DRUGS AND CENTRAL SYNAPTIC TRANSMISSION

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Preface

The nature of synaptic transmission in the central nervous system and its role in relation to the mode of action of drugs affecting brain function is of considerable current and widespread interest. Thus, the presence in New Delhi, on the occasion of the 26th International Congress of Physiological Sciences in October 1974, of a group of research workers in the various aspects of brain research, provided a unique opportunity to bring them together at a satellite symposium, to discuss this topic and present their most recent findings. This book therefore contains the material presented at this recent international symposium which was held at the Central Drug Research Institute in Lucknow from 28th to 30th October 1974, under the title "*Use of Pharmacological Agents in the Elucidation of Central Synaptic Transmission*". The symposium was jointly sponsored by the Central Drug Research Institute and the Indian Pharmacological Society.

Even though drugs have been used as tools in pharmacological, physiological and neurochemical studies for a long time, the symposium was perhaps the first interdisciplinary meeting where this usage of drugs was the main theme. The disciplines represented at the meeting included (besides classical pharmacology and physiology) electrophysiology, neurochemistry, biochemistry, behavioural sciences, etc. An important feature of the book is that it contains a series of articles (Nos. 1–4) reviewing some specific major aspects of central synaptic transmission, in each case by a well-known investigator in the area concerned. These reviews contain the data presented as special lectures at the symposium. In total, the book contains 32 papers (Nos. 5–36) presented at the symposium, incorporating original observations (including hitherto unpublished results) of the participants. Since all the papers have been given by invited participants there has been coverage of all the major aspects of synaptic transmission, and yet there is little duplication or overlap of data. The present volume thus combines the virtues of a well-planned book with those of a multi-disciplinary symposium.

We should like to thank the Council of Scientific and Industrial Research, New Delhi, India, and the Ministry of Health, Government of Uttar Pradesh, Lucknow, India, for financial support for the symposium. We are grateful to Dr Nitya Nand, Director of the Central Drug Research Institute, Lucknow, for actively helping in the planning and organisation of the symposium, and for making available the excellent facilities of the Institute. We should also like to

express our gratitude to Professor K. P. Bhargava, Head of the Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow, Dr J. D. Kohli, Industrial Toxicology Research Centre, Lucknow, and Professor R. L. Singhal, Department of Pharmacology, Medical School, Ottawa, Canada, for their individual help in numerous ways in the organisation of the symposium. Our thanks are due to Miss Diane Collins for her help in editing and collating the manuscripts and to Mrs Susan Green for the index. Finally, we must acknowledge the excellent co-operation of all the contributors in keeping both the time schedule as well as the proof schedule for their contributions.

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Introduction

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The subject of this International Symposium is pharmacological but it also deals with a fundamental physiological problem. Associated biochemical, histochemical and electrophysiological studies as well as a combination of techniques, have all aided in the elucidation of central synaptic transmission. Present at the Symposium were eminent scientists from different disciplines whose main concern has been the study of central synaptic transmission. The central theme in all studies has been the use of pharmacological tools in different neurobiological situations, each one adding knowledge to the understanding of central synaptic function.

The transmission at synapses in the nervous system is now universally accepted to be 'chemical'. The criteria for assigning a neurotransmitter function to a specific chemical substance at a particular synapse were laid down soon after the convincing demonstration of the neurotransmitter role of acetylcholine at a peripheral synapse by Otto Loewi in 1921. The first use of a pharmacological agent in the elucidation of synaptic transmission was made in these classical studies of cholinergic transmission at the cardiac end of the vagus nerve. The identification of acetylcholine was aided by the use of a pharmacological agent, eserine, an inhibitor of cholinesterase. The continued use of eserine has been instrumental in the discovery of the transmitter role of acetylcholine at central sites. When McIntosh and Oborin (1953) found that acetylcholine is released from the surface of the cortex in quantities which vary with cerebral activity, it was again by preserving the released substance with eserine that the experiments were possible.

To assign a neurotransmitter role to a biogenic substance, it is necessary to demonstrate its release from activated nerve endings and exogenous application of the chemical must exhibit postsynaptic actions similar to those produced by nerve activation. Furthermore, the effects of the nerve activation and chemical application should be blocked by appropriate antagonists. The enzymes of synthesis and degradation should be identified in the neuronal tissue.

The multiplicity of synapses and the complexity of their arrangement in the central nervous system has been the biggest hurdle in providing direct proof for neurohumoral transmission. The techniques which have been used successfully for determining the identity of chemical substances released at peripheral synapses are difficult if not impossible to apply to the central nervous system (CNS). Nevertheless, overwhelming indirect proof has been accumulated for acetylcholine, noradrenaline, dopamine and 5-hydroxytryptamine (serotonin, 5-HT) at central synapses. All these putative neurotransmitters have been demonstrated in the central nervous system, and enzymes for their synthesis and degradation have been identified. Furthermore, agents have been developed which block the synthesis or degradation of these neurotransmitters. More or less specific agents are known which block the postsynaptic action of the neurotransmitters. In fact the range of activity of pharmacological tools has expanded and we now have precursors of the neurotransmitters which readily cross the blood–brain barrier and raise the concentrations of the biogenic substances in specific neurones. Pharmacological agents can activate specific noradrenergic, dopaminergic, cholinergic and serotonergic receptors. Thus, ample pharmacological proof exists for several putative neurotransmitters. Similar evidence is accumulating in favour of histamine, amino acids and other biogenic substances as central synaptic transmitters.

A wide array of pharmacological agents which could be used as valuable tools for investigating synaptic transmission are now available. They can interfere with almost every step of synaptic transmission such as transport, synthesis, storage, release, postsynaptic action and inactivation of the putative neurotransmitters. The steps in the biosynthesis and metabolism of specific neurotransmitters are now much better understood. This wealth of information was mostly obtained from studies on peripheral synapses but is being successfully employed for the study of central synaptic transmission. The rate limiting enzyme, tyrosine hydroxylase, in the biosynthesis of catecholamines can be selectively inhibited, by α-methylparatyrosine (α-MPT), resulting in a marked drop in neuronal catecholamine levels (Spector, Sjoerdsma and Udenfriend, 1965). Similarly, diethyldithiocarbamate (DDC) is an agent which inhibits the enzyme dopamine-β-hydroxylase thus reducing concentrations of noradrenaline in the brain while raising those of dopamine (Carlsson, Lindqvist, Fuxe and Hökfelt, 1966). Again, we have agents like MK-486, which inhibit the peripheral decarboxylase enzyme, and thus make available greater amounts of precursors of neurotransmitters for conversion to the neurotransmitters in the brain. Parachlorophenylalanine (*p*-CPA) is a potent inhibitor of tryptophan hydroxylase and leads to a profound reduction in brain 5-HT levels (Koe and Weissman, 1966). Its actions are not specific, however, as *p*-CPA can compete with transport mechanisms for tryptophan, phenylalanine and other amino acids (Grahame-Smith, 1971) and also produce small depletions of brain catecholamines (Koe and Weissman, 1966; Miller, Cox, Snodgrass and Maickel,

1970). The difficulty in finding a drug that acts exclusively on serotinergic mechanisms remains the crucial limitation to pharmacologically based investigations of 5-HT mediated neural function. In fact, one can generalise as well as emphasise that pharmacological tools, though versatile in their range of activity, are not entirely specific.

Of even greater interest is the unique possibility of selectively destroying noradrenergic and dopaminergic neurones in the brain by intraventricular or intracerebral injections of 6-hydroxydopamine (Uretsky and Iversen, 1969; 1970). Protriptyline and imipramine can efficiently protect the noradrenergic neurones and so leave the dopaminergic neurones to selective destruction by 6-hydroxydopamine (Evetts and Iversen, 1970). Intraventricular injection of 5,6-dihydroxytryptamine leads to a destruction of serotonergic nerve terminals although the destructive effects of this compound on serotonergic neurones does not seem to be as efficient as that of 6-hydroxydopamine on catecholaminergic neurones (Baumgarten, Björklund, Lachenmayer, Nobin and Stenevi, 1971).

The first and still the best-documented example of a cholinergic synapse in the mammalian CNS is that between the collaterals of motor axons and the Renshaw cells of the spinal cord. This was made possible by the microiontophoretic technique, in which minute quantities of drugs are applied directly into the immediate vicinity of single neurones, thus overcoming diffusional and enzymatic barriers which normally restrict access of substances to neuronal receptors. By the multibarrel microiontophoretic technique, Curtis and Eccles (1958) obtained pharmacological proof for cholinergic transmission at the Renshaw cell synapse. Carbachol mimicked the excitatory actions of acetylcholine and cholinesterase inhibitors potentiated these effects, while both excitatory responses were antagonised by dihydro-β-erythroidine, a nicotinic receptor antagonist. Ryall reports (paper 7) on the importance of muscarinic receptors on the Renshaw cell.

The technique of microiontophoresis has aided the recognition of certain amino acids as natural agonists and established their selective antagonists. It is surmised that the amino acids function as synaptic transmitters at the primary sensory and inhibitory synapses where no other substance has yet been implicated. Curtis (paper 1) and other participants present reports on the transmission of amino acids at certain synapses in the CNS. Few of the papers deal with microiontophoretic studies with monoamines, although Bradley (paper 29) and Herz (paper 30) discuss morphine action at the neuronal level.

In spite of an immense amount of data which has accumulated from microiontophoretic studies, the difference in the technical factors make it difficult to interpret the results. Careful and critical evaluation is necessary to draw valid conclusions.

The excellent work on histochemical fluorescence by Andén and his colleagues in Sweden, has provided proof of monoaminergic transmission in the CNS. The monoaminergic neurone systems have been mapped out (Andén, Dahlström,

Fuxe and Larsson, 1965). Andén (paper 3) has now produced selective lesions of nigrostriatal dopaminergic neurones, the bulbo-spinal noradrenergic and serotonergic neurones and subsequently studied drug effects. By this technique, functions of each neurone system can be studied separately. Lal (paper 17) has similarly studied the interrelationship of cholinergic and dopaminergic systems.

At a symposium on 'Brain Histamine' in Paris (July, 1974) good evidence was presented for a synaptic transmitter role for histamine. We have shown the presence of H_1 and H_2 receptors, at least, in the area postrema. Schwartz (paper 36) provides further pharmacological proof for histaminergic transmission at central synapses.

Drugs have been localised to structures in the CNS by intraventricular injection or perfusion and similar techniques which limit their actions to the CNS. These studies have been employed extensively to delineate the role of brain neurotransmitter systems in the control of different physiological functions. Schmitt reports (paper 4) studies on the adrenergic and cholinergic mechanisms in the regulation of cardiovascular function. Saxena and Bhargava, working in Rotterdam, have provided further evidence for the existence of β-adrenoceptors in the hypothalamus concerned with the release of adrenal catecholamines. Similarly, intraventricular injection or perfusion of the ventricular system has been used for the study of neurone systems in the control of body temperature, antidiuretic hormone secretion, aggressive behaviour, reward behaviour, stereotypy and learning.

Furthermore, pharmacological actions of drugs and toxins active in the CNS have been studied to implicate a biochemical parameter of synaptic transmission. Thus, the analgesic action of morphine, the anticonvulsant action of diphenylhydantoin and the convulsant action of strychnine and nicotine are reported. Similarly the neurochemical effects of Δ^9-tetrahydrocannabinol and the toxins of *Lathyrus sativa* and cobra venom are discussed.

The biochemical approaches to the problem of central synaptic transmission have also been quite ingenious. Costa reports (paper 2) a new technique of microdissection of diencephalic nuclei and the study of turnover rates of putative neurotransmitters. The biochemical regulation of cholinergic and adrenergic transmitter synthesis are also discussed by Bhagat (paper 12) and Rama Sastry (paper 5). Sabelli (paper 14) presents evidence for a new modulator transmitter, 2-phenylethylamine, in the nigro-striatal dopaminergic system.

Let us hope that the studies presented here will further substantiate the identity of chemical transmitters involved at particular synapses, and will elucidate the nature of the receptors concerned. These studies are ultimately likely to be of therapeutic significance in modulating transmitter imbalance characterised by disease. The pharmacological studies, therefore, may have far reaching consequences.

REFERENCES

Andén, N-E., Dahlstrom, A., Fuxe, K. and Larsson, K. (1965). 'Mapping out of catecholamine and 5-hydroxytryptamine neurons innervating the telencephalon and diencephalon', *Life Sci.*, **4,** 1275–1279

Baumgarten, H. G., Bjorklund, A., Lachenmayer, L., Nobin, A. and Stenevi, U. (1971). 'Long lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine', *Acta physiol. scand.* (Suppl.), **373,** 3–15

Carlsson, A., Lindqvist, M., Fuxe, K. and Hokfelt, T. (1966). 'Histochemical and biochemical effects of diethyl dithiocarbamate on tissue catecholamines', *J. Pharm. Pharmac.*, **18,** 60–62

Curtis, D. R. and Eccles, R. M. (1958). 'The excitation of Renshaw cells by pharmacological agents applied electrophoretically', *J. Physiol., Lond.*, **141,** 435–445

Evetts, K. D. and Iversen, L. L. (1970). 'Effect of protriptyline on the depletion of catecholamines induced by 6-hydroxydopamine in the brain of the rat', *J. Pharm. Pharmac.*, **22,** 540–543

Grahame-Smith, D. G. (1971). 'Studies *in vivo* on the relationship between brain tryptophan, 5-HT synthesis and hyperactivity in rats with a MAO inhibitor and L-tryptophan', *J. Neurochem.*, **18,** 1053–1066

Koe, B.K. and Weissman, A. (1966). '*p*-Chlorophenylalanine: A specific depletor of brain serotonin', *J. Pharmac. exp. Ther.*, **154,** 499–516

McIntosh, F. C. and Oborin, P. E. (1953). 'Release of acetylcholine from the cerebral cortex. 19th Intern. Physiol. Congr. Therien Fre'res, Montreal, 580 (abstract)

Miller, F. D., Cox, R. H., Snodgrass, W. R. and Maickel, R. P. (1970). 'Comparative effects of *p*-chlorophenylalanine, *p*-chloroamphetamine and *p*-chloro-N-methylamphetamine on rat brain norepinephrine, serotonin and 5-hydroxyindole acetic acid', *Biochem. Pharmac.*, **19,** 435–442

Spector, S., Sjoerdsma, A. and Udenfriend, S. (1965). 'Blockade of endogenous norepinephrine synthesis by α-methyltyrosine an inhibitor of tyrosine hydroxylase', *J. Pharmac. exp. Ther.*, **147,** 86–95

Uretsky, N. J., and Iversen. L. L. (1969). 'Effects of 6-hydroxydopamine on noradrenaline containing neurones in the rat brain', *Nature.* **221,** 552–560

Uretsky, N. J. and Iversen, L. L. (1970). 'Effect of 6-hydroxydopamine on catecholamine containing neurones in the rat brain', *J. Neurochem.*, **17,** 369–378