ELSEVIER

Brain Research 938 (2002) 81–86

BRAIN RESEARCH

www.elsevier.com/locate/bres

Short communication

Polyamines and central nervous system injury: spermine and spermidine decrease following transient focal cerebral ischemia in spontaneously hypertensive rats

Rao Muralikrishna Adibhatla[a,b,c,*], James F. Hatcher[a], Kurt Sailor[a], Robert J. Dempsey[a,c]

[a]*Department of Neurological Surgery, H4-330, Clinical Science Center, 600 Highland Avenue, University of Wisconsin-Madison, Madison, WI 53792-3232, USA*

[b]*Cardiovascular Research Center, University of Wisconsin, Madison, WI 53792, USA*

[c]*Veterans Administration Hospital, Madison, WI, USA*

Accepted 19 December 2001

Abstract

Polyamines (putrescine, spermidine and spermine) are ubiquitous cellular components, but their specific role in central nervous system (CNS) injury has yet to be characterized. CNS injury results in increased activities of ornithine decarboxylase and spermidine/spermine-N^1-acetyltransferase, and accumulation of putrescine. The present study determined the polyamine profile in three models of CNS injury, in two different species (gerbil and rat) and two strains of rats (Sprague–Dawley and spontaneously hypertensive): (1) transient focal cerebral ischemia in spontaneously hypertensive rats (SHR); (2) traumatic brain injury in Sprague–Dawley rats; and (3) transient forebrain ischemia in gerbils. While there was a significant increase in putrescine in all three models, spermine and spermidine levels were unaltered in forebrain ischemia and traumatic brain injury. However, transient focal cerebral ischemia shows depletion of spermine and spermidine levels in injured hemisphere compared to contralateral region. Exogenous spermine significantly restored the spermine as well as spermidine levels in the ipsilateral hemisphere after transient focal cerebral ischemia, but did not alter putrescine levels or the ratio of spermidine to spermine. The loss of spermine in particular, may have several consequences that contribute to ischemic injury, including destabilization of chromatin, decreased mitochondrial Ca^{2+} buffering capacity, and increased susceptibility to oxidative stress. Based on our and other studies, we propose a tentative antioxidant mechanism of spermine neuroprotection.

Theme: Ischemia

Topic: Disorders of the nervous system

Keywords: Antioxidant; Fe^{2+} chelation; Hydroxyl radical; Putrescine; Radical scavenger; Spermidine/spermine-N^1-acetyltransferase; Transient forebrain ischemia; Traumatic brain injury

Spermine phosphate, a naturally occurring polyamine, was first discovered by Leeuwenhoek in 1678. Polyamines (putrescine, spermidine and spermine) are ubiquitous components of all eukaryotic cells [8,11,36,39,42,47]. Central nervous system (CNS) injury results in alteration of polyamine metabolism [31,39], particularly increases in activities of ornithine decarboxylase (ODC) and spermidine/spermine-N^1-acetyltransferase (SSAT) and putrescine accumulation [31,39,40], but the precise role of polyamines in CNS injury [6,12,13,31,37,40] has yet to be defined [16,21,31,39].

Spermine is considered to act as an antioxidant [24,26,27] and free radical scavenger [17,22,23]. Administration of spermine provided neuroprotection in CNS

Abbreviations: AdoMet, *S*-adenosyl-L-methionine; ODC, ornithine decarboxylase; $OH^{\cdot}$, hydroxyl radical; PAO, polyamine oxidase; SSAT, spermidine/spermine-N^1-acetyltransferase; *t*BCAO, transient bilateral carotid artery occlusion; TBI, traumatic brain injury; *t*MCAO, transient middle cerebral artery occlusion

*Corresponding author. Tel.: +1-608-263-1791; fax: +1-608-263-1409.

E-mail address: adibhatl@neurosurg.wisc.edu (R.M. Adibhatla).

0006-8993/02/$ – see front matter
PII: S0006-8993(02)02447-2

injury models [10,15], however, neither the tissue polyamine status nor the mechanism of neuroprotection is known in these studies. The present study determined the polyamine profiles in three models of CNS injury: (1) transient middle cerebral artery occlusion (*t*MCAO) in spontaneously hypertensive rats (SHR); (2) traumatic brain injury (TBI) in Sprague–Dawley rats; and (3) transient bilateral carotid artery occlusion (*t*BCAO) in gerbils. While there was a significant increase in putrescine in all three models, only *t*MCAO resulted in significant loss of spermine and spermidine in injured hemisphere compared to contralateral region. Exogenous spermine significantly restored the spermine as well as spermidine levels in the ipsilateral hemisphere after *t*MCAO, but did not alter putrescine levels or the spermidine to spermine ratio.

The following materials were obtained from the indicated suppliers: 1,7-diaminoheptane, and dansyl chloride (Sigma Chemical Company, St. Louis, MO), HPLC grade solvents (Fisher Scientific, Pittsburgh, PA), and BondElut C_{18} columns (Varian Associates, Harbor City, CA). 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* (US Department of Health and Human Services Pub 85-23, 1985) and were approved by the animal care committee of the University of Wisconsin-Madison. During surgical procedures, animals were anesthetized with 1.5% halothane in a 50:50 mixture of N_2O:O_2.

MCAO was induced in spontaneously hypertensive male rats (SHR) using intraluminal suture occlusion as described previously [13,40]. After 2 h, the suture was removed and reperfusion was continued for up to 24 h. Spermine (10 mg/kg i.p.) or saline was administered to animals just after the end of MCAO in SHR. This dose has been shown to provide significant reduction in infarct volume in *t*MCAO of male Wistar rats [10].

TBI was induced in male Sprague–Dawley rats (300–350 g) using a controlled cortical impactor (6 mm diameter tip, 3 m/s velocity, 2 mm deformation) described earlier [4,12].

Male Mongolian gerbils (50–80 g) bilateral common carotid arteries were occluded using aneurysm clips for 10 min and reperfused for up to 24 h as described [1,40]. Shams underwent the same surgical procedure except the vessels were not occluded. Body temperature was maintained at 37–38 °C during ischemia and reperfusion.

Tissue polyamines were determined in the brain regions susceptible to the particular CNS injury by HPLC against internal (1,7-diaminoheptane) and external standards using a Hewlett-Packard 1046A fluorescence detector with excitation 340 nm and emission 515 nm as described earlier [38,40]. The recovery of the internal standard in samples was 95–98%. All the measurements were expressed as means±standard deviation (S.D.). Data were analyzed using ANOVA with the Bonferroni multi-group post-test to compare differences between the groups. A value of $P<0.05$ was considered significant.

We have not found it necessary to perfuse animals with saline to remove blood prior to brain tissue collection. Normal blood levels of polyamines were determined to be: putrescine 2.12±1.04, spermidine 38.01±6.42 and spermine 2.49±1.70 μM. This is consistent with data from a recent clinical study of stroke patients showing detectable amount of spermidine in erythrocytes with low-to-undetectable levels of putrescine and spermine [14]. Since the cerebral blood volume is about 34 μl/g brain [45], the blood contributes 1% or less to the tissue polyamines.

Tissue polyamine levels at 6 and 24 h following CNS injury are presented in Table 1. Putrescine levels were significantly elevated in the ipsilateral hemisphere by 6 h in *t*MCAO ($P<0.01$) and TBI ($P<0.05$) compared with contralateral region. In both models putrescine showed further increases at 24 h which were significantly ($P<0.01$) elevated compared with contralateral region and with the ipsilateral region at 6 h. In *t*BCAO there was no increase in putrescine at 6 h; however, significant increase ($P<0.01$ compared to sham) was observed at 24 h in both cortex and hippocampus.

Spermine and spermidine showed a significant ($P<0.01$) decrease in the ipsilateral vs. contralateral cortex at 6 and 24 h after *t*MCAO. Spermine and spermidine showed a similar, though less pronounced decrease in ipsilateral vs. contralateral striatum, which was significant ($P<0.01$) at 24 h. In TBI and *t*BCAO there were no significant changes in spermine or spermidine levels in injured tissue compared to corresponding contralateral region or sham, respectively at 6 or 24 h.

Based on the observed decrease in spermine and spermidine in *t*MCAO (Table 1) and a study demonstrating reduction in cortical infarct volume by exogenous spermine (10 mg/kg) in transient focal cerebral ischemia [10], we determined the effects of spermine administration on brain polyamine levels in *t*MCAO. Administration of spermine (10 mg/kg i.p. in saline) immediately after the onset of reperfusion significantly ($P<0.01$) restored the levels of spermine and spermidine in the ipsilateral cortex and striatum at 24 h after *t*MCAO compared to saline treatment (Table 1). No significant changes in putrescine levels were observed.

CNS injury results in alteration of polyamine metabolism, including induction of ODC and accumulation of putrescine [5,19,31,37–39]. In *t*BCAO or TBI, spermine and spermidine levels show no significant changes (Table 1) [19,35,46]. However, depletion of spermine and spermidine occurs both in *t*MCAO (Table 1) and permanent focal cerebral ischemia [41]. Factors that may affect the levels of spermine and spermidine are: (1) depletion of *S*-adenosyl-L-methionine (AdoMet); (2) decreased activity of AdoMet decarboxylase [32]; (3) induction of the SSAT/polyamine oxidase (PAO) interconversion pathway [3,40]; (4) activation of tissue transglutaminase [34,42]; and/or (5) increased clearance into the blood from severely injured neurons [31]. The finding that delayed neuronal death occurs several days after *t*BCAO and TBI, while the

Table 1
Tissue polyamine levels (nmol/g tissue) at 6 and 24h after CNS injury (n=8 per group)

Model/ species	Region	Time (h)	Putrescine		Spermidine		Spermine	
			Contra-	Ipsi-	Contra-	Ipsi-	Contra-	Ipsi-
*t*MCAO in SHR (n=8)	Cortex	6	7.61±0.3	35.3±4.0[a]	338.4±18.5	248.0±27.9[a]	265.0±14.0	207.6±22.7[a]
		24	12.8±3.6	64.0±13.7[a,c]	343.5±26.7	246.5±30.3[a]	258.0±29.4	191.7±12.1[a]
	Striatum	6	12.3±2.8	26.1±6.7[a]	529.7±56.5	469.8±57.1	207.8±37.8	180.7±5.5
		24	23.0±9.4	51.4±8.8[a,c]	552.3±46.3	433.0±91.7[a]	233.6±13.4	164.1±37.8[a]
	Spermine treated							
	Cortex	24	10.7±2.5	74.2±9.6[a]	338.5±9.1	298.1±55.0[d]	253.7±7.5	229.2±33.9[d]
	Striatum	24	10.7±4.9	52.5±26.9[a]	543.5±11.2	493.7±77.4[d]	220.5±7.4	208.9±15.8[d]
Sham/SHR	Cortex		7.32±0.5		330.7±21.6		267.2±17.9	
	Striatum		11.3±1.8		523.6±51.9		210.2±40.2	
TBI in SD rat (n=8)	Cortex	6	14.1±4.4	48.8±15.9[a]	403.3±74.1	417.5±62.8	210.9±25.3	230.0±51.3
		24	7.5±3.0	75.4±15.3[a,c]	340.1±30.7	344.3±15.3	252.2±25.5	253.0±7.5
	Hippocampus	6	15.9±1.5	34.3±5.0[b]	383.5±24.3	376.8±13.2	222.0±15.6	211.6±6.3
		24	12.1±8.3	77.6±22.3[a,c]	401.6±54.0	368.5±46.4	286.2±51.4	256.6±39.9
Sham/SD rat	Cortex		8.7±2.7		342.1±33.1		228.2±26.8	
	Hippocampus		11.1±7.6		385.6±60.2		249.2±50.9	
*t*BCAO in Gerbil (n=8)			Sham	Ischemic	Sham	Ischemic	Sham	Ischemic
	Cortex	6		9.0±1.9		245.8±19.6		293.0±14.6
		24	4.1±0.2	27.6±10.2[a,c]	221.5±12.7	241.8±22.8	272.5±14.9	287.0±18.7
	Hippocampus	6	7.0±0.1	9.9±1.5	327.2±23.2	355.5±21.8	308.9±21.8	323.4±25.4
		24		33.0±14.4[a,c]		319.5±37.0		294.8±18.4

[a] $P<0.01$ compared to contralateral or sham.
[b] $P<0.05$ compared to contralateral.
[c] $P<0.01$ compared to 6 h ischemic/6 h ipsilateral.
[d] $P<0.01$ compared to ipsilateral regions at 24 h, saline treated.

impact of *t*MCAO is more immediate (as indicated by infarction 24 h after injury) may be a reason for loss of spermine and spermidine after *t*MCAO. Release of polyamines from injured brain tissue into peripheral blood was suggested by recent clinical studies showing elevated spermidine levels in erythrocytes of stroke patients [14].

Following CNS injury, activity of AdoMet decarboxylase was shown to decrease [20,32], while those of transglutaminase [34] and SSAT increased [3,40]. The decrease in polyamine synthesis and induction of the interconversion pathway contribute to accumulation of putrescine, a common feature of CNS injury that was observed in all three models (Table 1). Conversion of spermine and spermidine via SSAT/PAO results in production of by-products hydrogen peroxide and 3-acetamidopropanal which can contribute to ischemic injury [30,40,43]. Treatment with the polyamine analog MDL 72527 [N^1, N^4-bis-(2,3-butadienyl)-1,4-butadiamine], a specific PAO inhibitor, demonstrated increased formation of N^1-acetylspermidine and suggested the contribution of this pathway [40] to putrescine accumulation and development of infarction after *t*MCAO [13]. However, the levels of putrescine and the increase in N^1-acetylspermidine [40] could not fully account for the loss of spermine/spermidine, suggesting that other mechanisms also contribute to the depletion.

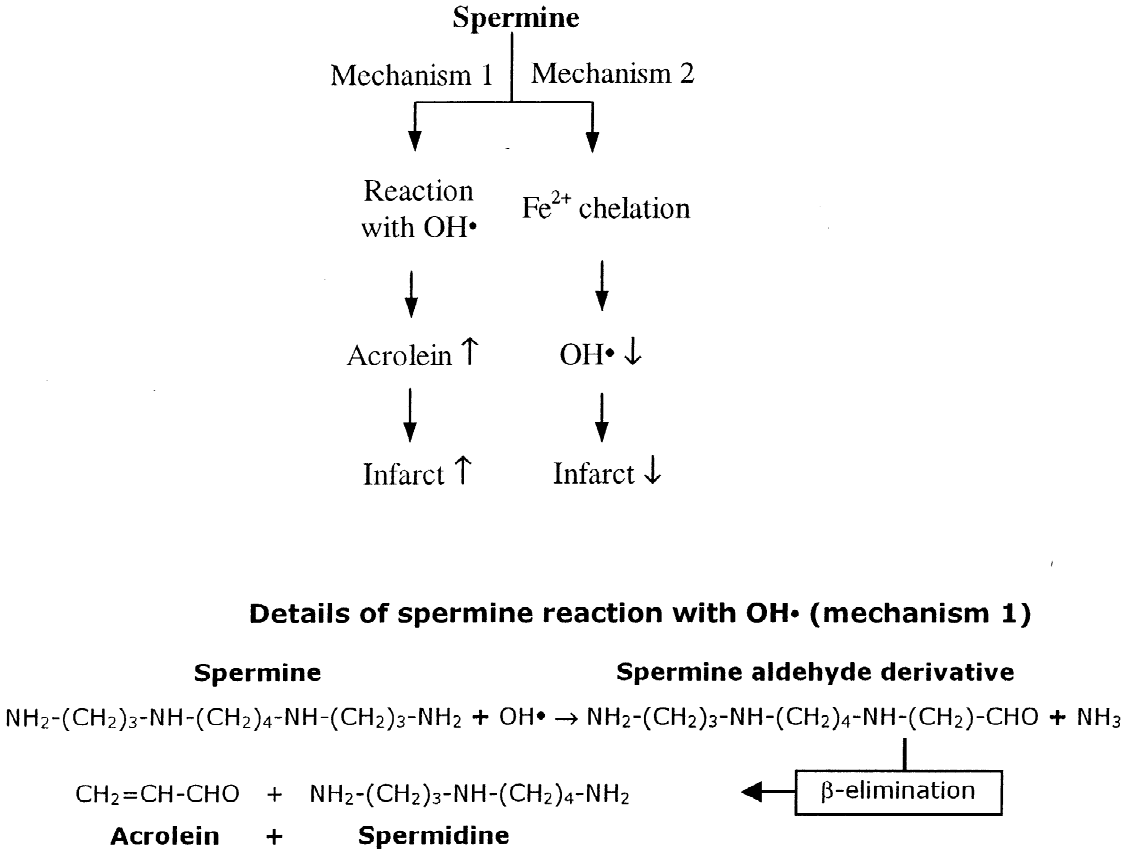
One previous study showed a decrease in spermine but not spermidine in gerbil hippocampus following 5 min forebrain ischemia and 4 days reperfusion. There were no changes in spermine or spermidine levels in the cortex or hippocampus following 10 min forebrain ischemia with 24 h reperfusion in gerbils [33], consistent with our results (Table 1). Similarly, following 30 min forebrain ischemia in rats, there were no significant changes in spermidine or spermine in cortex or hippocampus at 24 h reperfusion; however, a decrease in spermine and spermidine was observed in the caudoputamen [35].

Significant decreases in spermine and spermidine occurred only in *t*MCAO; the mechanism or combination of factors causing this depletion is unknown at present. In this respect, one study reported a decrease in spermidine but not spermine levels in cortex and striatum following 30 min focal cerebral ischemia (no reperfusion) in rat, with no changes in spermine and spermidine levels over 24 h reperfusion [32]. This disparity could be due to the difference in ischemic severity. In our studies, 1 h *t*MCAO also induced decreases in spermine and spermidine (data not shown) but to a lesser extent compared to 2 h *t*MCAO (Table 1). Some of these differences could also arise from variable responses of different strains of rats. Unfortunately, due to the variations in the duration and type of CNS injury, the brain regions analyzed for polyamines, the timing after the injury, and the fact that the strain of rat was not designated in some studies [32,35], there is insufficient data in the literature to draw any definite conclusions.

The loss of spermine and spermidine, particularly spermine, may have several consequences that contribute to ischemic injury, including destabilization of chromatin [7], decreased mitochondrial Ca^{2+} buffering capacity [32], and increased susceptibility to oxidative stress [27]. Ischemia–reperfusion results in the generation of reactive oxygen species (ROS) including hydroxyl radical ($OH^{\cdot}$) that contributes to ischemic injury [1,2,9]. Iron released from ferritin during ischemia–reperfusion promotes the formation of $OH^{\cdot}$ [50]. Spermine was reported to act as a potent antioxidant either by scavenging oxygen radicals [17] or through chelation of Fe^{2+} that catalyzes $OH^{\cdot}$ generation [27–29,48] via the Fenton reaction [18,50]. The reaction of spermine with $OH^{\cdot}$ results in a spermine aldehyde derivative (Scheme 1) [17]; this product is the same as that formed by serum amine oxidases and undergoes non-enzymatic, pH dependent β-elimination to form acrolein and spermidine (Mechanism 1 of Scheme 1) [25]. This reaction could account for the loss of spermine but is expected to increase spermidine levels. However, in *t*MCAO, spermidine levels do not increase, but instead decline and further conversion of spermidine to putrescine accounts for only part of the decrease in spermine and spermidine (Table 1).

Acrolein formed as a result of spermine reaction with $OH^{\cdot}$ should increase ischemic injury [49]. Treatment with spermine significantly decreased infarct volume in transient focal cerebral ischemia [10], a result that does not support a significant reaction of spermine with $OH^{\cdot}$ [17]. On the other hand, chelation of Fe^{2+} by spermine [27] would prevent formation of $OH^{\cdot}$ via the Fenton reaction [18,49] without the formation of toxic byproducts. Spermine has been shown to chelate with Cu^{2+} and the structure of the complex has been described [28]. While the structure of the spermine–Fe^{2+} chelate has not been published, evidence for its formation has been presented [28] and its structure may be similar to that of the spermine–Cu^{2+} complex. Under physiological conditions, the nitrogens of spermine are protonated, and presumably formation of a complex with metal ions would involve displacement of the positively-charged protons.

Two anti-oxidant pathways have been proposed for spermine, one of which produces the toxic aldehyde acrolein. Based on this, a tentative antioxidant action of spermine in ischemic neuroprotection is outlined in Scheme 1. It is conceivable that spermine is involved in both pathways (reaction with $OH^{\cdot}$ and chelation of Fe^{2+}). The rate-constants for reactions with $OH^{\cdot}$ indicate that spermine does not significantly react with $OH^{\cdot}$ and that much more efficient radical scavengers are present in vivo



Scheme 1. Antioxidant pathways of spermine and a tentative neuroprotective action. ↑ indicates increase; ↓ indicates decrease.

Table 2
Spermidine/spermine ratios in contralateral vs. ipsilateral regions, and ipsilateral/contralateral ratios for spermidine and spermine at 24 h after *t*MCAO, treatment with saline or spermine ($n=8$ per group)

Region	Treatment	Spermidine/spermine		Ispi-/contra-	
		Contra-	Ipsi-	Spermidine	Spermine
Cortex	Saline	1.33±0.05	1.29±0.07	0.72±0.04	0.74±0.03
	Spermine	1.33±0.06	1.30±0.05	0.88±0.07	0.90±0.04
Striatum	Saline	2.36±0.23	2.64±0.38	0.78±0.06	0.70±0.05
	Spermine	2.47±0.18	2.36±0.20	0.91±0.04	0.95±0.07

[27]. This would argue in favor of Fe^{2+} chelation by spermine as its anti-oxidant role (Mechanism 2 of Scheme 1).

Exogenous spermine significantly restored the spermine and spermidine levels after *t*MCAO (Table 1). The fact that spermidine levels also increased suggests conversion of spermine by SSAT/PAO. The mechanism whereby spermine enters the brain is unclear, but could involve passive transport following disruption of the blood–brain barrier. Alternatively, there is evidence of an active polyamine transport system [44], although it has not been well characterized in mammalian systems [8].

Table 2 shows that the spermidine/spermine ratios were the same in ipsilateral vs. contralateral regions, indicating that the same proportion of spermidine and spermine was depleted from the ischemic regions. At 24 h after *t*MCAO, the ratios of spermidine and spermine in the ipsilateral vs. contralateral cortex and striatum declined to 0.70–0.78 (Table 2). Following treatment with spermine, the ratios of spermidine and spermine in ipsilateral vs. contralateral regions were restored to 0.88–0.95. The spermidine to spermine ratio was unaltered in both the ipsilateral and contralateral regions and independent of saline or spermine treatment (Table 2).

It is not known if exogenous spermine will provide neuroprotection in the other two injury models (TBI and *t*BCAO, in which polyamines were not depleted). One study showed that spermine was neuroprotective in forebrain ischemia of gerbil [15]. The polyamines were not measured in this study, but based on our data and those of Paschen et al. [33], it is likely that polyamines were not altered by ischemia. Thus we speculate spermine may be neuroprotective also in models where polyamine depletion does not occur. Further studies are necessary to confirm in vivo antioxidant properties of spermine, such as chelation of Fe^{2+}, to establish the neuroprotective nature of spermine and to define its mechanism of action.

Acknowledgements

This study was supported by start-up funding and Medical School Research grant (161-9904) from University of Wisconsin to R.M. Adibhatla and grants from NIH (RO1 NS 28000, PO1 NS31220), and American Heart Association Grant-in-Aid (0151424Z) to R.J. Dempsey.

References

[1] R.M. Adibhatla, J.F. Hatcher, R.J. Dempsey, Effects of citicoline on phospholipid and glutathione levels in transient cerebral ischemia, Stroke 32 (2001) 2376–2381.
[2] R.M. Adibhatla, J.F. Hatcher, R.J. Dempsey, Citicoline: neuroprotective mechanisms in cerebral ischemia, J. Neurochem. 80 (2002) 12–23.
[3] G.N. Babu, K.A. Sailor, D. Sun, R.J. Dempsey, Spermidine/spermine N^1-acetyl transferase activity in rat brain following transient focal cerebral ischemia and reperfusion, Neurosci. Lett. 300 (2001) 17–20.
[4] M.K. Baskaya, A. Dogan, A.M. Rao, R.J. Dempsey, Neuroprotective effects of citicoline on brain edema and blood–brain barrier breakdown after traumatic brain injury, J. Neurosurg. 92 (2000) 448–452.
[5] M.K. Baskaya, A.M. Rao, M.R. Prasad, R.J. Dempsey, Regional activity of ornithine decarboxylase and edema formation after traumatic brain injury, Neurosurgery 38 (1996) 140–145.
[6] M.K. Baskaya, A.M. Rao, L. Puckett, M.R. Prasad, R.J. Dempsey, Effect of difluoromethylornithine treatment on regional ornithine decarboxylase activity and edema formation after experimental brain injury, J. Neurotrauma 13 (1996) 85–92.
[7] H.S. Basu, L.J. Marton, Biological and therapeutic implications of the effects of polyamines on chromatin condensation, in: R.A. Casero (Ed.), Polyamines: Regulation and Molecular Interaction, Vol. 1, Springer Verlag, New York, 1995, pp. 101–128.
[8] R.A. Casero Jr., P.M. Woster, Terminally alkylated polyamine analogues as chemotherapeutic agents, J. Med. Chem. 44 (2001) 1–26.
[9] P.H. Chan, Reactive oxygen radicals in signaling and damage in the ischemic brain, J. Cereb. Blood Flow Metab. 21 (2001) 2–14.
[10] B.A. Coert, R.E. Anderson, F.B. Meyer, Exogenous spermine reduces ischemic damage in a model of focal cerebral ischemia in the rat, Neurosci. Lett. 282 (2000) 5–8.
[11] S.S. Cohen, A Guide to the Polyamines, Oxford University Press, Oxford, 1998, pp. 1–624.
[12] A. Dogan, A.M. Rao, M.K. Baskaya, J. Hatcher, C. Temiz, V.L.R. Rao, R.J. Dempsey, Contribution of polyamine oxidase to brain injury after trauma, J. Neurosurg. 90 (1999) 1078–1082.
[13] A. Dogan, A.M. Rao, J. Hatcher, V.L.R. Rao, M.K. Baskaya, R.J. Dempsey, 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 (1999) 765–770.
[14] T. Els, J. Bruckmann, G. Röhn, M. Daffertshofer, J.S. Mönting, R.I. Ernestus, M. Hennerici, Spermidine: a predictor for neurological

outcome and infarct size in focal cerebral ischemia?, Stroke 32 (2001) 43–46.
[15] G.M. Gilad, V.H. Gilad, Polyamines can protect against ischemia-induced nerve cell death in gerbil forebrain, Exp. Neurol. 111 (1991) 349–355.
[16] G.M. Gilad, V.H. Gilad, Polyamines in neurotrauma. Ubiquitous molecules in search of a function, Biochem. Pharmacol. 44 (1992) 401–407.
[17] H.C. Ha, N.S. Sirisoma, P. Kuppusamy, J.L. Zweier, P.M. Woster, R.A. Casero Jr., The natural polyamine spermine functions directly as a free radical scavenger, Proc. Natl. Acad. Sci. USA 95 (1998) 11140–11145.
[18] E.D. Hall, Lipid peroxidation, in: K.M.A. Welch, L.R. Caplan, D.J. Reis, B.K. Siesjo, B. Wier (Eds.), Primer on Cerebrovascular Disease, Academic Press, San Diego, 1997, pp. 200–204.
[19] C.M. Henley, C. Muszynski, L. Cherian, C.S. Robertson, Activation of ornithine decarboxylase and accumulation of putrescine after traumatic brain injury, J. Neurotrauma 13 (1996) 487–496.
[20] C.M. Henley, K. Wey, A. Takashima, C. Mills, E. Granmayeh, I. Krishnappa, C.S. Robertson, *S*-adenosylmethionine decarboxylase activity is decreased in the rat cortex after traumatic brain injury, J. Neurochem. 69 (1997) 259–265.
[21] R.A. Kauppinen, L.I. Alhonen, Transgenic animals as models in the study of the neurobiological role of polyamines, Prog. Neurobiol. 47 (1995) 545–563.
[22] A. Khan, P. Mascio, M. Medeiros, T. Wilson, Spermine and spermidine protection of plasmid DNA against single-strand breaks induced by singlet oxygen, Proc. Natl. Acad. Sci. USA 89 (1992) 11428–11430.
[23] A. Khan, Y. Mei, T. Wilson, A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen, Proc. Natl. Acad. Sci. USA 89 (1992) 11426–11427.
[24] M. Kitada, Y. Naito, K. Igarashi, S. Hirose, Y. Kanakubo, H. Kitagawa, Possible mechanism of inhibition by polyamines of lipid peroxidation in rat liver microsomes, Res. Comm. Chem. Pathol. Pharmacol. 33 (1981) 487–497.
[25] Y. Lee, L.M. Sayre, Reaffirmation that metabolism of polyamines by bovine plasma amine oxidase occurs strictly at the primary amino termini, J. Biol. Chem. 273 (1998) 19490–19494.
[26] E. Lovaas, Hypothesis: spermine may be an important epidermal antioxidant, Medical Hyp. 45 (1995) 59–67.
[27] E. Lovaas, Antioxidative and metal-chelating effects of polyamines, Adv. Pharmacol. 38 (1997) 119–149.
[28] E. Lovaas, G. Carlin, Spermine: an anti-oxidant and anti-inflammatory agent, Free Radic. Biol. Med. 11 (1991) 455–461.
[29] M.R. Marzabadi, E. Lovaas, Spermine prevent iron accumulation and depress lipofuscin accumulation in cultured myocardial cells, Free Radic. Biol. Med. 21 (1996) 375–381.
[30] D.M.L. Morgan, Polyamine oxidases—enzymes of unknown function?, Biochem. Soc. Trans. 26 (1998) 586–591.
[31] W. Paschen, Polyamine metabolism in different pathological states of the brain, Mol. Chem. Neuropath. 16 (1992) 241–271.
[32] W. Paschen, Polyamine metabolism in reversible cerebral ischemia, Cerebrovasc. Brain Metab. Rev. 4 (1992) 59–88.
[33] W. Paschen, J. Hallmayer, G. Mies, Regional profile of polyamines in reversible cerebral ischemia of Mongolian gerbils, Neurochem. Pathol. 7 (1987) 143–156.
[34] W. Paschen, G. Röhn, R. Schmidt-Kastner, Transglutaminase activity in reversible cerebral ischemia in the rat, Neurosci. Lett. 110 (1990) 232–236.
[35] W. Paschen, R. Schmidt-Kastner, B. Djuricic, C. Meese, F. Linn, K.-A. Hossmann, Polyamine changes in reversible cerebral ischemia, J. Neurochem. 49 (1987) 35–37.
[36] A.E. Pegg, P.P. McCann, Polyamine metabolism and function, Am. J. Physiol. 243 (1982) C212–221.
[37] A.M. Rao, M.K. Baskaya, M.E. Maley, M.R. Prasad, R.J. Dempsey, Ornithine decarboxylase activity and edema formation in cerebral ischemia of conscious gerbils, J. Neurochem. 65 (1995) 2639–2643.
[38] A.M. Rao, J.F. Hatcher, M.K. Baskaya, R.J. Dempsey, Simultaneous assay of ornithine decarboxylase and polyamines after central nervous system injury in gerbils and rats, Neurosci. Lett. 256 (1998) 65–68.
[39] A.M. Rao, J.F. Hatcher, R.J. Dempsey, Polyamine response to CNS injury: for better or for worse?, Rec. Res. Develop. Neurochem. 2 (1999) 517–532.
[40] A.M. Rao, J.F. Hatcher, A. Dogan, R.J. Dempsey, Elevated N^1-acetylspermidine levels in gerbil and rat brains after CNS injury, J. Neurochem. 74 (2000) 1106–1111.
[41] D. Sauer, P. Martin, P.R. Allegrini, R. Bernasconi, H. Amacker, G.E. Fagg, Differing effects of α-difluoromethylornithine and CGP 40116 on polyamine levels and infarct volume in a rat model of focal cerebral ischaemia, Neurosci. Lett. 141 (1992) 131–135.
[42] R.G. Schipper, L.C. Penning, A.A.J. Verhofstad, Involvement of polyamines in apoptosis. Facts and controversies: effectors or protectors?, Sem. Cancer Biol. 10 (2000) 55–68.
[43] N. Seiler, Oxidation of polyamines and brain injury, Neurochem. Res. 25 (2000) 471–490.
[44] W.W. Shin, W.F. Fong, S.F. Pang, P.C. Wong, Limited blood–brain barrier transport of polyamines, J. Neurochem. 44 (1985) 1056–1059.
[45] R.P. Shockley, J.C. LaManna, Determination of rat cerebral cortical blood volume changes by capillary mean transit time analysis during hypoxia, hypercapnia and hyperventilation, Brain Res. 454 (1988) 170–178.
[46] E. Shohami, J.L. Nates, L. Glantz, V. Trembovler, Y. Shapira, U. Bachrach, Changes in brain polyamine levels following head injury, Exp. Neurol. 117 (1992) 189–195.
[47] C.W. Tabor, H. Tabor, Polyamines, Ann. Rev. Biochem. 53 (1984) 749–790.
[48] B. Tadolini, Polyamine inhibition of lipoperoxidation. The influence of polyamines on iron oxidation in the presence of compounds mimicking phospholipid polar heads, Biochem. J. 249 (1988) 33–36.
[49] K. Uchida, Current status of acrolein as a lipid peroxidation product, Trends Cardiovasc. Med. 9 (1999) 109–113.
[50] W.H. Ying, S.K. Han, J.W. Miller, R.A. Swanson, Acidosis potentiates oxidative neuronal death by multiple mechanisms, J. Neurochem. 73 (1999) 1549–1556.