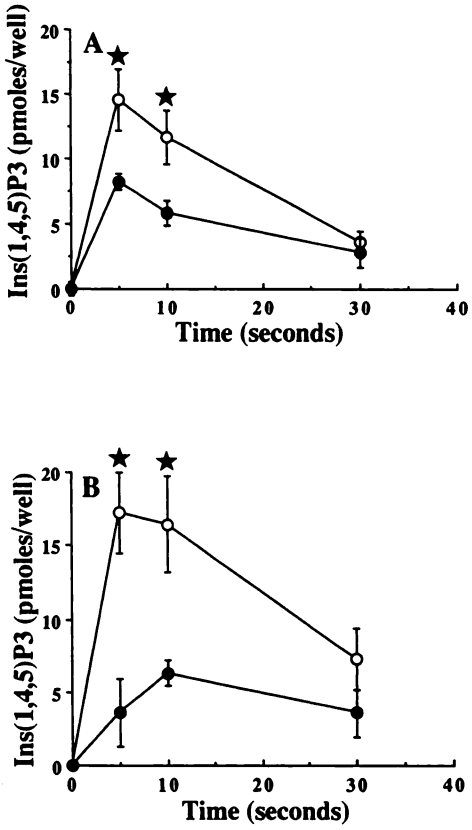

Fig. 2. Homologous experimental design. Effect of preincubation with 30 μM 2MeSATP (A) or 300 μM UTP (B) on stimulation of Ins(1,4,5)P_3 by incubation with the same agonist for the period of time shown. Preincubations were for 5 min, followed by a 2-min wash and incubation with agonist for the time shown. ○, Preincubation with no agonist; ●, preincubation with the agonist indicated. Data are pooled from three separate experiments (mean ± standard error, three determinations). ★, $p < 0.05$ (significant difference from preincubation with agonist).

UTP in the preincubation. It can be seen from Fig. 4 that the two responses showed similar sensitivities to increasing concentrations of UTP; the IC_{50} for concentrations of UTP in the preincubation inhibiting subsequent stimulation by 2MeSATP was 62 ± 4.6 μM, whereas with stimulation by UTP it was 76.3 ± 3.8 μM (three separate paired experiments). This indicates that this is true heterologous desensitization.

Effects of PKC stimulation and inhibition. We previously reported that treatment with the PKC-stimulating phorbol ester PMA significantly attenuated the response to 2MeSATP but not that to UTP (21). For example, 2MeSATP (30 μM) gave a stimulation of 4.86 ± 0.55 pmol/well when 100 nM PMA was included during the 10-min preincubation and 5-sec stimulation, compared with a stimulation of 13.79 ± 1.68 pmol/well in the absence of PMA; with UTP (300 μM) the corresponding figures were 14.87 ± 1.46 pmol/well with PMA and 9.69 ± 2.37 pmol/well without PMA (data in each case had unstimulated controls deducted; three determinations from a single representative experiment). To further investigate a role for PKC in desensitization, we used Ro 31–8220 (a relatively selective inhibitor) (4) at 10 μM. This is a concentration previously shown to be the minimum required to effectively inhibit PMA- or agonist-stimulated PKC-dependent events in these cells (26).[2] Fig. 5, A and B, shows that when the PKC inhibitor Ro 31–8220 (10 μM) was included in the preincubation the

[1] J. R. Purkiss and M. R. Boarder, unpublished observations.

[2] J. R. Purkiss and M. R. Boarder, unpublished observations.

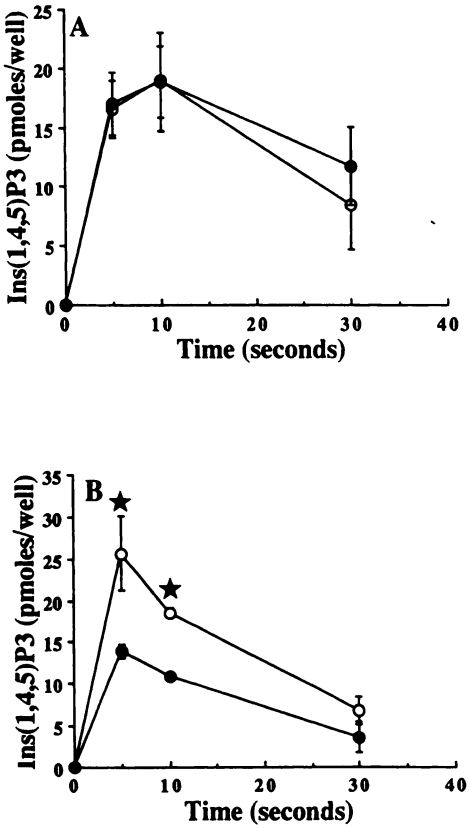

Fig. 3. Heterologous experimental design. Effect of preincubation with 2MeSATP (30 μM) and stimulation with UTP (300 μM) (A) or preincubation with UTP and stimulation with 2MeSATP (B). Preincubations were for 5 min, followed by a 2-min wash and stimulation with the second agonist for the time shown. ○, Preincubation with no agonist; ●, preincubation with the agonist indicated. Data are pooled from three separate experiments (mean ± standard error, three determinations). ★, $p < 0.05$ (significant difference from preincubation with agonist).

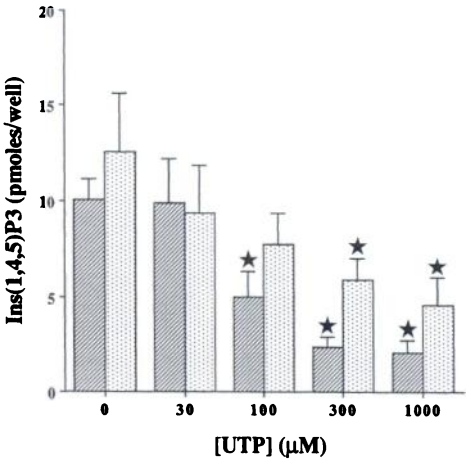

Fig. 4. Preincubations (5 min) with different concentrations of UTP and subsequent response to stimulation with 300 μM UTP (▨) or 30 μM 2MeSATP (□). Results are mean ± standard error from three separate experiments. ★, $p < 0.05$ (significant difference from preincubation with no UTP).

response to 5-sec stimulation with 2MeSATP, but not with UTP, was significantly enhanced. These results show that 2MeSATP stimulation is modulated by PKC activation, inhibiting the 2MeSATP response via a short feedback loop. In contrast, the response to UTP is apparently not sensitive to

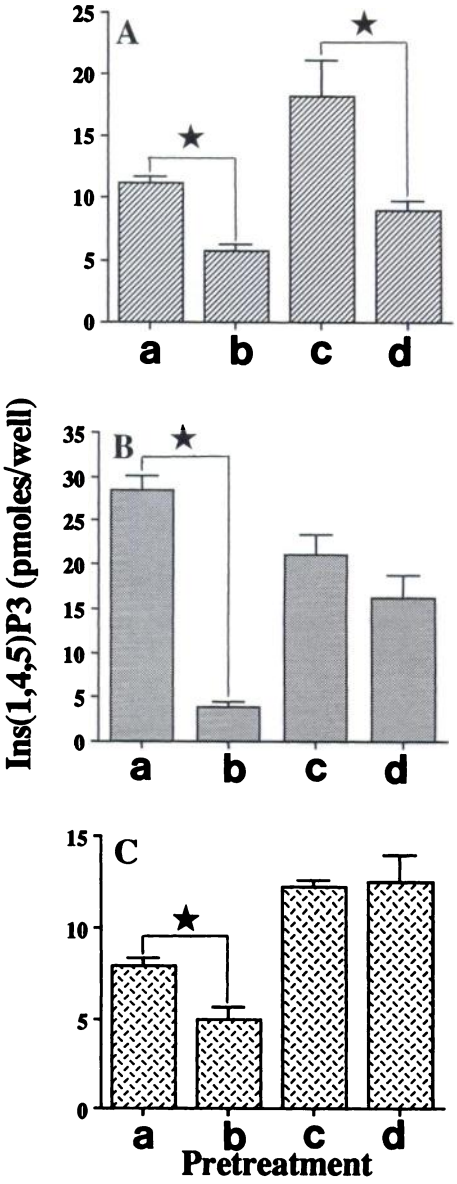

Fig. 5. Effect of Ro 31–8220 (10 μM) on desensitization. *a-d*, Preincubation conditions; *a* and *c*, without agonist; *b* and *d*, with agonist in the preincubation; *a* and *b*, without Ro 31–8220; *c* and *d*, with 10 μM Ro 31–8220 in the preincubation. A, 2MeSATP (30 μM) as the agonist for both preincubation and incubation; B, UTP (300 μM) in both the preincubation and the incubation; C, UTP in the preincubation and 2MeSATP in the incubation. Data are mean ± standard error (three determinations) from a single experiment representative of three or four separate experiments. Some normalized data pooled across the experiments are presented in the text. ★, $p < 0.05$.

regulation by this inhibitory feedback loop. This is consistent with our earlier data (21).

To determine whether PKC mediates components of the desensitization described above, we included Ro 31–8220 in the preincubations along with the desensitizing agonist. Fig. 5A shows that homologous desensitization with 2MeSATP was retained in the presence of the inhibitor. PKC inhibition during the preincubation raised the subsequent response to 2MeSATP, consistent with the data described above, but did not inhibit the desensitization caused by preincubation with 2MeSATP. Pooled across three experiments, the effects of additions to the preincubation on the subsequent response to 30 μM 2MeSATP were as follows: Ro 31–8220 (10 μM), 157.7 ± 5.5%; 2MeSATP

(30 μM), 44.7 ± 9.4%; 2MeSATP and Ro 31–8220, 103.4 ± 17.9% (data are percentage of the response to 30 μM 2MeSATP when only BSS was present in the preincubation). Desensitization in the presence of the PKC inhibitor was not significantly different from that in its absence.

In contrast to this, the inclusion of Ro 31–8220 in the preincubation did attenuate the homologous desensitization with UTP. The inhibitor had no significant effect when present alone during the preincubation, but the response to UTP was partially restored when Ro 31–8220 was included with UTP in the preincubation (Fig. 5B). We investigated the dependence of this effect on the concentration of Ro 31–8220 and showed that the concentration dependency was the same as that for inhibition of PMA-stimulated events (see above), reaching a maximal effect by 10 μM (data not shown). Pooled across three experiments, the effects of additions to the preincubation on the subsequent response to 300 μM UTP were as follows: Ro 31–8220 (10 μM), 84.4 ± 5.5%; UTP (300 μM), 10.17 ± 5.2%; UTP and Ro 31–8220, 54.4 ± 10.7% (data are percentage of the response to 300 μM UTP when only BSS was present in the preincubation). Desensitization in the presence of the PKC inhibitor was significantly different from that in its absence ($p < 0.01$). It can be concluded that those actions of UTP in the preincubation that generated the subsequent desensitization were sensitive to PKC inhibition.

When similar experiments were undertaken to investigate the involvement of PKC in the heterologous desensitization by UTP of subsequent responses to 2MeSATP, the results again showed that desensitization caused by UTP was sensitive to the presence of Ro 31–8220. The response to 2MeSATP was enhanced by the presence of the inhibitor alone during the preincubation and was inhibited by the presence of UTP during the preincubation but was restored when both UTP and inhibitor were present (Fig. 5C).

Discussion

The endothelial cells that line the vasculature play a dynamic role in the maintenance of various functions, including those related to blood clotting on the luminal side and smooth muscle contraction on the abluminal side. These fuctions of endothelium are regulated by various cell surface receptors. One of the mechanisms whereby platelets regulate the endothelium is by release of ATP/ADP and activation of endothelial purinergic receptors. These receptors, which are responsible for endothelium-dependent relaxation (27), prostaclyclin production (28), calcium mobilization (29), and phospholipid breakdown (30, 31), have been classified as P_{2Y} purinoceptors, largely because they respond to 2MeSATP. However, it has recently become apparent that an additional class of receptors that are sensitive to the pyrimidine UTP as well as purines, called the nucleotide or P_{2U} receptors, are present on many cell types (20), including BAECs, where they coexist with P_{2Y} receptors (18, 19). There are, therefore, two PIC-linked receptors for the same endogenous agonist, ATP, on these cells. The results presented in this article indicate the existence of complex regulatory mechanisms between the two types of receptor. The significance of these observations for regulation of vascular endothelium lies in our findings that not only the biochemistry (21) but also the nature of the vasodilator response (22) varies between P_{2Y} and nucleotide receptors.

Studies on receptors for ATP are limited by the tools available. In this context the lack of suitable radioligand binding data and of selective antagonists is significant. Using agonists acting at other types of receptors, we found that acetylcholine gave no PIC response (consistent with other studies on cultured endothelial cells), whereas noradrenaline, bradykinin, angiotensin II, and histamine each gave responses that were considerably smaller than those generated by ATP.[3] This made the interpretation of cross-desensitization experiments with these other agonists inconclusive. We have therefore limited the present report to studies with purinergic agonists and UTP.

The mechanisms that underly the desensitization of responses to agonists acting at PIC-linked receptors are complex and poorly understood (10). Here we measure the formation of $Ins(1,4,5)P_3$, the primary water-soluble product of PIC acting on phosphatidylinositol 4,5-bisphosphate; desensitization therefore relates to events proximal to the receptor, encompassing the receptor/G protein/PIC complex. Although other reports have implicated PKC activation in such desensitization (e.g., Ref. 31), in the present report we provide a unique example of dissociation of the ability of PKC activation by agonists to cause transient desensitization and the role of a persistent PKC-independent mechanism in long term desensitization. The data presented show that stimulation of $Ins(1,4,5)P_3$ formation by P_{2Y} receptor activation is inhibited by PMA and enhanced by Ro 31–8220. These results show that a short PKC-dependent feedback loop operates with P_{2Y} agonists, attenuating the PIC response. We show here that elevation of the response by Ro 31–8220 occurs when the inhibitor is present during the 5-min preincubation, and 5-sec stimulation with 2MeSATP in the absence of Ro 31–8220 and occurs after a 2-min wash. This presumably means that PKC inhibition persists through this 2-min wash period. A consequence of this is that, when both agonist and Ro 31–8220 were present during the preincubation, the effect of the wash was to remove the desensitizing agonist but not to relieve the PKC inhibition. Under these circumstances the presence of the inhibitor caused an enhanced reponse to the 5-sec stimulation. However, we did not see a loss of desensitization; in the presence of the inhibitor, inclusion of the agonist in the preincubation caused an attenuation of response similar to that observed when the inhibitor was not present.

This situation is difficult to understand. The 2MeSATP response was regulated by a PKC inhibitory loop activated by the agonist, but on second presentation of the agonist the desensitization was not contributed to by PKC. The most likely explanation for these results is that the PKC feedback loop was only transiently activated by the agonist. Thus, when stimulation was for 5 sec the response was enhanced by Ro 31–8220. However, by the end of the preincubation and 2-min wash period the effect of this first exposure to 2MeSATP on PKC activation was no longer apparent. Another desensitization mechanism, which was PKC independent and which persisted through the 5-min preincubation and the 2-min wash, then operated.

One observation to emerge is that whether a desensitization is seen to be PKC dependent depends on the experimental protocol used. Many published studies on desensitization use long pre-exposures (1–2 hr is not unusual). Here we have shown two desensitization mechanisms operating in the first few minutes, with one (PKC dependent) fading out as another (PKC independent) persists over a period of minutes. Clearly, the studies with long pre-exposures would fail to see the transient PKC component.

In contrast, UTP stimulation was not affected by PMA or Ro 31–8220. This suggests that the nucleotide receptor has no

[3] G. F. Wilkinson and M. R. Boarder, unpublished observations.

short PKC feedback loop. It was unexpected, therefore, that nucleotide homologous desensitization was inhibited by Ro 31–8220. There are two possible explanations. Firstly, the effect of the drug may have been due to actions other than PKC inhibition. This possibility cannot be excluded, although this drug is relatively selective for PKC, compared with cAMP- and Ca^{2+}/calmodulin-dependent protein kinases (25), and the concentration-response curve for inhibition of homologous desensitization lies in the same range (1–10 μM)[4] as that seen for inhibition of other PKC-dependent effects. Secondly, PKC activation may be necessary for expression of homologous desensitization but alone is not sufficient to attenuate the response. This explanation assumes that the 5-min preincubation with UTP sets in motion events leading to a persistent desensitization that requires not only PKC activation but also other changes occurring upon activation of the nucleotide receptor. This desensitizing mechanism must have a slow onset; otherwise, we would see evidence of a PKC-dependent short feedback loop with stimulation by UTP.

With respect to the heterologous desensitization, 5-min preincubation with 2MeSATP was insufficient to attenuate the subsequent response to UTP, but UTP preincubation was adequate to attenuate the subsequent response to 2MeSATP; this latter desensitization was dependent on PKC, as judged by the effect of Ro 31–8220. Nucleotide receptor activation therefore is able to desensitize both nucleotide and P_{2Y} receptors by a mechanism that is apparently dependent on PKC. We can therefore propose that there is a form of desensitization that is activated by nucleotide receptors but not P_{2Y} receptors, is dependent on PKC but requires the recruitment of other components, and has a slow onset but a persistent effect on both nucleotide and P_{2Y} receptors.

The further resolution of the events leading to desensitization will probably require the study of isoforms of PKC (e.g., PMA and Ca^{2+} sensitive and insensitive) involved in the receptor-stimulated responses. At present, obtaining such data is not possible.

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