Progress in Drug Research

Fortschritte der Arzneimittelforschung

Progrès des recherches pharmaceutiques

Editor:
Ernst Jucker

28

Birkhäuser Verlag, Basel · Boston · Stuttgart

Progress in Drug Research
Fortschritte der Arzneimittelforschung
Progrès des recherches pharmaceutiques
Vol. 28

Progress in Drug Research
Fortschritte der Arzneimittelforschung
Progrès des recherches pharmaceutiques
Vol. 28

Edited by · Herausgegeben von · Rédigé par
Ernst Jucker, Basel

Authors · Autoren · Auteurs
B. K. Bhat, M. Seth and A. P. Bhaduri, M. H. Bickel,
Jürgen Drews, Fred Samson and J. Alejandro Donoso,
D. L. Swallow, William J. Wechter and Barbara E. Loughman,
G. B. West, Archie F. Wilson

1984

Birkhäuser Verlag
Basel · Boston · Stuttgart

ISBN 3-7643-1556-3
Printed in Western Germany

Foreword

Volume 28 of 'Progress in Drug Research' contains 8 articles, a subject index for this volume, an alphabetic subject index for volumes 1–28, and an author and subject index for all the volumes which have so far been published. The contributions of volume 28 are particularly concerned with biogenic amines, with immunology and the pharmacology of the immune system, with antiviral agents, with amino-quinoline antimalarials, with the axoplasmic transport, with drug treatment of asthma and with the role of adipose tissue in the distribution and storage of drugs.

The authors have tried, and I think they have succeded, not only to summarize the current status of particular fields of drug research, but also to provide leads for future research activity. The articles of this volume will be of special value to those actively engaged in drug research, and to those who wish to keep abrest of the latest developments influencing modern therapy. In addition, it is believed that the 28 volumes of 'Progress in Drug Research' now available represent a useful reference work of encyclopedic character.

The editor would like to take the occasion of the publication of this volume to express his gratitude both to the authors and to the readers. The authors have willingly undertaken the great labor of writing significant topical contributions, and many readers have helped the editor with criticism and advise. With these thanks, the editor would like to express his gratitude to the publisher, Birkhäuser Verlag Basel, particularly to Messrs. C. Einsele and A. Gomm, and their associates for the excellent cooperation.

Bâle, June 1984 Dr. E. Jucker

Vorwort

Der 28. Band der «Fortschritte der Arzneimittelforschung» umfasst 8 Beiträge und enthält ausserdem ein Stichwortverzeichnis dieses Bandes sowie ein Sachverzeichnis, nach Gebieten geordnet, der bisher erschienenen Bände und einen Autoren- und Artikelindex der Bände 1–28. Die Artikel des vorliegenden Bandes befassen sich mit verschiedenen aktuellen Problemen der Arzneimittelforschung, wobei der Schwerpunkt in den Gebieten der Immunologie, der Virus-Krankheiten und der Malaria liegt. Ausserdem liegen ein interessanter Überblick über biogene Amine vor sowie Abhandlungen über den Axoplasmatic-Transport, die Rolle der Adipose-Gewebe in der Verteilung und Lagerung von Arzneimitteln und über Behandlung von Asthma. Wiederum wurde Wert gelegt auf Beiträge mit spezifischer und gezielter Richtung sowie auf solche mit einer das gesamte Gebiet der Arzneimittelforschung tangierenden Thematik.

Die Autoren dieses Bandes haben wiederum versucht, ihr Fachgebiet prägnant und übersichtlich darzustellen, die neuesten Entwicklungen aufzuzeigen und darüber hinaus auch in die Zukunft weisende Betrachtungen anzustellen. So dürfte auch der 28. Band der Reihe dem aktiven Forscher, sei es in der Industrie oder an der Hochschule, von Nutzen sein und demjenigen, der sich über die neuesten Entwicklungen orientieren und auf dem laufenden halten will, eine gute Hilfe bieten. Die Reihe «Fortschritte der Arzneimittelforschung» stellt heute mit den vorliegenden 28 Bänden sicherlich ein wertvolles Nachschlagewerk mit enzyklopädischem Charakter dar.

Der Herausgeber möchte hiermit den Autoren und den Lesern der «Fortschritte der Arzneimittelforschung» seinen Dank abstatten; den Autoren für die grosse bei der Abfassung der Artikel geleistete Arbeit, den Lesern für ihre Kritik und Anregungen. Die vielen Zuschriften und die Rezensionen helfen entscheidend mit, die Reihe auf einem hohen Niveau zu halten und den heutigen, sich stets verändernden Bedingungen der Arzneimittelforschung anzupassen. Dank sei auch dem Birkhäuser Verlag, insbesondere den Herren C. Einsele und A. Gomm, sowie ihren Mitarbeitern für die ausgezeichnete Zusammenarbeit und für die sorgfältige Ausstattung des Werkes ausgesprochen.

Basel, Juni 1984 Dr. E. Jucker

Contents · Inhalt · Sommaire

Biogenic amines and drug research

By G. B. West
Department of Paramedical Sciences, North-East London Polytechnic, Romford Road, London E15 4LZ, UK

1 Introduction

From time to time during the course of basic biochemical and pharmacological research, discoveries are made which reveal the order and elegance of what seems to be the bewildering array of molecules responsible for the life of even the simplest cell. The isolation of deoxyribonucleic acid (DNA) in 1869 and the elucidation of its composition and structure more than 80 years later, for example, revealed not only that this molecule was to be found in the cells of every living thing from bacteria to man but also that the genetic code is uniform throughout life. This led to a tremendous breakthrough in cellular biology, and present-day concepts of protein synthesis and cancer chemotherapy, among others, are rooted in our understanding of DNA.

Similar stories can be told in the biological areas of naturally-occurring amines such as adrenaline, acetylcholine, 5-hydroxytryptamine, histamine and many others. Identification of a chemical as part of a research project has been followed by many years of investigation until its function was elucidated. Being a pupil of Sir Jack Gaddum for several years, my own chief interests have naturally been in this area of local hormones as he himself was influenced by the research of Sir Henry Dale in the early part of this century.

In the present chapter, personal research approaches towards finding a physiological function of these amines are described and discussed, where possible, in the light of modern knowledge.

2 Catecholamine research

The discovery that any chemical is a natural constituent of living tissue has always led to an increase in experimental work on that subject. Adrenaline, for example, was long regarded as both the specific hormone of the adrenal medulla and the chemical transmitter in the sympathetic nervous system. Its synthesis in 1904 ended, for practical purposes, biochemical research in that particular field of medical science. There were difficulties, however, in accepting adrenaline as the transmitter of sympathetic nerve impulses until Bacq in 1934 put forward the view that noradrenaline (and not adrenaline) might be the transmitter substance in adrenergic nerves.

Adrenaline is a secondary amine, having one hydrogen atom in the amine group substituted by a methyl group. Noradrenaline differs

from adrenaline in lacking this methyl group and hence is a primary amine. Euler in 1946 was the first to show by biological methods that a substance closely resembling noradrenaline was present in extracts of both fresh ox and cow spleen as well as in extracts of the heart of the ox, horse and cat and of nerves of the sympathetic chain.

Our own study began in 1947 by comparing first the activities of the two amines on a large number of animal tissues so that methods for distinguishing them in extracts and perfusates of tissues by parallel quantitative assays could be devised [1]. Ratios of equi-active doses were determined, the highest values showing adrenaline to be the more active on the cat and rat non-pregnant uteri as well as in the perfused frog heart preparation. Noradrenaline, on the other hand was always more potent as a vasopressor agent than was adrenaline (table 1). Al-

Table 1
Ratio of noradrenaline dose to equi-active dose of adrenaline on different preparations (adapted from[1]).

Test object	Ratio	Excitor (E) or inhibitor (I) action
Cat, blood pressure	0.8	E
Cat, pregnant uterus	0.8	E
Cat, nictitating membrane	1.2	E
Rabbit, ileum	2.0	I
Rat, ileum	3.0	I
Rabbit, pregnant uterus	4.0	E
Cat, non-pregnant uterus	10.0	I
Frog, perfused heart	33.3	E
Rat, non-pregnant uterus	100.0	I

though Barger and Dale in 1910 had reported that a dose of ergot extract, sufficient to reverse the pressor effect of adrenaline in the spinal cat, did not reverse that of noradrenaline, we later were able to find the conditions necessary for showing a vasodepressor action with noradrenaline by anaesthetizing cats with chloralose and using anti-adrenaline agents like dibenamine [2, 3].

It was generally conceded in 1946 that adrenaline injected into the portal vein of mammals produced a smaller rise of blood pressure than when it was injected into other veins like the femoral or jugular. The amine was inactivated in the liver by the enzyme, monoamine oxidase. A comparison was therefore made first of the activities of adrenaline and noradrenaline on the blood pressure after injecting them intraportally, intrajugularly and intra-arterially in the absence and presence of

an inhibitor of monoamine oxidase (guanidine). Then the study was extended to include the effect during and after hepatic nerve stimulation [4]. By choosing the dose of guanidine which did not potentiate the pressor action of noradrenaline in cats but significantly potentiated that of adrenaline, we showed that the effects of hepatic nerve stimulation on the blood pressure were also not potentiated. Further similarities between the responses after hepatic nerve stimulation and those of intraportal noradrenaline were recorded using other monoamine oxidase inhibitors [5]. When the splenic nerve was stimulated, a similar but smaller activity (less than 0.5 μg/ml) indicative of noradrenaline was obtained. However, when the hypogastric and inferior mesenteric sympathetic nerves were stimulated, both amines appeared in the plasma [6].

The technique of exhausting the adrenal medulla of mammals of their amines by injections of insulin has been used by many groups of workers to study possible precursors of adrenaline. When I tried the technique in rabbits, noradrenaline with adrenaline appeared in the gland extract [7]. In pigeons, total activity was dramatically reduced but the percentage of noradrenaline was unchanged, whereas in chicken the relative noradrenaline content as well as the total activity were reduced [8, 9]. So different responses can be obtained in different animal species, even after the same type of treatment.

2.1 Catecholamines in the adrenal gland

Reports in the literature up to 1950 showed that the noradrenaline content of the adrenal medulla varied from species to species. I had found only minute amounts of noradrenaline in the adrenal glands of rabbits and guinea-pigs but the predominatory amine in pigeon and chicken glands was shown to be noradrenaline. So, an extensive investigation was made with adrenal glands of 17 species including man (table 2). We showed that there was some relationship between total activity and relative noradrenaline content in these adult adrenal glands, the greater the activity, the higher usually was the noradrenaline content. Other workers had suggested that the relative size of the cortex might determine how far methylation of noradrenaline proceeded and it was apparent from our results that the smaller the relative size of the medulla, the greater was the degree of methylation [10]. For example, the ratio of cortex to medulla in guinea-pigs was high (60) and only traces

Table 2
Catecholamines in adrenal glands of adult animals (adapted from [10]).

Species	Total activity (mg/g)	Noradrenaline (%) in the total
Fowl	10.10	80
Dogfish	3.30	73
Pigeon	3.00	55
Frog	3.10	55
Pig	2.15	49
Cat	0.97	41
Sheep	0.75	33
Cow	1.75	29
Dog	1.50	27
Mouse	1.00	25
Horse	0.84	20
Man	0.60	17
Hare	0.35	12
Rat	1.23	9
Hamster	0.40	8
Rabbit	0.48	2
Guinea-pig	0.15	2

of the total catecholamines in the gland were noradrenaline, whereas in the dog or pig (with ratio values of 5) the noradrenaline content was above 25%. In dogfish, however, where the medullary tissue can be completely separated from the cortical tissue, both amines were found. Therefore, methylation of noradrenaline does not always require the proximity of cortical hormonal tissue to medullary cells [11].

In man, about 17% of the total activity in the adrenal gland was shown to be noradrenaline yet it was not known whether or not this proportion varied with disease or age. We analysed glands of many adult patients but only found a wide range of total activity, probably because these represented exhausted glands [12]. The most surprising result however, came from our analysis of the glands of 32 children aged less than 70 days. In these [10], more than 90% of the total catecholamine was noradrenaline (fig. 1). In fact, the relative amounts of the amines in human embryonic tissue were about the same as those reported in adult medullary tumours, a few of which had been analysed by that time. Tumour cells, like embryonic cells, are relatively undifferentiated cells and so human embryonic tissue provided material which might allow the identification of precursors of adrenaline [13], using both paper chromatography of extracts and bioassay techniques. By choosing suitable solvents, it was possible to separate the then-known precursors (table 3). However, only adrenaline and noradrenaline

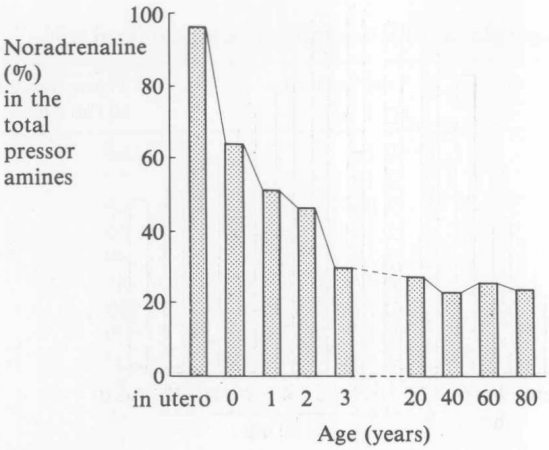

Figure 1
Influence of age on the relative noradrenaline content of human adrenal glands (adapted from [10]).

were identified [14], the glands of 2-year-olds being composed of equal quantities of the two amines and no other catecholamine (fig. 1). The most complete changeover, however, was found when rabbits of different ages were used, for a gland of entirely noradrenaline in utero changed to one of entirely adrenaline in adults, the process of methylation occurring soon after birth (fig. 2) [15].

Table 3
Identification of catecholamines and their possible precursors by paper chromatography (adapted from [14]). Solvent was butanol: acetic acid: water.

Compound	R_f-value	Colour reaction with developer			
		KIO_3	$K_3Fe(CN)_6$	Nitraniline	Ninhydrin
Adrenaline	0.36	Pink	Pink	Grey-blue	Purple
Noradrenaline	0.28	Violet	Rose	Grey-blue	Brown
Dopamine	0.39	Brown	Brown	Grey-blue	Brown
DOPA	0.21	Grey	Grey	Grey-blue	Purple
DOPS	0.15	Brown	Brown	Grey-blue	Purple
Tyramine	0.58	0	0	Grey-blue	Purple
Tyrosine	0.30	0	0	Purple	Purple
Phenylalanine	0.51	0	0	Rose	Purple

2.2 Accessory chromaffin tissue

Extra-adrenal chromaffin-staining cells in the abdominal sympathetic plexuses of human foetuses are known as the Organs of Zuckerkandl

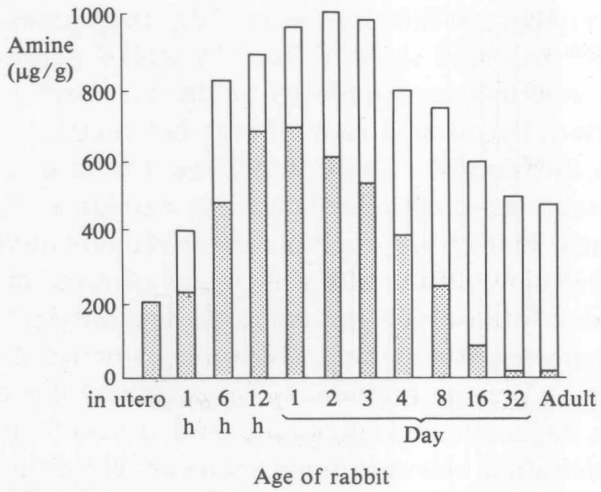

Figure 2
Influence of age on the catecholamine content (μg/g) of adrenal glands of rabbits. Shaded areas are noradrenaline; open areas are adrenaline (adapted from [15]).

and they are situated along the aorta near to the origin of the inferior mesenteric artery. They originate from the neural crest in common with adrenal medullary cells and develop up to the age of 12 months and then degenerate. We decided to extract them and assay for catecholamines [12]. Their content of noradrenaline [16] far exceeded, in absolute amounts, that in the adrenal glands (fig. 3). This suggested

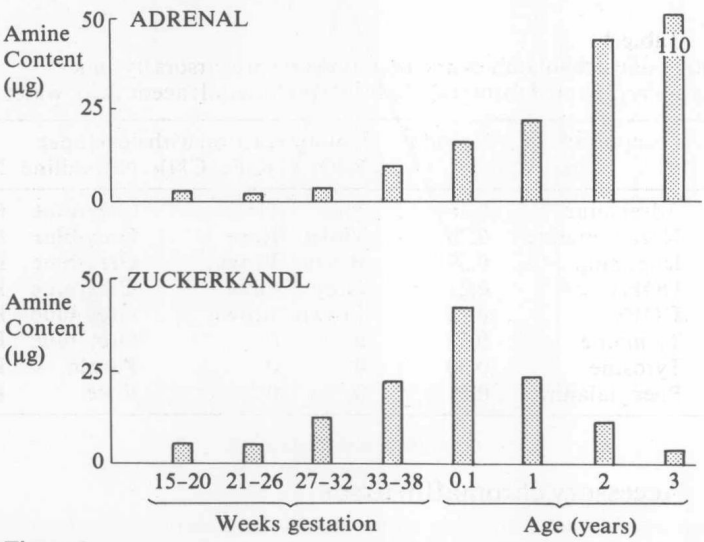

Figure 3
Influence of age on the absolute amount of catecholamine (μg) in one adrenal gland and one organ of Zuckerkandl in human foetuses and babies (adapted from [16]).

that they play an important function early in life, for the concentration of noradrenaline increased steadily from 16 weeks' gestation until birth and there was increased intensity of the chromaffin reaction. During this period, the adrenal medulla was functionally immature. Soon after birth, the Organs of Zuckerkandl began to fibrose, with loss of chromaffin-staining properties and decrease in pharmacological activity. By the third year of life when mature adrenal medullary cells were rapidly developing, fibrosis of the Organs of Zuckerkandl was very marked and only traces of pressor amines were found [16].
Other mammals possess retroperitoneal tissues composed of chromaffin cells containing pressor substances. We examined this accessory tissue in rabbits, dogs, cats, and guinea-pigs and in all cases only noradrenaline was identified, with maximum values about the time of birth [17]. Again, the possibility exists that this tissue helps to maintain blood pressure in utero and in early life.

2.3 Adrenal medullary tumours

Tumours of the chromaffin cells of the adrenal medulla have been known for more than a hundred years, and the pathological and clini-

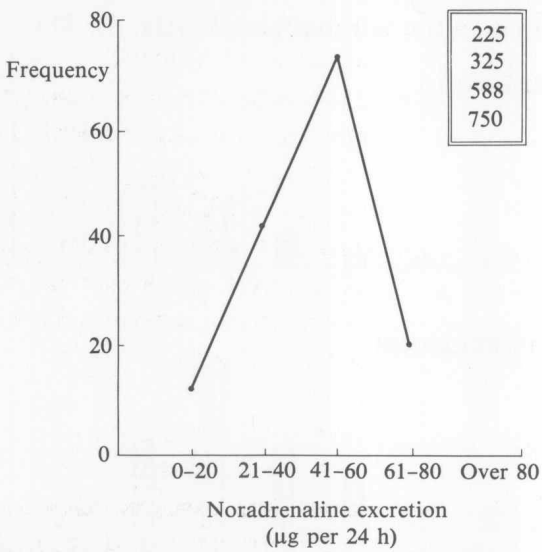

Figure 4
Frequency distribution of noradrenaline excretion (μg/day) in the urine of 200 human cases of hypertension. The four individual values exceeding 80 μg per day (contained within the frame) were indicative of a phaeochromocytome; this was confirmed in each case (adapted from [10]).