The inducible isoform II of nitric-oxide synthase (iNOS) was recently cloned from brain and identified in astroglial cells. Induced nitric oxide biosynthesis occurs in brain cells only if extracellular cerebrospinal fluid contains L-arginine. This study demonstrates for the first time that induced iNOS activity is strictly dependent on concomitant induction of an alternatively spliced transcript of the cat-2 gene encoding high affinity L-arginine transporter System y* in cultured rat astrocytes. Inhibition profiles of radiolabeled L-arginine and L-leucine uptake identified the dominance of Na+-independent transport System y* serving cationic amino acids, with insignificant activities of Systems y L, b0,+t, or B0,+t. A reverse transcription-polymerase chain reaction/sequencing/cloning strategy was used to identify a single 123-base nucleotide sequence coding the high affinity domain of alternatively spliced CAT-2 (not CAT-2a) in astrocytes activated by lipopolysaccharide/interferon-γ. Using this sequence as a cDNA probe, it was determined that CAT-2 mRNA, iNOS mRNA, and System y* activity were concomitantly and strongly induced in astrocytes. Constitutive CAT-1 mRNA was weakly present in neurons and astrocytes, was not inducible in either cell type, and contributed <3% to total System y* activity. Although astroglial iNOS $K_m$ = 10 μM L-arginine for intracellular substrate, hyperbolic kinetics of inducible iNOS activity measured as a function of extracellular L-arginine concentration gave $K_m$ = 50 μM L-arginine with intact cells. The same $K_m$ = 50 μM was obtained for induced membrane transport System y* activity. iNOS activity was reduced to zero in the absence of extracellular L-arginine uptake via System y*. These findings expand the current understanding of NO biosynthesis modulation and implicate a coordinated regulation of intracellular iNOS enzyme activity with membrane L-arginine transport in brain.

The abbreviations used are: iNOS, inducible isoform II of nitric-oxide synthase; LPS, lipopolysaccharide; IFN-γ, interferon-γ; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; NMG, N-methyl-D-glucamine; kb, kilobase(s); RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s).

Controlled augmentation of nitric oxide (NO) biosynthesis is responsible for a variety of important physiological events in animals. In the central nervous system, NO is a double-edged sword, whereby excessive synthesis contributes to the pathophysiology of AIDS dementia, multiple sclerosis, and other neurodegenerative diseases (1). The mechanism by which cells achieve high levels of NO remains poorly understood, with current research efforts focused on signaling/transcription/translation of the inducible isoform II of nitric-oxide synthase (iNOS). This study addresses induced NO biosynthesis in the brain from the alternative perspective that modulating the accessibility of extracellular L-arginine (in cerebrospinal fluid) to intracellular iNOS is a critical step in controlling NO production. L-Arginine can move across plasma membranes via transport Systems denoted by y*, b0,+t, B0,+t, or y L, although not all transporters are expressed in every cell type.

Nitric oxide (NO) is generated in cells of the central nervous system by two isoforms of nitric-oxide synthase. Low levels of NO are produced by Ca2+-dependent isoform I, which is constitutively expressed in both neurons and glia (1). Large amounts of NO are synthesized by the inducible isoform II of nitric oxide synthase (iNOS) following brief activation with bacterial antigens and/or cytokines (1–8). iNOS was recently cloned from brain, and it is localized to astrocytes and microglia, based on in situ hybridization and histochemical staining of brain tissue (1, 2–5). NO synthesis by iNOS is sustained in brain cells in vitro and in vivo only if L-arginine exists at physiological concentrations in the extracellular cerebrospinal fluid (1, 9–11), but the molecular mechanism underlying this observation is unknown (12). Sustained high levels of NO synthesis is a poignant issue in the brain because this organ lacks the urea cycle enzymes carbamoyl-phosphate synthetase and ornithine carbamoyl transferase, which are required to maintain adequate intracellular synthesis of L-arginine (13–15). L-Arginine is the sole metabolic precursor for NO biosynthesis (1, 2). The molecular mechanism of L-arginine transport regulation is unknown, although outside the brain it has been observed that cationic amino acid uptake is stimulated in NO-producing macrophages activated by LPS/IFN-γ (16–19).

EXPERIMENTAL PROCEDURES

Preparation of Cells—Brain astroglial cultures were prepared (20) from 1-day-old WKY rats (Harlan Sprague-Dawley, Indianapolis, IN). Briefly, the hypothalamic/brain astrogial cultures were prepared (20) from 1-day-old WKY rats (Harlan Sprague-Dawley, Indianapolis, IN).
placed in an isotonic saline solution, pH 7.4, containing streptomycin (100 µg/ml), penicillin G (100 units/ml), and amphotericin-B (0.25 mg/ml). Pia mater and blood vessels were carefully removed, and the remaining brain tissue was minced into 2-mm³ pieces. Minced tissue was then dissociated by trituration at 37°C in 20 ml of isotonic saline solution containing 0.25% trypsin (150 units/ml) and DNase I (8 mg/ml). Dissociated cells were then added to an equal volume of DMEM (Life Technologies, Inc.) containing 10% (v/v) FBS (Life Technologies, Inc.). This suspension was then centrifuged at 1000 x g for 5 min, and the cell pellet was washed twice with 50 ml of DMEM containing 10% FBS. The final cell pellet was resuspended in DMEM containing 10% FBS to a density of 1 x 10⁶ cells/ml, and 10 ml of this suspension were added to poly(L-lysine)-coated 100-mm tissue culture dishes. Primary cultures were maintained in a humidified incubator (5% CO2/95% air) at 37°C, and use was confirmed in cells prepared from at least three separate brain preparations.

Transport and Nitrite Measurements—Initial rates of [³H]-labeled amino acid uptake were measured during a 0–5-min interval, as described previously (22). Our astroglial cell transport buffer contained 1.8 mM CaCl₂, 0.8 mM MgSO₄, 2.7 mM KCl, 1.2 mM KH₂PO₄, 154 mM NaCl, 5.5 mM D-glucose, and 25 mM HEPES/Tris, pH 7.3. For uptake measured in the presence of sodium, 154 mM NaCl replaced NMG chloride. Nitrite concentrations were quantitated using the Greiss reagent (23). Radio-labeled [³H]amino acid concentrations ranged from 10⁻⁴ M to 2 mM (4 µCi/ml, Amersham Corp.).

LPS/IFNγ Treatment—Cell cultures were treated with DMEM/10% FBS lacking (control) or containing 0.1 mg/ml bacterial LPS (Escherichia coli serotype 055:B5; Sigma stock #L2628) plus 100 units/ml IFNγ (rat recombinant; Life Technologies, Inc.). At t = 0, the growth medium was aspirated and replaced with transport media, and cultures were permitted to incubate at 37°C for the times shown. After each period, total RNA was isolated and evaluated by Northern blot analysis, or initial rates of [³H]-labeled arginine uptake via System y⁺ were measured.

Northern Blot Analysis—Cells were washed with phosphate-buffered saline, then lysed directly in 100-ml culture dishes with GTC lysis buffer (4 M guanidine thiocyanate, 0.1 M dithiothreitol, 0.5% sarcosyl, and 20 mM sodium acetate, pH 5.2). Total RNA was prepared by the cesium chloride method (24), and the concentration was estimated by absorbance at 260/280 nm. For Northern analysis, 20 µg of total RNA was denatured in formamide, formaldehyde, MOPS, pH 7.0, sodium acetate, EDTA, and ethidium bromide at 65°C for 10 min. The RNA was fractionated by size on a 1% agarose, 6% formaldehyde, and MOPS buffer and transferred to GeneScreen Plus membranes (DuPont NEN). The membranes were hybridized overnight at 61°C in the above hybridization solution with ³²P-labeled cDNA probes. Some blots were stripped and reprobed according to the manufacturer’s recommendation. The membranes were washed under high stringency conditions (0.04 M sodium phosphate and 0.1% SDS at 65°C four times for 10 min each) and then subjected to autoradiography with an intensifying screen at 85°C. Bands representing transcripts were quantitated using a PhosphorImager and/or NIH Image Analysis software. Band densities were normalized to cathepsin B transcripts that hybridized to a 1.8-kb cDNA cathepsin B probe or ribosomal 18 S/28 S bands visualized by ethidium bromide. Each ³²P-labeled cDNA (50 ng) was the product of random primer extension.

cDNA Clones and Probes—CAT-1, CAT-2, and CAT-2a cDNA probes were prepared from inserts in pSP72 plasmids. The full-length CAT-1 cDNA insert (a gift from J. Cunningham) was directionally subcloned into the pJET vector into the plasmid pSP72 at the BamHI and EcoRI restriction sites. The full-length murine CAT-2 insert (a gift from C. MacLeod) was isolated using an EcoRI digestion. Subcloning into pSP72 of a 132-bp sequence containing the 123-bp sequence of the alternatively spliced region specific for murine CAT-2 was achieved using endogenous BamHI and PvuII sites, and subcloning of the rat 122-bp sequence (pBS1R11A; see below) into the 5 Prime → 3 Prime, Inc. (Boulder CO) pCNTR vector was accomplished by blunt end ligation. The RT-PCR product from rat astroglial RNA (see below) was sequenced, then directly cloned (pBS1R11) into an Invitrogen (San Diego, CA) pCR2.1 TA cloning vector, with the sequence confirmed using M13(-20) primer. As a CAT-2a control, the full-length murine CAT-2a insert (a gift from J. Cunningham) was subcloned into pSP72 at BglII and PstI sites. A full-length iNOS 4.0-kb murine cDNA probe (25) was obtained as a gift from J. Cunningham given to M. Waters in the laboratory of H. Nick. The rat astroglial iNOS cDNA has been subsequently cloned (5), giving 93% identity with the murine iNOS cDNA sequence. The plasmid containing rat cathepsin B (a gift of S. Jin Chan) was digested with EcoRI, providing a 1.8-kb cDNA fragment.

RT-PCR—Total RNA was reverse transcribed using Stratagene re-
verse transcriptase with specific antisense oligonucleotide 5'-TATCCA-GACCTCCTTTGGCGGTGC-3' (sequence from C. MacLeod). Conditions for PCR amplification of the resulting first-strand DNA template were 94 °C denaturing, 58 °C annealing, 72 °C extension, 35 cycles using Taq DNA polymerase, and 1.5 mM MgCl₂. PCR products were purified using Millipore Ultrafree-MC filters, then applied directly to 1% agarose gels with loading buffer, and further purified from cut-out agarose bands using Millipore Durapore filters, according to the manufacturer’s instructions. DNA samples were sequenced in the DNA Sequencing Core Laboratory of the Florida Interdisciplinary Center for Biotechnology Research. Sequencing was accomplished by using the Taq DyeDeoxy Terminator and DyePrimer Cycle Sequencing protocols developed by Applied Biosystems using fluorescently-labeled deoxynucleotides and primers. The labeled extension products were analyzed on an Applied Biosystems Model 373A DNA sequencer.

RESULTS

Plasma Membrane Transport System Identification—The inhibition profiles described in Fig. 1 established that System y’ is the predominant L-arginine transport system in these cells. System y’ is inhibited by cationic amino acid analogues (including N⁰-monomethyl-L-arginine but not N⁰-nitro-L-arginine methyl ether) in the absence of Na⁺ and is inhibited by neutral amino acids (such as L-leucine) or analogues in the presence of Na⁺ (17, 22, 26). The same inhibition profile was obtained in LPS/IFNγ-treated astrocytes. Preloading cells with 2 mM of these analogues did not influence L-arginine uptake. However, preloading with analogue concentrations above 10 mM resulted in significant trans-stimulation effects, consistent with System y’ (17, 26). Neither extracellular nor pre-loaded intracellular L-citrulline influenced L-arginine uptake (data not shown). In contrast to System y’, sodium-independent System L mediates no cationic amino acid transport but instead serves only large branched-chain neutral amino acids such as leucine; System L is defined by uptake inhibition by the synthetic analogue 2-amino-5-bicyclo[2.2.1]heptane-2-carboxylate (17, 22, 26). Thus, in Fig. 3 measurement of [³H]leucine uptake via transport System L served as a control for cationic amino acid-independent neutral amino acid uptake in astroglia.

Astroglial System y’ Encoded by CAT-2 or CAT-1—Physiological membrane transport System y’ activity is encoded by both the cat-I and cat-2 genes (16, 27, 28). The presence of CAT transcripts in rat brain cells was initially established by Northern blot analysis of total RNA from astroglial and neuronal cell cultures. The data of Fig. 2 demonstrate the existence of CAT-1 mRNA in rat neurons and astroglia. CAT-1 mRNA was not increased by LPS/IFNγ in either cell type, corroborating previous observations that CAT-1 is constitutively expressed in most tissues studied (17, 22, 26). CAT-2 was not detected in untreated neurons and was marginally detectable in unactivated astrocytes. However, the data of Fig. 2 demonstrate that CAT-2 mRNA was strongly induced in astrocytes by LPS/IFNγ. The faint CAT-2 mRNA signal in neuronal cultures exposed to LPS/IFNγ was attributable to the 5% astroglial cells present in the neuronal dishes (20, 21). The full-length CAT-2 probe gave results similar to those obtained using a specific 132-bp cDNA probe containing the sequence described in Fig. 3B. The role of astrocyte CAT-2 was subsequently pursued.

Sequence of Brain Alternatively Spliced CAT-2—Next, we determined which of the mutually exclusive alternatively spliced isoforms of induced CAT-2/2a (high affinity isoform CAT-2 or low affinity isoform CAT-2a) was expressed in rat astrocytes. This was accomplished by sequencing the nucleotide region that differs in the two isoforms (Fig. 3). Total RNA from LPS/IFNγ-activated rat astroglia was reverse transcribed using a specific antisense primer oligonucleotide (primer P1; 5'-GTAGGCTGAAACCCTGTCCTTGC-3') with antisense primer P1. These primers hybridized to sequences common to both mCAT-2 (based on a portion of exon 3 from murine lymphoma mCAT-2 (16, 30)) and mCAT-2a (from murine liver) (28), flanking the region of alternative splicing. Following separation on 1% agarose, the single-band PCR product cDNA was purified and sequenced by primer walking using primers P1 and P2 and then sense P3 (5'-AGGGACGCTGCTGGTGTC-3').
The sequence was directly cloned into pBS1R11. As controls, full-length mCAT-2 and mCAT-2a cDNAs (25) were also amplified by PCR using these primers. As shown in Fig. 3, a specific 123-base DNA sequence (subclone pBS1R11A) was nested within a 787 nucleotide sequence obtained by RT-PCR using the primers that flanked the region of alternative splicing. This sequence encodes the \( L^{-}\text{arginine high affinity site of the CAT-2 isoform polypeptide, which is expressed in } \textit{Xenopus oocytes as cationic amino acid transport System } y_1 \text{ activity} \) (16, 28, 29, 31).

Induced \( L^{-}\text{Arginine Transport—} \) The time course of \( CAT-2 \) mRNA accumulation was investigated by Northern analysis of total RNA from LPS/IFN-\( \gamma \)-induced astroglial cells (Fig. 4, A and B). Using either a 132-bp cDNA probe containing the sequenced alternatively spliced portion described in Fig. 3B or a full-length mouse mCAT-2 cDNA (16, 28–30), the same results were obtained. The data of Fig. 4 shows that the stimulation of steady-state \( CAT-2 \) mRNA followed a time course similar to that for \( iNOS \) mRNA, with a peak at 12 h then subsequent decrease through 72 h.

\( \text{L-Arginine uptake rates via transport System } y_1 \) activity increased in LPS/IFN-\( \gamma \)-activated cells, as shown in Figs. 4 and 5. The time course of transport induction (Fig. 4C) was similar to that for \( CAT-2 \) and \( iNOS \) mRNAs (Fig. 4B), although the peak in physiological \( L^{-}\text{arginine uptake activity} \) occurred at 24 h, lagging \( CAT-2 \) mRNA by about 12 h. This delay shift likely represents the time required for transporter polypeptide translation and trafficking to the plasma membrane; actinomycin D (1 \( \mu \)g/ml) present during LPS/IFN-\( \gamma \) treatment prevented increases in \( L^{-}\text{arginine transport} \) (452 ± 15 versus 64 ± 5 pmol/mg/min; \( p < 0.05 \)) and blocked nitrite release (88 ± 5 versus 0.3 ± 0.1 pmol/mg/24 h; \( p < 0.05 \)).

Extracellular \( L^{-}\text{Arginine Kinetics of Induced Membrane Transporter and } iNOS \) Activity—The kinetics of extracellular [\( ^3H \)]L-arginine transport were measured in control and stimulated rat brain astroglial cells, as shown in Fig. 5. Initial rates of \( Na^+ \)-independent [\( ^3H \)]L-arginine uptake via System \( y_1 \) were measured in NMG uptake buffer over the concentration range 0.01–1 mM L-arginine (21 °C). In both cases (Fig. 5), data were fit to hyperbolic kinetics by nonlinear regression analysis; the maximal transport rate for control cells was \( V_{\text{max}} = 734 ± 21 \) pmol/min/mg protein with apparent \( K_m = 44 ± 4 \) \( \mu \)M L-arginine, whereas LPS/IFN-\( \gamma \)-treated cultures gave \( V_{\text{max}} = 1513 ± 42 \) pmol/min/mg protein and apparent \( K_m = 54 ± 3 \) \( \mu \)M L-arginine. For all experiments, the overall mean apparent \( K_m = 49 ± 5 \) \( \mu \)M L-arginine.
The kinetics of NO\textsubscript{2}\textsuperscript{−} released from astroglial cells were also measured as a function of extracellular L-arginine concentration, as shown in Fig. 6. Cells were treated for 24 h in normal DMEM/FBS growth media containing or lacking LPS/IFN\textgamma, then initial rates of nitrite release were measured over a 7-h period after cells were washed and exposed to a defined DMEM growth media (37 °C) containing L-arginine concentrations ranging from 0–2 mM. For LPS/IFN\textgamma-stimulated cells, nonlinear regression analyses of the empirical data of Fig. 6 were fit to single hyperbolic functions $V_{\text{max}} = \frac{[\text{L-Arg}]}{K_m + [\text{L-Arg}]}$, giving $K_m = 46 \pm 3 \mu M$ L-arginine and $V_{\text{max}} = 73 \pm 14 \text{mmol/mg protein/7 h}$. This data also gave a single linear component when re-plotted on an Eadie-Hofstee plot (data not shown), with a slope parallel to that for L-arginine linear component when re-plotted on an Eadie-Hofstee plot. Initial rates of nitrite release were linear from 0–7 h, following a 24-h period of exposing cells to LPS/IFN\textgamma. In an independent attempt to obtain kinetics based on NO\textsubscript{2}\textsuperscript{−} release during the entire 0–24 h LPS/IFN\textgamma treatment period often inappropriately reported in the literature, a complex nonhyperbolic kinetic model resulted. This was the consequence of a 9-h lag period within the 24-h exposure period following addition of LPS/IFN\textgamma, during which time there is no detectable physiological expression of NO\textsubscript{2}\textsuperscript{−} release or L-arginine transport (data and kinetic model not shown).

**DISCUSSION**

Transport System y\textsuperscript{+} Is Induced in Brain Astroglia—This study concerns induced L-arginine plasma membrane transport and its role in intracellular iNOS activity in brain astrocytes. There are four physiological transport Systems (y\textsuperscript{+}, b\textsuperscript{+}, B\textsuperscript{cat}, and y\textsuperscript{−}L) that can mediate cationic amino acid transport across plasma membranes in a variety of cell types (reviewed in Refs. 17 and 26). Because these transport systems are not all expressed in every cell type, we initially determined their presence or absence in our rat brain hypothalamic/brainstem astroglial cell membranes (20). Based on well known criteria identifying each system (17, 22, 26), the inhibition profiles described in Fig. 1 established that System y\textsuperscript{+} is the predominant L-arginine transport system in these cells. The lack of sodium-stimulated leucine or arginine uptake and the lack of sodium-independent leucine-inhibitable L-arginine uptake (Fig. 1) rules out significant, if any, contribution by System y\textsuperscript{−}L, b\textsuperscript{+}, or B\textsuperscript{cat} to total L-arginine uptake in astrocytes.

Physiological transport System y\textsuperscript{+} activity is encoded by the cat-1 and cat-2 (cationic amino acid transporter) genes (27), with cDNA clones denoted as CAT-1, CAT-2, and CAT-2a (16, 27). Three different polypeptides encoded by these genes each catalyze sodium-independent cationic amino acid uptake. CAT-1 is constitutively expressed in most cell types except liver, where it is absent (16, 17, 28). CAT-2 and CAT-2a are distinct proteins encoded by two mature mRNAs originating from one primary transcript of the cat-2 gene (16, 17, 28, 30). These isoforms result from the mutually exclusive alternative splicing of the transcript in a tissue-specific manner (30). For example, liver expresses only CAT-2a and not CAT-2 mRNA; lymphocytes express CAT-2 (originally named tea gene; T-cells early activation) but not CAT-2a mRNA (16, 17, 28). Such splicing has not been explored in the rat brain.

Alternatively Spliced CAT-2 Is Induced in Rat Brain Astrocytes—Using PCR primers that flanked the region of alternative splicing, the sequenced 123-nucleotide region of Fig. 3B was determined to be 99% identical to mouse lymphocyte CAT-2 (16) and 98% identical to an analogous portion within a human intestinal CAT-2 clone(2). The sequences of Fig. 3 represent the first report of CAT-2 in the rat. The rat brain (Fig. 3B) and mouse lymphocyte sequences differ in only one base within this alternatively spliced region, with rat “A” replacing mouse “G” at nucleotide position 1162. This results in a single amino acid conservative substitution of “I” in rat for “V” in mouse (16) within the predicted 41-amino acid polypeptide sequence (Fig. 3B). This sequence encodes the L-arginine high affinity site of the CAT-2 isoform, which is expressed in Xenopus oocytes as transport System y\textsuperscript{+} activity (16, 28, 31). The rat astroglial amino acid sequence of Fig. 3B is only 49% identical to the...
optimal alignment of the alternatively spliced region of the CAT-2a low affinity isoform (28).

For L-arginine uptake kinetics, the parallel straight lines of the Eadie-Hofstee plot of Fig. 5 demonstrated the presence of a single prominent high affinity transport System y’ in both stimulated and control cells. LPS/IFNγ increased the transport $V_{\text{max}}$, while the apparent $K_m$ remained unchanged (overall $K_m = 49 \pm 5 \mu M$ L-arginine). In mouse lymphocytes, expression of CAT-2 results in high affinity ($K_m = 40–100 \mu M$) System y’ uptake (16, 28). The CAT-2a transporter isoform has a much lower substrate affinity ($K_m = 5 \mu M$) and is expressed only in liver and muscle (28, 30). Inasmuch as cerebrospinal fluid L-arginine concentrations are $<100 \mu M$ (32, 33), CAT-2 is the expected physiologically relevant isoform, with negligible or no anticipated contribution by CAT-2a. Indeed, the transport data of Figs. 1, 4C, and 5 provide physiological evidence for the existence of the high affinity CAT-2 mRNA containing the 123-nucleotide rat astroglial sequence obtained in Fig. 3B.

Induced iNOS Depends on Induced CAT-2 Encoding High Affinity System y’ in Astrocytes—In LPS/IFNγ-activated astroglial cells, CAT-2 mRNA, iNOS mRNA, and high affinity System y’ activity were concomitantly induced (Figs. 4 and 5). In astrocytes, the intracellular iNOS enzymatic activity apparent $K_m = 16 \mu M$ L-arginine (4). In contrast to this, the data of Fig. 6 gave an apparent $K_m = 46 \mu M$ L-arginine for NO biosynthesis from extracellular substrate; nitrite production was a hyperbolic function of extracellular [L-arginine] such that up-regulated nitrite production was reduced to zero when extracellular [L-arginine] = 0 mM. Brain cells lack the appropriate enzymes required to maintain adequate intracellular synthesis of L-arginine for induced NO production (13, 14, 15). The extracellular fluid concentration of L-arginine that is required for sustaining glial intracellular iNOS production of NO ranges from 20–80 mM (1, 7, 9, 10, 11, 32, 33). This range brackets the $K_m = 49 \mu M$ L-arginine measured (Fig. 5) for astroglial membrane System y’ transport activity.

Even at extracellular L-arginine concentrations $\geq 1 \text{mM}$, total nitrite production in unstimulated control cells was $<3\%$ that in LPS/IFNγ-treated cells (Fig. 6). The paucity of nitrite production in the absence of induced CAT-2 (Figs. 2, 4, and 5) points to a negligible contribution by CAT-1 to overall System y’ activity. Constitutive CAT-1 mRNA was weakly detectable and was not induced by LPS/IFNγ (Fig. 2). Thus, even if CAT-1 mRNA were expressed and contributed to baseline System y’ activity $V_{\text{max}}$ with an apparent $K_m$ coincidently equal to CAT-2 (Fig. 5), the contribution by CAT-1 to the induced uptake kinetics is trivial relative to CAT-2.

In conclusion, our results demonstrate that induced NO biosynthesis is dependent on induced expression of high affinity transport System y’ activity encoded by alternatively spliced CAT-2 in cultured astroglial cells. In light of empirical observations that brain iNOS activity requires extracellular L-arginine, these data support the hypothesis that modulating iNOS activity via access to extracellular L-arginine is an essential step to ultimately controlling NO biosynthesis within these cells. The results further suggest merit in pursuing studies concerning the coordination of signaling and promoter regions of iNOS and CAT-2 in brain and possibly other cells outside the central nervous system.

Acknowledgments—We thank M. S. Kilberg and C. MacLeod for critical reading of the manuscript and J. Bosworth for technical assistance. We thank H. Nick, S. Shanker, W. Wakeland, R. D. Allison, D. Purich, Y. Hong, D. Lu, J. Streit, D. Feldman, and W. Farmerie for helpful discussions.

REFERENCES

1. Brosnan, C. F., Battistini, L., Raine, C. S., Dickson, D. W., Casadevall, A., and Lee, S. C. (1994) Dev. Neurosci. 16, 152–161
2. Forsterrmann, U., Closs, E. L., Pullock, J. S., Nakane, M., Schwartz, P., Gath, I., and Kleiner, H. (1994) Hypertension 23, 1121–1131
3. Simmons, M. L., and Murphy, S. J. (1993) Neurochemistry 59, 897–905
4. Galea, E., Feinstein, D. L., and Reis, D. J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10945–10949
5. Galea, E., Reis, D. J., and Feinstein, D. L. J. (1994) Neurosci. Res. 37, 404–414
6. Feinstein, D. L., Galea, E., and Reis, D. J. (1994) Ann. NY Acad. Sci. 738, 325–328
7. Park, S. K., Geyrlich, D., Lin, H. L., and Murphy, S. (1994) Neuropharmacology 33, 1419–1423
8. Simmons, M. L., and Murphy, S. (1993) Eur. J. Neurosci. 5, 825–831
9. Rao, B. L., Audet, R. M., and Butterworth, R. F. J. (1995) Neurochemistry 65, 677–678
10. Schmidlin, A., and Wiesinger, H. (1994) Glia 11, 262–268
11. Schmidlin, A., and Wiesinger, H. J. (1995) Neurochemistry 65, 590–594
12. Travia, J. (1994) Science 260, 970–972
13. Masters, B. S. (1994) Annu. Rev. Nutr. 14, 131–145
14. Alano, E., Garcia-Perez, A., Buejo, J., and Rubio, V. (1991) Neurochem. Res. 16, 787–794
15. Buniatian, H. C. (1971) in Handbook of Neurochemistry (Lajtha, A., ed) Vol. V, pp. 235–247, Plenum Publishing Corp., New York
16. MacLeod, C., Finley, K. D., and Kakuda, D. K. (1994) J. Exp. Biol. 19, 109–121
17. Malandro, M., and Kilberg, M. S. (1996) Annu. Rev. Biochem. 65, 305–330
18. Bogle, R. G., Baydoun, A. R., Pearson, J. D., Mnneda, S., and Mann, G. (1992) Biochem J. 284, 15–18
19. Baydoun, A. R., and Mann, G. E. (1994) Biochem. Biophys. Res. Commun. 200, 726–731
20. Raizada, M. K., Phillips, M. I., Crews, F. T., and Summers, C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 4655–4659
21. Summers, C., Tang, W., Zelena, B., and Raizada, M. K. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 7567–7571
22. Pan, M., Malandro, M., and Stevens, B. R. (1995) Am. J. Physiol. 268, F459–4676
23. Ding, A. H., Nathan, C. F., and Stuehr, D. J. (1988) J. Immunol. 141, 2407–2412
24. Fredrick, M., Ausubel, R. B., Kingston, R. E., Moore, D., Seidman, J., Smith, J., and Struhl, K (1992) Short Protocols in Molecular Biology, 2nd Ed., pp. 4.5–4.6, John Wiley & Sons, New York
25. Lyons, C. R., Orloff, G. J., and Cunningham, J. M. (1992) J. Biol. Chem. 267, 6370–6374
26. Kilberg, M. S., Stevens, B. R., and Novak, D. (1993) Annu. Rev. Nutr. 13, 137–165
27. Reizer, J., Finley, K., Kakuda, D., MacLeod, C. L., Reizer, A., and Saier, M. H. (1993) Proteins Sci. 20, 30–30
28. Closs, E. L., Lyons, C., Kelly, C., and Cunningham, J. M. (1993) J. Biol. Chem. 268, 20796–20801
29. Kakuda, D. K., Finley, K. D., Dionne, V. E., and MacLeod, C. L. (1993) Transgene 1, 91–101
30. Finley, K. D., Kakuda, D. K., Barriques, A., Kleeman, J., Huynh, P. D., and MacLeod, C. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9378–9382
31. Van Winkle, L. J., Kakuda, D. K., and MacLeod, C. L. (1996) Biochem. Biophys. Acts 1233, 213–216
32. Lemar, J., Herranz, A. S., Herreras, O., Abraira, V., and Martin del Rio, R. (1986) Brain Res. 384, 145–155
33. Wood, J. H. (1982) Handbook of Neurochemistry (Lajtha, A., ed) 2nd Ed., Vol. 1, pp. 415–487, Plenum Press, New York
Induced Nitric Oxide Synthesis Is Dependent on Induced Alternatively Spliced CAT-2 Encoding L-Arginine Transport in Brain Astrocytes
Bruce R. Stevens, Donald K. Kakuda, Kan Yu, Michael Waters, Chi B. Vo and Mohan K. Raizada

J. Biol. Chem. 1996, 271:24017-24022.
doi: 10.1074/jbc.271.39.24017

Access the most updated version of this article at http://www.jbc.org/content/271/39/24017

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 9 of which can be accessed free at http://www.jbc.org/content/271/39/24017.full.html#ref-list-1