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Characterization of Dopaminergic Receptors in Dispersed Bovine Parathyroid Cells

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SUMMARY

BROWN, E. M., M. F. ATTIE, S. REEN, D. G. GARDNER, J. KEBABIAN AND G. D. AURBACH. Characterization of dopaminergic receptors in dispersed bovine parathyroid cells. *Mol. Pharmacol.* **18:** 335–340 (1980).

Several dopaminergic ligands were assessed for their effects on cAMP accumulation, adenylate cyclase activity, and parathyroid hormone (PTH) release from dispersed bovine parathyroid cells. Dopamine, 6,7-dihydroxy-1,2,3,4 tetrahydronaphthalene (ADTN), 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine (SKF-38393), and apomorphine increased cAMP content 30-, 25-, 10-, and 2- to 3-fold with K_a's of 0.6, 0.5, 1, and 1–3 μM, respectively. These same agents also stimulated adenylate cyclase activity up to 4-fold and PTH release 1.3- to 3-fold. With the exception of lisuride (K_i = 15 nM), ergot derivatives were of relatively low potency (K_i's = 0.4–20 μM) in inhibiting dopamine-stimulated cAMP accumulation. Similar K_i's were observed for effects on dopamine-stimulated adenylate cyclase activity and PTH release. Interactions of these ligands with the dopamine receptor could be clearly differentiated from effects of β-adrenergic or α-adrenergic receptors also known to modulate cAMP accumulation and secretion in this cell type. These results demonstrate the presence of a D-1 dopaminergic receptor on bovine parathyroid cells and document further the relationship in this receptor subclass between adenylate cyclase activity, cAMP accumulation, and a biologic response.

INTRODUCTION

We have previously shown (1) that dispersed bovine parathyroid cells have a dopaminergic receptor associated with agonist-induced elevations in cAMP content and parathyroid hormone (PTH)[1] release. Recently, it has been suggested (2) that there are two broad subclasses of receptors for dopamine. One category of receptor, designated as D-1, can enhance either intracellular cAMP content or adenylate cyclase activity in broken cell preparations, while the second category of receptor, designated as D-2, appears to achieve its physiological effects by mechanisms unrelated to enhanced formation of cyclic AMP. Several ergot derivatives discriminate between these two categories of receptor. Ergots such as dihydroergocryptine or bromergocryptine are potent agonists, mimicking the effects of dopamine upon the D-2 receptor; however, these agents are predominantly antagonists of low potency upon the D-1 receptor. In order to categorize further the parathyroid dopamine receptor, we have detailed the interactions of various dopaminergic ligands with this system. The effects of agonists and antagonists on cAMP accumulation, adenylate cyclase activity, and PTH release substantiate the D-1 characteristics of this receptor, the role of cAMP in mediating its biologic response, and its differentiation from other catecholamine receptors on this cell type (3, 4).

MATERIALS AND METHODS

Dispersed bovine parathyroid cells were prepared as described (5). In brief, minced bovine parathyroid tissue was incubated with 0.2% collagenase (Worthington, type I) and 50 μg/ml DNase (Sigma, DNH-II); cells were obtained by pipetting the minced tissue through a 10-ml disposable plastic pipet (Falcon). The cell suspension was then sedimented and resuspended two or three times at 50 *g* in Eagle's medium No. 2 (bicarbonate deleted) with 0.5 mM $MgSO_4$ and 20 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) ("standard medium"), as well as 1.5 mM $CaCl_2$ and 2% heat-inactivated bovine serum albumin (BSA). Incubations were carried out at

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[1] Abbreviations used: PTH, parathyroid hormone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; BSA, bovine serum albumin; PCA, perchloric acid; ADTN, 6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene; SKF-38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3 benzazepine.

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37°C with a shaking incubator (Dubnoff-Precision Scientific Instruments) in 20-ml plastic disposable scintillation vials (Beckman) with 0.5- to 1.0-ml volume of standard medium containing 1.0 mM $CaCl_2$ and 0.2% heat-inactivated BSA.

PTH release was determined by radioimmunoassay as described (5), following sedimentation of the cell pellet for 1–2 min at 500*g* in a desk-top centrifuge (Sorvall GLC-1). Total (cells plus medium) cAMP was determined by radioimmunoassay with a modification of the method of Harper and Brooker (6), following extraction with 5% (v/v) perchloric acid (PCA), neutralization with $KHCO_3$, and acetylation. Of the total cAMP 80–90% is intracellular after 5 min of incubation with a variety of agonists (7).

For adenylate cyclase assays, particulates of parathyroid cell lysates were prepared from freshly dispersed cells as described (M. F. Attie, E. M. Brown, *et al.*, submitted for publication). In brief, dispersed cells were resuspended in hypotonic medium (30 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM EGTA, and 2 mM mercaptoethanol). Following sedimentation at 1000*g* for 10 min, the pellet was resuspended in the same volume of 10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, and 2 mM mercaptoethanol. Following a second sedimentation at 1200*g* for 10 min, the pellet was suspended in 50 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM dithiothreitol, and 0.25 M sucrose and frozen under liquid nitrogen.

Adenylate cyclase assays were performed with a modification of the method of Salomon *et al.* (8). The assay mixture, with a final volume of 0.07 ml, contained 50 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 10 mM KCl, 1 mM EGTA, 5 mM cAMP, 1 mg/ml bovine serum albumin, 0.125 mM [α-^{32}P]ATP (8×10^5 cpm/tube), 5 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase, and 5–15 μg of lysate protein. Incubations were carried out for 10 min at 37°C and terminated by the addition of 0.1 ml of 50 mM Hepes, pH 7.5 with 2.2 mM ATP and [^{3}H]cAMP (2×10^5 cpm/ml) followed by boiling for 3 min. cAMP was recovered through sequential Dowex and alumina columns (9). Recoveries for [^{3}H]cAMP were consistently 60–85%.

Cell counts were determined with a hemocytometer immediately prior to use. Reagents were of the best grade commercially available. Dopaminergic ligands were obtained from the following sources: dopamine—Sigma Chemical Company, St. Louis, Missouri, ADTN—Burroughs Wellcome Company, Research Triangle Park, North Carolina; SKF-38393—Smith Kline and French Laboratories, Philadelphia, Pennsylvania; apomorphine—Merck, Sharp and Dohme, West Point, Pennsylvania; α-flupenthixol—H. Lundbeck & Company, Ltd., DK-2500, Valby, Denmark; lisuride—Schering Pharmaceuticals Bloomfield, New Jersey; lergotrile—Eli Lilly and Company, Indianapolis, Indiana; molindone—Endo Laboratories, Garden City, New York; dihydroergocryptine and bromergocryptine—Sandoz Pharmaceuticals, East Hanover, New Jersey.

RESULTS

Effects of dopaminergic agonists and antagonists on cAMP accumulation. As reported previously (1), dopamine stimulated cAMP accumulation maximally 30-fold in dispersed bovine parathyroid cells with a K_a of 0.6 μM. ADTN, SK-38393 (10), and apomorphine, which are dopaminergic agonists in other systems, also elevated cAMP content with K_a's of 0.5, 1 and 3 μM, respectively (Fig. 1). Maximal stimulation, however, was less than that observed with dopamine (25-, 10-, and 2- to 3-fold, respectively). In the cases of dopamine and apomorphine, cAMP accumulation at 10^{-4} M was less than or equal to that observed at 10^{-5} M (data not shown). SKF-38393- and ADTN-stimulated cAMP accumulation were inhibited 90% by 10^{-5} M α-flupenthixol, a potent dopaminergic antagonist in this system (1), but less than 20% by 10^{-5} M (−)-propranolol (not shown). Lergotrile and bromergocryptine, potent D-2 agonists (2), caused only a slight, if any, elevation in cAMP content (10–30% above control). Lisuride, a potent D-2 agonist (2), and molindone, a D-2 antagonist (2), caused no significant cAMP accumulation.

Dopamine-stimulated cAMP accumulation was inhibited by lisuride, lergotrile, molindone, dihydroergocryptine, and bromergocryptine with K_i's of 15 nM, 0.7 μM, 0.4 μM, 0.9 μM, and 20 μM, respectively (Fig. 2A). The low potency of bromergocryptine was not due to a slow time course for binding to the dopamine receptor, since preincubation of parathyroid cells with this ligand for up to 1 h prior to the addition of dopamine did not alter the K_i. The competitive interaction of lisuride and dopamine was suggested by the apparent decrease in potency of dopamine in the presence of increasing concentrations of lisuride, without a decrease in maximal cAMP accumulation (Fig. 2B). The K_i for lisuride derived by Schild plot analysis (11) of the data in Fig. 2B was also 15 nM.

Effects of dopaminergic ligands on adenylate cyclase activity. The activation of adenylate cyclase in parathyroid cell lysates by agonists or the inhibition of dopamine-stimulated activity by various antagonists are shown in

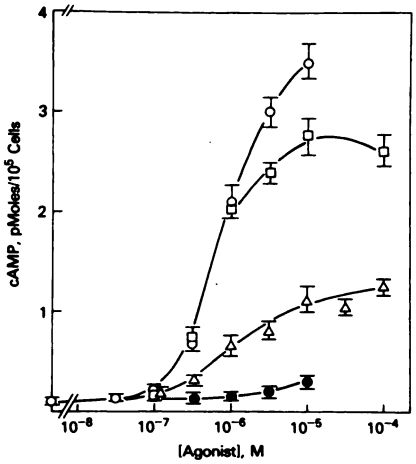

FIG. 1. *Effects of dopaminergic agonists on cAMP accumulation*
Dispersed parathyroid cells (300,000–600,000/ml) were incubated at 37°C for 5 min with increasing concentrations of dopamine (○), ADTN (□), SKF-38393 (△), or apomorphine (●) in standard medium with 1.0 mM calcium. The reaction was terminated by the addition of 5% PCA (final v/v) and cAMP was determined by radioimmunoassay as described under Materials and Methods. Results indicate the mean ± SEM for duplicate determinations on each of three independent incubation vials.

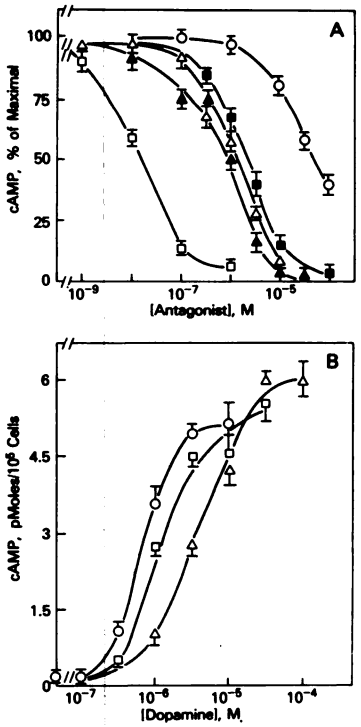

FIG. 2A. *Effects of dopaminergic antagonists on dopamine-stimulated cAMP accumulation*

Dispersed parathyroid cells (300,000–400,000/ml) were incubated for 5 min at 37°C in standard medium with 1.0 mM calcium as well as 10^{-6} M dopamine and increasing concentrations of lisuride (□), molindone (▲), lergotrile (△), dihydroergocryptine (■), or bromergocryptine (○). Samples were processed and cAMP determined as in Fig. 1. Points represent the mean ± SEM for three independent observations. Data are expressed as percentage of cAMP observed with dopamine alone.

FIG. 2B. *Effect of lisuride on dopamine-stimulated cAMP accumulation*

Dispersed cells (300,000/ml) were incubated as Fig. 2A with increasing concentrations of dopamine alone (○), or dopamine plus 10^{-8} M lisuride (□) or 10^{-7} M lisuride (△). cAMP was determined as in Fig. 2A. Points are the mean ± SEM for three independent observations.

Figs. 3A and B. GTP was employed in the assay buffer in order to enhance enzyme activation by dopamine (M. F. Attie, E. M. Brown, *et al.*, submitted for publication). Maximal stimulation by dopamine was 4-fold, while those due to ADTN, SKF-38393, and apomorphine were 3.8-, 4.2-, and 2.0-fold, with K_a's of 4, 3, and 2 μM. Lisuride, lergotrile, molindone, dihydroergocryptine, and bromergocryptine inhibited dopamine-stimulated adenylate cyclase activity with K_i's of 0.16, 0.4, 0.9, 3.3, and 2.1 μM, respectively.

Effects of dopaminergic ligands on PTH release. The stimulation of PTH release by dopaminergic agonists and the inhibition of dopamine-stimulated secretion by various antagonists are shown in Figs. 4 and 5. PTH release was maximally stimulated two- to threefold by dopamine, as reported previously (2). ADTN, SKF-38393, and apomorphine stimulated PTH release to 100, 70, and 30% of the level observed for dopamine with K_a's of 0.13, 0.3, and 1–3 μM, respectively. Lisuride, lergotrile, and molindone did not alter PTH release by themselves, but inhibited dopamine-stimulated PTH release with K_i's of 0.1, 0.9, and 0.7 μM, respectively. Because of the low potency of bromergocryptine, and since it was necessary to dissolve this agent as well as dihydroergocryptine in organic solvents, the effects of these ligands on secretion were not tested. A comparison of the effects of the various ligands tested on cAMP accumulation, adenylate cyclase activity, and PTH release is shown in Table 1.

Effects of dopaminergic ligands on other catecholamine receptors. Dispersed parathyroid cells have been shown to possess β-adrenergic (3) and α-adrenergic receptors (4). Since ligands interacting with catecholamine receptors frequently are not specific for a single type of receptor, the effects of the ligands employed above were tested on β-adrenergic and α-adrenergic receptors as well. Interactions of antagonists with β-adrenergic receptors were assessed by effects on cAMP accumulation stimulated by isoproterenol, a nearly pure β-adrenergic agonist in this system (3) (Fig. 6). Only lisuride inhibited isoproterenol-stimulated cAMP accumulation at concentrations of less than 10^{-5} M.

The interactions of dopamine antagonists with α-adrenergic receptors were assessed by the potentiation of (−)-epinephrine-stimulated cAMP accumulation. In dispersed bovine parathyroid cells, α-adrenergic agonists inhibit cAMP accumulation enhanced by β-adrenergic or

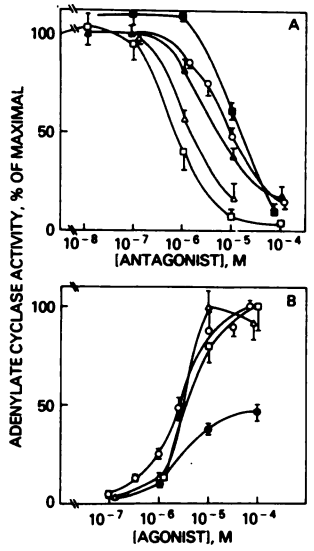

FIG. 3. *Effects of dopaminergic agonists and antagonists on bovine parathyroid adenylate cyclase*

(A) Inhibition of dopamine-stimulated adenylate cyclase activity by dopaminergic antagonists. Adenylate cyclase activity was determined following incubation of cell particulates for 10 min at 37°C with 10^{-5} M dopamine, 10^{-5} M GTP, and increasing concentrations of lisuride (□), molindone (△), lergotrile (△), dihydroergocryptine (■), or bromergocryptine (○). Points represent the mean ± SEM for triplicate incubations. Adenylate cyclase activity for GTP alone was 410 and for dopamine + GTP 1170 pmol/mg protein/10 min. (B) Stimulation of adenylate cyclase by dopamine agonists. Particulates of parathyroid cell lysates were incubated for 10 min at 37°C with increasing concentrations of dopamine (○), ADTN (□), SKF-38393 (△), or apomorphine (●) as well as 10^{-5} M GTP, and adenylate cyclase was determined as under Materials and Methods. Points represent the mean ± SEM for triplicate incubations. Adenylate cyclase activity for GTP alone was 280 and for 10^{-4} M dopamine + GTP 1100 pmol/mg protein/10 min.

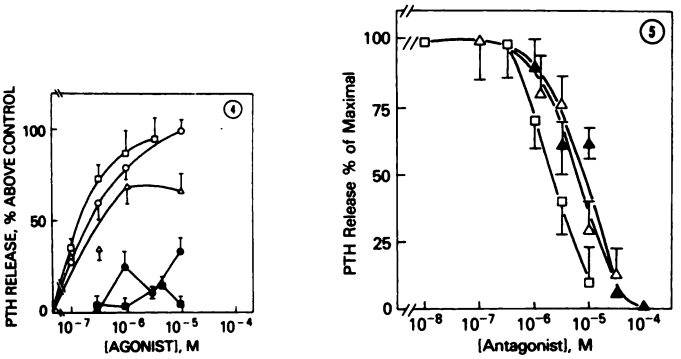

FIG. 4. *Effects of dopaminergic agonists on PTH release*

Dispersed parathyroid cells (400,000–500,000/ml) were incubated for 5 min at 37°C in standard medium with 1.0 mM calcium and increasing concentrations of dopamine (□), ADTN (○), SKF-38393 (△), lergotrile (■), or apomorphine (●). PTH was determined by radioimmunoassay on supernatant samples as under Materials and Methods. Points represent the mean ± SEM of PTH determined from three independent incubation vials, each analyzed in duplicate. Data are expressed as percentage increase in secretion over control. Dopamine-stimulated and control PTH release were 6.6 and 3.3 ng/10^5 cells/5 min.

FIG. 5. *Effects of dopaminergic antagonists on dopamine-stimulated PTH release*

Dispersed cells (400,000–500,000/ml) were incubated for 5 min at 37°C in standard medium with 1.0 mM calcium as well as 3×10^{-6} M dopamine and increasing concentrations of lisuride (□) or 1×10^{-6} M dopamine and increasing concentrations of molindone (▲) or lergotrile (△). Points represent the mean ± SEM for PTH in triplicate incubation vials determined as in Fig. 4. Data are expressed as percentage of dopamine-stimulated PTH release. Dopamine-stimulated and control PTH release were 5.0 and 2.6 ng/10^5 cells/5 min with 3×10^{-6} M dopamine and 7.3 and 3.3 ng/10^5 cells/5 min with 10^{-6} M dopamine.

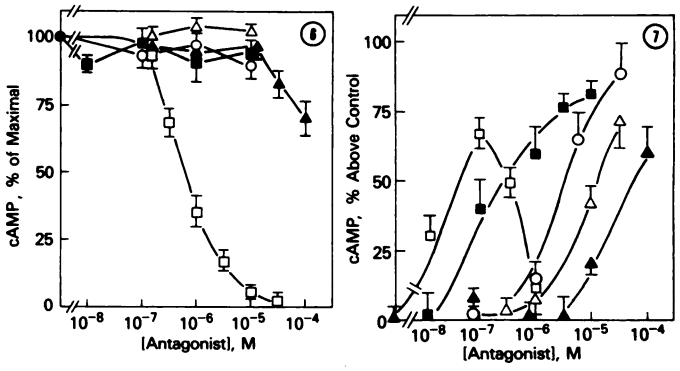

FIG. 6. *Effects of dopaminergic antagonists on isoproterenol-stimulated cAMP accumulation*

Dispersed cells (150,000–250,000/ml) were incubated for 5 min at 37°C in standard medium with 1.0 mM calcium, 10^{-6} M (−)-isoproterenol, and increasing concentrations of lisuride (□), molindone (▲), lergotrile (△), dihydroergocryptine (■), or bromergocryptine (○). Samples were processed and cAMP determined as in Fig. 1. Results are expressed

as percentage of the value for cAMP observed with isoproterenol alone (4.4 to 6.0 pmol/10^5 cells/5 min); points represent mean ± SEM for triplicate incubation vials.

FIG. 7. *Effects of dopaminergic antagonists on (−)epinephrine-stimulated cAMP accumulation*

Dispersed cells (200,000–300,000)/ml) were incubated for 5 min at 37°C in standard medium with 1.0 mM calcium, 3×10^{-6} M (−)-epinephrine and increasing concentrations of lisuride (□), molindone (▲), lergotrile (△); dihydroergocryptine (■), or bromergocryptine (○). Samples were processed and cAMP determined as in Fig. 1. Results are expressed as percentage above the value for cAMP observed with (−)-epinephrine alone (1.5 to 2.8 pmol/10^5 cells/5 min); points represent the mean ± SEM for triplicate incubation vials.

dopaminergic ligands (4). This inhibition is competitively antagonized by α-adrenergic blockers such as phentolamine. Thus, stimulation of cAMP accumulation by (−)-epinephrine is the sum of its stimulatory β- and inhibitory α-adrenergic properties. α-Adrenergic antagonists, therefore, potentiate the stimulatory β-adrenergic effect of epinephrine nearly to the level observed with isoproterenol (4).

TABLE 1

K_a or K_i of various dopaminergic agonists or antagonists for effects on cAMP accumulation, adenylate cyclase activity, or PTH release from dispersed bovine parathyroid cells

Agonist or antagonist	K_a[a] or K_i[b] (μM)		
	cAMP accumulation	Adenylate cyclase	PTH release
Dopamine	0.6	3	0.2
ADTN	0.5	4	0.15
SKF-38393	1	3	0.3
Lisuride	0.015	0.16	0.09
Molindone	0.4	0.9	0.7
Lergotrile	0.7	0.37	0.9
Dihydroergocryptine	0.9	3.3	ND[c]
Bromergocryptine	21	2.1	ND

[a] Defined as the concentration of agonist resulting in one-half of the maximal response.

[b] Calculated from the relationship $K_i = IC_{50}/(1 + [S]/K_a)$, where IC_{50} is the concentration of antagonist half-maximally inhibiting the response due to a concentration $[S]$ of dopamine. K_a is defined as in footnote *a* for effects of dopamine on cAMP accumulation, adenylate cyclase, or PTH release. In the case of lisuride, K_i was also calculated according to the Schild plot (11) from data in Fig. 2B. Results indicate the mean of two or more experiments.

[c] Not done.

All of the dopaminergic antagonists tested in this manner (Fig. 7) showed evidence of being α-adrenergic antagonists as well. The augmentation of (−)-epinephrine-stimulated cAMP content by lergotrile and bromergocryptine could not be explained by their own weak effects on cAMP accumulation, since these agents by themselves increased cAMP by less than 0.1 pmol/10^5 cells. In the cases of dihydroergocryptine and bromergocryptine, ethanol itself, which was employed to enhance solubility of the ligands, raised cAMP levels and these increases were subtracted to determine the effect due to the α-adrenergic antagonism per se. A comparison of the relative potency of the various ligands as α-adrenergic, β-adrenergic, and dopaminergic antagonists is shown in Table 2. Because of being both an alpha- and beta-adrenergic antagonist, lisuride caused a biphasic effect on epinephrine-stimulated cAMP accumulation (see Discussion). Phentolamine has no inhibitory effect on dopaminergic-stimulated effects in this system at concentrations as high as 10^{-5} M (data not shown).

DISCUSSION

In dispersed bovine parathyroid cells, there is a close relationship between intracellular cAMP content and parathyroid hormone release for agents either stimulat-

TABLE 2

Comparison of the interactions of various dopaminergic antagonists with bovine parathyroid dopaminergic, β-adrenergic, or α-adrenergic receptors

K_i's represent values derived from effects on cAMP accumulation in intact parathyroid cells

Antagonist	K_i[a] (M) for		
	Dopaminergic receptor	β-Adrenergic receptor	α-Adrenergic receptor
Lisuride	0.015	0.055	0.01[b]
Molindone	0.4	[c]	3.6
Lergotrile	0.7	[d]	0.9
Dihydroergocryptine	0.9	[d]	0.018
Bromergocryptine	20	[d]	0.3

[a] K_i was calculated as in Table 1, based on the following K_a's: Dopamine—0.6 μM, (−)-isoproterenol—0.1 μM, (−)-epinephrine—0.3 μM (as α-adrenergic agonist).

[b] Because of the biphasic effects of lisuride, the results are expressed as an IC_{50}.

[c] 30% Inhibition at 10^{-4} M.

[d] No inhibition at 10^{-5} M.

ing or inhibiting secretion (7). In addition, this cell type has beta-adrenergic (3), alpha-adrenergic (4), and dopaminergic (1) receptors. This system, therefore, has several advantages for studying this regulation by dopaminergic receptors of cyclic nucleotide metabolism and cellular function.

Several dopaminergic ligands, which increase adenylate cyclase activity in other tissues such as striatum (12, 13) or retina (14), also enhance cAMP accumulation, adenylate cyclase activity, and secretion of PTH in dispersed bovine parathyroid cells. Dopamine and ADTN are full agonists in this system, while SKF-38393 and apomorphine are partial agonists.[2] It is of interest that maximal stimulation by partial agonists was not equivalent for cAMP accumulation, adenylate cyclase activity, and hormone release. For SKF-38393, maximal stimulation of these parameters (as percentage of that observed with dopamine) was 30, 100, and 70%, respectively; for apomorphine, it was 10, 50, and 30%. However, because of the log-linear relationship in this system between increases in cAMP accumulation and PTH release (7), small changes in cAMP content may be associated with large changes in secretion. Thus, SKF-38393, while causing only 30% of the maximal cAMP response of dopamine, results in a nearly equivalent increase in PTH secretion. In fact, 30–50% of the maximal cAMP response to isoproterenol or dopamine is sufficient to stimulate secretion maximally (7). The large secretory response to small changes in cAMP content also helps to explain why agonists have a slightly lower and antagonists a slightly higher K_a or K_i for effects on PTH release as compared with cAMP accumulation (7).

The reasons underlying the greater intrinsic activity of partial agonists in activating adenylate cyclase relative to cAMP accumulation are not completely clear. It has previously been shown, however, that guanine nucleotides significantly enhance dopamine-stimulated adenylate cyclase in this system (Attie, Brown, *et al.*, submitted for publication). Since adenylate cyclase assays in the present studies were carried out with saturating concentrations of GTP, it is possible that the "coupling" between receptor binding and adenylate cyclase was enhanced. A similar effect has been noted in other systems (15), where agonists such as dopamine, not normally stimulating cAMP accumulation in intact cells, become partial agonists for β-adrenergic-mediated activation of adenylate cyclase in the presence of Gpp(NH)p. An additional effect of guanine nucleotides (15) is to lower the K_a of agonists toward, although not necessarily to, the values observed in intact cells. This phenomenon may explain why K_a's of agonists for adenylate cyclase are three- to eightfold higher than those for cAMP accumulation, while K_i's of antagonists for both parameters are generally in good agreement.[3] In any case, it is clear that the intrinsic efficacy and potency of a ligand must be defined in terms of the particular response employed to measure it (receptor binding, cAMP accumulation, adenylate cyclase activity, biologic response, etc.).

Kebabian and Calne (2) have recently subclassified dopaminergic receptors in part on the basis of changes in cAMP content or adenylate cyclase activity. D-1 receptors are associated with agonist-induced increases in these parameters, suggesting that bovine parathyroid cells possess this class of dopamine receptor. The affinities and relative intrinsic activities of dopamine, ADTN, and apomorphine in this system are similar to those observed in caudate nucleus (12) or retina (14). SKF-38393, which may be a specific D-1 agonist (10), was also a partial agonist in the caudate nucleus, although it had an apparent affinity nearly 100-fold greater than that of dopamine. The reasons for the lower potency of this ligand in the present studies are not clear.

In D-2 systems, ergot derivatives are generally agonists of high (nanomolar) potency. Such agents, on the other hand, have been antagonists of lower (micromolar) potency in D-1 systems, The potency of ergot derivatives as dopamine antagonists in dispersed parathyroid cells is between 10^{-7} and 10^{-5} M; there is reasonably good agreement between K_i's derived from effects on cAMP accumulation, adenylate cyclase activity, or hormone release. The difference in potency of bromergocryptine in parathyroid cells (K_i = 20 μM for cAMP accumulation) and in the mammotroph (16), a D-2 system (K_i = 2.9 nM for binding), is particularly striking. Thus, results with antagonists are also consistent with the D-1 classification of the bovine parathyroid dopamine receptor.

The presence of distinct α-adrenergic, β-adrenergic,

[2] We have reported that apomorphine was a pure antagonist of dopamine-stimulated cAMP accumulation (1), while in the present study a weak stimulatory effect was noted (two- to threefold). This stimulatory effect was apparently not observed previously because it was less than 10% of the effect of dopamine or epinine.

[3] Lisuride and bromergocryptine showed approximately a 10-fold difference in potency between K_i's derived from effects on cAMP accumulation vs adenylate cyclase activity. This discrepancy most likely reflects the differences in experimental design: cAMP accumulation was performed with intact cells in plastic scintillation vials and 5-min incubation; adenylate cyclase assays were carried out with cell lysates in glass tubes and a 10-min incubation.

and dopaminergic catecholamine receptors on a single cell type allows for comparison of the interaction of different ligands with these receptors. The stimulatory effects of dopamine, ADTN, and SKF-38393 appear to be mediated nearly exclusively through dopaminergic receptors since they are inhibited by the potent dopamine antagonist α-flupenthixol but not the β-adrenergic anatogonist (−)-propranolol. Possible interactions of these agents with the α-adrenergic receptor were not tested.

All dopaminergic antagonists employed in these studies were also α-adrenergic antagonists. The relative potencies of these ligands, however, at the two receptors differed considerably. Molindone, for example, was the second most potent dopaminergic antagonist of cAMP accumulation (K_i = 0.4 μM), but the least potent α-adrenergic antagonist (K_i = 3.6 μM). Dihydroergocryptine was a relatively weak dopaminergic antagonist (K_i = 0.9 μM), but a potent α-adrenergic antagonist (K_i = 0.018 μM). Only lisuride interacted with all three receptors with significant potency. Its inhibition of both α- and β-adrenergic receptors apparently accounts for the biphasic effects of this ligand on epinephine-stimulated cAMP. At low concentrations, reversal of the inhibitory α-adrenergic effects of epinephrine results in an increase in cAMP, while at higher concentrations, inhibition of the stimulatory β-adrenergic effects of the catecholamine produces a decrease in cAMP levels. These results point out the multiple pharmacologic actions of these agents. Moreover, in attempting to assess interactions with a single class of receptors, it is important to define and correct for effects on other receptors.

The present studies further define the presence and characteristics of a D-1 dopaminergic receptor on bovine parathyroid cells and its differentiation from other catecholamine receptors on this cell type. The close correspondence between cAMP levels, adenylate cyclase activity, and the biologic secretory response in this system strongly support the role of adenylate cyclase and cAMP in mediating the biologic response of dopamine in the D-1 receptor.

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