MOLECULAR PHARMACOLOGY, 19:372–378

Binding of [^{3}H]Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene to Rat Striatal Membranes

Effects of Purine Nucleotides and Ultraviolet Irradiation

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Received September 15, 1980; Accepted December 30, 1980

SUMMARY

ZAHNISER, N. R., K. A. HEIDENREICH, AND P. B. MOLINOFF. Binding of [^{3}H]amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene to rat striatal membranes: effects of purine nucleotides and ultraviolet irradiation. *Mol. Pharmacol.* 19:372–378 (1981).

The binding of the rigid dopamine analogue [^{3}H]amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthalene ([^{3}H]ADTN) to rat striatal membranes was characterized in the presence and absence of purine nucleotides. At 37°, when assays were carried out in the presence of ATP or GTP, the binding of [^{3}H]ADTN increased for approximately 5 min and then progressively declined. However, when assays were carried out at 20°, [^{3}H]ADTN binding reached equilibrium and was stable in both the absence and presence of purine nucleotides. Scatchard analysis of binding isotherms showed that the affinity of the binding sites for [^{3}H]ADTN decreased by approximately 3-fold in the presence of either 0.3 mM ATP or GTP. An unexpected finding was that the density of binding sites increased by approximately 5-fold. In contrast, the nonhydrolyzable purine nucleotide analogues guanylylimidodiphosphate and adenylylimidodiphosphate did not affect the binding of [^{3}H]ADTN. The pharmacological specificity of [^{3}H]ADTN binding, determined either in the absence or presence of ATP or GTP, was characteristic of binding to dopamine receptors in that dopamine, epinine, and (±)-ADTN were more potent than (−)-norepinephrine or (−)-epinephrine in inhibiting [^{3}H]ADTN binding, and binding was inhibited by a variety of neuroleptics including (+)-butaclamol and (α)-flupenthixol. However, there were effects of purine nucleotides on the affinities of the receptor for the partial agonist apomorphine and for a variety of antagonists. In the presence of nucleotides, antagonists were 2 to 10 times less potent in inhibiting [^{3}H]ADTN binding, whereas apomorphine was 300-fold less potent. Exposure of striatal membranes to UV irradiation (λ_{max} = 310 nm) for 30 min reduced [^{3}H]ADTN binding observed in the absence of nucleotide by 60% and eliminated the increase in binding observed in the presence of nucleotide. Neither [^{3}H]spiroperidol binding nor dopamine- or ADTN-stimulated adenylate cyclase activity was affected by this treatment. The results suggest that, in the presence of ATP or GTP, [^{3}H]ADTN binds to a second class of binding sites in the striatum. These additional sites (B_{max} ~ 20 pmoles/mg of protein) are not associated with either [^{3}H]spiroperidol binding or dopamine-stimulated adenylate cyclase activity.

INTRODUCTION

The rigid dopamine analogue ADTN[2] is a potent agonist at striatal dopamine receptors. ADTN (Fig. 1) is equipotent with dopamine in stimulating striatal adenylate cyclase activity (1) and in inhibiting the firing of striatal neurons (2). When injected intrastriatally into rats, ADTN produces mild stereotypy but does not affect locomotor activity (3). Recently [^{3}H]ADTN of high spe-

This research was supported by United States Public Health Service Grant NS 15756.

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[2] The abbreviations used are: ADTN, amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; Gpp(NH)p, guanylylimidodiphosphate; App(NH)p, adenylylimidodiphosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid.

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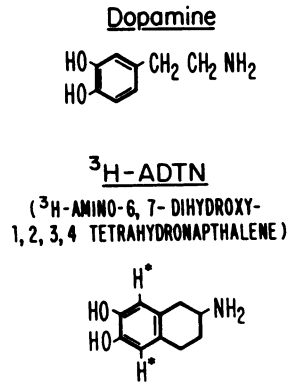

FIG. 1. *Structures of dopamine and [^{3}H]ADTN*

cific activity has become commercially available and has been used to label striatal dopamine receptors (4, 5).

It has been suggested that dopamine receptors, including those in the striatum, can be classified into two groups according to whether or not their effects are mediated by activation of adenylate cyclase (6). These two putative classes of dopamine receptors can be distinguished both biochemically and pharmacologically. Dopamine is a more potent agonist at receptors which are not coupled to adenylate cyclase (D-2 receptors) than at those which are coupled (D-1 receptors; see ref. 6). Apomorphine and ergot alkaloids are full agonists at D-2 receptors but partial agonists or antagonists at D-1 receptors (ref. 1; see ref. 6). In the striatum, neither the presynaptic dopamine receptors on terminals of corticostriatal neurons nor those on terminals of nigrostriatal neurons appear to act through changes in adenylate cyclase activity (7). In contrast, activation of postsynaptic dopamine receptors on striatal perikaria does increase adenylate cyclase activity (8). It has been suggested that the binding of [^{3}H]ADTN in the striatum is confined almost entirely to postsynaptic sites, which are thought to be linked to adenylate cyclase (9). This conclusion was based on the observation that [^{3}H]ADTN binding was not detected following the administration of ibotenic acid, which destroys neuronal perikaria. In the same study, neither 6-hydroxydopamine-induced lesions of the nigrostriatal pathway nor cortical ablations altered [^{3}H]ADTN binding (9). These results also suggest that the binding sites are not associated with nerve terminals. However, results from other experiments suggest that ADTN does bind to receptors on the terminals of corticostriatal, presumably glutamatergic, fibers. In these experiments ADTN was shown to be equipotent with dopamine in inhibiting K^+-evoked glutamate release from rat striatal slices (10).

A variety of techniques has been used in studies with a number of receptor systems to differentiate agonist from antagonist binding sites (11–15). For example, the affinity of striatal dopamine receptors for agonists, but not antagonists, is decreased in the presence of GTP or Gpp(NH)p. This effect is selective for guanine nucleotides, which are much more potent than adenine nucleotides (see 11). Another approach has been to alter selectively agonist or antagonist binding using physical methods such as temperature (14, 15). It has been reported that dopamine agonist binding sites in the striatum are selectively labile to increased temperature (15). In the current experiments the effects of various agents, including purine nucleotides, on [^{3}H]ADTN binding were examined. The effects of UV irradiation on the binding of [^{3}H]ADTN and [^{3}H]spiroperidol, as well as on the activity of dopamine-sensitive adenylate cyclase, were compared. In the presence of a maximally effective concentration of either GTP or ATP, the number of binding sites for [^{3}H]ADTN was increased by approximately 5-fold. These additional binding sites were destroyed by UV irradiation and did not appar to be associated with either [^{3}H]spiroperidol binding sites or with dopamine-stimulated adenylate cyclase activity.

MATERIALS AND METHODS

Materials. The sources of the drugs used were as follows: [1-*phenyl*-4-^{3}H]spiroperidol, [5,8-^{3}H]ADTN, and cyclic [^{3}H] AMP were obtained from New England Nuclear Corporation, Boston, Mass.; dopamine, epinine, (−)-norepinephrine, (−)-epinephrine, GTP, GDP, ATP, ADP, AMP, cyclic AMP, and adenosine from Sigma Chemical Company, St. Louis, Mo.; GTP, Gpp(NH)p, and App(NH)p from Boehringer Mannheim Biochemicals, Indianapolis, Ind.; (±)-ADTN from Burroughs Wellcome Company, Research Triangle Park, N. C.; apomorphine from Merck, Sharp and Dohme, Rahway, N. J.; (+)- and (−)-butaclamol from Ayerst Laboratories, New York, N. Y.; (α)- and (β)-flupenthixol from H. Lundbeck and Company A/S; spiroperidol from Janssen Pharmaceutica Inc., Beerse, Belgium; bromocriptine from Sandoz Pharmaceuticals, East Hanover, N. J.; and phentolamine from Ciba Pharmaceutical Company, Summit, N. J. All other chemicals used were reagent grade. [α-^{32}p]-ATP was prepared according to the method of Walseth and Johnson (16).

Binding assays. The number and characteristics of striatal dopamine receptors were determined using the antagonist [^{3}H]spiroperidol (17) and the agonist [^{3}H] ADTN (4). Striata from male Sprague-Dawley rats were homogenized in 300 volumes of 20 mM Hepes buffer (pH 7.5, adjusted with tetramethylammonium hydroxide at 25°) containing 1 mM $MnCl_2$ for 10 sec at speed 4.5 with a Brinkman Polytron. $MnCl_2$ was included since it increased the density of [^{3}H]ADTN binding sites 3-fold either in the absence or presence of nucleotide (see also ref. 4). Membrane pellets prepared by centrifugation at 20,000 × g for 10 min at 4° were washed by resuspension and centrifugation. Radioligands, drugs, and nucleotides were dissolved in 2.8 mM ascorbic acid containing BSA, 10 μg/ml. The final concentration of ascorbic acid (0.43 mM) did not affect the K_D values or the densities of binding sites for [^{3}H]spiroperidol or [^{3}H]ADTN. In control experiments the concentration of free [^{3}H]ADTN (>90%) was not altered by purine nucleotides in either the absence or presence of BSA. The concentration of [^{3}H]ADTN (25 Ci/mmole) ranged from 0.5–15 nM and the final concentration of [^{3}H]spiroperidol (26 Ci/mmole) ranged from 0.05–0.6 nM. Nonspecific binding of [^{3}H]-spiroperidol was defined in the presence of 2 μM (+)-

butaclamol at 37° and 30 μM (+)-butaclamol at 20°. Specific binding was 75–90% of total binding. For samples incubated with [^{3}H]ADTN at 37°, nonspecific binding was defined with 3 μM (+)-butaclamol; at 20° it was defined with 30 μM (+)-butaclamol. Specific binding of [^{3}H]ADTN constituted approximately 70% of total binding in the presence of nucleotides and 30% in the absence of nucleotides. The absolute amount of nonspecific binding (picomoles per milligram) was not affected by the presence or absence of nucleotides.

Assays were initiated by addition of 850-μl aliquots of resuspended striatal membranes (0.15–0.2 mg of protein) to 150 μl of ascorbate-BSA containing appropriate ligands and drugs. Samples were incubated for 20 min at 37° or for 45 min at 20°. Reactions were terminated by the addition of 10 ml of ice-cold 10 mM Tris buffer (pH 7.5) and samples were immediately filtered through glass-fiber fibers (Schleicher and Schuell, No. 30). Each filter was washed with an additional 10 ml of cold wash buffer. Radioactivity was determined by liquid scintillation spectrometry in 3 ml of a Triton X-100/toluene-based fluor. The counting efficiency for tritium was approximately 32%.

Protein concentrations were determined according to the method of Bradford (18) using BSA as a standard. The concentrations were approximately 40% lower than those determined using the method of Lowry *et al.* (19).

Adenylate cyclase activity. Adenylate cyclase activity was measured using the method of Salomon *et al.* (20) as modified by Minneman *et al.* (21). Assays contained 50 mM Hepes (pH 7.5, adjusted with NaOH at 25°); 1 mM $MgCl_2$; 0.25 mM ATP; 5 mM cyclic AMP; 1 mM theophylline; 10 mM creatine phosphate; creatine kinase 0.1 mg/ml; and 1–2 million cpm [α-^{32}P]ATP. Dopamine-sensitive or ADTN-sensitive adenylate cyclase activity was determined in the presence of 100 μM dopamine or (±)-ADTN. Reactions were initiated by the addition of tissue homogenates (1:30 in 2 mM Hepes containing 2 mM EGTA; 40 μg of protein per assay). The final volume of the reaction mixture was 0.1 ml. Samples were incubated at 30° for 10 min. Reactions were terminated by addition of 0.1 ml of 50 mM Tris-maleate (pH 7.5) containing 5 mM ATP and 10% sodium dodecyl sulfate. Samples were then placed in a boiling water bath for 10 min to solubilize proteins and minimize clogging of the columns. Cyclic [^{3}H]AMP (~5000 cpm) was added to each sample to monitor recovery during the column chromatographic isolation procedure. All results have been corrected for recovery (typically 70–75%).

Exposure to UV irradiation. Tissue was exposed to UV irradiation at room temperature prior to initiation of assays. For most of the experiments in which binding was determined, aliquots (1 ml) of the tissue suspension (1:300) were pipetted into plastic cluster trays and placed 14.5 cm below a 20-W fluorescent lamp (Westinghouse FS20) with an energy emission band of 228–350 nm (λ_{max} = 310 nM). The temperature of the samples during UV irradiation ranged from 22° to 29°. For experiments in which both adenylate cyclase activity and binding were to be determined, the tissue was exposed to UV irradiation as a more concentrated suspension (1:30). This necessitated lowering the fluorescent lamp to 12.5 cm above the samples. With respect to [^{3}H]ADTN binding, these conditions with the more concentrated tissue were found to produce results identical with those produced by the conditions used with the more diluted tissue.

RESULTS

The time course of [^{3}H]ADTN binding was examined in both the absence and presence of purine nucleotides (Fig. 2). In the absence of ATP (or GTP) at 37°, [^{3}H]ADTN binding equilibrated within the first 10 min and was stable for at least 45 min of incubation (Fig. 2). However, when samples were incubated at 37° in the presence of nucleotide, [^{3}H]ADTN binding to striatal membranes did not appear to reach a steady state (Fig. 2). Binding increased for approximately 5 min and then progressively declined. This effect was not specific for adenine nucleotides in that GTP was as effective as ATP in producing this effect (data not shown).

To try to stabilize [^{3}H]ADTN binding in the presence of nucleotides, experiments were carried out at 20°. At this lower temperature, binding reached equilibrium within 45 min in either the presence or absence of nucleotides (Fig. 2). A surprising result observed in experiments carried out at 20° was a very marked increase in the amount of [^{3}H]ADTN bound in the presence of ATP. Scatchard analysis (23) of binding isotherms performed in the absence and presence of 0.3 mM ATP showed that the affinity of rat striatal dopamine receptors for [^{3}H]ADTN decreased 3-fold in the presence of nucleotide (Fig. 3; Table 1). Under the same conditions the number of binding sites increased 5-fold (Table 1). Scatchard plots were linear in either the presence or absence of a purine nucleotide (Fig. 3). Similar results were obtained using concentrations of [^{3}H]ADTN as high as 30 nM;

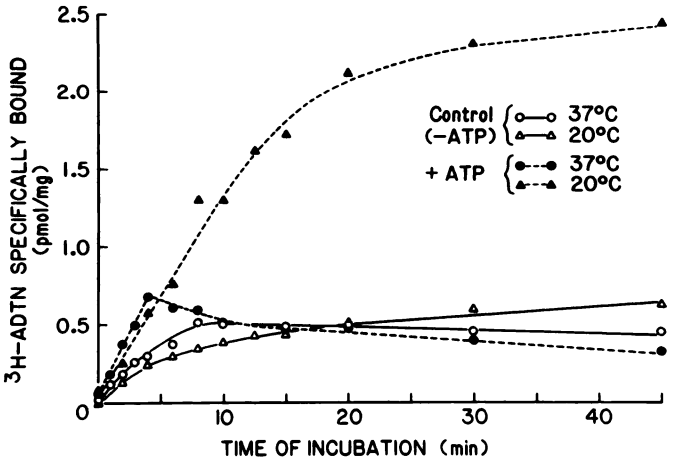

FIG. 2. *Effect of purine nucleotides on the association of [^{3}H]ADTN with rat striatal membranes at 37° and 20°*

Membranes were incubated in 20 mM Hepes (pH 7.5) containing 1 mM $MnCl_2$ with 5 nM [^{3}H]ADTN in the absence (○, △) and presence (●, ▲) of 0.3 mM ATP at 37° (○, ●) or 20° (△, ▲) for the times indicated. The data shown are mean values from three separate experiments (standard errors of the mean, not shown, were less than 15%). In the presence of ATP at 37°, the amounts of [^{3}H]ADTN specifically bound at 15, 20, 30, and 45 min of incubation were significantly lower ($p < 0.05$) than that at 4 min as determined by Dunnett's multiple-comparison test (22) following analysis of variance.

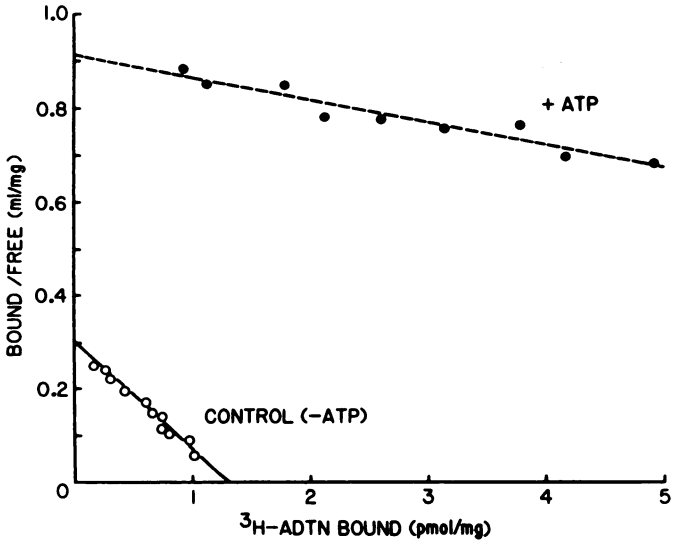

FIG. 3. *Scatchard analysis of [^{3}H]ADTN binding to rat striatal membranes at 20° in the absence and presence of 0.3 mM ATP*

Tissue was prepared in 20 mM Hepes (pH 7.5) containing 1 mM $MnCl_2$. Assays contained 0.5–10 nM [^{3}H]ADTN, and specific binding was defined using 30 μM (+)-butaclamol. The values shown are means (n = 3) at each concentration of [^{3}H]ADTN. The calculated affinities and densities of binding sites were as follows: control K_D value 4.5 nM, B_{max} 1.3 pmoles/mg of protein; plus ATP K_D value 23 nM, B_{max} 19 pmoles/mg of protein.

however, even in the presence of ATP, specific binding was poor (10–20%) at these high concentrations.

An increased number of binding sites was also observed in the presence of GTP. ATP and GTP had EC_{50} values of approximately 0.03 mM, and both nucleotides were maximally effective at concentrations of 0.3 mM. ADP and GDP were partially effective in producing an effect like that of the nucleotide triphosphates, but AMP, adenosine, and cyclic AMP were without effect. The nonhydrolyzable purine analogues Gpp(NH)p and App(NH)p did not substitute for GTP or ATP (data not shown). This finding suggests that a phosphorylation reaction or an energy-dependent process may be responsible for the marked increase in [^{3}H]ADTN binding observed at 20° in the presence of GTP or ATP. However, inclusion of 1 mM cyclic AMP to stimulate cyclic AMP-dependent protein kinase did not potentiate the [^{3}H]ADTN binding observed in the presence of ATP. Furthermore, the inhibitor of cyclic AMP-dependent protein kinase described by Walsh and colleagues (24) did not block the ATP-induced increase in [^{3}H]ADTN binding. Ouabain (0.1 mM) used to inhibit sodium-potassium-dependent ATPase activity did not alter the response to ATP.

The pharmacological specificity of [^{3}H]ADTN binding in either the absence or presence of nucleotide was characteristic of binding to dopamine receptors (Table 2). Dopamine was 3- to 5-fold more potent than either (−)-norepinephrine or (−)-epinephrine in inhibiting [^{3}H]ADTN binding. Although both (−)-norepinephrine and (−)-epinephrine were relatively potent inhibitors of [^{3}H]ADTN binding, the *alpha*-adrenergic receptor antagonist phentolamine did not inhibit [^{3}H]ADTN binding. This finding suggests that [^{3}H]ADTN was not labeling *alpha*-adrenergic receptors. [^{3}H]ADTN binding was not inhibited nonspecifically by catecholamines, since pyrocatechol was ineffective in blocking [^{3}H]ADTN binding. Concentrations of dopamine, (±)-ADTN, and (+)-butaclamol from 3 to 30 μM inhibited [^{3}H]ADTN binding to approximately the same extent (Fig. 4). At higher concentrations they appeared to inhibit nonspecific binding.

The addition of purine nucleotides, however, did result in changes in the potencies of dopamine receptor agonists and antagonists. Epinine and (±)-ADTN were 2 and 7 times, respectively, less potent in inhibiting [^{3}H]ADTN

TABLE 1

Effects of temperature and ATP on dopamine receptors

Membranes from homogenates of rat striatum were prepared in 20 mM Hepes (pH 7.5) containing 1 mM $MnCl_2$. Assays contained 0.5–15 nM [^{3}H]ADTN or 0.05–0.6 nM [^{3}H]spiroperidol. At 20°, samples were incubated in the absence or presence of 0.3 mM ATP. At 37° in the presence of ATP, [^{3}H]ADTN binding was unstable. The values shown are from Scatchard analyses and are the means ± standard error of the mean of three to six determinations.

	K_D value	B_{max}
	nM	*pmoles/mg*
37° Incubation		
[^{3}H]ADTN	4.0 ± 0.90	1.4 ± 0.21
[^{3}H]Spiroperidol	0.19 ± 0.01	1.2 ± 0.11
20° Incubation		
[^{3}H]ADTN	6.8 ± 1.1	3.4 ± 0.93
[^{3}H]ADTN + ATP	19 ± 1.7[a]	18 ± 2.9[a]
[^{3}H]Spiroperidol	0.67 ± 0.05	1.0 ± 0.05
[^{3}H]Spiroperidol + ATP	0.54 ± 0.05	1.1 ± 0.14

[a] $p < 0.05$ compared with control.

TABLE 2

Inhibition of [^{3}H]ADTN binding to rat striatal membranes in the absence and presence of ATP

Striatal membranes, homogenized in 20 mM Hepes containing 1 mM $MnCl_2$, were incubated with 3–5 nM [^{3}H]ADTN in the presence of various concentrations of the compounds listed below for 45 min at 20°. K_D values were calculated from IC_{50} values using the method of Cheng and Prusoff (25). K_D values for [^{3}H]ADTN used in these calculations were 5.7 nM in the absence of ATP and 19.3 nM in the presence of 0.3 mM ATP. The results presented are means ± standard error of the mean for three to seven determinations.

Compound	K_D values	
	−ATP	+ATP
Dopamine	29 ± 6.1	18 ± 1.5
(±)-ADTN	6.6 ± 2.9	49 ± 8.4
Epinine	29 ± 5.1	56 ± 8.1
Apomorphine	19 ± 8.8	5,600 ± 1,300
l-Norepinephrine	82 ± 23	48 ± 7.6
l-Epinephrine	150 ± 29	53 ± 19
Pyrocatechol	>100,000	>100,000
Spiroperidol	6.8 ± 0.93	93 ± 16
(+)-Butaclamol	41 ± 23	420 ± 180
(−)-Butaclamol	>30,000	4,300 ± 910
(*α*)-Flupenthixol	81 ± 38	450 ± 130
(*β*)-Flupenthixol	600 ± 110	850 ± 80
Bromocriptine	3,600 ± 530	9,500 ± 3,700
Phentolamine	1,900 ± 900	9,700 ± 2,000

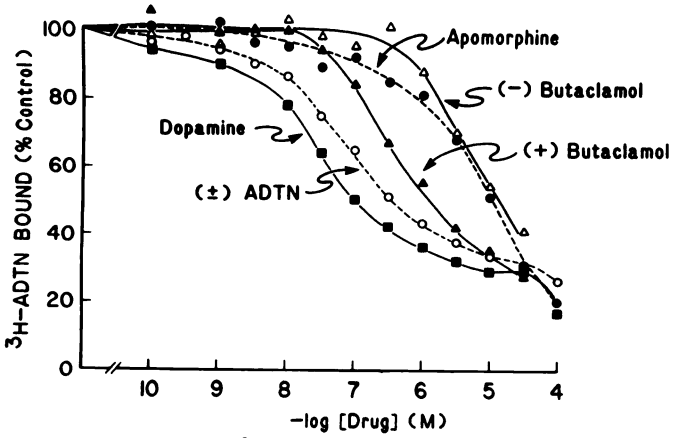

FIG. 4. *Inhibition of [^{3}H]ADTN binding to rat striatal membranes at 20° in the presence of ATP by dopamine receptor agonists and antagonists*

Tissue was homogenized in 20 mM Hepes (pH 7.5) containing 1 mM $MnCl_2$. Assays were carried out in the presence of 0.3 mM ATP. Results are expressed as percentage of [^{3}H]ADTN bound in the absence of competing drug and are the means of four to six independent determinations.

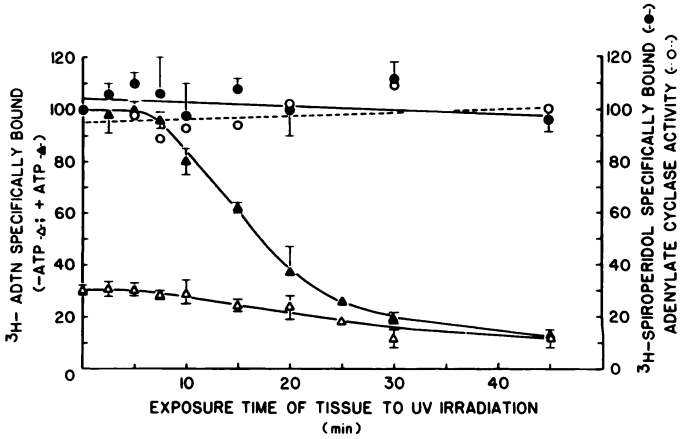

FIG. 5. *Binding of [^{3}H]ADTN and [^{3}H]spiroperidol and dopamine-stimulated adenylate cyclase activity following exposure of striatal membranes to UV irradiation*

For binding experiments, 1-ml aliquots of striatal homogenates in 20 mM Hepes (pH 7.5) containing 1 mM $MnCl_2$ were exposed to UV irradiation (λ_{max} = 310 nm) for the times indicated. Control tissue not exposed to UV irradiation was kept at room temperature for equivalent periods of time. After exposure of the tissue to UV irradiation, samples were incubated with 5 nM [^{3}H]ADTN in the absence (△——△) or presence (▲——▲) of 0.3 nM ATP at 20° or with 0.6 nM [^{3}H]spiroperidol in the presence (●——●) of 0.3 mM ATP. The results shown are expressed as the percentage of radioligand specifically bound in the presence of 0.3 mM ATP in tissue not exposed to UV irradiation. The values are the means ± standard error of the mean for three independent determinations. For adenylate cyclase determinations, rat striatal homogenates were exposed to UV irradiation at room temperature for the times indicated. Control tissue was kept at room temperature for equivalent periods of time. Tissue samples were then assayed for adenylate cyclase activity in the absence or presence of 100 μM dopamine as described under Materials and Methods. Basal adenylate cyclase activity was 40 pmoles of cyclic AMP/min/mg of protein, and a 2-fold stimulation resulted from the presence of dopamine. The results shown (○- - -○) are expressed as the percentage of dopamine-stimulated activity in tissue that had not been exposed to UV irradiation. Values are the means of two to four determinations from two separate experiments.

binding in the presence of ATP, whereas dopamine was 2-fold more potent (Table 2). The most marked difference in potency was observed in studies with the partial agonist apomorphine, which was 300-fold less potent when ATP was present than in its absence. Dopamine receptor antagonists also appeared to be less potent (6- to 14-fold) in inhibiting [^{3}H]ADTN binding in the presence of ATP. The ratios of the K_D values for (+)- and (−)-butaclamol and (α)- and (β)-flupenthixol were decreased in the presence of ATP (Table 2).

A variety of physical and chemical manipulations has been used in studies of a number of receptor systems to differentiate agonist from antagonist binding sites (11–15). In the current experiments, striatal homogenates were exposed to UV irradiation to try to differentiate classes of dopamine receptors in the striatum. Tissue homogenates were exposed to UV irradiation prior to initiating normal binding assays. Exposure of the tissue to UV irradiation resulted in a time-dependent decrease in [^{3}H]ADTN binding measured in either the absence or presence of ATP (Fig. 5). The effect of ATP on [^{3}H]ADTN binding was completely abolished by 30 min of UV irradiation. The affinity for [^{3}H]ADTN and the density of binding sites which remained after 30 min of UV irradiation in the assays containing ATP were similar to those determined in assays carried out in the absence of ATP (Table 3). In contrast, [^{3}H]spiroperidol binding either in the absence or presence of ATP was stable in tissue exposed to UV irradiation (Fig. 5; Table 3). Basal, dopamine-stimulated (Fig. 5), or (±)-ADTN-stimulated (data not shown) adenylate cyclase activity was not affected by exposure of the tissue to UV irradiation for up to 45 min.

DISCUSSION

Guanine nucleotides have been reported to decrease the amount of [^{3}H]ADTN binding in striatum (4, 5, 26). This effect has been ascribed to a GTP-induced decrease in the affinity of the receptor for [^{3}H]ADTN without a change in the number of sites. However, a potential problem with these experiments is that the samples were incubated for 10 min at 37°. The results reported here (Fig. 2) show that, at 37° in the presence of either GTP or ATP, the binding of [^{3}H]ADTN is unstable. Thus, the amount of [^{3}H]ADTN binding measured in the presence of a purine nucleotide would depend on the time of incubation at 37°.

In the absence of added nucleotide, equilibrium binding of [^{3}H]ADTN to washed striatal membranes resulted in linear Scatchard plots. This result is consistent with binding to a single class of sites. On the other hand, binding to a second site with an affinity similar to that of the first site would not result in detectably curved Scatchard plots. Hill plots (27) are also used to analyze equilibrium binding data with regard to the possible existence of multiple binding sites. Low Hill coefficients are used as an indicator of multiple sites or cooperativity. In the absence of purine nucleotides, accurate determination of Hill coefficients was not possible since specific

TABLE 3

Effect of UV irradiation on the affinity and density of striatal dopamine receptors

Membranes from homogenates of rat striatum were prepared in 20 mM Hepes (pH 7.5) containing 1 mM $MnCl_2$. Aliquots of tissue (1 ml) were exposed to UV irradiation (λ_{max} = 310 nm) for 30 min at room temperature. Samples not exposed to UV irradiation were kept at room temperature for 30 min. Incubations with either 0.5–15 nM [^{3}H]ADTN or 0.05–0.6 nM [^{3}H]spiroperidol in the absence or presence of 0.3 mM ATP were carried out for 45 min at 20°. Values shown are the means ± standard error of the mean for four to twelve determinations.

	K_D value	B_{max}
	nM	*pmoles/mg*
[^{3}H]ADTN		
Control	5.3 ± 0.43	2.9 ± 0.33
Control + UV	2.7 ± 0.84	1.2 ± 0.31[a]
ATP	19 ± 3.5	30 ± 6.5
ATP + UV	5.1 ± 1.2[a]	2.6 ± 0.83[a]
[^{3}H]Spiroperidol		
ATP	0.58 ± 0.43	1.1 ± 0.06
ATP + UV	0.52 ± 0.08	0.94 ± 0.14

[a] $p < 0.05$ when compared with tissue not exposed to UV irradiation.

binding was only 30% of total binding. Thus, it has not been clearly established whether [^{3}H]ADTN binds only to a single site in the absence of purine nucleotides.

In the presence of ATP or GTP, a marked increase in the density of [^{3}H]ADTN binding sites was observed. These findings differ from previously published reports which described specific guanine nucleotide effects on receptors (see ref. 11). Approximately 5 times more binding sites were detected in the presence of either GTP or ATP than in their absence. The physiological significance of the increased number of [^{3}H]ADTN binding sites observed in the presence of nucleotide is unknown. It is interesting to note that dopamine was the most potent compound tested in terms of competing with [^{3}H]ADTN for these binding sites. However, the pharmacological profile of the nucleotide induced sites was different from that observed in the absence of GTP or ATP (Table 2). The affinity of the nucleotide-dependent site for [^{3}H]ADTN was lower than that determined in the absence of nucleotide. Thus, under equilibrium conditions in the presence of nucleotides, [^{3}H]ADTN should bind to the sites characterized in both the absence and presence of nucleotides. Scatchard plots were still apparently linear. Analysis of simulated data showed that the differences in the number and the affinity of the binding sites observed in the absence and presence of ATP and GTP were not large enough to produce curvilinear Scatchard plots. On the other hand, Hill coefficients for both agonist and antagonist inhibition of [^{3}H]ADTN binding (4 nM) were less than 1, suggesting binding to more than one site. The low Hill coefficients were apparent despite linear Scatchard plots, because at the low concentrations of ligand used for displacement studies a larger proportion of the total binding was associated with the site having a higher affinity. The observed differences in the pharmacological profiles and the ratios of the activities of the active and inactive isomers of butaclamol and flupenthixol (Table 2) also support the hypothesis that [^{3}H]ADTN binds to a pharmacologically distinct site in the presence of purine nucleotides. This same ability of ATP to increase the density of [^{3}H]ADTN binding sites has not been observed in anterior pituitary or retina, other tissues that are thought to contain dopamine receptors.[3]

It has recently been proposed, on the basis of studies of specific lesions, that [^{3}H]ADTN binding in the striatum is entirely to postsynaptic binding sites (9). However, another report suggests that ADTN binds to receptors on terminals of corticostriatal neurons as well as to postsynaptic receptors (10). The fact that all of the nucleotide-induced and up to 60% of the nonnucleotide-associated [^{3}H]ADTN binding sites were lost in our studies using UV irradiation without any loss in dopamine-stimulated adenylate cyclase activity (Fig. 5) suggests that the majority of the [^{3}H]ADTN binding sites are not coupled to this enzyme. It is not clear whether or not the [^{3}H]ADTN binding sites which remain following UV irradiation are coupled to dopamine-sensitive adenylate cyclase. Some ADTN binding must be coupled to adenylate cyclase, however, since ADTN and dopamine are equipotent in stimulating adenylate cyclase (1). Furthermore, ADTN-stimulated adenylate cyclase activity remains in tissue exposed to UV irradiation.

Evidence has been presented that [^{3}H]spiroperidol binds to both presynaptic and postsynaptic receptors (7, 28–30). Since ADTN inhibits [^{3}H]spiroperidol binding (17), it is likely that ADTN and spiroperidol label some common receptor sites. Under equilibrium conditions in the absence of purine nucleotides, [^{3}H]spiroperidol and [^{3}H]ADTN appear to bind to approximately the same number of sites (Table 1). However, it has not been established that these ligands bind to identical sets of sites in the striatum. In the presence of purine nucleotides there is little doubt that [^{3}H]spiroperidol and [^{3}H]ADTN bind to different sites. In the first place, [^{3}H]ADTN binds to many more sites than does [^{3}H]spiroperidol. Second, the pharmacological profiles of the sites labeled by [^{3}H]ADTN when nucleotide was added appear to be different from those labeled by [^{3}H]spiroperidol. For example, the ratios of the affinities of active isomers as compared with inactive isomers of (+)- and (−)-butaclamol and (α)- and (β)-flupenthixol derived from inhibition of [^{3}H]spiroperidol binding were much greater than those derived from inhibition of [^{3}H]ADTN binding (ref. 17, Table 2). The additional binding sites labelled by [^{3}H]ADTN when assays were carried out in the presence of ATP were completely labile to UV irradiation. UV irradiation of the tissue did not affect [^{3}H]spiroperidol binding in the absence or presence of ATP or the activity of dopamine-stimulated adenylate cyclase. The binding of [^{3}H]dopamine, but not [^{3}H]spiroperidol, can be decreased by 80–90% following exposure of striatal membranes to 53° for 2 min (15). The current results are consistent with this finding but cannot be explained by thermal lability, since the temperature of the exposed membranes never exceeded 29° during the irradiation procedure. It has recently been reported that UV irradiation resulted in a selective loss of saxitoxin binding sites in rat synapto-

[3] R. Huff, personal communication.

somes with the rate of loss being independent of temperature (31). Whether the loss in binding sites during UV irradiation involves a direct modification of a moiety in the binding site or perturbation in the tertiary or quaternary structure of the molecule is not known.

The results of the present study provide evidence that, in the presence of purine nucleotides, [^{3}H]ADTN binds to a site in the rat striatum which differs from [^{3}H]ADTN binding sites detected in the absence of nucleotides and which is not associated with either [^{3}H]spiroperidol binding or with dopamine-sensitive adenylate cyclase. The fact that neither Gpp(NH)p or App(NH)p affected [^{3}H]ADTN binding suggests that GTP and ATP are being used in a phosphorylation reaction that exposes an additional binding site for [^{3}H]ADTN. If the mechanism involved is mediated via phosphorylation, it is unlikely that it is a cyclic AMP-dependent phosphorylation. Neither cyclic AMP nor the Walsh inhibitor (24) had any effect on the ATP-induced increase in [^{3}H]ADTN binding sites. Furthermore, the phosphorylation reaction would have to occur under conditions used in this study, that is, in the presence of manganese and GTP. Clement-Cormier (32) has recently reported a dopamine-dependent increase in phosphorylation of a 50,0000 mol wt protein solubilized from striatal membranes. This phosphorylation also appears to involve a cyclic AMP-independent protein kinase. The possible mechanisms by which the number of striatal binding sites for [^{3}H]ADTN is increased in the presence of purine nucleotides remain to be investigated.

ACKNOWLEDGMENTS

We thank Ms. Candace Collamer for excellent secretarial assistance and Drs. Barry Wolfe, Ken Minneman, Rita Huff, Rick Rabin, and Greg Weiland for their helpful discussions. Dr. James Maller generously donated the Walsh inhibitor.

REFERENCES

1. Miller, R., A. Horn, and L. Iversen. Effects of dopamine-like drugs on rat striatal adenyl cyclase have implications for CNS dopamine receptor topography. *Nature* (*Lond.*) **250**:238–241 (1974).
2. Woodruff, G. N., A. O. Elkhawad, A. R. Crossman, and R. J. Walker. Further evidence for the stimulation of rat brain dopamine receptors by a cyclic analogue of dopamine. *J. Pharm. Pharmacol.* **26**:740–741 (1974).
3. Elkhawad, A. O., and G. N. Woodruff. Studies on the behavioral pharmacology of a cyclic analogue of dopamine following its injection into the brains of conscious rats. *Br. J. Pharmacol.* **54**:107–114 (1975).
4. Creese, I., and S. H. Snyder. Dopamine receptor binding of ^{3}H-ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene) regulated by guanyl nucleotides. *Eur. J. Pharmacol.* **50**:459–461 (1978).
5. Creese, I., T. Prosser, and S. H. Snyder. Dopamine receptor binding: specificity, localization and regulation by ions and guanyl nucleotides. *Life Sci.* **23**: 495–500 (1978).
6. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature* (*Lond.*) **277**:93–96 (1979).
7. Schwarz, R., I. Creese, J. T. Coyle, and S. H. Snyder. Dopamine receptors localised on cerebral cortical afferents to rat corpus striatum. *Nature* (*Lond.*) **271**:766–768 (1978).
8. Kebabian, J. W., G. L. Petzold, and P. Greengard. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similaritv to the "dopamine receptor." *Proc. Natl. Acad. Sci.* (*U. S. A.*) **69**:2145–2149 (1972).
9. Fuxe, K., H. Hall, and C. Köhler. Evidence for an exclusive localization of ^{3}H-ADTN binding sites to postsynaptic nerve cells in the striatum of the rat. *Eur. J. Pharmacol.* **58**:515–517 (1979).
10. Rowlands, G. J., and P. J. Roberts. Activation of dopamine receptors inhibits calcium-dependent glutamate release from cortico-striatal terminals in vitro. *Eur. J. Pharmacol.* **62**:241–242 (1980).
11. Rodbell, M. The role hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* (*Lond.*) **284**:17–22 (1980).
12. Pert, C. B., and S. H. Snyder. Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol. Pharmacol.* **10**:868–879 (1974).
13. Stadel, J. M., and R. J. Lefkowitz. Multiple reactive sulfhydryl groups modulate the function of adenylate cyclase coupled beta-adrenergic receptors. *Mol. Pharmacol.* **16**:709–718 (1979).
14. Weiland, G. A., K. P. Minneman, and P. B. Molinoff. Fundamental difference between the molecular interactions of agonist and antagonists with the β-adrenergic receptor. *Nature* (*Lond.*) **281**:114–117 (1979).
15. Lew, J. Y., and M. Goldstein. Dopamine receptor binding for agonists and antagonists in thermal exposed membranes. *Eur. J. Pharmacol.* **55**:429–430 (1979).
16. Walseth, T. F., and R. A. Johnson. The enzymatic preparation of (α-^{32}P)nucleoside triphosphates, cyclic (^{32}P)AMP, and cyclic (^{32}P)GMP. *Biochim. Biophys. Acta* **562**:11–31 (1979).
17. Zahniser, N. R., and P. B. Molinoff. Effect of guanine nucleotides on striatal dopamine receptors. *Nature* (*Lond.*) **275**:453–455 (1978).
18. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
20. Salomon, Y., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541–548 (1974).
21. Minneman, K. P., L. R. Hegstrand, and P. B. Molinoff. The pharmacological specificity of *beta*-1 and *beta*-2 adrenergic receptors in rat heart and lung *in vitro*. *Mol. Pharmacol.* **16**:21–33 (1979).
22. Dunnett, C. W. A multiple comparison procedure for comparing several treatments with a control. *J. Am. Statist. Assoc.* **50**:1096–1121 (1955).
23. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
24. Walsh, D. A., C. D. Ashby, C. Gonzalez, D. Calkins, E. H. Fischer, and E. G. Krebs. Purification and characterization of a protein inhibitor of adenosine 3′,5′-monophosphate-dependent protein kinases. *J. Biol. Chem.* **246**:1977–1985 (1971).
25. Cheng, Y.-C., and W. H. Prusoff. Relationship between the inhibition of constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
26. Creese, I., T. B. Usdin, and S. H. Snyder. Dopamine receptor binding regulated by guanine nucleotides. *Mol. Pharmacol.* **16**:69–76 (1979).
27. Hill, A. V. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J. Physiol.* (*Lond.*) **40**:iv–vii (1910).
28. Schwarz, R., and J. T. Coyle. Striatal lesions with kainic acid: neurochemical characteristics. *Brain Res.* **127**:235–249 (1977).
29. Nagy, J. I., T. Lee, P. Seeman, and H. C. Fibiger. Direct evidence for presynaptic and postsynaptic dopamine receptors in brain. *Nature* (*Lond.*) **274**:278–281 (1978).
30. Reisine, T. D., J. I. Nagy, H. C. Fibiger, and H. I. Yamamura. Localization of dopamine receptors in rat brain. *Brain Res.* **169**:209–214 (1979).
31. Weigele, J. B., and R. L. Barchi. Ultraviolet irradiation produces loss of saxitoxin binding to sodium channels in rat synaptosomes. *J. Neurochem.* **35**:430–435 (1980).
32. Clement-Cormier, Y. C. Dopamine receptor: isolation, purification and regulation, in *Receptors for Neurotransmitters and Peptide Hormones* (G. Pepeu, M. J. Kuhar, and S. J. Enna, eds.). Raven Press, New York, 159–167 (1980).

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