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Effect of Acrylamide on Energy-Linked Functions in Rat Brain

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Acrylamide (ACR) is an extensively used monomer in the production of polymers and derivatives in the plastic industry (Spencer and Schaumberg 1974). Exposure of humans to ACR produces a dying back type of neuropathy in both, occupational and non-occupational situations. Several experimental and clinical studies have provided evidence for central nervous system disturbances in both animals and man. Individuals affected at the work place complained of loss of sensation in the fingers, fatigue and weight loss. Depletion of brain biogenic amines in certain localised areas and its effect on neurotransmitter receptors have been reported following ACR exposure (Dixit et al. 1981; Agrawal et al. 1981). Alterations in the steady state concentration of cellular glutathione has also been proposed as a causative factor of its neurotoxicity (Dixit et al. 1980; Das et al. 1982). Though in the past, several hypothesis have been put forth concerning the mechanism of action of ACR neurotoxicity, yet the exact mechanism has not been established. The available literature on ACR reveals that its effect on energy metabolism has been poorly studied (Sabri and Spencer 1980). Our earlier studies in this direction showed that ACR altered the activity of succinic dehydrogenase (SDH) and had no effect on adenosine triphosphatase in vivo in developing and adult rat brain (*Husain et al.* Unpublished data).

A rapidly firing nerve terminal demands energy for its various tasks of transmitter release, membrane potential maintenance and vesicle movement etc. Any mitochondrial malfunction may lower this supply causing abberant neuromuscular transmission, which may finally result in motor incoordination. The present study was therefore designed to explore the in vitro effect of ACR on rat brain mitochondrial swelling and activities of two energy linked enzymes SDH and ATPase in mitochondrial preparations of various areas of the rat brain.

MATERIALS AND METHODS

Adult male albino rats were killed by cervical dislocation

brains were removed and cerebellum, pons medulla and cerebral cortex were dissected out (Glowinski and Iverson 1966). Intact mitochondria was prepared by homogenizing in 0.32 M Sucrose followed by centrifugation at 13000 x g for 10 minutes (Lovtrup and Zelander 1962). Mitochondrial swelling was measured by the method of (Tedeschi and Harris 1955). The ATP hydrolysis medium in a final concentration consisted of potassium chloride (100 mM), magnesium chloride (14 mM), ethylene diamine tetraacetic acid (1 mM), glycyl glycine (16.7 mM), sodium ATP (3 mM) and sucrose (15 mM). The incubation mixture was heated to 37°C and then placed in a cuvette followed by addition of 50 ulitre mitochondrial suspension. The light scattering of the incubation mixture (total volume 3.0 ml) was measured at 550 nm with a spectronic 20, spectrophotometer (Bausch and Lamb). Readings were taken at 20 S interval until constant values were observed. The test compound was added, the mixture stirred and the absorbance recorded until again constant. Reading of the test cuvette was balanced against one containing the ATP hydrolysis medium only.

ATPase was assayed according to the method of (Myers and Slater 1957). Enzyme activity was expressed as nmoles Pi liberated/min./mg protein. Inorganic phosphorous was estimated according to the method of Fiske and Subba Row (1925).

SDH activity was measured by a modification of the method of Slater and Bonner (1952). Enzyme activity is expressed as nmoles of Ferricyanide reduced/mt/mg protein.

Protein was estimated according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

The entire experimental procedure was repeated 3 times. Acrylamide used was obtained from Sigma (USA). All the other chemicals used were of the purest grade commercially available.

RESULTS AND DISCUSSION

Figure 1 illustrates induction of mitochondrial swelling by varying, concentration of ACR (5-17 mM) in different brain regions such as cerebellum, pons medulla and cerebral cortex. Addition of ACR caused a rapid decrease in absorbance at 550 nm. Maximum swelling appeared in the pons medulla and cortex region. Table 1 shows the effect of ACR on SDH activity. A concentration dependent decrease was noted in enzyme activity. Highest inhibition appeared at 0.5 mM concentration of ACR. No significant alteration in adenosine triphosphatase activity was noted

in any of the brain regions (data not shown).

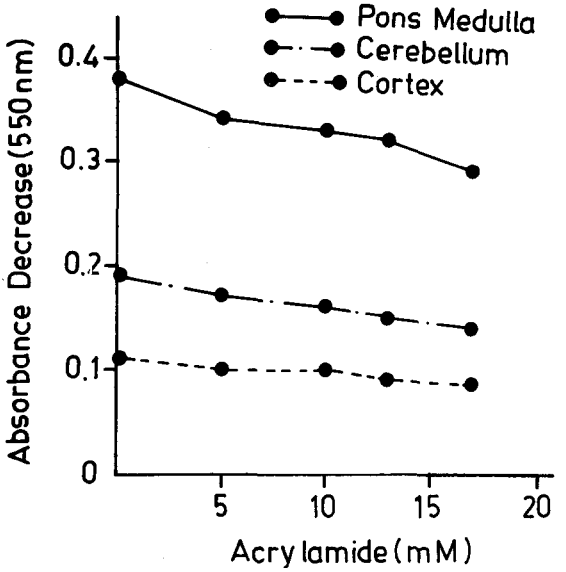

Figure 1. Effect of Acrylamide on mitochondrial swelling in brain regions

Curve 1, 2 and 3 show the concentration dependent changes in the absorbance decrease caused by ACR (Fig. 1).

ACR caused drastic swelling of mitochondria, the initial swelling was fast followed by a low speed swelling. Maximum swelling appeared in the pons medulla region. It is accepted that oxidative phosphorylation is dependent on the membranous structure of mitochondria. The induction of swelling indicates the lytic action of ACR on mitochondrial inner membrane. Due to lysis of the inner mitochondrial membrane the morphological integrity may be disrupted and uncoupling of mitochondrial respiration may occur. ACR may interact with phospholipids of mitochondrial inner membrane, this may also be a causative factor of induced swelling (Howland and Lowndes 1984).

Succinic dehydrogenase, a mitochondrial inner membrane enzyme being a part of the Krebs Cycle and Electron Transport Chain (ETC), showed a concentration dependent decrease in all the brain regions. Pronounced effects appeared in pons medulla and cortex regions of the brain. Inhibition of this enzyme results in decreased NADH formation for synthetic processes and oxidation by ETC is decreased with the result a decreased formation of ATP is possible. A reduced supply of energy from mitochondria would result in a compromise in these processes and would thus interfere with neuromuscular transmission. The apparent loss of the cofactor (NAD)

Table 1. Effect of ACR on *succinic dehydrogenase activity in brain regions

Brain part	Control	Acrylamide concentration (mM)A				
		0.30	0.35	0.50	0.45	0.50
Cerebellum	0.194	0.168 (13.4)	0.163 (15.97)	0.155 (20.10)	0.155 (20.10)	0.150 (22.7)
Medulla & pons	0.102	0.102 (-)	0.102 (-)	0.078 (23.52)	0.069 (32.35)	0.062 (29.21)
Cortex	0.118	0.099 (16.10)	0.106 (10)	0.093 (21.18)	0.087 (26.27)	0.068 (42.37)

A = Final concentration
Values in parenthesis denote percent inhibition.
*nmoles of Ferricyanide reduced/mt/mg protein.

from the mitochondria, perhaps as a consequence of mitochondrial swelling may account for the greater inhibition of SDH by ACR. ACR has a high affinity for thiols and therefore this may lead to perturbed axonal glycolysis and energy production.

In general a correlation between altered neurotransmitter levels and disturbed energy metabolism can be proposed. Although these correlations cannot be interpreted as convincing evidence. In our earlier in vivo studies a marked decrease in catecholamine content and serotonin levels was noted in pons medulla and cortex regions (*Husain et al. 1985*). These areas are linked to motor function and this could be one of the major factors responsible for ACR induced hind limb paralysis.

From the foregoing discussion we can draw the conclusion that disturbed bioenergetic capacity of neurons via disruption in mitochondrial function may partly account for many of the toxic actions of ACR. Further studies are needed in this direction.

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