Biochem. J. (1990) 272, 217–221 (Printed in Great Britain)

Homologous desensitization of ATP-mediated elevations in cytoplasmic calcium and prostacyclin release in human endothelial cells does not involve protein kinase C

Thomas D. CARTER,*‡ Joshua S. NEWTON,†§ Ron JACOB†‖ and Jeremy D. PEARSON*¶
*Section of Vascular Biology, MRC Clinical Research Centre, Harrow, Middx. HA1 3UJ, U.K.,
and †Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts. AL6 9AR, U.K.

Single human umbilical-vein endothelial cells in culture loaded with the Ca^{2+}-sensitive dye fura-2 exhibited characteristic increases in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) in response to extracellular ATP. The rapid decline of $[Ca^{2+}]_i$ to pre-stimulated levels in the continued presence of ATP, with in most cells no sustained or oscillatory increase in $[Ca^{2+}]_i$, indicated desensitization. This was agonist-specific, and contrasted with the $[Ca^{2+}]_i$ response to histamine, though each agonist mobilized Ca^{2+} from the same internal store. In populations of cells, when desensitization was variably induced by a second challenge with ATP after different times, desensitization of the initial peak $[Ca^{2+}]_i$ was directly related to desensitization of prostacyclin release. This was not affected by treatment with the protein kinase C inhibitor staurosporine, under conditions where a similar degree of desensitization of peak $[Ca^{2+}]_i$ induced by phorbol 12-myristate 13-acetate was blocked. Sequential addition of ATP to cell populations cumulatively desensitized the peak elevation of $[Ca^{2+}]_i$, but did not block the second, sustained, phase of the response. We conclude that desensitization of prostacyclin synthesis by ATP is likely to be due to uncoupling of the P_{2Y} purinoceptor from phosphoinositidase C, but does not involve protein kinase C activation.

INTRODUCTION

ATP acting at P_{2Y} purinoceptors potently stimulates release of prostacyclin (PGI_2) and endothelium-derived relaxing factor (EDRF) from endothelial cells [1]. PGI_2 release is rapid in onset but transient, lasting only a few minutes even in the continued presence of ATP, and shows marked desensitization on subsequent challenge with ATP, but not with unrelated agonists [2]. In contrast, EDRF release can be maintained for many minutes in the continued presence of ATP [3].

We have previously demonstrated that ATP-stimulated PGI_2 release from human umbilical-vein endothelial cells is triggered by elevations of $[Ca^{2+}]_i$ [4], and that ATP-induced elevations of $[Ca^{2+}]_i$ in pig aortic endothelial cells show marked homologous desensitization [5]. Thus the tachyphylaxis of ATP-induced PGI_2 release may result from an inability to raise $[Ca^{2+}]_i$ after prior exposure of the cells to ATP, though the mechanisms that regulate this response are unknown.

Although protein kinase C (PKC) does not appear to play any role in triggering PGI_2 release induced by ATP in human umbilical-vein endothelial cells [6], activation of this enzyme by tumour-promoting phorbol esters or by synthetic diacylglycerols has been shown to inhibit agonist-stimulated phosphoinositide hydrolysis and $[Ca^{2+}]_i$ mobilization in endothelial cells [6–8]. This has led to the suggestion that desensitization of agonist-induced PGI_2 release may involve feedback inhibition by PKC [7,8]. Two lines of evidence, however, argue against this. First, although activators of PKC decrease agonist-induced elevations of $[Ca^{2+}]_i$ in human umbilical-vein endothelial cells, we have shown that they actually enhance the release of PGI_2 in response to these agonists [6]. Second, phorbol-ester-induced desensitization of agonist-stimulated phosphoinositide hydrolysis is reversed by the PKC inhibitor H-7, whereas homologous desensitization is not [9].

We have now studied the desensitization of ATP-induced elevations of $[Ca^{2+}]_i$ in single cells, which is important, since qualitatively different responses from those observed in populations of cells can occur [10]. These differences arise because measurements in cell populations represent an averaged result from the summation of individual responses that are heterogeneous in time and extent. We then carried out experiments with populations of cells where $[Ca^{2+}]_i$ and PGI_2 release were measured concomitantly, to investigate whether the relationship between $[Ca^{2+}]_i$ and PGI_2 synthesis is maintained during homologous desensitization and to determine whether PKC activation is involved.

METHODS

Human umbilical-vein endothelial cells were isolated and cultured as previously described [11]. Cells from confluent primary cultures were detached by brief trypsin treatment, and seeded on to coverslips. For single-cell experiments, cells were seeded on to 24 mm-diameter no. 1 coverslips and used 3–4 days later at sub-confluent density. For population experiments, cells were seeded at near confluent density on 22 mm × 11 mm coverslips and used 1–2 days later. Cells were loaded with fura-2 as previously described [4,12].

$[Ca^{2+}]_i$ was measured in single cells by using an inverted microscope (Zeiss Axiomat) equipped with a glycerol-immersion ×40 Nikon fluorite objective. The cells were maintained at

Abbreviations used: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; PGI_2, prostacyclin; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; EDRF, endothelium-derived relaxing factor.

‡ Present address: Division of Neurophysiology and Neuropharmacology, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.
§ Present address: Division of Biomedical Science, King's College, Strand, London WC2R 2LS, U.K.
‖ Present address: Division of Biomedical Science, King's College, Kensington, London W8 7AH, U.K.
¶ To whom correspondence should be addressed.

37 °C, superfused at 0.3 ml/min with Hepes-buffered physiological saline (145 mM-NaCl, 5 mM-KCl, 1 mM-$MgCl_2$, 1 mM-$CaCl_2$, 10 mM-glucose, 10 mM-Hepes, pH 7.4) containing 0.1 % BSA, and fluorescence in response to epi-illumination was detected as previously described [12].

$[Ca^{2+}]_i$ and PGI_2 release in populations of cells on coverslips in a fluorimeter cuvette were determined exactly as previously described [4,11]. Desensitization of the ATP response was achieved in these experiments by pretreating the cells with 10 μM-ATP for 5 min at 37 °C before transferring the coverslip to a cuvette with fresh buffer containing no ATP. Cells were re-challenged with ATP 2–20 min later.

Phorbol esters and histamine were from Sigma; ATP and staurosporine were from Calbiochem.

RESULTS

ATP-stimulated elevations of $[Ca^{2+}]_i$ in single cells

Single endothelial cells responded to extracellular application of 1–500 μM-ATP (in buffer containing 1 mM extracellular Ca^{2+}) with a rapid elevation of $[Ca^{2+}]_i$, from resting levels of ~ 100 nM to 1–2 μM (17 cells). In most of the cells studied (12/17), this initial peak then declined back to resting levels within ~ 90 s (Fig. 1*a*), but in a smaller proportion of the cells either the decline in $[Ca^{2+}]_i$ was protracted (Fig. 1*b*), or complex oscillations in $[Ca^{2+}]_i$ were seen (Fig. 1*c*). The peak $[Ca^{2+}]_i$, the decay rate, and the occurrence or frequency of oscillations were not obviously related to the concentration of ATP applied.

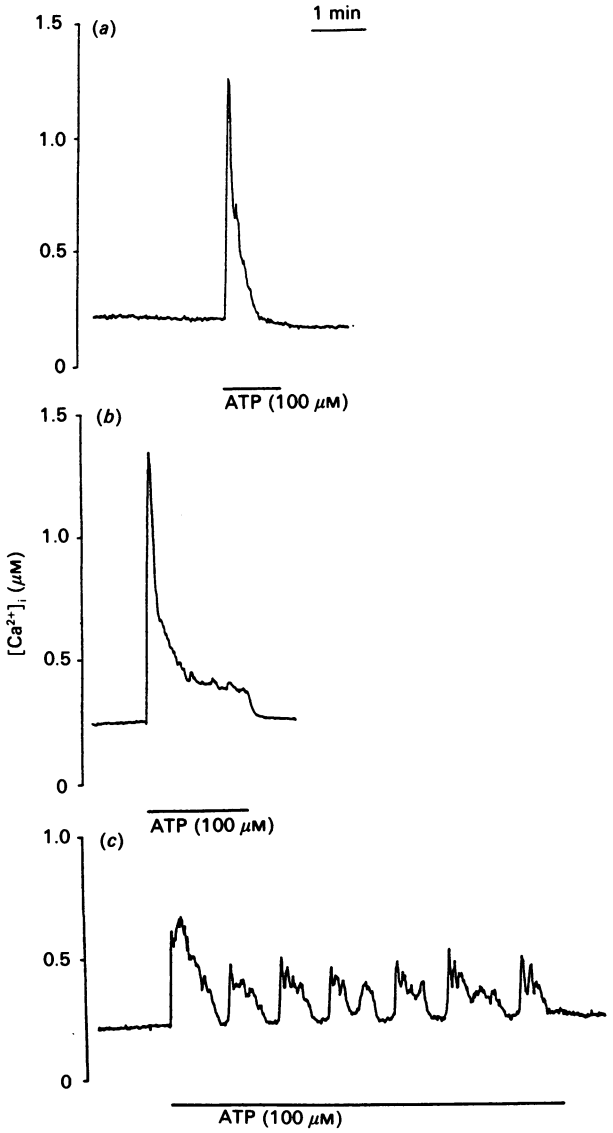

Fig. 1. Representative recordings of ATP-induced changes in $[Ca^{2+}]_i$ from single human umbilical-vein endothelial cells in culture

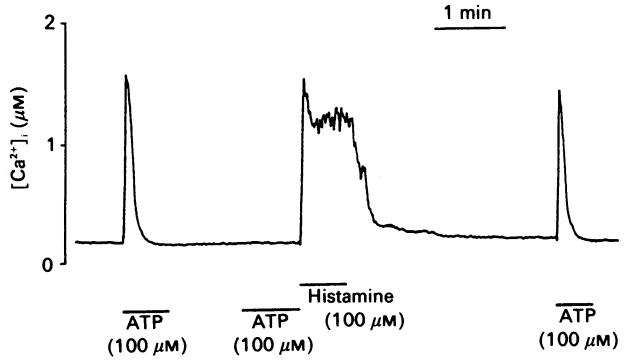

Fig. 2. Recording of $[Ca^{2+}]_i$ from a single human umbilical-vein endothelial cell in culture demonstrating homologous desensitization of the response to ATP

This experiment was repeated four times with equivalent results.

Fig. 2 shows an example of a cell in which the $[Ca^{2+}]_i$ response to a short exposure (< 1 min) to 100 μM-ATP was rapid and transient. Re-exposure of the same cell to ATP 2 min after the first dose resulted in no $[Ca^{2+}]_i$ response, although exposure to 100 μM-histamine immediately after this resulted in a typical $[Ca^{2+}]_i$ response [10]. An interval of 5 min between successive challenges with ATP was sufficient to allow recovery of the response to ATP in this cell (Fig. 2).

The kinetics of the $[Ca^{2+}]_i$ elevations induced by ATP and by histamine were significantly different; in particular, as we have previously shown [10], the response to histamine was maintained in the continued presence of the agonist when extracellular Ca^{2+} was present. Differences in the kinetics of the ATP and histamine responses could reflect the utilization of distinct pools of intracellular Ca^{2+}. To test this, cells were exposed to 100 μM-histamine in the absence of extracellular Ca^{2+}. After $[Ca^{2+}]_i$ had returned to resting levels, reflecting the complete discharge of Ca^{2+} from histamine-sensitive stores, ATP was unable to elevate $[Ca^{2+}]_i$ (Fig. 3).

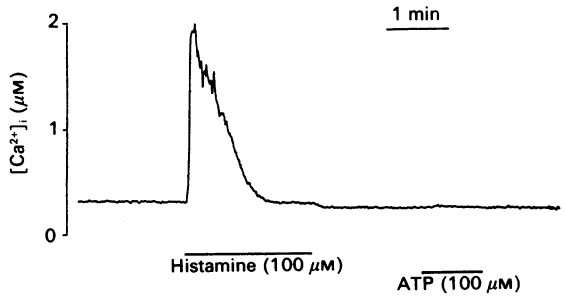

Fig. 3. Changes in $[Ca^{2+}]_i$ recorded from a single human umbilical-vein endothelial cell in culture, demonstrating that histamine and ATP evoke Ca^{2+} release from the same internal pool

This experiment was repeated four times with the same result.

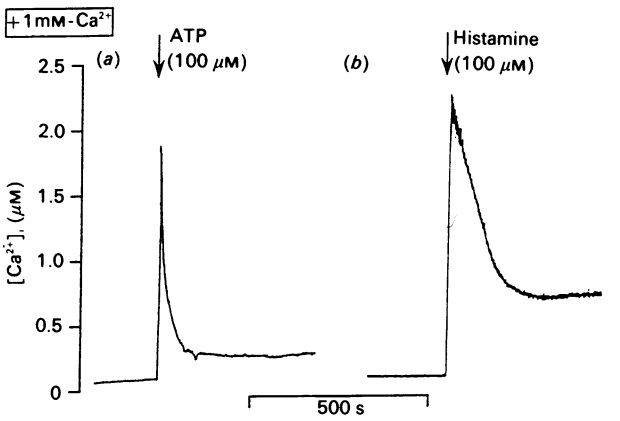

Fig. 4. Representative records of the changes in $[Ca^{2+}]_i$ measured in populations of human umbilical-vein endothelial cells in culture in response to ATP (100 μM) or histamine (100 μM)

This experiment has been repeated more than 30 times with the same results.

Table 1. Peak $[Ca^{2+}]_i$ responses in human umbilical-vein endothelial cells pretreated with phorbol ester or ATP in the absence or presence of staurosporine (90 nM) and then re-challenged with ATP (10 μM)

Values are means ± S.E.M. ($n = 4$). Cells were exposed for 7 min to vehicle alone (0.1 % dimethyl sulphoxide) or PMA, or for 5 min to ATP plus 2 min wash, before re-challenging with ATP. Staurosporine was added 2 min before other treatments.

	$[Ca^{2+}]_i$ (nM)	
Pretreatment	Control	+Staurosporine
Vehicle alone	980±25	940±20
PMA (10 nM)	475±70	960±50
ATP (10 μM)	360±35	310±40

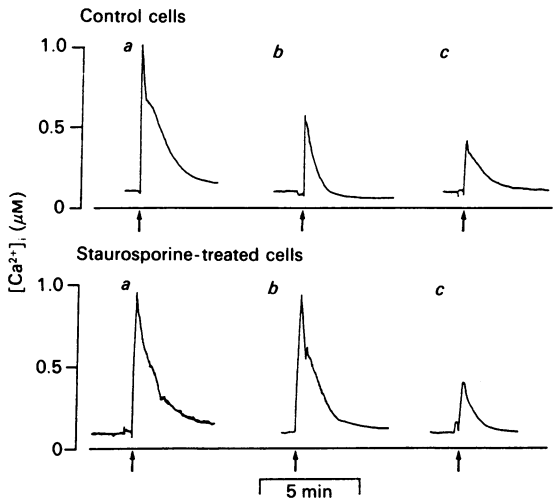

Fig. 5. Records of the changes in $[Ca^{2+}]_i$ measured in populations of human umbilical-vein endothelial cells in culture in response to ATP (10 μM)

Cells were pretreated for 7 min with vehicle (0.1 % dimethyl sulphoxide) alone (*a*), for 7 min with 10 nM-PMA (*b*), or for 5 min with 10 μM-ATP, followed by a 2 min wash (*c*), and then challenged with 10 μM-ATP (arrows). The upper records are from control cells and the lower records are from cells in the presence of 90 nM-staurosporine, which was added 2 min before the other treatments.

ATP-stimulated elevations of $[Ca^{2+}]_i$ measured in populations of endothelial cells

Fig. 4 gives examples of the $[Ca^{2+}]_i$ responses to ATP (100 μM) and, for comparison, to histamine (100 μM) that were consistently observed in cell populations. Each agonist yielded a qualitatively similar response, consisting of a rapid initial transient followed by a maintained plateau, but exposure to ATP produced a faster decay of the initial peak and a lower plateau level of $[Ca^{2+}]_i$.

To investigate the possible role of PKC in the homologous desensitization of the $[Ca^{2+}]_i$ response to repeated challenge with ATP, we designed experiments in which a similar degree of desensitization was induced by brief pretreatment with phorbol 12-myristate 13-acetate (PMA). The results are summarized in Table 1, with examples of the responses shown in Fig. 5. When cells were exposed to 10 μM-ATP either after 7 min exposure to 10 nM-PMA, or at 2 min after previous exposure to 10 μM-ATP for 5 min, the initial $[Ca^{2+}]_i$ peaks were similarly decreased (Table 1 and traces *b* and *c* respectively, in Fig. 5, upper panel). The desensitization induced by ATP was agonist-specific: previous exposure to 10 μM-histamine had no effect on the subsequent response to ATP, or vice versa (results not shown). The PKC-inactive phorbol ester 4α-phorbol didecanoate (10 nM) was without effect (results not shown).

Cells were then exposed to increasing concentrations (15–90 nM) of the PKC inhibitor staurosporine [13] for 2 min before adding PMA. The lowest dose that completely blocked the effect of PMA on a subsequent ATP response was 90 nM (Fig. 5, trace *b*, lower panel). In contrast, similar pretreatment with staurosporine had no effect on homologous desensitization induced by ATP (Table 1, and Fig. 5, trace *c*, lower panel).

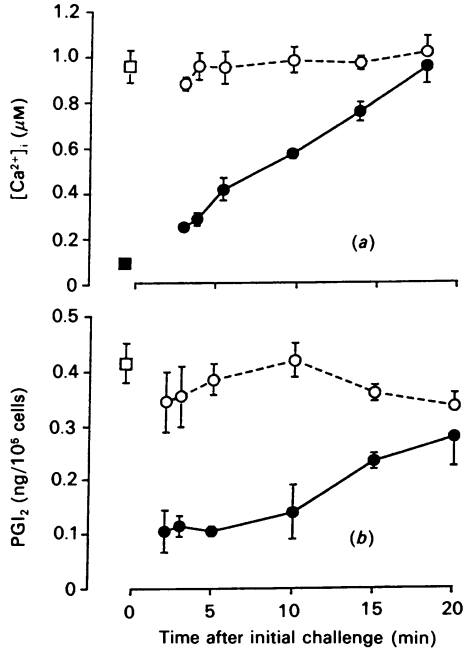

Fig. 6. Time course of recovery of endothelial-cell responsiveness to ATP

Cells were pretreated with 10 μM-ATP for 5 min, rinsed and then exposed again to 10 μM-ATP after time intervals of 2–20 min. Panel (*a*) shows the peak $[Ca^{2+}]_i$ in response to the second exposure to ATP; panel (*b*) shows the associated PGI_2 release from the same cells 5 min after the second challenge. ●, Test cells; ○, cells manipulated in the same way as test cells, but not pretreated with ATP; □, cells challenged with ATP without any pretreatment; ■, resting $[Ca^{2+}]_i$. Points are means; error bars are S.E.M. of 3 observations, or 18 observations for resting $[Ca^{2+}]_i$.

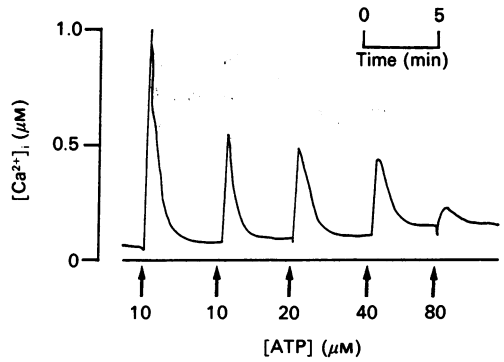

Fig. 7. Continuous record of $[Ca^{2+}]_i$ from a population of endothelial cells exposed to sequential additions of ATP without rinsing

This experiment was repeated four times with the same result.

Neither resting $[Ca^{2+}]_i$ nor the response to ATP was affected by staurosporine (Fig. 5, trace *a*, lower panel).

Kinetics of recovery from homologous desensitization

Cells were pre-exposed to 10 μM-ATP, and the interval between the first and second exposures to ATP was varied from 2 to 20 min. Sub-samples of buffer were taken from the fluorimeter cuvette immediately before and 5 min after the second exposure to ATP, for assay for PGI_2. Fig. 6 demonstrates that the peak elevation of $[Ca^{2+}]_i$, markedly decreased when the second challenge with ATP was 2 min after the first, recovered in a linear fashion with time, such that a full response was seen ~ 20 min after the initial exposure to ATP. PGI_2 release was similarly markedly inhibited, but recovered, after an initial lag period of ~ 10 min, to reach control levels by ~ 20 min (Fig. 6).

Effect of cumulative additions of ATP on $[Ca^{2+}]_i$ measured in populations of cells

When endothelial-cell $[Ca^{2+}]_i$ was monitored continuously, during sequential additions of increasing concentrations of ATP, the peak $[Ca^{2+}]_i$ achieved was progressively further desensitized, but in contrast the sustained elevated phase of the response increased (Fig. 7).

DISCUSSION

Responses of single endothelial cells to ATP

In most of the cells studied, the $[Ca^{2+}]_i$ response to ATP consisted of a single peak that returned to pre-stimulated levels within ~ 90 s in the continued presence of the agonist. In only a small proportion of cells was either a protracted decay in $[Ca^{2+}]_i$ or oscillatory changes of $[Ca^{2+}]_i$ observed (Fig. 1). Agonist-induced $[Ca^{2+}]_i$ oscillations are now known to be a widespread phenomenon in non-excitable cells [14]. We previously showed that, in striking contrast with the responses to ATP reported here, individual human umbilical-vein endothelial cells reproducibly respond with persistent $[Ca^{2+}]_i$ oscillations, whose frequency is dose-related, when exposed to histamine [10]. Again in contrast with their response to histamine, these cells are temporarily desensitized in a homologous manner when exposed to repeated challenges with ATP (Fig. 2). Another indication of rapid desensitization in the response to ATP is the prompt return of $[Ca^{2+}]_i$ to pre-stimulated levels in the continued presence of the agonist. This occurs more rapidly than the return to basal values during histamine stimulation in Ca^{2+}-free medium (cf. Figs. 1 and 3), which reflects the time taken for the cell to pump out Ca^{2+}. The more rapid return to a basal value in the presence of ATP indicates that exposure to ATP releases less of the stored Ca^{2+} than does exposure to histamine, presumably because of desensitization. Any alternative explanation based on the idea that ATP utilizes a separate intracellular store of Ca^{2+}_i was ruled out by the results of the experiment shown in Fig. 3, though it is likely that ATP cannot maximally mobilize Ca^{2+}_i to the same extent as with histamine.

Responses of endothelial-cell populations to ATP

The $[Ca^{2+}]_i$ response to ATP measured in populations of cells differs from that seen in single cells. The initial event is a rapid, transient, elevation of $[Ca^{2+}]_i$, which then declines within 2–3 min to a sustained elevated plateau that is maintained for many minutes, and each phase of the response is similarly dose-related to the concentration of ATP applied (Fig. 4 and ref. [4]). The magnitude of the sustained phase of the response (maximally 0.2–0.3 μM: ref. [4]) is lower than that seen in single cells exhibiting a slow decay of $[Ca^{2+}]_i$ (Fig. 1). Thus the response observed in populations of cells reflects a summation of individual heterogeneous responses, in which a minority of cells do, but most do not, exhibit some kind of sustained $[Ca^{2+}]_i$ response (either maintained or oscillatory).

Homologous desensitization

In endothelial cells, as in some other cell types, activators of PKC attenuate phosphoinositide turnover and mobilization of Ca^{2+}_i stimulated by agonists, guanosine 5′-[γ-thio]triphosphate or fluoride; this has led to the suggestion that homologous desensitization of agonist-evoked PGI_2 release involves a feedback mechanism dependent on PKC [7,8,15,16]. However, we have shown previously that exogenous activation of PKC enhances PGI_2 release in response to a variety of agonists, including ATP, despite a decrease in the magnitude of the changes in $[Ca^{2+}]_i$ [6]. It has also recently been found that desensitization of endothelial phosphoinositide metabolism induced by pretreatment with PMA, but not desensitization induced by prior exposure to an agonist (thrombin or histamine), was reversed by the PKC inhibitor H-7 [9]. It is not clear why Haldorsson & Thorgeirsson [9] noted desensitization in response to histamine: we have shown that histamine-induced elevations of $[Ca^{2+}]_i$ do not desensitize rapidly in human endothelial cells [10], and the results reported by Pollock *et al.* [17] suggest that this is also the case for histamine-stimulated phosphoinositide turnover.

Having established that prior exposure to ATP resulted in a marked decrease in subsequent ATP-stimulated increases in $[Ca^{2+}]_i$, we pretreated endothelial cells with PMA under conditions that produced a similar extent of desensitization of the $[Ca^{2+}]_i$ response to ATP. Under these conditions the PKC inhibitor staurosporine was able to block completely the effect of PMA, but did not alter the extent of homologous desensitization induced by ATP (Table 1 and Fig. 5). The selectivity of this effect, coupled with the lack of effect of staurosporine alone on the response to ATP, provides strong evidence that PKC is not involved in regulating homologous desensitization of ATP-stimulated elevations of $[Ca^{2+}]_i$ in endothelial cells. Similar observations have been made by using different agonists in other cell types [18,19].

To determine directly whether the inability of ATP to mobilize Ca^{2+}_i in desensitized cells is functionally related to the decrease in PGI_2 release, we measured $[Ca^{2+}]_i$ and PGI_2 release concomitantly in cells desensitized to different extents by varying the time between the first and second exposures to ATP. The amount of PGI_2 release by these cells in response to a single challenge with ATP is determined predominantly or exclusively by the extent to which $[Ca^{2+}]_i$ is elevated above a threshold of

$\sim 0.8\ \mu M$ [4]. Fig. 6 shows that the $[Ca^{2+}]_i$ response recovered linearly to reach control levels 20 min after the initial exposure to ATP. This interval is considerably longer than that observed in some single cells (Fig. 1), and presumably reflects the time required for all the cells in the population to resensitize. PGI_2 release also returned to control values by 20 min, but there was a lag of ~ 10 min before any recovery was seen (Fig. 6), which corresponds to the time during which ATP was unable to elevate $[Ca^{2+}]_i$ above the activation threshold for PGI_2 release. These results provide the first direct evidence that desensitization of ATP-induced PGI_2 release results from an inability to raise $[Ca^{2+}]_i$ sufficiently to trigger PGI_2 release, and also show that PKC activation is not involved.

The lack of any quantitative perturbation in the relationship between $[Ca^{2+}]_i$ and PGI_2 release in homologously desensitized cells, in contrast with the effects of PMA [6], makes it doubtful that PKC activation occurs to a significant degree when these cells are stimulated with ATP. A recent study has shown that in 3T6 mouse fibroblasts ATP induces phosphoinositide turnover and Ca^{2+}_i mobilization without activating PKC [20], though similar experiments have not yet been carried out with endothelial cells. Demolle *et al.* [21] found that ionophore A23187, but not PMA, produced a similar pattern of protein phosphorylation in bovine aortic endothelial cells to that produced by ATP, suggesting that ATP-stimulated phosphorylation events are mediated via a Ca^{2+}-dependent kinase distinct from PKC. Whether one or more of these phosphorylations are involved in the mechanism of desensitization remains to be determined.

Sequential addition of increasing concentrations of ATP, with a first dose of $10\ \mu M$, gave an initial elevation of $[Ca^{2+}]_i$ to $\sim 1.0\ \mu M$. The second and subsequent additions gave progressively lower initial peaks that were well below the activation threshold for PGI_2 release in these cells, consistent with the inability to generate cumulative dose–response curves for the release of this mediator. At the same time, the sustained elevated phase of the response increased as more ATP was added (Fig. 7). EDRF release from endothelial cells is well sustained in response to ATP [3], and cumulative dose–response curves for ATP-induced endothelium-dependent vasodilatation can be generated [22]. EDRF generation, like the second phase of the $[Ca^{2+}]_i$ response, requires extracellular Ca^{2+} and is blocked by PMA [23,24]. These data strongly suggest that the sustained phase of the response is related to EDRF production, and that this requires lower elevations of $[Ca^{2+}]_i$ than are necessary for PGI_2 synthesis.

The lack of progressive desensitization of the sustained phase of the response when ATP was added sequentially may be because it is not directly mediated by receptor activation. Hallam *et al.* [25] first showed that in populations of human umbilical-vein endothelial cells bivalent-cation influx occurs in the absence of continued receptor occupation if the internal store of Ca^{2+} is not refilled. This idea is substantiated by studies on single human umbilical-vein endothelial cells, which show that the degree to which the internal store is depleted of Ca^{2+} regulates the rate of bivalent-cation entry [12]. The results in Fig. 7 are consistent with this model. Thus, if sequential additions of ATP result in further discharge of the internal store, the cells compensate by increasing Ca^{2+} influx. Further comparative experiments with single cells and cell populations are needed to resolve this point.

We conclude that homologous desensitization of ATP-stimulated PGI_2 release is due to a similar desensitization of the ability of ATP to induce discharge of Ca^{2+} from internal stores. Although this process is likely to be due to uncoupling of the P_{2Y} purinoceptor from phospholipase C, perhaps by action on a G-protein, it does not involve activation of PKC. In contrast with the homologous desensitization of Ca^{2+} mobilization induced by ATP, Ca^{2+} entry is not blocked, which may be relevant to the ability of ATP to induce more prolonged release of EDRF.

T.D.C. held an MRC/Smith, Kline & French Partnership research studentship. J.S.N. holds an SERC/S,K & F CASE award. We thank the Delivery Suite, Northwick Park Hospital, for their continued expert help in the collection of umbilical cords.

REFERENCES

1. Pearson, J. D. & Gordon, J. L. (1989) Biochem. Pharmacol. **38**, 4157–4163
2. Toothill, V. J., Needham, L., Gordon, J. L. & Pearson, J. D. (1988) Eur. J. Pharmacol. **157**, 189–196
3. Kelm, M., Feelisch, M., Spahr, R., Piper, H., Noake, E. & Schrader, J. (1989) Biochem. Biophys. Res. Commun. **154**, 236–244
4. Carter, T. J., Hallam, T. J., Cusack, N. J. & Pearson, J. D. (1988) Br. J. Pharmacol. **95**, 1181–1190
5. Hallam, T. J. & Pearson, J. D. (1986) FEBS Lett. **207**, 95–99
6. Carter, T. D., Hallam, T. J. & Pearson, J. D. (1989) Biochem. J. **262**, 431–437
7. Holdorsson, H., Kjeld, M. & Thorgeirsson, G. (1988) Arteriosclerosis **8**, 147–154
8. Brock, T. A. & Capasso, E. A. (1988) J. Cell. Physiol. **136**, 54–62
9. Haldorsson, H. & Thorgeirsson, G. (1989) Biochem. Biophys. Res. Commun. **161**, 1064–1069
10. Jacob, R., Merritt, J. E., Hallam, T. J. & Rink, T. J. (1988) Nature (London) **335**, 40–45
11. Hallam, T. J., Pearson, J. D. & Needham, L. (1988) Biochem. J. **251**, 243–249
12. Jacob, R. (1990) J. Physiol. (London) **421**, 55–77
13. Tamaoki, T., Nomoto, H., Takahashi, I., Kata, Y., Morimoto, M. & Tomita, F. (1986) Biochem. Biophys. Res. Commun. **135**, 397–402
14. Jacob, R. (1990) Biochim. Biophys. Acta **1052**, 427–438
15. Luckhoff, A., Zeh, R. & Busse, R. (1988) Pflugers Arch. **417**, 654–658
16. Vojno-Yasentskaya, T. A., Tkachuk, V. A., Cheknyova, E. G., Panchenko, M. B., Grigorian, G. Y., Vavrek, R. J., Stewart, J. M. & Ryan, U. S. (1989) FASEB J. **3**, 44–51
17. Pollock, W. K., Wreggett, K. A. & Irvine, R. F. (1989) Biochem. J. **256**, 371–376
18. Sugiya, H. & Putney, J. W. R. (1988) Biochem. J. **256**, 677–680
19. Crouch, M. F. & Lapetina, E. G. (1989) J. Biol. Chem. **264**, 584–588
20. Gonsalez, F. A., Rozengurt, E. & Heppel, L. A. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 4530–4534
21. Demolle, D., Lecomte, M. & Boeynaems, J.-M. (1988) J. Biol. Chem. **263**, 18459–18465
22. Martin, W., Cusack, N. J., Carleton, J. S. & Gordon, J. L. (1985) Eur. J. Pharmacol. **108**, 295–299
23. Long, C. J. & Stone, T. W. (1985) Blood Vessels **22**, 205–208
24. Lewis, M. J. & Henderson, A. H. (1987) Eur. J. Pharmacol. **137**, 167–171
25. Hallam, T. J., Jacob, R. & Merritt, J. E. (1988) Biochem. J. **255**, 179–184

Received 2 April 1990/18 June 1990; accepted 7 August 1990