Vol. 100, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1981 Pages 1-7

LIPOXYGENASE-DERIVED PRODUCTS OF ARACHIDONIC ACID MEDIATE STIMULATION OF HEXOSE UPTAKE IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

David A. Bass*, Michael J. Thomas*, Edward J. Goetzl+
Lawrence R. DeChatelet*, and Charles E. McCall*

*Departments of Medicine and Biochemistry
Bowman Gray School of Medicine
Winston-Salem, NC 27103
and
+Howard Hughes Medical Institute Laboratory at
Harvard Medical School and Departments of Medicine
Harvard Medical School and The Brigham and Women's Hospital
Boston, MA 02115

Received February 17,1981

SUMMARY

Chemotactic factors, arachidonic acid and leukocyte-derived lipoxygenase products of arachidonate (5-hydroperoxy-, 5-hydroxy- and 5,12-dihydroxy-eicosatetraenoic acids) stimulated stereospecific uptake of ^{3}H-2-deoxyglucose by human neutrophils. However, of all stimuli tested, only the effects of 5-hydroxy-eicosatetraenoic acid were not inhibited by 5,8,11,14-eicosatetraynoic acid at concentrations which also inhibit release of arachidonate metabolites from leukocytes. The data suggest that 5-hydroxy-eicosatetraenoic acid mediates the coupling of membrane receptor stimulation to the functional response of hexose uptake in human neutrophils.

INTRODUCTION

The mechanism(s) by which stimulation of appropriate membrane receptors of mammalian cells are coupled to augmented hexose uptake are not well defined. We recently found that chemotactic factors (1,2) or the calcium ionophore A21387 (3) stimulate uptake of the glucose analog, ^{3}H-2-deoxyglucose by human polymorphonuclear leukocytes (PMNL). Others have demonstrated that similar stimuli cause neutrophils to release arachidonic acid (AA) from membrane phospholipids (4), presumably by activation of phospholipase A_2 or C. The AA can then be metabolized by lipoxygenases to form hydroperoxy-eicosatetraenoic

ABBREVIATIONS: AA, arachidonic acid; ETYA, 5,8,11,14-eicosatetraynoic acid; 5-HETE, 5-L-hydroxyeicosatetraenoic acid; 5-HPETE, 5-L-hydroperoxyeicosatetraenoic acid; 5,12-diHETE, 5(S), 12(R)-dihydroxy-6,8,10 (two trans, one cis), 14-cis-eicosatetraenoic acid; fMLP, N-formyl-methionyl-leucyl-phenylalanine; PMNL, polymorphonuclear leukocytes.

0006-291X/81/090001-07$01.00/0
Copyright © 1981 by Academic Press, Inc.
All rights of reproduction in any form reserved.

acids, which are then converted to monohydroxy- and dihydroxy-eicosatetraenoic acids (HPETEs, HETEs and diHETEs, respectively) and by cyclooxygenases to form prostaglandins and thromboxanes (5-10). Certain of these products, e.g. 5-HETE, are reincorporated in part into cell membranes and might directly mediate subsequent functional responses, perhaps by altering membrane fluidity (8). Previous studies demonstrated that AA stimulated deoxyglucose uptake and that inhibitors of AA metabolism, such as 5,8,11,14-eicosatetraynoic acid (ETYA), prevented deoxyglucose uptake stimulated by either AA or by the chemotaxin, N-formyl-methionyl-leucyl-phenylalanine (fMLP) (2); these observations indicated that the release and metabolism of endogenous AA might be a critical prerequisite for the stimulation of hexose uptake. The inhibitory profiles suggested that the relevant metabolism of AA most probably involved the lipoxygenase pathway. We now report that exogenous PMNL-derived lipoxygenase products of AA stimulate deoxyglucose uptake in PMNL. That the stimulatory effect of 5-HETE alone is not prevented by the inhibition of lipoxygenation in PMNL, suggests that this specific endogenous product mediates augmented hexose uptake in PMNL stimulated by diverse agents.

METHODS

Preparation of hydroxylated arachidonate derivatives. 5-L-hydroperoxy-eicosatetraenoic acid (5-HPETE), 5-L-hydroxyeicosatetraenoic acid (5-HETE) and 5(S),12(R)-dihydroxy-6,8,10(two-trans, one-cis),14-cis-eicosatetraenoic acid (5,12-diHETE or leukotriene B_4) were extracted from purified human PMNL which had been incubated for 20 min at 37°C with 1 mg AA/10^8 PMNL and 10 μM A23187 in the presence of 10 μM indomethacin, and were purified by sequential silicic acid column chromatography and reverse-phase high pressure liquid chromatography (5,10,11). Identities of purified products were confirmed by U.V. absorption spectroscopy and by mass spectrometry; products were quantitated by optical density at the characteristic wavelengths (7,11).

Assay of hexose uptake. Deoxyglucose uptake was measured as described (2), by incubating 2 x 10^5 purified PMNL in 0.3 ml glucose-free Dulbecco's phosphate-buffered saline (PBS) containing 0.9 mM Ca^{++} and 0.5 mM Mg^{++} with stimuli and/or ETYA for 15 min at 37°C; uptake was initiated by adding 0.5 μCi ^{3}H-deoxyglucose. Following incubation for 60 min, the reaction was stopped by adding 1 ml of iced PBS; the cells were immediately washed by centrifugation and the radioactivity of the cell pellet determined. Results are expressed as c.p.m./2 x 10^5 PMNL. Triplicate determinations were performed on all PMNL preparations.

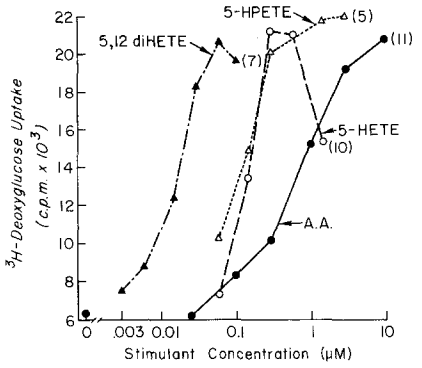

Figure 1. Stimulation of deoxyglucose uptake of PMNL by AA (●), 5-HPETE (Δ), 5-HETE(O) and 5,12-diHETE (▲). Points are means of the results of indicated number of studies (in parentheses) of separate PMNL preparations. At all points, the standard errors were less than 10% of the mean.

RESULTS

AA, 5-HPETE, 5-HETE and 5,12-diHETE stimulated deoxyglucose uptake in a concentration-related manner (Figure 1), with EC_{50}'s of 650 nM, 150 nM, 110 nM, and 17 nM, respectively. Stimulation of uptake by each of these agents required a stereospecific mechanism, since each was markedly inhibited by 10 mM D-glucose but unchanged by 10 mM L-glucose (Table 1).

Table 1

Stereospecificity of Deoxyglucose Uptake by PMNL

	^{3}H-2-Deoxyglucose Uptake with added hexose*:		
Stimulant	None	D-Glucose, 10 mM	L-Glucose, 10 mM
None	6.10 ± .04 (32)	0.31 ± .03 (16)	5.9 ± 0.6 (16)
AA, 1 μM	18.1 ± 0.6 (10)	0.27 ± .03 (10)	17.1 ± 0.9 (10)
5-HPETE, 0.3 μM	20.3 ± 1.9 (4)	0.52 ± .05 (4)	17.8 ± 2.8 (4)
5-HETE, 0.3 μM	25.9 ± 1.6 (4)	0.29 ± .02 (4)	26.7 ± 1.7 (4)
5,12-diHETE, 30 nM	19.7 (2)	0.65 (2)	19.3 (2)

*Deoxyglucose uptake measured as in figure 1 except hexose added as indicated to initial incubation medium. Data are means ± SEM of (n) determinations and are expressed as c.p.m. x 10^3/2 x 10^5 PMNL.

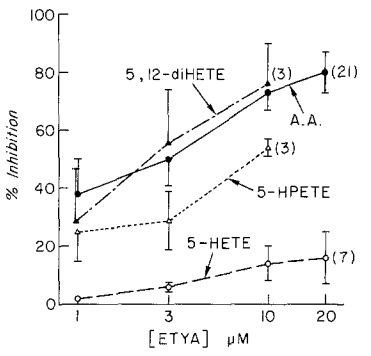

Figure 2. Inhibitory effect of varying concentrations of ETYA on the stimulation of deoxyglucose uptake by 1-3 μM AA (●), 0.3 μM 5-HPETE (Δ), 0.3 μM 5-HETE (O) and 30-60 nM 5,12-diHETE (▲). Mean deoxyglucose uptakes in the absence of ETYA for these experiments were AA, 17,016; 5-HPETE, 16,819; 5-HETE, 19,527; and 5,12-diHETE, 15,187 c.p.m./2 x 10^5 PMNL. Deoxyglucose uptake was measured as in Figure 1, except that ETYA, at the indicated concentrations, was present during the initial incubation of cells and stimuli. Data are means ± SEM for the indicated number (in parentheses) of separate PMNL preparations.

ETYA similarly inhibited the augmentation of deoxyglucose uptake by AA and 5,12-diHETE, with IC_{50}'s of 3.0 μM and 2.4 μM, respectively (Figure 2). Inhibition of the stimulation of deoxyglucose uptake by 5-HPETE required slightly higher concentration of ETYA (IC_{50} = 8.4 μM). In contrast, 5-HETE was only minimally inhibited by 1 to 20 μM ETYA (inhibition by 20 μM ETYA of 16 ± 8%, n=7).

DISCUSSION

PMNL require energy derived from glucose-6-phosphate for all functional responses. The hexose could be provided from extracellular supplies by transport (or diffusion) into the cell and subsequent phosphorylation, or from intracellular glycogen by activation of glycogen phosphorylase. Glycogen stores exist in PMNL and glycogen phosphorylase is activated during phagocytosis (12,13). However, the functional responses which precede phagocytosis, including adhesiveness and chemotaxis, may rely on extracellular hexose supplies. Thus, stimuli of chemotaxis and/or aggregation, including fMLP, C5a, arachidonic acid or low concentrations (20 nM-1 μM) of the ionophore A23187, augment uptake of extracellular hexose (1-3) and do not activate

glycogen phosphorylase (13). Indeed, rather than causing glycogen breakdown, fMLP activates glycogen synthase (unpublished observations), and, in rats, PMNL which were chemotactically attracted into a peritoneal exudate, had increased, rather than decreased, glycogen stores (14). These data suggest the presence of separate mechanisms for providing energy during specific functional responses, such as chemotaxis and phagocytosis. The mechanisms which couple membrane receptor stimulation to the appropriate process of hexose mobilization are not well defined. The present studies were directed to the hypothesis (2) that augmented uptake of extracellular hexose involves the release of endogenous arachidonic acid and its subsequent oxygenation.

Deoxyglucose was employed to measure hexose uptake since it enters other cells at the same sites as glucose by an apparently carrier-facilitated mechanism and is phosphorylated, but is very poorly metabolized (15) and thus accumulates within the cell. Therefore, the assay measures both transport and phosphorylation of deoxyglucose by specific mechanisms (16,17). Deoxyglucose uptake in PMNL was found to be saturable with increasing concentration of stimulant or substrate, stereospecifically inhibited by D-glucose, but not by L-glucose, and markedly suppressed by cytochalasin B (the small uptake of deoxyglucose in the presence of cytochalasin B reflects passive diffusion into the cells) [1,2]. Thus, uptake of deoxyglucose by PMNL requires a mechanism with characteristics similar to carrier-facilitated hexose transport in other cells stimulated by insulin (16,17). Within the cells, deoxyglucose is rapidly and nearly irreversibly phosphorylated by hexokinase. The rate of phosphorylation by hexokinase is similar for resting and stimulated PMNL and is unaffected by inhibitors of the oxygenation of arachidonic acid (1,2).

C5a, fMLP and the ionophore A23187 stimulate deoxyglucose uptake in PMNL (1-3), and have also been reported to cause release of AA and AA metabolites, with the greatest relative increase in AA metabolites hydroxylated at the 5 position (8,10). We therefore examined the stimulation of deoxyglucose uptake by AA, 5-HETE, 5-HPETE, and 5,12-diHETE. Each of these agents could act as

extracellular stimuli to cause enhanced, stereospecific uptake of deoxyglucose by PMNL (Figure 1). In this regard, 5,12-diHETE was the most potent.

Regarding the mechanisms of action of these stimuli, two possibilities require consideration: 1) Each could interact at the cell membrane to elicit the same cascade of receptor-function coupling mechanisms caused by other stimuli, such as fMLP. The response to such a product, presented as an extracellular stimulus, should be similarly inhibited by agents which inhibit the response to fMLP. 2) The product might serve as an intracellular mediator in the normal sequence of events leading to the activation of PMNL functions by other agonists. If, as proposed previously (2), this sequence involves the release and lipoxygenation of AA, then stimulation by an AA-derived mediator should not be blocked by concentrations of ETYA which block lipoxygenation of AA and thereby the stimulation of deoxyglucose uptake by fMLP or AA. We therefore examined the effects of ETYA on stimulation of deoxyglucose uptake by each of the AA derivatives.

ETYA prevents release of lipoxygenase-mediated AA derivatives from stimulated PMNL with 50% inhibition (IC_{50}) at concentrations of 3-8 μM (10,18). ETYA similarly inhibited the abilities of fMLP (2), AA and 5,12-diHETE (figure 2) to augment deoxyglucose uptake. Yet ETYA had little effect on deoxyglucose uptake stimulated by 5-HETE. Other substances which interact with AA metabolism, such as indomethacin, have been found to prevent the conversion of 5-HPETE to 5-HETE (19); such an effect could explain the inhibition of 5-HPETE by slightly higher concentrations of ETYA.

In summary, 5,12-diHETE was a potent extracellular stimulant of deoxyglucose uptake by PMNL. However, its effect was inhibited by ETYA at concentrations which inhibited to a similar extent the stimulatory effects of fMLP or AA. This suggests that 5,12-diHETE may act by the same mechanism as other chemotactic factors. In contrast, the observations that stimulation of PMNL causes the rapid endogenous production of 5-HETE (8,10) and that ETYA only minimally inhibited the stimulatory effect of 5-HETE (Figure 2), strongly

suggest that 5-HETE mediates a coupling of receptor stimulation to the functional response of hexose uptake by PMNL.

ACKNOWLEDGMENTS

We wish to thank Ms. Pamela Szejda and Sue Cousart for excellent technical assistance, and Ms. Vickie Cox for typing the manuscript. This work was supported, in part, by National Institutes of Health grants AI 14929, HL 16769, AI 09169, AI 10732 and HL 19777.

REFERENCES

1. McCall, C.E., Bass, D.A., Cousart, M.S. and DeChatelet, L.R. (1979) Proc. Nat. Acad. Sci. 76,5896-5900.
2. Bass, D.A., O'Flaherty, J.T., Szejda, P., DeChatelet, L.R. and McCall, C.E. (1980) Proc. Nat. Acad. Sci. 77,5125-5129.
3. Bass, D.A., O'Flaherty, J.T., Goetzl, E.J. DeChatelet, L.R. and McCall, C.E (1980) Prog. Lipid Res., in press.
4. Hirata, F., Corcoran, B.A., Venkatasubramanian, K., Schiffmann, E. and Axelrod, J. (1979) Proc. Nat. Acad. Sci. 76,2640-2643.
5. Goetzl, E.J. and Sun, F.F. (1979) J. Exp. Med. 150,406-411.
6. Borgeat, P., Hamberg, M. and Samuelsson, B. (1976) J. Biol. Chem. 251,7816-7820.
7. Borgeat, P. and Samuelsson, B. (1979) J. Biol. Chem. 254,2643-2646.
8. Stenson, W.F. and Parker, C.W. (1979) J. Clin. Invest. 64,1457-1465.
9. Spagnuolo. P.J., Ellner, J.J., Hassid, A. and Dunn, M.J. (1980) J. Clin. Invest. 66,406-414.
10. Goetzl, E.J. (1980) Immunol. 40,706-719.
11. Goetzl, E.J. and Pickett, W.C. (1980) Immunol. 125,1789-1791.
12. Saugmann, P. and Esmann, V. (1977) Biochem. Biophys. Res. Comm. 74, 1520-1527.
13. Neal, H.M., DeChatelet, L.R., McCall, C.E. and Bass, D.A. (1981) Fed. Proc., in press.
14. Scott, R.B. and Cooper, L.W. (1974) Br. J. Haematol. 26,485-496.
15. DeChatelet, L.R., Campbell, T.L., McCall, C.E., Shirley, P.S. and Swendsen, C.L. (1980) Inflammation 4,249-259.
16. Ciaraldi, T.P., Kolterman, O.G., Siegel, J.A. and Olefsky, J.M. (1979) Am. J. Physiol. 5,E621-E625.
17. Olefsky, J.M. (1978) Biochem. J. 172,137-145.
18. Stenson, W.F. and Parker, C.W. (1980) J. Immunol. 124,2100-2104.
19. Siegel, M.I., McConnell, R.T. and Cuatrecasas, P. (1979) Proc. Nat. Acad. Sci. 76,3774-3778.