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Polyamines in the brain: distribution, biological interactions, and their potential therapeutic role in brain ischaemia

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LIST OF ABBREVIATIONS:

AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

3-AP: 3-aminopropanal

Ca^{2+}: calcium

CNS: central nervous system

cdks: cyclin-dependent kinases

DFMO: Difluoromethylornithine

DNA: deoxyribonucleic acid

Kir: inwardly rectifying potassium channels

NMDA: N-methyl-D-aspartic acid

NR: NMDA receptor

ODC: ornithine decarboxylase

PAO: polyamine oxidase

SSAT: spermidine/spermine N^1-acetyltransferase

SOD: superoxide dismutase

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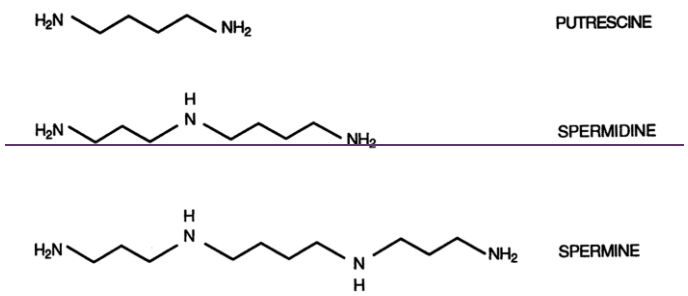
Abstract

The endogenous polyamines (spermine, spermidine, and putrescine) are present at~~in~~ relatively high concentrations in the mammalian brain and play crucial roles in a variety of ~~physiological processes including~~aspects of cell functioning~~, growth, and differentiation~~. Stroke is the third most common cause of death and the leading cause of disability among adults in the ~~W~~western world. Brain polyamine levels change dramatically following cerebral ischaemia. Polyamines may ~~take part~~be involved in the pathophysiological processes underlying brain ischaemia ~~and anoxia by~~ through several possible mechanisms. These include~~ing~~ direct effects on ~~various~~ ion channels and receptors modulating ~~sodium,~~ potassium, and most importantly calcium trafficking, or through the production of toxic metabolites. ~~Brain polyamine levels change dramatically following anoxia and ischaemia. This change is associated with neurotoxic effects through several mechanisms. In contrary, polyamine antagonists inhibit NMDA receptors, but may participate in several other processes and may potentially limit ischaemic injury.~~ Considerable evidence shows that the non-competitive polyamine antagonists, ifenprodil and eliprodil, are neuroprotective. Interestingly, novel polyamine analogues, such as N^1-dansylspermine, BU36b, and BU43b, have also recently been shown to have neuroprotective potential. The exact mechanisms of the neuroprotection afforded by the polyamine antagonists and their clinical applicability ~~need to be explored~~is worthy of further study.

1. Polyamine presentation, distribution and biological ~~effects~~interactions

Polyamines (putrescine, spermidine, and spermine), the naturally occurring di-, tri-, and tetra- amines, are constituents of all eukaryotic cells, including the cells of the vertebrate central nervous system (CNS) [1]. ~~They share this ubiquity with amino acids, proteins and nucleic acids. Structures of the most commonly occurring polyamines are shown in Figure 1.1.~~



~~Figure 1.1 Chemical structures of three polyamines [2].~~

The initial discovery of the polyamines was made by Antonie van Leeuwenhoek in 1678 when he isolated some 'three-sided' crystals from human semen [2]~~[3]~~. Tissue concentrations of the polyamines vary greatly throughout the body. Rapidly proliferating cells and tissues (embryos, tumours, etc) usually have higher putrescine and spermidine concentrations than corresponding non-growing tissues [3]~~(ref needed)~~. Due to the existence of the blood-brain barrier, the exchange of polyamines between blood and brain

is limited [3][4]. The vertebrate brain is an autonomous, nearly closed system with regard to polyamine metabolism [1]. As shown in table 1.1, brain concentrations of polyamines vary from region to region and putrescine levels are much lower than those of spermine and spermidine.

Brain area	Putrescine nmol/ g wet weight	Spermidine nmol/ g wet weight	Spermine nmol/ g wet weight
Frontal cortex	9.4±1.3	235 ±22	221±20
Rear Cortex		368±56	324±50
Olfactory Bulb	5.5±0.4	446±81	506±88
Hippocampus	7.1±1.2	420±107	334±64
Hypothalamus	22.9±2.0	591±109	253±54
Striatum		420±107	285±38
Midbrain	6.2±1.1	884±176	273±61
Medulla oblongata	3.7±0.8	1016±77	157±33
Cerebellum	13.0±1.3	674±58	381±47

Table 1.1 Tthe variation (mean ± SD) in the distribution of polyamines in different regions of rat brain. Putrescine data is adapted from Seiler and Schmidt-Glenwinkel, 1975 [4][5]. Spermidine and spermine data is adapted from Al-Deen, 1982 [5][6].

1.1 Biological effects interactions of polyamines

1.1.1 Polyamines as regulators of cell growth/cell death

Polyamines have long been known to act as intracellular growth factors, increasing the rate of cell growth. Normal cell growth is regulated in a cyclical manner by increases and decreases in kinases, cyclins, and cyclin-dependent kinases (cdks) [4][6][7]. Appropriate activation of the cdks and their partner cyclins is required for continual progression through the cell cycle. The cyclin/cdks exhibit cycle-specific regulation, and both polyamines and cyclin/cdks show phased changes throughout the cell cycle [2][3]. Intracellular polyamine concentrations have been reported to regulate important cellular checkpoints within the cell cycle, and this may, in part, explain why their concentrations are strictly controlled throughout the cycle [5][7][8]. A major portion of the pPolyamines is boundbind to rough endoplasmic reticulum and affect mammalian protein synthesis [6]. (ref needed). Polyamines also bind directly to DNA and modulate DNA-protein interactions [6][8][9]. In animal cells, the initiation of the cell growth always involves the stimulation of ornithine decarboxylase (ODC) [7][9][10]. Treatment of cells with a polyamine synthesis inhibitor depletes the polyamine pool and slows the cell growth [5][7][8].

Polyamines can also regulate cell death, particularly the cellular suicide known as apoptosis or programmed cell death [8][10][11]. The hallmarks of apoptosis include chromatin condensation, nuclear segmentation, cytoplasmic shrinkage, blebbing, and formation of apoptotic bodies [9][11][12]. It has been suggested that spermine and spermidine are involved inmay induce programmed cell death in mammalian embryos

through ~~the generation of~~ toxic degradation products ~~when they are metabolized~~generated by their oxidative catabolism. ~~Apoptotic effects may depend on the depletion of cellular polyamine pools or due to the formation of toxic polyamine catabolites.~~ Catabolism of ~~these~~ polyamines by polyamine oxidase produces hydrogen peroxide, which is extremely toxic to cells [10, 11]~~[12, 13][13, 14]~~. Another degradation product of polyamines, 3-aminopropanal (3-AP), is a lysosomotropic small aldehyde that causes apoptosis or necrosis of most cells in culture, apparently by inducing moderate or extensive lysosomal rupture [12]~~[14][15]~~. However, it is thought that the effects of the polyamines on apoptosis are far from simple with evidence suggesting both induction and inhibition of apoptosis following selective polyamine depletion [13, 14]~~[15, 16][16, 17]~~. ~~Packham and Cleveland demonstrated that enforced expression of c-myc caused increased ODC levels and apoptosis, both of which could be prevented by the ODC inhibitor difluoromethylornithine (DFMO) [18].~~

1.1.2 Polyamine interactions with cell membranes and the cytoskeleton

~~In addition to their interaction with DNA, p~~Polyamines have been found to interact with acidic phospholipids in membranes [15]~~[17][19]~~. It has been demonstrated that, by binding to the acidic phospholipids of the membrane, polyamines can influence the intracellular second messenger system based on phosphoinositide metabolism [15]~~[17][19]~~. Polyamines are also thought to be involved in the regulation of several membrane-bound enzymes, including adenylate cyclase and tissue transglutaminase [2]~~[3]~~.

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Polyamines have also been shown to promote tubulin polymerisation in tissue extracted from calf brain and may have a role in micortubule assembly[16] ~~(Wolff, J. Promotion of microtubule assembly by oligocations: co-operativity between charged groups, Biochemistry (1998), 37, 10722-10729).~~Spermine, which has four amino groups, promotes microtubule assembly more effectively than spermine, (3 amino groups), or putrescine (2 amino groups) [17] ~~(Anderson, P.J., Bardocz, S, Campos, R., Brown, DL, The effect of polyamines on tubulin assembly, Biochem Biophys Res Commun (1985), 132, 147-54).~~ In rat, polyamines were shown to be necessary for microtubule formation in gastric mucosa during healing [18] ~~(Banan, A., Mc Cormack, S.A.,& Johnson, L.R., Polyamines are required for microtubule formation during gastric mucosal healing, Am. J. Physiol. (1998), 274, G879-885).~~

1.1.~~2~~3 *Polyamines as regulators of ion channels*

The polyamines interact with, or regulate, several ion channels and receptors ~~taking part~~involved in the regulation of ~~Ca^{2+}~~calcium, sodium, and potassium homeostasis.

~~Among them are the ion channels such as inwardly rectifying potassium channels (Kir) and voltage-gated potassium channels, axonal sodium channels, voltage-activated and ligand-activated Ca^{2+} channels, inward rectifier Ca^{2+} channels, several ionotropic receptors such as NMDA (*N*-methyl-D-aspartate), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, quisqualate, and nicotinic acetylcholine receptors, gamma-aminobutyric acid (GABA) receptors, and a diverse group of G-protein-coupled receptors, among which Kir and voltage-activated Ca^{2+} and AMPA receptors, and NMDA receptors are probably the most studied targets.~~

There is recent evidence of interactions of the polyamines with strong inwardly rectifying potassium channels (particularly Kir 2.1) [19{Stanfield, 2003 #13, 20]. ~~(Oliver, D., Baukrowitz, T. & Fakler, B. (2000) Polyamines as gating molecules of inward rectifier K+ channels Eur. J. Biochem. 267 5824-5829; Stanfield, P.R. & Sutcliffe, M.J. (2003) Spermine is fit to block inward rectifier (Kir) channels J.Gen. Physiol. 122 481 – 484).~~ Indeed, the polyamines appear to be the most physiologically important blockers of the outward currents through Kir channels at μM concentrations [19] ~~(Oliver at al, 2000)~~ and therefore contribute to excitation of electrically excitable tissues. A rise in the concentration of intracellular polyamines would tend to increase the rectification of Kir channels and, hence, cause an increase in~~of~~ cellular excitability [21]~~[18]~~[20].

~~-~~Polyamines can also interact with voltage-activated Ca^{2+} channels [22]~~[19]~~[21]. It has been proposed that there are a number of intracellular and extracellular sites of action for polyamines ~~to inhibit and/or enhance the~~on voltage-activated Ca^{2+} channels [22]~~[19]~~[21]. Pullan reported that polyamines inhibit N-type (Cav 2.2) Ca^{2+} channels [23]~~[20]~~[22]. Recent evidence suggests a clear interaction of the polyamines at the α1 subunit of the L-type calcium channel (particularly Cav1.2 and 1.3), which may contribute to their biological effects. Herman demonstrated that putrescine increases L-type Ca^{2+} channel currents through the action of protein kinase C, but both spermidine and spermine are unable to produce this action [24]~~(reference needed)~~. Diltiazem and dihydropyridines, such as nitrendipine, bind to a site on the α1 subunit of the L-type Ca^{2+} channel and prevent Ca^{2+} influx. Polyamines have been shown to inhibit diltiazem and nitrendipine

binding to L-type Ca^{2+} channels in the brain and also inhibit [3H]nitrendipine (dihydropyridine) binding [24][25][29]. Polyamines also blocked an inward current activated by the dihydropyridine agonist BAYK 8644 in guinea-pig intestinal smooth muscle [25][29][24]. Dihydropyridines bind to the L-type Ca^{2+} channel and prevent Ca^{2+} influx. Thus polyamines could function endogenous modulators of L-type voltage-dependent calcium channels. Most recently, Doyle et al showed that antagonists of the α1 subunit of L-type ~~Ca^{2+}~~calcium channels can effectively

The polyamines can cause rectification of AMPA and kainate receptors through blocking the pore of the receptor channel and thus prevent the flux of sodium (and ~~Ca^{2+}~~calcium) [27, 28][~~24, 25~~][~~27, 28~~]. A subset of native ~~receptors of~~ AMPA and kainate receptors that show inward rectification, and are controlled by intracellular polyamines, have been shown to be ~~Ca^{2+}~~calcium permeable [29][~~26~~][~~29~~]. Therefore changes in levels of intracellular polyamines could alter ~~Ca^{2+}~~calcium flux and the excitability threshold at synapses containing polyamine-sensitive AMPA and kainate receptors. The block of AMPA and kainate channels would be reduced by a decrease in the concentration of polyamines, thereby increasing cell ~~Ca^{2+}~~calcium influx and excitability.

The NMDA receptor is the most extensively investigated glutamate receptor. Extracellular spermine has stimulatory effects on the NMDA receptor, although the degree of potentiation by spermine can var~~ied~~ greatly between individual neurons, suggesting NMDA receptors expressed on different neurons are differentially sensitive to spermine [30, 31][~~27, 28~~][~~30, 31~~]. It is thought that this variability is due to the existence of different molecular forms of the NMDA receptor and also partly due to the multiple

effects of spermine on the NMDA receptors. NMDA receptor channels gate ~~Ca^{2+}~~calcium and sodium and are blocked in a voltage-dependent manner by magnesium. They are composed of two copies of each of NR1 and NR2 subunits [32]~~(ref needed)~~. Multiple splice variants of NR1 and four different NR2 subunits exist, and both glutamate and glycine are essential for activation of the receptor channels [32]~~(ref needed)~~. The glycine binding site is located on the NR1 subunit and the glutamate binding site is located on the NR2 subunit [32]~~(ref needed)~~. Polyamines can act on both subtypes, but their effects are many ~~i~~fold. Spermine can potentiate NMDA currents in the presence of saturating concentrations of glycine (glycine independent stimulation). This~~It~~ involves an increase in the frequency of channel opening and a decrease in the desensitisation of NMDA receptors [33, 34]~~[29, 30]~~[32, 33]. Spermine also increases the affinity of the NMDA receptor for the co-agonist glycine (glycine-dependent stimulation) [31]~~[28]~~[31]. Spermidine has been shown to potentiate the seizure activity of NMDA in an apparently selective manner [35]~~[31]~~[34] and the intracerebroventricular injection of spermine causes tonic convulsions, which are mediated, at least in part, by the activation of the NMDA receptor [36]~~[32]~~[35]. When heteromeric NMDA receptors expressed from cloned subunits were studied in Xenopus oocytes, NR2 subunits were found to control the stimulatory effects of spermine at NMDA receptors [32]~~[33]~~[36].

In addition to stimulation, voltage-dependent inhibition by extracellular spermine was seen at native NMDA receptors in the absence of extracellular magnesium, possibly representing a direct block of the ion channel by spermine [30, 31]~~[27, 28]~~[30, 31]. Conceptually, this is similar to the block of Kir channels and AMPA channels by

intracellular polyamines. However, it is thought that in the case of NMDA receptors, it is extracellular spermine and not the intracellular polyamines that block the channels [29][~~26~~][~~29~~]. The voltage dependent inhibitory effects may include the interaction of spermine with negatively charged residues on the NMDA receptor, possibly close to the channel mouth, which impedes ion flow and thus reduces currents through NMDA channels [34][~~30~~][~~33~~]. The voltage-dependent block by extracellular spermine is weak and develops much more slowly than the block caused by physiological concentrations of extracellular magnesium, and therefore this action is thought to have negligible effect under physiological conditions. Indeed, using the NMDA receptors expressed in Xenopus oocytes, Williams [32][~~33~~][~~36~~] found that NR1A/NR2B controls both the stimulatory and inhibitory effects of spermine at the NMDA receptors but in the presence of extracellular magnesium, only stimulation by spermine was observed at NR1A/NR2B. It is thus suggested that stimulation, seen in the presence of physiological concentrations of Ca^{2+} and magnesium, may be the predominant effect of polyamines at NMDA receptors in the intact CNS [32][~~33~~][~~36~~].

Spermine and spermidine are present in the CNS and uptake and depolarization-induced release of polyamines from brain slices has been demonstrated [37, 38][~~34, 35~~][~~37, 38~~]. It is likely that sufficient polyamines could be released from neurons or glia into the synaptic cleft to influence the activation of NMDA receptors. Indeed, extracellular polyamine levels (as measured e.g. in microdialysis studies) are reported to be in the sub to low micromolar range [38, 39][~~35, 36~~][~~38, 39~~]. Very recently it has been demonstrated that low μM concentrations of spermine potentiated a guanidinosuccinate-evoked current

through the action of spermine on the polyamine binding site of the NMDA receptor complex [40]~~[37]~~[40]. It has been suggested that the polyamine transporters present in glial cells, neurons, and synaptic vesicles each have different properties and are, presumably, different molecular entities[41]~~[38]~~[41].

2. Role of the polyamines in the pathophysiology of brain ischemia ~~and anoxia~~

2.1 Change of polyamine profile following cerebral ischaemia

Intracellular polyamines do not per se exert toxic effects, as long as ~~physiological regulation~~the combined effect of ~~by~~ de novo synthesis, uptake, degradation, and release mechanisms maintains normal ~~occur at~~ physiological concentrations. ~~On the other hand, if extracellular polyamines undergo oxidative deamination, toxic products of this reaction (aldehydes and hydrogen peroxide) can cause cell damage and cell death. In brain ischaemia, following their release from necrotic cells, polyamines activate NMDA receptors and contribute to further tissue damage via glutamic acid-mediated excitotoxicity.~~

Following cerebral ischaemia, there are marked changes in polyamine~~s~~ levels. Thirty minutes of transient focal cerebral ischaemia in rats produced increases in cortical putrescine levels ~~at~~ 4 and 24 hours later with a significant three-fold increase achieved at the latter time. Significant increases in striatal putrescine were also reported after both 4 and 24 hours reperfusion following 30 minutes of focal cerebral ischaemia in rats in the same study (2.2- and 4-fold, respectively) [42]~~[39][42]~~. Baskaya reported a two-fold increase in putrescine in the penumbra after 6 h occlusion in a focal permanent cerebral ischaemia model in cats [43]~~(ref needed)~~. In the gerbil global ischaemia model, putrescine levels were shown to be ~~largely~~ increased throughout the brain after 3 bouts of

5 minutes ischaemia [43][~~40~~][~~43~~]. Increase of putrescine has been reported in cortex and striatum at both 6 hours (4.6- and 2.1-fold increase in~~for~~ cortex and striatum, respectively) and 24 hours (5- and 2.2-fold increase in~~for~~ cortex and striatum, respectively) ~~in~~ following~~a~~ transient focal cerebral ischaemia ~~model~~ in rats [44][~~41~~][~~2~~]. In the same study, a significant increase of putrescine was observed in both the cortex (6.9-fold) and hippocampus (4.7-fold) 24 hours following transient forebrain ischaemia in gerbils [44][~~41~~][~~2~~].

It has been proposed that the overshoot of putrescine may be due to an increase of ODC activity and/or the inhibition of S-adenosylmethionine decarboxylase activity [45][~~42~~][~~44~~]. ODC accounts for 30% of putrescine whereas the remaining 70% is formed through spermine-spermidine N^1-acetyltransferase/polyamine oxidase (SSAT/PAO) pathway [46, 47][~~43, 44~~][~~45, 46~~]. Inhibition of PAO with MDL 72527 significantly attenuated the putrescine levels at day 1 after transient forebrain ischaemia in gerbil cortex and hippocampus [48][~~45~~][~~47~~]. Although there is evidence demonstrating that under certain conditions PAO could directly act on spermine and spermidine to generate putrescine [49][~~46~~][~~48~~], the contribution of PAO via the direct oxidation of spermidine to putrescine may be minimal [48][~~45~~][~~47~~] and putrescine is largely formed by PAO actions on N^1-acetylspermidine. Therefore the induction of SSAT could also account for, at least partly, the increase in putrescine following cerebral ischaemia.

Despite the consistently observed increases in putrescine levels following ischaemia, changes in spermine and spermidine levels vary considerably depending on the types of

ischaemia and possibly the animal species. This may reflect the fact that spermine and spermidine synthesis requires the use of adenosylmethionine derived from adenosine triphosphate. Furthermore, the breakdown of spermine and spermidine by the interconversion pathway via acetylation involves the use of acetyl CoA [50][~~47~~][~~49~~]. Both adenosine triphosphate and acetyl CoA are affected under ischaemic conditions, but may be variously affected at different times and in different types of ischaemia [50][~~47~~][~~49~~]. A 27% and 22% decrease in spermidine was observed in the cortex and striatum, respectively, of rats following 30 minutes of focal cerebral ischaemia [42][~~39~~][~~42~~]. Spermine levels were also reduced in these tissues during the ischaemic period by 14% in the striatum and 22% in the cortex although the statistical significance was only seen in the latter structure. However, in the same study, no alterations in spermine or spermidine concentrations were observed at any of the recirculation times (2, 4, and 24 h) [42][~~39~~][~~42~~]. Following permanent focal ischaemia in rats, both spermine and spermidine levels were decreased 48 hours after permanent middle cerebral artery occlusion [51][~~48~~][~~50~~]. In a permanent focal ischaemia model in the cat, a non-significant decrease of spermidine levels (32%) was reported in the densely ischaemic core, while no alteration of spermine levels were observed [43][~~40~~][~~43~~]. In a study using a gerbil global ischaemia model, Paschen reported that spermine levels were significantly reduced in the hippocampal CA1-subfield after 5 minutes of ischaemia [52][~~(ref needed)~~]. Spermine levels were also reduced in the striatum and thalamus after 3-bouts of 5 minutes ischaemia in this model [52][~~49~~][~~51~~]. However, spermine and spermidine have also been reported to be increased following ischaemia. A small and non-significant increase in spermidine (20%) and spermine (11%) appeared 1-2 minutes after the occlusion and

spermidine and spermine showed a modest increase during the 15 minutes recirculation (with significance seen ~~with the increase of the~~for spermidine) in a gerbil global ischaemia model [53]~~[50]~~[52].

2.2 The role of the polyamines in cerebral ischaemia: a neuroprotective role?

Although the majority of evidence points to a role for the polyamines and polyamine metabolism in enhancing the neurodegeneration following cerebral ischaemia, there is still confusion over the role of polyamines because some studies have demonstrated that they, particularly spermine, are neuroprotective in~~after~~ cerebral ischaemia. Spermine has been reported to be neuroprotective in a model of forebrain ischaemia. Hippocampus and striatal cell loss in gerbils was significantly decreased by the intraperitoneal administration of spermine [54]~~[51]~~[53]. Farbiszewski demonstrated that, in a rat model of forebrain ischaemia, spermine significantly reversed the decrease in superoxide dismutase (SOD) activity in the cortex [55]~~[52]~~[54]. Spermine when given 30 minutes prior to ischaemia, showed a dose–dependent neuroprotective effect in a rat reversible focal cerebral ischaemia model [56]~~[53]~~[55]. Spermine has been shown to be of significant importance in the Ca^{2+}-buffering capacity of mitochondria [57]~~[54]~~[56]. Spermine can increase the rate of Ca^{2+} uptake of mitochondria by increasing the affinity of the uniporter for Ca^{2+}, therefore regulating ~~the~~ intracellular Ca^{2+} levels [58]~~[55]~~[57]. Also spermine can stabilize nucleic acids by its interaction with endonuclease or its substrate, DNA [59]~~[56]~~[58]. All of these factors could contribute to the neuroprotection observed in ischaemia with the exogenous polyamines and some novel polyamine

derivatives, which are thought to function in a similar manner [60][~~57~~][~~59~~]. More recently, a tentative antioxidant mechanism of spermine neuroprotection in ischaemia has been proposed [44][~~41~~][~~2~~]. Iron released from ferritin during ischemia–reperfusion promotes the formation of OH• ~~by catalyzing the OH• generation~~, which contributes to ischaemic injury [61, 62][~~58, 59~~][~~60, 61~~]. Spermine has been demonstrated to act as an antioxidant by scavenging oxygen radicals ~~possibly through chelation of iron~~ [63][~~60~~][~~62~~]. ~~Therefore, the authors suggested that spermine could afford its neuroprotection through this mechanism [2].~~ However, despite these few reports of neuroprotection by polyamines, the well-known stimulatory action of the extracellular polyamines on the NMDA receptor would suggest that polyamines would actually enhance the neurotoxicity following ischaemia. Therefore, the idea that exogenous polyamines are neuroprotective is far from convincing. Most evidence points towards a role of polyamines and polyamine metabolism in neurodegeneration following ischaemia ~~and is described below~~.

2.3 The role of the polyamines in cerebral ischaemia: a neurotoxic role?

It has been suggested that higher polyamines could be released from necrotic neurons into the extracellular space and cause multiple effects [52][~~49~~][~~51~~]. This may also explain the decrease of spermine and/or spermidine observed in some studies, following cerebral ischaemia, as polyamines released into the extracellular space then may be cleared by the blood circulation. Intracerebral putrescine levels are well-correlated with the extent of brain damage in experimental ischaemia studies, and therefore, it was suggested that

putrescine levels may be used as an endogenous molecular marker for this purpose. Following ischaemia, the polyamines released from necrotic neurons may diffuse to intact neurons in the vicinity and bind to the polyamine recognition site at the NMDA-receptor [52][~~49~~][~~51~~]. This binding could enhance the activational state of the NMDA receptor complex, potentiating the intracellular signal mediated by the NMDA receptor, and thereby increasinge excitotoxicity [64][~~61~~][~~63~~]. In fact, potassium-stimulated depolarization induces a Ca^{2+}-dependent release of polyamines from the cell, the re-uptake of which is nearly completely inhibited in the presence of high potassium concentrations [37, 65][~~34, 62~~][~~37, 64~~]. This observation indicates a release of polyamines from cells during conditions of energy depletion [52][~~49~~][~~51~~]. Indeed, Djuricic and co-workers observed that in hippocampal slices, polyamines are released from cells into extracellular compartments during a period of energy depletion produced by incubation in the absence of oxygen and glucose [42][~~39~~][~~42~~]. Carter reported that cortical spermidine, although not spermine, was released into the extracellular space following permanent focal cerebral ischaemia in rats. The amount of spermidine released was thought to be sufficient enough to enhance the activity of the NMDA receptor [66][~~63~~][~~65~~]. The increase of extracellular spermidine and spermine levels suggests that the higher polyamines may play an important role in the ischaemic damage. Furthermore, the NMDA antagonists, ifenprodil and eliprodil, are believed to produce their neuroprotection through blocking the polyamine recognition site [67][~~64~~][~~66~~]. Spermidine, ~~a polyamine modulatory site agonist,~~ potentiated NMDA- and glutamate-induced cytotoxicity in cultured rat cortical neurons [68][~~65~~][~~67~~]. In the same study,

spermidine also significantly reduced the protective effects of ifenprodil against NMDA- and glutamate-induced cytotoxicity [68][~~65~~][~~67~~].

~~Within a given brain structure, the p~~Postischaemic putrescine levels were found to correlate closely with the density of ischaemic cell injury and the time period of cerebral ischaemia [69][~~66~~][~~68~~]. The ODC inhibitor, DFMO, reduced the brain infarction volume by 57% after middle cerebral artery occlusion in rats [70][~~67~~][~~69~~]. The PAO inhibitor, MDL 72527 which blocks the overshoot of putrescine by 45% following cerebral ischaemia, has also been shown to have a neuroprotective effect against neuronal damage in a focal cerebral ischaemia model in rats [71][~~68~~][~~70~~].

In addition, polyamines have been found to influence the integrity of the blood-brain barrier [72][~~69~~][~~71~~]. Alteration of polyamine metabolism after cerebral ischaemia, particularly the increase of putrescine is an important factor in blood-brain barrier dysfunction and in the development of vasogenic oedema [43][~~40~~][~~43~~]. Intracerebroventricular or intracarotid injections of spermidine and spermine were found to disrupt the blood-brain barrier integrity within 15 minutes and were associated with the formation of vasogenic brain oedema. The breakdown of the blood-brain barrier and vasogenic oedema could be reversed by blocking ODC using DFMO in a focal cerebral ischaemia model in cats. Putrescine abolished the protective effect of DFMO [73][~~70~~][~~72~~]. In another study, DFMO treatment reduced both the ODC activity and oedema formation in a gerbil global cerebral ischaemia model, also indicating a role for polyamines in postischaemic oedema formation [74][~~71~~][~~73~~]. Lee showed that (-)-

epigallocatechin gallate reduced putrescine levels and attenuated neuronal damage after transient forebrain ischemia in gerbils [75][~~72~~][~~74~~].

During ischaemia, the change in polyamine levels may also have an effect on K^+ homeostasis. Intracellular polyamines, by blocking Kir channels, are of importance in controlling the resting membrane potentials and the excitability threshold for the initiation of action potentials as previously described. Johnson proposed that the increase of intracellular putrescine, which is a hallmark of cerebral ischaemic insult, would potentiate the polyamine-dependent Kir channel rectification resulting in an increased cellular excitability [21][~~18~~][~~20~~].

Also, the interconversion pathway of polyamines is proposed to be actively involved in the metabolism of polyamines following ischaemia. The increase of putrescine levels and the decrease~~s~~ of spermine/spermidine levels often observed, and recently, the increase of N^1-acetylspermidine reported [48][~~45~~][~~47~~] following ischaemia may implicate the activation of the interconversion pathway of polyamine metabolism. As previously described, oxidation of spermine or spermidine by tissue PAO leads to the formation of spermidine or putrescine, respectively. Hydrogen peroxide (H_2O_2) and 3-AP are also produced; both of which may induce cell death [2, 76][~~2, 73~~][~~3, 75~~]. PAO metabolism of spermine/spermidine was shown to have a cytotoxic effect in human umbilical vein vascular endothelial cell cultures [77][~~74~~][~~76~~]. Cerebral infarction volume, largely limited to the cerebral cortex, was significantly attenuated by intraperitoneal administration of aminoguanidine in a three-vessel occlusion model of focal cerebral

ischaemia in rats [78][~~75~~][~~77~~] and the neuroprotection by aminoguanidine (83% reduction in infarct volume) was suggested to be due to its PAO inhibitory activity [78][~~75~~][~~77~~]. Intracortical injection of either spermidine or spermine, but not putrescine, produced cerebral necrosis, which was inhibited by either systemic or cortical administration of aminoguanidine [21][~~18~~][~~20~~]. Intrastriatal injections of the same polyamines led to neuronal loss in a dose-dependent manner which could not be inhibited by a previous injection of MK-801, a potent NMDA receptor antagonist. Hence, the direct neurotoxic effects appear to be mediated, in part, through other mechanisms than NMDA-mediated neurotoxicity. The neuroprotective effect of the PAO inhibitor MDL 72527 observed in cerebral ischaemia models also supports the possibility that the cytotoxic intermediates produced by the polyamine interconversion pathway may be responsible for the observed cellular damage following cerebral ischaemia [71][~~68~~][~~70~~]. More recently, it is reported that spermine oxidase, and its product acrolein were significantly increased in the plasma of stroke patients and this correlated with the size of stroke ~~was nearly parallel with the multiplied value of acrolein and total PAO (acetylpolyamine oxidase plus spermine oxidase)~~ [79][~~76~~][~~78~~].

As described before, changes in levels of intracellular polyamines could alter Ca^{2+} flux and the excitability threshold at synapses containing polyamine-sensitive AMPA and kainate receptors. A decrease in the concentration of intracellular polyamines following ischaemia would reduce the block of AMPA and kainate channels, allowing more Ca^{2+} influx, resulting in increased excitability.

-Spermine has also been shown to be of significant importance in the Ca^{2+}-buffering capacity of mitochondria [57, 58]~~[54, 55]~~~~[56, 57]~~. Therefore during ischaemia, in regions where spermine levels are reduced, the Ca^{2+} buffering capacity of mitochondria may be disturbed, which could also contribute to the cellular damage [52]~~[49]~~~~[51]~~.

~~Cerebral ischemia itself induces brain PAO which leads to increased formation of cytotoxic end product 3-AP which in turn accumulated at concentrations lethal to neurons and glial cells and ignites caspase-1-dependent apoptosis.~~

3. Polyamine antagonists and cerebral ischaemia

Structural analogues that can inhibit the biological effects of the polyamines are considered to be polyamine antagonists. Some c~~C~~lose structural analogues of polyamines can cause inhibition of cell growth by depleting putrescine, spermidine, and spermine pools. This may occur due to the~~, by downregulating~~disregulation of ~~catabolic enzymes, and~~polyamine synthesis and catabolism ~~release~~ (ref needed), but not all antagonists have this effect on growth. ~~The structural analogues that do not inhibit growth due to perturbation of polyamine regulation and depletion are considered as polyamine antagonists. Many known polyamine derivatives usually share both properties at different portions.~~

~~As described previously, whether polyamines serve a neuroprotective or a neurodegenerative role remains unclear, although most evidence points towards a role in neurodegeneration.~~ The non-competitive polyamine antagonists, ifenprodil and its derivative eliprodil, have been shown to be effective neuroprotective agents in cerebral ischaemia models. This~~and the~~ neuroprotection was afforded through, at least partly, their action via the polyamine site at the NMDA receptor [80]~~[77][79]~~ although their other effects such as Ca^{2+} antagonism and their high affinity for σ receptors, could also contribute to the~~ir~~ neuroprotection [81-84]~~[78-81][80-83]~~. Historically, there ~~was~~ has been a lack of effective competitive polyamine antagonists to study. ~~It is interesting that~~

~~some old polyamine antagonists have been shown to have some agonist-like effects in vivo.~~-Arcaine, 1, 10-diaminodecane and diethylenetriamine were proposed as polyamine antagonists through the results of binding studies. All of these compounds reduced the increase in [^{3}H]-dizocilpine binding produced by polyamines [85-87]~~[82-84][84-86]~~. ~~Subsequently~~However, those polyamine antagonists were shown to have some agonist-like effects in vivo~~agonist effects~~. In vivo, administration of spermine intracerebroventricularly to Laca mice causes a distinct profile of two temporally distinct phases of behavioural effects. The first phase of effects develops within minutes of spermine administration and is short-lived, but consists behaviours such as upper-body scratching, face washing and clonic convulsions. A second wave of CNS excitation develops after approx. 2 hours. The animals develop a tremor, which gradually worsens and culminates in fatal tonic convulsions within 8h of administration [35, 87]. Both arcaine and 1, 10-diaminodecane were found to potentiate the first stage of the spermine-induced effects but inhibited the development of the second stage effects [88]~~[85][87]~~. Conversely, diethylenetriamine was effective against the development of the first stage of spermine induced effects, but potentiated the second stage of effects [88]~~[85][87]~~. More recently, a~~A~~rcaine and 1, 10-diaminodecane have been suggested to be inverse agonists of the polyamine site [86, 87]~~[83, 84][85, 86]~~. ~~Diethylenetriamine was found to increase [^{3}H]-dizocilpine binding in rat brain membrane, when tested under basal conditions [85]. Both arcaine and 1, 10-diaminodecane were found to potentiate the first stage of the spermine-induced effects but inhibited the development of the second stage effects [87]. Conversely, diethylenetriamine was effective against the development of the first stage of spermine-induced effects, but potentiated the second stage of effects [87].~~ Recently, a

novel polyamine analogue, N^1-dansyl-spermine, has been shown to be an effective polyamine antagonist and interest in this compound has been increasing. Chao demonstrated that N^1-dansyl-spermine blocked the recombinant NMDA receptor [89][~~86~~][~~88~~]. Later, this compound was shown to be a polyamine antagonist at the NMDA receptor as N^1-dansyl-spermine suppressed spermine induced CNS excitation and antagonised the spermine enhancement of NMDA-induced convulsions in mice [90][~~87~~][~~89~~]. More recently, N^1-dansyl-spermine has been reported to be neuroprotective in a global cerebral ischaemia model, and ~~focal~~ permanent and transient focal ischemia models. ~~, and It~~its neuroprotective effect has been suggested to be produced through an~~the~~ action at the polyamine site on the NMDA receptor [91-93][~~88-90~~][~~90-92~~]. The research on N^1-dansyl-spermine further confirms the neuroprotective possibilities of polyamine antagonists~~, particularly the polyamine antagonists at the NMDA receptor~~. BU36b and BU43b are novel polyamine analogues that have an inhibitory effect, in vitro, at the mammalian NR1/NR2B NMDA receptor subunit, which contains the polyamine site [32, 94][~~33, 91~~][~~36, 93~~]. In vivo, BU36b and BU43b were effective at antagonizing polyamine-induced CNS excitation in the form of tonic convulsions in mice [95][~~92~~][~~94~~]. Subsequently, both compounds were shown to be neuroprotective in both permanent and transient focal cerebral ischaemia models [96][~~93~~][~~95~~].

~~2.~~5~~4.~~ Polyamine involvement in traumatic spinal cord and brain injury

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Traumatic spinal cord injury is known to induce ischaemic cell death and alterations in polyamine metabolism have been observed. Following spinal cord compression injury, ODC and putrescine levels are increased in the injured spinal cord 4h later [97][~~94~~]

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(Mautes, AE, Paschen, W, Rohn, G & Nacimiento, AC, Changes in ornithine decarboxylase activity and putrescine concentrations after spinal cord compression injury in the rat, (1999), Neurosci. Lett. 264, 153-6).A further study showed increased ODC activity in transected spinal cord 6h after injury and a reduction towards normal levels 24h after injury, although activity was still significantly higher than normal [98](Gonzalez, 1995 #363) (Gonzalez, S, Coirini, H., Gonzalez Deniselle, MC, Gonzalez, S, Calandra, R & De Nicola, AF , Time – dependent effects of dexamethasone on glutamate binding, ornithine decarboxylase activity and polyamine levels in the transected spinal cord (1995), J. Steroid Biochem Molec Biol 55, 85-92).. -Putrescine levels were also increased above control levels 24h after injury in spinal cord tissue. No change was observed in spermine or spermidine levels [98](Gonzalez, 1995 #363) (Gonzalez et al, 1995).

Eliprodil has been shown to reduce lesion volume following lateral fluid percussion brain injury in rats when chronically administered for 7 days[99] (Toulmond S, Serrano, A, Benavides, J & Scatton, B (1993) Prevention by eliprodil (SL 82.0715) of traumatic brain damage in the rat. Existence of a large (18h) therapeutic window, Brain Res. 620, 32-41). A further study demonstrated that pericapillary astrocyte swelling, ODC and polyamine levels were raised 4h folowing cryo injury in the rat, an effect that was reduced by DFMO, ifenprodil and MK801. Addition of putrescine reversed the protective effect of DFMO [100] (Trout, JJ, Lu, CY, Goldstone, AD & Sahgal, S, (1995) Polyamines and NMDA receptors modulate pericapillary astrocyte swelling following cerebral cryo-injury in the rat, J. Neurocytol, 24, 341-6). ODC activity was raised 6h and 24h following

lateral controlled cortical – impact injury in rats, but had returned to normal 72h after injury in the cortex and hippocampus [101] ~~(Baskaya MK, Rao, AM, Prasad, MR, Dempsey, RJ (1996), Regional activity of ornithine decarboxylase and edema formation after traumatic brain injury, (1996) Neurosurgery, 38, 140-5)~~. A further study demonstrated that controlled cortical impact in the rat increased ODC activity and putrescine levels in the ipsilateral cortex, with no change in spermine or spermidine levels[102] ~~(Henley, CM, Muszynski, C, Cherian, L & Robertson, CS (1996), Activation of ornithine decarboxylase and accumulation of putrescine after traumatic brain injury, J. Neurotrauma, 13, 487-96)~~. In a further study, administration of MDL 72527 significantly reduced brain oedema, injury volume and putrescine levels 7 days after traumatic brain injury in the rat [103] ~~(Dogan, A, Rao, AM, Baskaya, MK, Hatcher, J, Temiz, C, Rao, VL & Dempsey, RJ (1999), Contribution of polyamine oxidase to brain injury after trauma, J. Neurosurg, 90, 1078-82)~~. Henley et al (1997) suggested that raised putrescine levels may be caused by reduced SAMdc activity from 1 to 72h after traumatic brain injury [104] ~~(Henley, C.M., Wey, K, Takashima, A, Mills, C, Granmayeh, E, Krishnappa, I & Robertson, CS (1997) J. Neurochem. 69, 259-65)~~. Chronic ifenprodil treatment over a 6h period following cortical impact injury in rats reduced injury volume measured 7 days later [105] ~~(Dempsey, RJ, Baskaya, MK & Dogan, A (2000) Attenuation of brain edema, blood brain barrier breakdown, and injury volume by ifenprodil, a polyamine site N-methyl-D-Aspartate receptor antagonist, after experimental traumatic brain injury in rats, Neurosurgery, 47, 404-6)~~.

One obstacle to axonal regeneration is the presence of axonal regeneration inhibitors, such as myelin associated glycoprotein (MAG) and Nogo [106] ~~(Cai D, Deng, K, Mellado, W, Lee, J, Ratan, RR & Filbin, MT (2002), Arginase I and polyamines act downstream from cyclic AMP in overcoming inhibition of axonal growth MAG and myelin in vitro, Neuron, 35, 711-719)~~. Increased levels of cAMP overcome the inhibition by MAG and enhance regeneration of spinal axons through a mechanism that involves protein synthesis [106] ~~(Cai et al, 2002)~~. It has been suggested that cAMP may upregulate arginase I, a rate limiting enzyme which catalyses the conversion of arginine to ornithine, and therfore may affect polyamine synthesis. A recent study showed that elevation of cAMP levels in cerebellar neurones induces Arg I mRNA and the protein witihn 3 h, and the effect is sustained for at least a further 21h [106] ~~(Cai et al, 2002)~~. Putrescine levels were increased in these cells 6h and 18h after cAMP administration, reflecting an effect on polyamine synthesis. Induction of Arg I in the absence of cAMP elevation was found to be sufficient to overcome axonal outgrowth by MAG and myelin. Application of a polyamine synthesis inhibitor, either MOHA (ArgI inhibitor) or DFMO inhibited the induction of axonal regeneration which suggests an involvement of polyamine synthesis in the regeneration process. Application of putrescine restored the regenerative capacity of cAMP[106] ~~(Cai et al, 2002)~~,.

45. Conclusion and future directions

Generally speaking, mounting evidence suggests that polyamines are involved in ischaemia through mechanisms such as (1) the enhancement of NMDA receptor activation; (2) the activation of voltage dependent Ca^{2+} channel activity; and (3) the reduction in the integrity of blood-brain barrier; and (4) the production of toxic metabolites. Therefore, an effective polyamine antagonist could provide neuroprotection as discussed above. The future direction of polyamine research in ischaemia would be to synthesize and screen effective polyamine antagonists. The magnitude of the neuroprotective effect may increase if an antagonist can inhibit several distinct targets in the ischaemic cascade. For novel antagonists which have already been shown to be neuroprotective in animal models, it is important to investigate the clear mechanisms those compounds and more clinical relevant studies shall be conducted, i.e. extending their therapeutic time-windows.

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Brain area	Putrescine nmol/ g wet weight	Spermidine nmol/ g wet weight	Spermine nmol/ g wet weight
Frontal cortex	9.4±1.3	235 ±22	221±20
Rear Cortex		368±56	324±50
Olfactory Bulb	5.5±0.4	446±81	506±88
Hippocampus	7.1±1.2	420±107	334±64
Hypothalamus	22.9±2.0	591±109	253±54
Striatum		420±107	285±38
Midbrain	6.2±1.1	884±176	273±61
Medulla oblongata	3.7±0.8	1016±77	157±33
Cerebellum	13.0±1.3	674±58	381±47

Table 1.1 The variation (mean ± SD) in the distribution of polyamines in different regions of rat brain. Putrescine data is adapted from Seiler and Schmidt-Glenwinkel, 1975 [107]. Spermidine and spermine data is adapted from Al-Deen, 1982 [108].

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