Biochemical Pharmacology, Vol. 44, No. 3, pp. 401–407, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
Pergamon Press Ltd

COMMENTARY

POLYAMINES IN NEUROTRAUMA

UBIQUITOUS MOLECULES IN SEARCH OF A FUNCTION

GAD M. GILAD* and VARDA H. GILAD
Neuropsychiatry Branch, NIMH Neurosciences Center at Saint Elizabeths, Washington, DC 20032, U.S.A.

The natural polyamines (PAs†) spermine $[NH_2\text{-}(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2]$, spermidine $[NH_2(CH_2)_4NH(CH_2)_3NH_2]$ and putrescine $[NH_2(CH_2)_4NH_2]$ are low molecular weight, aliphatic molecules that are positively charged at physiological pH values [1]. They are widely distributed in living organisms, from bacteria to humans, and can reach very high intracellular concentrations (in the millimolar range) [1]. Dividing cells make large and rapid changes in PA biosynthesis, and the depletion of PAs leads to retardation of cell proliferation and even to cell death [2, 3]. The existing evidence therefore supports the general notion that PAs are essential for cell division and growth; alas, in spite of a large volume of research, their specific role(s) in cellular functions still remains an enigma. Interestingly, PAs are found in abundance in the adult mammalian nervous system [4], where their synthesis can be greatly stimulated in response to stressful stimuli (e.g. glucocorticoid treatment [5] and electrical stimulation [6, 7]) or traumatic injuries (e.g. mechanical injuries [8–10], neurotoxins [11, 12] and ischemia [13]). In addition, pharmacological studies have shown that exogenous PAs interact directly with the nervous system to alter its functions [4]. The possibility that PAs are involved in neuronal function during the response to trauma has triggered a recent spurt of research. This commentary emphasizes the unique features of PA metabolism in the adult nervous system, highlights their proposed functions in cellular defense mechanisms, and suggests new perspectives to stimulate future pharmacological research.

Unique alterations in brain polyamine metabolism after trauma

A rapid, but short-lasting, increase in PA metabolism is the hallmark of cells responding to various traumatic stimuli. This indicates that the "polyamine response" can be induced by several different converging signaling pathways [1–3]. The inherent transient characteristic of the polyamine response is due to stringent control mechanisms of both biosynthetic and degradative enzymes [1, 14], as illustrated in Fig. 1. In general, the two rate-limiting enzymes in the biosynthetic pathway, ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (SAM-DC), which are characterized by extremely short half-lives (15–20 min) [1, 2], are induced in parallel, and lead to a sequential increase in all PAs. The rapid PA degradation is due to the induction of spermidine/spermine N^1-acetyltransferase (SSAT) which catalyzes the formation of N^1-acetylated PAs, the preferred substrates for oxidation by the constitutively expressed amine oxidases [14]. The coupling of the synthesizing and degradative pathways results in the reutilization of PAs (Fig. 1); this interconversion cycle has been demonstrated in the adult mammalian brain [14].

Peripheral nervous system. In peripheral neurons, nerve injuries elicit a full-fledged polyamine response where the amounts of all PAs are transiently elevated [10, 15]. Inhibition of PA biosynthesis results in selective death of the injured neurons, while intact neurons remain unaffected [16]. This indicates that the polyamine response is an essential part of the neuronal survival program after neurotrauma [16]. This assumption is supported by the finding that polyamine synthesis is increased in peripheral sympathetic neurons after treatment with nerve growth factor (NGF) [17], the survival factor of sympathetic neurons [18].

Central nervous system (CNS). In contrast to the periphery, the polyamine response to neurotrauma in the CNS is incomplete, resulting in increased levels of only putrescine; spermidine and spermine either do not change or may be transiently reduced [7, 13]. This anomaly is probably the result of a selective increase in ODC, while SAM-DC activity remains practically unchanged [6, 19], or even decreases [8]. In the adult brain, SAM-DC is a constitutively expressed enzyme of relatively high activity [20], while ODC remains inducible as in the developmental stage. This indicates an altered genomic regulation of PA metabolism in mature

* Corresponding author at present address: Faculty of Medicine, Technion–Israel Institute of Technology, Efron Street, P.O.B. 9649, Haifa 31096, Israel Tel (972) 4-545-111; FAX (972) 4-517-008.

† Abbreviations: PAs, polyamines; ODC, ornithine decarboxylase; SAM-DC, *S*-adenosylmethionine decarboxylase; SSAT, spermidine/spermine N^1-acetyltransferase; NGF, nerve growth factor; SAM, *S*-adenosyl-L-methionine; DFMO, α-difluoromethylornithine; NMDA, *N*-methyl-D-aspartate; and GABA, γ-aminobutyric acid.

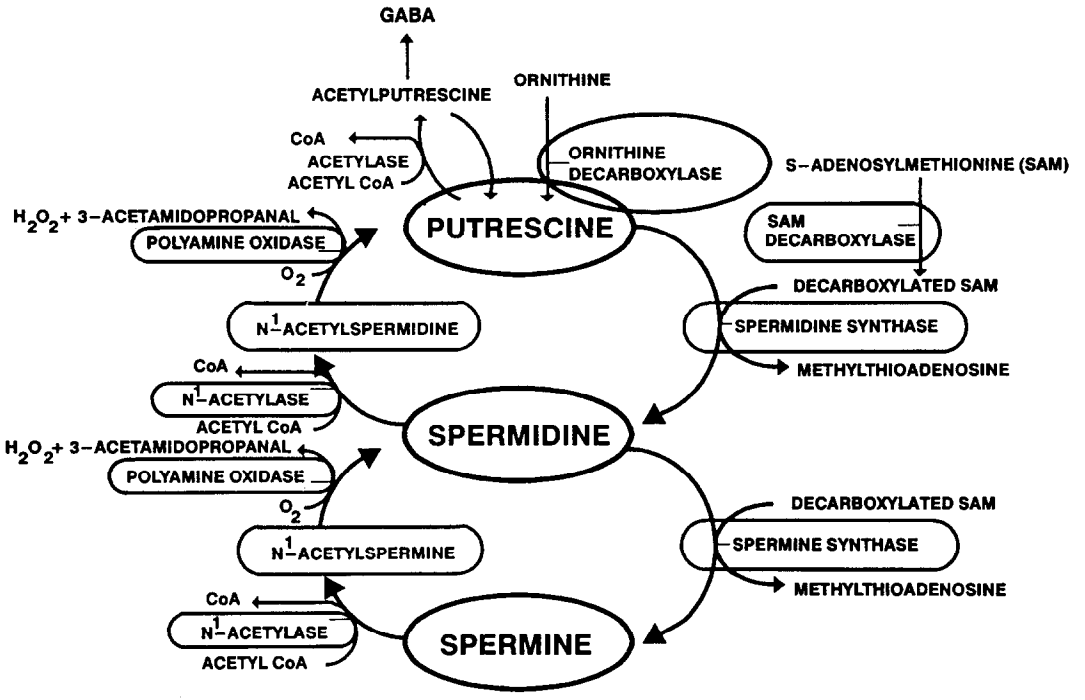

Fig. 1. Biochemical reactions in the control of polyamine metabolism. Clockwise, the biosynthetic pathway starts by putrescine formation (from ornithine) and continues with the sequential synthesis of spermidine and spermine, while the catabolic pathway results in the sequential reformation of spermidine and putrescine from spermine. The encircled polyamines and enzymes indicate their possible separate compartmentalization in the cell. N^1-Acetylase: spermidine/spermine N^1-acetyltransferase.

neurons. Still, it is surprising that elevated intracellular levels of putrescine, the natural activator of SAM-DC [21], do not lead to SAM-DC activation and to the sequential enhancement of PA synthesis after CNS trauma. Mature neurons are highly specialized, terminally differentiated cells with unique structural features that reflect sophisticated intracellular compartmentalization. A plausible explanation, therefore, is that differential intracellular compartmentalization of components in the PA metabolic pathway may restrict their accessibility to each other (see Fig. 1). In this manner, the interconversion cycle may become uncoupled at a critical point between putrescine formation and its conversion into spermidine. Another unique feature is the persistent elevation in putrescine, which may last for as long as 4 days [13], despite the fact that the increase in ODC activity is transient and subsides within 24 hr after the insult [6, 8, 10, 13]. Sequestration of putrescine in an isolated cellular compartment, rendering it unavailable for the metabolizing enzymes, may explain its long-term accumulation.

Interestingly, treatment with *S*-adenosyl-L-methionine (SAM) can protect neurons in the brain from cell death after ischemia [22, 23]. Besides being a general methyl donor [24, 25], SAM, of course, is also the source of the propylamine group for PA biosynthesis following its decarboxylation by SAM-DC (Fig. 1) [21, 26]. Therefore, in accordance with the theory of differential compartmentalization, if availability of SAM is a limiting factor, its increased availability, after treatment with exogenous SAM, may lead to the increased production of spermidine and spermine. Increased production of the PAs is postulated to play a role in cellular defense mechanisms.

Like ODC, SSAT is a short-lived protein [27] that is rapidly induced in the brain by stressful stimuli [28, 29]. This supports the notion that increased spermine and spermidine degradation, and the resultant re-conversion into putrescine (Fig. 1) may also contribute to the long-lasting increase in brain putrescine, and to a transient reduction in spermidine and spermine which may be observed after trauma [13,*].

It is conceivable that failure to initiate a complete polyamine response after trauma, and the resulting long-lasting, focal elevation of putrescine may become harmful [13]. Recent studies showed that

* Gilad GM, Gilad VH, Casero RA Jr and Globus MY-T, Polyamines in ischemia. Extracellular levels in rat brain and increased accumulation in gerbil brain slices. *Neurotrauma Soc Abstr*, St. Louis, MO, 1990.

treatment with α-difluoromethylornithine (DFMO), a specific ODC inhibitor, is neuroprotective against the neurotoxic effects of the glutamic acid agonist *N*-methyl-D-aspartate (NMDA) [12, 30]. In contrast, DFMO treatment cannot protect against neuronal damage after global [31] or focal* models of cerebral ischemia (stroke), whereas treatment with PAs can [31]. It is possible, therefore, that in drug-induced neurotoxicity DFMO may exert neuroprotective effects via mechanisms not related to polyamine metabolism.

A complete response with a transient increase in all PAs is therefore postulated to be crucial for overcoming cellular insults, and for survival. In other words, induction of the polyamine response after trauma is assumed to represent the attempt of injured neurons to initiate a protective program.

Uptake of extracellular PAs. It has been demonstrated in various systems that when an increased demand for PAs arises, not only does the biosynthetic capacity increase, but also the ability to take up extracellular PAs is greatly enhanced [32]. This, and the fact that extracellular PA levels are normally extremely low [33], are the rational for the use of exogenous PAs in an attempt to enhance survival and rescue neurons from degeneration after trauma. It has been observed that when exogenous PAs are given immediately and for a limited time (days) after the trauma, they can rescue peripheral and central neurons from various types of injuries, including axonal injuries, neurotoxins and ischemia [31, 34, 35]. Furthermore, it has been established that putrescine is an essential additive for neuron survival in defined growth media [36]. Thus, if after trauma, neurons are incapable of mounting a complete polyamine response, or are unable to synthesize sufficient amounts of PAs, they would benefit from the availability of extracellular PAs via the induced uptake system. These PAs would then behave as newly synthesized PAs and play their assumed part in cellular defense mechanisms.

Putative polyamine functions in cellular defense mechanisms

The strong electrostatic interactions between positively charged PAs, and negatively charged macromolecules (DNA, RNA and proteins) and membrane constituents (phospholipids and proteins) may be the basis for most of the actions ascribed to PAs [1, 33]. Thus, numerous biochemical effects were described when PAs were added to various experimental preparations [1, 2, 33], but the relevance of these effects to the actual function of PAs in the living cell is hard to ascertain [3, 33]. Nevertheless, several of the actions ascribed to PAs may assume importance for cell survival after trauma, and suggest that PAs may serve as endogenous neuroprotective agents.

Regulation of the cellular ionic environment. PAs can be considered natural organic cations. Acidosis is a typical consequence of brain injury and is probably the result of the cumulative effects of hypo-osmotic conditions (edema), massive loss of potassium ions, and lactic acid accumulation [37]. Post-traumatic increase in PA concentrations would therefore serve to counteract loss of potassium and to prevent acid stress. This defense mechanism has been suggested to occur in plants [38] and, evolutionarily, may be very old.

Regulation of signal pathways. Nonspecific electrostatic interactions of PAs with cellular membranes are probably the basis for their interference with ion fluxes [1, 33]. Recent studies, however, indicate the existence of specific membrane PA binding sites in the CNS [39, 40]. Furthermore, PAs were implicated in the specific modulation of the NMDA receptor–ionophore complex and its associated second messenger pathways [41–44], which may be involved in the post-trauma neurotoxic effects of the excitatory neurotransmitter glutamic acid [45]. The agonist (i.e. glutamate and glycine)-activated state of the NMDA receptor–ion channel complex permits Ca^{2+} influx and increased cGMP concentrations in neurons [43, 44]. In the activated mode, spermidine and spermine can enhance ligand binding at low concentrations, but this effect is diminished at higher concentrations [42]. In contrast, putrescine exerts only an inhibitory effect [42]. Modulation of the NMDA receptor would therefore be dependent on the availability and concentration of the specific polyamine. The unique alterations in brain PA metabolism (i.e. persistent elevations in putrescine and only minor changes in spermidine and spermine) therefore suggest that PAs would probably exert an antagonistic effect on the NMDA receptor after trauma. This issue is at present controversial [13, 31, 42–44].

Interestingly, putrescine metabolism can lead to the formation of γ-aminobutyric acid (GABA) (Fig. 1), an inhibitory neurotransmitter which may counteract the post-trauma neurotoxic effects of excessive stimulation by excitatory amino acids [46].

Several agents offering protection against neuronal cell death after ischemic insults have been found to be potent inhibitors of (Ca^{2+}/phospholipid-dependent) protein kinase C [47–50]. The PAs are not only potent inhibitors of protein kinase C [see Ref. 33 for review], but may also act indirectly to inactivate this enzyme by binding strongly to and inhibiting the hydrolysis of phosphatidylinositol [33, 51]. In this way, PAs may inhibit the formation of diacylglycerol and inositol phosphates and, in turn, prevent Ca^{2+} mobilization [52]. Thus, the depletion of free Ca^{2+} and diacylglycerol would leave protein kinase C inactivated.

Regulation of cellular Ca^{2+}. Besides interference with Ca^{2+} fluxes involved in signal transduction, PAs were found to interact with many of the cellular sites important in the control of Ca^{2+} homeostatis. Thus, PAs inhibit Ca^{2+}-transport ATPase and Ca^{2+} efflux from the endoplasmic reticulum [see Ref. 33 for review]. They also interact with calmodulin to prevent Ca^{2+} binding [53] and activate Ca^{2+} uptake by mitochondria [54, 55]. The latter action would be

* Bernasconi R, Sauer D, Martin P, Allegrini P, Amacker H and Fagg G, Lack of relationship between polyamine levels and infarct volume in the brain: Effects of CGP 40116 and difluoromethylornithine (DFMO). *Focus on Epilepsy, Satellite Symposium of the 3rd IBRO World Congress of Neuroscience, St. Adile, Quebec, Canada*, Abstr Vol: 9, 1991.

most significant for cellular defense, since under pathological conditions, accumulation and sequestration of Ca^{2+} by mitochondria are of paramount importance in protecting the cell against excess free Ca^{2+} [56]. Moreover, PAs have been shown to protect isolated mitochondria against damage, probably by regulating Ca^{2+} and phosphate uptake [54, 57].

Regulation of lipid peroxidation. The large lipid content and the high rate of oxidative metabolism make the nervous system especially vulnerable to free radical-induced damage [58]. Generation of excess free radicals after neurotrauma can lead to accelerated lipid peroxidation and, in turn, to membrane damage and cell death [59]. Polyamines were found to prevent lipid peroxidation in the liver after toxin treatments [60, 61], and they may conceivably exert a similar effect in the nervous system. This protective action is probably due to their avid binding to phospholipids [51], and not to their weak radical-scavenging property [62].

Interaction with nucleic acids. It has long been known that PAs bind avidly to RNA and DNA and that they do so in a specific manner [63]. In this capacity they may conceivably affect the general fidelity of protein synthesis under adverse conditions, but their exact function is unclear [1, 63]. Nevertheless, it is interesting to speculate that following neurotrauma, after which their accumulation in the cytoplasmic compartment is greatly increased, PAs are transported into the nucleus where they can alter genomic transcription and thereby serve to initiate a defense/survival program (reminiscent of the function ascribed to immediate early gene products [64]). In this capacity the PAs are poised to play an important role as intracellular messengers.

Noteworthy is the fact that in practically all the effects ascribed to PAs, the order of potency is increased with the length of the molecule and with the number of charges (i.e. putrescine < spermidine < spermine). Furthermore, in certain cases (e.g. modulation of the NMDA receptor [42]), putrescine may even exert effects opposite to those of spermidine and spermine.

Pharmacology of polyamines

Putative target tissues for exogenous PAs. In addition to direct actions on the injured neurons themselves, as implied above, it is also possible that PAs may exert their beneficial neuroprotective effects indirectly through interactions with other target cells. In theory, any cell affected by the traumatic insult that develops an increased demand for PAs would be a potential target for exogenous PAs. Such potential targets are glial and endothelial cells in the injured nervous system, denervated cells, and, perhaps, even peripheral endocrine cells which participate in the general reaction of the animal to the trauma. It is therefore noteworthy that a polyamine response is indeed induced in the cells of denervated target tissues after peripheral nerve injuries [65, 66]. Interaction of PAs with this potential target may lead to increased neurotrophic factor production and/or release, thereby enhancing neuron survival. If PAs were to act in this manner they could constitute a universal class of trophic factors. There is some indication that such an effect may occur in the peripheral sympathetic system where PA treatment leads to increased NGF accumulation in target tissues during development [67]. Although the cytoprotective effects of neurotrophic factors are well described, unfortunately their mechanisms of action are not yet clarified [18]. As mentioned above, however, NGF can stimulate PA biosynthesis in sympathetic neurons [17], implicating PAs as cellular mediators of NGF action. Taken together, the above observations suggest that exogenous PAs can bring about neuroprotection by acting on several target cells simultaneously.

Pharmacological effects on nervous system function. In laboratory animals, parenteral administration of increasing doses of spermine or spermidine leads to the depression of spontaneous motor activity, hypothermia, hypotension, hyperglycemia, sedation, analgesia, irritability, convulsion and, finally, death from respiratory arrest [4, 33, 68]. These effects are all nervous system related. Furthermore, similar effects can be produced by intracerebroventricular administration of PAs in the appropriate dosage [4, 33].

Further evidence for direct effects of PAs on neurotransmission function comes from their modulatory effects on neurotransmitter receptors in intact experimental systems. As mentioned above, PAs can modulate ligand binding and function of brain NMDA receptors [41–44], and while PAs do not alter GABA binding to synaptic vesicles [69], spermidine appears to modulate GABA receptor-mediated functions as expressed in *Xenopus* oocytes [70]. In addition, it has been suggested that PAs, specifically putrescine, through Ca^{2+} influx activation, may play a role not only in transduction of receptor-mediated signals [71], but also in the synaptic release of neurotransmitters [72].

Of the PA effects on the nervous system, two are of special interest for neuroprotection. First, since the development of hyperthermia after transient brain ischemia was demonstrated to be detrimental for neuron survival [73], the hypothermic effect of PAs may be critical for saving neurons from delayed cell death [31]. Second, the hypotensive effect of PAs may be beneficial after ischemia in reducing cerebral blood flow during the initial re-perfusion period.

In view of their limited transport into the intact brain [74], the question of whether sufficient amounts of PAs can reach the brain is an important one. It should be emphasized, however, that the blood–brain barrier is interrupted after CNS injuries. After ischemic insults the blood–brain barrier becomes compromised quite early and remains so for long periods [75]. Our findings indicate that polyamine transport into the brain is increased early after transient ischemia,* thus allowing administered PAs an easy access into the CNS.

Indications for drug development. The therapeutic window for PAs is probably limited to a short

* Gilad GM, Gilad VH, Casero RA Jr and Globus MY-T, Polyamines in ischemia. Extracellular levels in rat brain and increased accumulation in gerbil brain slices. *Neurotrauma Soc Abstr*, St. Louis, MO, 1990.

time interval (few days) early after the trauma [31, 34, 35, 76]. In general, it is advantageous to avoid lengthy drug treatment to prevent or reduce unwanted side-effects. One possible cause for toxic side-effects may be the oxidative deamination of exogenous PA by extracellular Cu^{2+}-containing amine oxidases, which results in the formation of aldehydes, hydroperoxide and ammonia [33], all of which are highly cytotoxic. Within the cell, however, these products (Fig. 1) are normally neutralized by suitable enzymatic processes [14, 33] (but see [77] and [78] for a hypothetical role of intracellular PAs in programmed cell death). In the extracellular compartment, oxidative deamination can be prevented with the enzyme inhibitor aminoguanidine [33], thus providing a pharmacological means to overcome toxic side-effects.

The development of PA analogues and derivatives as neuroprotective agents is justified by the positive findings in laboratory animals. Further drug development in this direction can take advantage of the already available numerous analogues [e.g. Refs. 79 and 80] and conjugated derivatives [e.g. Refs. 81–83] which may prove active and more lypophilic. In addition, a class of naturally occurring PA-conjugates, the wasp and spider toxins, was demonstrated recently to modulate glutamate receptor binding [84, 85], and may indicate an interesting avenue for drug development [e.g. Refs. 86 and 87 (patent applications)].

Summary

In spite of their abundance, the function of PAs in the adult nervous system remains enigmatic. It is postulated that after trauma, the induction of polyamine metabolism (i.e. the polyamine response), which is inherently transient, is an integral part of a protective biochemical program that is essential for neuronal survival. Several functions ascribed to PAs may assume importance in cellular defense. Thus, regulation of the ionic environment, modulation of signal pathways, control of cellular Ca^{2+} homeostasis, inhibition of lipid peroxidation, and interaction with nucleic acids are all putative sites for PA action. During maturation, the CNS, unlike the peripheral nervous system, undergoes changes which result in the expression of an incomplete polyamine response after trauma. This may be due to an altered pattern of gene expression, and/or restrictive compartmentalization of the PAs and their metabolizing enzymes. Induction of this partial polyamine response after injury results in a sustained accumulation of putrescine, which by itself may be harmful, without the concomitant increase in spermidine and spermine. Administration of exogenous PAs after trauma exerts a neuroprotective effect. Exogenous PAs are postulated to gain access into cells via an induced uptake system after trauma, and function similarly to newly synthesized PAs. Besides the injured neurons themselves, tissues which are connected or associated with these neurons may be potential targets where PAs could act to stimulate neurotrophic factor production. Based on the neuroprotective effects of PAs in laboratory animals and on their proposed role in mechanisms of neuronal survival, the development of PA-based compounds as therapeutic neuroprotective agents should be pursued.

REFERENCES

1. Tabor CW and Tabor H, Polyamines. *Annu Rev Biochem* **53**: 749–790, 1984.
2. Pegg AE and McCann PP, Polyamine metabolism and function. *Am J Physiol* **243**: C212–C221, 1982.
3. Janne J, Alhonen L and Leinonen P, Polyamines: From molecular biology to clinical applications. *Ann Med* **23**: 241–259, 1991.
4. Shaw GG, The polyamines in the central nervous system. *Biochem Pharmacol* **28**: 1–6, 1979.
5. Cousin MA, Lando D and Moguilewsky M, Ornithine decarboxylase induction by glucocorticoids in brain and liver of adrenalectomized rats. *J Neurochem* **38**: 1296–1304, 1982.
6. Pajunen AEI, Hietala OA, Virransalo E-L and Piha RS, Ornithine decarboxylase and adenosylmethionine decarboxylase in mouse brain—Effect of electrical stimulation. *J Neurochem* **30**: 281–283, 1978.
7. Russell DH and Gfeller E, Distribution of putrescine, spermidine and spermine in rhesus monkey brain; Decrease in spermidine and spermine concentrations in motor cortex after electrical stimulation. *J Neurobiol* **5**: 349–354, 1974.
8. Dienel GA and Cruz NF, Induction of brain ornithine decarboxylase during recovery from metabolic, mechanical, thermal, or chemical injury. *J Neurochem* **42**: 1053–1061, 1984.
9. Desiderio MA, Zini I, Davalli P, Zoli M, Corti A, Fuxe K and Agnati LF, Polyamines, ornithine decarboxylase, and diamine oxidase in the substantia nigra and striatum of the male rat after hemitransection. *J Neurochem* **51**: 25–31, 1988.
10. Gilad GM and Gilad VH, Early rapid and transient increase in ornithine decarboxylase activity within sympathetic neurons after axonal injury. *Exp Neurol* **81**: 158–166, 1983.
11. de Vera N, Artigas F, Serratosa J and Martínez E, Changes in polyamine levels in rat brain after systemic kainic acid administration: Relationship to convulsant activity and brain damage. *J Neurochem* **57**: 1–8, 1991.
12. Porcella A, Carta C, Fage D, Voltz C, Lloyd KG, Serrano A and Scatton B, The effects of *N*-methyl-D-aspartate and kainate lesions of the rat striatum on striatal ornithine decarboxylase activity and polyamine levels. *Brain Res* **549**: 205–212, 1991.
13. Paschen W, Schmidt-Kastner R, Hallmayer J and Djuricic B, Polyamines in cerebral ischemia. *Neurochem Pathol* **9**: 1–20, 1988.
14. Seiler N and Bolkenius FN, Polyamine reutilization and turnover in brain. *Neurochem Res* **10**: 529–544, 1985.
15. Sabri MI, Soiefer AI and Spencer PS, Axotomy produces selective increase of spermine in DRG. *Am Soc Neurochem Abstr* **19**: 89, 1988.
16. Gilad GM and Gilad VH, Polyamine biosynthesis is required for survival of sympathetic neurons after axonal injury. *Brain Res* **273**: 191–194, 1983.
17. Hendry IA and Bonyhady R, Retrogradely transported nerve growth factor increased ornithine decarboxylase activity in the rat superior cervical ganglia. *Brain Res* **200**: 39–45, 1980.
18. Calissano P, Cayyaneo A, Biocca S, Aloe L, Mercanti D and Levi-Montalcini R, The nerve growth factor. Established findings and controversial aspects. *Exp Cell Res* **154**: 1–9, 1984.
19. Kleihues P, Hossmann K-A, Pegg AE, Kobayashi K and Zimmermann V, Resuscitation of the monkey

brain after one hour complete ischemia. III. Indications of metabolic recovery. *Brain Res* **95**: 61–73, 1975.
20. Gilad GM and Kopin IJ, Neurochemical aspects of neuronal ontogenesis in the developing rat cerebellum: Changes in neurotransmitter and polyamine synthesizing enzymes. *J Neurochem* **33**: 1195–1204, 1979.
21. Pegg AE and Williams-Ashman HG, Stimulation of the decarboxylation of *S*-adenosylmethionine in mammalian tissues. *Biochem Biophys Res Commun* **30**: 76–82, 1968.
22. Matsui Y, Kubo Y and Iwata N, *S*-Adenosyl-L-methionine prevents ischemic neuronal death. *Eur J Pharmacol* **144**: 211–216, 1987.
23. Sato H, Hariyama H and Moriguchi K, *S*-Adenosyl-L-methionine protects the hippocampal CA1 neurons from the ischemic neuronal death in rat. *Biochem Biophys Res Commun* **150**: 491–496, 1988.
24. Cantoni GL, Activation of methionine for transmethylation. *J Biol Chem* **189**: 745–755, 1951.
25. Hirata F and Axelrod J, Phospholipid methylation and biological signal transmission. *Science* **209**: 1082–1087, 1980.
26. Pegg AE and Williams-Ashman HG, On the role of *S*-adenosylmethionine in the biosynthesis of spermidine by rat prostate. *J Biol Chem* **244**: 682–693, 1969.
27. Persson L and Pegg AE, Studies on the induction of spermidine/spermine N^1-acetyltransferase using specific antiserum. *J Biol Chem* **259**: 12364–12367, 1984.
28. Ientile R, De Luca G, Di Giorgio RM and Macaione S, Glucocorticoid regulation of spermidine acetylation in the rat brain. *J Neurochem* **51**: 677–682, 1988.
29. Gilad GM, Gilad VH, Wyatt RJ and Casero RA Jr, Chronic lithium treatment prevents the dexamethasone-induced increase of brain polyamine metabolizing enzymes. *Life Sci* **50**: PL-149–PL-154, 1992.
30. Markwell MAK, Berger SP and Paul SM, The polyamine synthesis inhibitor α-difluoromethylornithine blocks NMDA-induced neurotoxicity. *Eur J Pharmacol* **182**: 607–609, 1990.
31. Gilad GM and Gilad VH, Early polyamine treatment enhances survival of sympathetic neurons after postnatal axonal injury or immunosympathectomy. *Dev Brain Res* **38**: 175–181, 1988.
32. Seiler N and Dezeure F, Polyamine transport in mammalian cells. *Int J Biochem* **22**: 211–218, 1990.
33. Seiler N, Pharmacological properties of the natural polyamines and their depletion by biosynthesis inhibitors as a therapeutic approach. *Prog Drug Res* **37**: 107–159, 1991.
34. Gilad GM and Gilad VH, Treatment with polyamines can prevent monosodium glutamate neurotoxicity in rat retina. *Life Sci* **44**: 1963–1969, 1989.
35. Gilad GM and Gilad VH, Polyamines can protect against ischemia-induced nerve cell death in gerbil forebrain. *Exp Neurol* **111**: 349–355, 1991.
36. Bottenstein J, Skaper S, Varon S and Sato G, Selective survival of neurons from chick embryo sensory ganglionic dissociates utilizing serum-free supplemented medium. *Exp Cell Res* **125**: 183–190, 1980.
37. Katayama Y, Becker DP, Tamura T and Hovda DA, Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *J Neurosurg* **73**: 889–900, 1990.
38. Flores HE, Polyamines and plant stress. In: *Stress Response in Plants: Adaptation and Acclimation Mechanisms* (Eds. Alscher RG and Cumming JR), pp. 217–239. Wiley-Liss, New York, 1990.
39. Gilad GM and Gilad VH, Polyamine uptake, binding and release in rat brain. *Eur J Pharmacol* **193**: 41–46, 1991.
40. Mantione CR, Demirgoren S and London ED, Specific binding of [^{3}H]spermidine to membranes of rat brain. *Eur J Pharmacol* **180**: 393–394, 1990.
41. Ransom RW and Stec NL, Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor–ion channel complex by L-glutamate, glycine, and polyamines. *J Neurochem* **51**: 830–835, 1988.
42. Williams K, Romano C, Dichter MA and Molinoff PB, Modulation of the NMDA receptor by polyamines. *Life Sci* **48**: 469–479, 1991.
43. Rao TS, Cler JA, Oei EJ, Emmett MR, Mick SJ, Iyengar S and Wood PL, The polyamines, spermine and spermidine, negatively modulate *N*-methyl-D-aspartate (NMDA) and quisqualate receptor mediated responses *in vivo*: Cerebellar cyclic GMP measurements. *Neurochem Int* **16**: 199–206, 1990.
44. Singh L, Oles R and Woodruff G, *In vivo* interaction of a polyamine with the NMDA receptor. *Eur J Pharmacol* **180**: 391–392, 1990.
45. Olney JW, Excitatory amino acids and neuropsychiatric disorders. *Biol Psychiatry* **26**: 505–525, 1989.
46. Ylinen AMA, Miettinen R, Pitkanen A, Gulyas AI, Freund TF and Reikkinen PJ, Enhanced GABAergic inhibition preserves hippocampal structure and function in a model of epilepsy. *Proc Natl Acad Sci USA* **88**: 7650–7653, 1991.
47. Saitoh T, Masliah E, Jin L-W, Cole GM, Wieloch T and Shapiro IP, Protein kinases and phosphorylation in neurologic disorders and cell death. *Lab Invest* **64**: 596–616, 1991.
48. Hara H, Onodera H, Yoshidoma M, Matsuda Y and Kogure K, Staurosporine, a novel protein kinase C inhibitor, prevents postischemic neuronal damage in the gerbil and rat. *J Cereb Blood Flow Metab* **10**. 646–653, 1990.
49. Zivin JA, Kochhar A and Saitoh T, Phenothiazines reduce ischemic damage to the central nervous system. *Brain Res* **482**: 189–193, 1989.
50. Schengrund C-L, The role(s) of gangliosides in neural differentiation and repair: A perspective. *Brain Res Bull* **24**: 131–141, 1990.
51. Schuber F, Influence of polyamines on membrane functions. *Biochem J* **260**: 1–10, 1989.
52. Berridge MJ, Inositol triphosphate and diacyglycerol: Two interacting second messengers. *Annu Rev Biochem* **56**: 159–193, 1987.
53. Walters JD and Johnson DJ, Inhibition of cyclic nucleotide phosphodiesterase and calcineurin by spermine, a calcium-independent calmodulin antagonist. *Biochim Biophys Acta* **957**: 138–142, 1988.
54. Toninello A, Miotto G, Siliprandi D, Siliprandi N and Garlid K, On the mechanism of spermine transport in liver mitochondria. *J Biol Chem* **263**: 19407–19411, 1988.
55. Jensen JR, Lynch G and Baudry M, Polyamines stimulate mitochondrial calcium transport in rat brain. *J Neurochem* **48**: 765–772, 1987.
56. Meldrum BS, Simon RP, Swan J, Evans MC and Griffiths T, Calcium loading of mitochondria in ischemia and status epilepticus: Its reversibility and significance for pathological outcome. In: *Calcium Entry Blockers and Tissue Protection* (Eds. Godfraind T, Vanhoutte PM, Govoni S and Paoletti R), pp. 183–195. Raven Press, New York, 1985.
57. Phillips JE and Chaffee RRJ, Restorative effects of spermine on oxidative phosphorylation and respiration in heat-aged mitochondria. *Biochem Biophys Res Commun* **108**: 174–181, 1982.
58. LeBel CP and Bondy SC, Persistent protein damage despite reduced oxygen radical formation in the aging rat brain. *Int J Dev Neurosci* **9**: 139–146, 1991.
59. Hall ED and Braughler JM, Role of lipid peroxidation in post-traumatic spinal cord degeneration: A review. *CNS Trauma* **3**: 281–294, 1987.

60. Ohmori S, Misaizu T, Kitada M, Kitagawa H, Igarashi K, Hirose S and Kanakubo Y, Polyamine lowered the hepatic lipid peroxide level in rats. *Res Commun Chem Pathol Pharmacol* **62**: 235–249, 1988.
61. Tadolini B, Polyamine inhibition of lipoperoxidation: The influence of polyamines on iron oxidation in the presence of compounds mimicking phospholipid polar heads. *Biochem J* **249**: 33–36, 1988.
62. Bors W, Langerbartels C, Michel C and Sandermann H Jr, Polyamines as radical scavengers and protectants against ozone damage. *Phytochemistry* **28**: 1589–1595, 1989.
63. Marton JL and Feuerstein BG, Polyamine–DNA interactions: Possible site of new cancer chemotherapeutic intervention. *Pharmacol Res* **3**: 311–317, 1986.
64. Curran T, Rauscher FJ III, Cohen DR and Franza BR, Beyond the second messenger: Oncogenes and transcription factors. *Cold Spring Harbor Symp Quant Biol* **III**: 769–777, 1988.
65. Hopkins D and Manchester KL, The influence of nerve section on the metabolism of polyamines in rat diaphragm muscle. *Biochem J* **196**: 603–610, 1981.
66. Kaminska AM, Stern LZ and Russell DH, Polyamine metabolism in muscle: Differential response to tenotomy and denervation in the soleus and gastrocnemius muscle of adult rats. *Exp Neurol* **78**: 331–339, 1981.
67. Gilad GM, Dornay M and Gilad VH, Polyamines induce precocious development in rats: Possible interaction with growth factors. *Int J Dev Neurosci* **7**: 641–653, 1989.
68. Evans EA Jr, Vennesland B and Schneider JJ, Effect of spermine on tissue oxidation. *Proc Soc Exp Biol Med* **41**: 467–470, 1939.
69. Kuriyama K, Robers E and Vos J, Some characteristics of binding of γ-aminobutyric acid and acetylcholine to a synaptic vesicle fraction from mouse brain. *Brain Res* **9**: 231–245, 1968.
70. Brackley P, Goodnow R Jr, Nakanishi K, Sudan HL and Usherwood PNR, Spermine and philanthotoxin potentiate excitatory amino acid response of *Xenopus* oocytes injected with rat and chick brain RNA. *Neurosci Lett* **114**: 51–55, 1990.
71. Koenig H, Goldstone A and Lu CY, Polyamines regulate calcium fluxes in a rapid membrane response. *Nature* **305**: 530–534, 1983.
72. Bondy SC and Walker CH, Polyamines contribute to calcium-stimulated release of aspartate from brain particulate fraction. *Brain Res* **371**: 96–100, 1986.
73. Kuroiwa T, Bonnekoh P and Hossmann K-A, Prevention of postischemic hyperthermia prevents ischemic injury of CA1 neurons in gerbils. *J Cereb Blood Flow Metab* **10**: 550–556, 1990.
74. Shin W-W, Fong W-F, Pang S-F and Wong PC-L, Limited blood–brain barrier transport of polyamines. *J Neurochem* **44**: 1056–1059, 1985.
75. Dietrich WD, Halley M, Valdes I and Busto R, Interrelationships between increased vascular permeability and acute neuronal damage following temperature-controlled brain ischemia in rats. *Acta Neuropathol (Berl)* **81**: 615–625, 1991.
76. Kauppila T, Stenberg D and Porkka-Heiskanen T, Putative stimulants for functional recovery after neural trauma: Only spermine was effective. *Exp Neurol* **99**: 50–58, 1988.
77. Coffino P and Poznanski A, Killer polyamines? *J Cell Biochem* **45**: 54–58, 1991.
78. Morris DR, A new perspective on ornithine decarboxylase regulation: Prevention of polyamine toxicity is the overriding theme. *J Cell Biochem* **46**: 102–105, 1991.
79. Bergeron RJ, Neims AH, McManis JS, Hawthorne TR, Vinson JRT, Bortell R and Ingeno MJ, Synthetic polyamine analogues as antineoplastics. *J Med Chem* **31**: 1183–1190, 1988.
80. O'Sullivan MC, Golding TB, Smith LL and Wyatt I, Molecular features necessary for the uptake of diamines and related compounds by the polyamine receptor of rat lung slices. *Biochem Pharmacol* **41**: 1839–1848, 1991.
81. Edwards ML, Snyder RD and Stemerick DM, Synthesis and DNA-binding properties of polyamine analogues. *J Med Chem* **34**: 2414–2420, 1991.
82. Schmid N and Behr J-P, Location of spermine and other polyamines on DNA as revealed by photoaffinity cleavage with polyaminobenzenediazonium salts. *Biochemistry* **30**: 4357–4361, 1991.
83. Xiao L, Swank RA and Matthews HR, Photoaffinity polyamines: Sequence-specific interactions with DNA. *Nucleic Acids Res* **19**: 3701–3708, 1991.
84. Jackson H and Usherwood PNR, Spider toxins as tools for dissecting elements of excitatory amino acid transmission. *Trends Neurosci* **11**: 278–283, 1988.
85. Eldefrawi AT, Eldefrawi ME, Konno K, Mansour NA, Nakanishi K, Oltz E and Usherwood PNR, Structure and synthesis of a potent glutamate receptor antagonist in wasp venom. *Proc Natl Acad Sci USA* **85**: 4910–4913, 1988.
86. Usherwood PNR, Bycroft BW, Blagbrough IS and Mather AJ, New phenyl-substituted polyamine—e.g. N-4-hydroxycinnamoyl spermine amid derivatives are useful in treating psychosis or neurological disorders. Patent Application, PCT International: WO 89GB1004, 1989.
87. Nakanishi K, Eldefrawi AT, Eldefrawi ME and Usherwood PNR, *N*-3-Phenyl-propionyl-substituted spermine and related polyamine derivatives—Antagonists of glutamate binding to receptors, useful as insecticides and for treating e.g. neurodegenerative disorders. Patent Application, PCT International: WO 89US482, 1989.