The Effects of Acrylamide Treatment upon the Dopamine Receptor

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The Effects of Acrylamide Treatment upon the Dopamine Receptor. AGRAWAL, A. K., SQUIBB, R. E., AND BONDY, S. C. (1981). Toxicol. Appl. Pharmacol. 58, 89–99. The binding of tritiated spiroperidol to striatal membranes prepared from acrylamide-treated male 6-week-old rats and matched controls has been studied. This binding has high-affinity characteristics and is stereospecific and reversible. Equilibrium was attained within 15 min at 37°C. The extent of binding was much more pronounced in the striatum than in any other brain region and was not detectable within the cerebellum or spinal cord. Regional distribution of binding and competition studies with other pharmacologic agents suggested a correspondence between the location of ligand–membrane complex formation and the dopamine receptor. Twenty-four hours after a single oral administration of acrylamide there was a significant increase in [3H]spiroperidol binding at all acrylamide doses tested (50, 100, and 200 mg/kg body weight). However, there was no significant change in striatal dopamine levels of treated animals. Kinetic analysis of animals treated with 100 mg acrylamide/kg suggested increased affinity of receptors of treated animals toward the labeled ligand. Receptor density was only slightly elevated in experimental animals. Effects were also studied in rats that had received 10, 20, or 30 mg/kg acrylamide daily for 10 days. Twenty-four hours after the last dose there was a major increase in spiroperidol binding in treated animals. Scatchard plot analysis again revealed that this change was largely attributable to a change in the dissociation constant of the binding interaction but also there was an increase in the overall number of receptor sites. Normal values were restored within 8 days after cessation of dosing. This illustrates that, under the conditions of this experiment, the effect of acrylamide on a central neurotransmitter system is reversible.

Acrylamide is a widely used industrial material and cases of poisoning resulting from exposure to this agent have been reported (Spencer and Schaumburg, 1974). This chemical appears to damage nerve tissue selectively and has been known for some time to cause peripheral neuropathies (Fullerton and Barnes, 1966; Pleasure et al., 1970; Schaumberg et al., 1974). More recently central nervous system involvement in acrylamide poisoning has also been reported in experimental animals (Ghetti et al., 1973; Gipon et al., 1977; Schotman et al., 1978; Schaumburg and Spencer, 1978). Acrylamide poisoning leading to distinct central nervous system behavioral deficits and encephalopathy has also been reported (Fujita et al., 1961; Igisu et al., 1975).

Many of the peripheral signs of acrylamide poisoning appear to involve excess activity of the sympathetic nervous system (Auld and Bedwell, 1967). These include pupil dilation, excess salivation, distension of the urinary bladder, and tremor (Fullerton and Barnes, 1966; Thoman et al., 1974). In this study we have examined the effect of acrylamide on a central catecholamine system involved in motor control; the striatal dopaminergic pathways. This system was found to be sensitive to both single
and repeated administrations of acrylamide and the evoked neurochemical changes appeared to be reversible.

METHODS

Six-week-old male Sprague–Dawley and Fischer rats were used in this study. 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. The doses of acrylamide used in the single administration studies were 50, 100, and 200 mg/kg body weight. Repeated administration studies utilized doses of 10, 20, and 30 mg/kg body weight, administered daily for 10 days. After decapitation, brain regions were dissected by the method of Iversen and Glowinski (1966). The spinal cord was then inserted in the caudal end of the isolated segment, brain regions were sliced by the method of Jacobowitz and Richardson (1978). This involved the preparation of a fluorescent derivative by oxidation. Dopamine was assayed by the method of Jacobowitz and Richardson (1978). This involved the preparation of a fluorescent derivative by oxidation. Dopamine concentration was measured relative to a set of known standards with an emission wavelength of 385 nm using an excitation wavelength of 320 nm.

The effects of the single exposure to acrylamide on striatal dopamine content and binding of $[$H$]$spiroperidol and the effects of repeated exposure to acrylamide on body weights, striatal wet weights, and striatal dopamine contents and binding of $[$H$]$spiroperidol binding at 24 and 168 hr postdosing was assessed for statistical significance using a one-way analysis of variance. Differences between treatment groups were assessed using Fisher’s least significant difference test. 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 simultaneously maintained and membrane preparations and binding studies were always conducted at the same time. Since handling alone may alter striatal dopamine binding capacity (Corda et al., 1980), care was taken to ensure equal handling of all rats. The Scatchard plots presented are representative and each series was carried out on three separate occasions. The magnitude of acrylamide effects was very similar in each comparison.

| Competing ligand     | pmol spiroperidol bound/100 mg protein |
|----------------------|---------------------------------------|
| None                 | 22.9 ± 1.6                            |
| $10^{-6}$ M Haloperidol | 5.1 ± 0.3                             |
| $10^{-6}$ M Ergocryptine    | 20.3 ± 1.5                            |
| $10^{-6}$ M Alprenolol      | 23.2 ± 5.3                            |

* Incubation was at 37°C for 15 min. Standard errors of the mean are given. Further details are presented in the text.
RESULTS

a. Binding Characteristics

The interaction between striatal membranes and [3H]spiroperidol was over 75% specific (Table I) and was temperature dependent (Fig. 1a). Binding reached maximal values within 15 min at 37°C and could be reversed by subsequent addition of 10⁻⁶ M haloperidol (Agrawal and Bondy, 1980). The amount of membrane preparation used
Fib. 2. Regional distribution of specific spiroperidol binding sites within rat brain. Membranes were prepared from 6-week-old male Sprague–Dawley rats and incubated for 15 min at 37°C. Standard errors are shown.

b. The Effect of Short-Term Single Dose Exposure to Acrylamide

Acrylamide was administered to 6-week-old male Sprague–Dawley rats (n = 8 rats per group) and striatal tissue was dissected out 24 hr later. There was a significant increase in [3H]spiroperidol binding [F(3,24) x 32.38; p < 0.05] at all doses of acrylamide used relative to control values (Fig. 3). Detailed kinetic analysis was then carried out on animals treated with the nonlethal dose of 100 mg/kg. The binding of increasing amounts of spiroperidol was plotted by the method of Scatchard (1949). While there is much evidence for multiple classes of dopamine receptor (Kebabian and Calne, 1979; Thal et al., 1978) restriction of spiroperidol concentrations to 10−8 M or less allowed the determination of high-affinity binding sites with little interference by lower affinity sites. The affinity of binding of receptors from treated animals was 0.58 × 10−9 M, considerably greater than the control Kd value of 0.74 × 10−9 M (Fig. 4). Rats dosed with acrylamide also exhibited an in-
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crease in the receptor site density (48 pmol bound/100 mg protein). The striatal dopamine content of acrylamide-treated rats was not significantly higher than control values. However, acrylamide-treated animals showed a clear trend toward increased levels of dopamine within the striatum (Table 2).

c. The Effect of Repeated Treatment with Acrylamide

Six-week-old male Fischer rats \( (n = 16 \) per group) were treated with various amounts of acrylamide administered daily for 10 days by gavage. Twenty-four hours and 8 days after the last dose, eight animals from each group were killed and the striatal tissue dissected out. All doses of acrylamide used caused a major rise in the specific binding of \( [\text{H}] \)spiroperidol to striatal membranes 24 hr after dosing. However, within 8 days of cessation of acrylamide dosing, normal values were obtained (Fig. 5). The body weights of animals dosed at all levels tested were temporarily reduced \( [F(3,60) = 8.83; p < 0.01] \) (Table 3). Striatal wet weight also appeared reversibly reduced in treated animals (Table 3). This unexpected effect was detected by post hoc study of results. The dissection had been previously carried out "blind," i.e., without knowledge of which were control and which were treated animals. Since protein concentration in the striatum was unaffected by acrylamide treatment at all concentrations reported here, this effect could not clearly be attributed to dehydration. However, it is known that acrylamide-

FIG. 3. Effect of a single acrylamide treatment upon the striatal binding of \( [\text{H}] \)spiroperidol. Assays were carried out 24 hr after administration of acrylamide to 6-week-old male Sprague–Dawley rats. Bars indicate standard error. Eight rats were used in each treatment group. * Differs significantly from control value (Fisher's least significant difference test, \( p < 0.05 \)).
Scatchard analysis of the group treated with the lowest dose of acrylamide (10 mg/kg) showed that major changes were present in both the dissociation constant which was significantly decreased in dosed animals (from $0.99 \times 10^{-9}$ to $0.70 \times 10^{-9}$ M) and in the receptor site density which was increased in experimental animals (from 32.9 to 40.9 pmoles/100 mg protein) (Fig. 6).

**DISCUSSION**

Alterations in the striatal dopamine receptor in response to pharmacological agents or surgical procedures have been previously reported (Creese et al., 1977; Muller and Seeman, 1977; Rosengarten and Friedhoff, 1979; Schwartz et al., 1978). These changes generally involve modula-

**TABLE 2**

| Acrylamide dose (mg/kg body weight) | Dopamine (ng/gm wet tissue) |
|------------------------------------|-----------------------------|
| 0                                  | 4623 $\pm$ 423              |
| 50                                 | 5687 $\pm$ 310              |
| 100                                | 6062 $\pm$ 420              |
| 200                                | 5624 $\pm$ 658              |

* Standard errors of the mean are given.
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Fig. 5. Effect of repeated treatment with acrylamide upon the striatal binding of spiroperidol. Assays were performed 24 hr or 8 days after completion of a course of 10 doses of acrylamide over a 2-week period to 6-week-old male Fischer rats. Eight rats were used in each group. Standard errors are given. * Differs significantly from corresponding value for untreated rats (Fisher's least significant difference test, $p < 0.05$). Solid bars, 24 hr after completion of treatment; hatched bars, 8 days after completion of treatment.

The possibility exists that our observations can be explained in terms of a shift in the proportions of the varying receptor species that can bind spiroperidol. At least three classes of dopamine receptor appear to exist with relatively high-affinity binding characteristics (Tye et al., 1977; Thal et al., 1978; Kebabian and Calne, 1979; Titeler...
TABLE 3
WEIGHTS OF RATS AND THEIR STRIATA 24 HR OR 7 DAYS AFTER REPEATED ACRYLAMIDE DOSINGa

| Acrylamide dose (mg/kg body weight) | Body weight (gm) | Striatal wet weight (mg) |
|-----------------------------------|------------------|-------------------------|
| 24 hr after last dose             |                  |                         |
| 0                                 | 200 ± 2          | 79 ± 3                  |
| 10                                | 194 ± 2a         | 66 ± 3b                 |
| 20                                | 188 ± 2a         | 68 ± 3b                 |
| 30                                | 187 ± 2a         | 71 ± 2b                 |
| 7 days after last dose            |                  |                         |
| 0                                 | 216 ± 4          | 82 ± 3                  |
| 10                                | 222 ± 2          | 80 ± 2                  |
| 20                                | 219 ± 1          | 76 ± 5                  |
| 30                                | 219 ± 1          | 79 ± 4                  |

a The dosing regimen is described in the text and in Fig. 6. Eight animals were used in each group.

b Differs significantly from the corresponding value for untreated animals (Fisher's least significant difference test, p < 0.05).

et al., 1980). Increased information concerning heterogeneity of receptor classes and specificity of the binding reaction complicates interpretation of shifting binding profiles. However, the elevation of striatal dopamine binding ability occurs consequent to acrylamide treatment.

Since our membrane preparations are lysed and washed, it is unlikely that our results are attributable to the presence of free acrylamide. It is conceivable that acrylamide may modify the receptor by covalently binding to proteins. However, we have found that acrylamide, when added directly to incubation tubes at a concentration of 1×10⁻⁵ M, had no effect upon spiroperidol binding (unpublished result). Thus, the effects that we are reporting are more likely to be caused secondarily in response to changed activity of the dopamine system.

The rather rapid response of spiroperidol binding sites to acrylamide is not surprising since receptors can be modified within hours after physiological perturbation (Paul and Skolnick, 1978).

While no significant difference was found in the striatal level of dopamine of acrylamide-treated rats, these animals tended to

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**Fig. 6.** Scatchard analysis of striatal binding of [3H]spiroperidol to membranes prepared from rats 24 hr after completion of a course of 10 doses of acrylamide (10 mg/kg body weight each time) over a 2-week period, to 6-week-old male Fischer rats. Data are from eight animals in each group. ●, Experimental rats; ○, control rats. Curve derived from linear regression analysis.
have higher dopamine levels than controls. A larger number of animals might have revealed a significant difference. Such an increase concurrent with increased dopamine binding may reflect a relatively quiescent dopamine system with relatively low rates of dopamine turnover. At present we are conducting determinations of levels of striatal dihydroxyphenylacetic acid in treated animals in order to estimate rates of dopamine catabolism.

In several respects, the effect of acrylamide upon the striatal dopamine receptor resembles the effect of neuroleptic agents (Creese et al., 1978). Thus, the adult response to haloperidol or to acrylamide is to increase striatal binding capacity toward dopamine antagonists. However, if either haloperidol (Rosengarten and Friedhoff, 1979) or acrylamide (Agrawal and Squibb, in preparation) is administered prenatally, a reduction of striatal dopamine binding sites can occur in the offspring.

Recently, the occurrence and reversibility of behavioral and morphological changes associated with single and repeated administration of acrylamide to rats has been reported (Tilson et al., 1979; Tilson and Cabe, 1979). In the repeated dosing studies, the morphological changes in PNS and CNS tissue samples and the functional deficits had, by 5 weeks after cessation of dosing, completely reversed. The selection of the doses used in the present report was based on their studies.

The data from the current studies suggest that dopaminergic neurons may be especially vulnerable to acrylamide. This specificity has been substantiated by a series of studies analogous to those reported here but measuring six neurotransmitter or neuromodulator sites within the brain (Agrawal et al., 1980; Bondy et al., 1980). It is known that the dopamine system is especially sensitive to a variety of neurotoxic agents including manganese (Goldman, 1972) and lead compounds (Bondy et al., 1979). It is important to delineate further the selectivity of the effects reported here by assay of receptors for other neurotransmitters in acrylamide-treated animals.

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