POLYAMINE RESPONSE TO CNS INJURY: FOR BETTER OR FOR WORSE?

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Running title: Polyamines in CNS Injury

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ABSTRACT

The polyamine system is very sensitive to different pathological states of the brain and is perturbed after CNS injury. The "polyamine response" results in significant increases in ornithine decarboxylase (**ODC**) and spermidine/spermine-N^1-acetyltransferase (**SSAT**) activities, and the accumulation of putrescine, but not spermidine or spermine, after CNS injury.

Studies have shown induction of ODC and subsequent alterations in polyamine metabolism, particularly an increase in putrescine levels, to be important factors in the development of blood-brain barrier dysfunction, edema, and neuronal injury after CNS trauma. Inhibition of ODC by α-difluoromethylornithine (**DFMO**) protected the brain from CNS injury. Specific inhibition of polyamine oxidase (**PAO**) reduced the tissue putrescine levels, edema and infarct volume after CNS injury. These studies indicated that putrescine and the toxic by-products hydrogen peroxide and 3-acetamidopropanal formed by the SSAT/PAO pathway may contribute to apoptotic and necrotic neuronal death after CNS trauma. It has been reported that spermine itself induces apoptosis by caspase-3 activation and cytochrome c release from mitochondria.

Recent studies have not demonstrated a definitive role of polyamines in neuronal injury. Over-expression of ODC in transgenic animals and a large increase in cerebral putrescine levels either reduced or had no effect on the neuronal injury. In other experiments, DFMO exacerbated the neuronal injury. Exogenous administration of naturally occurring polyamines after CNS injury either induced apoptosis or provided neuroprotection.

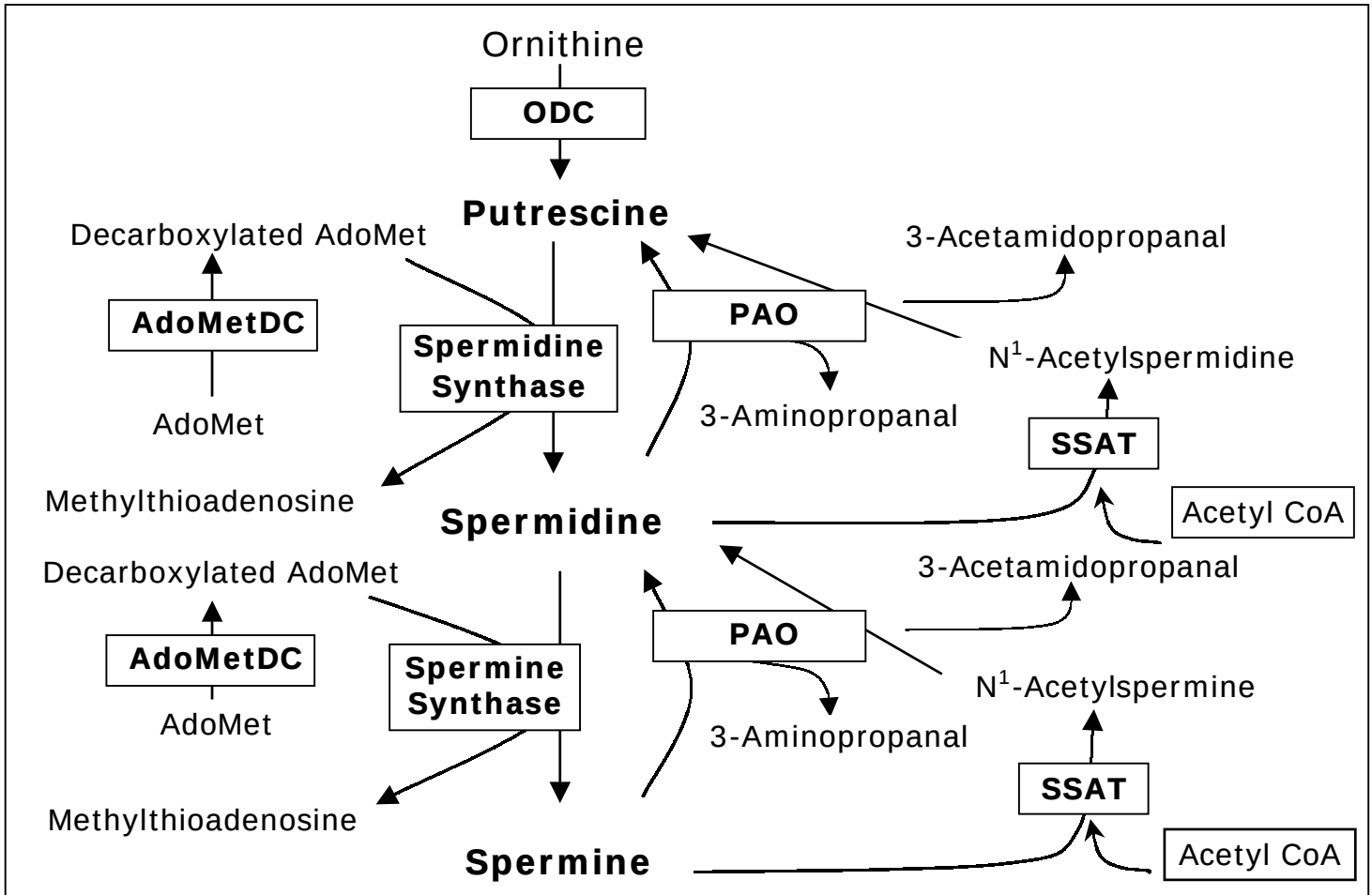
The role of endogenous polyamines in the normal and pathological states of the brain remains uncertain. Whether induction of ODC, SSAT and the alteration of polyamine levels after CNS trauma are adaptive neuroprotective responses or cause neurotoxicity is a controversial issue and needs to be resolved.

INTRODUCTION

Putrescine, spermidine, and spermine are

endogenous polyamines essential for cellular growth, proliferation, regeneration and differentiation [1-3]. Under physiologic pH, the polyamines exist as poly-cationic molecules that bind to proteins and nucleic acids. While the molecular targets and cellular mechanisms have not been completely defined, polyamines are known to bind to and modify the structure of DNA [4], modulate glutamate binding to the NMDA ionophore receptor complex [5-7], and alter specific K^+ channels [8-10].

The metabolism and catabolism of polyamines is highly regulated by the concerted action of six enzymes [1,11-14] (Scheme **1**). The *de novo* synthesis of polyamines is regulated by ornithine decarboxylase (**ODC**), S-adenosyl-L-methionine decarboxylase (**AdoMetDC**), and spermidine/spermine synthases. Of these, ODC is the rate-limiting step for the conversion of ornithine to putrescine. Spermidine/spermine N^1-acetyltransferase (**SSAT**) and polyamine oxidase (**PAO**) regulate the inter-conversion of spermine/spermidine back to spermidine/putrescine [15-17]. In this review, the term 'polyamine oxidase' refers to the enzyme that catalyses the cleavage of spermine/spermidine and their N^1-acetyl derivatives to produce spermidine and putrescine respectively. The PAO reaction is very slow with the parent polyamines, as the preferred substrates for PAO are the N^1-acetyl derivatives formed by SSAT (Scheme **1**) [1]. SSAT is thus considered to be the rate-limiting step in the inter-conversion of polyamines [15,18]. In normal brain tissue it has been estimated that ODC accounts for 30% of



Scheme 1. Polyamine metabolism. Ornithine decarboxylase (ODC); S-adenosyl-L-methionine decarboxylase (AdoMetDC); Spermidine/spermine-N^1-acetyltransferase (SSAT); Polyamine oxidase (PAO).

putrescine, whereas the rest is formed through the SSAT/PAO pathway [19,20].

POLYAMINES IN CNS INJURY: NEUROPROTECTIVE OR NEUROTOXIC?

Beneficial effects of polyamines have been attributed to: 1) anti-apoptotic [21] and antioxidant [22] properties 2) altered neuronal excitability by putrescine [23], 3) chromatin stabilization [24] and altered "polyamine response" [25].

Several mechanisms of polyamine-dependent neuronal injury have been proposed: 1) overactivation of calcium fluxes and neurotransmitter release in areas with an over-production of putrescine [26]; 2) over-stimulation of the NMDA receptor complex [5-7] caused by a release of polyamines into the extracellular space during or after CNS trauma; 3) inward rectification of potassium channels [11,27,28]; and 4) production of hydrogen peroxide, 3-acetamidopropanal and 3-aminopropanal through the inter-conversion of spermine and spermidine into putrescine via SSAT/PAO [2,13,20,29,30].

Many conditions need to be fulfilled for polyamines to be lethal to neurons. Polyamines must be present in sufficient amount; they must be N^1–acetylated and then oxidized to generate hydrogen peroxide; and target cells must be present that are sufficiently sensitive to that toxic product. Alternatively, unmodified polyamines must be presented to diamine oxidases [31] to produce hydrogen peroxide through terminal oxidative mechanisms [30].

STUDIES WITH DFMO PROVIDED AMBIGUOUS RESULTS. The cerebral ODC/polyamine system is very sensitive to both physiological [32] and pathological stimuli [33,34] including CNS injury. The main modifications are significant increases in ODC activity and putrescine concentration, with minor or no variations in spermidine and spermine levels [13,35]. Alterations in polyamine metabolism correlated with the degree of CNS injury and have been implicated in neuronal degeneration [11,13,14,16,35-44].

Previous work from our laboratory has shown that induction of ODC and subsequent alterations in polyamine metabolism, particularly an increase in putrescine levels, are important factors in blood-brain barrier (**BBB**) dysfunction and the development of vasogenic edema after CNS injury [16,40,42,43,45-55].

In the gerbil model of global ischemia, ODC immunoreactivity localizes to those hippocampal CA_1 pyramidal neurons [56] that are destined to undergo delayed neuronal death [57,58]. α-Difluoromethyl-ornithine (**DFMO**), which reduces the synthesis of polyamines by irreversibly inhibiting ODC, has been reported to be a neuroprotective agent following NMDA-infusions, calpain-induced spectrin break-down and against ischemic neuronal damage [14,40]. The neuroprotective effect of DFMO has been regarded as strong evidence favoring the hypothesis that putrescine is a causative factor for neuronal damage. A putative mechanism, through which elevated putrescine could exacerbate neuronal degeneration, has been thought to be its effect on the NMDA receptor [14,59,60].

Studies with DFMO have not, however, afforded unambiguous data for the role of ODC and putrescine accumulation in CNS injury. In *in vitro* studies, DFMO prevented NMDA-induced neurotoxicity in cultured mouse cortical neurons [61], but did not provide neuroprotection against glutamate-induced neuronal death in cultured rat cerebellar granule neurons [62]. In the first study, only the NMDA-sensitive subclass of

glutamate receptors were stimulated to induce neuronal damage, while in cells exposed to glutamate, all glutamate receptor subclasses (NMDA-, AMPA-, kainate-, and metabotropic-) were activated. It is possible, though speculative, that DFMO acts at the NMDA receptor and this effect is overwhelmed by the more diverse injury induced by glutamate.

In some *in vivo* studies, DFMO provided protection from ischemic brain injury [40,44,63], while others have shown either no effect [64] or worsening of injury [23,65,66] after cerebral ischemia.

It is not known whether the dose, timing and duration of DFMO treatment can account for these apparent discrepancies. Two studies, one showing beneficial [63] and another demonstrating adverse [23] effects, administered DFMO prior to ischemia. However, the dose, route and duration of DFMO pretreatment differed. In one study [63], DFMO was administered sub-cutaneously for 3-days, whereas in the other study [23], DFMO was administered in the drinking water for 7-days prior to induction of ischemia.

Putrescine can also be formed by decarboxylation of arginine to agmatine followed by de-amination. While agmatine occurs mainly in plants and bacteria, recent data indicate that in mammalian tissues, including brain, ODC can also decarboxylate arginine [67]. Prolonged administration of DFMO may result in accumulation of arginine, the substrate for nitric oxide synthase (**NOS**). NOS produces the free radical, nitric oxide (**NO•**), which can react with the superoxide anion radical to form peroxynitrite and initiate oxidation of proteins and lipids. It is conceivable that long-term administration of DFMO could have adverse effects by increasing NO• production.

IS ODC ACTIVATION A NEUROADAPTIVE RESPONSE? Cerebral ODC expression with its effect on putrescine level is a sensitive marker for CNS trauma. There are two at least partly contradicting points of view on the functional significance of high putrescine levels in brain. One point of view is that the commonly observed increase in ODC activity and resulting large increase in brain putrescine is a cause of the neuronal damage. The role of putrescine in neuronal necrosis can be deduced only indirectly from 1) the close relationship between putrescine accumulation and the extent of necrosis, 2) the known activities of putrescine, and 3) the observation that pharmacological prevention of ischemia-induced neuronal necrosis also reduces the post-ischemic over production of putrescine [13,14,40].

The other point of view is that these biochemical factors do not cause neuronal damage even if they supersede it [33], or that the increase in putrescine content associated with neuronal damage is a neuroprotective measure rather than a cause of the damage [23,33,68]. Studies using transgenic animals over-expressing ODC have indicated that elevated ODC activity and putrescine accumulation exhibit either no effect or neuroprotection in transient focal cerebral ischemia [23,33,69-71]. In the transgenic animals, the extensive stimulation of ODC and accumulation of endogenous putrescine did not influence long-term recovery and did not exacerbate cell damage, suggesting that under these conditions these two biochemical factors are not involved in the cascade of neuronal injury [33]. In line with this view are recent findings indicating that life-long over expression of ODC in transgenic mice did not lead to neuronal degeneration [72] and that polyamines promote regeneration of injured axons of

cultured rat hippocampal neurons [73].

The results obtained with ODC transgenic animals support the conclusion that elevated putrescine levels have either no effect on ischemic cell damage or provide neuroprotective action. These studies appear to contradict earlier studies in which direct infusion of putrescine into the brain induced neuropathological lesions indistinguishable from ischemic damage [33,74, 75]. Conversely, in other studies, intracortical [29] or intra-striatal [76,77] microinjections of putrescine were not neurotoxic, suggesting that the effects of exogenous polyamines in the brain are unpredictable. It is also conceivable that endogenously produced putrescine acts differently than that exogenously administered [33].

A number of cellular biochemical issues need to be addressed in the transgenic animals to explain some of the unexpected data. In transgenic animals over-expressing ODC, spermidine and spermine pools were not significantly altered despite substantial increases in putrescine levels and spermidine synthase activity in the brain [33]. The elevated putrescine levels were not due to alterations of SSAT and PAO since these enzymes showed similar activity between the syngenic and transgenic animals. It has been hypothesized that the lack of alteration of spermidine/spermine pools in the transgenic animals [78] indicated that putrescine, possibly through sequestration into vacuoles [79,80] or through some other unknown mechanism, is rendered unavailable for further conversion to spermidine and spermine [33]. Polyamine sequestration, however, is a passive, chemical phenomenon that cannot be wholly over-ridden by biological mechanisms. This is a chemical rather than a regulatory phenomenon, and it may owe nothing to a sophisticated control system [79]. Understanding these mechanisms is crucial for the future progress of this line of research as the proportion of 'free' or metabolically active putrescine must be known before conclusive hypotheses are formulated. Thus, while the polyamine response is now recognized to be a critical reaction of neurons to injury, whether this process is neurotoxic or neuroprotective is currently an issue of debate.

SPERMIDINE AND SPERMINE: PRO-APOPTOTIC OR ANTI-APOPTOTIC?

IFENPRODIL PROVIDED NEUROPROTECTION AFTER TRANSIENT CEREBRAL ISCHEMIA. Spermidine and spermine bind to a polyamine recognition site on the NMDA ionophore receptor complex and increase glutamate binding [5-7]. Ifenprodil is an NMDA receptor polyamine binding site antagonist. The precise mechanism underlying the effects of ifenprodil on NMDA receptors is not clear. Experimental results indicate that ifenprodil may interact directly with the ion channel site of the NMDA receptor as a channel blocker like phencyclidine or dizolcipine [81]. Binding studies with [^{3}H]-ifenprodil have revealed high-affinity binding of ifenprodil to the polyamine site of the NMDA receptor [82], and thus it may act to cause strong suppression of the channel-gating process [48,59,81, 83-89].

Administration of ifenprodil significantly reduced the infarct volume following middle cerebral artery occlusion (**MCAO**) [48,86,88] and the neuroprotective effects may be related to its antagonist action at the polyamine modulatory site of NMDA receptor. On the contrary, recent studies attributed anti-apoptotic properties of spermine to its action at NMDA receptor sites [21], while others reported a pro-apoptotic role of spermine in cerebellar granule neurons, which was independent of its actions at NMDA receptors [90].

Experimental studies in animals have demonstrated that systemic treatment with the naturally occurring polyamines can protect neurons after CNS injury [25,65]. Spermine has been shown to have anti-oxidant properties through scavenging of free radicals [22]. Spermine also stabilizes chromatin, thus preventing endonuclease-mediated DNA fragmentation and apoptosis in thymocytes [24]. However, in other studies, significant cortical cell death was induced by micro-injections of spermidine or spermine, which was attributed to metabolism by PAO and formation of cytotoxic 3-aminopropanal [29]. In other studies, spermine-induced neurotoxicity was not blocked by inhibitors of polyamine catabolism and the toxicity was attributed to spermine itself [90].

SSAT/PAO CONTRIBUTE TO CNS INJURY. While ODC is considered the rate-limiting enzyme in polyamine biosynthesis [91], it is not the sole pathway accounting for putrescine levels in tissues. Activities of AdoMetDC, 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 [34,39,42,92]. At 1-day after CNS insult, putrescine accumulates even while ODC activity is declining to near basal levels. This suggests that activation of inter-conversion pathway enzymes, SSAT [93] and PAO [94], which convert spermine to spermidine and spermidine to putrescine, probably is a major factor in putrescine accumulation at 1-day. SSAT is normally present at very low levels, but is highly inducible in response to various pathological injuries. Activation of SSAT after transient ischemia has been indicated by increased SSAT *m*RNA [93] and accumulation of the SSAT product N^1-acetylspermidine after CNS injury [16].

The inter-conversion of spermidine/spermine to putrescine/spermidine is accomplished through N^1-acetylation by SSAT and cleavage by PAO. The amino-propyl function is liberated as 3-acetamido-propanal (Scheme **1**).

Recent studies have provided evidence that PAO may oxidize spermine/spermidine directly, bypassing the SSAT pathway (Scheme **1**), and producing 3-amino-propanal as the aldehyde byproduct. Since the N^1-acetyl derivatives of spermine and spermidine are the preferred substrates for PAO [1], this raises the question of the relationship between these two oxidative reactions and their relative importance in normal and pathological conditions.

The cytotoxicity of 3-aminopropanal is believed to be mediated by cleavage to acrolein [29,95]. It has not been established whether 3-acetamidopropanal undergoes a similar reaction to produce acrolein, and thus its cytotoxicity may be considerably different from the non-acetyl analog. If this is the case, the SSAT/PAO system may represent a regulatory pathway for the cell to generate putrescine from spermine/spermidine without the consequences of 3-aminopropanal formation. However, the two pathways (PAO acting directly on spermidine/spermine *vs* the SSAT/PAO pathway) can only be distinguished by the formation of the respective aldehyde products (Scheme **1**). This issue has not been addressed in the literature.

AMINE OXIDASES MAY CONTRIBUTE TO POLYAMINE TOXICITY. Polyamines are catabolized via two distinct pathways: PAO and the terminal catabolic pathway. The terminal catabolic pathway involves the oxidative deamination of polyamines by copper-containing amine oxidases, generating compounds that cannot be reconverted into polyamines. Both PAO and the terminal oxidases generate potentially toxic reactive oxygen species (**ROS**), such as hydrogen

peroxide, that can damage proteins, DNA and lipids. Several reports have suggested that polyamine toxicity is a direct result of ROS produced by polyamine catabolism [29,37,96,97].

POLYAMINES INDUCE CASPASE ACTIVATION. Spermine induced cell death in cerebellar granule neurons, which was not blocked by the amine oxidase inhibitors aminoguanidine and N^1,N^4-bis-(2,3-butadienyl)-1,4-butadiamine (**MDL 72527**) [90]. This supported the hypothesis that toxicity was due to spermine rather than a catabolite. Consistent with this interpretation, spermine triggered release of cytochrome c from mitochondria and caspase-3 activation [98,99]. Caspase activation was not prevented by anti-oxidants or inhibition of polyamine oxidase [98,99], indicating that spermine itself was responsible for the toxicity. These data suggested that elevated free cytosolic polyamines may act as transducers of a death message [98,99].

In these studies, spermine and spermidine were exogenously added. The contribution of these effects to neuronal death remains an issue since spermidine and spermine levels show no significant change after CNS trauma [16,33,37,38,47,100]. Under physiological conditions, the polyamines exist as poly-cationic molecules that are bound to cellular components, with very low levels existing unbound [1]. It has been suggested that cellular injury may trigger a translocation of polyamines from intracellular stores into the cytosol and these effects could contribute to induction of apoptosis [99], without altering the total polyamine content of the cells.

EXTRACELLULAR POLYAMINES DID NOT INCREASE FOLLOWING CNS INJURY. There are few reports on extracellular polyamines after transient and focal cerebral ischemia. Extracellular polyamine levels are comparable to the low levels in other extra-cellular compartments (human cerebral spinal fluid and serum), and do not change during permanent focal ischemia or the initial reperfusion period after global ischemia [47,101]. Thus any changes in free versus bound polyamines after CNS trauma are not reflected by excretion into the extracellular compartment.

EFFECTS OF PHARMACOLOGICAL AGENTS ON POLYAMINE METABOLISM IN CNS INJURY

CNS INJURY MODELS.

Transient cerebral ischemia: Male Mongolian gerbils (50-80 g) common carotid arteries were exposed through a neck incision and occluded using aneurysm clips for 10-min under halothane anesthesia and reperfused for 1-day as described [100,102, 103]. Body temperature was maintained at 37-38°C during ischemia and reperfusion.

Focal cerebral ischemia: MCAO was induced for 2-hr in spontaneously hypertensive rats (**SHR**) using intraluminal suture occlusion as described previously [37,104].

Traumatic brain injury (TBI) was induced in male Sprague-Dawley rats under halothane anesthesia using a controlled cortical impactor (6-mm diameter tip, 3-m/sec velocity, 2-mm deformation) as described earlier [36,45,46].

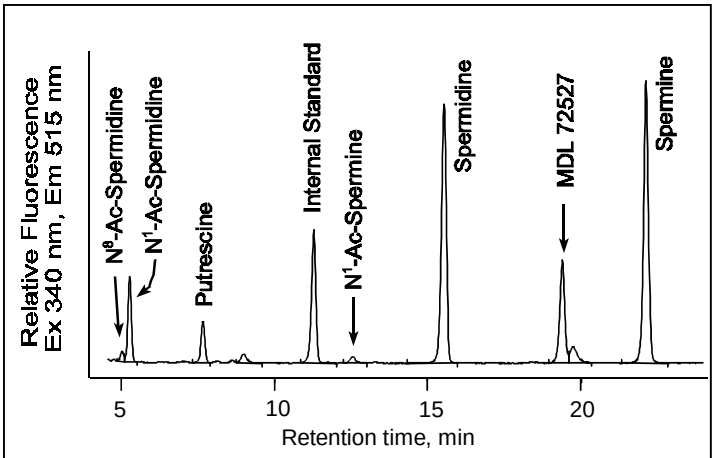


Figure 1. HPLC profile of polyamines.

ACCUMULATION OF N^1-ACETYLSPERMIDINE PARALLELED THE DECREASE IN PUTRESCINE AFTER MDL 72527 TREATMENT. Brain levels of putrescine, spermidine, spermine, N^8-acetylspermidine, N^1-acetylspermidine, N^1-acetylspermine and the polyamine analog MDL 72527 were measured by high-performance liquid chromatography (**HPLC**) (Fig. **1**).

Putrescine formation occurs partly by the actions of ODC on ornithine. ODC activity is induced at 6-h to 8-h following CNS injury. In contrast, putrescine levels were only marginally increased at 6-h after injury, but were significantly elevated at 1-day, at which time ODC activity had declined to near sham levels [16,34,36,100]. This suggested that pathways other than ODC, such as SSAT/PAO, determined the elevated putrescine levels at 1-day.

Blockade of PAO with MDL 72527 resulted in a decrease in putrescine to sham levels (Fig. **2**) with a corresponding increase in N^1-acetylspermidine (Fig. **3**) [16], the intermediate product formed by SSAT (Scheme **1**). Other studies have demonstrated that PAO is completely inhibited within 30-min following treatment with MDL 72527, and a linear increase in N^1-acetylspermidine levels over time paralleled the decrease in putrescine in normal brain tissue [19,20].

To determine if the increased putrescine formation at 1-day after CNS injury was due to SSAT, we calculated the change in putrescine due to injury compared to the increase in N^1-acetylspermidine following MDL 72527 treatment. The changes in putrescine levels due to CNS injury (Δputrescine) were calculated as the difference between injured (ischemic or ipsilateral) and control (sham or contralateral) levels. Some N^1-acetylspermidine is formed by SSAT in normal brain, but does not accumulate in the absence of PAO inhibition due to rapid conversion to putrescine. The formation of N^1-acetylspermidine due to CNS injury was therefore determined as the increase (ΔN^1-acetylspermidine) in injured brain tissue

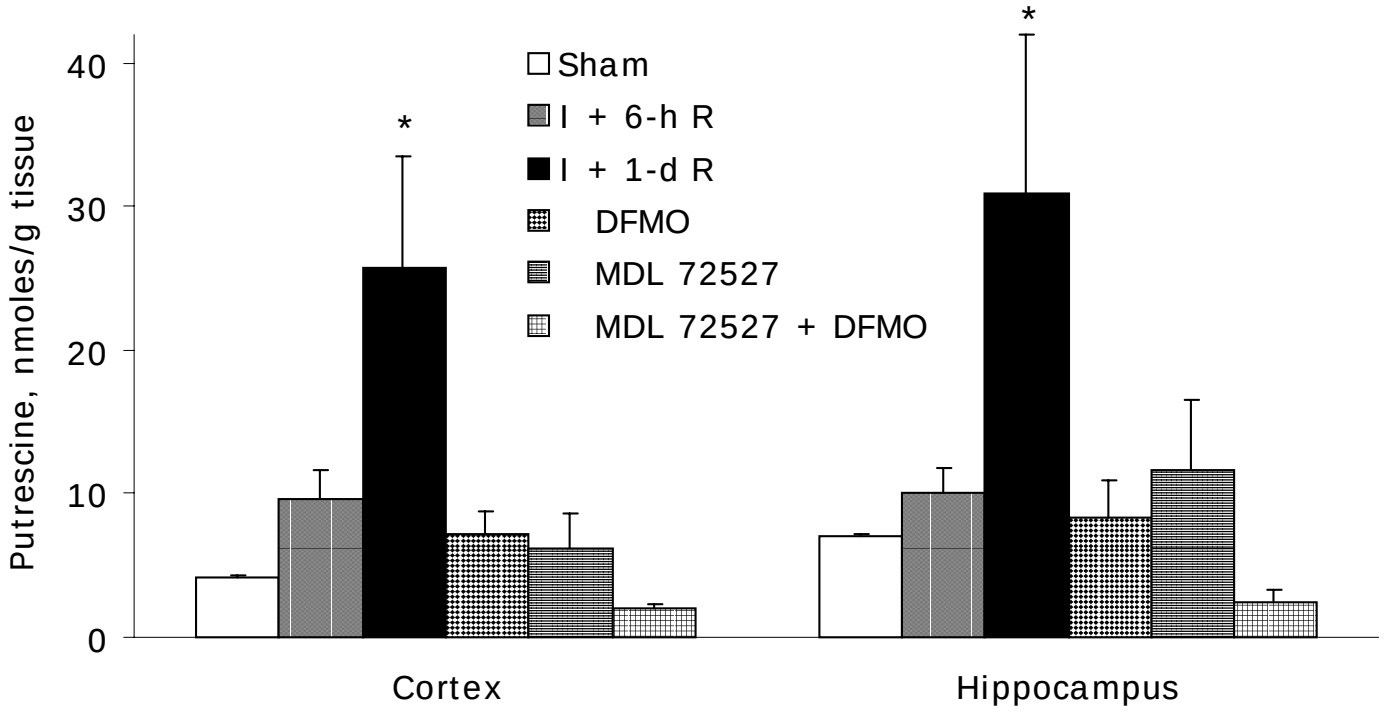


Figure 2. Putrescine levels in gerbils after 10-min forebrain ischemia and 1-day reperfusion. * $p<0.05$ compared to shams.

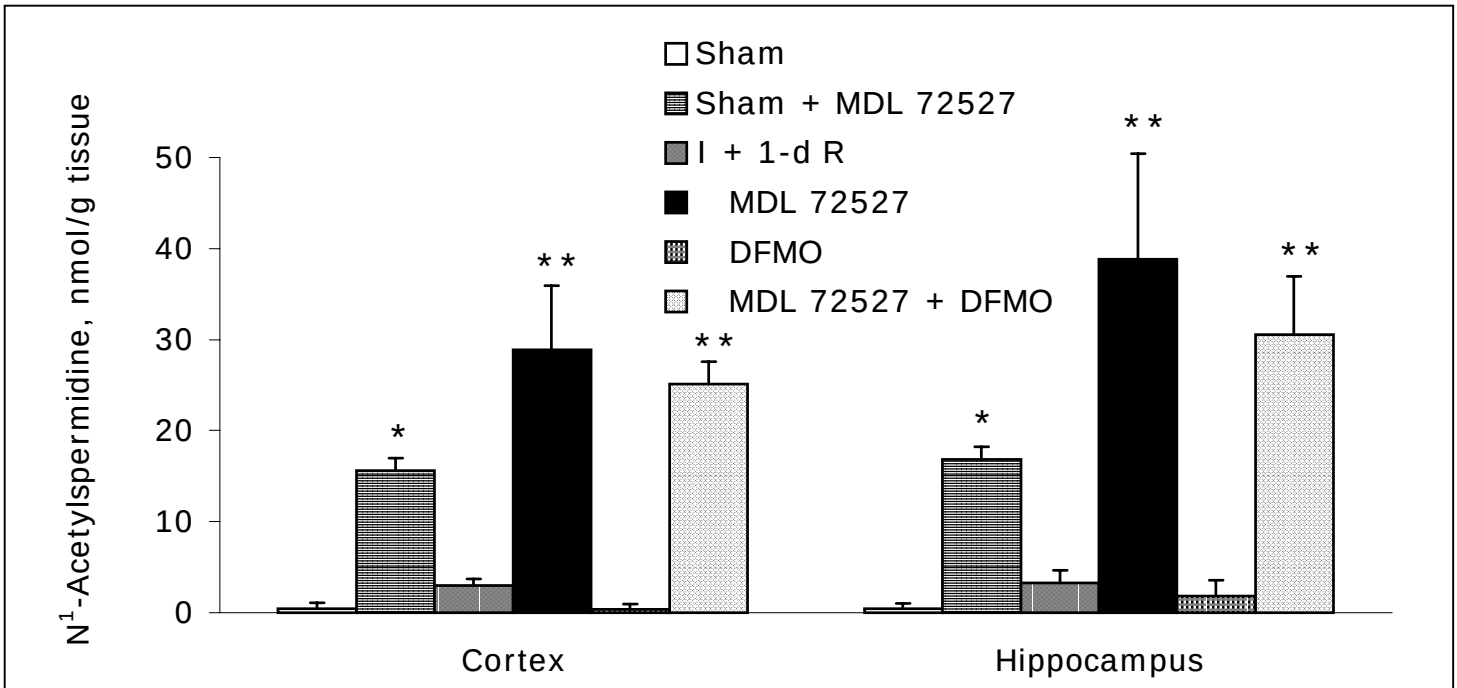


Figure 3. N^1-acetylspermidine levels in gerbils after 10-min forebrain ischemia and 1-day reperfusion. * $p<0.05$ compared to untreated shams; ** $p<0.05$ compared to MDL 72527-treated shams.

following MDL 72527 treatment compared to the corresponding MDL 72527 treated controls. These results are presented in Table **1**.

In gerbil forebrain ischemia and MCAO in SHR, there were no significant differences between Δputrescine and ΔN^1-acetyl-spermidine, suggesting that the increased putrescine after CNS injury was mediated predominantly by SSAT. In TBI, ΔN^1-acetylspermidine was less than Δputrescine in the cortex and hippocampus; but only achieved statistical significance ($p<0.05$) for the hippocampus. However, in TBI, MDL 72527 treatment reduced putrescine levels by approximately 50% [38], whereas putrescine levels were reduced to near sham in the gerbil (Fig. **2**) or to

Table. 1. Relationship between putrescine and N^1-acetylspermidine after CNS injury.

Type of injury	Region	Δputrescine [3]	ΔN^1-acetylspermidine [3]
Transient forebrain ischemia [1] (n=5)	Cortex	21.7 ± 7.7	13.3 ± 7.0
	Hippocampus	23.9 ± 11.1	22.0 ± 11.0
Focal transient cerebral ischemia [2] (n=9)	Cortex	18.8 ± 6.2	20.1 ± 8.0
	Striatum	29.3 ± 12.1	21.5 ± 12.0
Traumatic brain injury [2] (n=7)	Cortex	57.8 ± 26.4	33.1 ± 23.1
	Hippocampus	59.5 ± 21.0	27.4 ± 11.1*

[1] Δputrescine = putrescine (ischemic – sham); ΔN^1-acetylspermidine = N^1-acetyl-spermidine (ischemic with MDL 72527 - sham with MDL 72527).

* $p<0.05$ compared to Δputrescine.

[2] Δputrescine = putrescine (ipsilateral – contralateral); ΔN^1-acetylspermidine = N^1-acetyl-spermidine (ipsilateral – contralateral) following MCAO or TBI and MDL 72527 treatment.

[3] Tissue levels were determined as nmoles/g wet weight tissue.

contralateral levels in the MCAO model [37]. In TBI, the 50% decrease in putrescine in the ipsilateral regions after MDL 72527 treatment corresponds with the increase in N^1-acetylspermidine. Thus, in all the models, the decrease in putrescine after MDL 72527 treatment corresponds with the increased formation of N^1-acetylspermidine. These data indicate that the additional putrescine is largely formed by PAO action on N^1-acetylspermidine, and the direct oxidation of spermidine to putrescine by PAO, as suggested by Ivanova et al [29], appears to be negligible.

In vitro studies showed N^8-acetylspermidine protected low K^+-induced apoptosis in rat cerebellar granule cells [105]. Our *in vivo* studies showed that N^8-acetylspermidine levels were very low and were not altered following CNS injury or treatment with MDL 72527 [16].

TREATMENT WITH DFMO ALSO DECREASED PUTRESCINE TO SHAM LEVELS. DFMO treatment decreased putrescine to sham levels at 1-day after transient ischemia (Fig. **2**), but did not result in any accumulation of N^1-acetylspermidine (Fig. **3**), suggesting that DFMO did not block PAO. So far the only known effect of DFMO is the inhibition of ODC; however, it is possible that DFMO blocked SSAT, preventing N^1-acetylspermidine and consequently putrescine formation. To determine if DFMO altered SSAT activity, N^1-acetylspermidine levels were determined following treatment with MDL 72527 and DFMO. In these studies, we did not measure SSAT enzymatically since the assay may overestimate activity [106,107].

When gerbils were treated with the combination of DFMO and MDL 72527, putrescine further decreased to below sham levels (Fig. **2**). The combination showed approximately the same level of N^1-acetylspermidine compared to MDL 72527 alone, indicating that DFMO did not have a significant effect on SSAT. These data indicate that DFMO did not decrease putrescine levels through inhibition of either PAO or SSAT. Since ODC appears to have only a minor contribution to putrescine formation at 1-day, the mechanism by which DFMO acts to decrease putrescine levels at 1-day cannot be fully explained at this time. Other pathways that would affect putrescine levels are tissue excretion, metabolism to spermidine, and conversion to γ-aminobutyric acid (GABA) initiated by diamine oxidases.

CDP-CHOLINE AND POLYAMINE METABOLISM. Cytidine 5'-diphosphocholine (**CDP-choline**) is a rate-limiting intermediate in the biosynthesis of phosphatidylcholine (**PtdCho**) from 1,2-diacylglycerol (**DAG**) [103]. Increases in DAG and intracellular Ca^{++} activate protein kinase C, which in turn may regulate induction of ODC [108]. CDP-choline may decrease the DAG levels by stimulating the synthesis of PtdCho from DAG [109], thereby attenuating protein kinase C activation and the subsequent activation of ODC [110]. Choline liberated from CDP-choline can be converted to S-adenosyl-L-methionine (**AdoMet**) [42,103] *via* the pathway:

CDP-choline → Choline → Betaine → Methionine → AdoMet

Decarboxylated AdoMet serves as the aminopropyl donor for the synthesis of putrescine/spermidine to spermidine/spermine (Scheme **1**). Through these pathways, CDP-choline may alter ODC activity and polyamine synthesis.

CDP-CHOLINE ATTENUATED ODC ACTIVITY AND PUTRESCINE LEVELS: CDP-choline treatment after transient cerebral ischemia in gerbils attenuated ODC activity [111] (Fig. **4**) and putrescine levels after 6-h and 1-day reperfusion (Fig. **5**). CDP-choline had

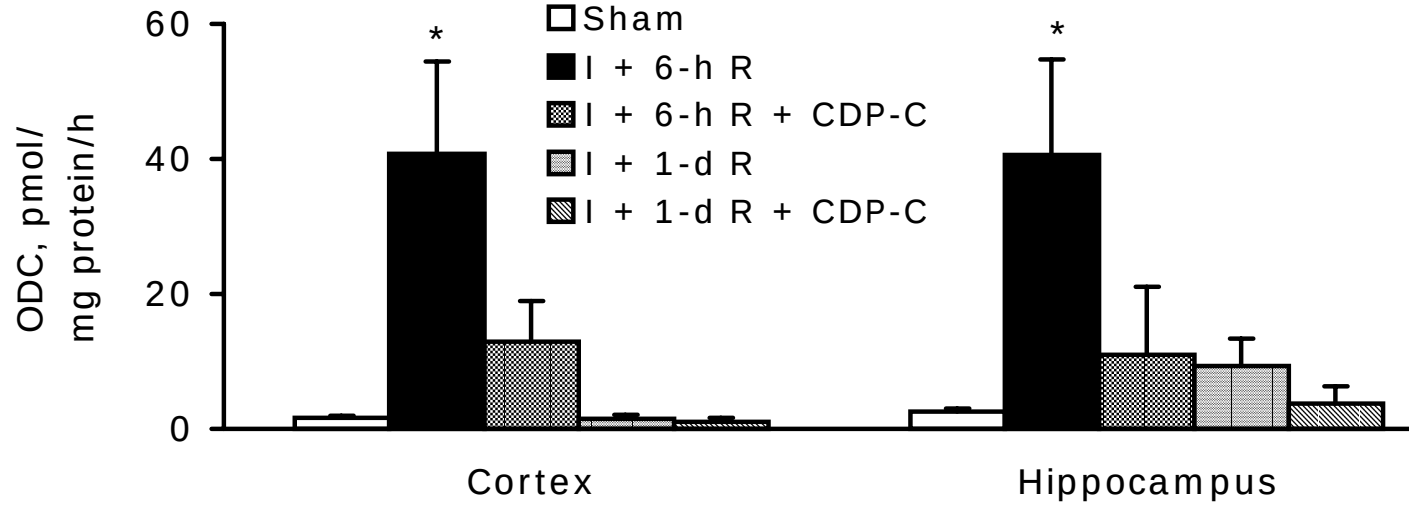


Figure 4. Effect of CDP-choline on ODC activity at 6-h and 1-day after transient forebrain ischemia in gerbil. * $p<0.05$ compared to shams.

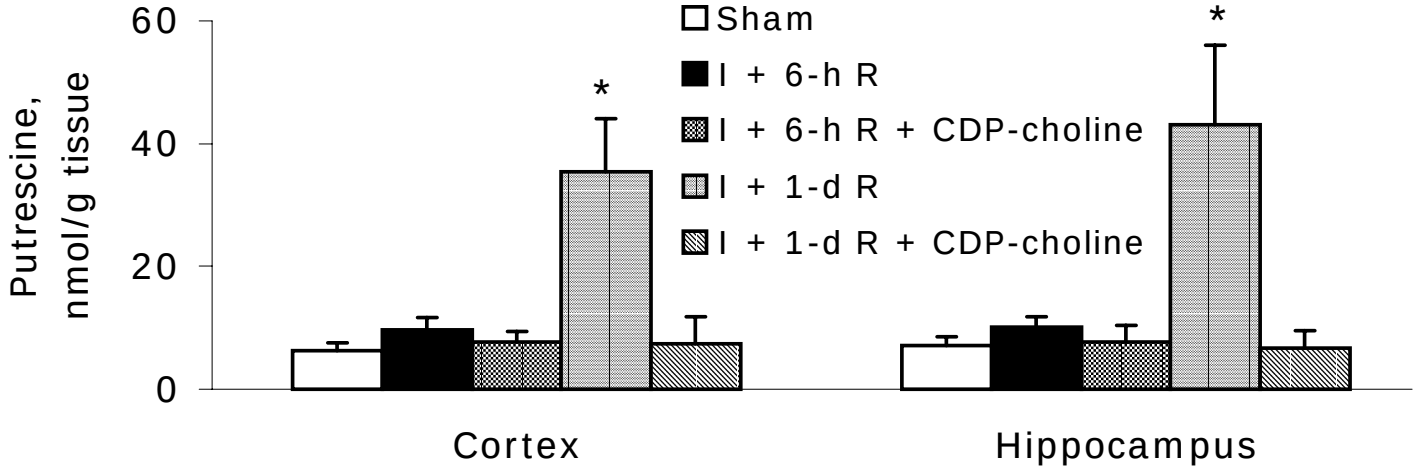


Figure 5. Effect of CDP-choline on putrescine levels following 10-min transient forebrain ischemia in gerbil. * $p<0.05$ compared to shams.

relatively little effect on ODC activity at 1-day after transient ischemia, since ODC activity had declined to near sham levels at this time (Fig. **4**).

CDP-choline could have attenuated the putrescine levels at 1-day through increased spermidine synthesis, however, spermidine/spermine levels (~ 280 nmol/g wet tissue without or with CDP-choline treatment) were not significantly altered. One explanation is that even the elevated putrescine levels at 1-day after ischemia are less than 10% of the total spermidine and spermine pool. Thus, the increased synthesis from putrescine could be absorbed without a significant effect on the much larger spermidine/spermine pools.

CDP-CHOLINE PROTECTED CA_1 HIPPOCAMPAL NEURONS AFTER TRANSIENT CEREBRAL ISCHEMIA. Gerbils treated with CDP-choline or AdoMet after 10-min ischemia and 6-7-day reperfusion showed significant neuroprotection in the CA_1 region [42,103]. CDP-choline neuroprotection could be multi-factorial: 1) stabilizing the membrane through increased conversion of DAG to PtdCho and bio-synthesis of AdoMet,

2) AdoMet serves as the methyl donor for the conversion of phosphatidylethanolamine to PtdCho and the aminopropyl donor for the synthesis of spermidine from putrescine, 3) minimizing arachidonic acid release is likely to have decreased oxygen radical generation associated with oxidative metabolism of arachidonic acid, and 4) CDP-choline may also increase sphingomyelin synthesis and decrease levels of apoptosis-inducing ceramide [103]. Alteration of polyamine metabolism by CDP-choline after CNS injury has not been previously reported. Since the role of ODC induction and putrescine accumulation as causal factors in neuronal injury remains controversial, the contribution of altered polyamine metabolism in neuroprotection by CDP-choline remains an open question.

CONCLUSIONS

ISCHEMIC INJURY TO THE BRAIN IS MULTI-DIMENSIONAL. Due to the multiple pathways involved in ischemic injury (i.e., release of excitatory amino acids and activation of their receptors, alteration of calcium channels, activation of phospholipases, cyclooxygenases and lipoxygenases, generation of free radicals and peroxidation of lipids, modification of polyamine metabolism), no single pathway is likely to completely account for the injury following CNS trauma [103,112]. There is general agreement that polyamines are essential components for cell growth and differentiation and their metabolic pathways have been elucidated. However, their physiological functions still remain enigmatic. Further understanding of the roles of polyamines in normal and pathological states may help to resolve the apparently conflicting data in this complex field.

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