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Effect of Acrylamide on Glutathione-S-Transferase Activity in Different Regions of Rat Brain

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Acrylamide (ACR), a widely used monomer in the plastics and polymer industry, is a potent neurotoxin (Spencer et al., 1974, NIOSH Publication 1976). Exposure to ACR leads to peripheral neuropathy of the nervous system in humans and animals (Spencer et al., 1974). Previous studies have shown that ACR is excreted as biliary glutathione-S-conjugates (Edwards, 1975). Studies from our laboratory have indicated that ACR reacts with glutathione both non-enzymically and enzymically. The enzymic reaction was catalyzed by liver and brain cytosolic glutathione-S-transferase(s) (GST) where ACR acted as a substrate (Dixit et al., 1981). ACR treatment was also found to decrease the GST activity of liver and brain in rats (Das et al., 1981a; Das et al., 1982a).

The toxicity of a xenobiotic is dependent, at least in part, upon the balance between the toxication-detoxication processes operating in the tissue. The various brain regions are structurally and physiologically different from each other and show significant variations in chemical and biochemical contents. We have recently observed that various rat brain regions have different levels of GST and aryl hydrocarbon hydroxylase activities (Das et al., 1981b; Das et al., 1982b). The information about the effect of acrylamide, a potent neurotoxic, on GST activity in different brain regions may therefore provide a better understanding of its neurotoxic effects. Therefore in the present study activity of GST and contents of glutathione were studied in different brain regions of control and acrylamide exposed rats.

MATERIALS AND METHODS

Male Wistar albino rats (140 ± 15g) of the Industrial Toxicology Research Centre animal breeding colony, maintained under standard laboratory conditions and raised on ad libitum commerical pellet diet (Hind Lever, Bombay) and water were used. 180 animals were divided into two equal groups. One group of animals was treated with acrylamide (i.p. 50mg/kg body weight in 0.5ml of 0.15M NaCl) while another group received the vehicle alone. Thirty animals each from control and experimental groups were sacrificed on day 3, 6 and 10 of the experiment by cervical dislocation. Brain regions were separated by the method of Glowinski and Iversen (1966). The tissues of 5 animals were pooled and for each group six

such pools were made. The tissues were homogenized in 4 volumes of 0.1M phosphate buffer, (pH 7.4), containing 0.15M KCl. The homogenates were centrifuged at 14,000 x g for 20 min in a Remi K-24 refrigerated centrifuge and the supernatants were used as the enzyme source for the estimation of GST activity.

The activity of GST using 1-chloro 2,4-dinitrobenzene as substrate was determined by the method of Habig et al. (1974) as described earlier (Das et al., 1962b). Glutathione content in whole homogenates of different brain regions was measured according to the method of Ellman (1959). Protein content in the samples was estimated by the method of Lowry et al. (1951) using bovine serum albumin as reference standard.

RESULTS AND DISCUSSION

The effect of ACR on the GST activity of different brain regions is summarized in Table-1. After 3 days of ACR treatment there was no significant change in the activity of GST in any brain region. The ACR treatment for 6 days caused an increase in GST activity in cerebellum and hypothalamus. After 10 days of ACR exposure a significant decrease in GST activity in all brain regions, except frontal cortex, was observed. Signs of complete hind limb paralysis were also observed in ACR treated animals at this time period (data not shown).

Data presented in Fig-1 show the effect of ACR treatment on GST content of the brain. Since in general no effect of ACR was found on the activity of GST at earlier dosing times the GSH contents were estimated only after 10 days of ACR treatment. The ACR treatment caused a significant decrease in GSH contents in all the brain regions with the exception of cerebellum and medulla & pons.

Cytosolic GST plays an important role in the detoxification of various electrophiles (Jerina and Bend, 1977). In our earlier studies ACR exposed rats showed an increase in hepatic GST at initial dosing and a decrease in the enzyme activity in liver and brain after prolonged treatments (Das et al., 1982a). Since anatomically distinct regions of rat brain possess significant activity of GST (Das et al., 1981a) the effect of ACR on GST activity was examined at different time periods in this study. The rats exposed to ACR for 6 days exhibited signficantly enhanced GST activity in cerebellum and hypothalamus. At this time period animals showed only either slight or no signs of hind limb paralysis. The increase in GST activiy of cerebellum and hypothalamus may be due to the substrate induction phenomenon, suggesting a potential for an increased detoxification of the neurotoxin since ACR can act as a substrate for liver and brain GST (Dixit et al., 1981a). After 10 days of ACR exposure GST activity decreased in all the regions of brain except the frontal cortex. It is interesting to note that inhibition of GST activity in different brain regions occurred at a time simultaneously to the onset of hind limb paralysis. These studies are consistent with earlier observations from our laboratory

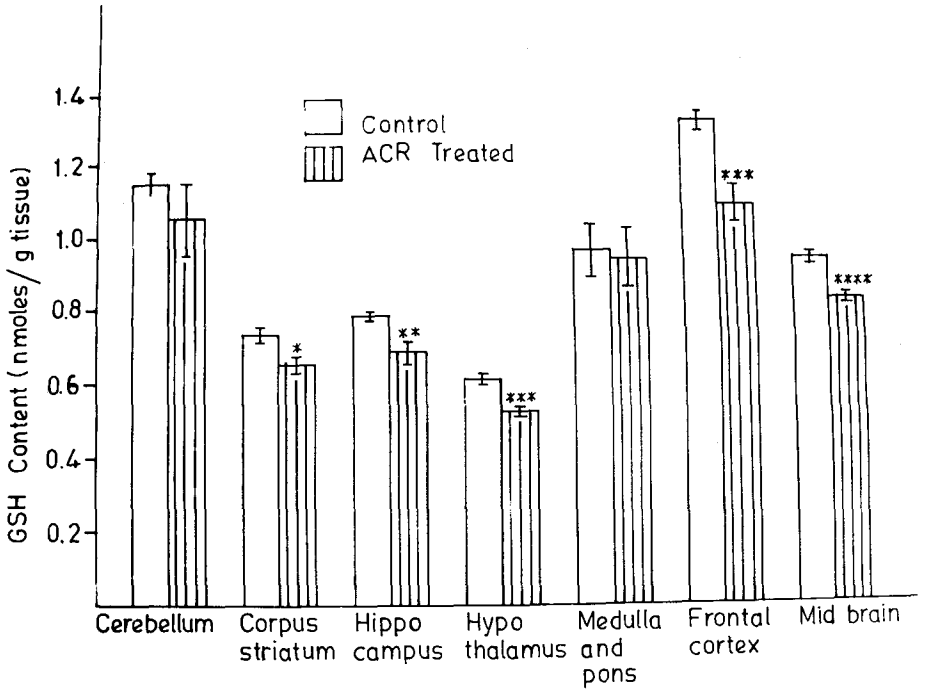

Figure - 1

Effect of acrylamide on glutathione content in different brain regions of rat. Value is mean ± S.E. of six determination on pools prepared from brain regions of five animals.

*P<0.05, **P<0.02, ***<0.01, ****P<0.001

as compared to control values.

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that whole brain GST activity is decreased in animals which show hind limb paralysis (Das et al., 1982a). The present results suggests that acrylamide is detoxified in early stages of exposure by binding with glutathione or GST without affecting enzmye activity while in later stages it inhibits the enzyme. Such suicidal inactivation of GST while removing ACR and other electrophiles has been reported previously (Dixit et al., 1981b; Smith et al., 1977). Glutathione plays a "Scavenger's" role in removing toxic electrohiles. The depletion of GSH in all brain regions except cerebellum and medulla and pons on exposure to ACR may at least in part be responsible for cumulative toxicity of ACR.

Table 1. Effect of acrylamide on glutathione-S-transferase activity in different brain regions of rat

Treatment	Cerebellulm	Corpus Striatum	Hippocampus	Hypothalamus	Medulla & pons	Frontal Cortex	Mid Brain
Control	187 ± 6	174 ± 6	214 ± 4	254 ± 13	136 ± 5	183 ± 4	154 ± 5
ACR 50mg/kgx3	192 ± 7	183 ± 7	218 ± 8	261 ± 9	140 ± 6	189 ± 8	149 ± 6
Control	193 ± 4	185 ± 9	223 ± 9	265 ± 13	142 ± 7	192 ± 6	161 ± 8
ACR 50mg/kgx6	214 ± 4*	200 ± 3	245 ± 10	304 ± 11*	166 ± 4	195 ± 4	180 ± 6
Control	183 ± 7	183 ± 12	217 ± 3	243 ± 11	127 ± 3	175 ± 13	151 ± 6
ACR 50mg/kgx10	140 ± 4*	128 ± 5*	137 ± 12*	178 ± 10*	105 ± 7*	161 ± 15	128 ± 4*

Data represents mean ± S.E. of six determinations in pools prepared from brain regions of five animals.

Treatment and killing was done as described under "Materials and Methods".

*$P < 0.05$, as compared to control values.

GST-n moles conjugate/min/mg protein.

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