Br. J. Pharmacol. (1989), **98**, 717–720

Cholera and pertussis toxins amplify prostacyclin synthesis in aortic smooth muscle cells

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Pretreatment of bovine aortic smooth muscle cells in culture with pertussis toxin (PT) or cholera toxin (CT) potentiated the synthesis of prostacyclin (PGI_2) induced by 5-hydroxytryptamine (5-HT) and phorbol-12-myristate, 13-acetate (PMA). The production of PGI_2 by explants from the bovine aortic media was also synergistically stimulated by 5-HT and CT, whereas PT was inactive. These data are consistent with the hypothesis that guanosine 5'-triphosphate binding proteins are directly involved in the control of phospholipases which release free arachidonic acid for prostaglandin synthesis.

Introduction There is growing evidence that the activity of phospholipases which release free arachidonic acid for prostaglandin synthesis can be controlled directly by guanosine 5'-triphosphate (GTP)-binding proteins (Burgoyne *et al.*, 1987). Among other arguments, this hypothesis is supported by experiments with cholera toxin (CT) and pertussis toxin (PT), which modulate the activity of some G proteins by catalyzing their ADP-ribosylation (Neer & Clapham, 1988). The action of these toxins on phospholipase A_2 activity and prostaglandin production is variable from one cell type to the other. The stimulation of prostaglandin E_2 (PGE_2) synthesis by α_1-adrenoceptor agonists is blocked by PT in FRTL5 rat thyroid cells (Burch *et al.*, 1986), while it is not modified by this toxin in MDCK canine kidney cells (Slivka & Insel, 1987). In the rod outer segments from the bovine retina, both PT and CT prevent the transducin-mediated activation of phospholipase A_2 by light (Jelsema, 1987). In contrast, CT and PT increase the basal production of PGE_2 in a murine macrophage line (Burch *et al.*, 1988) and potentiate the synthesis of prostacyclin (PGI_2) in bovine aortic endothelial cells stimulated by ATP or bradykinin (Pirotton *et al.*, 1987). In this study we have investigated the action of CT and PT on the production of PGI_2 by two distinct preparations of vascular smooth muscle: explants from the bovine aortic media, in which the cells keep a contractile phenotype and cultured cells obtained by outgrowth from these explants, which have modulated to the synthetic phenotype (Chamley-Campbell *et al.*, 1981).

Methods *Explants of the bovine aortic media* After removal of the intima and adventitia from the aorta, the media was cut into ± 1 mm squares (Ross, 1971); 4–5 such explants (20–50 mg wet weight) were put on 60 mm Petri dishes and cultured for 24 h at 37°C, under 5% CO_2/95% air, in a medium of the following composition: Dulbecco's modification of Eagle's medium (DMEM: 80%), Ham's F_{12} medium (20%), foetal calf serum (FCS: 20%), penicillin (100 u ml^{-1}), streptomycin (100 $\mu g\,ml^{-1}$), amphotericin B (2.5 $\mu g\,ml^{-1}$). PT and CT were added at the beginning and 2 h before the end of that 24 h period respectively. Then the medium was removed, the explants were rinsed and incubated for 60 min in DMEM containing cocaine 30 μM (Cory *et al.*, 1986), with or without 5-hydroxytryptamine (5-HT).

Bovine aortic smooth muscle cells Bovine aortic smooth muscle cells were obtained by outgrowth from media explants (Ross, 1971). The cells which had migrated out of the explants were subcultured in 35 mm Petri dishes in the complete culture medium (see above). Experiments were performed with confluent cells between passage 3 and 8. The cells were rinsed and incubated for 24 h in the complete culture medium (see above), except for the absence of FCS, in the presence of the agents to be tested. PT and CT were added 20 h and 1 h respectively before the start of the incubation with the agonists.

PGI_2 assay The production of PGI_2 was measured by radioimmunoassay of its stable degradation product, prostaglandin 6-keto-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in the incubation medium (Van Coevorden & Boeynaems, 1984).

Statistical analysis of the results was performed using the paired *t* test.

Materials CT and its subunits were obtained from Sigma Chem. Co., and PT from List Biological

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Laboratories. All the other reagents were purchased as previously described (Demolle & Boeynaems, 1988).

Results 5-HT (10 μM), adenosine 5'-O-(3-thiotriphosphate) (ATPγS, 100 μM), phorbol-12-myristate, 13-acetate (PMA, 50 nM) and A23187 (0.1 μM) stimulated the production of PGI_2 by bovine aortic smooth muscle cells, as previously described (Coughlin *et al.*, 1984; Demolle & Boeynaems, 1988; Demolle *et al.*, 1988). Pretreatment with CT (5 $\mu g\,ml^{-1}$, 2 h) or PT (200 $ng\,ml^{-1}$, 20 h) amplified the response to 5-HT (Figure 1a and b). In the absence of toxins, 5-HT induced a 2.5 fold stimulation of PGI_2 release (mean of 10 experiments, range 1–6, $P = 0.003$): this stimulation was increased to 10 fold by PT (mean of 5 experiments, range: 3–18, $P = 0.05$) and to 11 fold by CT (mean of 10 experiments, range: 5–24, $P = 0.001$). In some experiments where no stimulation could be detected in response to 5-HT, an effect was unmasked by PT or CT. Pretreatment with both toxins potentiated the release of PGI_2 in a more than additive way (Figure 1a). The toxins increased the basal release of PGI_2 in a few experiments (3 out of 10): mean stimulations of 1.5 fold and 2 fold were observed in response to CT and PT respectively. The stimulatory effect of PMA was also potentiated by PT (Figure 1b) and CT (not shown), whereas the toxins had no significant effect on the response elicited by ATPγS and ionophore A23187 (Figure 1b). A synergism between 5-HT and CT was also observed in explants from the bovine aortic media (Figure 1c, 8 experiments, $P = 0.02$), while PT was completely inactive (3 experiments, not shown). Neither the A nor the B subunit of CT was able to mimic the action of the holotoxin either in the media explants (Figure 1c), or in the cultured smooth muscle cells (not shown). Forskolin (50 μM), an agent known to increase adenosine 3':5'cyclic-monophosphate (cyclic AMP) levels in vascular cells, was inactive in both experimental systems (not shown).

Discussion Our data suggest that one or more GTP-binding proteins are directly involved in the control of PGI_2 synthesis by bovine aortic smooth muscle cells. Previous studies have provided evidence for a coupling between G protein and phospholipase A_2 (PLA_2). GTPγS and noradrenaline increased the release of arachidonate from membranes of FRTL5 cells in a synergistic way (Burch *et al.*, 1986). The activation of PLA_2 induced by light in rod outer segments of the retina was abolished by transducin depletion and restored by addition of exogenous transducin (Jeselma, 1987): this effect was

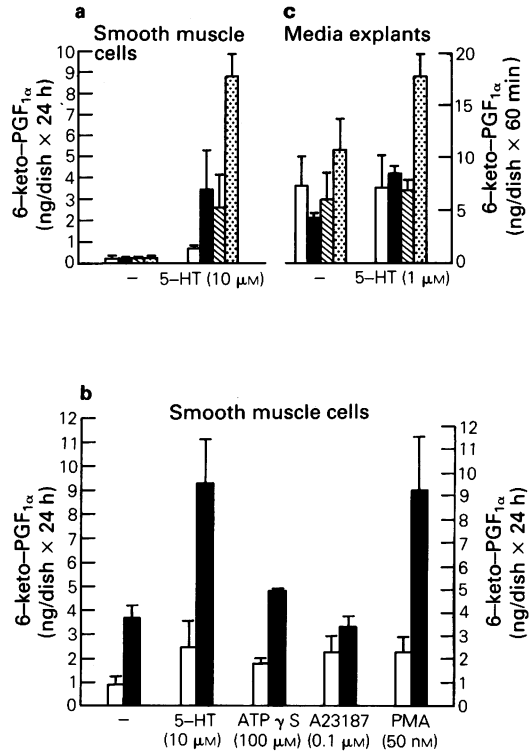

Figure 1 (a) Effect of pertussis toxin (PT) and cholera toxin (CT) on the production of prostacyclin (PGI_2) induced by 5-hydroxytryptamine (5-HT, 10 μM) in cultured bovine aortic smooth muscle cells. After a preincubation of respectively 20 h with PT (200 $ng\,ml^{-1}$) or 1 h with CT (5 $\mu g\,ml^{-1}$), the cells were incubated for 24 h in a culture medium without FCS, in presence of the various agents. Control: open columns; PT: solid columns; CT: hatched columns; PT + CT: stippled columns. (b) Selectivity of the PT action on the production of PGI_2 induced by 5-HT and PMA in cultured bovine aortic smooth muscle cells. The cells were incubated for 20 h with PT (200 $ng\,ml^{-1}$), prior to the addition of adenosine 5'-O-(3-thiotriphosphate (ATPγS, 100 μM), 5-HT (10 μM) A23187 (100 nM) or phorbol-12 myristate, 13-acetate (PMA, 50 nM): thereafter, the incubation was continued for 24 h, in the complete culture medium without FCS. Control: open columns; PT: solid columns. (c) Effect of CT on the production of PGI_2 by bovine aortic media explants stimulated by 5-HT: comparison between the holotoxin and its subunits. CT and its subunits A and B were added for 1 h to the tulture medium, each at 5 $\mu g\,ml^{-1}$. After removal of that medium and 2 washings, the explants were incubated for 1 h in presence of 5-HT (1 μM) and cocaine (30 μM). Control: open columns; CT, subunit A: solid columns; CT, subunit B: hatched columns; CT: stippled columns. For each panel, results represent the amount of 6-keto-PGF_1 accumulated in the medium (mean ± s.d. of triplicate determinations, in 1 representatative experiment out of 3).

mimicked by $\beta\gamma$-subunits and inhibited by the α-subunit (Jelsema & Axelrod, 1987). The GTP-binding proteins can be divided into four groups according to their susceptibility to ADP-ribosylation by the toxins: substrates for CT, for PT, for both toxins or for none (for review, see Neer & Clapham, 1988). PT and CT increased the basal production of PGE_2 in murine macrophages (Burch *et al.*, 1988) and potentiated the release of PGI_2 induced by ATP in bovine aortic endothelial cells (Pirotton *et al.*, 1987). Our results suggest that a similar mechanism operates in cultured bovine aortic smooth muscle cells, with some peculiarities. In smooth muscle cells, the toxins had little effect *per se*, whereas they increased the basal production of PGE_2 in murine macrophages (Burch *et al.*, 1988). In aortic endothelial cells, the toxins amplified the response to all the agonists tested (ATP, bradykinin, A23187); in smooth muscle cells, they were selective for some of them (5-HT, PMA). The action of PT and CT on PGI_2 synthesis in bovine aortic smooth muscle cells could be explained by the activation via ADP-ribosylation of a Gs-like protein, by the inhibition of a Gi-like protein (Jelsema, 1987) or by a combination of these 2 events, as suggested by Burch *et al.* (1988) to explain the synergism between PT and CT on PGE_2 synthesis in murine macrophages. Likewise, the effects of PT and CT on smooth muscle cells were more than additive.

In rat aortic myocytes, 5-HT, via 5-HT_2 receptors, induces an accumulation of inositol trisphosphate (Cory *et al.*, 1986), which results in an increase of cytosolic Ca^{2+}: this rise was inhibited by PT, which probably blocks the G protein coupling the 5-HT_2 receptor to phospholipase C (PLC) (Bruns & Marme, 1987). In FRTL5 cells, α_1-adrenoceptors are coupled to PLA_2 and PLC via different GTP binding proteins: only the G protein linked to PLA_2 was PT-sensitive (Burch *et al.*, 1986). The fact that PT potentiates the stimulatory effect of 5-HT on PGI_2 synthesis in bovine aortic smooth muscle cells is thus not incompatible with the observation that it blocks the rise of cytosolic Ca^{2+} induced by 5-HT in rat aortic smooth muscle cells.

In the explants of aortic media (contractile phenotype), only CT was able to potentiate the 5-HT effect on PGI_2 production, whereas in cultured smooth muscle cells (synthetic phenotype), PT was as effective as CT. Although, we cannot rule out the possibility that PT does not penetrate the media explants, its ineffectiveness is more likely to result from a differential sensitivity to PT of the G proteins involved in the control of PGI_2 production. For instance, PT inhibited PGE_2 synthesis induced by α_1-adrenoceptor agonists in FRTL5 rat thyroid cells (Burch *et al.*, 1986), whereas it was inactive in MDCK canine kidney cells (Slivka & Insel, 1987). The 5-HT receptor mediating the stimulation of PGI_2 synthesis in the media explants is different from the 5-HT_2 receptors involved in the cultured smooth muscle cells (Coughlin *et al.*, 1984; Demolle *et al.*, 1989). The mediators controlling PGI_2 production also seem to be specific for each system (Demolle & Boeynaems, 1988). Our observation of a differential action of the toxins constitutes an additional argument supporting the hypothesis that the modulation from a contractile to a synthetic phenotype is accompanied by a fundamental change in the mechanisms which control PGI_2 production.

This work was supported by grants from the 'Ministère de la Politique Scientifique' ('Action Concertée') and the 'Fonds de la Recherche Scientifique Médicale'.

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(Received March 31, 1989
Accepted August 10, 1989)