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Biochemistry

Dopaminergic stimulation of cyclic AMP accumulation and parathyroid hormone release from dispersed bovine parathyroid cells

(dopamine/dispersed cells)

E. M. BROWN*, R. J. CARROLL, AND G. D. AURBACH

Metabolic Diseases Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

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ABSTRACT **The effects of dopaminergic agonists and antagonists have been studied in dispersed bovine parathyroid cells. Dopaminergic agonists caused a transient 20- to 40-fold increase in cellular cyclic AMP and a 2- to 3-fold increase in parathyroid hormone release. Dose-response relationships were similar for cyclic AMP accumulation and hormone release, whether studied by increasing agonist concentration or by increasing concentration of antagonist with constant agonist. The effects on the dopamine receptor could be differentiated from those of the previously characterized β-adrenergic receptor by specific inhibitors. These results appear to represent proof with a homogeneous cell population that dopaminergic receptors linked to adenylate cyclase can regulate a secretory process mediated by cyclic AMP. This system should be useful in further studies on dopamine receptors and should provide a valid tool for determining interactions of radiolabeled ligands with such receptors.**

Specific dopaminergic receptors have been found in various tissues (1–6). Studies in central (7, 8) and peripheral (9) nervous tissue have shown an accumulation of cyclic AMP (cAMP) in response to dopaminergic agonists specifically inhibitable by compounds of the phenothiazine and butyrophenone classes and clearly differentiated from effects due to other biogenic amines. More recently, studies with radiolabeled agonists and antagonists (10, 11) have suggested that it is possible to identify dopamine receptors by direct binding methods and to correlate effects on binding with alterations in biologic activity or cAMP accumulation.

In most of these studies, preparations from heterogeneous cell populations represented by whole organs, tissue slices, homogenates, or membrane fractions had been used, and functional capacity of the cells had not been determined. Thus, it has been difficult to correlate alterations in biological function directly with changes in cAMP, the presumed intracellular mediator of these effects. We recently described the preparation of viable dispersed bovine parathyroid cells which retain many of the morphologic and functional properties recognized physiologically (12). We now show that this cell preparation contains a dopamine receptor that is unequivocally differentiated from the β-adrenergic receptor previously identified in this tissue (13–16). Alterations in cellular cAMP correlate directly with changes in parathyroid hormone (PTH) release, the biologic consequence of increased cAMP in this cell type. Dispersed bovine parathyroid cells thus provide a useful model for studying the interaction of dopaminergic agonists and antagonists with target tissues.

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MATERIALS AND METHODS

Dispersed bovine parathyroid cells were prepared as described (12) by digestion with collagenase and DNase. Incubations were carried out in 20-ml polypropylene scintillation vials (Beckman) in a 37° metabolic shaker (Dubnoff-Precision Scientific Instruments); the medium was Eagle's medium number 2 (bicarbonate deleted) supplemented with 0.02 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.47), 0.5 mM $MgSO_4$, variable $CaCl_2$ as indicated, and 0.2% heat-inactivated bovine serum albumin ("standard medium"). Agonists and antagonists were added directly to the cell suspension (100,000–400,000 cells per ml). In time-course experiments, separate vials were used for each time point.

Medium was separated from cells in 400-μl Microfuge tubes (Beckman) and assayed for PTH with a guinea pig anti-bovine-PTH antiserum (12). cAMP was extracted with 5% (wt/vol) perchloric acid, neutralized with potassium bicarbonate, acetylated, and determined by radioimmunoassay with a modification of the method of Harper and Brooker (17). In several experiments, intracellular and extracellular cAMP were determined separately after sedimentation of the cell pellet for 30 sec in a Microfuge (Beckman, model B).

Cell counts with a hemocytometer were carried out just prior to incubation. Cell viability (trypan blue exclusion) was routinely 95–100%. Reagents were of the best grade commercially available and were obtained from sources previously cited (13). Epinine was a generous gift of K & K Laboratories, Inc., Plainview, NJ. Fluphenazine was donated by E. R. Squibb and Sons, Princeton, NJ, chlorpromazine by Smith, Kline and French Laboratories, Philadelphia, PA, and spiroperidol by Janssen Pharmaceuticals, Beerse, Belgium. α- and β-flupenthixol and apomorphine were generous gifts of J. Kebabian.

RESULTS

Effects of Dopaminergic Agonists and Antagonists on cAMP Accumulation. cAMP accumulation was stimulated 20- to 40-fold by 10 μM dopamine (Fig. 1). Intracellular cAMP reached a maximum between 5 and 10 min and gradually returned to baseline over the next 30–60 min. Extracellular cAMP increased progressively from 0 to 120 min, reaching a maximum between 60 and 120 min. Because intracellular cAMP was maximal and 75% or more of the cyclic nucleotide was intra-

Abbreviations: cAMP, cyclic AMP; PTH, parathyroid hormone; IC_{50}, concentration of antagonist resulting in 50% inhibition of cAMP accumulation.

* Address reprint requests to: Dr. E. M. Brown, Bldg. 10, Room 9D-20, National Institutes of Health, Bethesda, MD 20014.

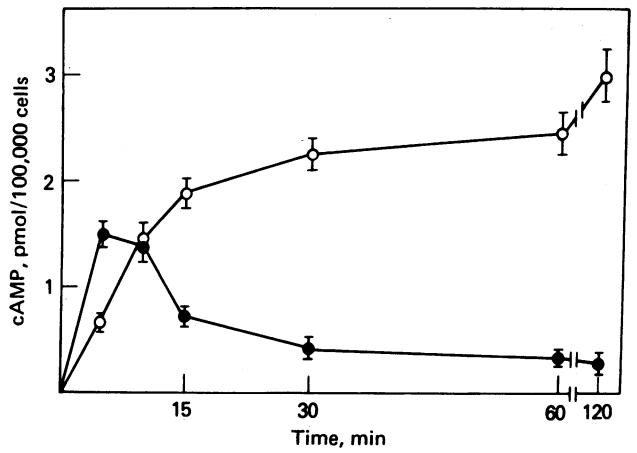

FIG. 1. Time course of dopamine (10 μM) stimulation of intracellular and extracellular cAMP. Dispersed parathyroid cells (400,000/ml) were incubated at 37° in standard medium with 1.0 mM $CaCl_2$ for the times indicated. The cells were then sedimented as described in *Materials and Methods* and intracellular (●) and extracellular (○) cAMP were determined separately by radioimmunoassay. The points represent mean ± SEM for six determinations (duplicate samples from each of three incubation vessels) with one batch of cells.

cellular at 5 min, dose-response relationships for various agonists and antagonists were studied at this time interval.

A representative example of the accumulation of cAMP in isolated parathyroid cells in response to dopaminergic agonists is shown in Fig. 2. In a number of experiments, dopamine and epinine (*N*-methyldopamine) were approximately equipotent and resulted in nearly equivalent maximal cAMP accumulation. Apomorphine, on the other hand, caused no detectable alterations in cAMP.

The accumulation of cAMP in response to 1 μM dopamine was inhibited by the potent phenothiazines fluphenazine and chlorpromazine with 50% inhibition concentrations (IC_{50}) of about 0.1 and 0.3 μM (Fig. 3). α-Flupenthixol was the most potent compound tested with IC_{50} of about 60 nM. In contrast, the *trans* form of this compound (β-flupenthixol) had only 1% of the potency of α-flupenthixol. Although inactive as an agonist, apomorphine was an effective antagonist of dopaminergic stimulation of cAMP, with an IC_{50} of about 1 μM (Fig. 3). The butyrophenone spiroperidol had relatively low potency with an IC_{50} of 10 μM (not shown). In the same experiment, the β-adrenergic antagonist (−)propranolol at 10 μM inhibited cAMP accumulation 15% or less. The α-adrenergic inhibitor phentolamine had no effect on dopamine-stimulated cAMP accumulation at 10 μM.

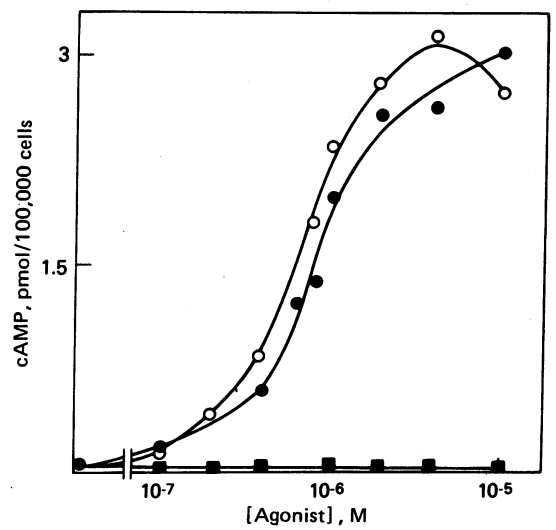

FIG. 2. Stimulation of cAMP accumulation by dopaminergic agonists. Dispersed parathyroid cells (200,000/ml) were incubated as in Fig. 1 at 37° with increasing concentrations of dopamine (●), epinine (○), or apomorphine (■). After 5 min, the reaction was stopped with 5% perchloric acid, and cAMP was determined as in *Materials and Methods*. Points represent the mean of six determinations as for Fig. 1.

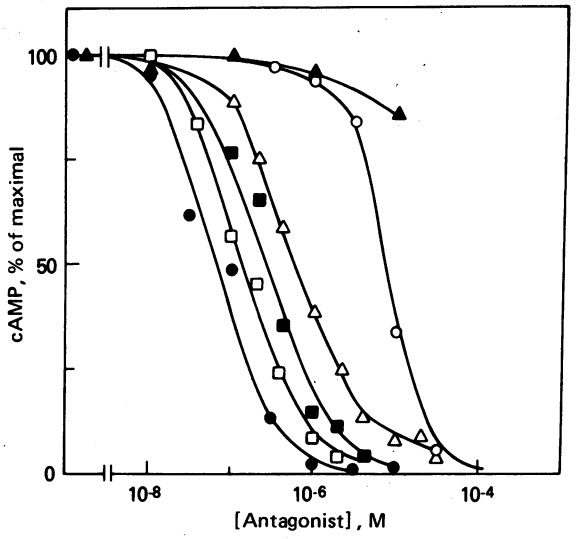

FIG. 3. Inhibition of dopamine-stimulated cAMP accumulation by dopaminergic agonists. Dispersed cells (150,000/ml) were incubated as in Fig. 1 for 5 min at 37° with 1 μM dopamine and increasing concentrations of α (●) or β-flupenthixol (○), flupenazine (□), chlorpromazine (■), apomorphine (△), or (−)propranolol (▲). cAMP was then extracted with 5% perchloric acid and determined as in *Materials and Methods*. Points represent the mean of six determinations as in Fig. 1.

Correlations of cAMP Accumulation with PTH Release. PTH release was stimulated 2- to 3-fold by dopamine at 1–10 μM (Figs. 4 and 5). The time course of PTH release was analogous to that of cAMP accumulation (Fig. 4). An increase in

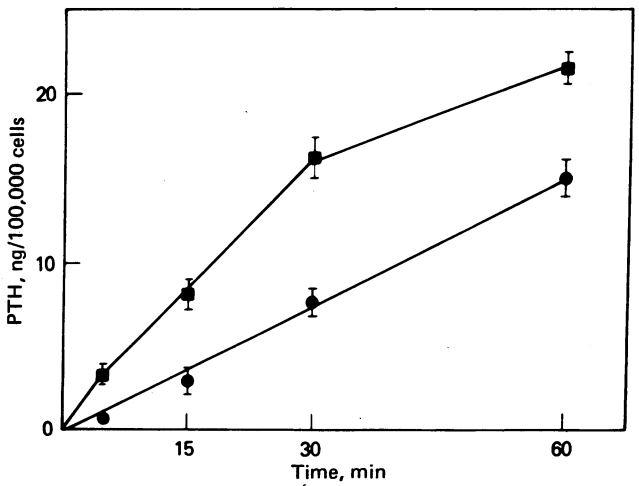

FIG. 4. Time course of stimulation of PTH release by dopamine. Dispersed cells (400,000/ml) were incubated at 37° in standard medium with 1.0 mM $CaCl_2$. At the times indicated, samples were collected and assayed for PTH by radioimmunoassay as described in *Materials and Methods*. Points indicate mean ± SEM of six determinations as for Fig. 1. ●, 1.0 mM Ca; ■, 1.0 mM Ca plus 1 μM dopamine.

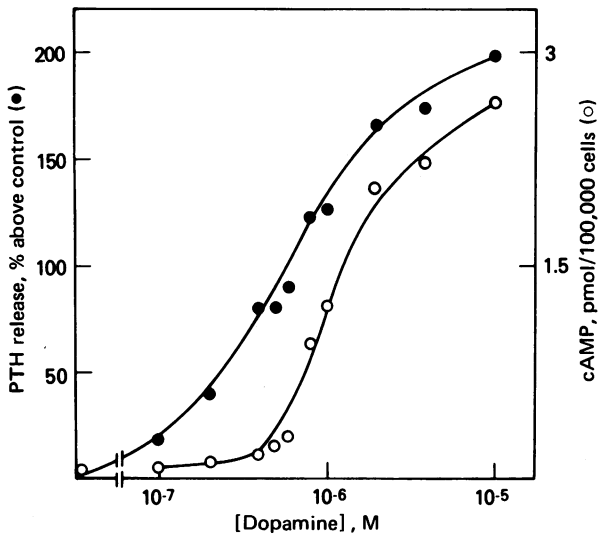

FIG. 5. Correlation of effects of dopamine on cAMP accumulation and PTH release. Dispersed cells (400,000/ml) were incubated for either 5 min (cAMP) (○) or 15 min (PTH) (●) at 37° in standard medium with 1.0 mM $CaCl_2$. Samples were then collected and assayed for cAMP or PTH as described in Figs. 1 and 4. Points indicate the mean of six determinations as for Fig. 1.

PTH release was clearly detectable at 5 min and persisted for 30–45 min.

In order to characterize more completely the relationship between cAMP accumulation and PTH release, these two parameters were directly compared in a single batch of cells incubated with increasing concentrations of dopamine (Fig. 5). PTH release was studied at 15 min, because release was nearly linear at this point and the larger absolute increment over control at 15 min compared to 5 min facilitated measurement of immunoreactive PTH. Increases in PTH release and cAMP accumulation were nearly parallel although PTH release was stimulated by approximately 50% lower concentrations of dopamine.

A similar relationship was observed between inhibition of

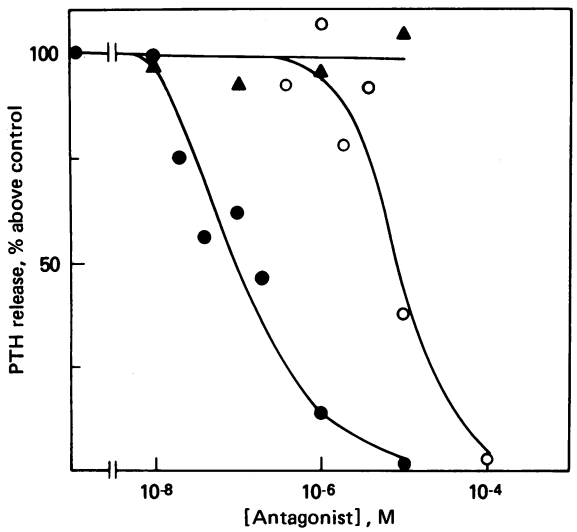

FIG. 6. Effects of dopaminergic inhibitors on dopamine-stimulated PTH release. Dispersed parathyroid cells (300,000/ml) were incubated in standard medium with 1.0 mM $CaCl_2$ for 15 min at 37° with 1 μM dopamine and increasing concentrations of α-flupenthixol (●), β-flupenthixol (○), or (−)propranolol (▲). Samples were assayed for PTH as in Fig. 4. Points represent the mean of six determinations as for Fig. 1.

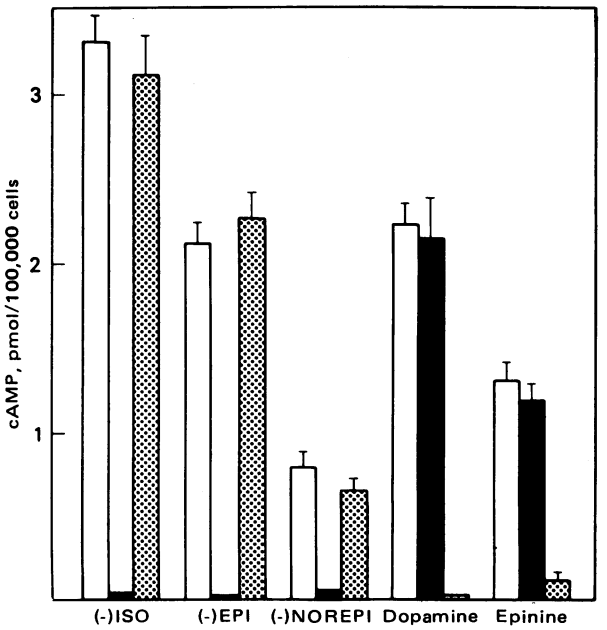

FIG. 7. Specificity of β-adrenergic and dopaminergic stimulation of cAMP accumulation. The agents, 0.2 μM (−)isoproterenol [(−)-ISO], 0.3 μM (−)epinephrine [(−)EPI], 2 μM (−)norepinephrine [(−)NOREPI], 1 μM dopamine, and 1 μM epinine were incubated for 5 min at 37° as in Fig. 3 with 150,000 isolated cells per ml, either alone (open bars), with 1 μM (−)propranolol (solid bars), or with 10 μM α-flupenthixol (stippled bars). cAMP was extracted with perchloric acid and determined as in *Materials and Methods*. Data are shown as mean ± SEM of six determinations as for Fig. 1.

dopamine-stimulated cAMP accumulation and PTH release (Fig. 6). Approximately equal IC_{50} values were observed for effects on cAMP and PTH with α- and β-flupenthixol. (−)-Propranolol had no significant effect on dopamine-stimulated PTH release at concentrations as high as 10 μM.

Differentiation of Dopamine and β-Adrenergic-Stimulated cAMP Accumulation. The β-adrenergic agonists (−)isoproterenol, (−)epinephrine, and (−)norepinephrine stimulated cAMP accumulation 10- to 40-fold (Fig. 7). This stimulation was inhibited virtually completely by propranolol but not significantly by 10 μM α-flupenthixol. In contrast, cAMP accumulation stimulated by dopamine and epinine was inhibited 95–100% by α-flupenthixol but not significantly by 1 μM (−)propranolol.

DISCUSSION

We showed previously a close relationship between effects of β-adrenergic agonists and antagonists on cAMP accumulation and PTH release from dispersed bovine parathyroid cells (13). These results indicated that this system might be a useful model for investigating the mechanism of action of various hormones and neurotransmitters that act through cAMP.

In the process of screening a number of such compounds, dopamine was found to stimulate cAMP accumulation markedly. Like β-adrenergic agonists (unpublished data), dopamine caused a transient increase in intracellular cAMP accompanied by a larger and more prolonged increase in extracellular cAMP, indicating a significant release of cellular cyclic nucleotide into the medium. Maximal concentrations of cAMP achieved with dopamine or the nearly equipotent agonist epinine were approximately equivalent to those seen with (−)isoproterenol. PTH release followed a time course similar to that of intracellular cAMP and was also stimulated to a similar degree, as observed previously with β-adrenergic agonists (13).

A close relationship was also observed between cAMP accumulation and PTH release in dose-response studies with dopamine agonists and antagonists. Increasing concentrations of dopamine stimulated PTH release with a 2-fold higher potency than for stimulation of cAMP accumulation. A similar shift in the dose-response for PTH was observed with β-adrenergic stimulation (unpublished data), confirming that maximal effects of PTH are obtained with agonist concentrations resulting in a submaximal cAMP response. A close correlation also was observed with inhibition of dopamine-stimulated PTH release and cAMP accumulation.

Although apomorphine caused no effect on cellular cAMP content *per se*, it effectively inhibited cAMP accumulation due to either dopamine or epinine. Its potency as an inhibitor ($IC_{50} \cong 1\ \mu M$) was comparable to that observed in other systems, although apomorphine generally has had the properties of an agonist or partial agonist. The low potency of spiroperidol in this system contrasts with certain systems (11) in which the potency of the butyrophenones is comparable to that of the phenothiazines. In certain other cases (3, 10), however, the most potent phenothiazines have been up to 100-fold more potent than spiroperidol, similar to the ratio observed here.

Effects of dopamine on cAMP content and PTH release could be differentiated unequivocally from those due to β-adrenergic stimulation. Whereas the potent β-adrenergic blocker (−)-propranolol essentially completely inhibited cAMP accumulation due to β-adrenergic agonists, the dopaminergic blocker α-flupenthixol had no effect. Dopamine- or epinine-stimulated cAMP accumulation, on the other hand, was inhibited totally by the dopaminergic antagonist and not at all by the β-adrenergic blocker.

Studies on dopaminergic systems in isolated cell populations are few (5, 6), and the current results represent the sole description of a homogeneous cell preparation with a secretory function mediated by a dopaminergic receptor coupled through the adenylate cyclase-cAMP mechanism. Earlier reports that dibutyryl cAMP or aminophylline cause secretion of PTH (15) indicate that cAMP generated in parathyroid cells mediates PTH release in response to dopamine as well as other potential or known secretogogues. In the current experiments, the specificity of the dopamine effect on cAMP and secretion was substantiated further by results with stereospecific (α- versus β-flupenthixol) inhibition of dopaminergic–receptor interaction. These general findings suggest that isolated parathyroid cells should be useful for further studies on the dopamine receptor and provide a system allowing validation of radiolabeled ligand interaction with this receptor.

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