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Authors

Bondy, SC
Tilson, HA
Agrawal, AK

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Neurotransmitter Receptors in Brain Regions of Acrylamide-Treated Rats.

I: Effects of a Single Exposure to Acrylamide¹

A. K. AGRAWAL, P. K. SETH, R. E. SQUIBB, H. A. TILSON
L. L. UPHOUSE AND S. C. BONDY

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

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AGRAWAL, A. K., P. K. SETH, R. E. SQUIBB, H. A. TILSON, L. L. UPHOUSE AND S. C. BONDY *Neurotransmitter receptors in brain regions of acrylamide-treated rats. I Effects of a single exposure to acrylamide* PHARMAC. BIOCHEM. BEHAV 14(4)527-531, 1981.—A single oral dose of acrylamide (25 or 50 mg/kg) increased the level of striatal ³H-spiroperidol binding in six week old male rats. This enhanced dopamine receptor activity was specific since treatment caused no significant changes in glycine, serotonin, and muscarinic cholinergic binding. At the highest acrylamide dose tested (100 mg/kg), elevations of the medullary glycine and frontal cortical serotonin receptors were also found. Pretreatment of animals with a blocker of hepatic mixed function oxidase (SKF 525a) or a thiol blocker (methylmercuric chloride) prevented the acrylamide-induced elevation of striatal spiroperidol binding, indicating that the causative agent was a secondary metabolite of acrylamide. Apomorphine-induced motility was significantly attenuated by 24 hr predosing with acrylamide, suggesting a change in the sensitivity of the dopamine receptor. The behavioral relevance of observed biochemical changes was thus shown by the altered response of treated animals to apomorphine.

Neurotransmitters Receptors Acrylamide Rat brain

ACRYLAMIDE has been shown to cause damage to the peripheral nervous system, in humans and experimental animals [11, 16, 18, 21]. Other signs of acrylamide intoxication such as tremor, pupillary dilatation, and excess salivation suggest hyperactivity of the catecholaminergic system [4]. More recently central nervous tissue has also been shown to be injured by systemically administered acrylamide [3, 18, 19]. In this laboratory, the striatal dopamine receptor of animals given a single dose of acrylamide has been shown to be increased in density and to have a heightened affinity for a labeled neuroleptic agent. These effects are greater following a two week dosing schedule containing 10 separate exposures to acrylamide [13].

The purpose of this work was to determine the specificity of the effect of a single oral dose acrylamide treatment upon the dopamine receptor. In addition, further information was sought concerning the nature of the active agent causing changes within the brain after exposure to acrylamide. These data indicate the dopamine receptors to be unusually susceptible to acrylamide exposure. The toxic effect appeared to be due to a metabolically formed derivative of acrylamide rather than directly to the unaltered molecule. Moreover,

acrylamide-treated rats were also found to be less sensitive to the behavioral effects of apomorphine, indicating that acrylamide might have altered the functional sensitivity of the dopamine receptor.

METHOD

Binding Assay

Six-week old male Fischer rats were used in this study. 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. This procedure combined with the prior freezing step causes major lysis of structural cell components such as mitochondria or nerve endings. The final pellet was suspended in the tris-HCl pH 7.4 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

¹Send reprint requests to: Dr. Stephen C. Bondy, Head, Neurochemistry Workgroup, Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709

TABLE 1
PHARMACOLOGICAL AGENTS USED IN CEREBRAL RECEPTOR STUDIES

Labeled Ligand	Specific activity (Ci/mmol)	conc (nM)	Unlabeled competitor	conc (μ M)	Receptor species assayed
[1-phenyl-4 3 H] sprioperidol	23	1 0	Haloperidol	1 0	Dopamine
DL-[benzyl-4,4'- 3 H] quinuclidinyl benzilate	29 4	1.0	Atropine	1 0	Acetylcholine
(methyl- 3 H)-diazepam	73	0 7	Diazepam	1 0	Benzodiazepine
[methylene- 3 H (N)]-muscimol	7 3	8 0	GABA	1 0	GABA
[G- 3 H]-strychnine sulfate	13	6 0	Strychnine	10 0	Glycine
[1.2 3 H (N)]-serotonin	29 8	3 1	Serotonin	1 0	Serotonin

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 5.7×10^{-3} M ascorbic acid. The amount of tissue used per tube corresponded to 5–10 mg original wet weight and contained 300–400 μ g protein as determined by the method of Lowry *et al.* [14]. 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 twice rapidly with 5 ml tris buffer. In the case of strychnine binding assay, only one wash was used. Filter discs were then dried and counted in 5 ml of a scintillation mixture (Aquasol, New England Nuclear, Boston, MA) using a scintillation counter (Packard Tri Carb 2660) at an efficiency of 38–43%.

Table 1 presents the final concentrations of radioactive ligands used, the receptor class that they were intended to assay for, and the final concentrations of competing compounds that were present in control incubations in order to determine the extent of non-specific binding. Control incubations were carried out simultaneously with the experimental series containing unlabeled competing ligand. The level of non-specific binding to tissue was between 5% and 29% of total binding. The method used was essentially similar to other filtration binding methods [22]. 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 [15]. The accepted level of significance in all cases was $p < 0.05$ using a two-tailed test. There was always experimental variance between various groups of control animals tested in different weeks. For this reason all treated and control rats that were to be compared were maintained simultaneously and membrane preparations and binding studies were always conducted in parallel. Each data point represents values derived from 6–8 individual animals.

Drug and Acrylamide Administration

Methylmercuric chloride (CH_3HgCl) was injected intraperitoneally (14 mg/kg) daily for three days preceding

acrylamide (100 mg/kg body weight) administration. 2-diethylaminoethyl-2, 2-diphenylvalerate HCl (SKF 525A), was injected twice intraperitoneally (50 mg/kg) at 10 a.m. and 4 p.m. on the day before acrylamide dosing of 100 mg/kg body weight. Acrylamide dissolved in water was administered orally by gavage in a volume of 5 ml/kg body weight. Control rats received an equivalent volume of distilled water. Animals were killed 24 hr after administration of acrylamide in all cases.

Apomorphine Challenge

Thirty male Fischer rats were randomly assigned to receive either 0, 25, or 100 mg/kg of acrylamide by gavage in a volume of 5 ml/kg body weight. Twenty-four hr later, the rats were put individually into plastic domiciliary cages having 0.5 inches of corn cob bedding and a wire screen lid; they were then placed inside light and sound attenuating chambers containing activity monitors (Automex, Columbus Instruments, Columbus, Ohio). The animals were allowed to acclimate for 10 min and then injected IP with 1 mg/kg of apomorphine hydrochloride dissolved in isotonic saline. Apomorphine-induced motility was measured for a period of 1 hr.

In a second experiment, 20 rats received either 0 or 100 mg/kg of acrylamide by gavage and were challenged with 1 mg/kg of apomorphine as described in the previous experiment. In the present study, the animals were also assessed for apomorphine-induced stereotypy using a rating scale similar to that of Reinstein *et al.* [17]; activity was also measured by the automated activity monitors. Behavioral observations were recorded at 1 min intervals by an observer unaware of pretreatment of the animals (i.e., acrylamide or vehicle pretreatment). Behavioral ratings of forward-crawling, paddling, wall climbing, forward-sniffing, and rearing were combined for a quantitative measure of "Activity". "Inactivity" was defined as sitting, lying, displaying odd posture, and sitting-sniffing. A third category, "sniffing", consisted of ratings of forward and sitting-sniffing.

The total number of counts accumulated by the automex monitor were square root transformed [12] and were tested for statistical significance using a one-way analysis of variance, post hoc comparisons between groups were made

TABLE 2
EFFECT OF 24 HOUR EXPOSURE TO ACRYLAMIDE ON
CEREBRAL RECEPTORS

Receptor	Region	Dose (mg/kg)			
		0	25	50	100
Dopamine	Striatum	334±13	413±11*	417±11*	481±32*
Muscarinic	Striatum	527±26	490±25	479±21	549±33
Cholinergic					
Benzodiazepine	Frontal	76± 6	79± 6	63± 5	80± 4
	Cortex				
GABA	Cerebellum	640±64	768±48	576±48	544±32
Glycine	Medulla	558±96	636±24	654±42	690±35*
5HT	Frontal	66± 5	72± 5	70± 4	84± 7*
	Cortex				

Binding expressed as pmol/g protein±S E

*Differs from zero dose ($p < 0.05$, Fisher's Least Significant Difference Test) Experimental details are given in the text.

using Fisher's Least Significant Difference Test [15]. In the case where there were only two treatment groups, Student's *t*-test was used to test for differences between means [15]. All tests were two-tailed and $p < 0.05$ was set as the accepted level of significance.

The number of movements rated as "activity", "inactivity", or "sniffing" were accumulated for the 60 min observation period. Differences between treatment groups were tested for statistical significance using two-tailed Mann-Whitney U-tests [20]. Correlation between activity counts measured by the automex with behavioral categories of "activity", "inactivity", and "sniffing" were determined using Spearman's Rank correlation coefficient [20].

RESULTS AND DISCUSSION

An increase in ^3H -spiroperidol binding within the corpus striatum of treated animals was found 24 hr after administration of acrylamide at all dose levels studied. However, at the two lower doses, the intensity of binding of several other labeled ligands was not significantly changed (Table 2). The lack of an effect upon the striatal muscarinic receptor showed that it was the dopamine binding site rather than the entire striatum that was initially the most sensitive to the treatment. This selectivity was lost at the highest dose used (100 mg/kg). In this latter case, increased glycinergic and serotonergic binding were also apparent. These data suggest that the dopaminergic circuitry may be unusually susceptible to disruption by acrylamide treatment.

Twenty-four hr after the administration of acrylamide, the number of activity counts generated during the 10 min acclimation was not affected significantly (Fig. 1). However, pretreatment with acrylamide significantly affected the number of apomorphine-induced activity counts (Fig. 1). Animals receiving 25 or 100 mg/kg acrylamide had significantly fewer counts than those receiving distilled water. The animals receiving the higher dose of acrylamide also differed significantly from the animals receiving 25 mg/kg.

In the second experiment in which a rating scale was used, acrylamide pretreatment was also found to antagonize the effects of apomorphine (Fig. 2). The number of counts

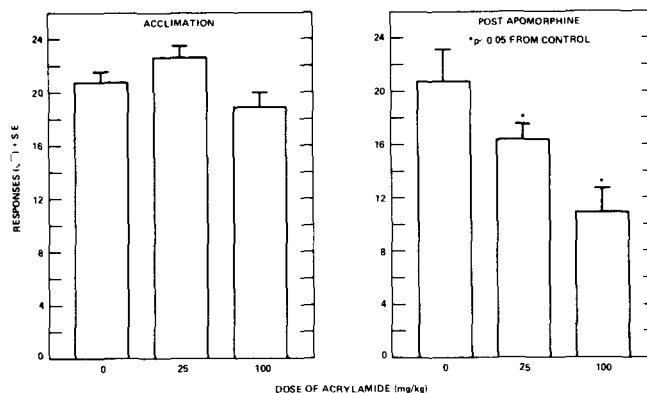


FIG. 1. The effects of acrylamide pretreatment on motor activity 10 min before injection (acclimation) and for 1 hr after injection of 1 mg/kg apomorphine. Data are average counts (square root transformed) \pm SE for 10 rats per group. Overall significance was assessed by a one-way analysis of variance. Asterisks indicate significant difference from control (0 mg/kg) (Fisher's Least Significant Difference Test, $p < 0.05$).

rated as "activity" or "sniffing" were significantly decreased by acrylamide pretreatment, while the number of ratings in the "inactivity" category were significantly increased by acrylamide. The number of activity counts measured by the automex was positively correlated with ratings of "activity" ($r = .572$, $p < 0.05$) and "sniffing" ($r = .482$, $p < 0.05$), but was inversely correlated with "inactivity" ($r = -.594$, $p < 0.05$).

The elevation of dopamine receptors in conjunction with a reduced behavioral response to a dopaminergic agonist can best be explained in terms of destruction of or damage to the dopamine neurons of the nigrostriatal pathways. This would reduce the magnitude of stereotypic induction by apomorphine and also explain the receptor increase in terms of a

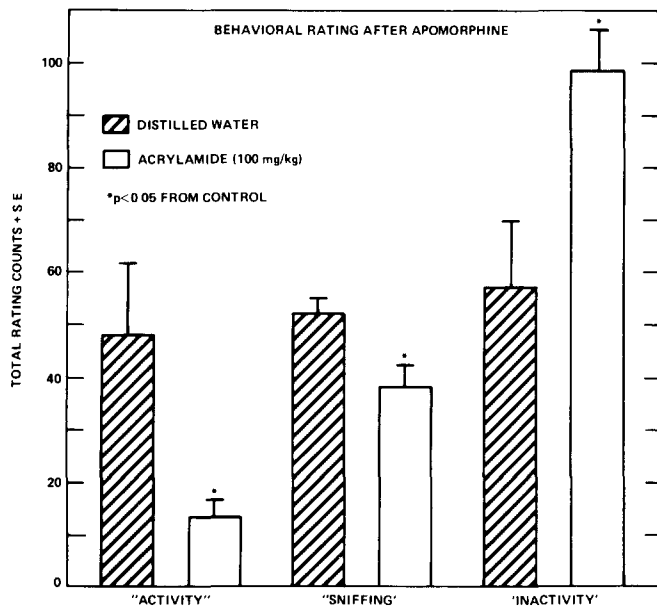


FIG. 2 The effects of acrylamide on apomorphine-induced motility as measured by a rating scale described in the text. Data are average counts \pm SE taken over a 60 min observation period. There were 10 animals per group. Asterisks indicate a significant difference from control (Mann-Whitney U-test, two-tailed, $p < 0.05$)

denervation supersensitivity of the postsynaptic cell. The correlation of transmitter receptor binding data with behavioral changes should be viewed with caution, since some active neuroleptics such as disulfuride bind poorly to the dopamine receptor while certain psychogenically inactive butyrophenones compete strongly with ^3H -spiroperidol binding [13].

Another study was directed toward ascertaining whether it was acrylamide, one of its metabolites or a conjugate involving interaction with sulfhydryl groups that was the causative agent in effecting the observed changes. This was carried out by pretreatment of rats with an inhibitor of hepatic induction of mixed function oxidases (SKF 525A) or an agent reacting with thiol groups (methylmercuric chloride). The acrylamide-induced changes of striatal dopaminergic receptors were completely prevented by either SKF 525A or methylmercuric chloride. However, neither agent had, by itself, any significant effect upon the dopamine receptor (Table 3). The results with SKF 525A suggested that the effect of acrylamide on the striatum was not direct but involved the production of an active agent from acrylamide by catabolic enzymes. The results with the methylmercuric

TABLE 3
EFFECT OF PRETREATMENT WITH SKF 525A OR METHYLMERCURIC CHLORIDE UPON THE ACRYLAMIDE-INDUCED ELEVATION OF STRIATAL SPIROPERIDOL BINDING

	^3H -spiroperidol bound \pm S.E (pmol/g protein)
SKF 525A series	
Control	364 \pm 23
Acrylamide	453 \pm 27*
SKF 525A, then acrylamide	351 \pm 26
SKF 525A	379 \pm 20
Methylmercuric chloride series	
Control	246 \pm 12
Acrylamide	303 \pm 12*
Methylmercuric chloride then acrylamide	255 \pm 14
Methylmercuric chloride	244 \pm 9

*Differs from control ($p < 0.05$, Fisher's Least Significant Difference Test). The acrylamide dose level was 100 mg/kg body weight. Experimental details are given in the text.

chloride might be due to blockage of interaction of acrylamide with certain sulfhydryl residues [7]. It may be that the effect of acrylamide upon the dopamine receptor induces reaction of key sulfhydryl residues with acrylamide or an acrylamide-metabolite. This is in contrast with reports that SKF 525A pretreatment worsens the peripheral effects of and the lethality of acrylamide [9]. However, phenobarbital induction of hepatic enzymes does not alter the rate of onset of acrylamide induced peripheral neuropathy in hens [8]. Thus, acrylamide may act directly as a toxic substance but some of its effects may also be attributed to metabolic breakdown products. The lack of effect of 10^{-5} M acrylamide *in vitro* upon the striatal binding of ^3H -spiroperidol [Agrawal, A.K., unpublished result] substantiates this idea. The relation between the neurotoxicity of acrylamide and its capacity to link to -SH residues remains unclear since N(hydroxymethyl) acrylamide which apparently does not injure the peripheral nervous system, is as avid as acrylamide in attaching itself to -SH groups [9]. However, the effects of this derivative upon the central nervous system have not been determined. The nature of the chemicals produced by acrylamide catabolism is presently being studied.

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