Hellman, B. (1977) *Proc. FEBS Meet. 42*, 41.
Hellman, B., Sehlin, J., & Täljedal, L.-B. (1976) *Science (Washington, D.C.) 194*, 1421.
Herman, L., Sato, T., & Hales, C. H. (1973) *J. Ultrastruct. Res. 42*, 298.
Horrocks, W., Jr., & Sudnick, D. (1979) *J. Am. Chem. Soc. 101*, 334.
Horrocks, W., Jr., & Collier, W. (1981) *J. Am. Chem. Soc. 103*, 2856.
Horrocks, W., Jr., Holmquist, B., & Vallee, B. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4764.
Howell, S. L., Young, D. A., & Lacy, P. E. (1969) *J. Cell Biol. 41*, 167.
Howell, S. L., Montague, W., & Tyhurst, M. (1975) *J. Cell Sci. 19*, 395.
Howell, S. L., Tyhurst, M., Duvefelt, H., Andersson, A., & Hellerstrom, C. (1978) *Cell Tissue Res. 188*, 107.
Hudecek, J., Anzenbacher, P., & Kalous, V. (1979) *FEBS Lett. 100*, 374.
Klöppel, G., & Bommer, G. (1979) *Diabetes 26*, 585.
Kohnert, K.-D., Hahn, H. J., Gylfe, E., Borg, H., & Hellman, B. (1979) *Mol. Cell. Endocrinol. 16*, 205.
Kropp, J. L., & Windsor, M. W. (1965) *J. Chem. Phys. 42*, 1599.
Kropp, J. L., & Windsor, M. W. (1966) *J. Chem. Phys. 45*, 761.
Malaisse, W. J., Herchuelz, A., Levy, J., Somers, G., Devis, G., Ravazzola, M., Malaisse-Lagae, F., & Orci, L. (1975) in *Calcium Transport in Contraction and Secretion* (Carafoli, E., Ed.) p 211, North-Holland, Amsterdam.
Matthews, B. W., & Weaver, L. H. (1974) *Biochemistry 13*, 1719.
Milthorpe, R. K., Nichol, L. W., & Jeffrey, P. D. (1977) *Biochim. Biophys. Acta 495*, 195.
Moews, P. C., & Kretsinger, R. H. (1975) *J. Mol. Biol. 91*, 229.
Nieboer, E. (1975) *Struct. Bonding (Berlin) 22*, 1.
Pihl, E. (1968) *Acta Pathol. Microbiol. Scand. 74*, 145.
Pitts, J. E., Wood, S. P., Horuk, R., Bedarker, S., & Blundell, T. L. (1980) in *Insulin Chemistry, Structure and Function of Insulin and Related Hormones*, p 63, de Gruyter, New York.
Ravazzola, M., Malaisse-Lagae, F., Amherdt, M., Perrelet, A., McManse, W. J., & Orci, L. (1976) *J. Cell Sci. 27*, 107.
Reuben, J. (1975) *Naturwissenschaften 62*, 172.
Reuben, J. (1977) in *Calcium Binding Proteins* (Wasserman, R. H., Ed.) pp 21–28, North-Holland, New York.
Rhee, M., Horrocks, W., Jr., & Kosow, D. (1982) *Biochemistry 21*, 4524.
Schäfer, H.-J., & Klöppel, G. L. (1974) *Virchows Arch. A: Pathol. Anat. Histol. 362*, 231.
Schlichtkrull, J. (1956) *Acta Chem. Scand. 10*, 1455.
Sudmeier, J. L., Bell, S. J., Storm, M. C., & Dunn, M. F. (1981) *Science (Washington, D.C.) 212*, 560.
Wang, C. A., Leavis, P. C., Horrocks, W. DeW., Jr., & Gergely, J. (1981) *Biochemistry 20*, 2439.
Wollheim, C. B., Kicuchi, M., Renold, A. E., & Sharp, G. W. G. (1978) *J. Clin. Invest. 801*, 451.

Connective Tissue Activation: Stimulation of Glucose Transport by Connective Tissue Activating Peptide III†

C. William Castor,*,‡ Anne M. Furlong,‡ and Christin Carter-Su§
Rackham Arthritis Research Unit, Department of Internal Medicine, and Department of Physiology, The University of Michigan Medical School, Ann Arbor, Michigan 48109
Received May 3, 1984; Revised Manuscript Received October 5, 1984

ABSTRACT: Connective tissue activating peptide III (CTAP III), a human platelet derived growth factor, induced marked stimulation of 2-deoxy[^{14}C]glucose (2dG) uptake in cultures of human synovial cells, chondrocytes, and dermal fibroblasts. Cytochalasin B (2×10^{-5} M) blocked the mediator-induced increase in 2dG uptake; phlorhizin (8×10^{-4} M) partially inhibited this process. When cells were exposed to CTAP III (4×10^{-6} M) for 30 min prior to uptake assay, 2dG uptake was stimulated by 30–110%; greater stimulation (400–800%) occurred following 17–40-h preincubation with the mediator. A 17-h exposure to CTAP III similarly stimulated 3-*O*-methylglucose uptake by over 400%, suggesting that CTAP III stimulated 2dG uptake is mediated via changes in hexose transport. Cycloheximide clearly prevented the 17-h effects of CTAP III on 2dG uptake. Insulin (3×10^{-6} M) stimulated 2dG uptake 40–70% after 30-min preincubation with hormone; little effect was seen after 17-h preincubation. These data suggest that CTAP III stimulates glucose transport shortly after addition to target cells; the major stimulation observed after a 17-h incubation is consistent with the synthesis of new glucose transport protein.

The biologic activities of a human platelet derived growth factor, connective tissue activating peptide III (CTAP III), including promotion of DNA synthesis, stimulation of hyaluronic acid synthetase activity and hyaluronic acid synthesis, stimulation of sulfate incorporation into proteoglycans, and stimulation of prostaglandin E_2 (PGE_2) synthesis, plasminogen activator secretion, increased glucose uptake, and lactate formation (Castor et al., 1977, 1979; Sisson et al., 1980; Castor & Whitney, 1978; Castor & Pek, 1981; Ragsdale et al., 1982). The covalent structure of CTAP III has been reported (Castor et al., 1983b), and the evidence indicates that the N-terminal tetrapeptide and at least one of the two intrachain disulfide bonds are required for the biologic activity of this protein. While the significance of this molecule in human biology is

† This work was supported by U.S. Public Health Service Grants AM-10728 and AM-30516 and by the Michigan Chapter Arthritis Foundation. This is paper 29 in the series.
‡ Department of Internal Medicine.
§ Department of Physiology. Recipient of a Career Development Award from the Juvenile Diabetes Association.

0006-2960/85/0424-1762$01.50/0

yet unclear, its range of activities and the fact that it is secreted with the platelet α granule during physiologic platelet aggregation and release suggest a role in normal phenomena such as wound healing (Castor, 1981) as well as in disease processes involving chronic inflammation (Castor et al., 1979) and possibly in atherosclerotic vascular disease (Ross, 1976). Indeed, the plasma level of CTAP III/β-thromboglobulin (β-TG) antigen is known to be elevated in active rheumatoid arthritis, systemic lupus erythematosus, and other rheumatic afflictions (Myers et al., 1980 MacCarter et al., 1981).

A puzzling aspect of the mechanism of action of CTAP III has been the observation that the onset of the biological activities listed above tends to be delayed by 4–8 h after cell contact with the agonist. The possibility that changes in the membrane transport processes might be important early events seemed worthy of examination, particularly in view of a report that fibroblast growth factor (FGF) stimulated amino acid transport in mouse 3T3 cells within 2 min (Quinlan & Hochstadt, 1977). Further, epidermal growth factor (EGF) is known to stimulate glucose transport in these cells within 15 min (Barnes & Colowick, 1976).

The present study was designed to accomplish the following objectives: (1) to measure the effect of CTAP III on hexose transport in human connective tissue cells derived from synovium (from normal and arthritic individuals), cartilage, and skin; (2) to establish the time frame of putative biologic activity; and (3) to assess whether the effect(s) on hexose transport is (are) dependent upon protein synthesis.

Materials and Methods

Cell Cultures. Human synovial membrane was obtained at arthrotomy or amputation; a portion was fixed for routine histology, and the remainder was divided into small fragments to generate cell cultures by methods reported earlier (Castor, 1971). Synovial cell lines were derived from normal and rheumatoid synovial membrane specimens and from patients with degenerative joint disease or traumatic synovitis. Synovial cells from these latter two entities have similar growth and biochemical parameters (Castor, 1971). Fibroblasts from normal human dermis were grown in monolayer cultures from explants as previously described (Castor et al., 1962). Routine growth medium for connective tissue cells contained 80% synthetic medium 1066, 10% newborn calf serum (NBS), 5% fetal calf serum (FCS), and 5% heat-inactivated (56 °C, 1 h) human serum and was supplemented by L-glutamine, antibiotics, 0.02 M *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer, and Na_2CO_3. Cells were enumerated and sized with an electronic cell counter (Coulter Electronics, Hialeah, FL). Cells prepared for frozen storage after trypsin dispersal were resuspended in standard growth media containing 8% sterile dimethyl sulfoxide (Fisher Scientific Co., Fairlawn, NJ) at a concentration of (2.0–4.0) × 10^6 cells/mL. Human cell lines were recovered from frozen storage (−75 to −80 °F) for use in these studies as needed by quick thawing at 37 °C and immediate plating in fresh medium.

Chondrocyte cultures were derived from residual cartilage dissected from articular surfaces of patients undergoing joint replacement surgery. Cartilage was cut into small fragments and incubated with bacterial collagenase (Sigma type II, 300 units/mg), 4.0 mg/mL Eagle's synthetic medium containing 5% NBS, and antibiotics. Fragments were agitated in this solution for 2–16 h at 37 °C; cells released were washed twice with medium and plated in glass or plastic vessels containing CMRL 1066 (80%), NBS (10%), FCS), and human serum (HmS) 5%. Sequential 1- or 2-h digestions with collagenase yielded further harvest of viable chondrocytes.

Glucose Uptake Assay Using 2-Deoxy[^{14}C]glucose. (*A*) *Reagents.* 2-Deoxy-D-[^{14}C]glucose, (282.0 mCi/mmol) was purchased from New England Nuclear; Earle's balanced salt solution and Eagle's synthetic medium (ESM) were obtained from Grand Island Biological Co., and fetal calf serum (FCS) was purchased from Reheis. Human albumin (essentially fatty acid free), Hepes buffer, phlorhizin, and bovine insulin were purchased from Sigma Chemical Co. Indocin was a gift from Merck Sharp & Dohme. CTAP III, a growth factor from human platelets, was isolated as described earlier (Castor et al., 1977, 1979, 1983b). Several CTAP III preparations were used over the course of these studies; specific biologic activity is known to vary for reasons not yet clear.

(*B*) *Design of 2-Deoxy[^{14}C]glucose Uptake Experiments.* Cells were plated in polystyrene culture plates containing multiple flat-bottom wells (COSTAR 3596; Data Packaging, Cambridge, MA; growth surface area per well = 0.32 cm^2) at a density of 20 000 cells/well. Each well contained 100 μL of medium composed of 97% ESM and 3% FCS supplemented with Hepes buffer, L-glutamine, penicillin, streptomycin, sodium carbonate, erythromycin, and gentamicin (ESM_{97} FCS_3).

(*C*) *Preincubation with Mediator.* Plates were incubated 20–24 h at 37 °C to allow attachment and spreading of cells; then 20 μL of test materials was added to each well without medium change. The plates were incubated at 37 °C for a designated period of time (usually 30 min or 17 h) in the presence or absence of 40 μg/mL CTAP III or 3 μM insulin; after this incubation, medium was aspirated from each well.

(*D*) *Uptake Procedure.* Cell sheets in the wells were washed once with warm (37 °C), sterile, Earle's solution without glucose (supplemented with Hepes buffer and sodium carbonate), and 50 μL of 2-deoxy[^{14}C]glucose (2 μCi/mL in Earle's solution without glucose) was added to each well. Plates were incubated at 37 °C for a specified period of time, and the assay was terminated by aspirating the assay medium and washing the cells rapidly (3 times) with Earle's solution without glucose (4 °C). In some experiments, cytochalasin B (2×10^{-5} M) or phlorhizin (8×10^{-4} M) was included in the 2dG assay medium. After the uptake period and wash of the cell sheet, 100 μL of 0.3 N NaOH was added to each well, and the plates were incubated a further 1.5 h at 37 °C. The cell lysate was then spotted onto 1.5-in. squares of Whatman 3MM chromatography paper, dried, and counted in a Beckman 7000 liquid scintillation counter using a toluene/1,4-bis[2-(5-phenyloxazolyl)]benzene–2,5-diphenyloxazole (POPOP–PPO) system. Values reported are the mean ±1 SD of triplicate wells.

(*E*) *Design of 3-O-Methylglucose Uptake Studies.* The transport characteristics of 3-*O*-methylglucose (3-OMG) were examined in a fashion similar to that described for the 2dG assay. Cells (80 000/well) were plated in 24-well COSTAR culture plates in 500 μL of ESM_{97} FCS_3. After 24 h at 37 °C, the medium was replaced, and 100 μL of 0.15 M NaCl containing CTAP III, or 0.15 M NaCl alone, was added to each well; the cultures were then incubated for 17 h. Medium was then aspirated and each well washed once with 1.5 mL of Earle's solution without glucose at 21 °C; this was allowed to stand at room temperature for 15 min before initiation of the uptake study. The wash solution was then removed, and Earle's solution without glucose (250 μL) containing 3-*O*-[*methyl*-^{14}C]methyl-D-glucose (2 μCi/mL) (Pathfinder's Laboratory) and unlabeled 3-OMG (0.2 mM) were added as each well was individually studied. Each microculture was incubated with 3-OMG for the designated time at 21 °C;

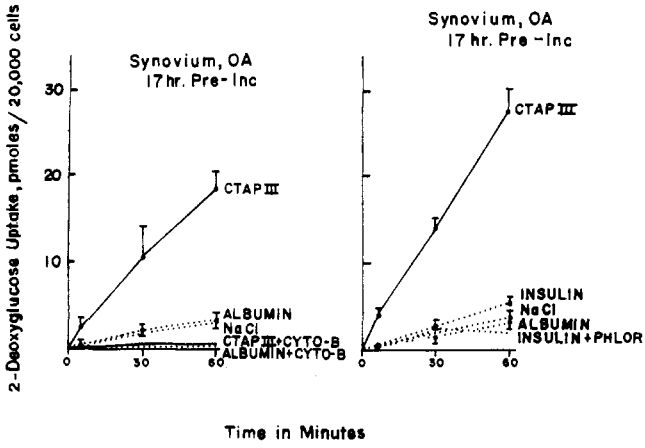

FIGURE 1: Stimulation of 2dG uptake in synovial cells by CTAP III and insulin. Cultured synovial cells from a patient with osteoarthritis (OA) were incubated for 17 h in the absence or presence of 40 μg/mL CTAP III or 3 μM insulin. Cytochalasin B (2×10^{-5} M) or phlorhizin (8×10^{-4} M) was added at the time transport was initiated. Control substances, in this and subsequent figures, were albumin (40 μg/mL) and NaCl (0.15 M).

uptake was terminated by aspirating the medium and washing the wells 3 times, rapidly, with Earle's solution containing cytochalasin B (5×10^{-6} M), but without glucose, at 4 °C, and then 200 μL of 0.1% sodium dodecyl sulfate (SDS) was added to each well. The assay termination procedure required approximately 5 s per well. To control for trapping of isotope, a group of microcultures was exposed to isotope at 4 °C; isotope was removed immediately and the cell sheet washed rapidly 3 times at 4 °C. Cytochalasin B was present in the isotope solution as well as the washing solution (Earle's without glucose). After the cells were incubated with SDS for 2.0 h at 37 °C, 150 μL of lysate was spotted on paper and counted in a scintillation counter. Values reported are the mean ± SE of triplicate wells.

RESULTS

Uptake of 2dG in Activated Connective Tissue Cell Cultures. Previous studies showed that incubation of human synovial cultures with CTAP III and other connective tissue activating peptides for 16–18 h leads to near-maximal activation of these cells in terms of capacity for synthesis of hyaluronic acid, proteoglycans, and prostaglandin E_2. In order to determine whether this activation might be related to or parallel an increase in the glucose transport capability of these cells, human synovial cells were incubated with CTAP III for 17 h prior to measurement of 2dG transport (Figure 1). A marked (4–8-fold) stimulation of 2dG uptake was observed in CTAP III treated cells compared to saline-treated controls. This stimulated rate of transport was not observed when transport was measured in the presence of cytochalasin B (Figure 1, left panel), suggesting that CTAP III alters protein-mediated transport rather than nonspecific diffusion. The stimulation was specific for CTAP III, since addition of a large amount of another protein (albumin) did not alter 2dG uptake. This increased rate of uptake also did not result from increased numbers of cells, since the amount of cell protein remains the same for control and CTAP III treated cells (data not shown). The effect of insulin on glucose transport in these cells following a 17-h incubation (Figure 1, right panel) was not statistically significant ($p > 0.05$ and $p < 0.10$); this compares to the 4–8-fold stimulation induced by CTAP III. Both mediators were present in the assay medium.

Cultured synovial connective tissue cells from normal individuals and patients with osteoarthritis (OA) and rheumatoid

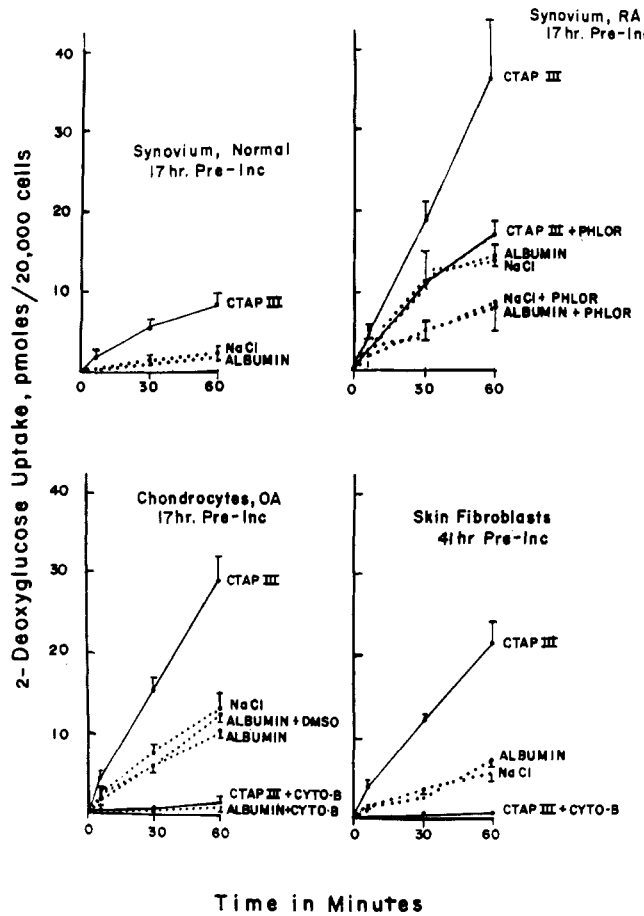

FIGURE 2: Effect of CTAP III on 2dG uptake in cultured synovial cells, chondrocytes, and skin fibroblasts. Cultured synovial cells from (top left panel) normal joints and (top right panel) patients with rheumatoid arthritis and (bottom left panel) chondrocytes from patients with osteoarthritis were incubated with CTAP III for 17 h prior to assay. Skin fibroblasts were incubated with CTAP III for 41 h prior to assay (bottom right panel). Dimethyl sulfoxide (Me_2SO) (0.2% v/v in the wells) was used as a solvent for cytochalasin B; this was controlled by showing no difference in albumin with and without Me_2SO.

arthritis (RA), chondrocytes from osteoarthritic patients, and normal skin fibroblasts all showed time-dependent 2dG uptake (Figure 2). The variation in uptake rates between cell lines was no greater than the variation observed when different batches of a particular cell line were plated on different days. Because of day to day variability, the 2dG uptake rates and relevant variables were evaluated simultaneously within the format of a single assay to reduce unexplained variations related to the cell culture process. The result observed with activated normal synovial cells was replicated with rheumatoid synovial cells, osteoarthritic chondrocytes, and normal skin fibroblasts as shown in Figure 2. Stimulated uptake of the glucose analogue 2dG was inhibited by cytochalasin B (Figure 2, bottom panels) and substantially reduced by the addition of phlorhizin (Figure 2, top right panel).

The experiments described above employed 33–40 μg of CTAP III/mL, concentrations shown to induce maximal activation in other systems. It is pertinent to note, however, that lesser concentrations of CTAP III also stimulated 2dG uptake (Figure 3). As shown in Figure 3, increasing concentrations (0.3–3.0 μg of CTAP III/mL preincubated with synovial cells for 17 h resulted in progressive enhancement of 2dG uptake.

Because 2dG is phosphorylated by cells, it was important to distinguish whether CTAP III stimulated hexose transport or hexokinase activity. The transport of 3-OMG (a nonme-

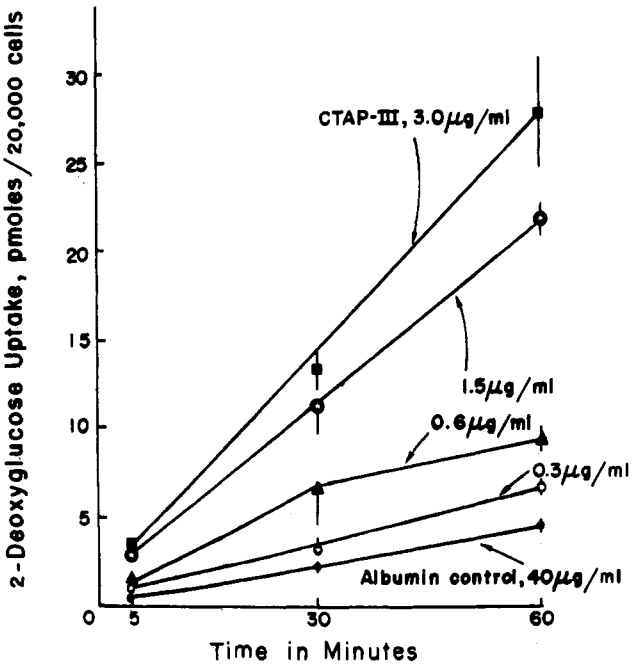

FIGURE 3: Dose dependence of CTAP III effect on 2dG uptake. Synovial cells from a patient with OA were incubated for 17 h with the designated concentration of CTAP IH.

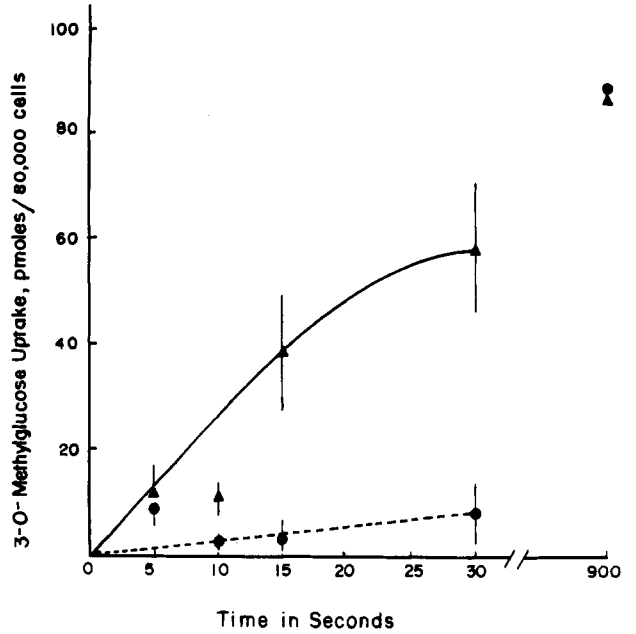

FIGURE 4: CTAP III stimulation of 3-OMG uptake. OA synovial cells were incubated for 17 h with CTAP III prior to the transport assay. The amount of radioactivity associated with control and CTAP III treated cells was the same after 30 min.

tabolized analogue of D-glucose) was measured in control and CTAP III activated synovial cell cultures (Figure 4). These studies showed marked enhancement (10-fold) of labeled 3-OMG uptake by activated cells compared to control cells. Although we cannot rule out an effect of CTAP III on hexokinase activity, the fact that CTAP III stimulates 3-OMG at least as much or more than 2dG uptake (observed in Figures 1–3) strongly suggests that the CTAP III induced stimulation of 2dG uptake represents increased hexose transport.

In order to determine whether CTAP III alters the maximal rate of 2dG uptake (V_{max}) or the concentration at which the half-maximal rate of 2dG uptake is obtained (K_t), initial rates of 2dG uptake (V_i) were estimated by using synovial cells treated with CTAP III or its vehicle for 17 h. The initial rates

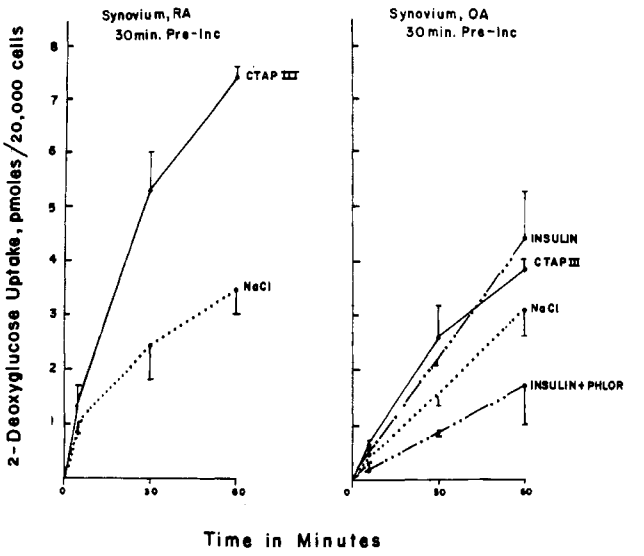

FIGURE 5: CTAP III stimulated and insulin-stimulated 2dG uptake after 30-min incubation. Synovial cells from patients with rheumatoid arthritis and osteoarthritis were incubated with CTAP III and/or insulin for 30 min prior to assay.

of uptake were estimated from the linear portion of the 2dG uptake curves determined from multiple early time points (2, 5, and 10 min) at 2dG concentrations of 0.125, 0.1, 0.5, 1, 5, and 10 mM. These initial entry rates were corrected for diffusional entry by subtracting the rate of 2dG uptake in the presence of cytochalasin B (20 μM) at each concentration of 2dG. These rates (V_i) were graphed vs. $V_i/[2dG]$ (Eadie–Hofstee plot), and the V_{max} (y intercept) and K_t (negative slope) were determined by using linear regression analysis. In one experiment, the calculated V_{max} of control cells was 13 pmol min^{-1} (20 000 cells)$^{-1}$, and the K_t was 2.7 mM. These values compared to a V_{max} of 37 pmol min^{-1} (20 000 cells)$^{-1}$ and a K_t of 1.1 mM for CTAP III treated cells. In a second experiment, the V_{max} of control cells was calculated to be 19 pmol min^{-1} (20 000 cells)$^{-1}$ and the K_t was 4.2 mM compared to a V_{max} of 102 pmol min^{-1} (20 000 cells)$^{-1}$ and a K_t of 5.8 mM. Thus, in both experiments, CTAP III significantly increased the V_{max} of transport. K_t was not altered in a consistent fashion.

Evidence for Increased 2dG Uptake as an Early Event in Connective Tissue Cell Activation. In order to determine whether this large stimulation of hexose transport occurred as an early or delayed event following CTAP III addition to cells, synovial cells were incubated in the presence and absence of CTAP III for only 30 min prior to 2dG transport measurements (Figure 5, left panel). CTAP III caused a small initial increment in 2dG uptake after as little as 30-min preincubation. This modest early stimulation of 2dG uptake was seen in 13 of 14 experiments. In contrast to the large difference between the transport effects caused by CTAP III and insulin in the 17-h incubations (Figure 2, top right panel), the short-term effects of these two agents were quantitatively similar (Figure 5, right panel). The early effect of both CTAP III (data not shown) and insulin (Figure 5, right panel) was inhibited by phlorhizin.

Protein Synthesis Requirement for Increased Carbohydrate Uptake in Activated Fibroblast Cultures. To test whether protein synthesis was required for CTAP III action on 2dG transport, synovial cells were incubated for 17 h with CTAP III in the presence and absence of cycloheximide (Figure 6). Control cells to which cycloheximide was added generated virtually the same 2dG uptake curve as that shown for CTAP III plus cycloheximide (data not shown); no morphologic

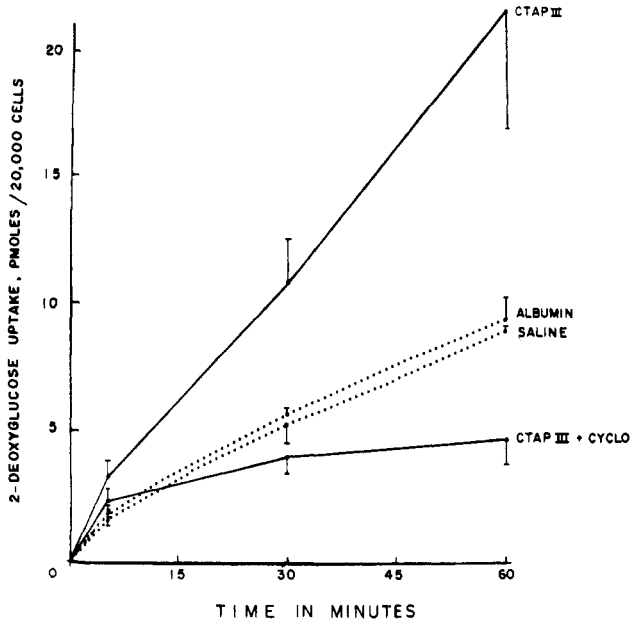

FIGURE 6: Cycloheximide-prevented CTAP III stimulation of 2dG uptake. Synovial cells (RA) were incubated with CTAP III in the presence or absence of cycloheximide (10 μg/mL) for 17 h prior to assay. Vehicles with cycloheximide exhibited the same 2dG uptake profile as CTAP III and cycloheximide.

changes from cycloheximide were noted. Clearly, cycloheximide prevented any stimulation of 2dG uptake by CTAP III, suggesting that CTAP III may increase hexose transport in the activated cell cultures by increasing the synthesis of glucose transport proteins. Whether or not the very early stimulation of 2dG uptake noted in Figure 5 requires protein synthesis is not yet clear. While a 30-min preincubation with cycloheximide does not reduce CTAP III stimulated 2dG transport rates to control levels, interpretation is complicated by the finding that a 30-min incubation with cycloheximide alone increases hexose transport (data not shown).

Discussion

The glucose transport system in representative human connective tissue cell types, including synovial cells, chondrocytes, and skin fibroblasts, appears to be a facilitated diffusion system (Figures 1–6) similar to that found in other nonepithelial cell types. The transport system has an affinity for both 3-OMG and 2dG and is inhibited by both cytochalasin B and phlorhizin, with cytochalasin B being the more potent inhibitor. In 17-h incubations, CTAP III stimulates the V_{max} of glucose transport in these cell types by a mechanism that requires protein synthesis (Figure 6); whether the 30-min stimulation of transport by CTAP III also requires protein synthesis is unclear. Thus, stimulation of glucose transport by CTAP III has properties in common with the enhanced glucose transport seen in response to glucose starvation or viral transformation (increased V_{max}, delayed onset, dependence on protein synthesis) (Kletzien & Perdue, 1975; Pessin et al., 1982; Kawai & Hanafusa, 1971; Salter & Weber, 1979; Klip, 1982). In contrast, insulin causes a rapid (within minutes) increase in transport which is independent of protein synthesis (Klip, 1982).

That enhanced hyaluronic acid (HA) synthesis induced by a connective tissue activating agent (as CTAP III) is dependent on adequate glucose concentrations in the target cell's microenvironment (Castor, 1972) is hardly surprising. Incremental energy is required for enhanced synthesis of extracellular matrix macromolecules, and in the case of hyaluronic acid, the carbon chain of the polymer itself is assembled from glucose without scission of the hexose. Thus, it is reasonable to find that fully activated (CTAP III for 17 h) connective tissue cells, which are committed to enhanced replication and increased glycosaminoglycan synthesis, are supported by marked enhancement in glucose transport capability.

Whether CTAP III is an important regulator of glucose homeostasis in man is uncertain. In the present study, maximally stimulating concentrations of CTAP III appeared to be as effective as maximally stimulating concentrations of insulin in promoting 2dG uptake by human connective tissue cells in short-term assays; when exposure to CTAP III was prolonged, the platelet factor was a more potent regulator of glucose transport than insulin. In considering the physiologic and pathologic implications of these data, it is pertinent to note that roughly 75% of the wet weight of human body mass is of mesenchymal origin and that skeletal tissues, skin, and fascia (excluding muscular components) account for over one-third of the body mass (Dempster, 1955; Vierordt, 1893). The likelihood that CTAP III might have special significance in relation to a large responsive target cell mass clearly also hinges on the ambient concentrations of the agonist and its specific activity. From the average circulating platelet mass (1.2×10^{12} platelets), the mean platelet survival time (10 days), and the platelet content of CTAP III (50 μg of CTAP III/10^9 platelets) (Castor et al., 1981), one may estimate that approximately 6.0 mg of CTAP III is released from platelets daily. While conventional polyclonal antisera do not distinguish between CTAP III and its degradation product, β-TG, they may be used to measure CTAP III/β-TG antigen. If the measured plasma half-time (100 min) (Dawes, 1978) for the CTAP III/β-TG antigen is generally applicable, one may estimate the steady-state concentration of CTAP III/β-TG antigen in plasma and extracellular water to be approximately 30 ng/mL. In fact, the measured plasma levels of the CTAP III/β-TG antigen have been reported as 30.7 ± 13.7 ng/mL (Dawes, 1978) and 29 ± 6 ng/mL (Myers et al., 1980; MacCarter et al., 1981). Although extensive data on CTAP III/β-TG antigen concentrations in extracellular fluid are not available, measurements have been made with synovial fluid, which may be viewed as occupying a specialized connective tissue space. The median concentration of CTAP III/β-TG antigen in synovial fluid from 21 noninflammatory synovial effusions was reported to be 30 ng/mL (Myers & Christine, 1982).

The significance of nanogram concentrations of CTAP III/β-TG antigen in the plasma depends upon at least two kinds of information. The concentration estimates noted above are maximal estimates because there is no evidence yet that the circulating antigen is the active (CTAP III) form rather than the degraded (β-TG) form. Further, normal mean plasma concentrations are low compared to concentrations of CTAP III which stimulate 2dG uptake in fibroblast cultures. On the other hand, active connective tissue diseases characterized by vasculitis frequently manifest elevated plasma CTAP III/β-TG antigen levels in excess of 300 ng/mL (MacCarter et al., 1981), levels which have been shown to stimulate DNA synthesis in adult human connective tissue cells and, in this report, to stimulate hexose transport. Recently, it has become apparent that CTAP III may occur with subtle varieties of molecular heterogeneity (with variable biologic activity) and some subsets of CTAP III may be more potent than others (Castor et al., 1983a).

Thus, while CTAP III might play a role in normal glucose homeostasis, it seems likely that the major effects of CTAP

III would occur at sites of platelet aggregation and granule release (sites of injury) where higher (i.e., greater than 30 ng/mL) concentrations of active factor would be found. It is clear that CTAP III preparations from whole platelets or platelet releasates stimulate human connective tissue cells at microgram per milliliter concentrations (1–50 μg/mL), levels achievable in local microenvironments. We presently favor the idea that CTAP III acts primarily as an autacoid mediator at local sites where substantive platelet aggregation/release processes have given rise to higher concentrations of the agonist.

ACKNOWLEDGMENTS

We are pleased to acknowledge, with thanks, the expert assistance of Barbara King in the preparation of the manuscript and Virginia Castor in the preparation of the figures.

Registry No. CTAP III, 69344-77-0; 2dG, 154-17-6; 3-*O*-methylglucose, 146-72-5; insulin, 9004-10-8.

REFERENCES

Barnes, D., & Colowick, S. P. (1976) *J. Cell. Physiol. 89*, 633–640.
Castor, C. W. (1971) *J. Lab. Clin. Med. 77*, 65–75.
Castor, C. W. (1972) *J. Lab. Clin. Med. 79*, 285–301.
Castor, C. W. (1981) in *Handbook of Inflammation (Tissue Repair and Generation)* (Glynn, L. E., Ed.) pp 177–209, Elsevier North-Holland Biomedical Press, New York.
Castor, C. W., & Whitney, S. L. (1978) *J. Lab. Clin. Med. 91*, 811–821.
Castor, C. W., & Pek, S. (1981) *Arthritis Rheum. 24*, 504–509.
Castor, C. W., Prince, R. K., & Dorstewitz, E. L. (1962) *Lab. Invest. 11*, 703–713.
Castor, C. W., Ritchie, J. C., Scott, M. E., & Whitney, S. L. (1977) *Arthritis Rheum. 20*, 859–868.
Castor, C. W., Ritchie, J. C., Williams, C. H., Scott, M. E., Whitney, S. L., Myers, S. L., Sloan, T. B., & Anderson, B. (1979) *Arthritis Rheum. 22*, 260–272.
Castor, C. W., Cobel-Geard, S. R., Hossler, P. A., & Kelch, R. P. (1981) *J. Clin. Endocrinol. Metab. 52*, 128–132.
Castor, C. W., Hossler, P. A., & Walz, D. A. (1983a) *Clin. Res. 31*, 784A.
Castor, C. W., Miller, J. W., & Walz, D. A. (1983b) *Proc. Natl. Acad. Sci. U.S.A. 80*, 765–769.
Dawes, J., Smith, R. C., & Pepper, D. S. (1978) *Thromb. Res. 12*, 851–861.
Dempster, W. I. (1955) in Wright Air Development Center Technical Report 55-159, p 201.
Kawai, S., & Hanafusa, H. (1971) *Virology 46*, 470–479.
Kletzien, R. F., & Perdue, J. F. (1975) *J. Biol. Chem. 250*, 593–600.
Klip, A. (1982) *Life Sci. 31*, 2537–2548.
MacCarter, D. K., Hossler, P. A., & Castor, C. W. (1981) *Clin. Chim. Acta 115*, 125–134.
Myers, S. L., & Christine, T. A. (1982) *J. Rheumatol. 9*, 6–12.
Myers, S. L., Hossler, P. A., & Castor, C. W. (1980) *J. Rheumatol. 7*, 814–819.
Pessin, J. E., Tillotson, L. G., Yamada, K., Gitomer, W., Carter-Su, C., Mora, R., Isselbacher, K. J., & Czech, M. P. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 2286–2290.
Quinlan, D. C., & Hochstadt, J. (1977) *J. Cell. Physiol. 93*, 237–246.
Ragsdale, C. G., Castor, C. W., Roberts, D. J., & Swartz, K. H. (1982) *Arthritis Rheum. 25*, S100.
Ross, R. (1976) *Triangle (Engl. Ed.) 15*, 45–51.
Salter, D. W., & Weber, M. J. (1979) *J. Biol. Chem. 254*, 3554–3561.
Sisson, J. C., Castor, C. W., & Klavons, J. (1980) *J. Lab. Clin. Med. 96*, 189–197.
Vierordt, H. (1893) *Anatomische, Physiologische und Physikalische Daten und Tabellen zum Gebrauche fur Mediciner*, p 400, Fischer, Jena, West Germany.