Journal of Molecular Neuroscience

ISSN0895-8696/05/27:137–146/$30.00
DOI: 10.1385/JMN:27:01:137

ORIGINAL ARTICLE

Dietary Uridine-5′-Monophosphate Supplementation Increases Potassium-Evoked Dopamine Release and Promotes Neurite Outgrowth in Aged Rats

Lei Wang,[1] Amy M. Pooler,[1] Meredith A. Albrecht,[2] and Richard J. Wurtman*[,1,3]

[1]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02142; [2]Department of Anesthesia, Massachusetts General Hospital, Boston, MA; and [3]Division of Health Sciences and Technology, Harvard University, Massachusetts Institute of Technology, Cambridge, MA 02142

Abstract

Membrane phospholipids like phosphatidylcholine (PC) are required for cellular growth and repair, and specifically for synaptic function. PC synthesis is controlled by cellular levels of its precursor, cytidine-5′-diphosphate choline (CDP-choline), which is produced from cytidine triphosphate (CTP) and phosphocholine. In rat PC12 cells exogenous uridine was shown to elevate intracellular CDP-choline levels, by promoting the synthesis of uridine triphosphate (UTP), which was partly converted to CTP. In such cells uridine also enhanced the neurite outgrowth produced by nerve growth factor (NGF). The present study assessed the effect of dietary supplementation with uridine-5′-monophosphate disodium (UMP-2Na$^+$, an additive in infant milk formulas) on striatal dopamine (DA) release in aged rats. Male Fischer 344 rats consumed either a control diet or one fortified with 2.5% UMP for 6 wk, *ad libitum*. In vivo microdialysis was then used to measure spontaneous and potassium (K$^+$)-evoked DA release in the right striatum. Potassium (K$^+$)-evoked DA release was significantly greater among UMP-treated rats, i.e., 341 ± 21% of basal levels vs. 283 ± 9% of basal levels in control rats ($p < 0.05$); basal DA release was unchanged. In general, each animal's K$^+$-evoked DA release correlated with its striatal DA content, measured postmortem. The levels of neurofilament-70 and neurofilament-M proteins, biomarkers of neurite outgrowth, increased to 182 ± 25% ($p < 0.05$) and 221 ± 34% ($p < 0.01$) of control values, respectively, with UMP consumption. Hence, UMP treatment not only enhances membrane phosphatide production but also can modulate two membrane-dependent processes, neurotransmitter release and neurite outgrowth, in vivo.

DOI: 10.1385/JMN:27:01:137

Index Entries: Microdialysis; dopamine; neurite outgrowth; nucleoside; CDP-choline.

Introduction

The primary components of neuronal cell membranes are phospholipids, the most abundant of which is phosphatidylcholine (PC). PC synthesis via the Kennedy cycle (Kennedy and Weiss, 1956) is known to be regulated by the availability of its precursors (Araki and Wurtman, 1997, 1998); and the major rate-limiting precursor usually is cytidine-5′-diphosphate choline (CDP-choline) (Weiss, 1995). This compound, formed from cytidine triphosphate (CTP) and phosphocholine, combines with diacylglycerol (DAG) to form PC. Exposure of rat PC12 cells to the pyrimidine nucleoside uridine

*Author to whom all correspondence and reprint requests should be addressed. E-mail: dick@mit.edu

is known to increase their levels of CDP-choline (Richardson et al., 2003). It does so by increasing the formation of CTP, via the formation and subsequent amination of uridine triphosphate (UTP). Moreover the administration to gerbils of uridine (as uridine monophosphate [UMP]) elevates brain CDP-choline levels (Cansev et al., 2004), whereas giving rats oral CDP-choline, another source of uridine (Wurtman et al., 2000), increases brain PC production (Agut et al., 1993). These observations suggest that administration of uridine or other PC precursors might affect brain membrane levels and functions in vivo.

Thus, we explored whether UMP consumption might affect membrane-dependent synaptic processes in rat brain. We measured the content and release of the neurotransmitter dopamine (DA) in corpus striatum, and, as exogenous uridine is known to enhance neurite outgrowth in differentiated PC12 cells (Pooler et al., 2004), we also measured striatal levels of two structural neurofilament proteins, neurofilament 70 (NF-70) and neurofilament M (NF-M).

Ad libitum consumption of a UMP-enriched diet for 6 wk enhanced striatal DA release in proportion to the concomitant change it produced in striatal DA levels, and increased striatal levels of NF-70 and NF-M.

Materials and Methods

The following experiments were carried out under a protocol approved by the Institutional Committee on Animal Care of the Massachusetts Institute of Technology, in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals

Aged male Fischer 344 rats (22 mo old), obtained from the National Institute on Aging (Harlan Sprague-Dawley, Indianapolis, IN), were reared in a socially restricted (individual housing and minimal handling) environment, exposed to a 12-h light/dark cycle, and provided with food and water *ad libitum*.

Rats were fed a control laboratory diet (Teklad Global 16% protein rodent diet, TD.00217, Harlan Teklad, Madison, WI), or this diet fortified with UMP-2Na^+ (2.5%, TD.03398) for 1 or 6 wk. Three experiments were performed, using 13, 22, and 11 animals, respectively.

Chemicals and Solutions

Dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were obtained from Sigma (St. Louis, MO). Ringer's solution contained 147 m*M* NaCl, 2.7 m*M* KCl, 1.2 m*M* $CaCl_2$, and 0.85 m*M* $MgCl_2$. In the high-potassium-depolarizing variant, KCl was increased to 80 m*M* and NaCl was decreased to 69.7 m*M* to maintain osmolarity.

Microdialysis

Rats were anesthetized with a mixture of ketamine and xylazine (80 and 10 mg/kg body weight, respectively, ip) and placed in a Kopf stereotaxic frame. Surgical instruments were sterilized using a hot bead dry sterilizer or 70% ethanol. A small hole was drilled into the skull using a 2-mm trephine bone drill. A CMA/11 14/04 Cupr probe (O.D. 0.24 mm, 4 mm membrane length, 6000 Dalton, CMA Microdialysis, Sweden) was then implanted into the right striatum (anterior–posterior [AP]=+0.5, medial–lateral [ML]=–3.0 from bregma, dorsal–ventral [DV]=–7.3 mm from dura [Paxinos and Watson, 1986]), with the incisor bar set at –5.0 mm. Probes were secured permanently in position using dental cement and three anchor screws to the skull.

During perfusion, freely moving rats were kept in a circular bowl on a rotating platform, obviating the need for a liquid swivel (Wang et al., 2003). Experiments were performed 1 d after surgery. Ringer's solution was perfused continuously using fluorinated ethylene propylene (FEP) tubing and a gas-tight syringe (Exmire type I, CMA), at a constant rate of 1.5 μL/min, by a microinfusion pump (CMA/100). Dialysates were collected at 15-min intervals. Five microliters of 0.2 *M* $HClO_4$ (0.1 m*M* EDTA) was added to sampling vials prior to collection to prevent degradation of catecholamines. Samples obtained during the first hour of perfusion were discarded. Subsequently, three consecutive groups of samples (baseline, high K^+ stimulation, and recovery) were collected. The first two sampling sessions each lasted for 1 h and provided 4 samples; the third session, lasting 1.5 h, provided 6 samples.

Brain Dissection for Proteins and DA

After completion of each microdialysis experiment, rats were anesthetized with ketamine and xylazine (80 and 10 mg/kg, ip). Black ink was then pushed through the probe to stain the surrounding tissue and allow visual confirmation of probe location. After decapitation of the animals, brains were quickly

dissected on a chilled dissection board. The left striatum was snap-frozen in an Eppendorf tube and placed in liquid nitrogen for subsequent protein or DA assays. The right striatum was further dissected and the position of the probe verified.

Tissue DA Extraction

Striata were weighed, homogenized on ice (50 mg of tissue with 1 mL 0.2 *M* $HClO_4$, 0.2 μ*M* ascorbic acid, and 0.2 μ*M* EDTA) and vortexed for 10 s; a 10-μL aliquot was then taken for total protein determination (bicinchoninic acid protein assay, Sigma). The homogenates were then centrifuged (14,000 rpm for 15 min at 4°C) and filtered with Ultrafree-MC centrifugal filter units (Millipore, Milford, MA, 14,000 rpm/min at 4°C); a 1: 10 dilution (20 μL), made using the mobile phase, was then injected into the HPLC for measurement of catecholamines. Values from three repeated measures were averaged and normalized to the amounts of protein per sample.

DA Assay

Dopamine (DA) and its metabolites in dialysates and tissue samples were determined using an ESA Coulochem II 5100A detector ($E_1 = -175$ mV; $E_2 = 325$ mV; Eguard = 350 mV) with an ESA Microdialysis Cell (model 5014B, ESA, North Chelmsford, MA). The flow rate of the mobile phase (MD-TM, ESA) was 0.4 mL/min. The column (ESA MD 150, 3 × 150 mm, 3 μm) was kept at 40°C. Samples were injected and analyzed by Alltech AllChromsystem (Alltech, Deerfield, IL).

Western Blotting

Striatal tissues were placed in Eppendorf tubes containing 200 μL lysis buffer (60 m*M* Tris-HCl, 4% SDS, 20% glycerol, 1 m*M* dithiothreitol, 1 m*M* AEBSF, 8 μ*M* aprotinin, 500 μ*M* bestatin, 15 μ*M* E64, 200 μM leupeptin, 10 μ*M* pepstatin A). The samples were sonicated, boiled (10 min), and centrifuged (14,000*g* for 1 min at room temperature), and supernatant fluids were transferred to clean tubes and their total proteins determined using the bicinchoninic acid assay (Sigma, St. Louis, MO).

Equal amounts of protein (40 μg protein/lane) were loaded for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4–15% SDS PAGE; Bio-Rad, Hercules, CA). Prior to gel electrophoresis, bromphenol blue solution (0.07%) was added to each sample to indicate the location of the sample on the gel. Proteins were separated, transferred onto PVDF membranes (Immobilon-P, Millipore), and blocked with 5% bovine serum albumin (Tris-buffered saline [TBST]/0.15% Tween-20) for 1 h. After 3 × 10-min rinses in TBST, blots were incubated in TBST, along with various antibodies against the proteins of interest, including NF-70 and NF-M (1:2000 and 1:5000, respectively; Calbiochem, La Jolla, CA), at 4°C overnight on an orbital shaker. Protein–antibody complexes were detected and visualized using the ECL system (Amersham, Piscataway, NJ) and Kodak X-AR film, respectively, as suggested by the manufacturer. Films were digitized using a Supervista S-12 scanner with a transparency adapter (UMAX Technologies, Freemont, CA). Analysis was performed using the public domain NIH Image program (NIH v. 1.61).

Data Analysis

Statistical analyses were carried out using SPSS 12.0. Data were represented as mean ± S.E.M. Unpaired Student's *t* test, analysis of variance (ANOVA), and linear regression were used to assess statistical significance of effects. Tukey's honestly significant difference (HSD) post hoc analyses were used when appropriate. The significance level was set at $p < 0.05$.

Results

The control diet did not contain nucleosides or nucleotides, as demonstrated by HPLC analysis (data not shown). The 2.5% UMP diet provided approx 500 mg/kg per day of UMP-$2Na^+$, or approx 330 mg/kg per day of uridine.

Body Weight

In one experiment, rats randomly assigned to treatment or control groups were weighed after 0, 1, 2, 4, and 6 wk of UMP consumption. No significant differences in body weight were obtained between the groups, at the start ($t_{11} = 0.416$, $p > 0.05$) or end ($t_{11} = 0.321$, $p > 0.05$) of the 6-wk treatment period, that is, control rats ($n = 6$) weighed 453 ± 5 g at the start of the study and 457 ± 4 g after 6 wk; UMP-treated rats ($n = 7$) weighed 456 ± 8 g at the start of the study and 461 ± 11 g after 6 wk.

Dietary UMP Supplementation Enhanced K^+-Evoked DA Release in Striatum

Uridine-5′-monophosphate (UMP) supplementation did not affect basal DA levels in dialysates (control vs. UMP for 1 wk vs. UMP for 6 wk; 9.5 ± 1.1, 11.8 ± 2.0, and 9.6 ± 1.1 n*M*, respectively; $F_{2,19} = 0.98$, $p > 0.05$). The mean value of DA at baseline

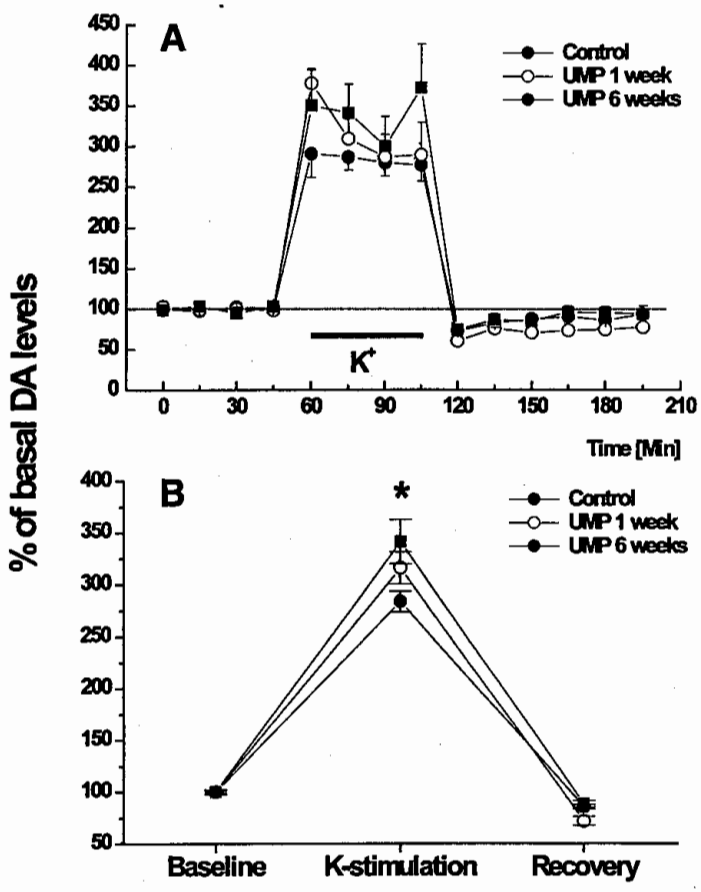

Fig. 1. Effect of high K^+ on DA release in animals pretreated with UMP. (**A**) Basal and potassium-evoked DA release from striata of rats that consumed UMP-containing or control diets for 1 or 6 wk prior to in vivo microdialysis. Animals were prepared and samples collected and assayed as described in the text. The baseline value (10.2 ± 0.4 n*M*, *n*=88; taken as 100%) was obtained by pooling four baseline measurements from each of 22 animals; UMP had no effect on basal (0–45 min) or poststmulation (recovery) (120–195 min) DA release. Potassium-evoked DA release in control animals (32.5 ± 1.7 n*M*, $n = 7$) was significantly greater than basal release ($p < 0.01$) and was further increased ($p < 0.05$) among animals that had consumed UMP for 6 wk. (**B**) Data from the three treatment groups in **A** were pooled and analyzed by ANOVA. DA release among animals receiving UMP for 6 wk (341 ± 21% of baseline) was significantly greater ($p < 0.05$) than that in control animals (283 ± 9%); the effect of consuming UMP for 1 wk was not significant. (*) $p < 0.05$ compared with corresponding controls.

was 10.2 ± 0.4 n*M* ($n = 22$ rats). Basal DA levels in the dialysates were stable after 1 h equilibration among the four consecutive samples obtained prior to K stimulation, varying from 10.1 ± 0.7 to 10.4 ± 0.8 n*M*.

Potassium (K^+)-evoked striatal DA release was significantly enhanced among rats supplemented with dietary UMP ($F_{2,266} = 3.36, p < 0.05$) (Fig. 1A,B). Among control rats not receiving UMP, K^+ depolarization increased DA release from 10.2 ± 0.4 to 32.5 ± 1.7 n*M* ($p < 0.01$). To calculate the effects of UMP treatments, baseline DA levels were obtained by pooling the four consecutive samples prior to K^+ stimulation and defined as 100%. Maximum DA release evoked by K^+ was 291 ± 29% of baseline in the control group, 377 ± 16% in the group given UMP for 1 wk, and 350 ± 45% in the group given UMP for 6 wk. Two-way ANOVA, which compared DA levels in the dialysates among treatment groups across time, indicated a significant effect of treatments ($p < 0.05$). Data were further grouped into baseline, K^+-stimulation, and recovery values, as shown in Fig. 1B. Average K^+-stimulated DA release was significantly enhanced in the group that received UMP for 6 wk (341 ± 21%), compared with control (283 ± 9%, $p < 0.05$). The group treated for 1 wk showed an insignificant increase in DA release (316 ± 15%). During the recovery period after K^+ stimulation, DA concentrations fell rapidly to 72–88% of basal values (Fig. 1A,B) and then returned to baseline. Uridine

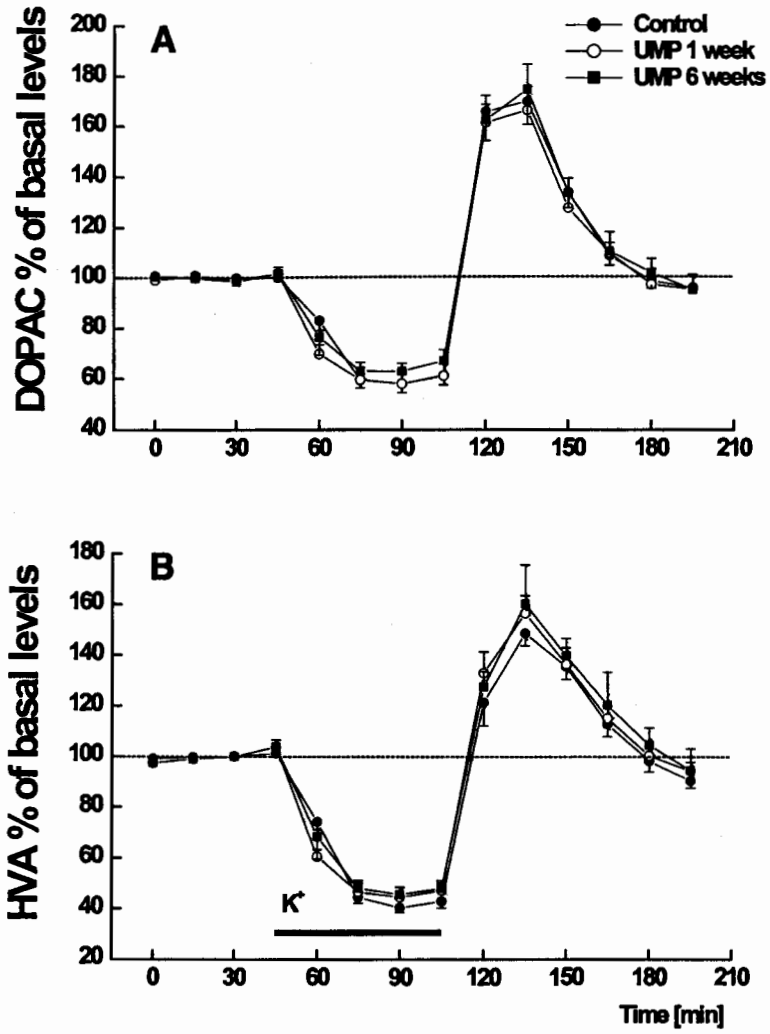

Fig. 2. Effect of high K^+ on DOPAC and HVA levels in animals pretreated with UMP. (**A**) Basal and potassium-depolarized DOPAC release from striata of rats that consumed UMP-containing or control diets for 1 or 6 wk prior to in vivo microdialysis. Animals were prepared and samples collected and assayed as in Fig. 1A. The baseline DOPAC value (612 ± 14 n*M*, $n = 88$; taken as 100%) was obtained by pooling 4 baseline measurements from each of 22 animals. Potassium-depolarized DOPAC release in control animals (65 ± 4%, $n = 7$) was significantly lower than basal release ($p < 0.01$); the effect of consuming UMP was not significant. (**B**) Basal and potassium-depolarized HVA release from striata of rats that consumed UMP-containing or control diets for 1 or 6 wk prior to in vivo microdialysis. The baseline HVA value (369 ± 7 n*M*, $n = 88$; taken as 100%) was obtained by pooling 4 baseline measurements from each of 22 animals. Potassium-depolarized HVA release in control animals (51 ± 4%, $n = 7$) was significantly lower than basal release ($p < 0.01$); the effect of consuming UMP was not significant.

5′-monophosphate (UMP) pretreatment did not affect these recovery values ($p > 0.05$).

Effects of UMP on Striatal DA Metabolites in Dialysates

Basal levels of DOPAC and HVA in dialysates were 612 ± 14 and 369 ± 7 n*M* ($n = 22$ rats), and were stable (all $p > 0.05$) in the four baseline measurements. There were no effects of UMP treatment on basal DOPAC and HVA levels (control vs. UMP for 1 wk vs. UMP for 6 wk; for DOPAC, 581 ± 28, 625 ± 16, 627 ± 25 n*M*; for HVA, 364 ± 15, 367 ± 12, 373 ± 12 n*M*; all $p > 0.05$).

Potassium (K^+) depolarization significantly decreased DOPAC and HVA to 65 ± 4% and 51 ± 4% of basal levels (all $p < 0.01$); UMP treatment had no effect on this response (62 ± 3% and 50 ± 2% in the group receiving UMP for 1 wk; 67 ± 2% and 53 ± 2%

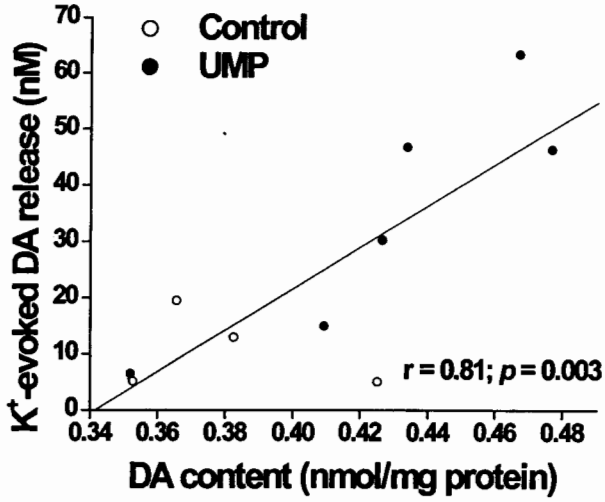

Fig. 3. Correlation between striatal DA content and K^+-evoked DA releases in each of 11 animals fed control ($n = 5$) or 2.5% UMP diets ($n = 6$) for 6 wk. Microdialysis experiments were carried out in right striata as described in the text, and whole left striata were obtained after dialysis to measure tissue DA content. There was a statistically significant correlation ($p < 0.01$) between each animalís K^+-evoked DA release and its striatal DA content postmortem. Data were also analyzed by linear regression, and K^+-evoked DA release was found to vary directly with striatal DA contents ($r = 0.81$).

in the group receiving UMP for 6 wk). During the immediate recovery period, DOPAC and HVA values were increased, peaking at 30 min after exposure to the high K^+ solution ended (DOPAC, 169 ± 9%; HVA, 149 ± 5%). These values returned to baseline after 75 min (Fig. 2A,B) and might have reflected recovery of the DA transporter after the end of depolarization, and uptake and metabolism of the accumulated extracellular DA. No significant differences in DOPAC or HVA levels between control and UMP-treated animals were observed at any time (Fig. 2).

Effects of UMP on Brain DA Contents in Striatum

An experiment was conducted on 11 aged Fischer 344 rats fed the control or 2.5% UMP diet for 6 wk. Microdialysis was carried out as in previous procedures, and whole left striata were obtained after dialysis to measure tissue DA content. There was a statistically significant correlation ($p < 0.01$) between K^+-evoked DA release in each animal and its striatal DA content postmortem (Fig. 3). Data were also analyzed by linear regression, and K^+-evoked DA release was found to vary directly with striatal DA content ($r = 0.81$).

Effect of UMP on Neurofilament Proteins in Striatum

Proteins (NF-70, NF-M) were measured in the contralateral, non-probe-lesioned striata. Six weeks of UMP treatment significantly increased striatal levels of both NF-70 and NF-M to 182 ± 25% ($F2,31 = 6.01$, $p < 0.05$) and 221 ± 34% ($F2,21 = 8.86$, $p < 0.01$) of control values (Fig. 4A,B). One week of treatment failed to increase levels of these proteins (82 ± 19% and 99 ± 14%; $p > 0.05$).

Discussion

These data show that K^+-evoked DA release is significantly increased in rats provided with a UMP-enriched diet for 6 wk, compared with that in rats provided with a control diet; this increase correlates with each animal's striatal DA level, as measured postmortem. Spontaneous release of DA is unaffected by UMP treatment. Striata of rats fed the UMP-enriched diet also display higher levels of two major neurofilament proteins (NF-70 and NF-M), suggesting that UMP also promotes neurite outgrowth in vivo as it does in differentiated PC12 cells (Pooler et al., 2005).

Oral uridine (in the form of UMP) most likely increases PC synthesis by stimulating the formation of CDP-choline. Oral UMP is rapidly adsorbed (Sonoda and Tatibana, 1978) and can provide many-fold increases in plasma uridine concentrations. In normal rats, the plasma uridine level is 1–5 μ*M*, and this uridine is thought to derive mainly from *de novo* synthesis or the salvage pathway (Gasser et al., 1981; Pizzorno et al., 2002). Following UMP administration by gavage to gerbils (1 mmol/kg, which is

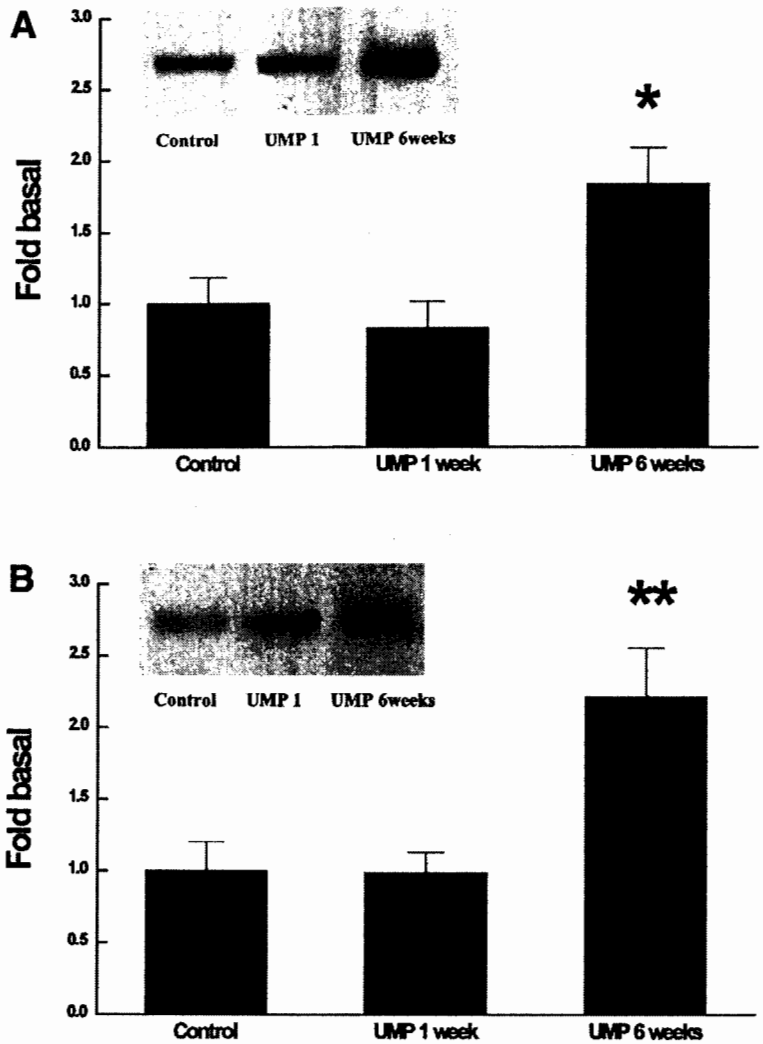

Fig. 4. Effect of UMP treatments on neurofilament protein levels in striatum. Proteins NF-70 (**A**) and NF-M (**B**) were measured in the contralateral, non-probe-lesioned striata after microdialysis experiments. UMP treatment (2.5%) for 6 wk significantly increased striatal levels of both NF-70 and NF-M, to 182 ± 25% ($p<0.05$) and 221 ± 34% ($p<0.01$) of control values. One week of treatment failed to increase levels of these proteins (82 ± 19% and 99 ± 14%). (*) $p<0.05$; (**) $p<0.01$ compared with controls.

similar to the dose of UMP consumed by rats in our experiment), plasma uridine levels rise by fivefold after 30 min (M. Cansev, pers. comm.). In human subjects, a smaller oral dose of UMP (2000 mg, approx 25 mg/kg) also raises plasma uridine threefold after 1–2 h (data not shown). Uridine can readily cross the blood–brain barrier via an unsaturated transport system (Cornford and Oldendorf, 1975) and thus increases brain uridine levels (Cansev et al., 2004). These elevations then increase brain levels of UTP, CTP (by amination of the UTP), and CDP-choline and finally enhance the synthesis of PC (Cansev et al., 2004). (As discussed below, UTP formed from uridine can also affect brain metabolism by directly activating P2Y receptors.)

Age-related reductions have been described in human (Kaasinen and Rinne, 2002) and rat (Kametani et al., 1995; Yurek et al., 1998) striatal DA levels and might explain the motor dysfunctions that can develop during aging. Thus, it is important to determine whether an increase in the synthesis of neuronal membrane as produced by uridine might also increase DA release. We postulate that the increase in DA release evoked by K^+ (Fig. 1B) is

caused by an increase in membrane synthesis. The time course of this phenomenon, being observed after 6 wk but not 1 wk of UMP consumption (Fig. 1B), is compatible with results of a previous report in which 6 wk of treatment with oral CDP-choline, a uridine source, was required to stimulate an absolute increase in brain PC levels (Agut et al., 1993). The increased membrane might allow for increased formation of synaptic vesicles, which could, among other things, protect DA from being metabolized by enzymes and cause more DA to be released in response to K^+ depolarization (Fig. 3). It might also increase the size or number of striatal DA synapses. Either mechanism could explain an increase in striatal DA content. Thus, the mechanism of K^+-evoked DA release might be related to the increased DA content.

Membrane phospholipids such as PC are needed for axonal and dendritic growth (Goldberg, 2003). Our data show that UMP treatment increases striatal levels of cytoskeletal proteins (NF-70 and NF-M; Fig. 4). These structural proteins, which are highly enriched in neurites, have been demonstrated to be reliable markers of neurite outgrowth (Lee et al., 1982); thus, their increases suggest increased branching or lengthening of axons or dendrites. Longer or more branched neurites might increase the number of contacts between the neurons and, consequently, the number of synapses that can be made between them. Neurofilament proteins also maintain the structural integrity of neurons and participate in intracellular axonal transport (Grafstein and Forman, 1980); these functions suggest that they might be involved in modulating neurotransmitter release.

Our findings are supported by an earlier report demonstrating that consuming a CDP-choline enriched diet increased K^+-evoked striatal DA release in rats (Agut et al., 2000). Both UMP and CDP-choline provide pyrimidine nucleosides (uridine and cytidine) to the blood and thereby promote brain CTP synthesis and, ultimately, brain membrane synthesis.

In addition to enhancing PC synthesis via the Kennedy cycle, UTP formed from uridine can also affect neuronal growth by interacting with P2Y receptors (Pooler et al., 2005). Activation of these G protein-coupled receptors can stimulate formation of downstream messengers, including IP_3, DAG, Ca^{2+}, and PKC, all of which can affect neurotransmitter release (Krugel et al., 2001; Shoji-Kasai et al., 2002) and neurite outgrowth (Sivasankaran et al., 2004). Hence, oral UMP might act via two complementary biochemical mechanisms.

We examined the effects of dietary UMP on stimulated DA release and neurofilament protein levels in aged, socially restricted rats. Our data show that UMP can, by either increasing CDP-choline (PC) synthesis or stimulating P2Y receptors, modulate both processes. If UMP has similar effects in humans, its consumption could slow age-related declines in striatal dopamine levels and perhaps ameliorate age-related motor dysfunction.

Acknowledgments

The authors thank Carol J. Watkins, Maya A. Hasan, Nayeli A. Dault, and Dr. Eline Van der Beek for their advice and excellent assistance. These studies were supported by grants from the National Institutes of Mental Health (grant no. 2 R01 MH028783-28) and the Center for Brain Sciences and Metabolism Charitable Trust.

References

Agut J., Lopez G-Coviella I., Ortiz J. A., and Wurtman R. J. (1993) Oral cytidine 5′-diphosphate choline administration to rats increases brain phospholipid levels. *Ann. N. Y. Acad. Sci.* **695**, 318–320.

Agut J., Ortiz J. A., and Wurtman R. J. (2000) Cytidine-5′-diphosphocholine modulates dopamine K+-evoked release in striatum measured by microdialysis. *Ann. N. Y. Acad. Sci.* **920**, 332–335.

Araki W. and Wurtman R. J. (1997) Control of membrane phosphatidylcholine biosynthesis by diacylglycerol levels in neuronal cells undergoing neurite outgrowth. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 11946–11950.

Araki W. and Wurtman R. J. (1998) How is membrane phospholipid biosynthesis controlled in neural tissues? *J. Neurosci. Res.* **51**, 667–674.

Cansev M., Modyanova N. N., Watkins C. J., and Wurtman R. J. (2004) Oral uridine-5-monophosphate (UMP) elevates brain CDP-choline and improves spatial memory in gerbils. Thirty-fourth Neuroscience Meeting Abstract, 435.15.

Cornford E. M. and Oldendorf W. H. (1975) Independent blood-brain barrier transport systems for nucleic acid precursors. *Biochim. Biophys. Acta* **394**, 211–219.

Gasser T., Moyer J. D., and Handschumacher R. E. (1981) Novel single-pass exchange of circulating uridine in rat liver. *Science* **213**, 777, 778.

Goldberg J. L. (2003) How does an axon grow? *Genes Dev.* **17**, 941–958.

Grafstein B. and Forman D. S. (1980) Intracellular transport in neurons. *Physiol. Rev.* **60**, 1167–1283.

Kaasinen V. and Rinne J. O. (2002) Functional imaging studies of dopamine system and cognition in normal aging and Parkinson's disease. *Neurosci. Biobehav. Rev.* **26**, 785–793.

Kametani H., Iijima S., Spangler E. L., Ingram D. K., and Joseph J. A. (1995) In vsivo assessment of striatal dopamine release in the aged male Fischer 344 rat. *Neurobiol. Aging* **16,** 639–646.

Kennedy E. P. and Weiss S. B. (1956) The function of cytidine coenzymes in the biosynthesis of phospholipids. *J. Biol. Chem.* **222,** 193–214.

Krugel U., Kittner H., Franke H., and Illes P. (2001) Stimulation of P2 receptors in the ventral tegmental area enhances dopaminergic mechanisms in vivo. *Neuropharmacology* **40,** 1084–1093.

Lee V., Trojanowski J. Q., and Schlaepfer W. W. (1982) Induction of neurofilament triplet proteins in PC12 cells by nerve growth factor. *Brain Res.* **238,** 169–180.

Paxinos G. and Watson C. (1986) The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press, San Diego, CA.

Pizzorno G., Cao D., Leffert J. J., Russell R. L., Zhang D., and Handschumacher R. E. (2002) Homeostatic control of uridine and the role of uridine phosphorylase: a biological and clinical update. *Biochim. Biophys. Acta* **1587,** 133–144.

Pooler A. M., Guez D. H., Benedictus R., and Wurtman R. J. (2004) Uridine enhances neurite outgrowth in NGF-differentiated PC12 cells. *Neuroscience,* in press.

Richardson U. I., Watkins C. J., Pierre C., Ulus I. H., and Wurtman R. J. (2003) Stimulation of CDP-choline synthesis by uridine or cytidine in PC12 rat pheochromocytoma cells. *Brain Res.* **971,** 161–167.

Shoji-Kasai Y., Itakura M., Kataoka M., Yamamori S., and Takahashi M. (2002) Protein kinase C-mediated translocation of secretory vesicles to plasma membrane and enhancement of neurotransmitter release from PC12 cells. Eur. *J. Neurosci.* **15,** 1390–1394.

Sivasankaran R., Pei J., Wang K. C., Zhang Y. P., Shields C. B., Xu X. M., and He Z. (2004) PKC mediates inhibitory effects of myelin and chondroitin sulfate proteoglycans on axonal regeneration. *Nat. Neurosci.* **7,** 261–268.

Sonoda T. and Tatibana M. (1978) Metabolic fate of pyrimidines and purines in dietary nucleic acids ingested by mice. *Biochim. Biophys. Acta* **521,** 55–66.

Wang L., Osborne P. G., Yu X., Shangguan D., Zhao R., Han H., and Liu G. (2003) Hyperoxia caused by microdialysis perfusion decreased striatalmonoamines: involvement of oxidative stress. *Neurochem. Int.* **42,** 465–470.

Weiss G. B. (1995) Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. *Life Sci.* **56,** 637–660.

Wurtman R. J., Regan M., Ulus I., and Yu L. (2000) Effect of oral CDP-choline on plasma choline and uridine levels in humans. *Biochem. Pharmacol.* **60,** 989–992.

Yurek D. M., Hipkens S. B., Hebert M. A., Gash D. M., and Gerhardt G. A. (1998) Age-related decline in striatal dopamine release and motoric function in Brown Norway/Fischer 344 hybrid rats. *Brain Res.* **791,** 246–256.