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Dibutyryl cytidine 3′:5′-cyclic monophosphate; an inhibitor of A23187-stimulated macrophage leukotriene B_4 synthesis

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Introduction

The second messenger functions of cyclic purine nucleotides (adenosine and guanosine 3′:5′-monophosphates) in regulating cellular changes have long been recognized. However little is known about the role of cyclic pyridine nucleotides. During the last ten years evidence has accumulated that cytidine 3′:5′-cyclic monophosphate (cCMP) can modulate cell functions, sometimes complementing, sometimes opposing, the affect of cAMP [1]. Dibutyryl (db) cAMP inhibits the calcium ionophore (A23187)-stimulated turnover of arachidonic acid (AA) using peritoneal macrophages [2]. It is possible, therefore, that db-cCMP could also modify macrophage eicosanoid release.

Materials and methods

The basic methods have been described in detail else where [2]. Briefly, macrophages were isolated from peritoneal washes by density gradient centrifugation, 4 d after an intraperitoneal injection of carrageenin, and suspended at 2×10^6 cells/ml in Dulbecco's modified Eagle's minimum essential medium. Aliquots (1 ml) of the cell suspension were incubated for 30 min at 37 °C, with or without A23187 (10^{-7} *M*), db-cAMP and db-cCMP (10^{-7} *M*). The tubes were then centrifuged and the supernatant fractions analysed for thromboxane (TX) A_2 (measured as TXB_2) and leukotriene (LT) B_4 by radioimmunoassay.

Results

A23187 stimulated TXB_2 and LTB_4 synthesis during the 30 min incubation period, (TXB_2, control = 1.05 ± 0.45, + A23187 = 7.24 ± 2.37*; LTB_4, control = < 0.02, + A23187 = 1.20 ± 0.44*; ng/2×10^6 cells, mean values of 2 experiments, $n = 6$, *$p < 0.05$ using Mann-Whitney U-test). In a separate series of experiments we investigated the effect of db-cAMP and db-cCMP on A23187-stimulated TXB_2 and LTB_4 synthesis. Both compounds (10^{-7} *M*) inhibited A23187-stimulated TXB_2 and LTB_4 formation, the production of TXB_2 being inhibited to a greater extent than that of LTB_4. The two cyclic-nucleotide derivatives were equally effective in inhibiting A23187-stimulated eicosanoid synthesis, at the concentration used (Table 1).

Table 1
The effect of db-cAMP and db-cCMP on A23187-stimulated TXB_2 and LTB_4 synthesis by carrageenin-elicited rat peritoneal macrophages.

Cyclic nucleotide (10^{-7} *M*)	Eicosanoid (ng/2×10^6 cells)	
	TXB_2	LTB_4
Control	25.30 ± 4.5	1.30 ± 0.08
db-cAMP	18.00 ± 4.0*	1.00 ± 0.22*
db-cCMP	13.50 ± 6.6*	0.98 ± 0.20*

Results are mean values from 3 experiments, $n = 7$, *$p < 0.05$ (Mann-Whitney U-test).

Discussion

Our results confirm an earlier report that db-cAMP inhibits macrophage AA turnover [2]. We have also shown, for the first time, that db-cCMP can modulate macrophage eicosanoid synthesis. The mode of action of the two cyclic compounds is not clear. db-cCMP has been reported to bind to the cytosolic cAMP-binding protein present in adrenal cell extracts [1]. It is possible therefore that both db-cAMP and db-cCMP also bound to macrophage cAMP-binding proteins, resulting in a reduced calcium influx, a decrease in phospholipase A_2 activity and fall in the concentration of free AA [3].

cCMP levels have been reported to be elevated during liver regeneration and in the white blood cells and urine of leukaemia patients. Furthermore, cytidylate cyclase activity was found to be elevated, and cCMP phosphodiesterase activity reduced, during proliferation of mouse myeloid tumours and in regenerative liver [1]. It is possible, therefore, that cCMP could be important for the regulation of cell growth. We are currently investigating the possible link between these effects and the inhibition of AA turnover.

Summary

db-cCMP inhibits A23187-stimulated TXB_2 and LTB_4 synthesis by rat carrageenin-elicited peritoneal macrophages *in vitro*.

Acknowledgements

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References

[1] T. R. Anderson, *Cyclic cytidine 3',5'-monophosphate (cCMP) in cell regulation.* Molecular and Cellular Endocrinology *28,* 373–385 (1982).

[2] G. R. Elliott, A. P. M. Lauwen and I. L. Bonta, *Prostaglandin E_2 inhibits, and indomethacin and aspirin enhance, A23187-stimulated leukotriene B_4 synthesis by rat peritoneal macrophages.* Br. J. Pharmacol. *96,* 265–270 (1989).

[3] E. G. Lapetina, *Regulation of arachidonic acid production: role of phospholipases C and A_2.* T.I.P.S. *3,* 115–118 (1982).