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DIFFERENT CONCENTRATIONS OF PERTUSSIS TOXIN HAVE OPPOSITE EFFECTS ON AGONIST-INDUCED PGE_2 FORMATION IN MESANGIAL CELLS

Detlef Schlondorff, Joseph A. Satriano, and Susan DeCandido

Department of Medicine,
Albert Einstein College of Medicine, Bronx, NY 10461

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Pertussis toxin may inactivate N proteins linked to phospholipase C. We examined the effect of pretreatment with pertussis toxin at different concentrations and times on agonist-induced PGE_2 synthesis in mesangial cells. Two to four hours with 10-50 ng/ml of pertussis toxin inhibited the response to angiotensin and platelet activating factor, but with a different sensitivity. This was associated with decreased [^{14}C] arachidonic acid release in prelabeled cells. The response to A23187 was unaltered. At high concentrations (1 to 5 ug/ml) pertussis toxin increased basal PGE_2 and the response to all agonists. Pertussis toxin pretreatment resulted in a dose-dependent ribosylation of a 40 kDa protein band. Thus, responses to different agonists have different sensitivity to pertussis toxin inhibition, which at high concentrations may even have opposite effects.

Recent evidence suggests that GTP binding proteins - N proteins - may also be involved in receptor-effector interactions that require phospholipase C activation for phosphatidylinositides as an initial step (1-2). This N protein(s) may be similar to the inhibitory subunit - N_i - of the adenylate cyclase complex, in that it can be inactivated by pertussis toxin via ADP-ribosylation (2). Nonetheless, this N protein appears distinct from N_i and may even comprise several types of N proteins (3).

Pretreatment with PT inhibits the effect of angiotensin II and platelet activating factor on release of arachidonic acid in 3T3 fibroblasts (4). In contrast in e.g. pituitary cells (5) and hepatocytes (6), AII effects on phospholipase C and physiological responses were not blocked by pretreatment with PT, in spite of 100% ADP-ribosylation by PT treatment. Thus in different cells some AII effects involve either no N protein or different N proteins not

ABBREVIATIONS: PT: pertussis toxin; PAF: platelet activating factor, alkyl-2-acetyl-sn-glycero-3-phosphocholine.

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all of which are inactivated by PT. AII and PAF stimulate PGE_2 synthesis by cultured mesangial cells via activation of phospholipase C and A_2 (7,8). Stimulation by A23187 requires only phospholipase A_2 activation (7). Recently, it has been reported that the AII-induced stimulation of phospholipase C and PGE_2 formation is inhibited by PT pretreatment in mesangial cells (9). We now examined the influence of different pretreatment protocols with PT on subsequent responses of mesangial cells to AII, PAF and A23187. While our results indicate that at certain concentrations PT pretreatment can indeed inhibit stimulation by angiotensin II and PAF, but not by A23187, the sensitivity of the PAF and angiotensin response to PT pretreatment differs. Furthermore, pretreatment with high concentrations (ug/ml) of PT resulted in unexpected stimulation of both basal PGE_2 synthesis and that in response to all three agonists.

MATERIALS AND METHODS

Rat mesangial cells were cultured as described (7,8). For each experimental series, aliquots of cells from 2nd to 3rd subculture were used. Mesangial cells in culture dishes were preincubated with PT in Hepes-buffered RPMI 1640 culture medium without fetal calf serum. PT, prepared as, and of the purty, as described by Tamura, et al (10) was from List Biological Laboratories, Campbell, CA). After medium removal the cells were washed twice, and incubated at 37°C with 1-2 ml of Krebs-Ringer's buffer and 2 mg/ml fatty acid-free BSA $\pm$ experimental agents. A basal was followed by a 15 min experimental period and PGE_2 in the buffer was determined by RIA as described (7,8).

Prelabeling of mesangial cells with [^{14}C] arachidonic acid was performed overnight as described (7). After prelabeling and washing the cells twice, 2 ml of RPMI medium without fetal calf serum and $\pm$ 50 ng/ml of PT were added to each well, and the plates were incubated for 2 hrs at 37°C. The cells were again washed, followed by addition of 3 ml fresh K.R. buffer $\pm$ experimental agents (as above). At the times indicated aliquots were removed for determination of radioactivity.

We verified that pretreatment of cells with PT did result in ADP ribosylation of N protein using the method of Nakamura and Ui (11). After preincubated $\pm$ PT and removal of medium, cells were homogenized in 1 ml of ice-cold homogenization buffer (25 mM tris HCl pH 7.4, 1 mM dithiothreitol, 1 m M EDTA containing 100 K units/ml of aprotenin). A 12,000 xg pellet fraction was incubated for 20 min at 30°C with 50 ul of buffer containing 100 mM potassium phosphate pH 7.4, 5 mM $MgCl_2$, 10 mM DTT, 20 mM thymidine, 2 mM ATP, 2 mM EDTA and 2 uM [α^{32}P] NAD (~1.5 million CPM/assay) and 50 ul of DTT-preactivated PT (15 ug/assay). Reaction products were dissolved in 0.1 ml of 1% SDS by boiling for 3 min and submitted to SDS-PAGE and autoradiography.

RESULTS AND DISCUSSION

Pretreatment with 10 to 50 ng/ml of PT for 1 to 4 hrs had no significant effect on basal PGE_2 production, but significantly blunted the response of AII

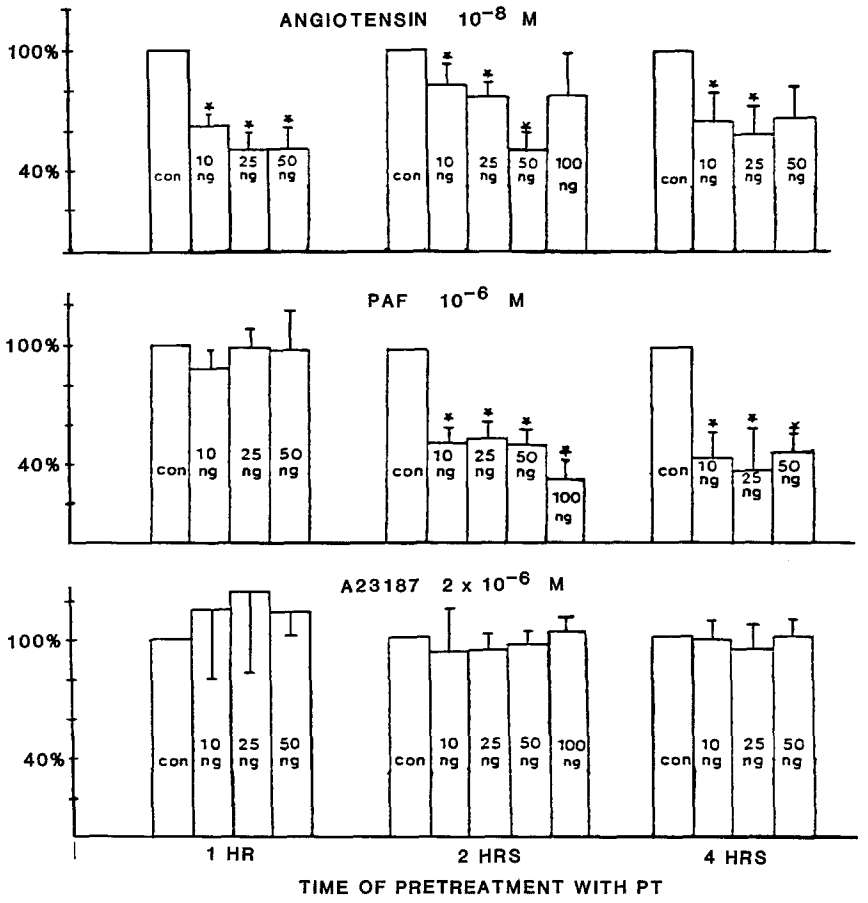

Fig. 1. Effects of pretreatment with pertussis toxin for various times and concentrations (given inside bars) on the subsequent stimulation of PGE_2 synthesis to angiotensin II, platelet activating factor and A23187. Results are means + SEM from 4 to 8 experiments for each concentration and time point and are expressed as percent of their respective controls, i.e. stimulation without PT pretreatment. Basal PGE_2 production was 100-300 pg/15 min and increased to 1000-2000 pg with AII, to 1500-3000 pg with PAF and 4000-8000 pg with A23187. Asterisks indicate $P < 0.05$ or better as compared to the respective controls.

(Fig. 1). Preincubation for 2 hrs with 100 ng/ml of PT or for 4 hrs with 50 ng/ml resulted, however, in an inconsistent degree of inhibition of the AII response.

The PGE_2 response to PAF showed a different sensitivity to PT pretreatment (Fig. 1). A 1 hr preincubation with PT had no effect, but after 2 and 4 hrs PT markedly inhibited the response to PAF. None of these PT-pretreatments did, however, influence the stimulation of PGE_2 by A23187. To test whether the effect of PT was related to inhibition of phospholipase, we examined cells

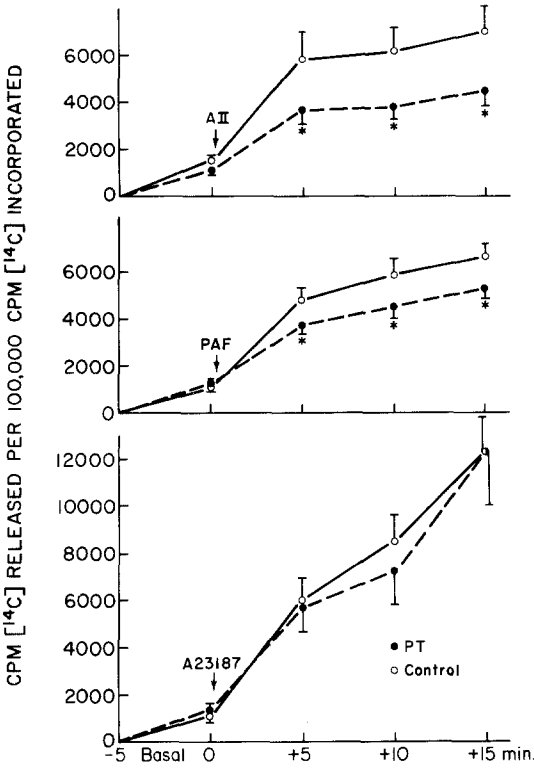

Fig. 2. Effect of 2 hour pretreatment with 50 ng/ml of pertussis toxin on release of [^{14}C] from cells prelabeled with [^{14}C] arachidonic acid. After determination of basal release the incubation buffer was changed to fresh buffer containing the experimental agents. [^{14}C] release was then determined for three consecutive 5 min periods in aliquots of incubation buffer. Results are means ± SEM of 5 sets of experiments for each agent. Asterisks indicate $P \leq 0.05$ or better as compared to respective controls.

prelabeled with [^{14}C] arachidonic acid. Pretreatment with 50 ng/ml of PT for 2 hrs did not influence basal or A23187-stimulated [^{14}C] release (Fig. 2), consistent with the results on PGE_2 synthesis under these conditions. Thus A23187 enhances phospholipase A_2 independent of N-protein linked activation (4). In contrast this PT-pretreatment significantly decreased the PAF- and AII-induced release of radiolabel (Fig. 2). Together with the findings on PGE_2 production (Fig. 1), this is consistent with PT inhibition of the initial step of the PAF and AII response, i.e. phospholipase C activation and subsequent release of arachidonic acid and PGE_2 formation (7,8). Pretreatment with higher PT (100 ng/ml) resulted in inconsistent inhibition of the AII response (Fig. 1). We, therefore, examined pretreatment with high (1-5 ug/ml) concentrations

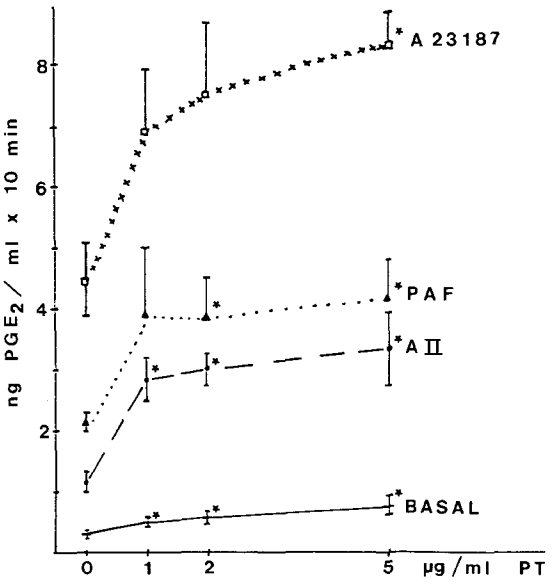

Fig. 3. Effect of 1 to 2 hour pretreatment without or with high concentrations (1-5 ug/ml) of pertussis toxin on PGE_2 production under basal conditions and with angiotensin II, platelet activating factor or A23187. Results are means ± SEM of 5 sets of experiments for each experimental agent (3 sets with 1 hour and 2 sets with 2 hour preincubation). Asterisks indicate $P < 0.05$ or better compared to respective values obtained without PT pretreatment.

of PT. Surprisingly, these high concentrations of PT enhanced basal PGE_2 synthesis and the stimulation by all three agents (Fig. 3). These findings cannot be explained by interference with an N subunit related to phospholipase C. (Cell damage was excluded as an explanation by over 95% trypan blue exclusion after PT pretreatment.)

Addition of saturating concentrations of PT (15 ug/assay) to membranes from control cells in the presence of [^{32}P] NAD resulted in radiolabeling of a band of about 40 kDa (Fig. 4). Two additional bands with molecular weights around 34 kDa showed faint [^{32}P] radioactivity. Pretreatment of intact cells with PT decreased the subsequent radiolabeling indicating prior ribosylation (Fig. 4). This was PT-dose-dependent with a decrease of labeling of: 20% after 25 ng/ml PT, 40% after 50 ng, 50% after 100 ng and 70% after 1 ug/ml. Thus failure to ribosylate cannot explain the stimulatory effect of high PT on PGE_2 synthesis (Fig. 3).

Two findings deserve discussion: The different AII and PAF responses to PT pretreatment and the paradoxical stimulation of PGE_2 production at high

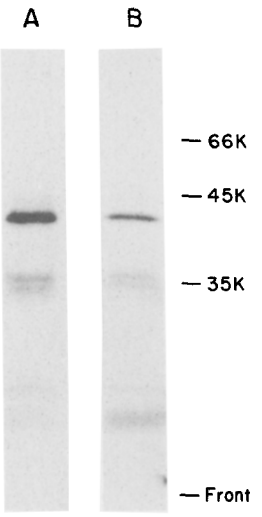

Fig. 4. Pertussis toxin-catalyzed radiolabeling of membrane preparation from mesangial cells pretreated for 2 hours in the absence (A) or presence (B) of 100 ng/ml pertussis toxin. Cells were then homogenized, a 12,000 xg pellet prepared and incubated with [^{32}P] NAD and saturating amounts of PT. The preparation was subjected to SDS-PAGE and autoradiography. Each lane contained comparable amounts of protein (5 ug). Position of molecular weight markers are indicated.

concentrations of PT. Concerning the first point; if one assumes that the PT effect was mediated by inactivation of an N protein linked to phospholipase C, the N proteins linked to PAF's and AII responses may have different susceptibilities to PT catalyzed ADP-ribosylation. In other words, different pools of N proteins would be involved in the PAF and AII response. This would not be detectable by the commonly employed ADP-ribosylation assay, as N proteins of 40 kDa would migrate together on SDS-PAGE. Recently, several immunologically different N proteins have been identified in a 40 kDa band of neutrophils (3). It appears that in mesangial cells only 20% to 40% ADP-ribosylation of the 40 kDa protein(s) is required to inhibit the response to AII or PAF, while higher degrees of ribosylation seem to override the inhibitory effect. It is interesting to note that pretreatment with similar, i.e. high concentrations of PT, leading to 100% ribosylation of N protein, caused no inhibition of AII's effect, but even a slight enhancement, in liver and pituitary cells (5,6). On the other hand Murayama and Ui (4) reported that PT pretreatment (100 ng/ml) in 3T3 fibroblasts inhibited AII- and PAF-induced arachidonate release and calcium influx, but not PAF-induced inositol release.

In neutrophils PT did, however, decrease phosphoinositide hydrolysis, but not calcium mobilization (12). Again, this may point to different receptor-effector interactions that are differentially affected by PT.

At present, we can only speculate about the second point, i.e. the stimulatory response to high dose PT pretreatment. Hypothetically, this may involve PT-induced changes in intracellular calcium or reincorporation of arachidonic acid into membrane phospholipids. Either of these possibilities could increase availability of arachidonate and hence PGE_2 production. Obviously mechanisms of action for PT unrelated to ADP ribosylation of N proteins have to be considered, too.

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