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STIMULATION OF HEXOSE TRANSPORT BY HUMAN POLYMORPHONUCLEAR LEUKOCYTES: A POSSIBLE ROLE FOR PROTEIN KINASE C

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SUMMARY: The protein C kinase activators 1-O-oleoyl, 2-O-acetylglycerol, 12-O-tetradecanoyl phorbol-13-acetate, and mezerein, stimulated deoxyglucose uptake in human neutrophils. The responses were stimulus specific since no effect was noted with the diether analogues 1-O-hexadecyl-2-O-ethylglycerol, 1-O-palmitoyl-2-O-acetyl or 1-O-palmitoyl-3-O-acetyl diesters of propanediol, or with 1,2-diolein. Stimulation of deoxyglucose uptake had the characteristics of carrier facilitated hexose transport. Stimulated uptake of deoxyglucose was inhibited by trifluoperazine (10-30 μM). Activation of protein kinase C therefore appears to trigger events involved in hexose transport.

Hexose transport may be linked with many cellular responses, including normal physiology and mitogenic capacity. Carrier-facilitated transport of hexoses into nonepithelial cells following cell-surface ligand interactions involves poorly understood transduction mechanisms. Whereas insulin, the classic inducer of hexose transport in smooth muscle and fat cells, does not stimulate hexose transport in human PMN , we found that C5a, the chemotactic oligopeptide FMLP, the ionophore A23187, AA and products of its metabolism (LTB_4, 5-HETE, 12-HETE), and PAF all cause PMN to take up ^{3}HdGlc (1,2,3).

ABBREVIATIONS:

OAG, 1-O-octadecenyl,2-O-acetylglycerol; PMA, 12-O-tetradecanoyl phorbol-13-acetate; EEG, 1-O-hexadecyl-2-O-ethylglycerol; 1,2PAP or 1,3PAP, 1-O-palmitoyl, 2-O-acetyl or 1-O-palmitoyl,3-O-acetyl diesters of propanediol; ^{3}HdGLC, ^{3}H-deoxyglucose; TFP, trifluoperazine; C-kinase, protein kinase C; DAG, diacyl glyceride; ITP, inositol triphosphate; AA, arachidonic acid; FMLP, N-formyl-methionyl-leucyl-phenylalanine; MEZ, mezerein.

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We suggested that hexose transport involves phospholipid mobilization and the metabolism of AA .

Recently more specific roles for phosphoglyceride metabolism in secondary messenger signal coupling have been advocated in a number of cells, including PMN (4). Stimulated PMN may rapidly metabolize PI leading to the formation of ITP and DAG (5); a specific lipase acting on DAG could then lead to release of AA. Alternatively, AA could be released more directly from phospholipids by phospholipase A_2. In either case, secondary messengers may involve: 1) calcium regulation by ITP; 2) direct activation of C kinase by DAG, a putative endogenous mediator which stereospecifically activates C kinase (6). AA also directly activates C-kinase in PMN (7) thus providing another putative endogenous mediator for C kinase derived from phosphoglyceride metabolism. C kinase as well as calcium may be important secondary messengers in signal-coupled physiologic responses of various cells (8). Examples in blood cells include platelet degranulation (9), PMN degranulation (10), and PMN oxidative metabolism (11). Recent studies demonstrate stimulation of hexose uptake in rat thymic lymphocytes by PMA; stimulation did not appear to be related to activation of Na+/H+ antiport or changes in cytoplasmic Ca++ (12). Phosphorylation reactions by C kinase were indirectly implicated.

Here we demonstrate that OAG, PMA, and MEZ, all of which directly stimulate C kinase, specifically enhance hexose transport in human PMN. OAG, per se, provides a powerful tool to determine whether exogenously added DAG can mimic the action of artificial activators of C kinase such as PMA and MEZ, thus establishing potential physiologic relevance to results obtained with PMA and MEZ. The data support the concept that C kinase plays a role in regulating carrier facilitated hexose transport.

MATERIALS AND METHODS

PMA, L-glucose, D-glucose, cytochalasin B, 1,2-diolein (Sigma Chemical Co., St. Louis, MO); A23187 (Calbiochem); FMLP (Peninsular Labs, Inc., San Carlos, CA); mezerein (Chem. for Cancer Research, Eden, MN) were purchased as indicated. Phosphate buffered saline, 0.9 mM Ca++/0.5mM Mg++ was employed in all assays.

Preparation of Glycerols:

OAG and EEG were purified and confirmed as described (13). For synthesis of 1-O-hexadecyl-3-O-acetyl-propanediol, the monopotassium akloxide of 1,3-propanediol was made by reaction of dry 1,3-propanediol (Aldrich Chemical Co.) with 1 molar equivalent of clean potassium metal by refluxing in xylene. After the reaction was complete, the light yellow solution was cooled to 70°C and 1 molar equivalent of hexadecyl methane sulfonate in zylene was added over a 30 min period. The mixture was then refluxed 12 hr. The solution was then cooled to room temperature, diluted with 5 volumes of ethyl ether and washed with the same volume of water. The ether phase was separated, the aquious phase was extracted with another equal volume of ether and the ether extracts combined. The solvent was removed and the product dried over P_2O_5. The 1-O-hexadecyl derivative of the propanediol was acetylated by reaction with a large excess of acetic anhydride in trimethylamine at room temperature for 6 hr. The final product was purified by thin-layer chromatography (Silica Gel G; developed in hexane/ethyl ether 1:1, v/v; Rf 0.5). For 1-O-hexadecyl-2-O-acetyl-propanediol, the 2-O-acetyl analog was synthesized in the same manner as the 3-O-acetyl analog with minor alterations. 1,2-Propanediol (Aldrich Chemical Co.) was reacted with 2 molar equivalents of clean potassium metal; 1 molar equivalent of hexadecyl methane sulfonate was added slowly over a 6 hr period. This procedure yielded predominately the 1-O-hexadecyl derivative with a minor amount of 2-O-hexadecyl derivative. The 1-O-hexadecyl derivative was purified from the mixture by thin-layer chromatography and acetylated in the same manner as described above; the final product was purified by thin-layer chromatography. All products were stored at -70°C in hexane. Before use, glycerols were freed of hexane under a stream of nitrogen and taken up in Hanks' buffer containing 2.5 mg/ml bovine serum albumin. All other stimuli were also dissolved in the same buffer. Stimuli and glycerols were diluted in the same buffer before being individually added to PMN suspensions. Glycerols were suspended in 250 µg of bovine serum albumin per ml. PMA was suspended in ethanol (.003%).

Preparation of PMNL

Heparinized blood was obtained from normal volunters and leukocyte-rich plasma was obtained by gravity sedimentation over Ficoll/diatrizoate (Isolymph, Gallard-Schlesinger, Carle Place, NY) as described (2). Leukocytes were centrifuged at 150 x g for 8 min, resuspended in Dulbecco's phosphate-buffered saline, layered over Isolymph, and centrifuged at 400 x g for 10 min. The granulocyte pellet was resuspended and washed by centrifugation in phosphate-buffered saline. The final preparations contained >90% PMN, the contaminating cells were eosinophils and lymphocytes. There were less than 2 platelets per 100 PMN in the preparations.

Assay of Hexose Uptake

[^{3}H]dGlc (specific activity, 50 Ci/mol; 1 Ci = 3.7 x 10^{10} becquerels) (New England Nuclear) was diluted to 50 µCi/ml in H_2O and stored at -20°C. As required, aliquots were further diluted to 5 µCi/ml with phosphate-buffered saline. Quadruplicate assays were performed in 1.5 ml polypropylene test tubes (Eppendorf, Brinkmann) in a final volume of 0.3 ml containing 2 x 10^5 PMN in phosphate-buffered saline/0.9 mM Ca++/0.5 mM Mg++. PMN were preincubated for 15 min with substances to be tested (putative stimuli and inhibitors) at 37°C in a shaking waterbath. Uptake was initiated by addition of 0.5 µCi [^{3}H]dGlc (10 nmol, with a total activity of 388,000 cpm) uptake was stopped after 60 min by addition of 1 ml of iced phosphate-buffered saline, and the samples were then centrifuged (Beckman Microfuge B) at 9000 x g for 20 sec. The pellets were washed with 1 ml of iced phosphate-buffered saline and again centrifuged. The supernatant was removed by aspiration, the pellet and tube were placed in scintillation vials containing 10 ml of Aquasol (New England

Nuclear), and radioactivity was determined on a Beckman scintillation spectrometer (Beckman, Palo Alto, CA). Values were expressed as cpm/2 x 10^5 PMN.

RESULTS AND DISCUSSION

OAG, PMA, and MEZ markedly enhanced the uptake of ^{3}HdGlc by human PMN (Figure 1). The increments were comparable to that observed with other agonists for ^{3}HdGlc transport (1,2,3). Uptake was structurally specific since DAG analogues, 1,2-diolein, and EEG and 1,2- and 1,3-PAP were completely inactive (Figure 2). Uptake was linear for 60 minutes (data not shown). OAG, PMA, and MEZ were nontoxic to PMN at the concentrations employed as measured by trypan blue exclusion.

^{3}HdGlc has been used as a measure of glucose transport because it enters cells at the same site as glucose by mechanisms with characteristics compatible with carrier-facilitated transport (14). Such stimulation of carrier facilitated transport of hexoses by insulin is characterized by being saturable, by inhibition by D-glucose, but not the L-glucose, and by inhibition with cytochalasin B, which competes with glucose on the transport glycoprotein. Our previous studies have indicated that deoxyglucose uptake in PMN similarly involves

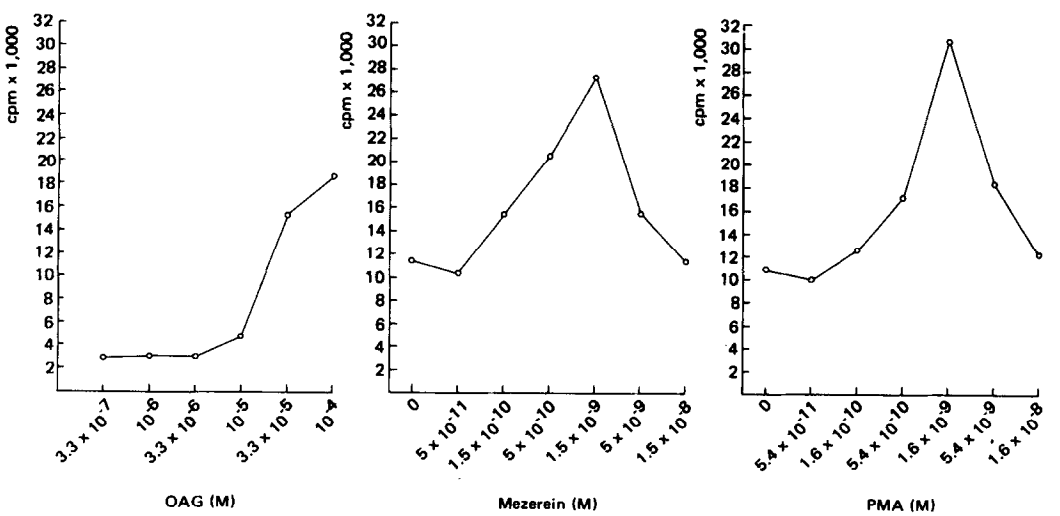


Figure 1: The effect of varying concentrations of C kinase activators OAG, MEZ, and PMA on hexose transport in human PMN. [^{3}H]dGLc was incubated with PMN at 37°C for 60 minutes and uptake expressed in cpm [^{3}H]dGlc per 2 x 10^5 PMN. Results represent one of at least three experiments with similar results, each performed in quadruplicate.

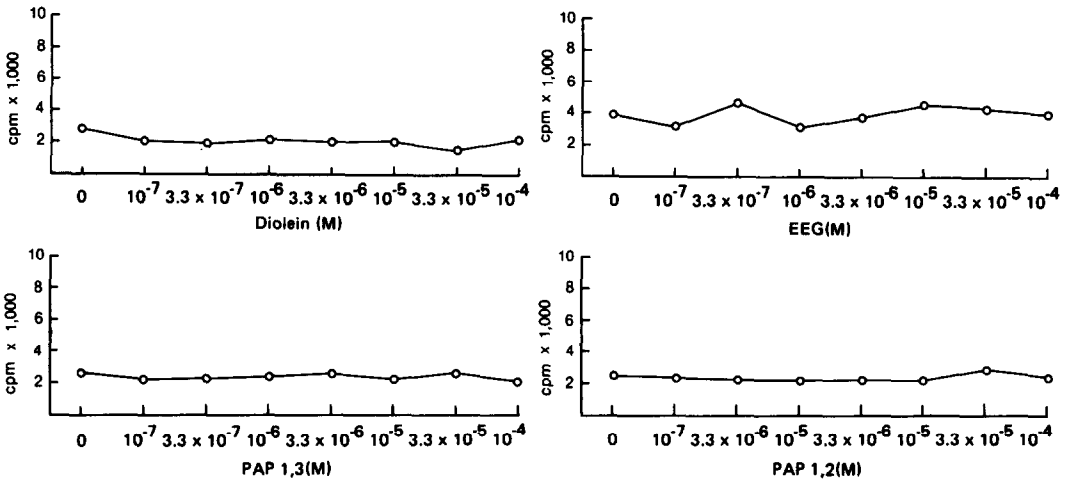
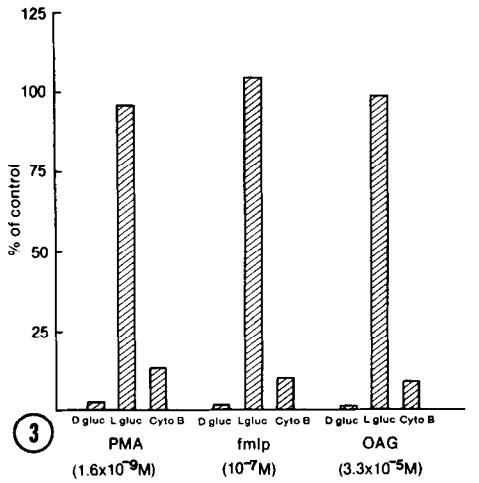


Figure 2: The effect of varying concentrations of DAG analogues EEG; 1,3 PAP; 1,2 PAP; and 1,2 diolein on hexose transport in human PMN. [^{3}H]dGLC was incubated with PMN at 37°C for 60 minutes and uptake expressed as cpm [^{3}H]dglc per 2 x 10^5 PMN. Results represent one of at least three experiments with similar results, each performed in quadruplicate.

carrier-facilitated transport and it is not dependent on extracellular calcium or magnesium (2). Substantiation of transport results using O-methyl glucose cannot be performed in PMN since this compound is freely permeable and is not taken in by PMN by carrier facilitated transport (unpublished observations). Figure 3 indicates that the enhanced uptake of ^{3}HdGlc by OAG and PMA was carrier facilitated since it was completely inhibited by 10 mM D-glucose and by cytochalasin B (5 μg/ml) but was unchanged by 10 mM L-glucose. Phloretin also inhibites uptake (unpublished observations).

TFP inhibits C kinase (15). Figure 4 demonstrates inhibition of OAG and PMA-induced uptake of ^{3}HdGlc uptake between 1-30 μM, concentrations which did not cause cell death; C-kinase is inhibited by similar concentrations (15). Further, TFP inhibits ^{3}HdGlc transport induced by FMLP and A23187 suggesting these agonists may also induce hexose transport via C kinase (Figure 4).

Beyond the clear findings that OAG, PMA, and MEZ stimulate hexose transport in concentrations which can directly activate C kinase in PMN, our data do not unambiguously implicate C kinase in the stimulus-coupling response of hexose transport. For example, intercalation of OAG, PMA, or MEZ into the membrane causing altered membrane fluidity and altered transport could occur.



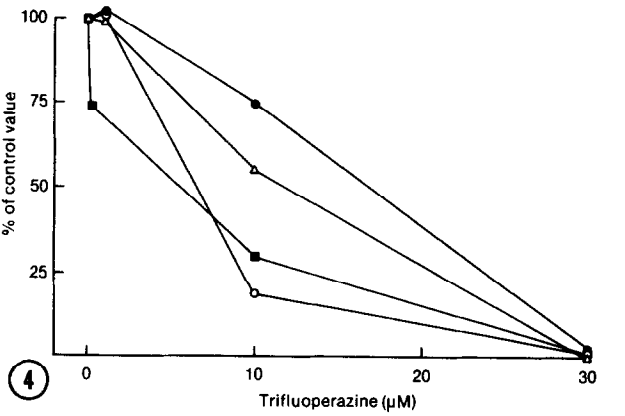


Figure 3: The effects of cytochalasin B (5 μg/ml), D-glucose (10 mM) and L-glucose (10 mM) on hexose transport in human PMN following stimulation by PMA, FMLP, or OAG. Results are expressed as percent of control. Results in cytochalasin B-treated PMN represent non-carrier mediated uptake.

Figure 4: Inhibition of hexose transport by varying concentrations of TFP. PMN were preincubated for 15 minutes with TFP and then stimulated with OAG (○—○, 3.3 x 10^{-5} M); PMA (■—■, 1.6 x 10^{-9} M); FMLP (●—●, 1 x 10^{-7} M); and A23187 (△—△, 1 x 10^{-7} M). By trypan blue exclusion, >95% of PMN were viable at all concentrations of TFP.

However, recent findings strongly argue against nonspecific effects on membranes by DAG: DAG interacts with C kinase partially purified from rat retina with stereospecificity for 1,2-sn-glycerol (16). This observation coupled with our observations that EEG, 1,2 and 1,3 PAPs, and 1,2-diolein do not enhance ^{3}HdGLC transport further support specificity.

Our findings indicate that activation of C kinase in PMN is sufficient to trigger enhanced hexose transport. More data, such as studies of specific phosphorylation reactions, will be required to determine whether C kinase activation is necessary for hexose transport in PMN. However, the inhibition by TFP of hexose transport induction by both OAG and PMA and by the agonists FMLP and A23187 supports the notion that C kinase is both sufficient and necessary for carrier facilitated hexose transport in human PMN.

These concepts may be extrapolated to stimulation of hexose transport in insulin sensitive tissues. Insulin rapidly leads to the metabolism of PI with the formation of ITP and DAG (17). DAG would be expected to activate C kinase. Moreover, A23187 mimics effects of insulin both in PMN (2) and in

insulin sensitive tissues (18). Finally, a role for an intracellular kinase in insulin-sensitive glucose transport has been proposed recently (18). We suggest C kinase serves such a role.

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