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Polyamines and brain injury

Review Article

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Summary. The cerebral ODC/polyamine system is disturbed by brain injury. The main modifications are important increases in ODC activity and putrescine concentration, with minor variations in spermidine and spermine concentrations. A great diversity of stimuli such as cerebral ischemia or overstimulation of the central nervous system by chemical or nonchemical agents can induce polyamine disturbances. Both the contribution of polyamines to the brain damage and their involvement in the repair mechanisms triggered after brain insults have been proposed.

Keywords: Amino acids – Putrescine – Spermidine – Spermine – Brain damage – Ischemia – Convulsion

In mammals, the polyamines putrescine spermidine and spermine are symthesized from ornithine. Their metabolism is complex and highly regulated by the enzymes, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase. The polyamines play important roles in cell multiplication and differentiation (Pegg and McCann, 1988; Seiler, 1990), and their metabolism suffers alterations in states involving proliferation and regeneration. The adult brain contains high concentrations of polyamines: about 10 nmol/g of putrescine, and 200 to 400 nmol/g of spermidine and spermine (Paschen et al., 1987; Hayashi et al., 1993; de Vera et al., 1995) but their physiological role is not well established. In recent years it has been found that the ODC/polyamine system is very sensitive to cerebral pathological states of different origin (Table 1). It is not clear whether polyamines have a role in these pathologies and what the role is for each polyamine.

Polyamines in cerebral ischemia

The disturbances of the polyamine system in cerebral ischemia have been studied extensively, especially by Paschen and coworkers (1987, 1988a,b,

Table 1. Pathological events inducing ODC/polyamine alterations

Brain ODC/polyamine changes in:
Brain injury produced by: • Metabolic stress: Ischemia, Hypoglycemia (Paschen et al. 1987, 1988a,b, 1991, 1992; Paschen, 1992) • Trauma: Criotrauma, Head injury, Mechanical lesions (Koenig et al., 1983, Shohami et al., 1992; Dienel and Cruz, 1984; Walsh et al., 1989; Zini et al., 1990).
Neuronal hyperactivity: • Convulsions (Bondy et al., 1987; Martínez et al., 1991; de Vera et al., 1991; Baudry et al., 1994) • Preseizure and subseizure states (Arai et al., 1990; Mialon et al., 1993; Giménez-Llort et al., 1995)

1991, 1992). In different models of ischemia it has been found that there is no modification of polyamine metabolism in the brain during ischemia, but after a period of recirculation there are: increases in ODC, a high increase in putrescine, (several times above the control values) in the ischemic vulnerable areas and a slight decrease in spermidine and spermine. The putrescine concentration correlates with the density of cell necrosis. An interesting point is that the increase in putrescine in the brain is found before the appearance of the ischemic brain damage.

Polyamines in neuronal hyperactivity

Another event that induces brain polyamine alterations is the overstimulation of the central nervous system. Thus, in several animal models of epilepsy, the increase in neuronal activity is followed by a disturbance to the ODC/polyamine system. A great diversity of stimuli involving different mechanisms can induce modifications in polyamines after the production of seizures. For example: excitatory amino acid agonists, insecticides or electrical stimulation (Table 2).

The polyamine response in the frontal cortex of adult rats 24h after the administration of kainic acid or lindane was a high increase in putrescine (Martínez et al., 1991). The time course of polyamine disturbances induced by kainic acid (de Vera et al., 1991) showed that the early changes were a decrease in spermidine and spermine in the frontal cortex and in the hippocampus (an area strongly involved in epilepsia) and later an increase in putrescine. In the same rats we found histological damage of different intensity depending on the behavioural alteration: from moderate spongiosis in the rats exhibiting minor behavioural alterations (wet dog shakes) to severe damage, with large areas of spongiosis and necrosis, in rats exhibiting status epilepticus. There is an association between the severity of the brain damage and the concentration of putrescine in the brain. Thus, the convulsions induced by kainic acid produce: a decrease in spermidine and spermine, a large increase in putrescine, and brain damage associated with the shooting of putrescine. These results are similar to those reported for the cerebral

Table 2. Different types of convulsants which induce brain ODC/polyamine system alterations

Convulsants	
Excitatory amino acids:	
• Kainic acid	Martínez et al., 1991; de Vera et al., 1991, Porcella et al., 1991, Facchinetti et al., 1992; Baudry et al., 1994
• NMDA	Porcella et al., 1991; Giménez-Llort et al., 1995
Insecticides:	
• Lindane	Martínez et al., 1991
Other chemical convulsants:	
• Pentylentetrazol	Martínez et al., 1991; Hayashi et al., 1993
Non-chemical convulsants:	
• Electrical stimulus	Pajunen et al., 1978; Bondy et al., 1987
• Hyperbaric oxygen	Mialon et al., 1993
• Kindling (electrical or chemical)	Hayashi et al., 1989, 1993

ischemic model, apart from the fact that we always found the brain damage and the shooting of putrescine simultaneously.

It has been proved that the shooting of putrescine seen after kainic acid is the result of both an increase in putrescine synthesis by ODC activation and an increase in the interconversion of spermine into spermidine, and then into putrescine (Baudry and Najm, 1994).

The main polyamine disturbance seen after convulsions is the increase in putrescine. Among the convulsants listed in Table 2, the highest concentration of putrescine in the brain is elicited by kainic acid: 20 times above control values (Martínez et al., 1991; de Vera et al., 1991). Apart from kainic acid and NMDA (de Vera et al., 1991; Porcella et al., 1991), which determines an early and slight decrease in spermidine and spermine, convulsants do not modify these polyamines, or induce a moderate increase.

On the other hand, it seems that not only convulsions but even neuronal activity of medium intensity can induce an increase in putrescine in the brain. Thus, after stereotypic NMDA-induced behaviour in rats (Giménez-Llort et al., 1995) there is an increase in putrescine in the brain and a similar increase is found in mice after preseizure clinical symptoms by hyperbaric oxygen (Mialon et al., 1993). In addition, subseizure activation of the Schaffer collateral commissural axonal system by electrical stimulation induces an increase in the ODC activity in several brain regions (Arai et al., 1990).

Proposed roles of polyamines in CNS

There are several proposed functions of polyamines in the central nervous system: nerve growth (Kauppila, 1992; Chu et al., 1995), regulation of calcium

fluxes (Iqbal and Koenig, 1985), and modulation of glutamatergic receptor (Markwell, 1990; Trout et al., 1993). Nevertheless, the effect of the polyamine disturbances after brain injuries is not clearly established and two opposite roles in brain injuries are suggested. It is proposed that polyamines are involved in the repair mechanisms activated following brain injury. Thus, there are some data showing that exogenous polyamines rescue neurons after ischemia (Gilad and Gilad, 1991) and recently it has been found that putrescine, spermidine and spermine have an axonal regeneration effect on injured neuron in cultures (Chu et al., 1995).

In contrast, other authors proposed that these compounds participate in the production of brain damage (Paschen, 1992; Paschen et al., 1988a,b, 1991, 1992). There are some processes involved in brain injuries in which polyamines can play a role: calcium homeostasis disturbances, blood-brain barrier breakdown, oxygen radical formation and glutamate receptor overactivation. So, polyamines increase the intracellular concentration of calcium (Koenig et al., 1983), and this event can provoke an abnormal release of neurotransmitters (Bondy et al., 1986). It is also proposed that polyamines are involved in the opening of the blood-brain barrier (Koenig et al., 1989), because the disturbances of the blood-brain barrier are blocked by the ODC inhibitor, α-difluoromethylornithine (DFMO). It is also suggested that the activation of the interconversion pathway described after kainic acid is common with other insults such as ischemia (Baudry and Najm, 1994) and it is proposed that the production of oxygen radicals by the activation of this pathway may contribute to the damage.

Polyamines and glutamate receptor

The participation of polyamines as modulators of the N-methyl-D-aspartate (NMDA) receptor activation would be in agreement with a role in the excitatory overactivation after brain insults. So, it is found *in vitro* (Ransom and Stec, 1988; McGurk et al., 1990) and *in vivo* (Singh et al., 1990; Chu et al., 1994), that spermidine and spermine activate the NMDA receptor, and, in theory, any release of these polyamines from neurons to the extracellular space may influence the NMDA receptor activation (Fage et al., 1992). There is evidence of this mechanism in ischemia (Paschen et al., 1992; Carter et al., 1995). However, as we have seen, putrescine is the polyamine that suffers major changes after brain insults, but its participation in the activation of the NMDA receptor seems to be the opposite of spermidine and spermine (Williams, 1989). Nevertheless, there is evidence that the inhibition of polyamine synthesis by DFMO protects against the neuronal damage induced by NMDA *in vivo* (Porcella et al., 1991) or *in vitro* (Markwel et al., 1990; Trout et al., 1993) and in experiments *in vitro* this neuroprotective effect is reversed by putrescine (Trout et al., 1993). The effects of polyamines on NMDA receptor activation are complex and it is suggested that there is more than one polyamine binding site. So, both the enhancing and the inhibitory effects of polyamines on the NMDA receptor channel function are suggested (Rock et al., 1995).

On the other hand, at high doses, putrescine injected into the animals is toxic: it produced mainly wet dog shakes and motor disorders, such as atony (de Vera et al., 1992; Camón et al., 1994), and the histological examination of the brain showed alterations such as cellular and perivascular edema (Genedani et al., 1987; de Vera et al., 1992).

In order to reconcile both the damaging and the protective roles of polyamines in brain injury the point of view of Lombardi et al. (1993) is interesting, suggesting that the effects of polyamines depend on their localization: thus, polyamines released from injured neurons or glia to the extracellular space may potentiate the excitotoxic action of glutamate on the NMDA receptor, and, on the contrary, the increase in intracellular ODC/ polyamines by a stimulus may have neuroreparative effects.

In conclusion, polyamines especially putrescine- are modified after brain insults and seem to be involved in events concerning both the brain damage production and the neuronal repair.

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