Neurochemical Research, Vol. 25, No. 5, 2000, pp. 561–566

Regulation of *Myo*-Inositol Homeostasis in Differentiated Human NT2-N Neurons*

James E. Novak,[1,2] Bernard W. Agranoff,[1,3] and Stephen K. Fisher[1,2,4]

(Accepted August 11, 1999)

We have investigated the possible role of second messengers on inositol homeostasis in NT2-N cells, human central nervous system neurons obtained by terminal differentiation of teratocarcinoma precursors. Uptake of inositol into NT2-N neurons was inhibited ~10% by protein kinase C (PKC) activation but was unaffected by either the presence of cyclic nucleotide analogs or changes in the intracellular concentration of Ca^{2+}. Efflux of inositol from NT2-N neurons was enhanced in hypotonic buffer but virtually eliminated by inclusion of the Cl^- channel blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid, a result which indicates the involvement of a volume-sensitive organic osmolyte-anion channel. Volume-sensitive inositol efflux was stimulated ~30% following activation of PKC or elevation of the cytosolic Ca^{2+} concentration but was unaffected by protein kinase A activation. These results suggest that whereas inositol uptake into NT2-N neurons is relatively refractory to regulation, volume-sensitive inositol efflux may be significantly affected by intracellular signaling events.

KEY WORDS: Inositol uptake; inositol efflux; neuron; second messenger; protein kinase C; calcium.

INTRODUCTION

Volume regulation in the human brain is essential to offset disturbances in blood osmolality. Although acute osmotic stress is countered by fluctuations in the intracellular concentration of electrolytes such as KCl, these fluctuations, if prolonged, would result in enzymatic dysfunction and disruption of membrane excitability (1). Consequently, the brain adapts to persistent osmotic stress by regulating the intracellular concentration of "compatible" organic osmolytes such as *myo*-inositol, sorbitol, taurine, betaine, and glycerophosphorylcholine (2–4). Among these osmolytes, inositol is thought to be one of the most critical in mammalian brain (4,5).

Dysregulation of inositol transport in the brain is implicated in the pathogenesis of a number of clinical disorders. For example, because of decreases in cerebral inositol concentration during prolonged hyponatremia, the rapid correction of blood osmolality with isotonic fluids may result in brain dehydration and myelinolysis; conversely, the rapid correction of chronic hypernatremia is frequently associated with life-threatening cerebral edema (6). These problems may, at times, be circumvented by the gradual correction of plasma tonicity (36 days) (5). Another instance of the pathophysiological role of inositol transport in brain is

[1] Neuroscience Laboratory and Mental Health Research Institute, University of Michigan, Ann Arbor, MI.
[2] Department of Pharmacology, University of Michigan, Ann Arbor, MI.
[3] Department of Biological Chemistry, University of Michigan, Ann Arbor, MI.
[4] Address reprint requests to: Dr. Stephen K. Fisher, 2014 Neuroscience Building, University of Michigan, 1103 E. Huron St., Ann Arbor, MI 48104-1687. Phone: (734) 763-4376 Fax: (734) 936-2690 E-mail: skfisher@umich.edu
* Special issue dedicated to Dr. Nicolas G. Bazan.

0364-3190/00/0500-0561$18.00/0

stroke. Local cerebral ischemia causes cytotoxic neuronal swelling and a reduction in cellular ATP (7). Since inositol efflux requires the nonhydrolytic association of ATP with the volume-sensitive organic osmolyte-anion channel (VSOAC), decreased cellular ATP availability is associated with decreased inositol release and exacerbates neuronal swelling (8).

In spite of its potential clinical relevance, the regulation of inositol transport in neurons of the human central nervous system (CNS) is unknown. Inositol transport is comprised of uptake, which is selective and actively driven by the Na^+/*myo*-inositol cotransporter, and efflux, which is nonselective and passively mediated by the Cl^- channel VSOAC. The diversity of regulatory mechanisms among cell types such as primary astrocyte (9), C6 glioma (10), retinal pigment epithelial (RPE) (11), bovine lens epithelial (12), inner medullary collecting duct (13,14), and Madin-Darby canine kidney (MDCK) (15) cells suggests that the modulation of inositol transport is cell type-specific, although protein kinases A and C as well as intracellular Ca^{2+} have emerged as frequent regulators. Notably, Ca^{2+} is a prime regulator of inositol release from the kidney (16) but plays little or no role in CNS glia (9,10). Since little is known of the regulation of inositol homeostasis in either primary culture CNS neurons or neuroblastoma cells, we have characterized inositol transport in NT2-N neurons. NT2-N cells are obtained from the human testicular teratocarcinoma cell line NT2, which is induced to irreversibly differentiate to a post-mitotic neuronal phenotype by means of retinoic acid treatment (17–19). NT2-N cells have a distinctly neuronal morphology, express a host of CNS neuron-specific proteins (19,20), and form functional synapses (21). We have recently shown that the neuronal differentiation of NT2 cells is accompanied by a 10-fold increase in inositol concentration, a result consistent with an increase in the uptake of inositol and a decrease in the efflux of this polyol (22). The present results indicate that, in these cells, it is primarily the efflux of inositol that is subject to regulation by protein kinase C (PKC) and intracellular Ca^{2+}.

EXPERIMENTAL PROCEDURE

Materials. *myo*-[^{3}H]Inositol (80 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). *myo*-Inositol, 4β-phorbol 12β-myristate 13α-acetate (PMA), 1,2-dioctanoyl-sn-glycerol (C8:0) (DiC8), 4α-phorbol 12,13-didecanoate (αPDD), N^6, 2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (dibutyryl cAMP, db-cAMP), 8-bromoguanosine 3′:5′-cyclic monophosphate (8Br-cGMP), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), L-glutamate, N-methyl-D-aspartate (NMDA), all *trans*-retinoic acid, uridine, 5-fluoro-2′-deoxyuridine, and cytosine β-D-arabinofuranoside were obtained from Sigma Chemical (St. Louis, MO). 2-Butyn-1-ammonium, *N,N,N*-trimethyl-4-(2-oxo-1-pyrrolidinyl) iodide (oxotremorine-M, oxo-M) was from Research Biochemicals International (Natick, MA). Bisindolylmaleimide I (BIM), ionomycin, and 1,2-bis(2-aminophenoxy)-ethane-*N,N,N′,N′*-tetraacetic acid / tetra(acetoxymethyl)-ester (BAPTA/AM) were from Calbiochem (La Jolla, CA). Powdered Dulbecco's modified Eagle's medium (DMEM), 0.25% (wt/vol) trypsin, 10X trypsin-EDTA [0.5% (wt/vol) trypsin, 5.3 m*M* EDTA], 1X Versene (0.2 g/l of EDTA in phosphate-buffered saline), and Hanks' balanced salt solution were purchased from GIBCO (Grand Island, NY). Fetal calf serum was obtained from Summit Biotechnology (Fort Collins, CO). Matrigel basement membrane matrix and poly-D-lysine hydrobromide were from Collaborative Biomedical Products (Bedford, MA). Tissue culture supplies were obtained from Corning Glass Works (Corning, NY) and Sarstedt (Newton, NC). Bicinchoninic acid protein assay reagents were from Pierce (Rockford, IL). UniverSol-ES liquid scintillation cocktail was obtained from ICN (Costa Mesa, CA). C6 glioma cells were a gift from Dr. W. R. Mancini (University of Michigan).

Cell Culture. Differentiated human NT2-N neurons were generated from NT2 teratocarcinoma cells as previously described (22). In brief, NT2 cells were treated with 10 μ*M* retinoic acid twice per week for five weeks. After retinoic acid treatment, cells were rinsed with 1X Versene, detached from 75-cm^2 flasks with 0.25% (wt/vol) trypsin, and replated into 225-cm^2 flasks (replate I). Two days later, cells were rinsed with Hanks' balanced salt solution and treated with 1X trypsin-EDTA. Flasks were struck repeatedly to remove NT2-N neurons, which were seeded on 6-cm dishes previously coated with 10 μg/ml poly-D-lysine and 1:36 Matrigel in DMEM (replate II). For the first three weeks after replate II, neurons were maintained in DMEM, supplemented with 10% fetal calf serum, that also contained 10 μ*M* 5-fluoro-2′-deoxyuridine, 10 μ*M* uridine, and 1 μ*M* cytosine β-D-arabinofuranoside. After three weeks, NT2-N cells were maintained in conditioned medium obtained from replate I.

Inositol Uptake. Inositol uptake was measured as described (22,23). NT2-N neurons, attached to 6-cm dishes, were washed with 4 × 2 ml of buffer A (142 m*M* NaCl, 5.6 m*M* KCl, 2.2 m*M* $CaCl_2$, 3.6 m*M* $NaHCO_3$, 1 m*M* $MgCl_2$, 30 m*M* HEPES, and 5.6 m*M* D-glucose, pH 7.4) and allowed to equilibrate in 2.55 ml of the same buffer at 37°C for 30 min. Reactions were initiated by the addition of 300 μl of drug and 150 μl of [^{3}H]inositol (250 μ*M;* 5 μCi/μmol) and allowed to proceed at 37°C for 1 h. To terminate the reactions, the buffer was rapidly aspirated and the cells washed with 5 × 2 ml of ice-cold buffer A containing 1 m*M* inositol and lysed with 2 × 1 ml of ice-cold 6% (wt/vol) trichloroacetic acid. Lysates were transferred to test tubes on ice and centrifuged at 2,400 *g* for 5 min at 4°C. Radioactivity in 0.5 ml of supernatant was determined by liquid scintillation spectrometry after the addition of 5 ml of UniverSol scintillation fluid. The pellet was dissolved in a solution of 5% (wt/vol) sodium dodecyl sulfate and 0.1 *M* NaOH and analyzed for protein.

Inositol Efflux. Efflux of inositol was determined essentially as described (22). NT2-N neurons, attached to 6-cm dishes, were prelabeled overnight with 5 μCi/ml *myo*-[^{3}H]inositol. Cells were then washed with 3 × 2 ml of isotonic (142 m*M* NaCl; 330–338 mOsm), hypotonic (67 m*M* NaCl; 202–216 mOsm), or intermediate-osmolality buffer A and incubated in 4 ml of drug in the same buffer at 37°C. Aliquots of buffer (50–100 μl) were periodically removed and radioactivity determined. To terminate the reactions, the buffer was rapidly aspirated and the cells washed with 3 × 2 ml of ice-cold buffer A and lysed with 2 × 1 ml of ice-cold 6% (wt/vol) trichloroacetic acid. Radioactivity in the supernatant was deter-

mined as previously described. Total [^{3}H]inositol originally present in the neurons was calculated as the sum of that recovered in the buffer and that remaining in the lysate at the end of the assay. Efflux at any given time was then expressed as the ratio of radioactivity present in buffer to total [^{3}H]inositol.

Data Analysis. Data shown represent mean ± SEM values for the number (n) of independent experiments done. Duplicate or triplicate values were obtained for each condition measured. Student's two-tailed *t* tests were used to evaluate the statistical differences between the means of paired or unpaired sets of data.

RESULTS

Inositol Uptake into NT2-N Neurons Is Inhibited by Protein Kinase C. The regulation of [^{3}H]inositol uptake into NT2-N cells was measured during 1-h incubations with various drugs and second messenger analogs. Uptake was not affected by either the muscarinic cholinergic agonist oxotremorine-M or by cell-permeable activators of protein kinases A and G (PKA and PKG), db-cAMP and 8Br-cGMP, respectively (Fig. 1). A small but significant reduction of inositol uptake (~11%) was observed, however, during the activation of PKC with 100 n*M* PMA. In addition, exposure of cells to 1 μ*M* PMA overnight to downregulate basal PKC activity (24) resulted in the stimulation of inositol uptake by approximately 12%. Twenty-four hour preincubation with hypertonic medium (~500 mOsm with NaCl) to stimulate transcription of the Na^+/*myo*-inositol cotransporter (25,26) did not affect the relative magnitude of these changes (data not shown). Neither the chelation of intracellular Ca^{2+} with BAPTA/AM nor the stimulation of Ca^{2+} influx with the Ca^{2+} ionophore ionomycin had significant effects on inositol uptake in NT2-N cells (Fig. 1). As reported for primary mouse astrocytes (27), neither acute (10 m*M*, 1 h) nor chronic (1 m*M*, 4 d) incubation with Li^+ caused a change in the rate of uptake.

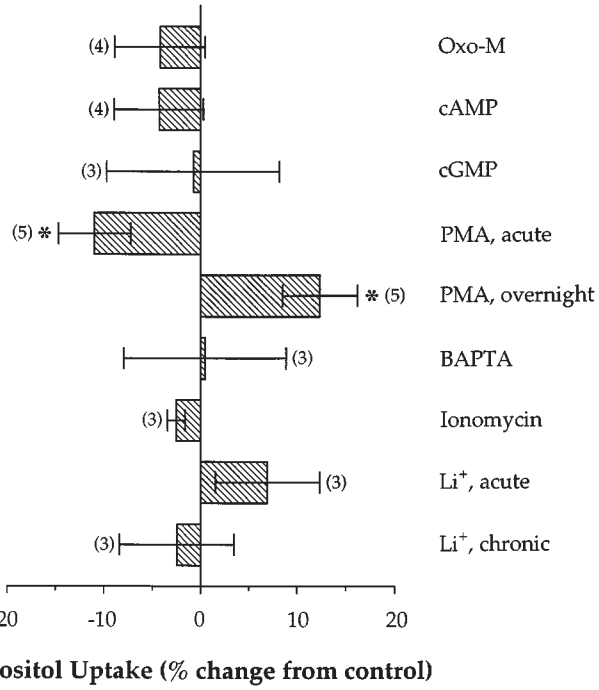

Fig. 1. Protein kinase C regulates *myo*-inositol uptake into NT2-N neurons. NT2-N neurons, adherent to 6-cm dishes, were washed four times with buffer and incubated with [^{3}H]inositol (250 μ*M;* 5 μCi/μmol) with or without the agents indicated for 1 h. Concentrations were as follows: Oxo-M, db-cAMP, 8Br-cGMP = 1 m*M;* PMA, acute = 100 n*M;* PMA, chronic = 1 μ*M* given overnight before the experiment; BAPTA/AM = 50 μ*M;* ionomycin = 1 μ*M;* Li^+, acute = 10 m*M;* Li^+, chronic = 1 m*M* given 4 d before the experiment. Results are expressed as the mean percentage ± SEM of inositol uptake in the presence of a given agent with respect to control for the number of experiments indicated in parentheses. *Different from control, $p < 0.05$.

Efflux of Inositol from NT2-N Neurons Is Mediated by VSOAC. Osmolyte efflux from most tissues occurs via the nonselective Cl^- channel VSOAC, whose presence is verified by the stimulation of efflux during hypotonic exposure and the inhibition of efflux by Cl^- channel blockers (5,9,28). To assess the volume sensitivity of inositol release from NT2-N cells, [^{3}H]inositol efflux was measured over the course of 6 h as a function of osmotic strength. Buffer osmolality was varied from 330 mOsm (isotonic) to 216 mOsm (hypotonic) by alteration of NaCl concentration; this range was within that previously used to demonstrate the effect of hypotonicity on inositol efflux (1,11,13,29). Inositol efflux was relatively unchanged from 330 to 300 mOsm, and although there was a tendency for increased efflux at 272 mOsm, this increase only reached significance at 6 h (Fig. 2). Significantly enhanced release was observed at 244 and 216 mOsm throughout the course of the assay. Since [^{3}H]inositol efflux was most prominent at 216 mOsm, this osmolality was used to test the effect of protein kinase activation and intracellular Ca^{2+} concentration in subsequent experiments. Incubation with 200 μ*M* DIDS, a Cl^- channel blocker that inhibits VSOAC-mediated osmolyte transport (9,13), resulted in the complete inhibition of volume-sensitive [^{3}H]inositol efflux from NT2-N neurons, in the presence or absence of PMA (see below). Collectively, these results indicate that volume-sensitive inositol efflux from NT2-N neurons is mediated by VSOAC.

Volume-Sensitive Inositol Efflux Is Stimulated by Protein Kinase C and Intracellular Ca^{2+}. Inositol efflux is regulated by PKA, PKC, and intracellular Ca^{2+} in numerous cell types (10,11,13,16). As a first analysis of the regulation of volume-sensitive inositol efflux from

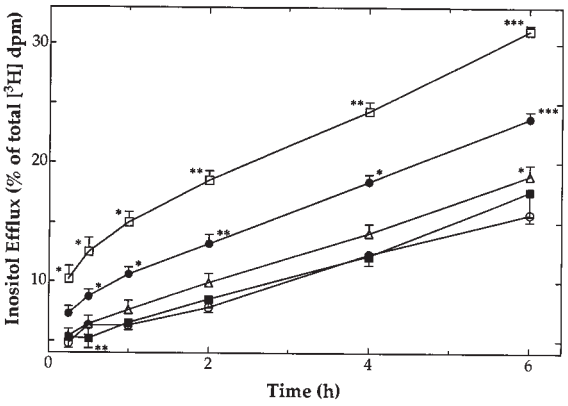

Fig. 2. Hypotonicity regulates *myo*-inositol efflux from NT2-N neurons. NT2-N neurons, adherent to 6-cm dishes, were incubated with medium containing 5 μCi/ml [^{3}H]inositol overnight. Cells were washed three times with buffer of varying osmolality and incubated at 37°C. Aliquots of extracellular buffer were periodically removed and radioactivity was determined. Data are expressed as [^{3}H]inositol recovered in the extracellular buffer relative to [^{3}H]inositol present initially in the cells. ○ = 330 mOsm (isotonic); ■ = 300 mOsm; △ = 272 mOsm; ● = 244 mOsm; □ = 216 mOsm. Results shown are mean ± SEM values for three separate experiments. *Different from isotonic control, $p < 0.05$. **Different from isotonic control, $p < 0.01$. ***Different from isotonic control, $p < 0.001$.

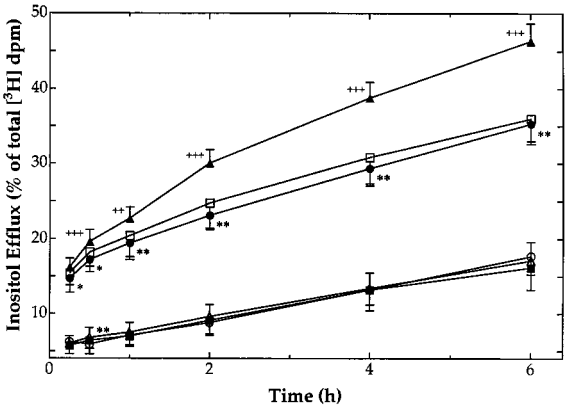

Fig. 3. Protein kinase C regulates volume-sensitive *myo*-inositol efflux from NT2-N neurons. [^{3}H]Inositol efflux from NT2-N neurons was determined as previously described. ○ = isotonic buffer (330–338 mOsm) alone; ■ = isotonic buffer + 1 m*M* db-cAMP; △ = isotonic buffer + 100 n*M* PMA; ● = hypotonic buffer (202–216 mOsm) alone; □ = hypotonic buffer + 1 m*M* db-cAMP; ▲ = hypotonic buffer + 100 n*M* PMA. Results shown are mean ± SEM values for three to six separate experiments. *Different from isotonic control, $p < 0.05$. **Different from isotonic control, $p < 0.01$. $^{++}$Different from hypotonic control, $p < 0.01$. $^{+++}$Different from hypotonic control, $p < 0.001$.

NT2-N neurons, cells were prelabeled overnight with [^{3}H]inositol, and efflux was subsequently measured over the course of 6 h in either isotonic (~330 mOsm) or hypotonic (~210 mOsm) buffer in the presence or absence of 1 m*M* db-cAMP or 100 n*M* PMA (Fig. 3). Under isotonic conditions, efflux of [^{3}H]inositol was unaffected by either PKA or PKC activation and occurred at the approximately linear rate of 2.1 ± 0.1%/h (n = 3–5), a value in accordance with the previously reported low rate of release from these cells (22). Under hypotonic conditions, as previously shown (see Fig. 2), inositol efflux from NT2-N neurons was significantly elevated at all time points. Although incubation with db-cAMP had no effect on hypotonic efflux, incubation with PMA caused a marked stimulation (~130% of hypotonic control at 6 h; n = 6).

The role of PKC in the activation of volume-sensitive inositol efflux was characterized in an additional series of measurements. Hypotonically-induced inositol efflux from neurons was unaffected by incubation with the muscarinic agonist oxo-M, although as previously observed, efflux was significantly increased in the presence of PMA ($p < 0.0001$; Fig. 4). Preincubation (1 h) with 5 μ*M* BIM, a competitive PKC antagonist, attenuated the PMA-mediated activation of volume-sensitive efflux at 6 h. Conversely, the nonphorbol PKC-activating diacylglycerol DiC8, although less efficacious than PMA, elicited an increase in inositol efflux at 6 h ($p < 0.03$), whereas the inactive phorbol ester αPDD had no effect at either time point ($p > 0.05$).

The regulation of inositol efflux from NT2-N neurons by Ca^{2+} was also determined. In the presence of 50 μ*M* BAPTA/AM, the hypotonic release of [^{3}H]inositol appeared to be increased in rate but not in magnitude, such that the large increase observed at 1 h had returned to control values by 6 h (Fig. 4). In contrast, 1 μ*M* ionomycin produced a sustained increase (~25%) in volume-sensitive inositol efflux at both time points. Similar data were obtained in the presence of the divalent cation ionophore A23187 (2.5 μ*M;* data not shown). Inositol efflux from NT2-N cells was not affected by the presence of 100 μ*M* or 1 m*M* concentrations of either glutamate or NMDA (data not shown).

DISCUSSION

myo-Inositol efflux from NT2-N neurons was sensitive to regulation by second messengers, whereas inositol uptake was relatively refractory to modulation. Neither the muscarinic agonist oxotremorine-M, nor cell-permeable activators of PKA or PKG, nor changes in the intracellular concentration of Ca^{2+} had any effect on neuronal inositol uptake. Conversely, muscarinic agonists, PKA activators, and Ca^{2+} ionophores have been

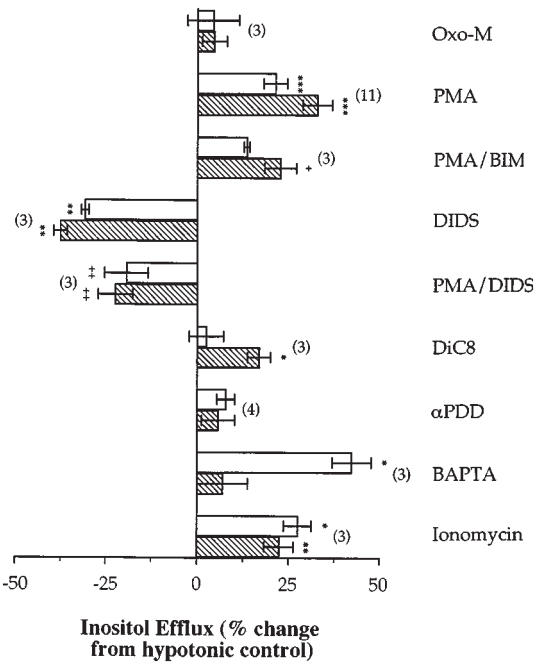

Fig. 4. Protein kinase C and Ca^{2+} stimulate, and DIDS inhibits, volume-sensitive *myo*-inositol efflux from NT2-N neurons. [^{3}H] Inositol efflux from NT2-N neurons was determined as previously described. Concentrations of Oxo-M, PMA, BAPTA/AM, and ionomycin were the same as described in the legend to Fig. 1. Other concentrations were as follows: PMA/BIM = 50 μ*M* BIM given 1 h before the experiment followed by 100 n*M* PMA given at the start of the assay; DIDS = 200 μM; PMA/DIDS = 200 μ*M* DIDS and 100 n*M* PMA given concurrently at the start of the assay; DiC8 = 50 μ*M;* αPDD = 100 n*M*. Results are expressed as the mean percentage ± SEM of inositol efflux in the presence of a given agent with respect to control for the number of experiments indicated in parentheses. Open bars, 1 h incubation. Hatched bars, 6 h incubation. *Different from hypotonic control, $p < 0.05$. **Different from hypotonic control, $p < 0.01$. ***Different from hypotonic control, $p < 0.001$. $^+$Different from PMA alone, $p < 0.05$. $^{++}$Different from PMA alone, $p < 0.01$.

reported to elicit either decreases (15,23) or increases (11) in the uptake of inositol into other cell types. Although inositol uptake into NT2-N neurons was inhibited by PKC activation, a result consistent among diverse cell types (11,15,23), this inhibition (10%) was less robust than has been reported elsewhere (20–30%). Taken collectively, these data indicate that the second messenger regulation of inositol uptake into NT2-N neurons is minimal compared to that in glia and non-neural cells.

Osmolyte efflux during a period of decreased tonicity is mediated by the volume-sensitive organic osmolyte-anion channel (VSOAC), which has not been definitively identified but has been characterized as a relatively nonselective, outwardly-rectified Cl^- channel (5,30). Involvement of VSOAC is frequently inferred from the stimulation of osmolyte efflux by hypotonicity and the inhibition of efflux by Cl^- channel blockers. NT2-N cells demonstrated [^{3}H]inositol efflux that increased during successive decreases in tonicity and whose volume-sensitive component was eliminated by 200 μ*M* DIDS. Thus, VSOAC most likely mediates hypotonic inositol efflux from human CNS neurons, as it does from human (5) and rat glia (9) as well as RPE (11) and inner medullary collecting duct cells (13).

Volume-sensitive [^{3}H]inositol efflux from NT2-N neurons was stimulated (125–130% of control) by PKC activation and increased intracellular Ca^{2+} concentration but not by PKA activation. Conversely, isotonic efflux was unaffected by PKC stimulation, a finding that emphasizes the importance of PKC signaling in the osmotic stress response. PKC activation increases organic osmolyte (10,11) as well as electrolyte (31) efflux in numerous cell systems and is thought to result in the phosphorylation of VSOAC itself or putative regulatory proteins such as pI_{Cln} (32; but see 33 and 34). Ca^{2+} typically regulates efflux of inositol in cells of renal (16) but not of glial (9,10) origin. In the presence of BAPTA/AM, [^{3}H]inositol efflux from NT2-N cells occurred more rapidly but to the same ultimate extent as the hypotonic control. Increased efflux at early time points has also been observed in C6 glioma cells pretreated with BAPTA/AM (10). In contrast, ionomycin generated a sustained increase (~25%) in inositol efflux from NT2-N neurons. By way of comparison, Strange et al. (10) had previously reported no effect of 1 μ*M* ionomycin on inositol efflux from C6 glioma cells, and we observed a decrease in volume-sensitive [^{3}H]inositol efflux from C6 glioma cells at 6 h under similar conditions (68 ± 6%, n = 3, $p < 0.05$; data not shown). This result suggests that neurons and glia are differentially regulated by intracellular Ca^{2+} with respect to volume-sensitive inositol efflux. Although activation of PKA stimulates volume-sensitive [^{3}H]inositol efflux in RPE cells (11), it is without effect in MDCK cells (16), as is observed in NT2-N neurons.

In summary, the regulation of inositol transport in differentiated human NT2-N neurons occurs primarily at the level of volume-sensitive efflux. Since the intracellular concentration of inositol in neurons seems to be maximal under isotonic conditions (~20 m*M*) (22), there may be a greater capacity to lose than to gain inositol with respect to the extracellular environment; hence, uptake is relatively refractory to regulation, whereas efflux is stimulated by hypotonicity and can be modulated by second messenger production. Small global decreases in tonicity, protracted over the course of weeks or months in hyponatremic patients, or large local increases in volume-sensitive inositol efflux within the brain during

stroke, could contribute to clinical pathophysiology. Further knowledge of these neuronal inositol transport processes may thus prove useful in the development of therapeutic agents to treat blood Na^+ imbalances and cerebral ischemia.

ACKNOWLEDGMENTS

This study was supported by NIMH grant MH 46252 (S.K.F. and B.W.A.) and NIH grant NS 23831 (S.K.F.). J.E.N. was supported by NIMH grant MH 12259 and NIH Training Grant GM 07767.

REFERENCES

1. Pasantes-Morales, H., Alavez, S., Sánchez-Olea, R., and Morán, J. 1993. Contribution of organic and inorganic osmolytes to volume regulation in rat brain cells in culture. Neurochem. Res. 18: 445–452.
2. Prockop, L. D. 1971. Hyperglycemia, polyol accumulation, and increased intracranial pressure. Arch. Neurol. 25:126–140.
3. Lien, Y-H. H., Shapiro, J. I., and Chan, L. 1990. Effects of hypernatremia on organic brain osmoles. J. Clin. Invest. 85:1427–1435.
4. Lee, J. H., Arcinue, E., and Ross, B. D. 1994. Brief report: organic osmolytes in the brain of an infant with hypernatremia. N. Engl. J. Med. 331:439–442.
5. Jackson, P. S. and Madsen, J. R. 1997. Identification of the volume-sensitive organic osmolyte/anion channel in human glial cells. Pediatr. Neurosurg. 27:286–291.
6. Gullans, S. R. and Verbalis, J. G. 1993. Control of brain volume during hyperosmolar and hypoosmolar conditions. Annu. Rev. Med. 44:289–301.
7. Nonaka, M., Yoshimine, T., Kohmura, E., Wakayama, A., Yamashita, T., and Hayakawa, T. 1998. Changes in brain organic osmolytes in experimental cerebral ischemia. J. Neurol. Sci. 157:25–30.
8. Jackson, P. S., Morrison, R., and Strange, K. 1994. The volume-sensitive organic osmolyte-anion channel VSOAC is regulated by nonhydrolytic ATP binding. Am. J. Physiol. 267:C1203–C1209.
9. González, E., Sánchez-Olea, R., and Pasantes-Morales, H. 1995. Inhibition by Cl^- channel blockers of the volume-activated, diffusional mechanism of inositol transport in primary astrocytes in culture. Neurochem. Res. 20:895–900.
10. Strange, K., Morrison, R., Shrode, L., and Putnam, R. 1993. Mechanism and regulation of swelling-activated inositol efflux in brain glial cells. Am. J. Physiol. 265:C244–C256.
11. Karihaloo, A., Kato, K., Greene, D. A., and Thomas, T. P. 1997. Protein kinase and Ca^{2+} modulation of *myo*-inositol transport in cultured retinal pigment epithelial cells. Am. J. Physiol. 273: C671–C678.
12. Reeves, R. E. and Cammarata, P. R. 1996. Osmoregulatory alterations in myo-inositol uptake by bovine lens epithelial cells. Invest. Ophthalmol. Vis. Sci. 37:619–629.
13. Ruhfus, B. and Kinne, R. K. H. 1996. Hypotonicity-activated efflux of taurine and myo-inositol in rat inner medullary collecting duct cells: evidence for a major common pathway. Kidney Blood Press. Res. 19:317–324.
14. Ruhfus, B., Tinel, H., and Kinne, R. K. H. 1996. Role of G-proteins in the regulation of organic osmolyte efflux from isolated rat renal inner medullary collecting duct cells. Eur. J. Physiol. 433:35–41.
15. Preston, A. S., Yamauchi, A., Kwon, H. M., and Handler, J. S. 1995. Activators of protein kinase A and of protein kinase C inhibit MDCK cell *myo*-inositol and betaine uptake. J. Am. Soc. Nephrol. 6:1559–1564.
16. Bagnasco, S. M., Montrose, M. H., and Handler, J. S. 1993. Role of calcium in organic osmolyte efflux when MDCK cells are shifted from hypertonic to isotonic medium. Am. J. Physiol. 264:C1165–C1170.
17. Andrews, P. W. 1984. Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev. Biol. 103:285–293.
18. Andrews, P. W., Damjanov, I., Simon, D., Banting, G. S., Carlin, C., Dracopoli, N. C., and Føgh, J. 1984. Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Lab. Invest. 50:147–162.
19. Pleasure, S. J., Page, C., and Lee, V. M.-Y. 1992. Pure, postmitotic, polarized human neurons derived from NTera 2 cells provide a system for expressing exogenous proteins in terminally differentiated neurons. J. Neurosci. 12:1802–1815.
20. Lee, V. M.-Y. and Andrews, P. W. 1986. Differentiation of NTERA-2 clonal human embryonal carcinoma cells into neurons involves the induction of all three neurofilament proteins. J. Neurosci. 6:514–521.
21. Hartley, R. S., Margulis, M., Fishman, P. S., Lee, V. M.-Y., and Tang, C.-M. 1999. Functional synapses are formed between human NTera2 (NT2N, hNT) neurons grown on astrocytes. J. Comp. Neurol. 407:1–10.
22. Novak, J. E., Turner, R. S., Agranoff, B. W., and Fisher, S. K. 1999. Differentiated human NT2-N neurons possess a high intracellular content of *myo*-inositol. J. Neurochem. 72:1431–1440.
23. Batty, I. H., Michie, A., Fennel, M., and Downes, C. P. 1993. The characteristics, capacity and receptor regulation of inositol uptake in 1321N1 astrocytoma cells. Biochem. J. 294:49–55.
24. Rasmussen, H., Isales, C. M., Calle, R., Throckmorton, D., Anderson, M., Gasalla-Herraiz, J., and McCarthy, R. 1995. Diacylglycerol production, Ca^{2+} influx, and protein kinase C activation in sustained cellular responses. Endocr. Rev. 16:649–681.
25. Paredes, A., McManus, M., Kwon, H. M., and Strange, K. 1992. Osmoregulation of Na^+-inositol cotransporter activity and mRNA levels in brain glial cells. Am. J. Physiol. 263:C1282–C1288.
26. Wiese, T. J., Dunlap, J. A., Conner, C. E., Grzybowski, J. A., Lowe, W. L., Jr., and Yorek, M. A. 1996. Osmotic regulation of Na-*myo*-inositol cotransporter mRNA level and activity in endothelial and neural cells. Am. J. Physiol. 270:C990–C997.
27. Wolfson, M., Hertz, E., Belmaker, R. H., and Hertz, L. 1998. Chronic treatment with lithium and pretreatment with excess inositol reduce inositol pool size in astrocytes by different mechanisms. Brain Res. 787:34–40.
28. Goldstein, L. and Davis, E. M. 1994. Taurine, betaine, and inositol share a volume-sensitive transporter in skate erythrocyte cell membrane. Am. J. Physiol. 267:R426–R431.
29. Bender, A. S. and Norenberg, M. D. 1994. Calcium dependence of hypoosmotically induced potassium release in cultured astrocytes. J. Neurosci. 14:4237–4243.
30. Jackson, P. S. and Strange, K. 1993. Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. Am. J. Physiol. 265:C1489–C1500.
31. Liedtke, C. M. 1995. The role of protein kinase C in α-adrenergic regulation of NaCl(K) cotransport in human airway epithelial cells. Am. J. Physiol. 268:L414–L423.
32. Reeves, R. E., Sanchez-Torres, J., Coca-Prados, M., and Cammarata, P. R. 1998. Expression of pI_{Cln} mRNA in cultured bovine lens epithelial cells: response to changes in cell volume. Curr. Eye Res. 17:861–869.
33. Li, C., Breton, S., Morrison, R., Cannon, C. L., Emma, F., Sánchez-Olea, R., Bear, C., and Strange, K. 1998. Recombinant pI_{Cln} forms highly cation-selective channels when reconstituted into artificial and biological membranes. J. Gen. Physiol. 112:727–736.
34. Patel, A. J., Lauritzen, I., Lazdunski, M., and Honoré, E. 1998. Disruption of mitochondrial respiration inhibits volume-regulated anion channels and provokes neuronal cell swelling. J. Neurosci. 18:3117–3123.