Neurotransmitter Receptors in Brain Regions of Acrylamide-Treated Rats. II: Effects of Extended Exposure to Acrylamide

S. C. BONDY,[1] H. A. TILSON AND A. K. AGRAWAL

Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences,
P.O. Box 12233, Research Triangle Park, NC 27709

BONDY, S. C., H A. TILSON AND A K. AGRAWAL. *Neurotransmitter receptors in brain regions of acrylamide-treated rats. II Effects of extended exposure to acrylamide* PHARMAC. BIOCHEM BEHAV. 14(4) 533–537, 1981.— Acrylamide was administered orally to 6 week old male rats in ten doses, spread over a two week period. At the two lower doses (5 and 10 mg/kg, total dose 50 and 100 mg/kg) effects on neurotransmitter receptor sites appeared confined to the striatum where both the dopamine and muscarinic acetylcholine receptors exhibited enhanced binding twenty four hours after the last acrylamide dose. Other receptor sites within the frontal cortex, cerebellum, and medulla were not significantly altered. At the highest dose (20 mg/kg, given ten times), increases were also found for frontal cortical serotonin, medullary glycine, and cerebellar GABA receptor sites. The only unaffected receptor found was the cortical site for benzodiazepene One week after the final acrylamide dose, the intensity of binding of all ligands studied was not significantly different in treated and control groups. Thus, effects appeared reversible Since striatal membrane protein concentration was reduced by treatment of rats with acrylamide, the observed increase in activity of muscarinic receptors could be best accounted for in terms of loss of striatal non-receptor protein rather than increased binding However, the magnitude of increased striatal ³H-spiroperidol binding in treated animals suggested an increase in overall binding capacity An effect on dopamine neurons was also suggested by a decreased responsiveness to apomorphine in rats treated with acrylamide at 10 mg/kg for 10 successive days; however, the effect had dissipated by 8 days after the final injection of acrylamide

Acrylamide   Neurotransmitters   Receptors   Dopamine

IN VIEW of its known neurotoxicity in humans, the effects of acrylamide treatment upon high affinity binding sites within the brain has been the subject of several studies from this laboratory [21]. Initial studies indicated an unusual sensitivity of the striatal dopamine receptor to systemic treatment of rats with acrylamide [4]. A single oral exposure to acrylamide caused a specific increase in the capacity of striatal membranes to bind ³H-spiroperidol. This increase could be correlated with behavioral changes and was attributed to the action of a metabolic breakdown product of acrylamide rather than to the intact chemical [2]. Gestational exposure to acrylamide also caused changes in the dopamine receptors of neonates [3].

The purpose of the present work was to determine whether repeated exposure to acrylamide would affect a wider range of transmitter binding sites than that observed after a single dose. The reversibility of any changes was also studied. Data were analyzed so as to reveal whether the overall binding capacity of the striatum was altered or whether acrylamide-induced changes reflected variation of the level of non-receptor protein within tissues.

METHOD

Six-week old male Fischer rats were used in this study. Acrylamide dissolved in water was administered orally by gavage daily in a volume of 5 ml/kg body weight. Control rats received an equivalent volume of distilled water. Animals were killed 24 hours or eight days after administration of acrylamide. After decapitation, brain regions were dissected by the method of Iversen and Glowinski [10]. A crude membrane fraction was prepared from frozen brain regions by homogenization of tissue in 19 volumes of 0.32 M sucrose followed by centrifugation (50,000 g, 10 min). The precipitate from this step was then homogenized in 40 mM tris pH 7.4 and recentrifuged. The final pellet was suspended in the tris-HCl buffer at a concentration representing 50 mg original tissue/ml.

Binding incubations were carried out in triplicate in a final volume of 1 ml containing 40 mM tris-HCl (pH 7.4) together with appropriate labeled and unlabeled pharmacological agents. The incubation mixture used in the assay of serotonin also included 10^{-5} M pargyline, 4×10^{-3} M $CaCl_2$ and

[1] To whom reprint requests should be addressed.

TABLE 1

REGIONAL RECEPTOR BINDING IN RATS RECEIVING TEN DOSES OF
ACRYLAMIDE OVER 14 DAYS

Receptor	Region	Acrylamide Dose (mg/kg)			
		0	5	10	20
Dopamine	Striatum	237±15	371±15*	314±17*	366±11*
Acetylcholine	Striatum	472±22	591±30*	594±26*	618±16*
Benzodiazepine	Frontal Cortex	43± 6	43± 5	34± 2	40± 4
GABA	Cerebellum	280±24	312±24	280±16	440±24*
Glycine	Medulla	420±18	456±12	450±18	492±36*
Serotonin	Frontal Cortex	65± 3	76± 6	69± 6	97± 5*

Binding expressed as pmol/g protein±S.E
*Differs from zero-dose ($p<0.05$, Fisher's Least Significant Difference Test)
Assays were conducted on membranes prepared from rats 24 hours after the last
acrylamide dose Experimental details are described in the text

$5-7\times10^{-3}$ M ascorbic acid. The amount of tissue used per tube corresponded to 5–10 mg original wet weight and contained 300–400 μg protein as determined by the method of Lowry et al [12]. At the end of a 15 min incubation at 37° samples were filtered on glass fiber discs (25 mm diameter, 0.3 μ pore size, Gelman Inc., Ann Arbor, MI) and washed rapidly two times with 5 ml tris buffer. In the case of the strychnine binding assay only one wash was used. Filter discs were then dried and counted in 5 ml of a scintillation mixture using a scintillation counter at an efficiency of 38–43%. Control incubations were carried out in order to determine the extent of non-specific binding simultaneously with the experimental series. The final concentration of unlabeled competing compounds in control incubations was 10^{-6} M. The assay of the dopamine receptor was performed using 10^{-9} M [1-phenyl-4^3H]-spiroperidol (23 Ci/mmol) as the binding ligand and haloperidol as the competing compound in control tubes. In a parallel manner 10^{-9} M DL [benzilic-4, $4'$-^{3}H] quinuclidinyl benzilate (29 Ci/mmol) was used to measure muscarinic sites with atropine as a competitor. Benzodiazepene sites were estimated with 0.7×10^{-9} M (methyl-^{3}H)-diazepam (73 Ci/mmol) and the unlabeled compound as competitor. 8.0×10^{-9} M [methylene-^{3}H(N)]-muscimol (7.3 Ci/mmol) and unlabeled GABA were used in GABA binding site assays. For the glycine site, non-radioactive strychnine was used to complete with 6.0×10^{-9} M [G-^{3}H]-strychnine sulphate (13 Ci/mmol) 3.1×10^{-9} M [1,2^{3}H(N)]-serotonin (30 Ci/mmol) was used together with unlabeled serotonin in the assay of this receptor. The method used was essentially similar to other filtration binding methods [23]. However, we felt it necessary to establish basic binding characteristics prior to studies on animals treated with acrylamide. These included delineation of saturability, specificity, reversibility, and regional distribution [1, 5, 6]. Differences between groups were assessed using Fisher's Least Significant Difference Test after a one-way analysis of variance. The accepted level of significance in all cases was $p<0.05$ using a one-tailed test. Each data point represents values drived from 6–8 individual animals processed separately.

In a separate experiment, 20 male Fischer rats were given either 0 or 10 mg/kg of acrylamide orally for a period of 10 consecutive days. Twenty-four hours and 8 days later, they were tested for responsiveness to the dopamine agonist apomorphine using a method described in detail elsewhere [2]. Motility was assessed using an automated monitor (Automex, Columbus Instruments, Columbus, Ohio). Rats were allowed to acclimate for a period of 10 minutes in plastic cages positioned atop of the monitors and then injected with 1 mg/kg of apomorphine, IP; motility was recorded for a period of 60 min.

RESULTS

Ten doses of acrylamide as low as 5 mg/kg resulted in significant elevations of ^{3}H-spiroperidol and ^{3}H-QNB binding to striatal membranes. Twenty-four hours after the last dose at 5 and 10 mg/kg levels, no other significant changes in receptor binding were observed, but at 20 acrylamide mg/kg, increased binding of several tritiated ligands were seen in cortex, cerebellum, and medulla (Table 1). The only receptor class examined that was not increased was the receptor for the non-neurotransmitter, benzodiazepene. All receptor sites thought to be directly associated with neurotransmission were elevated at the 20 mg/kg dose while no decreases in binding were observed.

An analysis was made of the total membrane protein recoverable from each brain area 24 hours after the last acrylamide dose. The concentration of membrane protein was reduced in the corpus striatum of acrylamide-treated rats but no major change was seen in protein content of other brain regions at any acrylamide dose used (Table 2). This difference between the striatum and other cerebral areas is additional evidence for the unusual sensitivity of the basal ganglia to the treatment with acrylamide. Taking into account the reduced striatal membrane content of treated animals, the observed changes in muscarinic binding are largely attributable to a loss of non-receptor protein rather than increased overall receptor capacity. However, the elevated dopaminergic binding in treated animals is too large to be totally accounted for in this manner. Together with previous work [4] the data indicate that a real increase in the dopamine receptor capacity occurs in treated animals.

TABLE 2

MEMBRANE PROTEIN CONTENT OF BRAIN REGIONS OF
ACRYLAMIDE-TREATED RATS

Acrylamide dose (given ten times) mg/kg	Brain region protein (μg/5 mg tissue$\pm$S.E)			
	Frontal	Cerebellum	Medulla	Striatum
0	204$\pm$14	318$\pm$ 4	373$\pm$17	367$\pm$16
5	188$\pm$ 6	322$\pm$ 7	369$\pm$ 8	299$\pm$ 4*
10	206$\pm$ 5	331$\pm$18	373$\pm$ 4	314$\pm$ 9*
20	188$\pm$ 5	313$\pm$ 7	374$\pm$10	285$\pm$ 6*

*Differs from zero-dose ($p<0$ 05, Fisher's Least Significant Difference Test). Experimental details presented in text. Measurements were made 24 hours after the last dose of acrylamide.
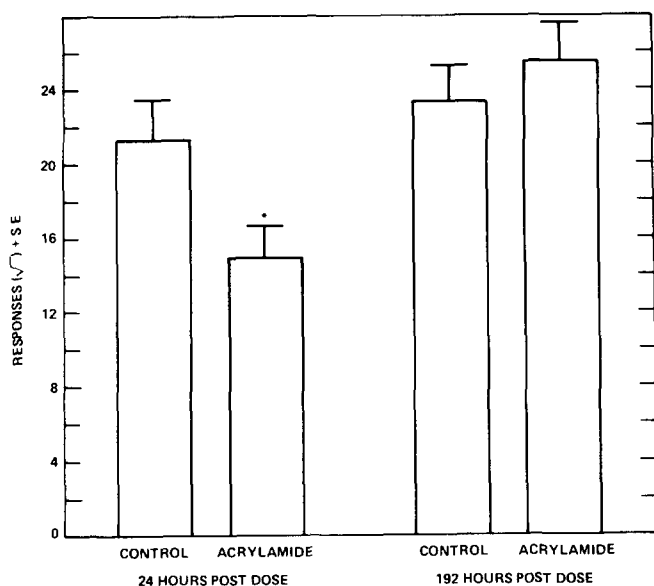

TABLE 3

STRIATAL RECEPTOR BINDING IN RATS RECEIVING TEN DOSES
OF ACRYLAMIDE OVER 14 DAYS

Receptor	Acrylamide dose (mg/kg)			
	0	5	10	20
Dopamine	299$\pm$ 5	309$\pm$16	352$\pm$ 7*	333$\pm$12*
Muscarinic Acetylcholine	333$\pm$23	347$\pm$16	365$\pm$38	399$\pm$23

Binding expressed as pmol/g protein$\pm$S.E
*Differs from zero-dose ($p<0$ 05, Fisher's Least Significant Difference Test). Binding assays were conducted on membranes prepared from rats 8 days after the last acrylamide dose. Experimental details are described in the text.

FIG 1 The effects of repeated exposure to acrylamide on responsiveness to apomorphine Rats were dosed with either 0 or 10 mg/kg of acrylamide, orally for 10 consecutive days Twenty-four and 192 hours later, they were challenged with 1 mg/kg of apomorphine, IP and motility recorded as described elsewhere [2]. Data are mean $\pm$ SE motility counts, square root transformed for 10 rats per group. The asterisk indicates significant difference from control (Student's t-test, $p<0.05$, two-tailed)

Animals treated with the intermediate dose of acrylamide (10 mg/kg, ten times) were tested for their responsiveness to the dopamine receptor agonist apomorphine (1 mg/kg). Experimental animals were significantly less susceptible to such drug-induced behavioral changes and the effect was reversed by 8 days after the last dose of acrylamide (Fig. 1). Thus, the observed alterations of striatal receptors in treated animals appeared to lead to modified behavior in response to a pharmacological challenge. Taken together, the data suggest an impaired functioning of dopamine neurons in treated rats. These observed differences in the apomorphine induced responses of treated rats were not detectable eight days after the last acrylamide dose. In a study involving greater expsoure to acrylamide, behavioral indices of peripheral nerve impairment were found no longer observed at 5 weeks, but were still present at 1 week after cessation of dosing [21].

All observed changes in striatal protein content were reversed within one week after cessation of the dosing schedule (data not presented). The binding of ligands to nonstriatal regions was also not significantly different in dosed and control animals eight days after the last acrylamide administration. However, at the two higher doses of acrylamide used, dopaminergic binding was still significantly elevated, and muscarinic binding tended to remain higher after this eight day interval (Table 3). This increase in acrylamide binding although significant was never greater than 18% and thus markedly less than the 32–57% increases found one day post-dosing Thus, much of the initial effect of acrylamide treatment appeared to be reversed, although a residual abnormality was still detectable.

DISCUSSION

Acrylamide treatment is known to rapidly depress the rate of protein synthesis in several tissues including brain [8,18]. However, this general effect may not totally account for the more localized reduction of striatal protein content that we have found Taken together with the binding data, our results imply an unusual susceptibility of the dopaminergic innervation of the striatum to acrylamide. The persistence of effects induced by the higher acrylamide doses could perhaps be related to the presence of residual acrylamide or metabolites, since this chemical is known to be present in nerve tissue for some weeks after exposure [9,17]. A similar reversibility has been found in young rats after gestation exposure to acrylamide [3] and after neonatal exposure (Gerhart and Tilson, unpublished observation). The central nervous system appears to possess sufficient regenerative capacity to at least partially restore normal metabolism after limited exposure to this toxicant. However, it is not known whether more prolonged acrylamide treatment could result in permanent damage to central nervous tissue. The repeated treatment reported here resulted in more widespread changes (both chemically and anatomically) than the single dose reported in the preceding chapter [2].

The increased striatal ^{3}H-spiroperidol binding level and concurrent decrease in responsiveness to apomorphine may appear to be paradoxical. However, our results are analogous to those reported by Owen et al. [14]. These authors found that chronic haloperidol treatment of rats lead to an increase of ^{3}H-spiroperidol binding sites in the striatum but a decrease in apomorphine induced stereotypy. Both their results and those reported in this paper might be best explained by postulating damage to, rather than hypoactivity of, the dopaminergic pathways. In this case the postsynaptic nerve cells might develop a supersensitivity but apomorphine would not be able to elicit a major response even in the

presence of an excess number of dopamine receptors. The specificity of the striatum may be due to some vulnerable biochemical aspect of the dopamine system. Another possibility is that the mean length of dopamine neurons is greater than that of neurons containing the other neurotransmitter species studied here Dopamine neurons run in relatively long tracts and shorter dopamine interneurons do not appear to exist. This excess length might cause such nerve cells to be more easily damaged by acrylamide. This could most readily be detected by receptor binding studies in areas where the target cells of tracts occupy a relatively compact area such as the striatum. This idea is supported by the known deleterious effect of acrylamide upon axonal transport processes in peripheral nerves [15]. Such impairment might be expected to be especially injurious to relatively long axons Defective axonal transport induced by acrylamide may be a secondary reflection of general damage to anabolic processes [22].

From this emerges the concept of a relation between the size of neurons and their vulnerability to a variety of toxicants. This relationship could in part explain the unusual sensitivity of dopamine neurons to toxicants such as manganese [7] and to anoxia [13]. The sensitivity of the optic nerve and long spinal tracts to various toxicants in humans and experimental animals, also supports this idea [11, 16, 20] The lack of a clear effect of acrylamide in vitro upon protein synthesis in the sciatic dorsal root ganglion [8], upon the striatal dopamine receptor (A. K. Agrawal, unpublished data) suggests that acrylamide itself is not neurotoxic directly This is supported by the observation that the effect of acrylamide treatment upon the dopamine receptor, is abolished by pre-treatment with an inhibitor of mixed function oxidases [2]. However, acrylamide has been found to be cytotoxic in chick ganglion cultures at relatively high concentrations above 10^{-5} M [18].

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