The Journal of Neuroscience
August 1986, 6(8): 2245-2251

Differential Down-Regulation of D1-Stimulated Adenylate Cyclase Activity in Rat Forebrain After *In Vivo* Amphetamine Treatments

Jill M. Roberts-Lewis, Patrick H. Roseboom, Lynn M. Iwaniec, and Margaret E. Gnegy
Departments of Psychology and Pharmacology, The University of Michigan, Ann Arbor, Michigan 48109

Amphetamine has complex behavioral actions in the rat that depend upon the release of dopamine in striatal and mesolimbic brain regions. To explore a possible role of the dopamine-sensitive cAMP second-messenger system in mediating these effects, we examined the effects of *in vivo* amphetamine treatments on the D1 receptor-coupled adenylate cyclase system in membranes from striatal and mesolimbic rat brain regions. The results show that amphetamine produces a regional, dose- and time-dependent down-regulation of adenylate cyclase activity. Intermediate and high doses of amphetamine (2.5 and 7.5 mg/kg, respectively), but not a low dose (1.0 mg/kg), resulted in a decrease in the apparent V_{max} and/or an increase in the apparent K_a for the selective D1 partial agonist, SKF38393, in striatal membranes 30 min after amphetamine treatment. Treatment of rats with 7.5 mg/kg amphetamine for 30 and 60 min, but not 10 min, similarly resulted in a down-regulation of D1-mediated adenylate cyclase activity in striatal membranes. In contrast, in mesolimbic tissues, no amphetamine treatment at any time resulted in an alteration of SKF38393-stimulated adenylate cyclase activity relative to saline controls. The results of behavioral analyses also showed that animals exhibiting intense stereotypies had significantly lower striatal apparent V_{max} values than did those animals engaged in moderate or no behavioral activity at the time of decapitation. These findings demonstrate that amphetamine treatments result in a down-regulation of striatal, but not mesolimbic, dopamine-sensitive adenylate cyclase activity that parallels the intense, stereotyped behaviors characteristic of dopaminergic activation in the striatum.

Amphetamine (AMPH), a phenylethylamine derivative, is a potent CNS stimulant with a multiplicity of behavioral and physiological effects (see Groves and Tepper, 1983, and Segal and Schuckit, 1983, for reviews). At lower doses of AMPH (1.0 mg/kg), rats exhibit enhanced locomotor activity and exploratory behaviors. As the dose is increased (e.g., 2.5 mg/kg), rats begin to show episodic bouts of repetitive head and limb movements (stereotypies). After a high dose of AMPH (5–7.5 mg/kg), a similar sequence of behaviors may be observed as a function of time after AMPH administration. Shortly after the drug injection (ca. 10 min), rats show a marked increase in rearing and sniffing, or locomotor activity, followed (ca. 30 min) by stereotyped head and limb movements. By 60 min after AMPH administration, most animals are engaged in intense, continuous stereotypy, head oriented down toward the cage floor, often accompanied by oral stereotypies such as licking or gnawing. These multiphasic time- and dose-dependent behavioral effects of AMPH appear to be due to differential stimulation of mesolimbic or striatal brain regions. Specifically, the mesolimbic brain regions are thought to have a role in mediating the locomotor hyperactivity and exploratory behaviors seen after low doses of AMPH, while the intense stereotypies elicited by higher doses of AMPH are believed to be largely mediated through the nigrostriatal pathway (Creese and Iversen, 1975; Kelly and Iversen, 1976; Kelly et al., 1975; Koob et al., 1978; Pijnenburg et al., 1976). A number of electrophysiological studies have also demonstrated biregional dose-dependent effects of AMPH that seemingly correspond to these behavioral phenomena (Groves and Rebec, 1976; Rebec and Segal, 1978; Rebec and Zimmerman, 1980). Moreover, AMPH also has biphasic dose- and time-dependent effects on striatal dopamine synthesis, which seem to parallel the behavioral and electrophysiological effects (Kuczenski, 1977).

It is well established that AMPH produces these behavioral and physiological effects primarily by enhancing dopamine release in the basal forebrain structures mentioned above (Besson et al., 1969, 1971; Chiueh and Moore, 1974; Moore, 1977; Philips and Robson, 1983; Roberts et al., 1975; and see above references). However, little is known about the subsequent mechanisms by which the enhanced release of dopamine may result in the characteristic effects of AMPH. There is some evidence that the cyclic nucleotide second messenger, cAMP, may be involved in the behavioral response to dopamine agonists (Fredholm et al., 1976; Heal et al., 1978; Lin et al., 1984; Miller and Kelly, 1975; Quenzer et al., 1977; Satoh et al., 1976). Dopamine can act upon at least 2 receptor subtypes in the brain that are linked to a cyclic nucleotide second-messenger system (Kebabian and Calne, 1979). The D1 dopamine receptor is positively coupled to adenylate cyclase, which stimulates the production of cAMP (Kebabian and Calne, 1979), while the D2 dopamine receptor is negatively coupled to adenylate cyclase and inhibits the formation of cAMP (Onali et al., 1984; Stoof and Kebabian, 1981). Both D1 and D2 receptors are present in the mesolimbic and striatal brain regions (Altar et al., 1985; Creese et al., 1983; Dawson et al., 1985; Miller et al., 1974; Ryan Jastrow et al., 1984; Schulz et al., 1984). Thus, because dopamine may stimulate receptors having functionally antithetical effects, it is important to be able to dissociate these receptor subtypes when evaluating treatments that affect dopaminergic neurotransmission. For example, an increase in the sensitivity of the D1 receptor may result in an increase in cAMP levels, whereas an increase in the sensitivity of the D2 receptor would have the opposite effect.

In the present study we examined the effects of *in vivo* AMPH treatments on the D1-coupled adenylate cyclase activity in rat striatal and mesolimbic brain regions, using the D1-specific partial agonist SKF38393. The results show that striatal, but not mesolimbic, tissues exhibit a dose- and time-dependent decrease in D1-mediated adenylate cyclase activity. These changes in striatal adenylate cyclase activity are associated with the behavioral response to AMPH.

Received Oct. 15, 1985; revised Jan. 24, 1986; accepted Feb. 20, 1986.

This study was supported by National Institute of Mental Health Grant 36044-05.

Correspondence should be addressed to Margaret E. Gnegy, Department of Pharmacology, 7427 Med. Sci. I, University of Michigan, Ann Arbor, MI 48109.

Copyright © 1986 Society for Neuroscience 0270-6474/86/082245-07$02.00/0

Table 1. Apparent kinetic constants for SKF38393-stimulated adenylate cyclase activity in rat striatal and mesolimbic membranes 30 min after treatment with different doses of AMPH or saline (control)

Dose AMPH[a] (mg/kg)	n	Apparent kinetic constant[b]: K_a (μM)	V_{max} (pmol/min/mg protein)
Striatum			
1.0	7	0.09 ± 0.01	206 ± 18
Control	7	0.07 ± 0.01	192 ± 13
2.5	7	0.07 ± 0.02	110 ± 10†
Control	7	0.06 ± 0.01	153 ± 10
7.5	7	0.08 ± 0.009‡	157 ± 14*
Control	7	0.05 ± 0.004	199 ± 17
Mesolimbic			
1.0	7	0.07 ± 0.01	96 ± 6
Control	7	0.06 ± 0.01	103 ± 11
2.5	4	0.06 ± 0.019	77 ± 7
Control	4	0.06 ± 0.019	107 ± 10
7.5	7	0.10 ± 0.01	81 ± 8
Control	7	0.08 ± 0.01	97 ± 12

[a] Rats were injected intraperitoneally with given doses of AMPH or an equivalent volume of saline and killed 30 min later. Striatal and mesolimbic membranes were prepared as described in Materials and Methods.

[b] Kinetic constants were determined by the method of Wilkinson (1961) and represent the mean ± SEM for the given number of rats (*n*). Control values between these experiments vary due to the different times at which they were performed over a 9 month period. Therefore, experiments with saline controls were always performed simultaneously with the given AMPH treatment, and direct comparisons were only made within the relevant experimental group. Significantly different from saline control at * $p < 0.05$, † $p < 0.01$, or ‡ $p < 0.005$.

Table 2. Basal and GTP-stimulated adenylate cyclase activity in rat striatal and mesolimbic membranes 30 min after treatment with different doses of AMPH or saline (control)

Dose AMPH[a] (mg/kg)	n	Adenylate cyclase activity[b] (pmol/min/mg protein): Basal	1 μM GTP
Striatum			
1.0	7	233 ± 22	470 ± 31
Control	7	216 ± 17	465 ± 30
2.5	7	218 ± 19	437 ± 18
Control	7	211 ± 15	462 ± 17
7.5	7	202 ± 28	464 ± 52
Control	7	186 ± 16	470 ± 39
Mesolimbic			
1.0	7	95 ± 13	209 ± 20
Control	7	89 ± 8	208 ± 16
2.5	4	101 ± 13	206 ± 16
Control	4	94 ± 12	202 ± 15
7.5	7	86 ± 10	214 ± 22
Control	7	96 ± 15	224 ± 30

[a] Rats were injected intraperitoneally with given doses of amphetamine or an equivalent volume of saline and killed 30 min later. Striatal and mesolimbic membranes were prepared as described in Materials and Methods. Experiments with saline controls were always performed simultaneously with the given dose of amphetamine.

[b] Adenylate cyclase activity measured in the absence (basal) and presence of 1 μM GTP is given as the mean ± SEM for the specified number of rats (*n*).

Materials and Methods

Female Holtzman rats, 150–250 gm (Madison, WI) were used in all of the experiments. Animals were injected with 1.0, 2.5, or 7.5 mg/kg *d*-amphetamine sulfate or an equal volume of physiological saline (vehicle), i.p., at specified times (10, 30, or 60 min) prior to decapitation. Control rats were always paired with AMPH-treated rats in the same experiment on any single day. Behavioral observations were made for a 1 min period immediately prior to decapitation; behavior was described by 1 of the following 3 categories: Still, asleep or awake but stationary; Moderate activity, intermittent or continuous sniffing, rearing, grooming, or ambulation; Intense stereotypy, continuous sniffing, and/or licking in one place with head oriented down toward the cage floor. The mesolimbic brain region, consisting of olfactory tubercle and nucleus accumbens, and the striatum were then rapidly dissected on ice and homogenized in 9 vol of ice-cold 10 mM Tris maleate buffer, pH 7.5, containing 1.2 mM EGTA and 1 mM $MgSO_4$. Crude membranes were prepared from the homogenates by centrifugation at 27,000 × *g* for 20 min. This procedure was repeated, and the final pellets were resuspended in the same buffer to a protein concentration of 5 mg/ml. Protein content was determined by the method of Lowry et al. (1951).

Adenylate cyclase assay

Adenylate cyclase activity was measured in an assay (0.2 ml vol) containing 80 mM Tris maleate buffer, pH 7.5, 5 mM $MgSO_4$, 2 mM cAMP, 4 mM phospho*enol*pyruvate, 10 μg of pyruvate kinase, 0.12 mM isobutylmethylxanthine, 100–150 μg of particulate membrane protein, 0.15 mM EGTA, and 1 mM [α-^{32}P]ATP (1 μCi/assay), with or without the addition of guanosine 5′-triphosphate (GTP) and SKF38393. Assays were incubated at 37°C for 5 min and the reaction was terminated by heating for 1 min at 95°C. A solution (0.2 ml) containing 20 mM ATP and 0.7 mM ^{3}H-cAMP was then added to each tube. The particulate material was pelleted by centrifugation at 5000 × *g* for 30 min, and the ^{32}P-labeled cAMP in the supernatant fraction was determined by the method of Krishna et al. (1968). Recovery of cAMP, estimated by the ^{3}H-cAMP, was consistently between 85 and 95%; all calculated enzyme activities were adjusted accordingly. GTP was required for activation by SKF38393. SKF38393-stimulated adenylate cyclase activity is defined as pmol cAMP/min/mg protein produced above that of GTP.

Data analysis

Kinetic constants were determined by the method of Wilkinson (1961), and statistical comparisons of the kinetic data were made using a Student's *t* test. Behavioral and biochemical comparisons were made using a 1-way analysis of variance.

Materials

α-^{32}P-ATP (specific activity, 38 Ci/mmol), and ^{3}H-cAMP were purchased from Amersham Searle Corp. (Arlington Heights, IL); cAMP, phospho*enol*pyruvate, and ATP were purchased from Sigma Chemical Co. (St. Louis, MO); pyruvate kinase was obtained from Boehringer Mannheim (Indianapolis, IN); GTP was purchased from International Chemical and Nuclear Corp (Irvine, CA); *d*-amphetamine sulfate was obtained through the University of Michigan Laboratory of Animal Medicine, and SKF38393 was graciously donated by Smith Kline and French Pharmaceuticals, Inc. (Philadelphia, PA).

Results

Dose-dependent effects of AMPH on adenylate cyclase activity in striatum

In vivo administration of AMPH resulted in dose-dependent changes in the ability of the D1 agonist SKF38393 to stimulate adenylate cyclase activity *in vitro.* While there was no apparent effect of *in vivo* treatment with 1.0 mg/kg AMPH on the ability of SKF38393 to stimulate adenylate cyclase activity (Fig. 1*A*, Table 1), treatments with 2.5 and 7.5 mg/kg AMPH did produce significant alterations in enzyme reactivity (Fig. 1, *B*, *C*; Table 1). Thirty minutes after 2.5 mg/kg AMPH (Fig. 1*B*; Table 1), the apparent V_{max} of SKF38393-stimulated adenylate cyclase

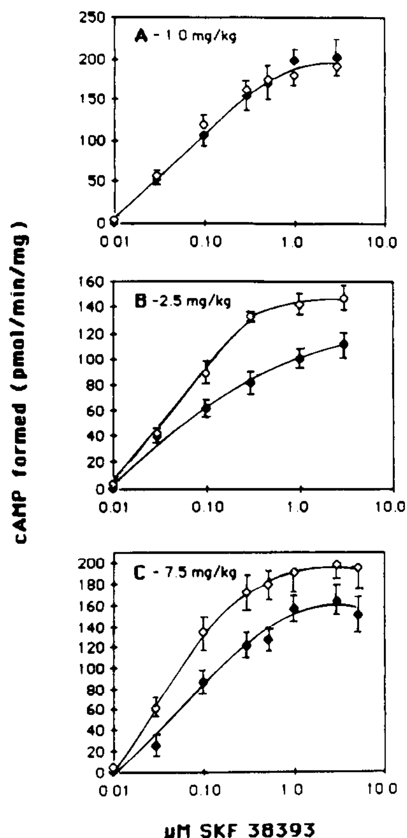

Figure 1. SKF38393-stimulated adenylate cyclase activity in striatal membranes prepared from rats receiving a 30 min treatment with (*A*) 1.0, (*B*) 2.5, or (*C*) 7.5 mg/kg AMPH (●), or an equivalent volume of saline (○). Each point represents the mean enzyme activity (pmol cAMP formed/min/mg protein) ± SEM for 5–7 animals. Mean basal and GTP-stimulated adenylate cyclase activities are shown in Table 2.

activity was significantly decreased in membranes from AMPH treated as compared to control rats. In contrast, there was no effect of 2.5 mg/kg AMPH on the apparent K_a for SKF38393. However, 30 min after administration of a higher dose of AMPH, 7.5 mg/kg (Fig. 1*C*; Table 1), the apparent K_a for SKF38393 was increased 60% over saline-treated control rats, and the apparent V_{max} was also significantly decreased. Despite the pronounced effects of *in vivo* AMPH treatments on SKF38393-stimulated adenylate cyclase activity *in vitro,* there were no alterations in basal adenylate cyclase activity or the activation by GTP as a result of any of these AMPH treatments (Table 2).

Dose-dependent effects of AMPH on adenylate cyclase activity in the mesolimbic region

In contrast to the down-regulation in striatum, AMPH treatments did not result in alterations in the affinity for agonist or maximum velocity of SKF38393-stimulated adenylate cyclase in mesolimbic membranes (Table 1). Basal and GTP-stimulated adenylate cyclase activity in membranes from the mesolimbic area were also unchanged by these AMPH treatments (Table 2).

Basal and GTP-stimulated adenylate cyclase activities in mesolimbic tissues were generally about 50% lower than those measured in striatal membranes, as were apparent V_{max} values for SKF38393-stimulated activity. However, there did not appear to be any differences between these 2 brain regions in the apparent K_a for SKF38393.

Table 3. Apparent kinetic constants for SKF38393-stimulated adenylate cyclase activity in striatal and mesolimbic membranes from rats which received 7.5 mg/kg AMPH or saline (control) at given times prior to decapitation

Treatment time[a] (min)	n	Apparent kinetic constant[b]: K_a (µM)	V_{max} (pmol/min/mg protein)
Striatum			
10	5	0.06 ± 0.01	135 ± 17
Control	5	0.09 ± 0.02	126 ± 19
30	7	0.08 ± 0.009†	157 ± 14*
Control	7	0.05 ± 0.004	199 ± 17
60	6	0.08 ± 0.01†	96 ± 4†
Control	6	0.04 ± 0.01	128 ± 7
Mesolimbic			
10	4	0.09 ± 0.01	70 ± 7
Control	4	0.06 ± 0.01	65 ± 5
30	7	0.10 ± 0.01	81 ± 8
Control	7	0.08 ± 0.01	97 ± 12
60	6	0.05 ± 0.01	51 ± 4
Control	6	0.08 ± 0.01	59 ± 3

[a] Rats were injected intraperitoneally with 7.5 mg/kg AMPH or an equivalent volume of saline and killed at given times after treatment. Striatal and mesolimbic membranes were prepared as described in Materials and Methods.

[b] Kinetic constants were determined by the method of Wilkinson (1961) and represent the mean ± SEM for the given number of rats (n). Control values between these experiments vary due to the different times at which they were performed over a 9 month period. Therefore, experiments with saline controls were always performed simultaneously with the given AMPH treatment, and direct comparisons were only made within the relevant experimental group. Significantly different from saline control at * $p < 0.05$, or † $p < 0.005$.

Time-dependent effects of 7.5 mg/kg AMPH on adenylate cyclase activity in striatum

The down-regulation of adenylate cyclase activity following AMPH was found to be time-dependent (Fig. 2; Table 3). At 10 min after *in vivo* treatment, there were no differences in SKF38393-stimulated adenylate cyclase activity in striatal membranes from rats treated with 7.5 mg/kg AMPH or saline (Fig. 2*A*; Table 3). However, at both 30 and 60 min after treatment, AMPH produced a significant decrease in SKF38393-stimulated adenylate cyclase activity in striatal membranes, as evidenced by an increase in the apparent K_a for agonist and a decrease in the apparent V_{max} (Figs. 1*C* and 2*B*; Table 3). Basal adenylate cyclase activity and the activation by GTP in striatal tissues from AMPH- and saline-treated rats were not significantly different at any time after *in vivo* treatment (Table 4).

Time-dependent effects of 7.5 mg/kg AMPH on adenylate cyclase activity in the mesolimbic region

In contrast to results obtained for striatum, there were no effects of *in vivo* treatment with 7.5 mg/kg AMPH on SKF38393-stimulated adenylate cyclase activity in mesolimbic membranes at any time after AMPH administration as compared to saline controls (Table 3). Neither were there any differences between AMPH or saline treatments on basal adenylate cyclase activity and the activation by GTP in mesolimbic tissues at any time after *in vivo* treatment (Table 4).

Relationship between behavior and AMPH treatment

As expected, dose- and time-dependent behavioral effects as described in the introduction were observed after AMPH treat-

Table 4. Basal and GTP-stimulated adenylate cyclase activity in striatal and mesolimbic membranes prepared from rats that received 7.5 mg/kg AMPH or saline (control) at different times prior to decapitation

Treatment time[a] (min)	n	Adenylate cyclase activity[b] (pmol/min/mg protein)	
		Basal	1 μM GTP
Striatum			
10	5	161 ± 19	377 ± 32
Control	5	143 ± 10	340 ± 25
30	7	202 ± 28	464 ± 52
Control	7	186 ± 16	470 ± 39
60	6	105 ± 7	261 ± 10
Control	6	106 ± 8	263 ± 9
Mesolimbic			
10	4	61 ± 11	141 ± 9
Control	4	60 ± 12	149 ± 34
30	7	86 ± 10	214 ± 22
Control	7	96 ± 15	224 ± 30
60	6	47 ± 9	117 ± 10
Control	6	41 ± 7	123 ± 9

[a] Rats were injected intraperitoneally with 7.5 mg/kg AMPH or an equivalent volume of saline and killed at given times afterward. Striatal and mesolimbic membranes were prepared as described in Materials and Methods. Experiments with saline controls were always performed simultaneously with the given AMPH treatment.

[b] Adenylate cyclase activity measured in the absence (basal) and presence of 1 μM GTP is given as the mean ± SEM for the specified number of rats (n).

ments. A 1-way analysis of variance comparing striatal apparent V_{max} values for the 3 behavioral categories revealed that animals engaged in the intense stereotypy 30 or 60 min after injection of 7.5 mg/kg AMPH had significantly lower apparent V_{max} values (101 ± 5.2 pmol/min/mg protein) than those animals that exhibited moderate (129 ± 7.7 pmol/min/mg protein) or no activity at the time of decapitation (141 ± 9.2 pmol/min/mg protein), which are typically seen after injections of saline, lower doses of AMPH, or 10 min after 7.5 mg/kg AMPH (Fig. 3).

Discussion

We have shown that the *in vivo* administration of AMPH to rats results in time- and dose-dependent down-regulation of dopamine-sensitive adenylate cyclase activity in membranes from striatal, but not mesolimbic, brain regions. Thirty minutes after administration, a low dose of AMPH did not affect striatal dopamine-sensitive adenylate cyclase activity, whereas increasing the dose of AMPH resulted in a down-regulation of this activity. Similarly, while no effects of a high dose of AMPH on SKF38393-stimulated adenylate cyclase activity were apparent in striatal membranes 10 min after treatment, a progressive down-regulation of this activity was observed as the time after AMPH injection was extended to 30 and 60 min. Thus, in the striatum, a well-pronounced dose- and time-dependent down-regulation of dopamine-sensitive adenylate cyclase activity occurred after *in vivo* treatments with AMPH. In sharp contrast to the striatum, dopamine-sensitive adenylate cyclase activity in membranes from the mesolimbic region was not affected by any *in vivo* treatment with AMPH.

The observed down-regulation of striatal adenylate cyclase activity could be due to a desensitization of this system at any of several levels. Alterations in receptor number or availability, affinity for agonist, or changes in the efficiency of receptor coupling to adenylate cyclase may all be implicated. It is unlikely

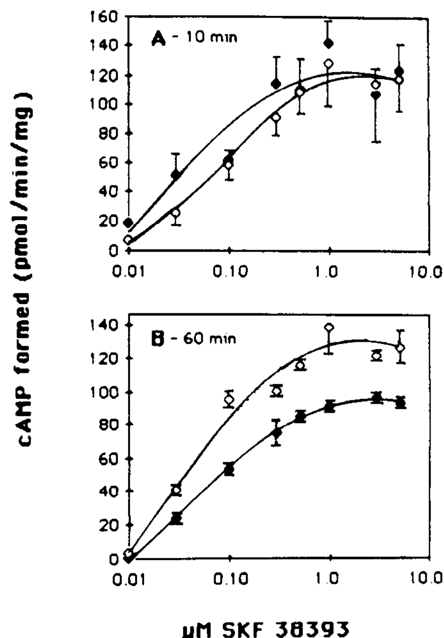

Figure 2. SKF38393-stimulated adenylate cyclase activity in striatal membranes prepared from rats receiving 7.5 mg/kg AMPH (●) or an equivalent volume of saline (○) 10 min (*A*) or 60 min (*B*) prior to decapitation. Each point represents the mean enzyme activity (pmol cAMP formed/min/mg protein) ± SEM for 5–7 animals. Mean basal and GTP-stimulated adenylate cyclase activities are shown in Table 4.

that these effects are due to a competition of agonist with endogenous released dopamine for the D1 receptor. We have found that the preparation of washed membranes for the cyclase assay removes at least 92% of the dopamine present in the homogenate. Assuming a dopamine concentration of about 120 ng/mg protein in the striatum, a maximum of approximately 60 nM residual dopamine could be carried into the assay. This concentration has no measurable effects on adenylate cyclase activity *in vitro* (Gnegy and Treisman, 1981). Moreover, such

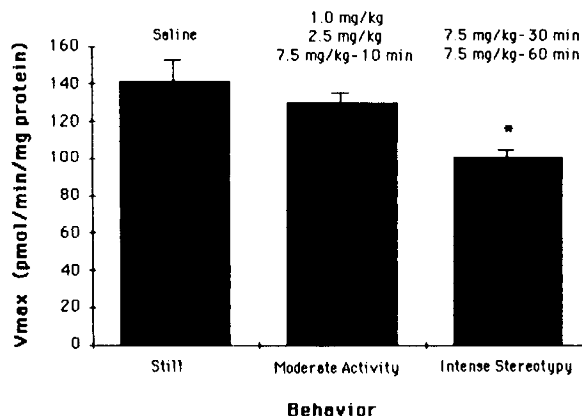

Figure 3. Mean striatal apparent V_{max} values (pmol cAMP formed/min/mg protein) ± SEM for each of the 3 behavioral categories described in methods. Following systemic treatment with AMPH or saline, behavioral observations were made for each rat 1 min prior to decapitation. Eight to 14 animals are represented in each behavioral category. The treatments with AMPH or saline that elicited behaviors in each category are indicated above the bars. •, V_{max} values in striata from animals exhibiting intense stereotypy were significantly lower than the V_{max} values obtained in striata from animals showing little or moderate behavioral activity at the time of decapitation ($p < 0.02$).

an effect would not explain the different results obtained in striatal and mesolimbic tissues.

The present findings are complemented by similar results obtained with *in vivo* AMPH treatments reported by Barnett and Kuczenski (1985). *In vitro* studies of agonist-induced receptor desensitization also provide results compatible with those presented here. In striatal slices preincubated with dopamine or D1 agonists, Memo et al. (1982) have demonstrated a selective D1-mediated desensitization of dopamine-stimulated adenylate cyclase activity that is characterized by an increase in the apparent K_d for agonist binding, as well as a decrease in the efficiency of the adenylate cyclase coupling system. Memo and Hanbauer (1984) have also shown that persistent stimulation of dopamine-sensitive adenylate cyclase activity in striatal slices results in the phosphorylation of specific membrane proteins, suggesting a possible means by which the dopamine receptor is desensitized after such treatments. Another possible mechanism for desensitization is receptor internalization, as demonstrated for the β-adrenergic receptor in non-neuronal cells (see Lefkowitz et al., 1980, and Perkins and Harden, 1984, for reviews). These studies may provide some insight into the mechanisms by which *in vivo* treatments with AMPH may regulate cAMP levels through the D1 receptor.

The difference observed between striatal and mesolimbic adenylate cyclase systems in response to AMPH treatment is intriguing, for it suggests that the same cAMP second-messenger system may exhibit fundamentally different regulatory properties, depending upon its location in the CNS. Such results could be due to a heterogeneous distribution of the D1 receptor in the basal forebrain (e.g., fewer D1 receptors in mesolimbic tissues). It could also be due to subtle differences in the D1 receptor complex *per se,* for example, a D1 receptor subtype which is desensitized by agonist versus a D1 subtype which is not. There may also be a variety of regional differences in the microenvironment surrounding the receptor–enzyme complex, such as the presence or absence within the membranes of certain neuroregulators that interact with the adenylate cyclase system. Dopamine turnover in the mesolimbic terminal region has been shown to differ from striatum in response to short- or long-term treatment with neuroleptics (e.g., Bartholini, 1976; Kaneno et al., 1978; Scatton et al., 1977). Moreover, basal rates of dopamine synthesis and metabolism are quite different in these 2 brain regions. In several studies, measures of dopamine synthesis were shown to be greater in striatum than mesolimbic tissues, while measures of dopamine utilization were greater in the mesolimbic regions than in striatum (e.g., Gundlach and Beart, 1981; Kuczenski, 1980; Pycock et al., 1980). Finally, recent studies have shown that dopamine uptake is differentially regulated in striatum and nucleus accumbens (Missale et al., 1985). These, as well as other presynaptic events, could influence the overall effect that AMPH may have in a given region, especially since there is evidence that AMPH preferentially releases newly synthesized, extragranular dopamine (Chiueh and Moore, 1975; Raiteri et al., 1979; Rutledge, 1978; Scheel-Kruger, 1971). Relatively high basal rates of dopamine release in the mesolimbic region (as estimated by dopamine utilization) could maintain a desensitized state of the D1 receptor (relative to striatal D1 receptors), and this might account for the lower basal and agonist-stimulated adenylate cyclase activities that we observed in this region. Moreover, a lower basal rate of dopamine synthesis in the mesolimbic region might result in relatively less dopamine released in response to AMPH (compared to striatum) and, hence, smaller effects of AMPH on adenylate cyclase activity.

We found that the dose- and time-dependent down-regulation of adenylate cyclase activity in the striatum was paralleled by a dose- and time-dependent behavioral response to AMPH. These results support the idea that the activation of striatal dopamine receptors is an important determinant of AMPH-elicited stereotypy and suggest that the striatal dopamine-sensitive cAMP second-messenger system may be involved in or affected by the biochemical cascade of events that result in stereotyped behaviors after high doses of AMPH. While this finding in no way suggests a direct correlation or causal relationship between these events, it does point to the fact that the D1-sensitive adenylate cyclase system shows dose-dependent responsivity to *in vivo* AMPH treatments which coincides with the dose-dependent behavioral effects. On the other hand, D1-mediated adenylate cyclase activity in the mesolimbic region was unaltered by AMPH treatments. Thus, it appears that the same second messenger system in the mesolimbic region may be uninvolved in, or differently affected by, the biochemical events that result in the behavioral response to AMPH. Together, these data show that the mesolimbic and striatal brain regions, which play different roles in the behavioral response to AMPH, can also be biochemically differentiated after behaviorally relevant doses of AMPH are administered. Assuming that dopamine release in the mesolimbic region is a crucial element of the behavioral response to low doses of AMPH (e.g., Kelly and Roberts, 1983; Kelly et al., 1975; Pijnenburg et al., 1976), it is possible that the negatively coupled D2 adenylate cyclase system may be involved preferentially in the behavioral response to low-dose AMPH. This idea is supported by a number of recent studies that have demonstrated the participation of both D1 and D2 receptors in orchestrating the full behavioral response to dopamine agonists (Braun and Chase, 1985; Herrera-Marschitz et al., 1984; Iorio et al., 1983; Mailman et al., 1984; Molloy and Waddington, 1984, 1985; O'Boyle et al., 1984; Yurek and Randall, 1985).

References

Altar, C. A., S. P. O'Neil, R. J. Walter, Jr., and J. F. Marshall (1985) Brain dopamine and serotonin receptor sites revealed by digital subtraction autoradiography. Science *228:* 597–600.

Barnett, J. V., and R. Kuczenski (1985) Changes in rat striatal dopamine-stimulated adenylate cyclase activity following acute amphetamine administration. Soc. Neurosci. Abstr. *11:* 922.

Bartholini, G. (1976) Differential effects of neuroleptic drugs on dopamine turnover in the extrapyramidal and limbic system. J. Pharm. Pharmacol. *28:* 429–433.

Besson, M. J., A. Cheramy, P. Feltz, and J. Glowinski (1969) Release of newly synthesized dopamine from dopamine containing terminals of the striatum of the rat. Proc. Natl. Acad. Sci. USA *62:* 741–748.

Besson, M. J., A. Cheramy, P. Feltz, and J. Glowinski (1971) Dopamine: Spontaneous and drug-induced release from the caudate nucleus in the cat. Brain Res. *32:* 407–424.

Braun, A. R., and T. N. Chase (1985) Obligatory D1–D2 receptor coactivation and the generation of dopaminergic behaviors. Soc. Neurosci. Abstr. *11:* 671.

Chiueh, C. C., and K. E. Moore (1974) In vivo release of endogenously synthesized catecholamines from the cat brain evoked by electrical stimulation and by D-amphetamine. J. Neurochem. *23:* 159–168.

Chiueh, C. C., and K. E. Moore (1975) d-Amphetamine-induced release of "newly synthesized" and "stored" dopamine from the caudate nucleus in vivo. J. Pharmacol. Exp. Ther. *192:* 642–653.

Creese, I., and S. D. Iversen (1975) The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Res. *83:* 419–436.

Creese, I., D. R. Sibley, M. W. Hamblin, and S. E. Leff (1983) The classification of dopamine receptors: Relationship to radioligand binding. Annu. Rev. Neurosci. *6:* 43–71.

Dawson, T. M., D. R. Gehlert, H. I. Yamamura, A. Barnett, and J. K. Wamsley (1985) D-1 dopamine receptors in the rat brain: Autoradiographic localization using [^{3}H]SCH-23390. Eur. J. Pharmacol. *108:* 323–325.

Fredholm, B. B., K. Fuxe, and L. Agnati (1976) Effect of some phosphodiesterase inhibititors on central dopamine mechanisms. Eur. J. Pharmacol. *38:* 31–38.

Gnegy, M. E., and G. Treisman (1981) Effect of calmodulin on do-

pamine-sensitive adenylate cyclase activity in rat striatal membranes. Mol. Pharmacol. *19:* 256–263.

Groves, P. M., and G. V. Rebec (1976) Biochemistry and behavior: Some central actions of amphetamine and antipsychotic drugs. Annu. Rev. Psychol. *27:* 91–127.

Groves, P. M., and J. M. Tepper (1983) Neuronal mechanisms of action of amphetamine. In *Stimulants: Neurochemical, Behavioral, and Clinical Perspectives,* I. Creese, ed., pp. 81–129, Raven, New York.

Gundlach, A. L., and P. M. Beart (1981) Differential effects of IV and IP muscimol on central dopamine metabolism. Psychopharmacology *74:* 71–73.

Heal, D. J., A. G. Phillips, and A. R. Green (1978) Studies on locomotor activity produced by injection of dibutryl cyclic 3′5′AMP into the nucleus accumbens of rats. Neuropharmacology *17:* 265–270.

Herrera-Marschitz, M., J. Hyttel, and U. Ungerstedt (1984) The dopamine D-1 antagonist SCH 23390 inhibits apomorphine but not pergolide induced rotation. Acta Physiol. Scand. *122:* 427–428.

Iorio, L. C., A. Barnett, F. H. Letiz, V. P. Houser, and C. A. Korduba (1983) SCH 23390: A potential benzazepine antipsychotic with unique actions on dopaminergic systems. J. Pharmacol. Exp. Ther. *226:* 462–468.

Kaneno, S., A. Watanabe, M. Torn, and Y. Shimazono (1978) Different dopamine stimulation of adenylate cyclase in striatum and mesolimbic area of rats treated with chronic chlorpromazine. Brain Res. *152:* 396–400.

Kebabian, J. W., and D. B. Calne (1979) Multiple receptors for dopamine. Nature *277:* 93–96.

Kelly, P., and S. D. Iversen (1976) Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in rats. Eur. J. Pharmacol. *46:* 45–56.

Kelly, P. H., and D. C. S. Roberts (1983) Effects of amphetamine and apomorphine on locomotor activity after 6-OHDA and electrolytic lesions of the nucleus accumbens septi. Pharmacol. Biochem. Behav. *19:* 137–143.

Kelly, P., P. Seviour, and S. D. Iversen (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. *94:* 507–522.

Koob, G., F. Riley, D. Smith, and T. Robbins (1978) Effects of 6-hydroxydopamine lesions of nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity and amphetamine anorexia in the rat. J. Comp. Physiol. Psychol. *92:* 917–927.

Krishna, G., B. Weiss, and B. B. Brodie (1968) A simple, sensitive method for the assay of adenyl cyclase. J. Pharmacol. Exp. Ther. *163:* 379–385.

Kuczenski, R. (1977) Biphasic effects of amphetamine on striatal dopamine dynamics. Eur. J. Pharmacol. *46:* 249–257.

Kuczenski, R. (1980) Amphetamine-haloperidol interactions on striatal and mesolimbic tyrosine hydroxylase activity and dopamine metabolism. J. Pharmacol. Exp. Ther. *215:* 135–142.

Lefkowitz, R. J., M. R. Wessels, and J. M. Stadel (1980) Hormones, receptors, and cyclic AMP: Their role in target cell refractoriness. Curr. Top. Cell. Regul. *17:* 205–230.

Lin, M. T., C. F. Chen, and K. S. Chuang (1984) Both dopaminergic and adrenergic receptors in the brain are involved in the behavioural excitation induced by dibutyryl 3′5′-adenosine monophosphate and aminophylline in the rat. Neuropharmacology *23:* 129–135.

Lowry, O. H., N. J. Rosebrough, L. A. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Mailman, R. B., D. W. Schulz, M. H. Lewis, L. Staples, H. Rollema, and D. L. DeHaven (1984) SCH-23390: A selective D-1 dopamine antagonist with potent D-2 behavioral actions. Eur. J. Pharmacol. *101:* 159–160.

Memo, M., and I. Hanbauer (1984) Phosphorylation of membrane proteins in response to persistent stimulation of adenylate cyclase linked to dopamine receptors in slices of striatum. Neuropharmacology *23:* 449–455.

Memo, M., W. Lovenberg, and I. Hanbauer (1982) Agonist-induced subsensitivity of adenylate cyclase coupled with a dopamine receptor in slices from rat corpus striatum. Proc. Natl. Acad. Sci. USA *79:* 4456–4460.

Miller, R. J., and P. H. Kelly (1975) Dopamine-like effects of cholera toxin in the central nervous system. Nature *255:* 163–166.

Miller, R. J., A. S. Horn, and L. L. Iversen (1974) The action of neuroleptic drugs on dopamine-stimulated adenosine-3′5′-monophosphate production in rat neostriatum and limbic forebrain. Mol. Pharmacol. *10:* 759–766.

Missale, C., L. Castelletti, S. Govoni, P. F. Spano, M. Trabucchi, and I. Hanbauer (1985) Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens. J. Neurochem. *45:* 51–56.

Molloy, A. G., and J. L. Waddington (1984) Dopaminergic behavior stereospecifically promoted by the D-1 agonist R-SK&F 38393 and selectively blocked by the D-1 antagonist SCH 23390. Psychopharmacology *82:* 409–410.

Molloy, A. G., and J. L. Waddington (1985) Sniffing, rearing and locomotor responses to the D-1 agonist R-SK&F 38393 and to apomorphine: Differential interactions with the selective D-1 and D-2 antagonists SCH 23390 and metoclopramide. Eur. J. Pharmacol. *108:* 305–308.

Moore, K. E. (1977) The actions of amphetamine on neurotransmitters: A brief review. Biol. Psychiatr. *12:* 451–462.

O'Boyle, K. M., M. Pugh, and J. L. Waddington (1984) Stereotypy induced by the D-2 agonist RU24213 is blocked by the D-2 antagonist Ro 22-2586 and the D-1 antagonist SCH 23390. Br. J. Pharmacol. *82:* 242P.

Onali, P., M. C. Olianas, and G. L. Gessa (1984) Selective blockade of dopamine D-1 receptors by SCH 23390 discloses striatal dopamine D-2 receptors mediating the inhibition of adenylate cyclase in rats. Eur. J. Pharmacol. *99:* 127–128.

Perkins, J. P., and T. K. Harden (1984) Mechanisms of desensitization of β-adrenergic receptors. In *Proc. 9th Int. Cong. Pharmacol.,* J. F. Mitchell, W. Paton, and P. Turner, eds., pp. 85–90, MacMillan, London.

Philips, S. R., and A. M. Robson (1983) Amphetamine-stimulated release of endogenous dopamine from the rat caudate nucleus in vivo. Neurochem. Res. *8:* 731–741.

Pijnenburg, A. J. J., W. M. M. Honig, J. A. M. Van der Heyden, and J. M. Van Rossum (1976) Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur. J. Pharmacol. *35:* 45–58.

Pycock, C. J., C. J. Carter, and R. W. Kerwin (1980) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical slices in the rat. J. Neurochem. *34:* 91–99.

Quenzer, L. F., C. L. Galli, and N. H. Neff (1977) Activation of the nigrostriatal dopaminergic pathway by injection of cholera enterotoxin into the substantia nigra. Science *195:* 78–79.

Raiteri, M., F. Cerrito, A. M. Cervoni, and G. Levi (1979) Dopamine can be released by two mechanisms differentially affected by the dopamine transport inhibitor, nomifensine. J. Pharmacol. Exp. Ther. *208:* 195–202.

Rebec, G. V., and D. S. Segal (1978) Dose-dependent biphasic alterations in the spontaneous activity of neurons in the rat neostriatum produced by D-amphetamine and methylphenidate. Brain Res. *150:* 353–366.

Rebec, G. V., and K. S. Zimmerman (1980) Opposite effects of D-amphetamine on spontaneous neuronal activity in the neostriatum and nucleus accumbens. Brain Res. *201:* 485–491.

Roberts, D. C. S., A. P. Zis, and H. C. Fibiger (1975) Ascending catecholamine pathways and amphetamine-induced locomotor activity: Importance of dopamine and apparent non-involvement of norepinephrine. Brain Res. *93:* 441–454.

Rutledge, C. O. (1978) Effect of metabolic inhibitors and ouabain on amphetamine- and postassium-induced release of biogenic amines from isolated brain tissue. Biochem. Pharmacol. *27:* 511–516.

Ryan Jastrow, T., E. Richfield, and M. E. Gnegy (1984) Quantitative autoradiography of [^{3}H]sulpiride binding sites in rat brain. Neurosci. Lett. *51:* 47–53.

Satoh, H., Y. Satoh, N. Notsu, and F. Honda (1976) Adenosine 3′5′-cyclic monophosphate as a possible mediator of rotational behavior induced by dopaminergic receptor stimulation in rats lesioned unilaterally in the substantia nigra. Eur. J. Pharmacol. *39:* 365–377.

Scatton, B., S. Bischoff, J. Dedek, and J. Korf (1977) Regional effects of neuroleptics on dopamine metabolism and dopamine-sensitive adenylate cyclase activity. Eur. J. Pharmacol. *44:* 287–292.

Scheel-Kruger, J. (1971) Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. Eur. J. Pharmacol. *14:* 47–59.

Schulz, D. W., S. D. Wyrick, and R. B. Mailman (1984) [^{3}H]SCH23390 has the characteristics of a dopamine receptor ligand in the rat central nervous system. Eur. J. Pharmacol. *106:* 211–212.

Segal, D. S., and M. A. Schuckit (1983) Animal models of stimulant-induced psychosis. In *Stimulants: Neurochemical, Behavioral, and Clinical Perspectives,* I. Creese, ed., pp. 131–167, Raven, New York.

Stoof, J. C., and J. W. Kebabian (1981) Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature *294:* 366–368.

Wilkinson, G. N. (1961) Statistical estimations in enzyme kinetics. Biochem. J. *80:* 324–332.

Yurek, D. M., and P. K. Randall (1985) The effects of selective dopamine receptor subtype manipulation on stereotypic behavior. Soc. Neurosci. Abstr. *11:* 686.