Journal of Neurochemistry
Lippincott Williams & Wilkins, Inc., Philadelphia

Elevated N^1-Acetylspermidine Levels in Gerbil and Rat Brains After CNS Injury

A. Muralikrishna Rao, James F. Hatcher, Aclan Doğan, and Robert J. Dempsey

Department of Neurological Surgery, University of Wisconsin; and Veterans Administration Hospital, Madison, Wisconsin, U.S.A.

Abstract: The polyamine system is very sensitive to different pathological states of the brain and is perturbed after CNS injury. The main modifications are significant increases in ornithine decarboxylase activity and an increase in tissue putrescine levels. Previously we have shown that the specific polyamine oxidase (PAO) inhibitor N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72527) reduced the tissue putrescine levels, edema, and infarct volume after transient focal cerebral ischemia in spontaneously hypertensive rats and traumatic brain injury of Sprague–Dawley rats. In the present study, N^1-acetylspermidine accumulation was greater in injured brain regions compared with sham or contralateral regions following inhibition of PAO by MDL 72527. This indicates spermidine/spermine-N^1-acetyltransferase (SSAT) activation after CNS injury. The observed increase in N^1-acetylspermidine levels at 1 day after CNS trauma paralleled the decrease in putrescine levels after treatment with MDL 72527. This suggests that the increased putrescine formation at 1 day after CNS injury is mediated by the SSAT/PAO pathway, consistent with increased SSAT mRNA after transient ischemia. **Key Words:** Cerebral ischemia—Spermidine/spermine-N^1-acetyltransferase—Polyamine oxidase—MDL 72527—Putrescine—Traumatic brain injury—Polyamine interconversion pathway—Polyamines—Ornithine decarboxylase.
J. Neurochem. **74,** 1106–1111 (2000).

Putrescine, spermidine, and spermine are endogenous polyamines essential for cellular growth, proliferation, regeneration, and differentiation (Pegg and McCann, 1982; Morgan, 1987; Marton and Pegg, 1995). The metabolism and catabolism of polyamines are highly regulated by the concerted action of six enzymes (Paschen, 1992*a*,*b*; Marton and Pegg, 1995). The metabolism of polyamines is regulated by ornithine decarboxylase (ODC) and *S*-adenosyl-L-methionine decarboxylase, together with spermidine/spermine synthases, of which ODC is the rate-limiting step for the conversion of ornithine to putrescine. Spermidine/spermine-N^1-acetyltransferase (SSAT) and polyamine oxidase (PAO) regulate polyamine catabolism and the interconversion of spermine/spermidine back to putrescine (Casero and Pegg, 1993; Woster, 1995). In normal brain tissue it has been estimated that ODC accounts for 30% of putrescine, whereas the remaining 70% is formed through the SSAT/PAO pathway (Seiler and Bolkenius, 1985; Seiler, 1995).

Alterations in polyamine metabolism have been implicated in neuronal degeneration after CNS injury (Paschen, 1992*a*,*b*; Schmitz et al., 1993; Kindy et al, 1994; Başkaya et al., 1996*b*; de Vera et al., 1997; Rao et al., 1997; Johnson, 1998; Doğan et al., 1999*a*,*b*). On the other hand, ODC activation and putrescine accumulation showed either no effect or neuroprotection in transient focal cerebral ischemia (Keinanen et al., 1997; Lukkarinen et al., 1997, 1998). Previous work from our laboratory has shown induction of ODC and subsequent alterations in polyamine metabolism, particularly an increase in putrescine levels, to be an important factor in blood–brain barrier dysfunction and the development of vasogenic edema after CNS injury (Dempsey et al., 1991; Kindy et al., 1994; Rao et al., 1995, 1997; Başkaya et al., 1997*b*,*c*). Inhibition of ODC by α-difluoromethylornithine either protected the brain from ischemic damage (Schmitz et al., 1993; Kindy et al., 1994) or enhanced the infarct volume in focal cerebral ischemia (Lukkarinen et al., 1998).

Several mechanisms of polyamine-dependent neuronal injury have been proposed: (a) overactivation of calcium fluxes and neurotransmitter release in areas with an overproduction of putrescine (Koenig et al., 1983); (b) overstimulation of the NMDA receptor complex caused by a release of polyamines into the extracellular space during or after CNS trauma; or (c) production of hydrogen peroxide and 3-acetamidopropanal through activation of the interconversion of spermine and spermidine

Received September 13, 1999; revised manuscript received October 8, 1999; accepted October 12, 1999.

Address correspondence and reprint requests to Dr. R. J. Dempsey at Department of Neurological Surgery, H4/336, Clinical Science Center, 600 Highland Avenue, University of Wisconsin–Madison, Madison, WI 53792-3232, U.S.A. E-mail: dempsey@neurosurg.wisc.edu

Abbreviations used: MCAO, middle cerebral artery occlusion; MDL 72527, N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine; ODC, ornithine decarboxylase; PAO, polyamine oxidase; SHR, spontaneously hypertensive rat; SSAT, spermidine/spermine-N^1-acetyltransferase; TBI, traumatic brain injury.

into putrescine via SSAT/PAO (Morgan, 1987, 1998; Paschen, 1992*a*; Seiler, 1995). SSAT is the rate-limiting step in the catabolism of polyamines (Casero and Pegg, 1993; Suppola et al., 1999), and the activation of SSAT after transient ischemia has been indicated by increased levels of SSAT mRNA (Zoli et al., 1996).

Our recent studies in transient focal cerebral ischemia in spontaneously hypertensive rats (SHRs) (Doğan et al., 1999*b*) and traumatic brain injury (TBI) in Sprague–Dawley rats (Doğan et al., 1999*a*) demonstrated that specific inhibition of PAO by N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine (MDL 72527) attenuated tissue putrescine levels, edema, and ischemic injury volume. The purpose of this study is to show the activation of SSAT by quantifying the accumulated N^1-acetylspermidine after CNS trauma. Here we report that MDL 72527 treatment (a) reduced the tissue putrescine levels in transient forebrain ischemia of the gerbil and (b) resulted in accumulation of N^1-acetylspermidine after CNS injury in the gerbil and the rat. The accumulation of N^1-acetylspermidine was greater in injured compared with sham or contralateral brain regions, which indicates that SSAT was activated (Casero and Pegg, 1993; Suppola et al., 1999) after CNS trauma. To the best of our knowledge, this is the first report showing the increased formation of N^1-acetylspermidine after CNS injury, in agreement with enhanced SSAT mRNA synthesis after transient ischemia (Zoli et al., 1996).

MATERIALS AND METHODS

The following materials were obtained from the indicated suppliers: N^1-acetylspermidine, N^1-acetylspermine, N^8-acetylspermidine, 1,7-diaminoheptane, and dansyl chloride from Sigma Chemical Co. (St. Louis, MO, U.S.A.), HPLC-grade solvents (Fisher Scientific, Pittsburgh, PA, U.S.A.), and Bond-Elut C_{18} columns (Varian Associates, Harbor City, CA, U.S.A.). MDL 72527 was a gift from Hoechst Marion Roussel (Cincinnati, OH, U.S.A.).

All surgical procedures were conducted according to the animal welfare guidelines set forth in the NIH *Guide for the Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services publication no. 85-23, 1985) and were approved by the animal care committee of the University of Wisconsin–Madison.

Transient cerebral ischemia

In male Mongolian gerbils (weighing 50–80 g) common carotid arteries were exposed through a neck incision and occluded using aneurysm clips for 10 min with the animal under halothane anesthesia and reperfused for 1 day as described (Rao et al., 1998, 1999*a*,*b*). Control animals underwent the same surgical procedure except the vessels were not occluded. Body temperature was maintained at 37–38°C during ischemia and reperfusion.

Focal cerebral ischemia

Middle cerebral artery occlusion (MCAO) was induced for 2 h in SHRs using intraluminal suture occlusion as described previously (Doğan et al., 1998, 1999*b*). After MCAO, the suture was withdrawn to restore cerebral blood flow. The animals were then permitted to recover from the anesthesia at room temperature.

TBI

Male Sprague–Dawley rats (weighing 300–350 g) were anesthetized with 1.5% halothane in a 50:50 mixture of N_2O/O_2. TBI was induced by using a controlled cortical impact device described earlier (Başkaya et al., 1996*a*,*b*, 1997*a*). Each rat in the experimental groups was injured with a 6-mm-diameter tip at a velocity of 3 m/sec and a 2-mm deformation, whereas rats in the sham group were subjected to the same surgical procedure but received no impact.

Physiological monitoring

Mean arterial blood pressure was monitored and maintained within physiological limits. Core and cranial temperatures were monitored with rectal and temporalis muscle probes and maintained at 37–38°C for rectal and at 36–37°C for temporalis muscle with a heating pad and a lamp.

MDL 72527 administration

MDL 72527 [100 mg (378 μmol)/kg i.p. (Doğan et al., 1999*a*,*b*)] or saline was administered to animals just after the end of occlusion in transient forebrain ischemia of gerbils, at the end of MCAO in SHRs, and at 5 min after TBI in Sprague–Dawley rats.

Tissue polyamine determination

The brain tissue was homogenized in 0.2 *M* perchloric acid. Homogenates were centrifuged at 16,000 *g* for 20 min. Polyamines in the supernatant were quantified as described earlier (Kabra et al., 1986) with minor modifications (Rao et al., 1998). The supernatants were dansylated and applied to Bond-Elut C_{18} columns (Varian Associates), and the dansylated polyamines were eluted with 1.5 ml of acetonitrile. Samples were diluted with an equal volume of 90% 10 m*M* sodium acetate (pH 4.5)/10% acetonitrile (solvent A), and 50 μl was injected into a Hewlett-Packard model 1100 high-performance liquid chromatograph. The instrument was fitted with a Waters 8-mm $\times$ 100-mm Nova-Pak column and radial compression module. The column was eluted with a mixture of 48% acetonitrile and 52% solvent A at a flow rate of 3 ml/min with a linear gradient to 90% acetonitrile from 0 to 25 min and 90 to 100% acetonitrile from 25 to 30 min. The uniqueness of this HPLC system is that quantification of putrescine, spermidine, N^1-acetylspermidine, N^8-acetylspermidine, spermine, N^1-acetylspermine, and MDL 72527 is accomplished in a single HPLC assay (Rao et al., 1998). The polyamines were quantitated against internal (1,7-diaminoheptane) and external standards using a Hewlett-Packard model 1046A fluorescence detector with λ_{ex} 340 nm and λ_{em} 515 nm. Polyamine levels were expressed as nanomoles per gram wet weight.

Statistical analysis

All the measurements were expressed as mean $\pm$ SD values. Data were analyzed using a one-factor ANOVA with the Bonferroni test to compare differences between the groups (GraphPad Software, San Diego, CA, U.S.A.). A value of $p < 0.05$ was considered significant.

RESULTS

MDL 72527 crosses blood–brain barrier and is present in brain 1 day after CNS injury

MDL 72527 levels in brain regions were measured by HPLC. The relative tissue levels of MDL 72527 in sham and CNS-injured animals were calculated in brain regions at 1 day as the percentage of administered dose

TABLE 1. *Tissue levels of MDL 72527 as a percentage of administered dose at 1 day in sham and CNS-injured animals*

Type of injury, region	% MDL 72527
Transient forebrain ischemia (n = 6)	
Sham	
Cortex	26.7 ± 3.4
Hippocampus	33.3 ± 3.1
Ischemic	
Cortex	19.0 ± 1.0[a]
Hippocampus	32.1 ± 4.0
Focal transient cerebral ischemia (n = 9)	
Contralateral	
Cortex	31.7 ± 11.4
Striatum	27.9 ± 7.1
Ipsilateral	
Cortex	17.9 ± 10.0[b]
Striatum	23.7 ± 6.9
TBI (n = 7)	
Contralateral	
Cortex	5.4 ± 1.1
Hippocampus	8.9 ± 1.9
Ipsilateral	
Cortex	4.5 ± 0.7
Hippocampus	7.2 ± 1.9

[a] $p < 0.01$ compared with sham cortex.
[b] $p < 0.05$ compared with contralateral cortex.

(378 μmol/kg) and are shown in Table 1. There was a trend toward lower levels of the drug in injured brain tissues compared with the corresponding noninjured (sham or contralateral) regions. These differences became statistically significant in ischemic gerbil cortex ($p < 0.01$, ischemic vs. sham) and SHR ipsilateral cortex after focal ischemia ($p < 0.05$, ipsilateral vs. contralateral).

Accumulation of putrescine is maximal at 1 day after transient forebrain ischemia of the gerbil

Putrescine levels after a 10-min transient forebrain ischemia followed by 6 h and 1 day of reperfusion in gerbils are shown in Fig. 1. There was a significant increase in putrescine levels at 6 h ($p < 0.05$ compared with sham); however, the increase at 1 day was much greater ($p < 0.01$ compared with sham), in agreement with other studies (Paschen, 1992*b*; Paschen et al., 1993).

MDL 72527 treatment resulted in decreased putrescine levels and increased N^1-acetylspermidine levels after CNS injury

Effects of MDL 72527 on putrescine levels after MCAO in SHRs (Doğan et al., 1999*b*) or TBI in rats (Doğan et al., 1999*a*) have been reported earlier.

Transient forebrain ischemia. Treatment with MDL 72527 significantly attenuated putrescine levels in gerbil cortex and hippocampus ($p < 0.01$ compared with untreated ischemic group; Fig. 1). The decrease in putrescine levels following treatment with MDL 72527 was reflected in an increase in N^1-acetylspermidine levels (Fig. 2A). To determine the effects of ischemia, N^1-acetylspermidine accumulation was measured in sham animals given MDL 72527. N^1-acetylspermidine accumulated in cortex and hippocampus of sham animals following treatment with MDL 72527 (Fig. 2A). MDL 72527 treatment resulted in significantly greater accumulation in ischemic cortex ($p < 0.05$) and hippocampus ($p < 0.01$) compared with drug-treated sham animals (Fig. 2A).

MCAO in SHRs. Figure 2B shows the N^1-acetylspermidine levels at 1 day after 2-h MCAO with and without MDL 72527 treatment. There was a significant accumulation of N^1-acetylspermidine in all brain regions of MDL 72527-treated SHRs compared with the saline group ($p < 0.01$). In the treated group, N^1-acetylspermidine levels were significantly higher in the ipsilateral regions ($p < 0.01$) compared with contralateral regions (Fig. 2B).

TBI in Sprague–Dawley rats. TBI after 1 day showed increased levels of N^1-acetylspermidine following MDL 72527 treatment (Fig. 2C). Similar to the trends observed after MCAO, there was significant accumulation of N^1-acetylspermidine in all regions of MDL 72527-treated rats compared with the saline group ($p < 0.01$). Also, in the treated group N^1-acetylspermidine levels were significantly elevated in ipsilateral regions ($p < 0.01$) compared with contralateral regions (Fig. 2C).

In all three models of CNS injury, MDL 72527 treatment attenuated tissue putrescine levels (Fig. 1) (Doğan et al., 1999*a*,*b*). Also, no changes in N^8-acetylspermidine levels or accumulation of N^1-acetylspermine were observed following treatment with MDL 72527 (data not shown).

DISCUSSION

SSAT/PAO may largely determine putrescine levels 1 day after CNS injury

CNS injury results in alteration of polyamine metabolism, including induction of ODC and accumulation of

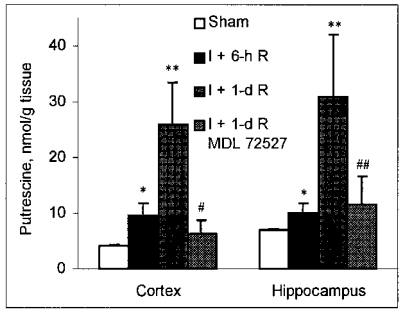

FIG. 1. Putrescine levels in gerbil cortex and hippocampus following a 10-min transient forebrain ischemia (I) and 6-h (n = 6) and 1-day (n = 5) reperfusion (R). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with sham (n = 6); $^{\#}p < 0.01$, MDL 72527-treated ischemic (n = 5) compared with untreated ischemic with 1-day R and not significant compared with sham; $^{\#\#}p < 0.01$, MDL 72527-treated ischemic (n = 5) compared with untreated ischemic with 1-day R and $p < 0.05$ compared with sham.

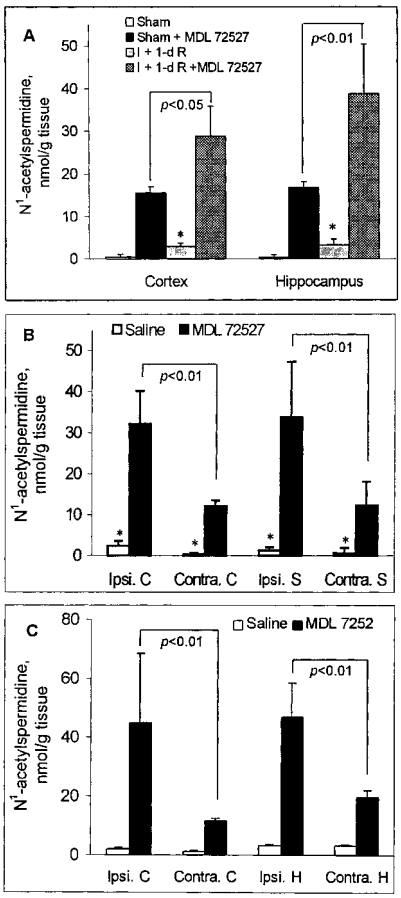

FIG. 2. A: N^1-Acetylspermidine levels in cortex and hippocampus after a 10-min transient forebrain ischemia (I) and a 1-day reperfusion (R) in gerbils. N^1-Acetylspermidine levels in sham animals were measured at 1 day after administration of MDL 72527. Accumulation of N^1-acetylspermidine was significantly greater in ischemic (n = 5) cortex ($p < 0.05$) and hippocampus ($p < 0.01$) compared with the corresponding tissues in MDL 72527-treated sham animals (n = 6). In the absence of PAO inhibition, there was a small but significant increase of N^1-acetylspermidine level in ischemic cortex and hippocampus (*$p < 0.05$) compared with the corresponding tissues in untreated sham animals (n = 6). **B:** N^1-Acetylspermidine levels after treatment with MDL 72527 or saline in cortex (C) and striatum (S) after a 2-h focal ischemia and 1-day reperfusion in SHRs. N^1-Acetylspermidine levels were significantly elevated in the ipsilateral (Ipsi.) regions ($p < 0.01$) compared with contralateral (Contra.) regions. There was significant (*$p < 0.01$) accumulation of N^1-acetylspermidine in all tissues of MDL 72527-treated SHRs (n = 9) compared with levels in corresponding regions of the saline group (n = 8). The N^1-acetylspermidine control values (in nmol/g of tissue) were as follows: cortex, 0.3 ± 0.2 (saline) and 9.6 ± 2.0 (MDL 72527); striatum, 0.6 ± 0.4. (saline) and 10.2 ± 1.8 (MDL 72527). **C:** N^1-Acetylspermidine levels after treatment with MDL 72527 or saline in cortex (C) and hippocampus (H) 1 day following TBI in Sprague–Dawley rats. N^1-Acetylspermidine levels were significantly elevated in the ipsilateral (Ipsi.) regions ($p < 0.01$) compared with contralateral (Contra.) regions. There was significant ($p < 0.01$) accumulation of N^1-acetylspermidine in all regions of MDL 72527-treated rats (n = 7) compared with levels in corresponding regions of the saline group (n = 8). The N^1-acetylspermidine control values (in nmol/g of tissue) were as follows: cortex, 0.37 ± 0.15 (saline) and 8.9 ± 2.2 (MDL 72527); hippocampus, 0.5 ± 0.32 (saline) and 16.2 ± 2.8 (MDL 72527).

putrescine (Dempsey et al., 1991; Paschen, 1992*a*; Kindy et al., 1994; Rao et al., 1995, 1998; Henley et al., 1996; Başkaya et al., 1996*a*,*b*). These changes correlate with the degree of injury and with neuronal death (Kindy et al., 1994; Henley et al., 1996; Ivanova et al., 1998). Although ODC is considered the rate-limiting enzyme in polyamine biosynthesis, it is not the sole pathway accounting for putrescine levels in tissues (Paschen et al., 1988). ODC activity peaks around 6 h after CNS injury (Paschen et al., 1993; Rao et al., 1995, 1998; Başkaya et al., 1996*a*,*b*) and declines at 1 day to near sham levels (Başkaya et al., 1996*a*,*b*; Rao et al., 1998), whereas putrescine levels are elevated at 1 day (Fig. 2) (Paschen et al., 1993; Rao et al., 1998). Activity of *S*-adenosyl-L-methionine decarboxylase, the enzyme that provides the aminopropyl groups necessary for the conversion of putrescine to spermidine and spermine, has been shown to decrease after CNS injury (Henley et al., 1997). Previous studies have indicated that in normal brain, ODC accounts for ~30% of putrescine, whereas the remaining 70% comes through SSAT/PAO (Seiler and Bolkenius, 1985; Seiler, 1995). This study suggests that SSAT/PAO may determine putrescine levels at 1 day, and therefore these experiments focused on the effects of the PAO inhibitor MDL 72527 1 day after CNS injury.

MDL 72527 attenuated putrescine accumulation

In the present study (Fig. 1) and previous studies (Doğan et al., 1999*a*,*b*), inhibition of PAO with MDL 72527 significantly attenuated putrescine levels at 1 day after CNS injury. This suggests that the induction of SSAT (Zoli et al., 1996) and PAO (Baudry and Najm, 1994; Hayashi and Baudry, 1995; Ivanova et al., 1998), which convert spermine and spermidine to putrescine, probably is a major pathway accounting for putrescine accumulation at 1 day. These studies did not attempt to correlate SSAT enzymatic activity to N^1-acetylspermidine levels because the SSAT assay may overestimate N^1-SSAT activity (Persson and Pegg, 1984; Gerner et al., 1993).

PAO inhibition produced accumulation of N^1-acetylspermidine

Following treatment with MDL 72527, PAO is completely inhibited within 30 min, and N^1-acetylspermidine levels increased linearly over time in normal brain tissue (Seiler and Bolkenius, 1985; Seiler, 1995). In our experiments, sham animals treated with MDL 72527 showed uptake of the drug into normal brain (gerbil shams, Table 1), indicating that the drug crosses the blood–brain barrier. Significantly lower levels of the drug were detected in the ischemic versus nonischemic cortex of MDL 72527-treated gerbils and in ipsilateral versus contralateral cortex of SHRs after MCAO. These differences were not significant for the gerbil hippocampus or striatum of SHRs (Table 1).

In the absence of MDL 72527 treatment, N^1-acetylspermidine levels remained low (Fig. 2), indicating conversion of N^1-acetylspermidine to putrescine by PAO.

Inhibition of PAO resulted in a significant accumulation of N^1-acetylspermidine at 1 day with a two- to threefold increase in ischemic versus sham (Fig. 2A) and in ipsilateral versus contralateral regions (Fig. 2B and C), indicative of a CNS injury-induced increase in SSAT activity. Because the N^1-acetylspermidine may represent the cumulative synthesis by SSAT over 1 day, the higher levels of N^1-acetylspermidine in injured brain tissue at 1 day do not necessarily indicate the time of SSAT induction during 1 day. Because MDL 72527 was administered intraperitoneally, there may be a contribution of serum N^1-acetylspermidine to the brain levels; it is not known if this contribution was uniform between injured and noninjured brain regions. SSAT enzyme has a rapid turnover and short half-life and may undergo transient short-term induction (Casero and Pegg, 1993; Seiler, 1995; Suppola et al., 1999). Recently, the SSAT mRNA level was shown to increase after ischemia and reached a maximum at 1 day (Zoli et al., 1996).

In these experiments, neither CNS injury nor MDL 72527 had any effect on N^8-acetylspermidine levels (data not shown). There was also no observable accumulation of N^1-acetylspermine following MDL 72527 treatment (data not shown). Seiler (1995) and Seiler and Bolkenius (1985) showed an ~20-fold greater increase in N^1-acetylspermidine compared with N^1-acetylspermine levels in normal brain following MDL 72527 treatment. This suggests that the kinetics of acetylation by SSAT are much greater for spermidine than spermine (Seiler and Bolkenius, 1985).

After a 10-min transient ischemia, putrescine levels showed a small but significant ($p < 0.05$) increase at 6 h (Fig. 2), which may be partly due to induction of ODC (Rao et al., 1998) and suggests SSAT is not substantially increased within 6 h after CNS injury. Putrescine levels are substantially elevated at 1 day, whereas ODC activity returns near to sham levels (Fig. 1) (Rao et al., 1998), suggesting that induction of SSAT accounts for the increase in putrescine.

Recent evidence demonstrated that under certain conditions PAO could directly act on spermine and spermidine to produce putrescine (Ivanova et al., 1998). Our data indicate that the putrescine is largely formed by PAO action on N^1-acetylspermidine and that the contribution of PAO direct oxidation of spermidine to putrescine appears to be minimal.

The interconversion of polyamines through SSAT/PAO (Casero and Pegg, 1993; Seiler, 1995; Suppola et al., 1999) results in toxic by-products, 3-acetamidopropanal and hydrogen peroxide, which may induce apoptosis after ischemia/reperfusion. The neuroprotective effects of MDL 72527, besides reducing the putrescine formation, may be due in part to attenuation of the toxic by-products. However, the role of polyamines, especially putrescine, in the pathology of CNS injury is not clear and needs to be further investigated.

Acknowledgment: This study was supported by startup funding from the University of Wisconsin (to A.M.R.) and grants from the National Institutes of Health (RO1 NS 28000 and PO1 NS31220) and Department of Veterans Affairs (to R.J.D.). We thank Dr. Böhme of Hoechst Marion Roussel Research Institute for kindly providing MDL 72527 and Dr. Hirak Basu of S'LIL Inc. for his helpful discussions.

REFERENCES

Başkaya M. K., Rao A. M., Prasad M. R., and Dempsey R. J. (1996*a*) Regional activity of ornithine decarboxylase and edema formation after traumatic brain injury. *Neurosurgery* **38,** 140–145.

Başkaya M. K., Rao A. M., Puckett L., Prasad M. R., and Dempsey R. J. (1996*b*) Effect of difluoromethylornithine treatment on regional ornithine decarboxylase activity and edema formation after experimental brain injury. *J. Neurotrauma* **13,** 85–92.

Başkaya M. K., Rao A. M., Doğan A., Donaldson D., and Dempsey R. J. (1997*a*) The biphasic opening of the blood–brain barrier in the cortex and hippocampus after traumatic brain injury in rats. *Neurosci. Lett.* **226,** 33–36.

Başkaya M. K., Rao A. M., Doğan A., Donaldson D., Gellin G., and Dempsey R. J. (1997*b*) Regional brain polyamine levels in permanent focal cerebral ischemia. *Brain Res.* **744,** 302–308.

Başkaya M. K., Rao A. M., Donaldson D., Prasad M. R., and Dempsey R. J. (1997*c*) Protective effects of ifenprodil on ischemic injury size, blood–brain barrier breakdown, and edema formaion in focal cerebral ischemia. *Neurosurgery* **40,** 364–370.

Baudry M. and Najm I. (1994) Kainate-induced seizure activity stimulates the polyamine inter-conversion pathway in rat brain. *Neurosci. Lett.* **171,** 151–154.

Casero R. A. Jr. and Pegg A. E. (1993) Spermidine/spermine N^1-acetyltransferase—the turning point in polyamine metabolism. *FASEB J.* **7,** 653–661.

de Vera N., Camon L., and Martinez E. (1997) Polyamines and brain injury. *Amino Acids* **12,** 1–7.

Dempsey R. J., Carney J. M., and Kindy M. S. (1991) Modulation of ornithine decarboxylase mRNA following transient ischemia in the gerbil brain. *J. Cereb. Blood Flow Metab.* **11,** 979–985.

Doğan A., Başkaya M. K., Rao V. L. R., Rao A. M., and Dempsey R. J. (1998) Intraluminal suture occlusion of the middle cerebral artery in spontaneously hypertensive rats. *Neurol. Res.* **20,** 265–270.

Doğan A., Rao A. M., Başkaya M. K., Hatcher J., Temiz C., Rao V. L. R., and Dempsey R. J. (1999*a*) Contribution of polyamine oxidase to brain injury after trauma. *J. Neurosurg.* **90,** 1078–1082.

Doğan A., Rao A. M., Hatcher J., Rao V. L. R., Başkaya M. K., and Dempsey R. J. (1999*b*) Effects of MDL 72527, a specific inhibitor of polyamine oxidase, on brain edema, ischemic injury volume, and tissue polyamine levels in rats after temporary middle cerebral artery occlusion. *J. Neurochem.* **72,** 765–770.

Gerner E. W., Kurtts T. A., Fuller D. J., and Casero R. A. Jr. (1993) Stress induction of the spermidine/spermine N^1-acetyltransferase by a post-transcriptional mechanism in mammalian cells. *Biochem. J.* **294,** 491–495.

Hayashi Y. and Baudry M. (1995) Effect of kainate-induced seizure activity on the polyamine interconversion pathway in juvenile rat brain. *Dev. Brain Res.* **87,** 96–99.

Henley C. M., Muszynski C., Cherian L., and Robertson C. S. (1996) Activation of ornithine decarboxylase and accumulation of putrescine after traumatic brain injury. *J. Neurotrauma* **13,** 487–496.

Henley C. M., Wey K., Takashima A., Mills C., Granmayeh E., Krishnappa I., and Robertson C. S. (1997) *S*-Adenosylmethionine decarboxylase activity is decreased in the rat cortex after traumatic brain injury. *J. Neurochem.* **69,** 259–265.

Ivanova S., Botchkina G. I., Alabed Y., Meistrell M., Batliwalla F., Dubinsky J. M., Iadecola C., Wang H. C., Gregersen P. K., Eaton J. W., and Tracey K. J. (1998) Cerebral ischemia enhances polyamine oxidation—identification of enzymatically formed 3-aminopropanal as an endogenous mediator of neuronal and glial cell death. *J. Exp. Med.* **188,** 327–340.

Johnson T. D. (1998) Polyamines and cerebral ischemia. *Prog. Drug Res.* **50,** 193–258.

Kabra P. M., Lee H. K., Lubich W. P., and Marton L. J. (1986) Solid-phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phase liquid chromatography: improved separation systems for polyamines in cerebrospinal fluid, urine and tissue. *J. Chromatogr.* **380,** 19–32.

Keinanen R., Miettinen S., Yrjanheikki J., and Koistinaho J. (1997) Induction of ornithine decarboxylase mRNA in transient focal cerebral ischemia in the rat. *Neurosci. Lett.* **239,** 69–72.

Kindy M. S., Hu Y., and Dempsey R. J. (1994) Blockade of ornithine decarboxylase enzyme protects against ischemic brain damage. *J. Cereb. Blood Flow Metab.* **14,** 1040–1045.

Koenig H., Goldstone A. D., and Lu C. Y. (1983) Blood brain barrier breakdown in brain edema following cold injury is mediated by microvascular polyamines. *Biochem. Biophys. Res. Commun.* **116,** 1039–1048.

Lukkarinen J., Grohn O. H. J., Sinervirta R., Jarvinen A., Kauppinen R. A., Janne J., and Alhonen L. I. (1997) Transgenic rats as models for studying the role of ornithine decarboxylase expression in permanent middle cerebral artery occlusion. *Stroke* **28,** 639–645.

Lukkarinen J. A., Kauppinen R. A., Grohn O. H., Oja J. M., Sinervirta R., Jarvinen A., Alhonen L. I., and Janne J. (1998) Neuroprotective role of ornithine decarboxylase activation in transient focal cerebral ischaemia: a study using ornithine decarboxylase-overexpressing transgenic rats. *Eur. J. Neurosci.* **10,** 2046–2055.

Marton L. J. and Pegg A. E. (1995) Polyamines as targets for therapeutic intervention. *Annu. Rev. Pharmacol. Toxicol.* **35,** 55–91.

Morgan D. M. (1987) Polyamines. *Essays Biochem.* **23,** 82–115.

Morgan D. M. L. (1998) Polyamine oxidases—enzymes of unknown function? *Biochem. Soc. Trans.* **26,** 586–591.

Paschen W. (1992*a*) Polyamine metabolism in different pathological states of the brain. *Mol. Chem. Neuropathol.* **16,** 241–271.

Paschen W. (1992*b*) Polyamine metabolism in reversible cerebral ischemia. *Cerebrovasc. Brain Metab. Rev.* **4,** 59–88.

Paschen W., Rohn G., Meese C. O., Djuricic B., and Schmidt-Kastner R. (1988) Polyamine metabolism in reversible cerebral ischemia: effect of alpha-difluoromethylornithine. *Brain Res.* **453,** 9–16.

Paschen W., Cleef M., Rohn G., Muller M., and Pajunen A. E. (1993) Ischemia-induced disturbances of polyamine synthesis. *Prog. Brain Res.* **96,** 147–160.

Pegg A. E. and McCann P. P. (1982) Polyamine metabolism and function. *Am. J. Physiol.* **243,** C212–C221.

Persson L. and Pegg A. E. (1984) Studies of the induction of spermidine/spermine *N*1-acetyltransferase using a specific antiserum. *J. Biol. Chem.* **259,** 12364–12367.

Rao A. M., Başkaya M. K., Maley M. E., Prasad M. R., and Dempsey R. J. (1995) Ornithine decarboxylase activity and edema formation in cerebral ischemia of conscious gerbils. *J. Neurochem.* **65,** 2639–2643.

Rao A. M., Başkaya M. K., Maley M. E., Kindy M. S., and Dempsey R. J. (1997) Beneficial effects of *S*-adenosyl-L-methionine on blood–brain barrier breakdown and neuronal survival after transient cerebral ischemia in gerbils. *Mol. Brain Res.* **44,** 134–138.

Rao A. M., Hatcher J. F., Başkaya M. K., and Dempsey R. J. (1998) Simultaneous assay of ornithine decarboxylase and polyamines after central nervous system injury in gerbils and rats. *Neurosci. Lett.* **256,** 65–68.

Rao A. M., Hatcher J. F., and Dempsey R. J. (1999*a*) CDP-choline: neuroprotection in transient forebrain ischemia of gerbils. *J. Neurosci. Res.* **58,** 697–705.

Rao A. M., Hatcher J. F., Kindy M. S., and Dempsey R. J. (1999*b*) Arachidonic acid and leukotriene C4: role in transient cerebral ischemia of gerbils. *Neurochem. Res.* **24,** 1225–1232.

Schmitz M. P., Combs D. J., and Dempsey R. J. (1993) Difluoromethylornithine decreases postischemic brain edema and blood–brain barrier breakdown. *Neurosurgery* **33,** 882–887.

Seiler N. (1995) Polyamine oxidase, properties and functions. *Prog. Brain Res.* **106,** 333–344.

Seiler N. and Bolkenius F. N. (1985) Polyamine reutilization and turnover in brain. *Neurochem. Res.* **10,** 529–544.

Suppola S., Pietila M., Parkkinen J. J., Korhonen V. P., Alhonen L., Halmekyto M., Porter C. W., and Janne J. (1999) Overexpression of spermidine/spermine N^1-acetyltransferase under the control of mouse metallothionein I promoter in transgenic mice: evidence for a striking post-transcriptional regulation of transgene expression by a polyamine analogue. *Biochem. J.* **338,** 311–316.

Woster P. M. (1995) *S*-Adenosylmethionine decarboxylase and spermidine/spermine-N^1-acetyltransferase—emerging targets for rational inhibitor design, in *Polyamines: Regulation and Molecular Interaction* (Casero R., ed), pp. 171–204. R. G. Landes Co., Austin, Texas.

Zoli M., Pedrazzi P., Zini I., and Agnati L. F. (1996) Spermidine/spermine N^1-acetyltransferase mRNA levels show marked and region-specific changes in the early phase after transient forebrain ischemia. *Mol. Brain Res.* **38,** 122–134.