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PREFACE

The appearance of Volume Ten of this Annual Series prompts us to pick up the retrospectroscope for a look at where we have gone in these turbulent, fascinating years. Perhaps the most apparent observation is that stability, which Windsor Cutting felt the series had achieved five years ago, has continued. Many of the titles in this volume are similar to those of Volume One, indicating that most areas of interest to pharmacologists contain unfinished business. On the other hand, when read in series the papers in any area reflect the progress of the past decade, which is principally the result of our increasing understanding of biological events at the molecular level and the development of new and powerful investigational techniques. For example, antiviral agents are receiving increasing attention, as are immunosuppressive and relatively specific carcinostatic compounds.

New topics have also appeared during the decade. The "pill" was first discussed in Volume Four, and various aspects of its pharmacology have appeared in four subsequent reviews. No one can accuse the Editorial Board of lack of interest in the important human functions! Developmental and perinatal pharmacology have become subspecialties and space-pharmacology and toxicology and behavioral pharmacology are with us to stay. Increasing complexity of our science is reflected in the tendencies of authors to restrict their reviews to specialized topics, and while this means that not all the research in a given area is reviewed each year, it is actually advantageous since in-depth reviews result. For a change of pace we can trace the development of modern pharmacology in the essays by eminent authors of the prefatory chapters and anyone who doesn't believe that pharmacologists have a sense of humor must contend with Carl Dragstedt!

Finally, to review the passing parade we welcome our new editor-in-chief at Annual Reviews, Robert R. Schultz, and wish Godspeed to Windsor Cutting who finds that building a new medical school in Hawaii is an all-consuming occupation. We are fortunate indeed to have had his help for a full ten years.

As always, sincere thanks are given to our capable assistant editor, Virginia Hoyle, and to the George Banta Company, Inc., our printers.

THE EDITORIAL BOARD

ERRATUM

Volume 9 (1969):

Page 356, line 29 *should read* . . . clearance and its conversion to oxipurinol. In single doses allopurinol has a . .

CONTENTS

PHARMACOLOGY AND MEDICINE

HIROSHI KUMAGAI

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The establishment and independence of pharmacology in Japan dates back some 50 years, when Professor Juntaro Takahashi took the pharmacological chair in the University of Tokyo, Medical Faculty. This was in 1919, when he came back from Germany, where he had studied pharmacology under the guidance of Professor Schmiedeberg.

The social structure at that time was so arranged that the traditional way of living and thinking of the Japanese leaders had been switched from that of medieval Japan to western. In order to take part as a member of the modern countries of the world, the government made every effort for the modernization of Japan and its people.

The core of the policy incorporated in the advancement of Japanese civilization was the encouragement of the scientific way of thinking. This policy led to the systematic organization of the educational system of Japan as a whole, and primary school education became compulsory. This led to an abrupt elevation of the standard of education in Japan, and aroused the desire and willingness of the countrymen for higher education. The policy of the government for the realisation of scientific Japan and the willingness of the Japanese people culminated in the establishment of the Imperial University of Tokyo. The faculty of medicine of the University of Tokyo was established in 1879, and the medical educational program was formulated after the German system. The role played by German professors, who were the first professors in clinical as well as in basic medical departments, was decisive in the further development of medical education and the medical service system in Japan. Among German professors, Erwin Baelz (1876–1905) was the most outstanding figure both as medical teacher and as educator in general. He, as a far-sighted man, had a deep sympathy for Japanese history and culture and he advised the Japanese Government to develop medical service for the people of Japan, that is to say, medical service by the Japanese for the Japanese. But his advice was not accepted by the Government. This resulted in the development of German medicine in Japan, without the philosophy and way of thinking that have been a part of the German culture.

German medicine cultivated in Japanese soil grew up abruptly and covered the whole area of the country. The number of medical schools, for instance, now amounts to 46, including national, prefectural, and private.

The principle and pattern of Japanese medical education have completely followed that of Germany, and the most outstanding feature that governed in medical education was the idea of the superiority of academic scientific research over medical practice. This concept undoubtedly contributed greatly to the progress and development of modern medical science in Japan, and is the backbone of present Japanese medicine. This means that more weight was laid on medical research than on the welfare of the public. This pattern of medical education resulted in the dissociation of medical education and medical service.

As for the development of pharmacology in Japan, the department of pharmacology of the faculty of medicine, of the Imperial University of Tokyo, served as a source of training of professors of pharmacology for other medical colleges and medical faculties. The first of this type was the Kyoto Imperial University. Professor Haruo Hayashi, Professor Juntaro Takahashi (the first holder of the pharmacology chair in the Imperial University of Tokyo), and Professor Morishima, jointly established the first pharmacological chair in the Kyoto Imperial University. The professor of pharmacology in medical faculties and colleges in Japan to-day is a descendant or a successor of either the school of Hayashi, or the school of Morishima. Generally speaking, the eastern half of Japan, including Tokyo, belongs to Hayashi's school, and the western half of Japan belongs to Morishima's school. Among forty-six medical faculties each have a pharmacological department with one professor's chair, while Tokyo and Kyoto University medical faculties, have two professors' chairs. The leading policy of the professors at the early stage of the development of pharmacology was to follow the research pattern of the German system, and it has been a custom for professors in charge of pharmacology to go to Germany and study pharmacology. As the medicine that prevailed in Japan before the introduction of western medicine had been Chinese medicine, much emphasis was laid on the discovery of active principles from Chinese drugs, that is to say the scientific evaluation of Chinese drugs by means of pharmacological tools. It was fortunate that the Japanese pharmacologists had pharmaceutical scientists as intimate friends in the University, and the professors in departments of pharmaceutical science of the Tokyo and Kyoto Imperial Universities were specialists, as well, in organic chemistry. Much effort was directed toward the discovery of active principles in the natural herbs that had traditionally been utilized in Chinese medicine.

Nonetheless, the scientific interests of organic chemists in their special field, and those of the pharmacologists, do not necessarily coincide; rather, there sometimes arose discrepancies between them. This situation stimulated the establishment of a pharmacological department in the faculty of pharmaceutical science in Tokyo University. I, as a professor of pharmacology of the medical faculty of Tokyo University, cochaired the first chair in 1959, and therefore I have to take responsibility for the development of pharmacology in the pharmaceutical science field. Since that time, to have a phar-

macological chair and department has become a requirement of every faculty and school of pharmaceutical science.

The Japanese Pharmacological Society was established in 1927, with membership composed solely of medical doctors. However, in 1959 membership was extended to scholars from pharmaceutical sciences. This led to a rapid increase in the number of members and size of the society. At an early stage of the establishment of pharmacological departments in faculties or colleges of pharmaceutical science, special efforts were directed to the teaching of biology to students who had specialized in chemistry rather than in biological sciences. Fortunately, because of the endeavours of the leaders concerned, the number of researchers in pharmacology from the field of pharmaceutical sciences increased steadily, and mutual understanding between medical pharmacologists and pharmaceutical pharmacologists become much improved. In my opinion, the research field covered by medical pharmacology may have to shift to a more physiological side, and that covered by pharmaceutical pharmacology more to structure-activity relationships of drugs or derivatives.

As the late Professor Gaddum has pointed out, "the pharmacologist borrows from physiology, biochemistry, pathology, microbiology and statistics, but he has developed one technique of his own, and this is the technique of bioassay." Bioassay without doubt is a technique of its own, however, technique must have a physiological meaning. In other words, we have to find out the biological mechanism that lies beneath the response of a tissue or an organ or a whole animal. This, as I believe, is the final goal that pharmacology has to aim at. Pharmacology by definition is the study of the responses of living matter to substances administered. Analysis and evaluation of responses to administered substances, functional as well as morphological, elicited by subcellular organelle, cell, tissue, organ, and especially the whole animal, chemical as well as physical, constitute the essential part of the study of the life phenomenon.

In my opinion, pharmacology is a science to elucidate the mechanism of the response of living matter to a given substance in its whole aspect. This, in turn, contributes to the elucidation of the physiology of living matter.

The Japanese Pharmacological Society, comprising medical, dental, veterinary and pharmaceutical pharmacologists, has now attained adulthood, nationally as well as internationally, both in size and in activity. Among driving forces that have caused the Japanese Pharmacological Society to develop rapidly, the introduction of American medical sciences after the end of World War II is decisive. Equipment and facilities came in torrents from the U.S.A. to Japan. Sophisticated young men visited the U.S.A. in enormous numbers spending one, two, or more years there, and they brought home the spirit of positiveness and independence from tradition, in every aspect of study and research.

Transfusion of American culture into postwar Japan brought progress in sciences together with confusion in social life. Among the changes and con-

fusions we have encountered in social life as well as in academic study is the breakdown of the family system of Japan. For good or evil, the family system traditionally was the backbone of Japanese culture in its every aspect, in pre-war days. The breakdown of this family system encouraged a spirit of independence and a sense of freedom from all traditional, social, and family life, especially in the younger generation.

The energy of the younger generation culminated in a wonderful increase in gross national production. It also applied to the advancement of scientific activity as evidenced in the increase in number of member Societies of the Japanese Association of Medical Sciences (in which I have been active as the vice president since 1963) from 34 to 62 during the period of 1947 to 1969.

On the other hand, our medical education system and postgraduate education system have remained unchanged since the beginning of the Meiji era—that is to say, during some 100 years.

Social as well as legal aspects of our medical education system must be adapted to the drastic social changes and to the social needs of post-war Japan. Taking the intern system as an example, there is no objection by graduate students or instructors to the intern system for training physicians. However, in Japan, sufficient money for teachers, equipment, and arrangements was never provided by the government. In other words, we imported the seed of the system of internship in postgraduate education but we failed to provide sufficient care and fertilizer to make it grow.

The Japanese people by nature are very keen in modeling foreign patterns in every aspect of human life, in production as well as in consumption. We imported Indian culture by way of China and created Japanese culture by modeling after it; however, in order to digest the foreign culture and to make it fit for the advancement and creation of our own culture, we have to provide every effort and means for the setting up of a nursery for growing foreign culture. If not, the foreign culture transplanted will be distorted or become the cause of confusion.

We Japanese have surely attained considerable economic development as viewed by our gross national production; however, we have many problems left to be solved, especially in medical education. To meet the ardent need for avoiding the confusion now raging in medical schools in our country, we have to work out the leading opinion as to medicine itself.

In this respect, I suggest that medicine is a science for the study of the laws of existence of the human race on this planet. In my opinion, to follow the natural law of survival or the struggle for existence is not sufficient for the ultimate welfare of the human race, we must aim at the co-existence and prosperity of all the human race.

For the realization of this principle, we have to work not only on human biology but also on human ecology. We have to keep in mind the fact that experimental biology and experimental medicine constitute only a part of medicine.

Recognition of this fact makes anyone who takes part in the study of

medicine be more modest in drawing conclusions from his experimental data and makes him careful not to distort the truth.

Pharmacology by definition is the study of the response of living matter to substances (chemical or physical) administered, and thus it belongs to experimental biology and experimental medicine, and indicates the position of pharmacology in medicine.

We have, however, an important but difficult problem to be solved regarding pharmacology in medicine. The problem may be written as follows: Is it possible or permissible in medicine to extrapolate pharmacological findings obtained in experimental animals to human beings?

The only thing we pharmacologists can do both from a scientific as well as a humanistic point of view is to work out a precise and elaborate spectrum of actions of a given drug on subcellular structures, cells, tissues, organs and in the whole animal in terms of metabolism, excitation, excitation-contraction, and excitation-secretion, and apply the spectrum to the human body, and evaluate its effect. It is to be noted that pharmacotherapy itself is a kind of human experiment.

As pointed out by the late Professor Gaddum, we pharmacologists have to borrow every means possible from the advanced fields of sciences, i.e., physics, chemistry, molecular biology, genetic biology, and so on, to elucidate the mechanism of drug action, which in turn reveals the mechanism of living matter.

To accumulate facts or phenomena about the whole animal behavior, and consolidate it into a system, is surely an important process in elucidating life's phenomena; however, phenomena have to be based on physical or physicochemical facts, in other words on a material basis. This way of my thinking or philosophy is the deduction from my experiences as a pharmacologist during the past 37 years.

At the onset of my academic life in 1932 I was engaged in the study of uterine activity in an unanesthetized bitch by means of a chronic uterine fistula. I worked with Professor Azuma, who was a pupil of the late Sir Henry Dale, at the Imperial University of Tokyo. By this method, studies were carried out to analyse the effects of hormones on uterine activity and to find out the water soluble principle of domestic ergots.

In doing this kind of experiment my interest became directed to the activity of smooth muscle.

This led to the study of muscle in more basic aspects, and a first step was set up at the "Conference on the chemistry of muscle contraction" in 1957 in Tokyo under my chairmanship. Together with my pupil, Professor Setsuro Ebashi, I conducted physicochemical study of muscle energetically and found a muscle relaxing factor which was proved to be in the endoplasmic reticulum. This later was proved to be a protein that accumulates calcium, and thus the role of calcium in muscle contraction was established. The development of the study of muscle contraction may be traced in "Molecular Biology of Muscle Contraction, 1965"—Elsevier, Amsterdam.

As a result of these basic studies concerning muscle contraction, the role

of Ca-ions as the basis of pharmacological action such as that of caffeine was clearly established. These findings are the outcome of contributions from colleagues from all over the world.

As I had a good successor, Professor Ebashi, in muscle study, I myself engaged in the study of the physiology and pharmacology of the central nervous system and especially the brain stem respiratory center and the unit discharge in inspiration and expiration, its localization, and its sensitivity to drugs.

Here also reigns my philosophy: phenomena must have as their basis a precise material background, that is to say, the respiratory center must have a neuro-cellular basis. In the study of the physiology and pharmacology of the respiratory center I am lucky to have had as my coworker Professor Fuminori Sakai, who succeeded to my chair in pharmacology at the University of Tokyo.

The scientific works in which I have been involved are limited and much is left to the hands of the worthy successors in my department.

In closing this manuscript, I emphasize the two important roles of the professor, one is to conduct academic study by himself, the other is to bring up competent and excellent successors. Scientific research must be carried out by cooperation not only on a global scale but also in the generational scale.

RECEPTOR MECHANISMS

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Introduction

Problems of receptor pharmacology have seemed stalemated for many years. Enormous quantities of data on drug potency have accumulated but it has proved singularly difficult to relate these to molecular events. Indeed it has become evident that structure activity studies involving a drug of uncertain conformation with a receptive system the nature of which we were almost totally ignorant was really not capable of yielding answers in molecular structural terms. The missing quantities are closer definition of the sites of drug action so that when comparing drugs we are certain that they are comparable, a knowledge of the structure of binding sites so that both components of the reaction should be known, and an understanding of the configuration of the drug in its bound state. Further, we must know what energy barriers must be surmounted in forming the drug receptor complex and what configurational changes result. Lastly the most pregnant question is how the receptor events are translated into physiological events. Advances in the biological sciences suggest that all these problems can now be solved with the available techniques, and in the period under review we are able to report a number of very encouraging advances. The problems are great but the task of isolating and characterizing a receptor now is surely no greater than was that of isolating a soluble enzyme forty years ago. This is not to suggest that with the characterization of even ten receptors pharmacology will become a simple science. Biological systems are intrinsically complicated and often devious, and pharmacological agents are often fearfully blunt weapons, but how helpful it would be to have at least a few problems solved at the molecular level to stiffen the foundations of our science.

In this review I have concentrated on the problems of the cholinoreceptor mainly because it is better studied than most other receptors and the problems it raises are therefore more continually before our eyes.

Structure and Conformation of Drugs

Until recently most attempts made by pharmacologists to characterize the spatial configurations of drugs were based on average values for bond lengths and angles derived from X-ray studies on other molecules together

with simple selection rules to derive the most probable conformation. It is heartening to see that a number of crystallographers are now taking an interest in drugs and we now have available reliable X-ray data on acetylcholine, methacholine, noradrenaline, bisoniums, histamine, and some related compounds (1–8). In the matter of bond lengths and angles there has so far been little novel revealed, but the question of conformation seems now to be yielding results consonant with other interpretations provided that reasonable precautions are taken. Few protein chemists still maintain that crystal structures are irrelevant to the behaviour of proteins in solution, but the retention of the dissolved conformation in the crystal depends on the massive character of the protein and the filling of the vacancies in the unit cell by solvent. In the case of small molecules the constraints of packing in the crystal lattice are more serious and more likely to be influenced by factors without biological significance such as the size of the anion. It is usually assumed that large anions are less likely to perturb the conformation of the drug cation than small anions. Nevertheless it is useful to have external supporting evidence of the preferred conformation.

Extensive use has been made of molecular orbital (MO) calculations (9–15) particularly by Kier. In this method the total energy of the molecule is evaluated by the linear combination of atomic orbitals. The calculation is carried through for all the combinations of torsion angles so that a plot of total molecular energy against torsion angle is obtained. The minimum energy corresponds to the preferred conformation. Other minima correspond to alternative less preferred conformations. In the case of muscaraine a sharp minimum is found at a torsion angle of 150° with respect to the ring. The torsion angle found agrees reasonably with the X-ray values (110°). The energy minimum is about 6 kcals/mol below the maximum, so that the conformation must be regarded as strongly preferred. In the case of acetylcholine, however, the α,β torsion angle shows a much smaller minimum of about 0.6 kcals/mol at 80°. This angle also agrees with the X-ray evidence. Less distinct minima are found for the ester linkage, but the preferred configuration for carbonyl-oxygen and oxygen-methylene links are both trans. The same technique has been applied to muscarone, nicotine, histamine, noradrenaline, ephedrine, and pralidoxime. The widely used MO method is recognized to be comparatively imprecise and is liable to overestimate the values of the energy barriers. An alternative theoretical approach in calculating peptide conformation (16–18) is to use van der Waal's interaction functions to compute the potential energy as a function of the torsion angles. For muscarine the torsional angle for minimum energy was 101° and the energy minimum 6.4 kcals/mol. This is in good agreement with the X-ray evidence. Application of this method to acetylcholine shows that there is little to choose between the conformations, the gauche conformation (107°) of $\alpha\beta$ bond being slightly preferred (0.4 kcal/mol). A gauche conformation of the oxygen-methylene bond was also slightly favoured. From the energies one can estimate the relative proportions of the four conformers as 0.40, 0.25, 0.21, 0.13.

These results suggest that acetylcholine in solution is a mixture of conformers undergoing inter-conversion and that it would be unwise to draw special pharmacological attention to the lowest energy state. Because of the existence of multiple conformations there is some importance in knowing how rapidly these are inter-convertible. Estimates of the stability of these conformations has been obtained by NMR relaxation measurements (19). The method measures the rate of rotation of molecular groups in solution and makes it possible to decide how much rotation of the group is caused by tumbling of molecules as a whole and how much by rotation around the bond axis. The energy barrier to rotation about C-C and C-N bonds in aliphatic molecules is sufficiently great that many rotations of the molecule occur for each bond rotation, but O-C bonds allow rotation around the bond nearly as easily as for rotation of the whole molecule. Applied to acetylcholine this means that the conformation of the acetyl group alters much more rapidly than that of the α-β bond, nevertheless the lifeline of the latter conformation is probably not more than a few microseconds. The conformations are thus very rapidly inter-converted. Nevertheless, considered in the time frame available for forming a complex with a receptor by collision the conformation of the α-β bond must be regarded as pre-existing and fixed whereas the acetyl conformation can change. In aromatic drugs it may be possible to determine the twist angle between the ring and a side chain by the ultraviolet spectrum (20). A study of the relationship between the conformation of choline aryl ethers and their nicotinic activity showed that activity was confined to those in which the ring and the side chain β-carbon were coplanar (21).

The latest attempt to avoid the uncertainties of multiple conformation by the use of rigid analogues of acetylcholine has been successful. The molecule was 1-acetoxy-cyclopropyl trimethylammonium. This molecule exists in four enantiomers, none of which is precisely the same as the acetylcholine conformers because of the distorted bond angles of the three membered ring. However, the trans (+) isomer was as active as acetylcholine on the ileum whereas the trans (−) isomer and the cis (±) were less than 1/200 as active (22, 23). A rather similar approach to the catecholamine receptor has been taken with the synthesis of stereospecific aminodecalols (24). The conformational similarities between muscarinic drugs and phospholipids, and betaine have been pointed out (25, 26) and a study of the binding of noradrenaline to phospholipid reported (27).

Attempts continue to put structure-activity studies on a more analytical basis. These consist mainly in correlating some chemical property of a drug series with activity. These may be Hammett substituent constituents, electronic densities derived from MO calculations, or oil-water partition coefficients or a combination of these (28–35). It appears that such measurements may have a considerable usefulness in planning synthetic programs, which is perhaps surprising in view of the omission of information on three-dimensional structures. For instance such studies fail to account for the pharma-

cological differences of enantiomers. Indeed the study of enantiomers remains a most sensitive test of drug specificity and has been especially of interest in β-adrenergic blocking agents (36–39). An excellent general review of stereospecificity in reactions of the cholinoreceptor and cholinesterase has also appeared (40).

A number of authors have seen the value of studying structure activity relationships against a well defined characteristic such as binding to a biological macromolecule. A readily available binder for parasympathomimetic molecules is acetylcholinesterase, and Belleau (41) has provided a thorough thermodynamic analysis of the binding of alkyltrimethylammoniums. He concludes that the binding of the ammonium head is mainly enthalpic but the binding of the lengthening alkyl chain is entropy controlled and hence primarily dependent on water structure. It is difficult to take this argument further because of the considerable uncertainties about the structures of liquid water; these are made clear in an excellent new book (42). The investigation of a distinct species of liquid water (polywater) may have relevance to water behaviour at interfaces and may well provide fuel for pharmacological imaginations (43). A related approach to the study of the structural correlates of activity is to generate binding macromolecules by making antibodies to an appropriate member of a drug series. Marlow et al. (44) have coupled the nicotinic drug cholinephenylether to protein and raised antibodies against it. The antibodies combined with a variety of drugs that react with cholinergic receptors, including both agonists and antagonists, both muscarinic and nicotinic drugs. While these results would appear to answer in the affirmative the question whether agonists and antagonists can really combine with the same site (45), the common feature of the drugs that combined was a basic group, so that the answer does not have the universality that is desirable. The same technique has been used to explore the binding of simple alkylamines (46) and to derive free energy components of binding. Again a low order of specificity is seen in the complex, and for the alkyl trimethylammonium group the results are in close accord with Belleau's results referred to above (41). The antibody technique has also been applied to the hallucinogenic methoxyphenyl alkylamines (47). Antigens were formed by coupling with polyglutamic acid by carbodiimide. Substantial differentiation between 2, 5 dimethoxy and 3, 4, 5 trimethoxy phenylalkylamines was achieved.

Since ideas of receptor activation are so strongly focused on conformation changes, it is important to keep under review the techniques that are useful in this field as well as the general results which are regularly reviewed in the companion volumes (48). Useful reviews on protein conformation (49), denaturation (50), and X-ray diffraction (51) have appeared. A technique that has come of age in biology this year is nuclear magnetic resonance (NMR). Jardetzky and his colleagues have completed an extensive series of studies on ribonuclease and staphylococcus nuclease and their reactions with inhibitors and have not only been able to identify binding

aminoacid residues but also to identity conformational conversions (52–55). With the technical development in the method a wide range of possibilities are opening up. Several direct applications to pharmacological problems of binding have appeared (56–59) and a comprehensive review is available (60).

Among the interesting conformational changes described in the period under review we may note that a large effect on the exchange of water with staphylococcus nuclease was found by the cooperative action of desoxythymidine diphosphate and Ca ions (61), that the kinetics of a fast allosteric process have been described (62), as well as an exceptionally slow conformational change ($t_{1/2}$~40 min) produced by 4'-(4-aminophenylazo) phenylarsonic acid with subtilisins (63). Of particular interest is the demonstration by Mansour and his colleagues that serotonin and adenosine 3' 5' phosphate are able to alter the conformation of phosphofructokinase from the fluke Fasciola hepatica (64).

Discrimination of Receptor Sites

A continuing problem of studies in drug receptors is the difficulty of being sure that a set of drugs are all operating on the same system. One group have used a battery of ten tests to evaluate agonists on smooth muscle (65, 66) and have come to the conclusion that indirect action may be important. This may be the recognized method of ganglionic stimulation typified by nicotine or by some other mechanism of neural release of acetylcholine that may mediate the actions of acetylcarbocholine and acetylsilicocholine. It seems improbable that neural mechanisms are typical of the action of partial agonists (67). Evidence based mainly on agonist structure-activity relationships using muscle contraction and potassium efflux as criteria of drug action on guinea pig ileum, indicates that these are mediated by distinct but related receptors (68) which nevertheless are not distinguished by antagonists. A comparable differentiation of the nicotinic receptors has been found in leech muscle by measuring pA_2 values of gallamine (69) and in the electroplax by the finding of supramaximal addition (70).

Considerable doubt has been thrown on the use of β-haloalkylamines as means of determining spare receptor ratios by the finding that a nonalkylating but slowly reversible component of the antagonism is present which is rapidly reversible by thiosulphate (71–73). After removal of the reversible component, no spare receptors were detectable in the vas deferens treated with SY 28. Similarly in intestinal muscle when flux and contraction were studied a parallel shift occurred in the contraction, but no shift in the flux dose response curve, whereas both shifted with atropine and benzylcholine mustard (68). Nevertheless, this method continues to be used and often gives most plausible results (74).

Another very interesting development has been the finding that the two autonomic transmitters may affect the release of each other, presumably by neural mechanisms. In the heart, acetylcholine causes a small increase in

noradrenaline output; this increase is greatly potentiated by atropine. On the other hand the considerable increase in noradrenaline output caused by dimethylphenylpiperazine (DMPP) is not increased by atropine. The DMPP stimulated output, however, can be inhibited by acetylcholine, methacholine, or pilocarpine and this inhibition is prevented by atropine. There is evidently a muscarinic inhibition of noradrenaline output by the adrenergic neurons in the heart (75). In the guinea pig ileum, on the other hand, adrenaline and noradrenaline reduce the acetylcholine output both at rest and in the electrically stimulated preparation and this effect is annulled by α-blockers. Phenylephrine and amphetamine also produce these effects, but isoprenaline, dopamine, and methoxamine do not (76).

In the field of adrenergic β-receptors some important new agonists have appeared having selectivity for bronchodilator and vascular effects. Sotorenol (77) and salbutamol (78) are simple variants of catecholamines, but trimetoquinol (79) is related to papaverine. Two other new classes of β-stimulants have also been described (80, 81). There have also been further studies on β-receptor antagonists in which specificity has been emphasized (82, 83). An entirely new type of irreversible α-blocker is of particular interest because it is of low intrinsic chemical reactivity (84).

Studies on synapses in invertebrates continue to turn up interesting variants on drug receptors. In the pleural ganglia of Aplysia some neurons show two phases of depolarization in response to presynaptic stimulation. Both early and late phases are mimicked by acetylcholine and carbachol, but nicotine, DMPP, oxotremorine, propionylcholine, methacholine, and tetramethylammonium produced only the early phase. Tubocurarine blocked the early phase but had no effect on the late phase. Atropine and other muscarinic antagonists were without effect on either phase (85). Later experiments revealed that β-methylxylocholine could block the late phase and this is especially remarkable since xylocholine itself was ineffective (86). It was also found possible to block the late phase by the intracellular injection of TEA (87). These studies introduce two new kinds of acetylcholine receptors into our repertory and remind us that the variety of receptors may be large.

Uptake Processes

Accumulative processes for acetylcholine and other synaptic elements have continued to be studied and as they involve a binding site are of interest in the consideration of drug receptors. The uptake of acetylcholine into cerebral cortex slices is inhibited when energy metabolism is inhibited, by treatment with phospholipase A or C and also by drugs related to acetylcholine, such as hemicholinium, atropine, choline, and eserine (88, 89). Further studies have been made of the uptake of carbachol and decamethonium by the cerebral cortex, and inhibition by morphine has been reported (90–92). It is doubtful if this is connected with the pharmacodynamic action of morphine.