



CRC

PHARMACEUTICAL  
CHEMISTRY  
*of*  
ADRENERGIC  
*and*  
CHOLINERGIC  
DRUGS

György Szász



CRC

PRESS

# Pharmaceutical Chemistry of Adrenergic and Cholinergic Drugs

Author

**György Szász, D.Sc.**

Director

Institute of Pharmaceutical Chemistry  
Semmelweis Medical University  
Budapest, Hungary

With contribution of

**Zs. Budvári-Bárány**



CRC Press, Inc.  
Boca Raton, Florida

**Library of Congress Cataloging in Publication Data**

Szász, György.

Pharmaceutical chemistry of adrenergic and cholinergic drugs.

Bibliography; p.

Includes index.

1. Autonomic drugs. 2. Chemistry, Pharmaceutical.

I. Title. [DNLM: 1. Sympathomimetics—Pharmacodynamics.

2. Sympatholytics—Pharmacodynamics. 3. Parasympatholytics—Pharmacodynamics. 4. Parasympathomimetics—

Pharmacodynamics. QV 129 S996p]

RS431.A98S93 1984 615'.78 84-4358

ISBN 0-8493-5158-8

This book represents information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Every reasonable effort has been made to give reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use.

All rights reserved. This book, or any parts thereof, may not be reproduced in any form without written consent from the publisher.

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

© 1985 by CRC Press, Inc.

International Standard Book Number 0-8493-5158-8

Library of Congress Card Number 84-4358

Printed in the United States

## PREFACE

The term pharmaceutical chemistry is not treated uniformly either in the literature or as far as the programs of higher education are concerned. Works under the title pharmaceutical chemistry in most cases cover problems of chemical structure — biological activity relationships, therapeutic applications, and the methods of synthesis. These are obviously the important features of pharmaceutical (medicinal) chemistry.

However, one can rarely encounter a treatment of the subject where pharmaceutical chemistry is introduced in a complex manner, i.e., all the pharmaceutically important properties are interpreted in the light of the chemical structure.

It is evident here that the term *pharmaceutically important properties* needs to be defined. These are the group of properties which are involved.

1. In the biological (therapeutic) activity; also the metabolic behavior
2. In the drug formulation, i.e., the preparation of dosage forms; also in the stability and degradation properties
3. In the physical and chemical methods of analysis; also quality control and analysis for diagnostic, toxicologic, etc. purposes

In other words, pharmaceutical chemistry is a complex science covering the chemical knowledge needed by all experts working with drug substances.

The above-mentioned general treatment of the structure-property relationships could help to make those employed in the various branches of the pharmaceutical profession, i.e., drug formulation, dispensing pharmacy, information service for patients and physicians, drug quality control, competent experts in a wide range of work relating to drugs.

This book is to be considered an attempt to introduce the application of the above concepts.

## THE AUTHOR

**György Szász, M. Pharm., D.Sc.**, is Head of the Department of Pharmaceutical Chemistry at Semmelweis Medical University, Budapest.

Dr. Szász was born in 1927 and received his pharmaceutical training at the Semmelweis Medical University between 1947 and 1952. In 1953 he joined the staff of the Department of Pharmaceutical Chemistry. He defended his thesis on the field of pharmaceutical analysis of amines and alkaloids and obtained his C.Sc. (1968) and D.Sc. (1980) scientific degrees. He is a member of the Committee of the Hungarian Academy of Sciences on Analytical Chemistry.

His major research interest is the structure-property relationship study among nitrogen-bridged compounds.

Dr. Szász published over 120 papers, several of them in western periodicals. He is also the Editor of the *Textbook of Pharmaceutical Chemistry* (Volumes 1 and 2, 3rd ed., Medicina, Budapest, 1983).

## TABLE OF CONTENTS

Chapter 1	
<b>Adrenergic Agonists.....</b>	1
I. Biogenic Catecholamines .....	1
A. History .....	1
B. Biosynthesis .....	1
C. Structure and Properties .....	10
1. Configuration, Conformation .....	10
2. Solubility .....	15
3. Basicity .....	16
4. Absorbance .....	17
D. Reactions, Analysis .....	17
1. Reactions .....	18
a. Complexation.....	18
b. Oxidation.....	19
c. Ether and Ester Formation.....	22
d. Reactions of the Amino Group .....	23
2. Instrumental Analysis .....	23
a. Thin-Layer Chromatography (TLC) .....	23
b. Gas Chromatography (GC) .....	27
c. High-Performance Liquid Chromatography (HPLC) .....	27
d. Electrochemical Analysis .....	28
e. Electron Excitation Spectroscopy .....	29
II. Sympathomimetics .....	29
A. Name, Structure, and Therapeutic Application .....	29
B. Physical Properties .....	29
C. Structure-Activity Relationship .....	30
1. The Amino Group .....	31
2. The Phenolic Hydroxy Group .....	32
3. The Alcoholic Hydroxy Group .....	34
D. Preparations .....	34
References.....	37
Chapter 2	
<b>Adrenergic Blocking Agents .....</b>	41
I. Introduction .....	41
II. $\alpha$ -Adrenergic Blocking Agents.....	41
A. $\beta$ -Haloalkylamines .....	41
1. About Mode of Action.....	41
2. About Antagonist-Receptor Interaction.....	48
3. Preparations.....	51
B. Imidazoline Derivatives .....	51
1. Mode of Action.....	51
2. Instability of the Imidazoline Ring.....	51
3. Preparations.....	51
C. Ergot Alkaloids.....	52
1. Origin.....	52
2. Structure and Activity.....	52
3. Properties .....	55
4. Analysis .....	55

5.	Preparations.....	57
III.	$\beta$ -Adrenergic Antagonists ( $\beta$ -Blockers) .....	57
A.	Historical .....	57
B.	Structure.....	58
C.	Structure-Activity Relationship .....	59
D.	Synthesis .....	63
1.	Propranolol, Oxprenol, and Pindolol.....	63
2.	Sotalol .....	64
E.	Properties, Analysis .....	65
1.	Basic Strength.....	65
2.	Solubility .....	65
3.	Melting Point .....	65
4.	Absorptivity.....	66
5.	Chromatography .....	66
6.	Chemical Reactions .....	70
F.	Metabolism.....	70
G.	Preparations .....	73
	References.....	73

### Chapter 3

	<b>Cholinergic Agonists .....</b>	77
I.	Introduction .....	77
II.	The Physiological Cholinergic Agent .....	77
A.	History, Action.....	77
B.	Properties.....	81
C.	Biosynthesis and Metabolism.....	82
III.	Structure-Activity Relationship.....	83
IV.	The Two Acetylcholinomimetics.....	87
A.	Muscarine .....	87
B.	Nicotine .....	90
V.	Choline Derivatives .....	91
VI.	Analysis (Synthesis, Metabolism).....	91
A.	Acetylcholine and Its Derivatives.....	91
B.	The Stigmines .....	92
1.	Physostigmine — (3 $\alpha$ S- <i>cis</i> -1,2,3,3a $\beta$ ,8a $\beta$ -Hexahydro-1,3a,8-Trimethylpyrrolo [2,3- <i>b</i> ]-Indol-5-ol Methylcarbamate) .....	92
2.	Neostigmine ( <i>M</i> -Hydroxyphenyl) Trimethylammonium Dimethylcarbamate .....	93
3.	Synthesis (Neostigmine) .....	95
4.	Metabolism .....	96
C.	Pilocarpine .....	96
1.	Pilocarpine Hydrochloride — (3S- <i>cis</i> )-3-ethyl-dihydro-4-[(1-Methyl-1H-imidazol-5-yl)methyl]-monohydro-)chloride .....	97
D.	Phosphate Esters .....	98
	References.....	101

### Chapter 4

	<b>Anticholinergic Agents .....</b>	103
I.	Introduction .....	103
II.	PNS-Spasmolytics .....	103
A.	Chemical Structure and Properties.....	103

1.	Amine Group .....	112
2.	The "Joining Block" and the Hb 3 Moiety .....	113
3.	Stereospecificity of the Tropane Moiety .....	113
B.	The Chemical Background of PNS-Anticholinergic (Spasmolytic) Activity .....	115
C.	Analysis .....	117
1.	Vitali-Morin Reaction .....	117
2.	Urethane-Formation, Fluorimetry .....	118
3.	Thin-Layer Chromatography .....	118
4.	Infrared Spectroscopy .....	119
5.	High Performance Liquid Chromatography .....	119
6.	Gas Chromatography .....	119
7.	Spectrophotometry .....	119
8.	Titrimetric Methods .....	122
D.	Preparation and Synthesis .....	122
III.	Antiparkinsonoids .....	125
A.	Structure .....	126
B.	Synthesis .....	126
IV.	Preparations .....	126
A.	Atropine Sulfate — ( $\pm$ )-1-Mondaza-3-hydroxy-[3,2,1]-Bicyclo-Octanotropic-Acid Ester, Sulfate (2:1) .....	126
B.	Atropine Methylnitrate .....	128
C.	Homatropine Hydrobromide — ( $\pm$ )-1-Mondaza-3-Hydroxy-[3,2,1]-Bicyclo-Octanemandelic Acid Ester, Hydro-Bromide .....	129
D.	Homatropinemethyl Bromide — <i>N</i> -Methylhomatropine Bromide .....	129
E.	Scopolamine Hydrobromide — 6 $\beta$ , 7 $\beta$ -Epoxy-1 $\alpha$ H, 5 $\alpha$ H-Tropan-3 $\alpha$ -Ol(-)Tropate Hydrobromide .....	129
F.	Methscopolamine Bromide — <i>N</i> -Methylscopolamine Bromide .....	129
G.	Clidinium Bromide — 3-Benzylxylo-1-Methyquinuclidinium Bromide .....	130
H.	Propantheline Bromide — (2-Hydroxyethyl) Diiso-Propyl Methylammonium Bromide Xanthene-9-Carboxylate .....	130
I.	Glycopyrrolate — 3-Hydroxy-1,1-Dimethylpyrrolidinium Bromide $\alpha$ -Cyclopentylmandelate .....	131
J.	Oxyphencyclimine Hydrochloride — (1,4,5,6-Tetrahydro-1-Methyl-2-Pyrimidinyl) Methyl $\alpha$ -Phenyl- Cyclohexaneglycolate Monohydrochloride .....	131
K.	Cyclopentolate Hydrochloride — 2-(Dimethylamino) Ethyl 1-Hydroxy- $\alpha$ -Phenyl-Cyclopentaneacetate Hydrochloride .....	131
	References .....	131
	Index .....	135

## Chapter 1

### ADRENERGIC AGONISTS

#### I. BIOGENIC CATECHOLAMINES

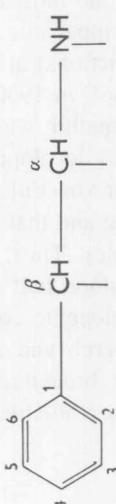
##### A. History

The hypertensive effect of an intravenous injection of a suprarenal gland extract was first described in 1895 by Oliver and Schäfer.<sup>1</sup> The proof that this action is due to the suprarenal medulla and not the whole suprarenal gland was provided by Langley<sup>2</sup> who demonstrated that the effect of the medulla extract was equivalent to that of electric stimulation of the sympathetic nerve. The synthetic adrenaline exhibits the same effect.<sup>3</sup> The name adrenaline was given by Takamine,<sup>4</sup> while earlier the same substance was named by Abel<sup>5</sup> as epinephrine. Takamine isolated this compound in pure form from a suprarenal extract and established its molecular formula in 1901. The synthesis of adrenaline (epinephrine) was achieved by Stoltz.<sup>6</sup> Its correct structural formula was published simultaneously by several scientists.<sup>7,8</sup> Cushny<sup>9</sup> recognized that the effect of synthetic epinephrine is only 50% of that of the natural substance. This drew attention to the stereochemistry of epinephrine and first demonstrated the importance of stereoselectivity in the interactions between drug molecules and receptors. In 1909, Flächer<sup>10</sup> succeeded in resolving the racemate components of synthetic epinephrine in the form of bitartrate salts. After the medium had been made alkaline with ammonia, the bases proved to be equipotent with natural epinephrine. These successes in chemical drug research encouraged further investigations in the field of physiology and new impetus was provided in the results of Loewy.<sup>11</sup> One of the most exciting principles leading to further development was undoubtedly the idea that sympathetic neurotransmission takes place chemically (i.e., by the mediation of chemical reactions) at the nerve endings (1921). The neurotransmitter was named sympathin by Cannon<sup>12</sup> in 1930. Due to the lack of versatile and sufficiently sensitive analytical methods, sympathin was believed to be a homogeneous single compound, for more than 10 years. The development in analytical methods and particularly chromatography, made it possible for von Euler<sup>13,14</sup> to show that sympathin is actually a mixture of adrenaline and noradrenaline and that the main factor in sympathetic neurotransmission is noradrenaline (norepinephrine). Thus, the physiological role and importance of this substance became properly understood half a century after its synthesis. That isopropyladrenaline (isoproterenol) is also a biogenic compound was recognized and experimentally verified only in 1957. The research and results relating to epinephrine and its congeners may be considered the major breakthrough in molecular biochemistry and pharmacology. The historical background is available in a much more detailed form in several monographs.<sup>15-18</sup>

##### B. Biosynthesis

The center of catecholamine biosynthesis is the medulla of the suprarenal gland. The pair of suprarenal glands is located at the height of thoracic vertebrae 11 and 12. They are orange organs, enclosed in the capsule of the kidneys and are pressed mildly to the upper pole of the kidney.<sup>19</sup> The weight of each of the human suprarenal glands is about 11 to 18 g. The suprarenal gland consists of two portions. The outer (yellow) is the cortex, which occupies the greater part of the organ, and the inner part (dark-red or brown) is the medulla. The catecholamines are concentrated in the medullary part of the glands (see Figure 1) in an amount of 0.5 to 0.7 mg/g, and comprise 90% epinephrine and 10% norepinephrine; isoproterenol is also produced, but only in a quantity of 0.1% (relative). The main particles within the medulla are the large chromaffine cells, arranged in a network. Epinephrine and

**Table 1**  
**ADRENERGIC AGONIST (SYMPATHOMIMETIC) AGENTS**

Generic name	Phenylalkylamine type	Proprietary name Synonyms	Therapeutic use	Doses
Dihydroxyphenylalkylamines Dopamine		3-OH,4-OH H H	H Intropin	$\alpha$ -Receptor stimulator, vaso-pressor, important in the treatment of some types of shock and of chronic refractory congestive heart failure
Norepinephrine bitartrate (USP)	3-OH,4-OH OH	H	H Arterenol, Noradrenalin, Levophedbitartrate, Sympathin	$\alpha$ -Receptor stimulator; vasopressor with little action on $\beta_2$ -receptor; of therapeutic value in hypotension due to shock
Epinephrine bitartrate (USP) Epinephrine (USP)	3-OH,4-OH OH	H	$\text{CH}_3$ Adrenalin, Suprarenin, Tonogen, Suprel, Vasotonin	I.v. 0.5-1 mg (strong diluted) with 0.2-0.5 $\mu\text{g}/\text{kg}/\text{min}$ velocity; checking blood pressure! I.m. s.c. 0.1-0.5 mg, 3 $\times$ I.v. 0.2 mg, 3 $\times$ (diluted and very slow!) Orally 1-2 mg, 3 $\times$
Norefrin	3-OH,4-OH OH	$\text{CH}_3$	H Cobeprin, Corbadrin, Isoadrenalin, Levonordefrin, Vasofren, Corbazil	$\alpha$ -Receptor stimulator, local vasoconstrictor in local anesthesia
Epinine Ethylnorepinephrine hydrochloride (USP)	3-OH,4-OH OH	H $\text{CH}_2\text{CH}_3$	$\text{CH}_3$ Deoxyepinephrin H Bronkophrine, Butane-frine, Ethymoradrenaline	Adrenergic agonist, bronchodilator, used in the treatment of daily asthma

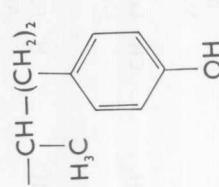
Isoproterenol hydrochloride (USP)	3-OH,4-OH OH H	$\text{CH}(\text{CH}_3)_2$	Isoprenalin	$\beta_2$ -Receptor stimulator; no action on $\alpha$ -receptors; relaxes the bronchial ( $\beta_2$ -receptor) and gastrointestinal smooth muscle; used as a cardiac stimulant too	I.v. 10-60 mg (repeatedly) Oral inhalation 80-160 mg
Isoetharine hydrochloride	3-OH,4-OH OH $\text{C}_2\text{H}_5$	$\text{CH}(\text{CH}_3)_2$	Asthmalitan Neoisuprel Dilabron	$\beta_2$ -Agonist for the treatment of bronchospasm with fewer cardiac side effects than isoproterenol	2.5-10 $\mu\text{g}/\text{kg}/\text{min}$ I.v. infusion
Dobutamine	3-OH,4-OH H H		Dobutrex	Selective $\beta_1$ -agonist acting directly on the receptors; cardiotonic effect based on inotropic activity	2.5-10 $\mu\text{g}/\text{kg}/\text{min}$ I.v.
Metaproterenol	3-OH,5-OH OH H	$-\text{CH}(\text{CH}_3)_2$	Orciprenaline Alupent Metaprel	$\beta_2$ -Adrenergic agonist; resistant to COMT therefore effective orally different from isoproterenol; used in treatment of bronchial asthma	Simple dose 0.65 mg Orally 20 mg 3-4 x daily
Terbutaline sulfate (USP)	3-OH,5-OH OH H	$\text{C}(\text{CH}_3)_3$	Bricanyl Brethine	Selective $\beta_2$ -stimulator; like metaproterenol, is resistant to COMT; used in bronchial asthma	S.c. 0.25 mg repeated max 1 x within 4 hr Orally 5 mg 3 x daily
Monohydroxyphenylalkylamines Phenylephrine hydrochloride (USP)	3-OH OH H	$\text{CH}_3$	Isonorepine Mesaton Meta-Synephrine Neo-Synephrine m-Sympatol	$\alpha$ - Receptor agonist; causes a rise in blood pressure; is a nasal decongestant and a pressor agent; also used in paroxysmal tachycardia and in ophthalmology as a mydriatic drug	I.m., S.c. 1-10 mg I.v. 100-500 $\mu\text{g}$

Table 1 (continued)  
ADRENERGIC AGONIST (SYMPATHOMIMETIC) AGENTS

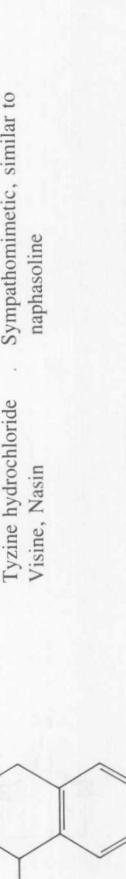
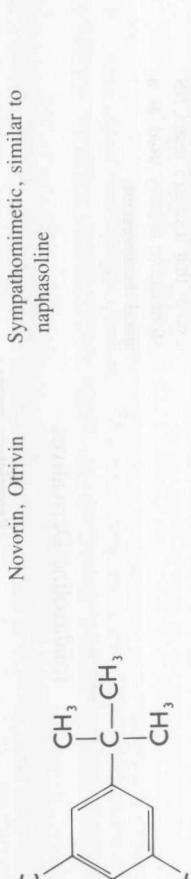
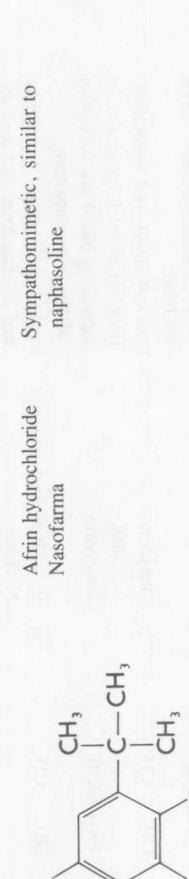
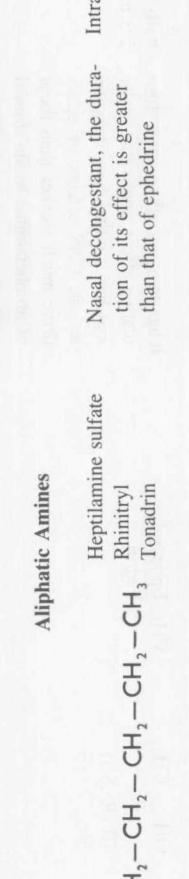
Generic name	Phenylalkylamine type	Proprietary name Synonyms	Therapeutic use	Doses
Metaraminol bitartrate (USP)	3-OH OH CH <sub>3</sub>	H Aminocardiol Isophenylephrine Pressorol Aramine	$\alpha$ -Receptor stimulator used mainly as a pressor agent against hypotension	1.m. 2-10 mg
Hydroxyamphetamine hydrochloride (USP)	4-OH H CH <sub>3</sub>	H Paredrine, Methyltyramin, Norpholedrin, Oxamphetamine	$\alpha$ -Receptor agonist, nasal decongestant and mydriatic	
Salbutamol	4-OH, 5-CH <sub>2</sub> OH OH H	C(CH <sub>3</sub> ) <sub>3</sub> Albuterol Vertilan	$\beta_2$ -Receptor agonist, bronchodilator and myorelaxant	Orally 2-4 mg 3-4 x daily
Methoxyphenylalkylamines Methoxamine hydrochloride (USP)	2-OCH <sub>3</sub> , 4-OCH <sub>3</sub>	H Methoxamine hydrochloride Mexan, Vasoxine	$\alpha$ -Receptor stimulator; has no stimulant action on the heart; used mainly as pressor agent in hypotensive states	1.m. 10-20 mg daily
Methoxyphenamine hydrochloride (USP)	2-OCH <sub>3</sub> H CH <sub>3</sub>	CH <sub>3</sub> Euspirol Ortoxin	$\beta_2$ -Receptor agonist, bronchodilator; used mainly in mild cases of asthma	Orally 50-100 mg every 3-4 hr if necessary
Phenylalkylamines Amphetamine sulfate (USP)	H CH <sub>3</sub>	H Benzedrine Aktedron	Although it has peripheral $\alpha$ and $\beta$ action, its powerful CNS stimulating action is used in therapy	
Methamphetamine hydrochloride (USP)	H CH <sub>3</sub>	CH <sub>3</sub> Deroxin Pervitin Oxydrin	Similar to amphetamine it has a CNS action, but in larger doses produces cardiac stimulation; mainly used for its central effect	I.m. 10-30 mg Orally 12.5-25 mg repeated
Mephentermine sulfate (USP)	H C(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>3</sub> Desoxyephedrin Wyamine Angiofen	It is a vasopressor also used topically as nasal decongestant max 1 x daily	max 1 x daily

Generic name	Chemical structure	Proprietary name Synonyms	Therapeutic use	Doses
Ephedrine hydrochloride (USP)	OH      CH <sub>3</sub>	CH <sub>3</sub> Ephedrol Fedrin	It has an $\alpha$ and a $\beta$ -receptor stimulating effect; applicable orally and has a rather long duration action; CNS effects of ephedrine much weaker than those of amphetamine; main clinical applications as bronchodilator, nasal decongestant and mydriatic	Orally 25-50 mg every 2-3 hr
Pseudoephedrine hydrochloride (USP)	OH      CH <sub>3</sub>	CH <sub>3</sub> Dofedrin Isofedrin Ro-Fedrin	Like ephedrine, is a bronchodilator but is much less active in increasing blood pressure; also used as a decongestant	
Phenylpropanolamine hydrochloride (USP)	OH      CH <sub>3</sub>	H Propadrine hydrochloride Midriatin Norephedrin	Its pharmacological effect is like that of ephedrine and it is approximately equal in potency except that it causes less CNS stimulation; mainly used as a nasal decongestant	

### Imidazoline Derivatives

Generic name	Chemical structure	Proprietary name Synonyms	Therapeutic use	Doses
Naphazoline hydrochloride (USP)		Naphazolin Pentazoline	Sympathomimetic; vasoconstrictor, similar to epinephrine in action; used as a nasal decongestant	Intranasal in 0.1% soln

Table 1 (continued)  
ABRENERGIC AGONIST (SYMPATHOMIMETIC) AGENTS

Generic name	Proprietary name Synonyms	Therapeutic use	Doses
Tetrahydrozoline hydrochloride (USP)	<b>Phenylalkylamine type</b> 	Tyzine hydrochloride Visine, Nasin	Sympathomimetic, similar to naphasoline
Xylomethazoline hydrochloride		Novorin, Otrivin	Sympathomimetic, similar to naphasoline
Oxymethazoline hydrochloride		Afrin hydrochloride Nasofarma	Sympathomimetic, similar to naphasoline
Tuaminoheptane (USP)	<b>Aliphatic Amines</b> 	Peptilamine sulfate Rhinitryl Tonadrin	Intranasal in 1% solution

Methylhexamine	$\begin{array}{c} \text{CH}_3 \\   \\ \text{CH}_3-\text{CH}_2-\text{CH}-\text{NH}_2 \\   \\ \text{CH}_3 \end{array}$	Forthane
Isomethopane hydrochloride	$\begin{array}{c} \text{CH}_3-\text{C}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}-\text{CH}_3 \\   \\ \text{CH}_3 \end{array}$	Ocit hydrochloride
Cyclopentamine hydrochloride	$\begin{array}{c} \text{CH}_2-\text{CH} \\   \\ \text{HNH}-\text{CH}_3 \\ \oplus \\ \text{Cyclopentane ring} \end{array}$	Clopante hydrochloride Cyclosan
Propylhexedrine	$\begin{array}{c} \text{CH}_2-\text{CH}-\text{CH}_3 \\   \\ \text{NH}-\text{CH}_3 \end{array}$	Benzedrex Benhaler
		Although it has moderate general sympathomimetic activity it is mainly used as an antispasmodic and a vasoconstrictor
		Its effects are similar to those of ephedrine, but it produces only slight cerebral excitation; orally it is more effective than ephedrine; mainly used as a nasal decongestant
		Sympathomimetic; has vasoconstrictive and a decongestant effect on the nasal membranes and produces scarcely any effect on the nervous system

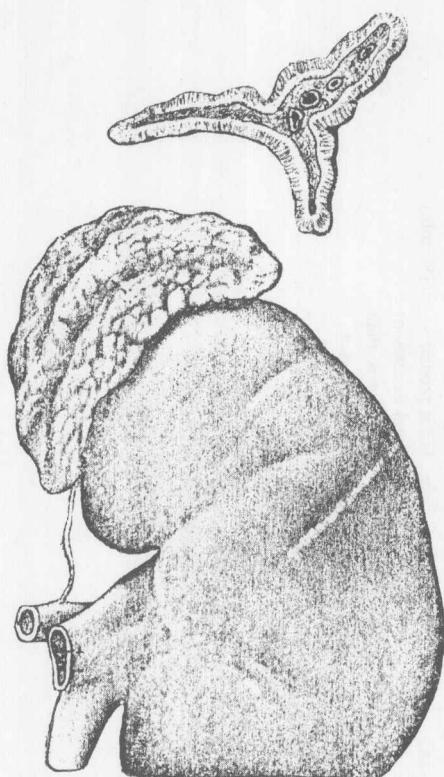


FIGURE 1. The left kidney with the suprarenal gland. Cross section of the suprarenal gland.

norepinephrine are found stored in these specific granules. During further research several other components have been shown to accompany to the catecholamines; recently enkephalins were identified.<sup>20,21</sup> The molar ratio of catecholamine and ATP has been found to be 4:1<sup>22</sup> and 4.5:1,<sup>23</sup> which is nearly equivalent with regard to the four negative charges of ATP and the one of catecholamine molecules. One characteristic feature of the cells of the medulla is that after treatment with a solution of a chromate (VI) salt, they are colored reddish brown due to the reaction of their chromaffine granule content.<sup>24</sup> These granules are about 200  $\mu\text{m}$  in size. This reaction is based on the oxidation of the catecholamines. The recognition of this chromium affinity led to the discovery of a chromaffine (paraganglionic) system. An interesting fact which awaits proper interpretation is that in neonates, the para-aortic body (a chromaffine group of cells at the crosspoint of the aorta and the base of the heart, about 1 cm in length) has a relatively high epinephrine content, many times that of the suprarenal glands.<sup>25</sup> Thanks to the rapid development in modern chromatographic and other separatory methods, all of the enzymes involved in the biosynthesis of catecholamines have been discovered and the biosynthetic pathways have also been elucidated. The key substance in the biosynthesis of catecholamines is L-tyrosine, (2) which is formed from the corresponding essential amino acid, L-phenylalanine (1) on the action of phenylalanine hydroxylase. This enzyme is located mostly in the liver and requires dihydrobiopterine (1a) as cofactor.<sup>26</sup> L-tyrosine is oxidized further to L-dihydroxyphenylalanine (3) by tyrosine hydroxylase (TH). The tissue distribution of this enzyme coincides with that of the catecholamines (adrenal medulla, brain, heart, etc.), indicating that the physiological role of this enzyme is mainly its contribution to the biosynthesis of catecholamines; the reaction involving the enzyme is found to be the rate-limiting factor.<sup>27</sup> This enzyme needs tetrahydropteridine (2a) as cofactor.

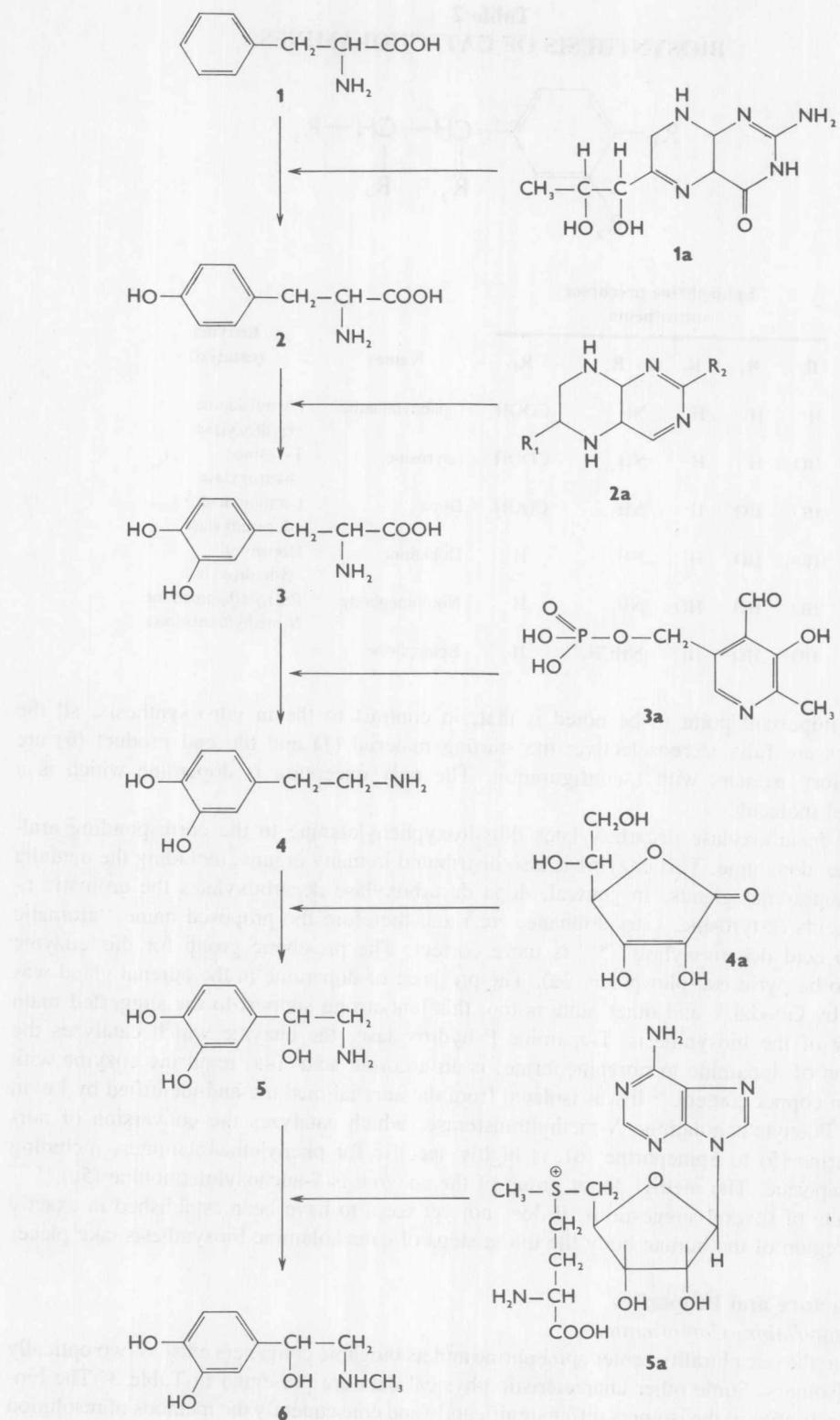


FIGURE 2. Biosynthesis of catecholamines.