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The stable prostacyclin-analogue, iloprost, unlike prostanoids and leukotrienes, potently stimulates cyclic adenosine monophosphate synthesis of primary astroglial cell cultures

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Abstract—The effect of different eicosanoids on adenosine-3', 5'-cyclic-monophosphate (cAMP) accumulation in primary astroglial cell cultures prepared from newborn rat brain was studied. The stable prostacyclin-analogue, iloprost, effectively stimulated cAMP synthesis in a concentratioin-dependent, saturable manner, the EC50 being about 3×10^{-8} M. Prostaglandin (PG) E_2 was less potent, without reaching plateau even at 10^{-5} M. Prostaglandins D_2 and $F_{2\alpha}$, and the stable thromboxane A_2-analogue, U 46619, as well as leukotrienes (LT) B_4, C_4, D_4 and E_4 were not effective and did not attenuate basal or isoprenaline (10^{-8} M)-stimulated astroglial cAMP formation. This is the first indication for the existence of a prostacyclin receptor coupled positively to the adenylate cyclase in astrocytes. Other eicosanoids are unlikely to be involved in receptor-mediated regulation of astroglial cAMP levels.

Prostanoids and leukotrienes are biologically active oxidation products of arachidonic acid (Needleman et al 1986). The synthesis of prostanoids (Wolfe 1982) and LTs (Simmet et al 1987) by brain tissue has been described. Except for prostacyclin, which is mainly of vascular origin (Wolfe 1982), cerebral prostanoids originate predominantly from mature astrocytes (Seregi et al 1987), while the cellular source of LTs in the brain remains to be elucidated. Prostanoids possess central effects of phsyiological relevance (Shimizu et al 1979; Wolfe 1982; Hertting et al 1985). Little is known however about the role of LTs in the brain (Moskowitz et al 1984). There is a growing interest concerning the biological response of astrocytes to putative signal-transducing substances (Murphy & Pearce 1987). Therefore, in this study, we have investigated the effects of prostanoids and LTs on receptor-coupled adenylate cyclase activity of astrocytes. As a model, primary astroglial cells cultures prepared from neonatal rat brain hemispheres were used.

Methods and materials

Details of the preparation and culture conditions as well as the morphological and immunocytochemical characterization of the cultures have been described recently (Keller et al 1985).

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Fourteen day old cultures were studied. For experimental incubations cells were washed in 0·01 M Na-phosphate buffer (pH 7·4) containing 0·15 M NaCl and 0·1% (w/v) glucose (solution A), and preincubated for 15 min at 37°C in 1 mL solution A supplemented with 10^{-5} M indomethacin and 5×10^{-4} M isobutylmethyl xanthine (solution B). Incubations were carried our for 3 min at 37°C in 1 mL solution B in the presence of the actual compounds to be tested. The inhibitors examined were present both during preincubation and incubation. The reaction was stopped by adding 0·1 mL 1·5 M $HClO_4$, and cultures were allowed to stand for 30 min at 4°C. Supernatants were transferred into plastic vials, neutralized by excess $CaCO_3$ (Tihon et al 1977) and centrifuged (2000 $g \times 10$ min, 4°C). Cyclic AMP concentrations were estimated from the resultant supernatants by a specific radioimmunoassay as described previously (Ortmann 1978). Protein was determined according to Lowry et al (1951).

Iloprost was kindly donated by Schering A.G. (Berlin, FRG), Leukotrienes C_4 and E_4 were the generous gift of Hoechst A.G. (Frankfurt, FRG). U 46619 [(15 S)-hydroxy-11α, 9α (epoxy methano) prosta-52, 13E-dienoic acid] was kindly supplied by Dr Th. Simmet. All other substances and chemicals were from commercial sources.

Results and discussion

Fig. 1. shows the effect of various prostanoids or their mimetics on basal cAMP formation of astrocytes.

In contrast to a human astrocytoma cell-line, where prostacyclin (PGI_2) proved to be a very weak stimulant of cAMP synthesis (Ortmann 1978), in the primary astroglial cell cultures, the stable PGI_2-mimetic agent iloprost (Schrör et al 1981) was the most effective substance in increasing cAMP accumulation. The effect of iloprost was concentration-dependent and reached plateau at 10^{-6} M. The EC50 value was about 3×10^{-8} M, which is close to that obtained for PGI_2 on platelet adenylate cyclase (Gorman et al 1977). These results strongly suggest that cultured astrocytes possess a prostacyclin receptor positively coupled to their adenylate cyclase activity. It may be of special interest since

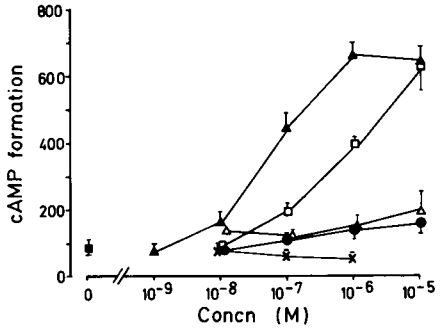

FIG. 1. Effect of (▲) iloprost, (×) U 46619 and prostaglandins (●) D_2, (□) E_2 and (△) $F_{2\alpha}$ on cyclic-3′, 5′-adenosine monophosphate formation (pmol cAMP (mg protein)$^{-1}$/3 min) in primary astroglial cell cultures. The results are the means ± s.e.m. of three independent experiments performed in triplicate. For experimental conditions see Methods.

astrocytic end-feet are in close proximity to brain capillaries, the major source of PGI_2 in the brain (Wolfe 1982). Among the primary PGs only PGE_2 stimulated astroglial cAMP synthesis (Fig. 1). This effect was also concentration-dependent but saturation was not reached even at a concentration as high as 10^{-5} M. This finding is in accordance with that obtained in primary cultures of perinatal mouse brain by VanCalker et al (1979).

Prostaglandin D_2, is the major prostanoid of the brain (Abdel-Halim et al 1977). In contrast to the observation that PGD_2 was a specific and potent activator of adenylate cyclase of a neuroblastoma cell line (Shimizu et al 1979), it failed to stimulate astroglial cAMP formation in the concentration range of 10^{-8}–10^{-5} M. Prostaglandin $F_{2\alpha}$ and the stable thromboxane A_2-mimetic agent U 46619 (Coleman et al 1981) proved also to be ineffective (10^{-8}–10^{-5} M) (Fig. 1).

Like in many other cells, the regulation of cAMP accumulation in astroglial cells is bimodal: some compounds increase while others inhibit basal and agonist-stimulated adenylate cyclase activity (VanCalker et al 1979; Evans et al 1984). Since PGD_2, $PGF_{2\alpha}$ and U 46619 did not increase cAMP synthesis, we further investigated whether they might be inhibitory to the isoprenaline-stimulated adenylate cyclase activity. Isoprenaline increased cAMP accumulation in a concentration-dependent manner (EC50 2×10^{-9} M), reaching plateau at 10^{-7} M. Neither PGD_2, nor $PGF_{2\alpha}$ or U 46619 (10^{-8}–10^{-5} M) attenuated the stimulatory effect (1178 ± 86 pmol cAMP (mg protein)$^{-1}$/3 min) of 10^{-8} M isoprenaline (data not shown).

Summarizing the effect of prostanoids on astroglial cAMP formation, we concluded that PGD_2, TXA_2, $PGF_{2\alpha}$ and PGE_2 which are actively synthesized by astrocytes (Seregi et al 1984; Keller et al 1985) did not specifically influence, while prostacyclin which is not of astroglial origin (Seregi et al 1984; Keller et al 1985) effectively stimulated, astroglial adenylate cyclase activity, most probably in a receptor-mediated manner.

As the existence of specific binding sites for LTC_4 in the brain (Schalling et al 1986) suggested that LTs might act via specific receptors, we have also investigated the effects of LTB_4, LTC_4, LTD_4 and LTE_4 on basal and evoked cAMP formation of primary astroglial cell cultures. None of the LTs tested in the concentration range of 10^{-8}–10^{-5} M affected basal or isoprenaline stimulated cAMP synthesis (data not shown).

In conclusion, the potent and saturable stimulatory effect of iloprost strongly suggests the existence of an astroglial prostacyclin receptor coupled positively to adenylate cyclase-mediated signal-transduction. The adenylate cyclase system of astrocytes does not seem to be a specific target of the other prostanoids or of the leukotrienes.

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