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Journal
Toxicology and Applied Pharmacology, 58(1)

ISSN
0041-008X

Authors
Agrawal, AK
Squibb, RE
Bondy, SC

Publication Date
1981-03-01

DOI
10.1016/0041-008x(81)90119-8

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TOXICOLOGY AND APPLIED PHARMACOLOGY 58, 89–99 (1981)

The Effects of Acrylamide Treatment upon the Dopamine Receptor

A. K. AGRAWAL, R. E. SQUIBB, AND S. C. BONDY

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

Received May 5, 1980; accepted August 5, 1980

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 [^{3}H]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

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TABLE 1

EFFECT OF α- AND β-ADRENERGIC ANTAGONISTS AND HALOPERIDOL UPON [^{3}H]SPIROPERIDOL BINDING TO STRIATAL MEMBRANES[a]

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

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

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 removed by sectioning the spine at the lumbar and upper cervical regions. An 18-gauge hypodermic needle was then inserted in the caudal end of the isolated segment and the cord expressed by injection of 0–32 M sucrose. 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 (50,000g, 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 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, appropriate pharmacological agents and [1-phenyl-4-^{3}H]spiroperidol (25.6 Ci/mmol) at a final concentration of 1.0 nm. In the case of Scatchard plots, spiroperidol concentrations were varied between 10^{-10} and 10^{-8} M. Spiroperidol binds to the dopamine receptor site with very high affinity and specificity (Burt *et al.*, 1976). The amount of tissue used per tube corresponded to 5 mg original wet weight and contained 300–400 μg protein as determined by the method of Lowry *et al.* (1951). Divalent metal ions have been used in some dopamine receptor studies (Creese *et al.*, 1978) but not in others (Fields *et al.*, 1977; Howlett and Nahorski, 1979). We omitted ions since the presence of 10^{-4} M Ca^{2+}, or $1-2 \times 10^{-2}$ M Na^{2+} did not enhance our binding values. At the end of incubation, samples were filtered on glass fiber discs (25 mm diameter, 0.3 μm pore size, Gelman Inc., Ann Arbor, MI) and washed twice rapidly with 5 ml Tris buffer. Filter discs were then dried and counted in a 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%. Specific binding was taken to consist of the [^{3}H]-spiroperidol binding that could be displaced by 10^{-6} M haloperidol, a structural analog of spiroperidol. These control incubations were carried out in triplicate simultaneously with the experimental series containing no haloperidol. Haloperidol was added to these tubes prior to addition of [^{3}H]spiroperidol. The level of nonspecific [^{3}H]-spiroperidol binding to striatal tissue was always below 23% of total binding. This method is essentially similar to other filtration-binding methods (Yamamura *et al.*, 1978). However, we felt it necessary to establish basal binding characteristics prior to studies on toxicant-treated animals.

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 [^{3}H]spiroperidol and the effects of repeated exposure to acrylamide on body weights, striatal wet weights, and striatal binding of spiroperidol 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.

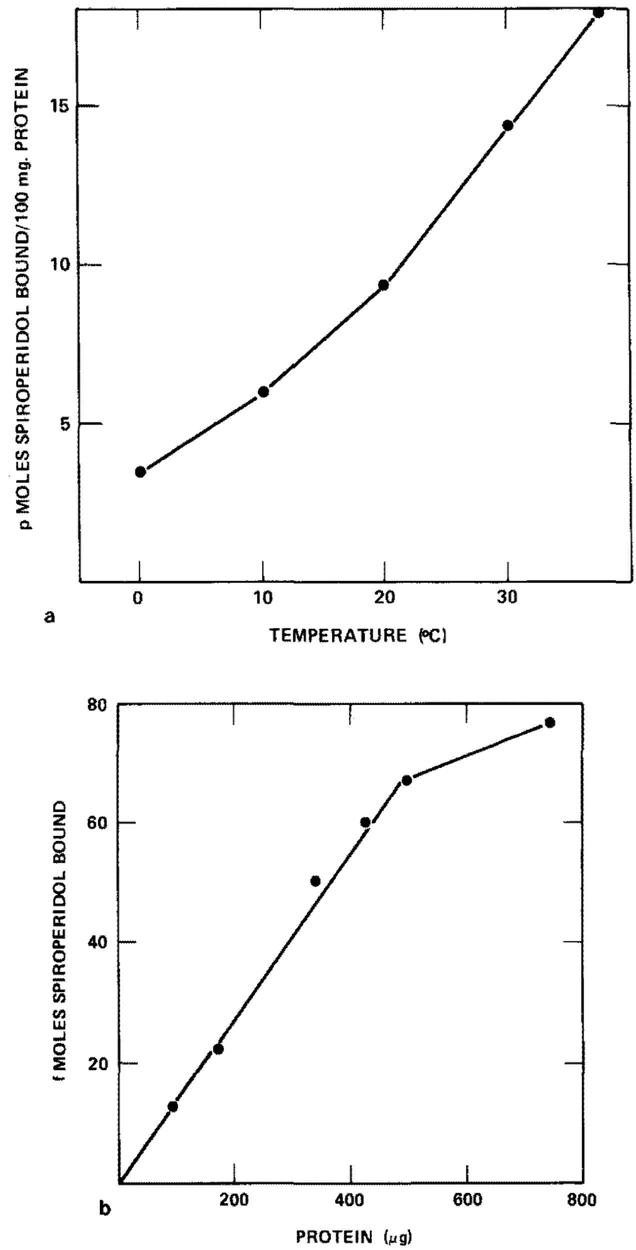

FIG. 1. (a) Effect of temperature upon specific binding of [^{3}H]spiroperidol to rat striatal membranes. Incubation was for 15 min. Each point represents the mean of three determinations. (b) Effect of varying membrane concentration upon the specific binding of spiroperidol to striatal receptor sites. Incubation was for 15 min at 37°C. Each point represents the mean of three determinations.

RESULTS

a. Binding Characteristics

The interaction between striatal membranes and [^{3}H]spiroperidol was over 75% specific (Table 1) 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

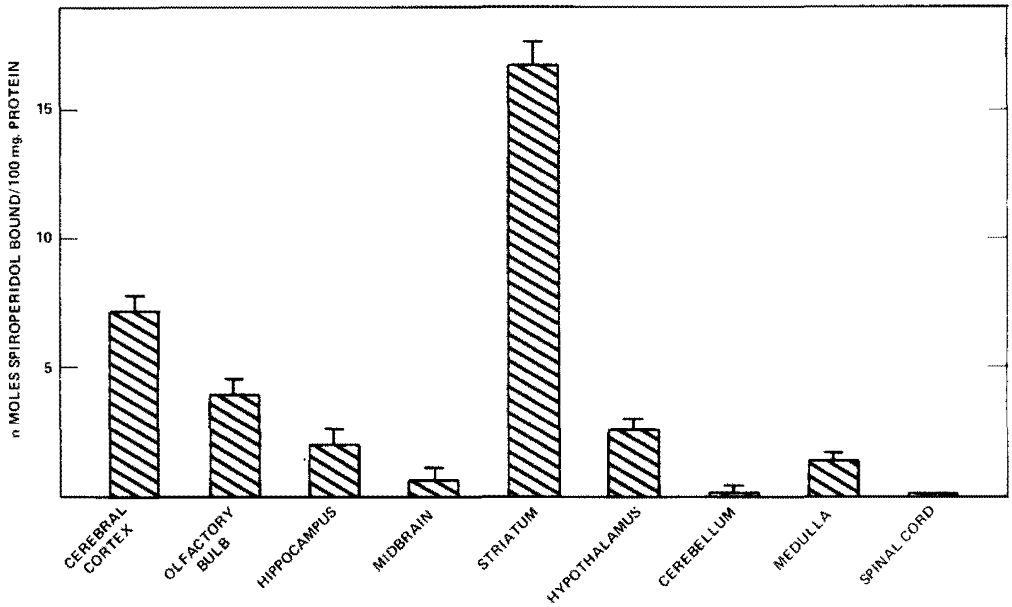

FIG. 2. Regional distribution of specific spiroperidol binding sites within rat brain. Membranes were prepared from 6-week-old male Sprague–Dawley rats and incubated for 15 min at 37°C. Standard errors are shown.

(300–400 μg protein) was limiting under our incubation conditions at 37°C (Fig. 1b). The regional distribution of activity was appropriate for that expected for the dopamine receptor in that it was highest in the striatum (Fig. 2). This specificity was further substantiated by the small effect of α- and β-adrenergic antagonists upon [^{3}H]spiroperidol binding (Table 1). The differential inhibitory effect of *d*- and *l*-butaclamol confirmed that binding was stereospecific (Bondy and Agrawal, 1980). Therefore, under our conditions, binding characteristics fullfilled several criteria suggesting that we were assaying the dopamine receptor. The striatal binding site density in untreated rats (32–42 pmol/100 mg protein) was in good agreement with results obtained from other workers (Burt *et al.*, 1976; Fields *et al.*, 1977; Thal *et al.*, 1979). Two additional washes of the striatal membranes did not further increase the binding of [^{3}H]spiroperidol. Thus, our preparative procedure had effectively removed any endogenous ligand or inhibitory soluble protein (Lahti *et al.*, 1978).

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 [^{3}H]spiroperidol binding [$F(3,24) \times 32.38$; $p < 0.05$] 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 K_D value of 0.74×10^{-9} M (Fig. 4). Rats dosed with acrylamide also exhibited an in-

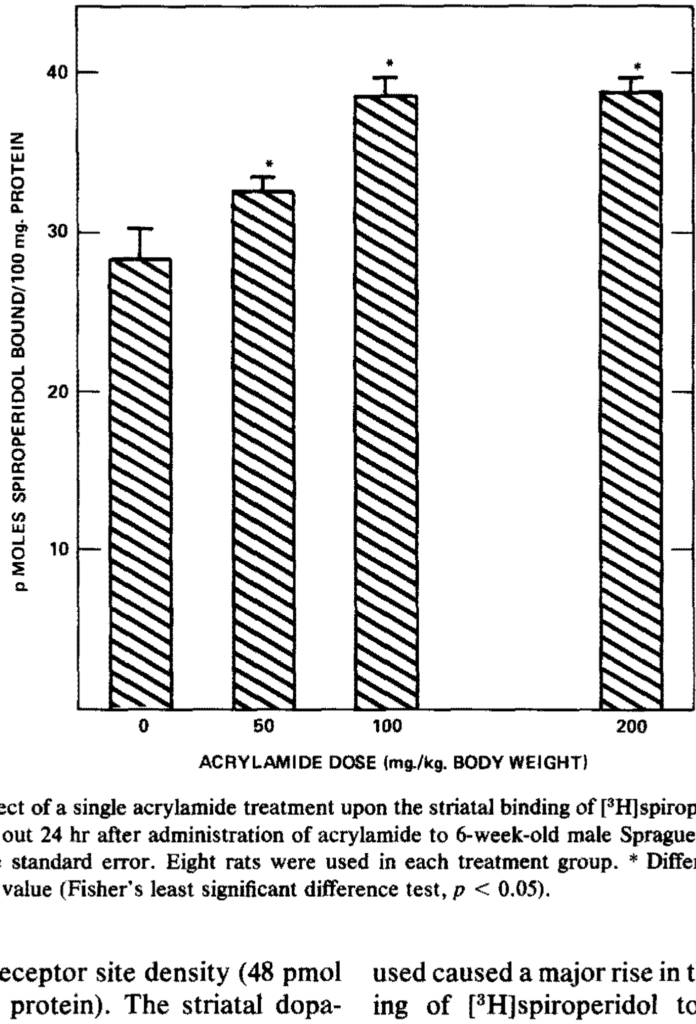

FIG. 3. Effect of a single acrylamide treatment upon the striatal binding of [^{3}H]spiroperidol. Assays were carried out 24 hr after administration of acrylamide to 6-week-old male Sprague-Dawley rats. Bars indicate standard error. Eight rats were used in each treatment group. * Differs significantly from control value (Fisher's least significant difference test, $p < 0.05$).

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 [^{3}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-

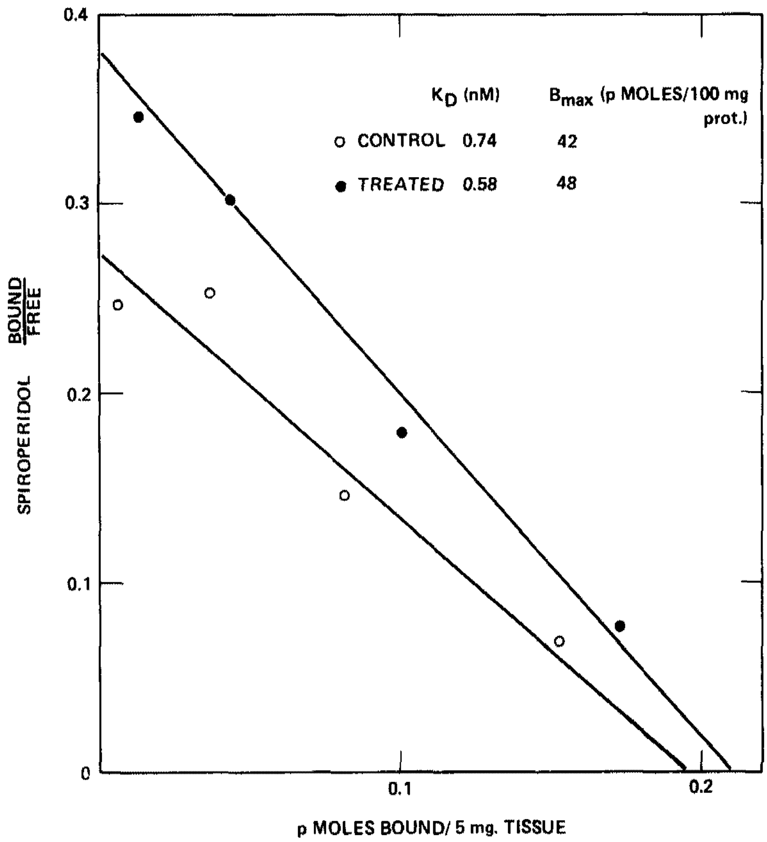

FIG. 4. Scatchard analysis of striatal binding of [^{3}H]spiroperidol to membranes prepared from rats 24 hr after administration of a single dose of acrylamide (100 mg/kg body weight). Data are from eight animals in each group. ●, Experimental rats; ○, control rats. Curve derived from linear regression analysis.

treated rats are polydipsic suggesting that a certain water inbalance may exist in these animals (Gipon *et al.*, 1977; Squibb and Tilson, unpublished observation).

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 0.99×10^{-9} to 0.70×10^{-9} M) and in the receptor site density which was increased in experimental animals (from 32.9 to 40.9 pmol/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

STRIATAL DOPAMINE CONTENT OF SPRAGUE–DAWLEY RATS 24 hr AFTER TREATMENT WITH A SINGLE DOSE OF ACRYLAMIDE[a]

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

[a] Standard errors of the mean are given.

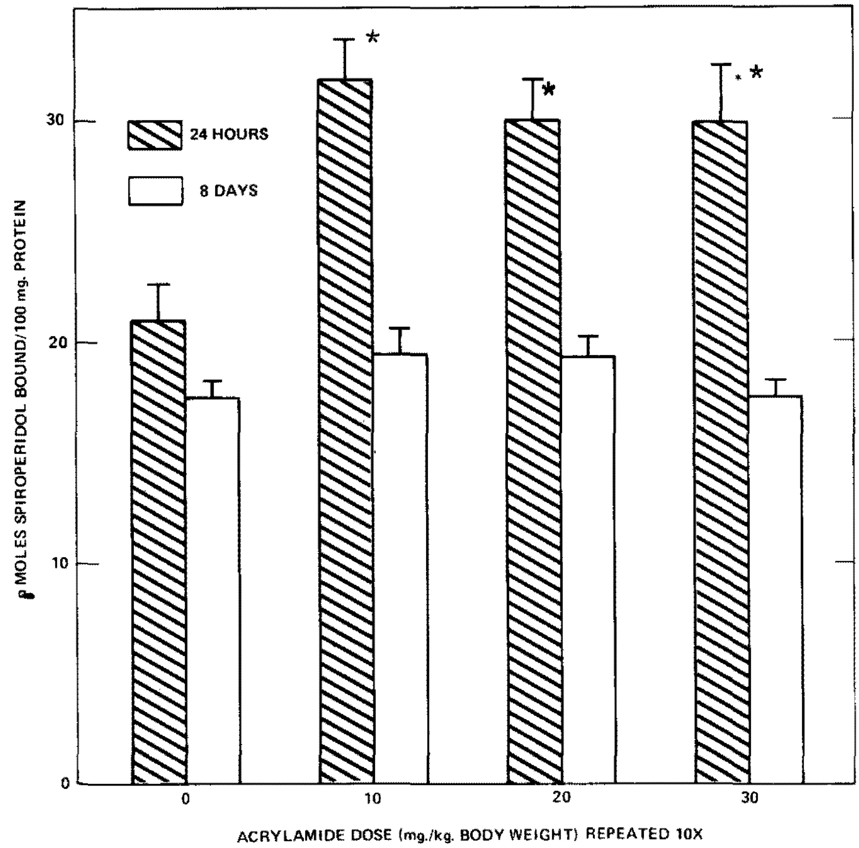

FIG. 5. Effect of repeated treatment with acrylamide upon the striatal binding of spiroperidol. Assays were performed 24 hr or 8 days after completion of a course of 10 doses of acrylamide over a 2-week period to 6-week-old male Fischer rats. Eight rats were used in each group. Standard errors are given. * 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.

tion of the total receptor number present while binding affinity is unaltered. This change in the density of binding proteins is a characteristic response, leading to denervation supersensitivity or hyperactivation subsensitivity in several neurotransmitter systems (Rosenberg and Chiu, 1979; Waddington and Cross, 1978; Crews and Smith, 1978; Schiller, 1979). Our results from acrylamide exposures are unusual in that the physical nature of the receptor as expressed by its dissociation constant is modified by the experimental treatment. Lesser changes in the amount of receptor present also occur. A somewhat parallel situation exists when *p*-chlorophenylalanine is administered to rats (Steigrad *et al.*, 1978). Initially, in the acute situation, the affinity of receptors for serotonin was increased, while after repeated administration of the drug, the number of receptor sites also increased. Also chronic *l*-DOPA therapy has been reported to increase both site density and receptor affinity (Wilner *et al.*, 1980).

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 DOSING[a]

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 ± 2[b]	66 ± 3[b]
20	188 ± 2[b]	68 ± 3[b]
30	187 ± 2[b]	71 ± 2[b]
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. Standard errors of the mean are given.

[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 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

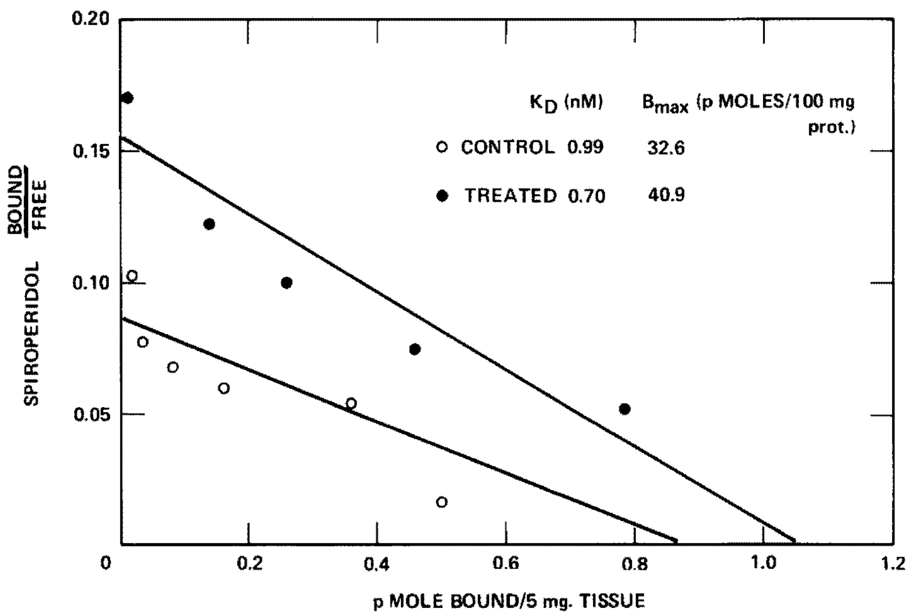

FIG. 6. Scatchard analysis of striatal binding of [^{3}H]spiroperidol to membranes prepared from rats 24 hr after completion of a course of 10 doses of acrylamide (10 mg/kg body weight each time) over a 2-week period, to 6-week-old male Fischer rats. Data are from eight animals in each group. ●, Experimental 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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