MOLECULAR PHARMACOLOGY, 20:319–325

Ca^{2+}-Dependent Regulation of Rat Caudate Nucleus Adenylate Cyclase and Effects on the Response to Dopamine

MICHAEL T. PIASCIK,[1] MARY F. PIASCIK, ROBERT J. HITZEMANN,[2] AND JAMES D. POTTER

Department of Pharmacology and Cell Biophysics and Department of Psychiatry, University of Cincinnati Medical Center, Cincinnati, Ohio 45267

Received November 11, 1980; Accepted April 21, 1981

SUMMARY

PIASCIK, M. T., M. F. PIASCIK, R. J. HITZEMANN, AND J. D. POTTER. Ca^{2+}-dependent regulation of rat caudate nucleus adenylate cyclase and effects on the response to dopamine. *Mol. Pharmacol.* **20**:319–325 (1981).

The Ca^{2+} dependence of rat caudate nucleus microsomal adenylate cyclase [ATP pyrophosphate-lyase (cyclizing) EC 4.6.1.1] was determined and compared with that of cortical microsomes. Both cyclase preparations exhibited a biphasic response to Ca^{2+} with no differences in the free Ca^{2+} concentrations required to stimulate (one-half maximum = 0.19 μM cortex; 0.2 μM caudate) and inhibit (one-half maximum = 1 μM cortex; 0.9 μM caudate) each cyclase system. Whereas the cortical activity was stimulated 7-fold by Ca^{2+}, the caudate activity exhibited only a 2-fold Ca^{2+}-induced enhancement of basal cyclase. This relative insensitivity of caudate adenylate cyclase is not due to the selective loss of calmodulin. Ca^{2+} concentrations (0.03–0.5 μM) which stimulate the cyclase and the addition of large excesses of calmodulin had no effect on the ED_{50} of dopamine. The abilities of Ca^{2+} and dopamine to stimulate caudate adenylate cyclase activity were additive over the concentration range of 0.03–0.5 μM Ca^{2+}. Ca^{2+} concentrations (>0.5 μM) which inhibit adenylate cyclase activity abolished the stimulatory effect of dopamine. Therefore, it is suggested that Ca^{2+} and dopamine, in a coordinated manner, can modulate the response of caudate adenylate cyclase.

INTRODUCTION

Catecholamines are thought to exert regulatory control over synaptic function, in part, by stimulation of a membrane receptor coupled to adenylate cyclase. Recent evidence has suggested that Ca^{2+} may also exert regulatory control over brain adenylate cyclase (1–3). The effect of Ca^{2+} on brain cyclase is biphasic in nature; stimulation occurs at low Ca^{2+} concentrations followed by inhibition at higher Ca^{2+} concentrations (1–3). The fact that both stimulation and inhibition occur at free Ca^{2+} levels thought to exist intracellularly (1) supports the concept that Ca^{2+} is an important regulator of brain cyclase. Whereas Ca^{2+} stimulation of cyclase activity is due to calmodulin (1–3), Ca^{2+}-dependent inhibition of cyclase activity is mediated by a Ca^{2+} binding site which may be distinct from calmodulin (4).

In the caudate nucleus, both dopamine and Ca^{2+} exert regulatory control over adenylate cyclase (5, 6). The Ca^{2+} stimulation is calmodulin-dependent (6). Gnegy *et al.* (7, 8) have suggested that a rise in caudate calmodulin content following chronic treatment with haloperidol can enhance cyclase activity and is responsible for dopamine receptor supersensitivity.

Although the Ca^{2+} regulation of adenylate cyclase is an attractive property consistent with the second messenger role of Ca^{2+}, little evidence has been obtained concerning the interaction between Ca^{2+} and dopamine in the regulation of caudate adenylate cyclase. Herein, we report the free Ca^{2+} concentrations required to stimulate and inhibit caudate cyclase activity as well as the interaction between Ca^{2+} and dopamine in the regulation of caudate adenylate cyclase.

METHODS

Isolation of microsomes from rat cortex and caudate nucleus. Male rats (150–200 g, Sprague-Dawley, Harlan Industries, Indianapolis, Ind.) were killed, the cortex was removed, and the caudate was dissected by the method of Glowinski and Iverson (9). Brain regions were homogenized in 9 volumes (w/v) of 5 mM Hepes[3] (pH 7.2)

This work was supported by National Institutes of Health Grants HL-22619(3A) and NS-16061.

[1] Present address: Department of Pharmacology, University of Kentucky, A. B. Chandler Medical Center, Lexington, Ky. 40536. This work was completed while M. T. Piascik was a United States Public Health Service Postdoctoral Fellow (HL-07382).

[2] Department of Psychiatry.

[3] The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

0026-895X/81/020319/07$2.00/0

containing 0.32 M sucrose. As determined by atomic absorption spectrophotometry, the buffer contained a 10 μM level of contaminating Ca^{2+}. The tissue was homogenized (eight strokes) in a Teflon-glass homogenizer at a rate of 150 rpm. The homogenate was centrifuged at 1,000 × *g* for 10 min and the supernatant was centrifuged at 17,000 × *g*, also for 10 min. The resulting supernatant was centrifuged at 100,000 × *g* for 1 hr and the final pellet containing the microsomes was resuspended in a minimal volume of 5 mM Hepes (pH 7.2) containing 0.32 M sucrose. The protein concentration was 5–10 mg/ml.

Assay of adenylate cyclase. Reaction mixtures (250 μl) contained 2 mM EGTA, 150 mM morpholinopropanesulfonic acid (pH 7.2), 83 mM Tris base, 1 mM Na_2ATP, 2 mM $MgCl_2$, 10 μM GTP, 10 mM theophylline, 1 mM cyclic AMP, 10 mM phosphocreatine, 12.5 μg of creatine phosphokinase, [^{3}H]cyclic AMP (10,000 cpm to monitor recovery), dopamine (when appropriate), and brain microsomal protein (0.08–0.3 mg/assay). Varying amounts of $CaCl_2$ were added to yield a specified free metal ion concentration (see below). These mixtures were incubated for 5 min prior to the addition of [α-^{32}P]ATP (1–2 × 10^6 cpm). The reaction was allowed to proceed for 10 min at 30° and was stopped by addition of 750 μl of 1% sodium dodecyl sulfate. [^{32}P]cyclic AMP was purified by the double-column procedure of Salomon *et al.* (10). The amount of radioactivity in the column eluents was determined by liquid scintillation spectrometry. Determinations were performed in triplicate. The rate of cyclic AMP formation was linear for at least 15 min at maximal stimulatory concentrations of Ca^{2+} and/or dopamine.

Calculation of free metal ion concentrations. By using a Perkin-Elmer atomic absorption spectrophotometer to determine the Ca^{2+} contamination of the assay reagents (10 μM) and employing a computer program, the concentrations of free Ca^{2+} were calculated for each addition of $CaCl_2$ to the adenylate cyclase assays. Logarithms of the association constants (11) used in the computer program were as follows: H^+ to $EGTA^{4-}$, 9.46; H^+ to $HEGTA^{3-}$, 8.85; H^+ to H_2EGTA^{2-}, 2.68; H^+ to H_3EGTA^{1-}, 2.00; Ca^{2+} to $EGTA^{4-}$, 11.00; Ca^{2+} to $HEGTA^{3-}$, 5.32; Mg^{2+} to $EGTA^{4-}$, 5.21; Mg^{2+} to $HEGTA^{3-}$, 3.37; H^+ to ATP^{4-}, 7.04; H^+ to $HATP^{3-}$, 3.93; Ca^{2+} to ATP^{1-}, 4.40; Ca^{2+} to $HATP^{3-}$, 2.48; Mg^{2+} to ATP^{4-}, 4.54; Mg^{2+} to $HATP^{3-}$, 2.73; and Na^+ to ATP^{4-}, 1.15. In all cases, contributions of other components in the reaction mixture to the concentration of free Ca^{2+} were considered and deemed negligible (e.g., Ca^{2+} to phosphocreatine = 60 M^{-1}). In the absence of added $CaCl_2$, the amount of free Ca^{2+} contaminating the reaction mixtures was approximately 10^{-10} M.

Protein determinations. Protein concentrations were determined by the method of Lowry *et al.* (12) using bovine serum albumin as standard.

Materials. Dopamine (Sigma Chemical Company, St. Louis, Mo.) solutions were made immediately before use in double-distilled water containing 1.0 mM ascorbate. The final ascorbate concentration in the reaction buffer was 100 μM and did not affect cyclic AMP formation rate. [^{3}H]cyclic AMP and [α-^{32}P]ATP were purchased from New England Nuclear Corporation (Boston, Mass.). Na_2ATP, cyclic AMP, EGTA, GTP, theophylline, morpholinopropanesulfonic acid, Hepes, Tris, phosphocreatine, and creatine phosphokinase were obtained from the Sigma Chemical Company. All other chemicals were of reagent grade.

Statistical analysis. When necessary, dose-response curves were normalized by conversion to probits and significant differences in ED_{50} were analyzed as described by Finney (13).

RESULTS

The Ca^{2+} dependence of both cortical and caudate microsomal adenylate cyclase activity was determined. Figure 1 shows that the cortical cyclase exhibits a typical biphasic response to Ca^{2+}, with both one-half maximal stimulation (0.19 μM) and inhibition (1 μM) occurring at concentrations of free Ca^{2+} thought to occur physiologically. The biphasic nature of the Ca^{2+} dose-response curve has previously been reported for brain cortex adenylate cyclase (1–3). The caudate adenylate cyclase also responds to Ca^{2+} in a biphasic manner, with one-half maximal stimulation and inhibition occurring at 0.2 and 0.9 μM, respectively. In both cortex and caudate, threshold levels of Ca^{2+} stimulation occur at approximately 0.03 μM with maximal stimulation at 0.5 μM free Ca^{2+}. Although cortical activity is stimulated 7-fold by Ca^{2+}, the caudate adenylate cyclase activity is stimulated only 2-fold. Since Ca^{2+}-dependent stimulation of adenylate cyclase is conferred by calmodulin (1–3), the difference in the Ca^{2+} enhancement of adenylate cyclase activity in cortex versus caudate might suggest that calmodulin is lost from caudate but not cortical microsomes during preparation. A calmodulin loss from caudate microsomes is unlikely, since the addition of saturating levels of calmodulin (>60 μg/ml) did not restore the caudate response to levels seen in cortex (Table 1). Large excesses of calmodulin caused only a moderate (7–17%) increase in Ca^{2+}-dependent cyclase activity in cortex as well as caudate. This suggests that both preparations were nearly saturated with endogenous calmodulin.

Figure 2 shows dose-response curves to dopamine at three different free Ca^{2+} concentrations, each of which produces stimulation of the adenylate cyclase activity. In addition to this Ca^{2+}-induced enhancement, dopamine also produces a concentration-dependent stimulation. The ED_{50} values (the concentration required to produce a 50% enhancement of cyclase activity) for dopamine extrapolated from Fig. 2 are 5, 7, 4, and 8 μM for 0, 0.13, 0.27, and 0.5 μM free Ca^{2+}, respectively. Log probit analyses (13) of these dose-response curves revealed no significant differences in the ED_{50} values.

Figure 3 reveals that at a Ca^{2+} concentration of 0.35 μM, the ED_{50} for dopamine is not significantly changed (as determined by probit analysis) in the presence or absence of a large excess (>60 μg of calmodulin per milligram of microsomal protein) of calmodulin.

The effect of a maximal stimulatory concentration of dopamine (50 μM) on the biphasic dose-response curve of Ca^{2+} is presented in Fig. 4. Dopamine enhanced basal activity nearly 2-fold but had no effect on the sensitivity (one-half maximal stimulation = 0.1 μM; one-half maximal inhibition = 0.9 μM) of the cyclase to Ca^{2+}. For Ca^{2+} concentrations which stimulated the cyclase (0.03–0.5 μM), the response to dopamine was additive with the Ca^{2+} effect. At Ca^{2+} concentrations which inhibited the cyclase (>0.5 μM), the system was unresponsive to do-

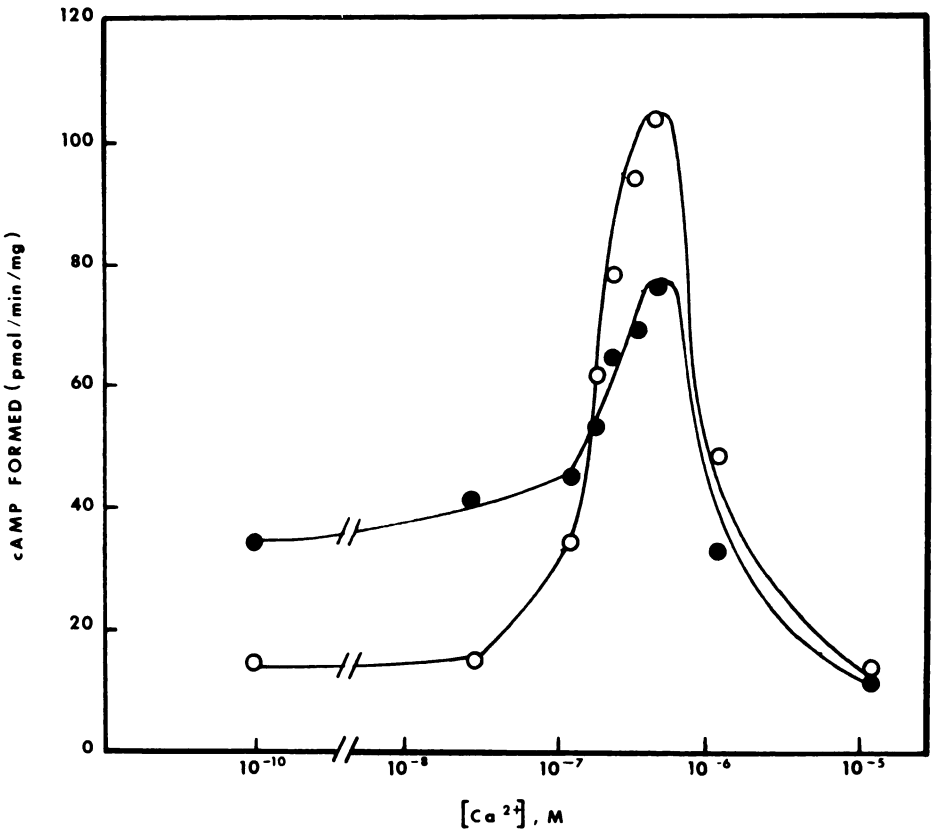

FIG. 1. *Effect of Ca^{2+} on the activity of rat cortex and caudate microsomal adenylate cyclase*

Assays were performed as described under Methods. Protein concentrations were 80–200 μg/assay for both cortex (○) and caudate (●). Each *point* represents the mean of triplicate determinations on each of four preparations. Error associated with each *point* was ≤10%. *cAMP*, cyclic AMP.

pamine. These findings are similar to those reported by Clement-Cormier *et al.* (15).

Figure 5 details the dose-response relationship of dopamine at two concentrations of Ca^{2+} which markedly inhibited cyclase activity. At both 1 and 10 μM free Ca^{2+}, no dopamine stimulation of caudate adenylate cyclase activity was observed.

DISCUSSION

In this study, the Ca^{2+} dependence of adenylate cyclase associated with rat caudate microsomes was examined.

TABLE 1

Effect of calmodulin on the microsomal adenylate cyclase of cortex and caudate[a]

	Cyclic AMP formation[b]	
	No calmodulin	10 μg Calmodulin/assay
	pmoles/min/mg	
Cortex	14.6 ± 2.3	13.6 ± 1.0
Cortex + 0.5 μM free Ca^{2+}	88.8 ± 7.5	95.9 ± 3.3
Caudate	34 ± 1.0	37 ± 1.1
Caudate + 0.5 μM free Ca^{2+}	65.7 ± 4.5	79 ± 2.3

[a] Membranes were isolated and adenylate cyclase was assayed as described under Methods. The assay volume was 250 μl. The protein concentration was approximately 100 μg/assay.

[b] Each point represents the mean and standard error of triplicate determinations on three preparations.

Cortical microsomes were prepared from the same brain to serve as an adenylate cyclase system which presents the characteristic biphasic response to Ca^{2+}. Cortical adenylate cyclase activity was stimulated (one-half maximum = 0.19 μM) and inhibited (one-half maximum = 1.0 μM) by free Ca^{2+} concentrations well within the physiological range. Caudate microsomes contained a calcium-dependent adenylate cyclase which was similarly affected by free Ca^{2+}. Values for one-half maximal stimulation (0.2 μM) and inhibition (0.9 μM) are in agreement with those seen in the cortex. The calcium requirements for rat microsomal cyclase are slightly different than those previously reported (1) for the adenylate cyclase in a crude, broken-cell preparation from guinea pig cortex (one-half maximal stimulation = 0.08 μM Ca^{2+}; one-half maximal inhibition = 0.3 μM Ca^{2+}). However, this slight discrepancy could reflect species variation or differences due to the purity of the preparation.

It is well established that the stimulatory phase of the Ca^{2+} dose-response in brain is due to calmodulin (1–3). Ca^{2+}, interacting with calmodulin in the cortical microsomes, produces a 7-fold enhancement of the basal adenylate cyclase activity (14.6 pmoles/min/mg), whereas the Ca^{2+}-calmodulin complex in caudate results in only a 2-fold increase in its basal cyclase activity (34 pmoles/min/mg). These data suggest that, relative to the cortex, the caudate adenylate cyclase is less sensitive to the stimulatory properties of Ca^{2+}. This lower sensitivity does not appear to be due to a selective loss of calmodulin

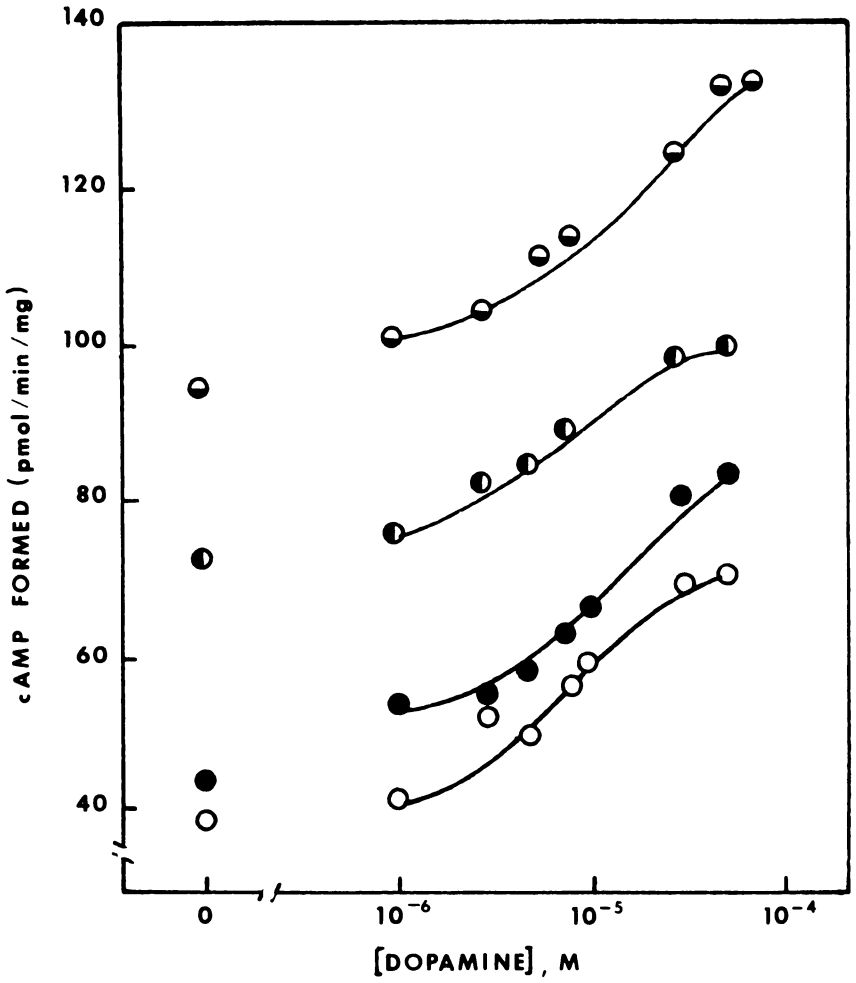

FIG. 2. *Effect of Ca^{2+} on the dopamine stimulation of rat caudate adenylate cyclase*

Assay of caudate microsomes was carried out as described under Methods. Free Ca^{2+} concentrations were 0 (○), 0.13 (●), 0.27 (◐), and 0.5 μM (◒). Each *point* represents the mean of triplicate determinations on two to five preparations. Error associated with each *point* was ≤10%. *cAMP*, cyclic AMP.

from the microsomes isolated from the caudate. The addition of a large excess of calmodulin to the caudate adenylate cyclase assays (Table 1) was not able to reconstitute a Ca^{2+} stimulatory effect to levels seen in the cortex. A lower Ca^{2+} sensitivity in caudate adenylate cyclase with respect to cortex has also been noted by Gnegy *et al.* (6).

Since calmodulin is an intracellular protein, it is reasonable to assume that the action of Ca^{2+} would occur via calmodulin on the cytoplasmic side (16) of the membrane. Dopamine initiates its effect via a cell surface receptor. This study indirectly characterizes the interaction between these two sites. The data in Figs. 2–4 indicate that Ca^{2+} and dopamine have no effect on the stimulatory properties of the other. Either agonist enhances basal caudate cyclase activity upon which the other can produce its typical dose-dependent stimulation of cyclase activity. No shift in sensitivity to the agonists is observed. Furthermore, a large excess of calmodulin enhanced basal activity without affecting the ED_{50} to dopamine. This would suggest that calmodulin and the dopamine receptor are noninteractive.

Recently, Gnegy *et al.* (7, 8) demonstrated an enhanced basal cyclase activity and a 4-fold decrease in the ED_{50} for dopamine concomitant with increases in caudate calmodulin content after 14 days of treatment with haloperidol. It has been proposed that these increases in the calmodulin content of caudate membranes after haloperidol treatment are responsible for receptor supersensitivity to dopamine (7, 8). The data reported here are not consistent with the above hypothesis. However, it must be cautioned that the results of Gnegy *et al.* (7, 8) were obtained following chronic treatment, and the effects of this treatment on calmodulin-cyclase coupling are unknown. If the effects are due simply to an enhanced level of caudate calmodulin, one might expect that an assay performed in the presence of high levels of calmodulin could mimic the supersensitive state with a resultant shift in the ED_{50} to dopamine. Figure 3 shows no such shift in ED_{50}. Calmodulin does not render the adenylate cyclase more sensitive to dopamine. In addition, other investigators have not been able to show increases in adenylate cyclase activity after chronic antipsychotic treatment (17, 18).

Dopamine stimulation of adenylate cyclase activity is seen only at Ca^{2+} concentrations between 0 and 0.5 μM.

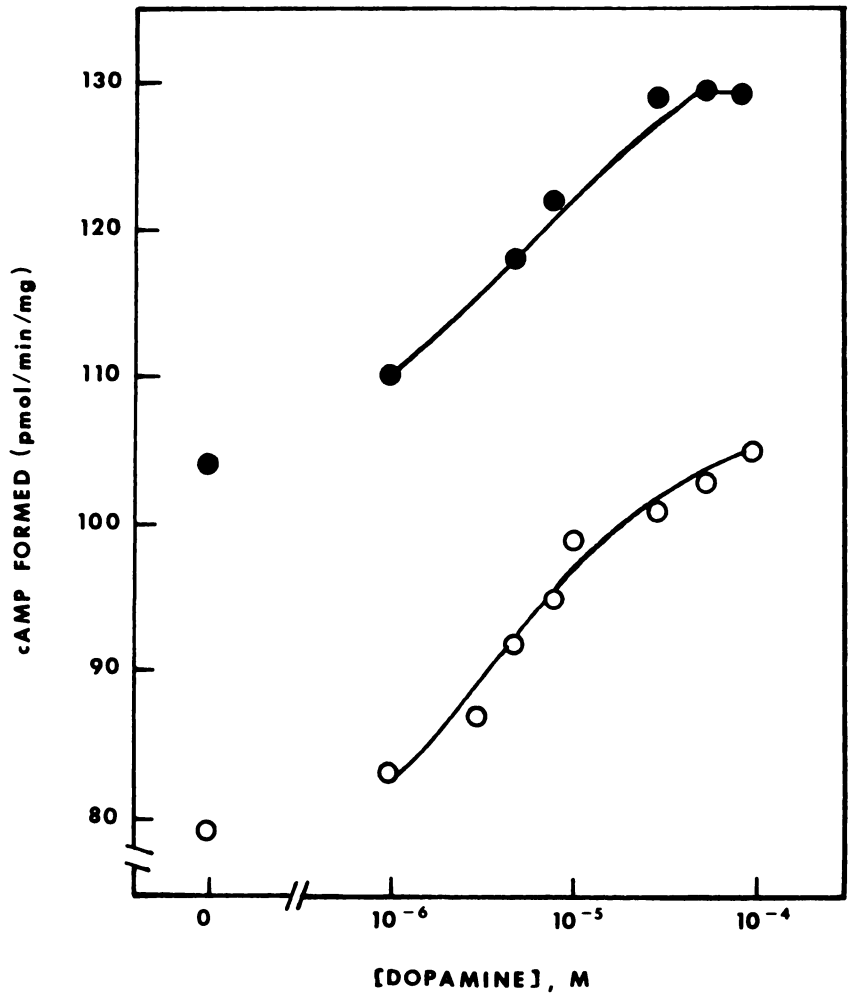

FIG. 3. *Effect of calmodulin on dopamine stimulation of adenylate cyclase*
Dose responses to dopamine were obtained in the presence of 0.35 μM free Ca^{2+} with (●) and without (○) 10 μg of purified calmodulin added to each assay. In the absence of 0.35 μM Ca^{2+}, calmodulin did not affect basal activity of the cyclase. Each *point* represents the mean of triplicate determinations on each of three preparations of caudate microsomes. Error associated with each *point* was ≤10%. *cAMP*, cyclic AMP.

Higher Ca^{2+} concentrations progressively inhibit the dopamine stimulation of cyclase activity (Figs. 4 and 5). Inhibition of adenylate cyclase is believed to occur by Ca^{2+} binding to a metal binding site distinct from calmodulin (4). Unlike calmodulin, this site can affect the response of the cyclase to dopamine. When this site is occupied by Ca^{2+}, the adenylate cyclase is refractory to the stimulatory effects of dopamine.

Thus, Ca^{2+}, acting at two distinct sites, can modify the response of caudate adenylate cyclase. When bound to calmodulin, low free Ca^{2+} concentrations can enhance cyclase activity (Figs. 1 and 4). However, at higher Ca^{2+} concentrations, when interacting at the inhibitory metal site, Ca^{2+} can inhibit all adenylate cyclase activity. The fact that this inhibition occurs over the concentration ranges of 0.5–10 μM suggests that Ca^{2+}-dependent inhibition of cyclase activity may be an important facet of Ca^{2+}-dependent regulation. Realizing that extrapolating data derived from broken-cell preparations to events occurring in the intact cell is tenuous, the following model for regulation of intracellular cyclic AMP levels in the caudate is proposed. Dopamine, by interacting with its receptor, initiates the stimulation of caudate adenylate cyclase with subsequent rises in intracellular cyclic AMP. At low free Ca^{2+} concentrations (0.03–0.5 μM), Ca^{2+} combines with calmodulin, producing an independent stimulation of cyclase activity. These effects might occur simultaneously to produce an additive effect. However, Ca^{2+} concentrations greater than 0.5 μM progressively inhibit all adenylate cyclase activity. Also, cyclic AMP phosphodiesterase has been shown to be active at Ca^{2+} concentrations which inhibit the cyclase (1). Thus, in a coordinated manner, Ca^{2+} and dopamine, acting individually or in concert, could regulate intracellular events in the caudate by modulating adenylate cyclase activity.

Whereas the model proposed above suggests the existence of a single population of caudate adenylate cyclase, the data presented herein are also consistent with the hypothesis of two distinct adenylate cyclase populations; one stimulated by Ca^{2+}, the other stimulated by dopamine (19). These populations could function independently to increase intracellular cyclic AMP levels, possibly in response to distinctly different stimuli. If such a distinct population did exist, then the data suggest that

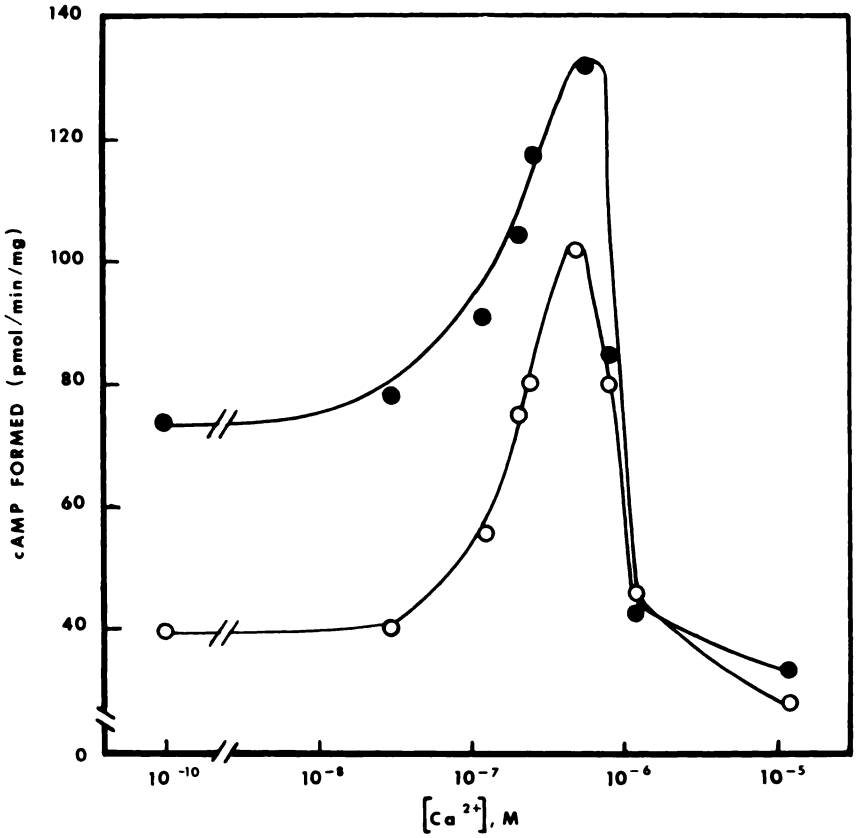

FIG. 4. *Effect of dopamine on the Ca^{2+} dependence of microsomal adenylate cyclase of rat caudate*

Ca^{2+} dependence of caudate adenylate cyclase was determined in the presence (●) and absence (○) of 50 μM dopamine. Each *point* represents the mean of triplicate determinations on each of four preparations. Error associated with each *point* was ≤10%. *cAMP*, cyclic AMP.

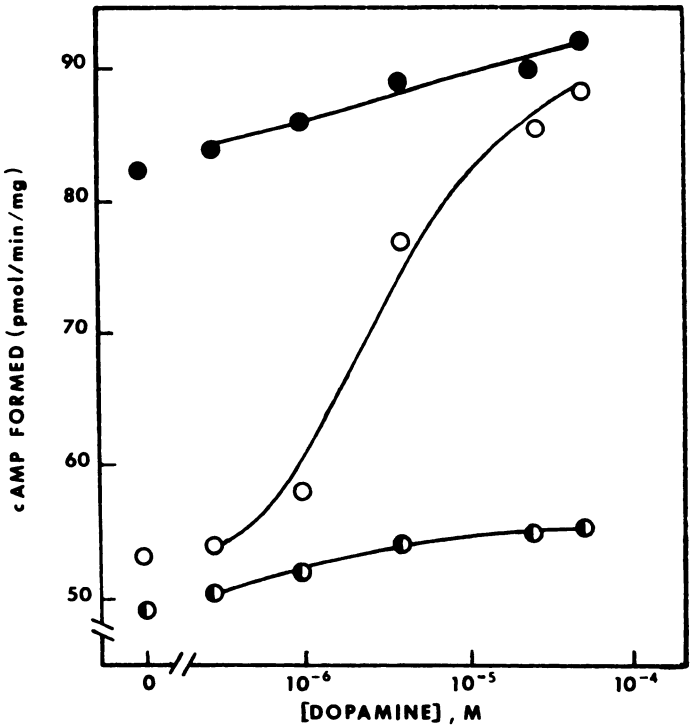

FIG. 5. *Effect of inhibitory Ca^{2+} concentrations on dopamine stimulation of adenylate cyclase*

The effect of various concentrations of dopamine was examined at Ca^{2+} concentrations which are inhibitory to adenylate cyclase. Ca^{2+} concentrations employed were 0 (○), 1 (●), and 10 μM (◐). Data are the mean of triplicate determinations on two preparations. Error associated with each *point* was ≤10%. *cAMP*, cyclic AMP.

the dopamine-sensitive form of the enzyme would be inhibited but not stimulated by Ca^{2+} (See Figs. 4 and 5). If both Ca^{2+} and dopamine-sensitive forms of the enzyme were present in the caudate, then our results imply that calmodulin is not associated with, or is unable to stimulate, the dopamine form of the enzyme. The fact that Ca^{2+} can inhibit dopamine-dependent stimulation of caudate adenylate cyclase (Figs. 4 and 5) suggests that if multiple forms of adenylate cyclase did exist, then both the Ca^{2+} and dopamine-sensitive forms would have an inhibitory Ca^{2+} binding site. Furthermore, both forms would be inhibited over the same range of Ca^{2+} concentrations (0.5–10 μM).

If both Ca^{2+} and dopamine-sensitive forms of caudate cyclase occurred on the same cell type, our results suggest that Ca^{2+} may be important in regulating cyclic AMP levels in the caudate. At low levels (0–0.5 μM), Ca^{2+} would combine with calmodulin to enhance the Ca^{2+}-sensitive form of caudate cyclase without affecting the dopamine-sensitive form of the enzyme. However, Ca^{2+} concentrations greater than 0.5 μM would progressively inhibit cyclic AMP (Ca^{2+}- and dopamine-stimulated) synthesis. If the Ca^{2+}- and dopamine-sensitive forms of cyclase occurred in discretely different areas of the caudate, the results presented here suggest that the effect of Ca^{2+} on cyclic AMP levels would depend on the form of cyclase present.

The results of the present study clearly illustrate that minor changes in the levels of free Ca^{2+} can have marked effects on the adenylate cyclase activity of caudate microsomes. Minor changes in intracellular free Ca^{2+} may

also have a marked effect on regulation in the intact cell. If true, this finding could provide an insight into the mechanism of action of various drugs, including the opiates and sedative-hypnotics, which are thought to involve changes in Ca^{2+} fluxes (20–23). Furthermore, studies have suggested that both the acute and chronic effects of the opiates involve changes in adenylate cyclase activity (24). Thus, these results could suggest that the mechanism by which opiates regulate cyclase activity may be coupled to the drug-induced changes in intracellular free Ca^{2+} concentration.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Henry Zot and the helpful supervision of manuscript preparation by Diane Reed, Deborah Turner, and Joyce Zerkle.

REFERENCES

1. Piascik, M. T., P. L. Wisler, C. L. Johnson, and J. D. Potter. Calcium-dependent regulation of guinea pig brain adenylate cyclase. *J. Biol. Chem.* **255**:4176–4181 (1980).
2. Brostrom, C. O., Y-C. Huang, B. M. Breckenridge, and D. J. Wolff. Identification of a calcium-binding protein as a calcium dependent regulator of brain adenylate cyclase. *Proc. Natl. Acad. Sci. U. S. A.* **72**:64–68, (1975).
3. Cheung, W. Y., L. S. Bradham, T. J. Lynch, Y. M. Lin, and E. A. Tallant. Protein activator of cyclic 3′,5′-nucleotide phosphodiesterase of bovine or rat brain also activates its adenylate cyclase. *Biochem. Biophys. Res. Commun.* **66**:1055–1062 (1975).
4. Potter, J. C., M. T. Piascik, P. L. Wisler, and C. L. Johnson. Calcium-dependent regulation of brain and cardiac muscle adenylate cyclase. *Ann. N. Y. Acad. Sci.* **356**:220–230 (1981).
5. Kebabian, J. W., G. L. Petzold, and P. Greengard. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat and its similarity to the "dopamine receptor". *Proc. Natl. Acad. Sci. U. S. A.* **69**:2145–2149 (1972).
6. Gnegy, M. E., T. Hultin, and G. Treisman. Effect of calmodulin on catecholamine linked adenylate cyclase activity in rat striatum and cerebral cortex, in *Receptors for Neurotransmitters and Peptide Hormones* G. Pepew, M. J. Kuhar, and S. J. Enna., (eds.) Raven Press, New York, 125–131 (1980).
7. Gnegy, M. E., P. Uzunov, and E. Costa. Participation of an endogenous Ca^{2+} binding protein activator in the development of drug-induced supersensitivity of striatal dopamine receptors. *J. Pharmacol. Exp. Ther.* **202**:558–564 (1977).
8. Gnegy, M. E., A. Lucchelli, and E. Costa. Correlation between drug-induced supersensitivity of dopamine dependent striatal mechanisms and the increase in striatal content of the Ca^{2+} regulated protein activator of cAMP phosphodiesterase. *Naunyn-Schmeideberg's Arch. Pharmacol.* **301**:121–127 (1977).
9. Glowinski, J., and L. L. Iverson. The disposition of [^{3}H]-norepinephrine in various regions of the brain. *J. Neurochem.* **13**:658–669 (1966).
10. Salomon, Y., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541–548 (1974).
11. Sillen, L. G., and A. E. Martell. *Stability Constants of Metal Ion Complexes*, Special Publication No. 17, Ed. 2. The Chemical Society, Burlington House, London (1964).
12. Lowry, O. H., H. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
13. Finney, D. J. *Probit Analysis.* Cambridge University Press, New York (1964).
14. Deleted in proof.
15. Clement-Cormier, Y. C., R. G. Parrish, G. L. Petzold, J. W. Kebabian, and P. Greengard. Characterization of a dopamine-sensitive adenylate cyclase in the rat caudate nucleus. *J. Neurochem.* **25**:143–149 (1975).
16. Roufogalis, B. D. Calmodulin: its role in synaptic transmission. *Trends Neurosci.* **3**:238–241 (1980).
17. Kreuger, B. K., J. Forn, J. R. Walters, R. H. Roth, and P. Greengard. Stimulation by dopamine of adenosine cyclic 3′,5′-monophosphate formation in rat caudate nucleus: effect of lesions of the nigroneostriatal pathway. *Mol. Pharmacol.* **12**:639–648 (1976).
18. Von Voigtlander, P. F., E. G. Losey, and H. J. Trozenberg. Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. *J. Pharmacol. Exp. Ther.* **193**:88–94 (1975).
19. Premont, J., M. Perez, and J. Bockaert. Adenosine-sensitive adenylate cyclase in rat striatal homogenates and its relationship to dopamine and Ca^{2+}-sensitive adenylate cyclases. *Mol. Pharmacol.* **13**:662–670 (1977).
20. Hood, W. F., and R. A. Harris. Effect of depressant drugs and sulfhydryl reagents on the transport of calcium by isolated nerve endings. *Biochem. Pharmacol.* **29**:957–959 (1980).
21. Harris, R. H., and W. F. Hood. Inhibition of synaptosomal calcium uptake by ethanol. *J. Pharmacol. Exp. Ther.* **213**:562–568 (1980).
22. Leslie, S. W., M. B. Freedman, R. E. Wilcox, and S. V. Elrod. Acute and chronic effects of barbiturates on depolarization-induced influx into rat synaptosomes. *Brain Res.* **185**:409–417 (1980).
23. Ross, D. H., and H. L. Cardenas. Nerve cell calcium as a messenger for opiate and endorphin actions, in *Neurochemical Mechanisms of Opiates and Endorphins* H. H. Loh and D. H. Ross, (eds.). Raven Press, New York, 301–336 (1978).
24. Sharma, S. K., W. A. Klee, and M. Nirenberg. Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc. Natl. Acad. Sci. U. S. A.* **72**:3092–3096 (1975).

Send reprint requests to: Dr. Michael T. Piascik, Department of Pharmacology, A. B. Chandler Medical Center, University of Kentucky, Lexington, Ky. 40536.