Brain Research, 371 (1986) 319–323
Elsevier

BRE 11627

Effect of Single and Repeated Doses of Acrylamide and Bis-Acrylamide on Glutathione-S-Transferase and Dopamine Receptors in Rat Brain

SRI PRAKASH SRIVASTAVA[1], MOHAMMAD I. SABRI[2], ASHOK K. AGRAWAL[1] and PRAHLAD K. SETH[1]

[1]Industrial Toxicology Research Centre, Mahatma Gandhi Marg, Lucknow-226001 (India) and [2]Institute of Neurotoxicology, Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461 (U.S.A.)

(Accepted August 27th, 1985)

Key words: acrylamide — bis-acrylamide — glutathione-S-transferase — glutathione — dopamine receptor — neurotoxicity — neuropathy — neurotoxin — axonopathy

The effect of single and repeated doses of acrylamide (a neurotoxin) and N,N'-methylene-bis-acrylamide (a non-neurotoxic analogue of acrylamide) on glutathione (GSH), glutathione-S-transferase (GST) and dopamine receptors has been studied in rat brain. In vitro, both acrylamide and bis-acrylamide decreased brain GSH content in a concentration-dependent manner. At equimillimolar concentrations (2–10 mM) bis-acrylamide was more effective than acrylamide in lowering GSH levels. In vitro, GST activity was also inhibited as a function of acrylamide concentration. A single dose of either acrylamide or bis-acrylamide depleted GSH content of rat brain in a concentration-dependent manner without inhibiting GST activity. Repeated administration of either acrylamide or bis-acrylamide in rats (50 mg/kg × 10 days) decreased GSH content in the brain but GST activity was inhibited only by acrylamide and not by bis-acrylamide. Single or repeated injections of acrylamide but not of bis-acrylamide increased brain dopamine receptors ([^{3}H]spiroperidol binding) in a concentration-dependent manner.

INTRODUCTION

Glutathione-S-transferases (GST) are a family of multifunctional enzymes that catalyze the conjugation of glutathione (GSH) with a wide variety of electrophilic compounds including acrylamide[9]. Toxicological studies have established that acrylamide produces distal axonopathy of the 'dying-back' type both in the central and peripheral nervous system[11,12,14,25,26]. Recent studies have shown that single and/or repeated injections of acrylamide altered retrograde axoplasmic flow[19,21], altered GSH levels and GST activity[6,30] and increased dopamine receptors in rat brain[2]. Acrylamide has also been shown to alter protein synthesis[27], inhibit glycolytic enzymes[17,18,23,24] and interfere with other biochemical processes[1,16]. Several non-neurotoxic analogues of acrylamide are also reported to deplete GSH levels[16] and produce biochemical changes similar to acrylamide. Hence, the mechanism of acrylamide neurotoxicity is not understood.

In the present study, the effect of single and repeated doses of acrylamide and bis-acrylamide (a non-neurotoxic analogue of acrylamide) has been studied on GSH and GST activity in rat brain. In addition, the effect of acrylamide and bis-acrylamide has been investigated on dopamine receptors in the same tissue. Preliminary data of this study have appeared elsewhere[22].

MATERIALS AND METHODS

In vivo treatment of animals with acrylamide and bis-acrylamide

Albino adult male rats of the Industrial Toxicological Research Center (ITRC) breeding colony were given food and water ad libitum throughout this study. In single exposure, 8 rats per group were given i.p. injections of acrylamide or bis-acrylamide at a dose of 100 and 200 mg/kg and in repeated exposure, 8 rats per group were injected with acrylamide or bis-acrylamide at a dose of 50 mg/kg for 10 consecutive

Correspondence: M.I. Sabri, Institute of Neurotoxicology, Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, U.S.A.

days. Control rats were injected with the vehicle (normal saline). The rats were killed by cervical dislocation after 2 h following single injections and 24 h after the last injection in the study of repeated exposure.

In vitro treatment

The in vitro effect of acrylamide and bis-acrylamide on brain GST activity was studied by adding varying concentrations of acrylamide or bis-acrylamide (2–10 mM) to the reaction mixture for enzyme assay. The effect of acrylamide and its analogue on GSH content was studied by incubating varying concentrations (2–10 mM) of these agents with the brain homogenates for 30 min at 37 °C. The activity of GST and contents of GSH was estimated as described below.

Assay of GSH and GST activity

The brains were quickly removed, blotted free of blood and washed with ice-cold saline. Tissues were homogenized in a glass tube with a Teflon pestle in 4 vols. of ice-cold 0.1 M phosphate buffer pH 7.4 containing 0.15 M KCl and centrifuged at 14,000 *g* for 20 min at 0 °C. The resulting supernatants were centrifuged at 104,000 *g* for 1 h in an MSE superspeed ultracentrifuge. The supernatant was carefully decanted and used for the measurement of GST activity.

GST activity was assayed according to the method of Habig et al.[15] using CDNB as a substrate. GSH content in brain homogenates was measured according to the method of Ellman[10]. Protein was determined according to the method of Lowry et al.[20].

Membrane preparation and binding assay

The rat brains were removed and the corpora striata dissected[13] and frozen at −70 °C[28]. Crude membranes from frozen corpus striatum were prepared following the method of Agrawal et al.[2]. Briefly the tissue was homogenized in 19 vols. of 0.3 M sucrose and centrifuged at 50,000 *g*. The pellet was washed twice with deionized water and suspended in cold 0.1 M Tris-HCl buffer pH 7.4 (50 mg/ml wet weight of tissue). Binding assays were performed on glass fiber discs as described earlier[3,28]. Briefly, the method consisted of incubation of membranes in triplicate (250–300 μg protein) at 37 °C for 15 min with 1 nM

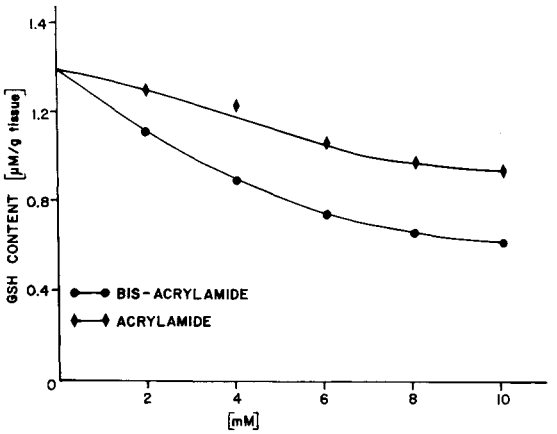

Fig. 1. In vitro effect of acrylamide and bis-acrylamide on brain glutathione content.

1-phenyl-4-[^{3}H]spiroperidol (NEN) in 40 nM Tris-HCl buffer pH 7.4 in a total volume of 1 ml. The reaction mixture was filtered through a glass fiber disc (0.3 μm pore size, 25 mm diameter, Gelman Inc., Ann Arbor, MI) and washed 3 times with 5 ml cold Tris-HCl. Filter dried discs were counted in the scintillation counter (Wallac LKB Rack II) with an efficiency of 50% for ^{3}H. To determine the extent of non-specific binding, parallel incubation was carried out in the presence of 10^{-6} haloperidol. Specific binding was calculated by subtracting non-specific binding from total binding obtained in the absence of haloperidol. Saturability, specificity, regional distribu-

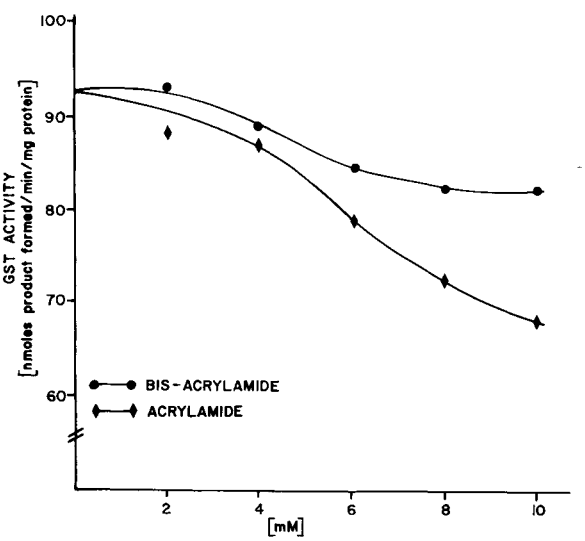

Fig. 2. In vitro effect of acrylamide and bis-acrylamide on brain glutathione-S-transferase activity.

TABLE I

Effect of a single injection of acrylamide or bis-acrylamide on glutathione and glutathione-S-transferase activity

Data represent mean ± S.E. of 4 animals.

Treatment	*GSH (μmol/ g tissue)*	*GST (nmol/min/ mg protein)*
Control	1.06 ± 0.026	135 ± 5
Acrylamide (100 mg/kg)	0.90 ± 0.017*	150 ± 6
Acrylamide (200 mg/kg)	0.81 ± 0.046*	153 ± 8
Bis-acrylamide (100 mg/kg)	0.85 ± 0.012*	128 ± 3
Bis-acrylamide (200 mg/kg)	0.74 ± 0.016*	128 ± 2

* $P < 0.05$ as compared to control values (Student's *t*-test).

tion and reversibility characteristic of spiroperidol binding were delineated prior to commencing this study[1].

RESULTS

The results of this study demonstrate that both acrylamide and bis-acrylamide conjugate the GSH under in vitro conditions (Fig. 1). The inhibitory effect of acrylamide and bis-acrylamide in depleting GSH content was a function of concentration (2–10 mM) of both compounds. In vitro, acrylamide caused a concentration-dependent decrease in the GST activity while bis-acrylamide failed to do so (Fig. 2).

Rats exposed to single doses of acrylamide or bis-acrylamide (100–200 mg/kg) showed no significant effect on GST activity but produced a significant decrease in GSH levels (Table I). Results of repeated exposure of acrylamide (50 mg/kg for 10 days) are summarized in Table II. The data show a significant decrease in GSH and GST levels in acrylamide-treated animals. Similar concentrations also caused a significant decrease in GSH level but failed to affect the GST activity.

TABLE II

Effect of repeated doses of acrylamide or bis-acrylamide on glutathione and glutathione-S-transferase activity of rat brain

Data represent ± S.E. of 6 animals.

Treatment	*GSH (μmol/ g tissue)*	*GST (nmol/min/ mg protein)*
Control	1.54 ± 0.055	140 ± 8
Acrylamide (50 mg/kg × 10 days)	1.22 ± 0.044*	113 ± 7*
Bis-acrylamide (50 mg/kg × 10 days)	1.27 ± 0.026*	138 ± 5

* Statistically significant from controls ($P < 0.05$).

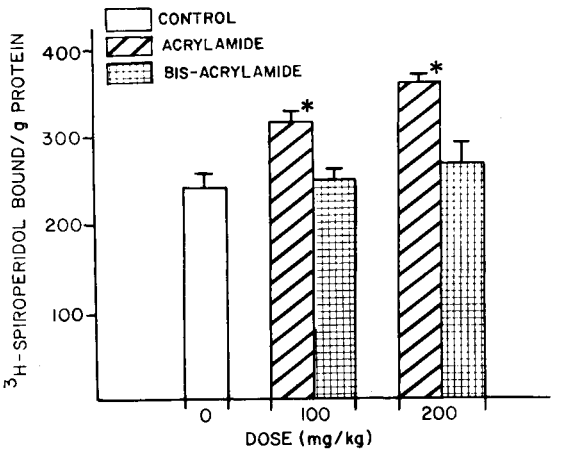

Fig. 3. Effect of single exposure of acrylamide and bis-acrylamide on [^{3}H]spiroperidol binding (pmol/g protein) in rat brain. * $P < 0.05$ as compared to controls by Student's *t*-test.

Single (100 and 200 mg/kg) and repeated (50 mg/kg × 10 days) exposure of acrylamide showed a significant increase of [^{3}H]spiroperidol binding in corpus striatal membranes (Figs. 3 and 4). Results of the Scatchard analysis showed a significant effect on the affinity of the receptor (K_d) without affecting the number of binding sites (B_{max}) (Table III). No significant effect of single and repeated exposure of bis-acrylamide on [^{3}H]spiroperidol binding was observed (Figs. 3 and 4).

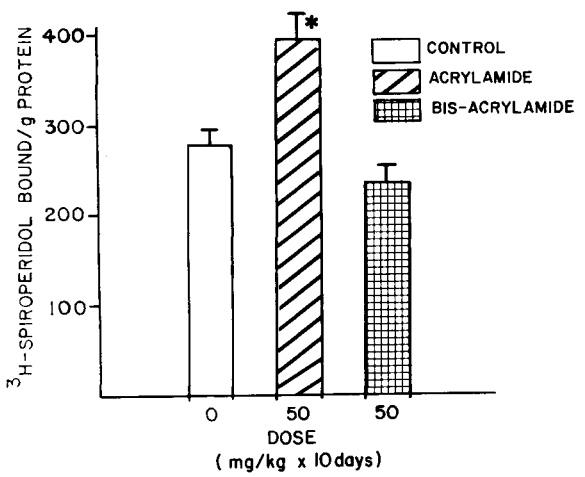

Fig. 4. Effect of repeated exposure of acrylamide and bis-acrylamide on [^{3}H]spiroperidol binding (pmol/g binding) in rat brain. * $P < 0.05$ as compared to controls by Student's *t*-test.

TABLE III

Effect of single and repeated injection of acrylamide on [^{3}H]spiroperidol binding in rat brain

Each set of data is the mean of 3 values from 3 separate experiments. K_d and B_{max} were calculated from linear regression lines.

Treatment	K_d *(nM)*	B_{max} *(pmol bound/g protein)*
Control	0.067 ± 0.038	525 ± 39
Single exposure (100 mg/kg)	0.42 ± 0.029	565 ± 52
Repeated exposure (50 mg/kg × 10 days)	0.39 ± 0.022	593 ± 53

DISCUSSION

The results of this study demonstrate that single or repeated injections of acrylamide or bis-acrylamide deplete GSH levels in rat brain. The degree of GSH depletion with bis-acrylamide is slightly greater than with equimillimolar concentrations of acrylamide. GST activity is inhibited only by acrylamide and bis-acrylamide has no effect on this enzyme. A significant increase in dopamine receptors has been observed in corpus striatum by single or repeated injections of acrylamide. Bis-acrylamide has no effect on dopamine receptors in rat brain.

The conjugation of GSH and the inhibition of GST activity has been earlier studied, but the specificity of GST inhibition by acrylamide has not been established. The inability of bis-acrylamide, a non-neurotoxic analogue of acrylamide, demonstrates the specificity and perhaps the involvement of GST in the expression of acrylamide neurotoxicity. GST catalyzes the reaction of acrylamide with glutathione to form sulfur-conjugates and thereby facilitate the elimination of acrylamide. But since acrylamide inhibits GST activity[6] the conjugation of acrylamide to GSH would be affected and the level of acrylamide or its metabolite will increase in the brain.

Acrylamide has been shown to bind covalently to proteins in nervous tissue predominantly by reaction with sulfhydryl groups[4,5] but the mechanism of action of acrylamide is unknown. The inhibition of certain sulfhydryl enzymes by acrylamide has been demonstrated both in vitro and in vivo[17,18,23,24]. Besides the sulfhydryl group, acrylamide has been shown to alkylate lysine, a property which may be linked to the abnormal accumulation of 10 nm neurofilaments (rich in lysine) in acrylamide-treated animals.

The observed low levels of GSH in acrylamide-treated animals appears to play a role in the expression of acrylamide neurotoxicity. This was demonstrated by Sharma and Obersteiner[29] that the neurotoxic action of acrylamide on chick ganglia tissue culture was rendered ineffective by the added glutathione into the culture medium. A similar conclusion can be drawn by the studies of Dixit et al.[7] in which the treatment of animals with diethylmaleate, a depleter of glutathione, prior to acrylamide administration, decreased the time of onset of acrylamide neuropathy in rats by 4 days.

The data of this study with bis-acrylamide suggests that depletion of GSH alone is perhaps not directly related to neurotoxicity of acrylamide, since bis-acrylamide, which depletes GSH levels in the brain, is non-neurotoxic to the rats.

Acrylamide has been shown to conjugate with tyrosine, a dopamine precursor, by Dixit et al.[8] and cause a significant increase in dopamine receptors[2]. The results of this study also show an increase in dopamine receptors in the brain following single or repeated acrylamide treatments with the neurotoxin acrylamide but not with the non-neurotoxic bis-acrylamide. The alteration in dopamine and dopamine receptor levels in the brains of rats treated with single or repeated doses of acrylamide appears to be specific, although the significance of these biochemical changes in relation to acrylamide neurotoxicity is not known and further studies in this direction may resolve the biochemical mechanism of acrylamide neurotoxicity.

ACKNOWLEDGEMENTS

The study was supported by the National Science Foundation, Washington, DC, and the Council of Scientific and Industrial Research, New Delhi, through an award to M.I.S. to visit India under the U.S.-India Scientists Exchange Program. The funding from NIH Grants NS19611 and OH-00851 is gratefully acknowledged. S.P.S. expresses thanks to the CSIR, New Delhi, for the award of Senior Research Fellowship. Thanks are also due to Mr. Umesh Prasad and Mr. Ashish Kumar for technical assistance and to Gloria Warkenthien and Jean Palladino for typing this manuscript.

REFERENCES

1 Agrawal, A.K. and Bondy, S.C., Characterization of catecholamine binding sites in the rat brain, *Neurotoxicology,* 2 (1981) 365–372.

2 Agrawal, A.K., Squibb, R.E. and Bondy, S.C., The effects of acrylamide treatment upon dopamine receptors, *Toxicol. Appl. Pharmacol.,* 58 (1981) 89–99.

3 Bennett, J.P., Methods in binding studies. In H.I. Yamamura, S.J. Enna and M.J. Kuhar (Eds.), *Neurotransmitter Receptor Binding,* Raven Press, New York, 1978, 57 pp.

4 Cavins, J.F. and Friedman, M., Specific modification of protein sulfhydryl groups with alpha, beta unsaturated compounds, *Fed. Proc. Fed. Am. Soc. Exp. Biol.,* 26 (1967) 822.

5 Cavins, J.F. and Friedman, M., New amino acids derived from reactions of E-amino groups in proteins with alpha, B-unsaturated compounds, *Biochemistry,* 6 (1967) 3767–3770.

6 Das, M., Mukhtar, H. and Seth, P.K., Effect of acrylamide on brain and hepatic mixed function oxidases and glutathione-S-transferase in rats, *Toxicol. Appl. Pharmacol.,* 66 (1982) 420–426.

7 Dixit, R., Husain, R., Seth, P.K. and Mukhtar, H., Effect of diethylmaleate on acrylamide induced neuropathy in rats, *Toxicol. Lett.,* 6 (1980) 417–421.

8 Dixit, R., Husain, R., Mukhtar, H. and Seth, P.K., Effect of acrylamide on biogenic amine levels, monoamine oxidase and cathepsin-D activity of rat brain, *Environ. Res.,* 26 (1981) 168.

9 Edwards, P.M., The distribution and metabolism of acrylamide analogues in rats, *Biochem. Pharmacol.,* 24 (1975) 1277–1282.

10 Ellman, G.L., Tissue sulphydryl groups, *Arch. Biochem. Biophys.,* 82 (1959) 70.

11 Fullerton, P.M. and Barnes, J.M., Peripheral neuropathy in rats produced by acrylamide, *Br. J. Ind. Med.,* 23 (1966) 210–221.

12 Gipon, L., Schotman, P., Jennekens, G.G.I. and Gispen, W.H., Polyneuropathies and CNS protein metabolism. I. Description of the acrylamide syndrome in rats, *Neuropathol. Appl. Neurobiol.,* 2 (1977) 115–123.

13 Iversen, L.L. and Glowinski, J.H., Regional studies of catecholamines in the rat brain: the disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the brain, *J. Neurochem.,* 13 (1966) 655–669.

14 Griffin, J.W., Price, D.L. and Drachman, D.B., Impaired axonal regeneration in acrylamide intoxication, *J. Neurobiol.,* 8 (1977) 355–370.

15 Habig, W.H., Pabst, M.J. and Jakoby, W.B., Glutathione-S-transferases in mercapturic acid formation, *J. Biol. Chem.,* 249 (1974) 7130–7139.

16 Hashimoto, K. and Aldridge, W.N., Biochemical studies in acrylamide, a neurotoxic agent, *Biochem. Pharmacol.,* 19 (1970) 2591–2604.

17 Howland, R.D., The etiology of acrylamide neuropathy: enolase, phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase activities in peripheral nerve, spinal cord, brain and skeletal muscle of acrylamide intoxicated cats, *Toxicol. Appl. Pharmacol.,* 60 (1981) 324–333.

18 Howland, R.D., Vyas, I.L. and Lowndes, H.E., The etiology of acrylamide neuropathy: possible involvement of neuron specific enolase, *Brain Research,* 190 (1980) 529–535.

19 Jakobsen, J. and Sidnius, P., Early and dose dependent decrease of retrograde axonal transport in acrylamide intoxicated rats, *J. Neurochem.,* 40 (1983) 447–454.

20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.,* 193 (1951) 265–275.

21 Miller, M.S. and Spencer, P.S., Single doses of acrylamide reduce retrograde transport velocity, *J. Neurochem.,* 43 (1984) 1401–1408.

22 Sabri, M.I., Shrivastava, S.P. and Seth, P.K., Effect of acrylamide on glutathione-S-transferase, *Trans. Am. Soc. Neurochem.,* 15 (1984) 174.

23 Sabri, M.I. and Spencer, P.S., Toxic distal axonopathy: biochemical studies and hypothetical mechanisms. In P.S. Spencer and H.H. Schaumburg (Eds.), *Experimental and Clinical Neurotoxicology,* Williams and Wilkins, Baltimore, 1980, pp. 206–219.

24 Sabri, M.I., In vitro and in vivo inhibition of glycolytic enzymes by acrylamide, *Neurochem. Pathol.,* 1 (1983) 179–191.

25 Schaumburg, H.H. and Spencer, P.S., Environmental hydrocarbons produce degeneration in cat hypothalamus and optic tract, *Science,* 199 (1978) 199–200.

26 Schaumburg, H.H., Wisniewski, H.M. and Spencer, P.S., Ultrastructural studies of the dying back process. I. Peripheral nerve terminal and axon degeneration in systemic acrylamide intoxication, *J. Neuropathol. Exp. Neurol.,* 33 (1974) 260–284.

27 Schotman, P., Gippon, L., Jennekens, F.G.I. and Gispen, W.H., Polyneuropathies and CNS protein metabolism. III. Changes in protein synthesis rate induced by acrylamide intoxication, *J. Neuropathol. Exp. Neurol.,* 37 (1978) 820.

28 Seth, P.S., Agrawal, A.K. and Bondy, S.C., Biochemical changes in the brain consequent to dietary exposure of developing and mature rats to chlordecone (Kepone), *Toxicol. Appl. Pharmacol.,* 59 (1981) 262–267.

29 Sharma, R.P. and Obersteiner, E.J., Acrylamide cytotoxicity in chick ganglia cultures, *Toxicol. Appl. Pharmacol.,* 42 (1977) 149–156.

30 Srivastava, S.P., Das, M., Mukhtar, H., Malhotra, O.P. and Seth, P.K., Effect of acrylamide on glutathione-S-transferase activity in different regions of rat brain, *Bull. Environ. Contam. Toxicol.,* 32 (1984) 166.