Dopamine Receptors

EDITED BY

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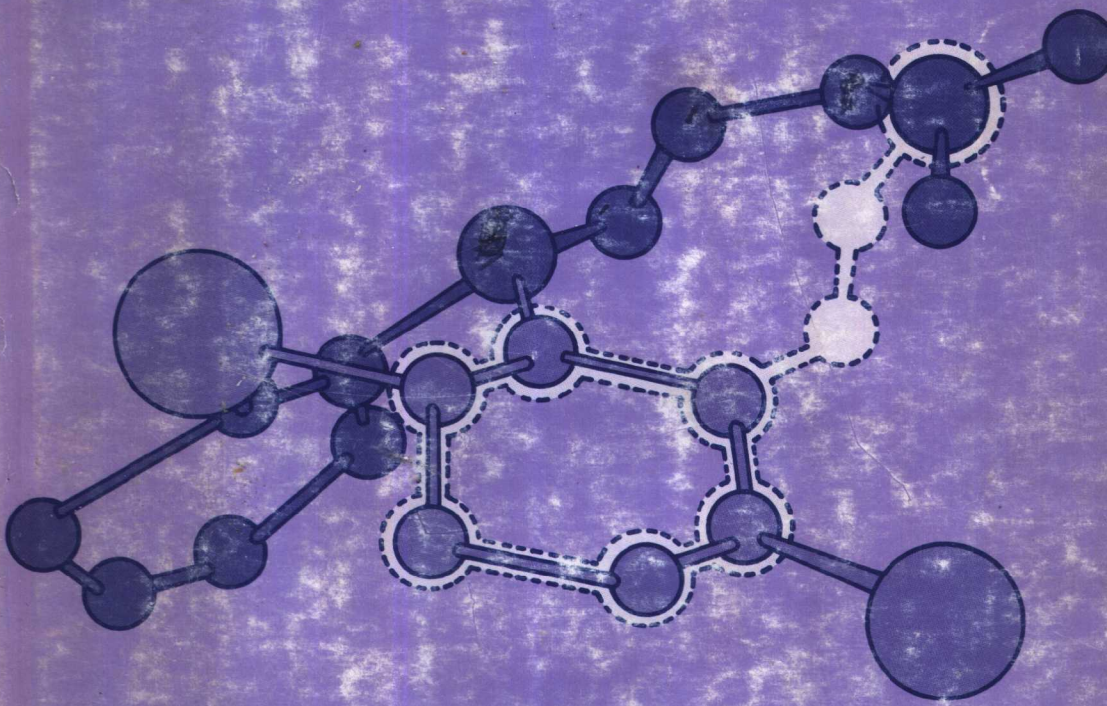

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Dopamine Receptors

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

EDITORS' PREFACE

THE INVITATION BY J. L. NEUMEYER and the Division of Medicinal Chemistry of the American Chemical Society to organize the symposia "Multiple Categories of Dopamine Receptors" and "Modulation of Dopamine Receptors" offered us the opportunity to highlight some of the recent advances in the understanding of dopamine receptors and the drugs interacting with these receptors. Several years ago, John Kebabian presented the "two dopamine receptor hypothesis." This hypothesis is the theme of the first symposium. Similarly, several years ago, Carl Kaiser participated in the discovery of SK&F 38393, a dopaminergic agonist relatively selective for the D-1 receptor. The second symposium focuses attention on the development of novel agonists for dopamine receptors and their use as therapeutic agents. Because some of these differentiate between different dopamine receptors, the concept of multiple categories of dopamine receptors is an integral part of the second symposium.

The requirement of the American Chemical Society that all material published by the Society be subjected to outside review offered an opportunity for us to solicit a second (and sometimes conflicting) opinion about the material presented in these symposia. It cannot be denied that the topic of dopamine receptor(s) remains an area of ongoing investigation, controversy, and disagreement. For many questions about dopamine receptors, the "final verdict" is not yet in. Indeed, if these symposia have delineated the areas of disagreement, the readers of this volume form the jury. For each disagreement or misunderstanding highlighted in this volume, the reader can review the ideas of the different authors and then decide which observations and interpretations are most helpful to their individual endeavors.

The editors are grateful to each of the authors of chapters and to those who contributed commentaries on these chapters. We also acknowledge with gratitude the financial support of Smith Kline & French Laboratories.

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PREFACE

BY LESLIE L. IVERSEN

THE CHAPTERS PRESENTED HERE REPRESENT an interesting state-of-the-art reflection of current trends in research on dopamine receptors. D. Calne and T. A. Larsen are probably accurate in suggesting that "dopamine has overtaken acetylcholine and norepinephrine as the most extensively investigated neurotransmitter in the nervous system." Their admirable survey of present and potential clinical uses of dopamine agonists and antagonists indicates that this research effort has indeed led to useful new therapeutic agents, including those acting on peripheral or endocrine targets as well as centrally acting drugs. In particular, the cardiovascular and endocrine effects of dopamine have attracted considerable interest, both from the basic and applied research viewpoints. Furthermore, S. Szabo and J. L. Neumeyer suggest that the actions of dopamine in the gastrointestinal tract, and its possible etiological role in peptic ulcers, will represent an important new focus for future work on the peripheral actions of dopamine.

The availability of peripheral models offers an important means of characterizing the pharmacological properties of dopamine receptors. In many cases it is possible to measure a clear-cut tissue response and, thus, to establish the agonist, partial agonist, and antagonist properties of test compounds. It is perhaps only now being recognized by neurochemists that receptors cannot be characterized fully in any other way. Ten years ago, with the discovery of new biochemical approaches to the study of dopamine receptors in CNS many of us were doubtless too optimistic in thinking that such approaches would lead to rapid progress in defining the characteristics of dopamine receptors. The fundamental problem in achieving such understanding, however, has been that we do not know what dopamine does as neurotransmitter in the various CNS pathways that contain it. Furthermore, there are no simple model systems that allow one to measure agonist and antagonist effects on CNS targets. We cannot know how reliable the results of neurochemical studies of dopamine receptors are if we have no biological response against which to assess the neurochemical data. A recent review (*1*) listed some 28 different applica-

tions of radioligand binding methods to the study of dopamine receptors in brain, that use more than twenty different agonist or antagonist radioligands. Remarkably little is said in the present volume about radioligand binding assays, and I suspect J. C. Stoof caught the mood of the meeting in stating: "Binding studies, although easy to perform, have yielded too many data, too many categories of dopamine receptors and too many controversies." The neurochemical emphasis has clearly shifted to "functional" assays, in which some biochemical response is measured, rather than simply occupation of receptor binding sites by ligands. The activation or inhibition of adenylate cyclase has proved a valuable model in this sense, and other biochemical responses may prove similarly useful (e.g., inhibition of peptide hormone or neurotransmitter release in response to dopamine agonists).

In this volume a good deal of emphasis is placed on studies of dopamine receptors in pituitary. This emphasis seems well justified. The clear-cut effects of dopamine in suppressing prolactin secretion from anterior lobe mammotrophs, and the inhibitory effects on secretion of α-MSH and related secretory products from intermediate lobe are important models for dopamine receptor studies. In both cases new evidence was put forward to support the hypothesis that the actions of dopamine on the secretory cells are mediated by inhibition of adenylate cyclase. This is far easier to demonstrate in the intermediate lobe, where all cells appear to respond to dopamine, than in the anterior lobe, where the dopamine-sensitive cells probably represent only a small minority.

In terms of multiple receptor categories, the suggestion made by Kebabian and Calne (*2*) of a distinction between D-1 and D-2 subtypes, based on whether the receptors lead to stimulation of adenylate cyclase or not, has been widely accepted. It now seems that in many cases (perhaps all) the D-2 sites are also coupled to adenylate cyclase, although in an inhibitory rather than stimulatory manner. All of the studies on pituitary place the dopamine receptors there clearly in the D-2 category. M. Caron et al., however, report interesting new results that indicate that these sites can exist in more than one form—with about half of the sites in the resting state in a form with high affinity for agonists and half in a low agonist affinity state. These forms can be interconverted, and guanyl nucleotide or NEM treatment shifts the population mainly to the agonist high affinity form. This may also help to explain the observations of Kebabian et al. that agonists were far more potent in intact pituitary cell preparations than in broken cell preparations.

There remain many difficulties in further understanding the nature of the different dopamine receptor categories. We continue to lack suitably selective agonists or antagonists for the D-1 and D-2 sites. In terms of agonists, bromocriptine and related ergoline derivatives are still the most selective D-2-stimulants, and the series of benzazepines related to SK&F 38393 are the most promising D-1-selective agents (J. Weinstock et al.). The discovery that benzazepines act in a stereochemically specific manner, and the resolution of the active and inactive stereoisomeric forms, offers further hope for more selective agonists in future. D. E. Nichols provides a detailed and thoughtful review of the medicinal chemistry aspects of dopamine agonist design. There is still no D-1-selective antagonist, although sulpiride and related benzamides are widely used as selective D-2-antagonists. The availability of at least one tissue model for D-1 receptors, the stimulatory effects of dopamine on parathyroid hormone secretion from bovine parathyroid cells, is of considerable importance, but we still have no corresponding model to elucidate the possible function of D-1 sites in the CNS.

There is also considerable difficulty in relating the biochemical classification of D-1 and D-2 sites to the pharmacologically defined dopamine receptor subtypes, described by L. Goldberg and J D. Kohli, on the basis of their painstaking analysis of agonist/antagonist actions on cardiovascular responses. They describe two subcategories of dopamine receptors, but these *do not* correspond readily to D-1 and D-2 sites. Thus, their "DA_1" receptors that cause relaxation of vascular smooth muscle have an agonist specificity similar to the D-1 sites: with an absolute requirement for a catechol grouping; rigid catechol analogues such as ADTN are fully active; ergolines are inactive. The responses are blocked by neuroleptics, but unlike the D-1 site, which is quite unresponsive to sulpiride, the DA_1 receptors are potently blocked by sulpiride. The "DA_2" receptors, mediating presynaptic control of norepinephrine release from sympathetic nerve terminals, resemble D-2 sites in their specificity, but again the comparison is not precise. Interestingly, the DA_2 sites can be stimulated even by some monophenolic agonists. This is intriguing because some of the newly described "autoreceptor agonists" in CNS, such as 3-PPP (*3*) are monophenolic structures. The topical question of whether the receptors located on the surface of dopamine neurons in CNS (autoreceptors) represent a unique pharmacological class does not perhaps receive as much attention in this volume as it should have, although the issue is discussed in some detail by J. C. Stoof. Although such receptors clearly resemble the D-2 class in many respects, there remains the suspicion that there may be some subtle differences.

Despite the large effort already directed to studies of dopamine and dopamine receptors it is clear that many questions remain unanswered. The area continues to be one of considerable promise and intellectual vigor and from this ferment useful new pharmacological and, possibly, new therapeutic tools may eventually emerge.

Literature Cited

1. Seeman, P. *Pharmac. Rev.* **1980**, *32*, 229–313.
2. Kebabian, J. W.; Calne, D. B. *Nature (London)* **1979**, *277*, 93–96.
3. Hjorth, S.; Carlsson, A.; Wikström, H.; Lindberg, P.; Sanchez, D.; Hacksell, U.; Arvidsson, L. E.; Svensson, U.; Nilsson, J. L. G. *Life Sci.* **1981**, *28*, 1225–1238.

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CONTENTS

D-1 Dopamine Receptor-Mediated Activation of Adenylate Cyclase, cAMP Accumulation, and PTH Release in Dispersed Bovine Parathyroid Cells

EDWARD M. BROWN and BESS DAWSON-HUGHES

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The evidence supporting the existence of a specific category of dopamine receptor on the parenchymal cells of the bovine parathyroid gland and the possible biochemical mechanisms by which dopamine stimulates the release of parathyroid hormone are reviewed. The dopamine receptor on the bovine parathyroid cell is compared to other dopamine receptors.

The parathyroid glands play a major role in normal calcium homeostasis (1). Calcium is generally recognized as the principal physiological regulator of the release of parathyroid hormone (PTH) (2). When the plasma concentration of ionized calcium decreases, PTH secretion increases. In turn, this PTH acts to raise plasma calcium by three mechanism: first, PTH increases renal tubular reabsorption of calcium; second, PTH enhances the release of skeletal calcium; and third, PTH increases gastrointestinal absorption of calcium by stimulating renal formation of 1,25 dihydroxyvitamin D. These three mechanisms elevate the plasma concentration of ionized calcium and consequently reduce the augmented secretion of PTH, thereby closing a negative feedback loop. The inhibitory effect of calcium upon PTH secretion contrasts with the stimulatory effect of calcium upon most other secretory systems (3). In addition to calcium, other factors also modify PTH secretion. Many of these factors change cellular cyclic adenosine 3'5', monophosphate (cAMP) levels at the same time that they modify PTH secretion (4,5,6).

This volume provides a forum in which it is appropriate to discuss the bovine parathyroid gland and the effects of dopamine upon this tissue. When administered intravenously to cows, dopamine raises the plasma content of immunoreactive PTH (7). This stimulatory effect of dopamine is partially blocked by pimozide, a dopamine antagonist, but is unaffected by propranolol, a beta-adrenergic antagonist. An understanding of

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the cellular mechanisms involved in mediating this dopamine-induced stimulation of PTH secretion is limited by the cellular heterogeneity of the bovine parathyroid gland (7) as well as by the temporal and spatial imprecision of intravenous infusions. Nevertheless, the *in vivo* results provide a standard against which *in vitro* results can be compared.

The cellular and molecular events involved in the dopamine-stimulated release of PTH can be clarified in experiments utilizing bovine parathyroid cells dispersed with collagenase and DNase (8). This dispersion procedure yields parenchymal cells with only a slight contamination by red blood cells. The parenchymal cells exclude trypan blue and appear normal by light and electron microscopy (8). These cells release PTH in a linear fashion for several hours; the release is inhibited by calcium and stimulated by dopamine and beta-adrenergic agonists at concentrations comparable to those used to elicit physiological responses *in vivo* (4,8).

Dopamine Enhances PTH Secretion in Dispersed Bovine Parathyroid Cells

Dopamine (1 μM) causes a transient 2 to 4-fold increase in the rate of release of immunoreactive PTH (IR-PTH) from dispersed bovine parathyroid cells (9). The stimulatory effect of dopamine is maximal after 5 minutes exposure and persists for approximately 30 minutes (Figure 1). Several compounds mimicking the effects of dopamine in other systems mimic the stimulatory effect of dopamine on IR-PTH release. Both 2-amino, 6,7-dihydroxy tetralin (6,7-ADTN) and SKF 38393 increase the release of IR-PTH to the same degree as does dopamine. The release of IR-PTH is half-maximally stimulated by dopamine, 6,7-ADTN and SKF 38393 at 0.2 μM, 0.15 μM and 0.3 μM, respectively (Figure 2) (10). In contrast, other dopaminergic agonists are substantially less potent than dopamine; both apomorphine and lergotrile elicit no more than 25% of the maximal response to dopamine, and lisuride is devoid of agonist activity. The dopamine-stimulated release of IR-PTH is inhibited in a stereospecific manner by the isomers of flupenthixol (9); cis-flupenthixol is approximately 100-fold more potent than its trans-isomer (calculated K_i's of 33 nM and 3,300 nM, respectively) (Figure 3).

Dopamine Enhances cAMP Accumulation in Dispersed Bovine Parathyroid Cells

Dopamine causes a 20 to 30-fold increase in the content of cAMP in dispersed bovine parathyroid cells (Figure 4) (9). Like the dopamine-stimulated enhancement of PTH release, the dopamine-stimulated increase in cAMP content is maximal after 5 to 10 minutes of exposure to 10 μM dopamine (9) and

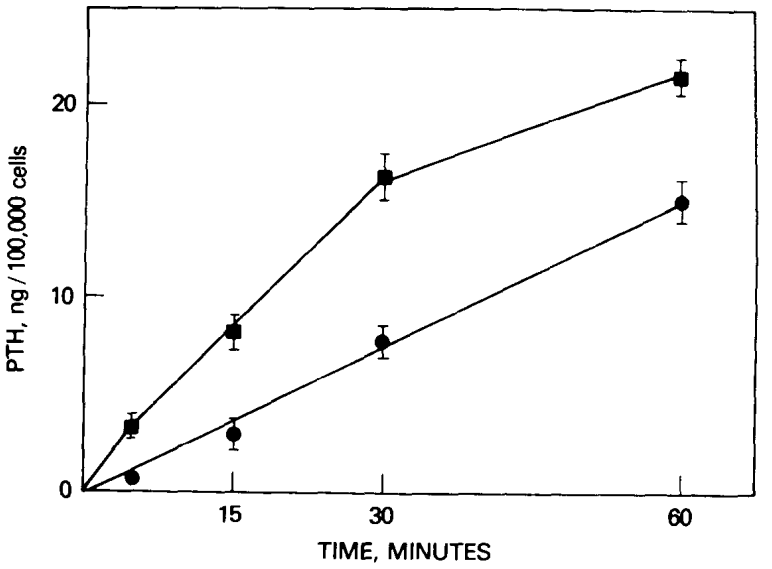

Figure 1. Stimulation of PTH release from dispersed bovine parathyroid cells by dopamine. Cells were incubated with (■) or without (●) 1 μM dopamine, and PTH release was determined by radioimmunoassay.

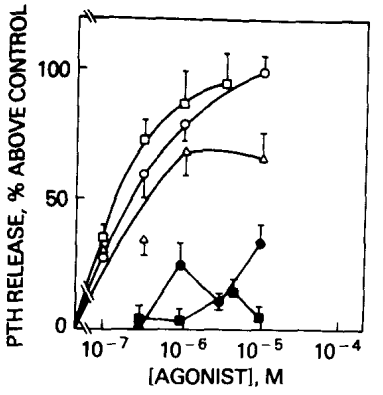

Figure 2. Stimulation of PTH release from dispersed bovine parathyroid cells by varying concentrations of dopamine (○), 6,7-ADTN (□), SKF 38393 (△), apomorphine (●), or dihydroergocryptine (■). (Reproduced with permission from Ref. 10. Copyright 1980, American Society for Pharmacology and Experimental Therapeutics.)

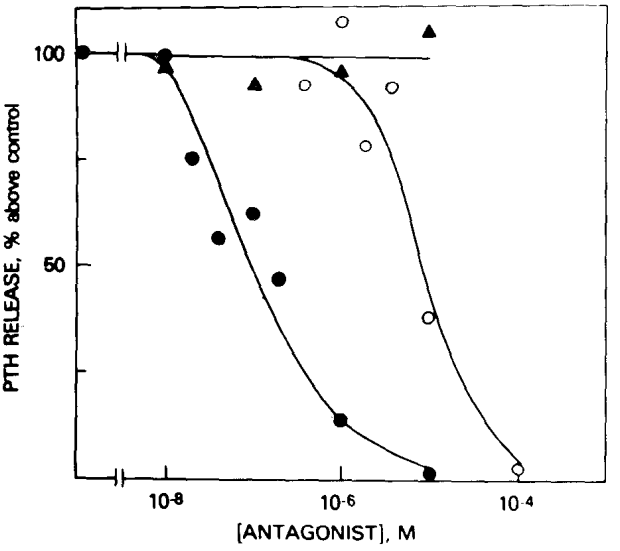

Figure 3. Inhibition of PTH-release stimulated by 1 μM dopamine by α-flupenthixol (●), β-flupenthixol (○), or (−) propranolol (▲).

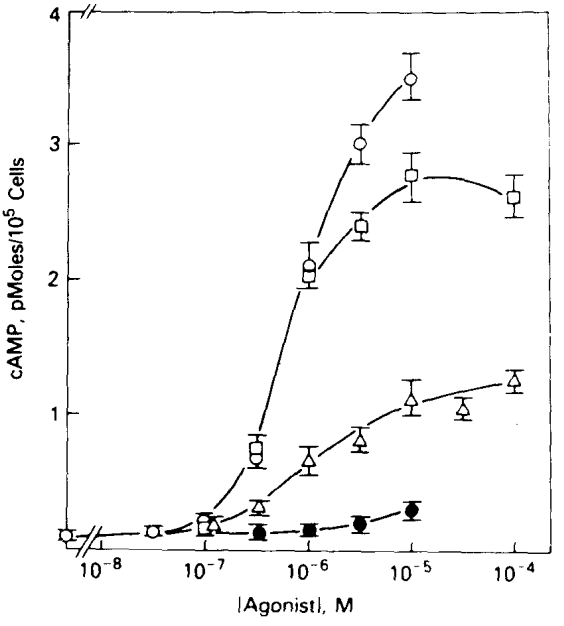

Figure 4. Stimulation of cAMP accumulation in dispersed bovine parathyroid cells by varying concentrations of dopamine (○), 6,7-ADTN (□), SKF 38393 (△), or apomorphine (●). (Reproduced with permission from Ref. 10. Copyright 1980, American Society for Pharmacology and Experimental Therapeutics.)