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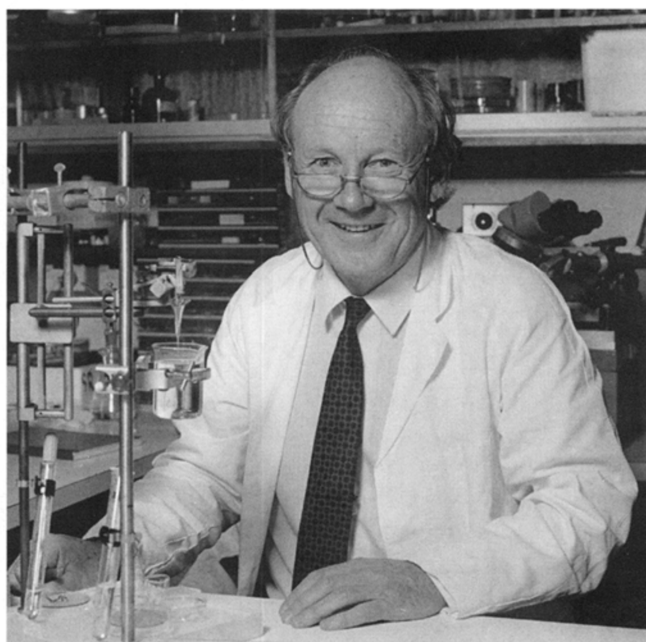

David R. Curtis

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Melbourne, Australia
June 3, 1927

Education:

University of Melbourne Medical School, M.B., B.S. (1950)
Australian National University, Canberra, Ph.D. (1957)

Appointments:

John Curtin School of Medical Research,
Australian National University (1954–2005)
Department of Physiology (1954–1973)
Department of Pharmacology
Professor and Head (1973–1988)
Division of Physiological Sciences,
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Director and Howard Florey
Professor of Medical Research (1989–1992)
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Fellow, Australian Academy of Science (1965)
Council (1974–1977)
Burnet Lecturer (1983)
President (1986–1990)
Fellow, Royal Society of London (1974)
Fellow, Royal Australasian College of Physicians (1987)
Companion, Order of Australia (1992)
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Australian Physiological Society
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David Curtis pioneered the use of microelectrophoretic techniques to examine the effects of potential synaptic transmitters on single identified neurons in the mammalian central nervous system (CNS) in vivo. He similarly examined the effects of compounds that blocked or enhanced synaptic transmission at the synapses of particular excitatory or inhibitory pathways. He thus contributed to the identification of a number of central transmitters, particularly glycine, gamma-aminobutyric acid (GABA), and aspartic and glutamic acids.

David R. Curtis

Family and Schooling

I was born at the beginning of the economic depression of the late 1920s–1930s. At this period our family circumstances were not particularly affluent because the salaries of public servants had been reduced by the government of the day. I was very fortunate, however, in having parents very aware of the value of education, although neither had attended a secondary school. My father, Edmund David Curtis (1900–1973), was the son of a building contractor. He had left school at the age of 16 to join the Postmaster General's Department (PMG), a Federal Department then responsible throughout Australia for all postal, telegraph, and telephone services, and later national AM and FM radio and television transmission.

By dint of night classes, correspondence courses, and in-service training, in what was a developing technological organization, my father progressed through the PMG to achieve engineering qualifications in 1924. At the time of his retirement in 1963, as Head of the PMG Engineering Division in Victoria and a Member of the Institution of Engineers Australia, he had participated in the replacement of Morse code instrumentation by teleprinters, in the transition from manual to automatic telephone exchanges, in the introduction of national radio and later television, and long-distance transmission by coaxial cable and by microwave radio. He had a particular interest in these developments as Head of the Research and Development Laboratories in Melbourne.

Hence I was strongly influenced by a home environment of scientific inquiry, particularly in matters electrical and mechanical. Education in mathematics and later physics and chemistry was stressed, and a well-equipped workshop was available at home. I have strong early memories of a childhood being involved in the construction of electrical, and later electronic, apparatus including radio receivers and audio amplifiers, and of mechanical equipment, as well as in the design and manufacture of items of wooden furniture. A major undertaking in 1943–1944 was my construction of a single channel ink-recording electrocardiograph, using thermionic tubes and other components then becoming available at Disposal Stores. All members of the family tested proved to be alive, and this project was important as an introduction to a maintained interest

in electronics, which was of inestimable value to my subsequent eventual career in neurophysiology.

Shortly after my birth my family had settled in the Melbourne suburb of Ormond, and from 1932 until 1938 I attended the local State Primary School. This was followed by 2 years at the Tooronga Road Central School in Malvern where I first came in contact with science. My academic achievements led to the award of a Junior Government Scholarship, which enabled me to attend the Melbourne Boys High School for 4 years.

This was a selective high school, and I was fortunate in having outstanding teachers in pure and applied mathematics, physics, and chemistry, and access to very good laboratories. In the December 1944 state-wide matriculation examination, a requirement for university entry, I gained Honors in both mathematical subjects and the State Exhibition in physics and was granted a Senior Government and a Gowrie Scholarship tenable at Melbourne University. These results qualified me for entry into either the Medical or Engineering courses. Biology had not been available as a subject at the Boys High School, but my parents had encouraged me to read a number of general biology text books. As a consequence, I chose Medicine rather than Electrical Engineering, a decision I have never regretted.

Melbourne University Medical School 1945–1950

This was then the only medical school in Melbourne. The physics and chemistry syllabuses in the first year of the course were less demanding than those for the matriculation examination. Thus, I was able to concentrate on zoology and botany, and the less academic aspects of university life. Professors influencing my interest in the nervous system in the preclinical years included Roy D. Wright (Physiology), whose somewhat obscure lectures necessitated long periods with textbooks, and Sydney Sunderland (Anatomy) who was an outstanding neuroanatomist. Pharmacology, a minor component of the physiology course, was more relevant to materia medica than to scientific pharmacology, and the CNS scarcely featured in Biochemistry.

My preclinical examination results included the Exhibition in physics and Honors in pathology, and my three clinical years were spent at the Royal Melbourne Hospital adjacent to the University, with shorter periods at the Royal Women's and the Children's Hospitals. At that time there were no university clinical departments in the teaching hospitals. Although some medical and surgical lectures were given at the University, the bedside teaching of medical students depended on honorary physicians, surgeons, and specialists appointed by the hospitals to care for patients.

Final examination results, which included Honors in Medicine and in Obstetrics and Gynecology, and the award of the degrees of Bachelor of Medicine (MB) and Bachelor of Surgery (BS), led to my appointment as a

Junior Resident Medical Officer (RMO) at the Royal Melbourne Hospital from January 1951.

Hospital Appointments 1951–1953

Life as a RMO in the then major City Hospital in the 1950s was never meant to be easy. Hours of duty were long, nights "off" were rare, and weekends off even rarer. Both my first year as a Junior RMO (1951) and a second as a Senior RMO were periods of intensive activity and learning in medical, surgical, emergency, and specialty wards. The only vacation I remember was an unpaid week in August 1951 when I married Lauri (nee Sewell), my most wonderful, understanding, and supportive wife.

Neurology and neurosurgery had been regarded as too specialized for medical students, but I was particularly fortunate in both my RMO years to enjoy terms in the Neurology/Neurosurgery Ward with two distinguished clinicians. The neurologist was Dr E. Graeme Robertson, a Melbourne graduate, who while training at the National Hospital for Nervous Diseases, Queen Square, London, had collaborated in research in the early 1930s with Derek Denny-Brown, a former colleague of John Eccles in Oxford and later Professor of Neurology at Harvard. The neurosurgeon Mr. Reginald S. Hooper, was also a Melbourne graduate and had trained in Oxford with Sir Hugh Cairns. His assistant, Mr. John B. Curtis (not related to my family) had also trained in Oxford. All were supportive and patient with a RMO whose interests were becoming centered on basic research rather than on further clinical involvement.

Third-year resident appointments at The Royal Melbourne Hospital were limited to potential physicians or surgeons training toward recognition as specialists. Hence I applied for the position of Neurological Registrar at the Alfred Hospital, which also was affiliated with Melbourne University. Here in 1953 I came under the influence of Dr. Leonard B. Cox and his assistant Dr. John A. Game who had trained at Queen Square, London.

Until their deaths I remained personally in touch with all five clinicians, particularly through meetings of the Australian Association of Neurologists and the Neurosurgical Society of Australasia. I later became an Honorary Member of both bodies.

This continuing excellent clinical experience, however, confirmed my resolve to undertake laboratory-based research relevant to neurological disorders rather than to be involved in clinical neurology or neurosurgery. Early in 1953 I read Sherrington's 1906 monograph *Integrative Action of the Nervous System* followed by his 1932 monograph *Reflex Activity of the Spinal Cord,* of which one of the coauthors was John Eccles. I failed to gain a grant from the National Health and Medical Research Council (NHMRC) to commence research in Professor Wright's Department of Physiology in 1954, and he suggested that I write to Eccles. Wright had played a major

role in the foundation in Canberra of the Australian National University (ANU) in 1946, and in the subsequent appointment in 1951 of Eccles to the Foundation Chair of Physiology in the University's John Curtin School of Medical Research (JCSMR).

Canberra

In 1901 the Commonwealth of Australia was created by the federation of the then six separate states, and, after much argument and negotiation, a site for the Australian Capital Territory was chosen in 1909, 240 km southwest of Sydney. Planning for the capital city began in 1911, and the name Canberra was selected in 1913. The Federal Parliament, which in the meantime met in Melbourne, moved to Canberra in 1927. At the time of the creation of the ANU in 1946 Canberra's population was 14,100, in 1953 it was 28,645, and by 2004 this had increased to 324,000.

John Curtin School of Medical Research

The ANU had been established in 1946 by the Federal Government as an all-research university (Foster and Varghese, 1996), and the JCSMR was one of four original Research Schools. John Curtin had been Prime Minister from 1941, when planning for the ANU had begun, until his death in 1945. Sir Howard Florey, an Australian who was then Professor of Pathology at Oxford, had a major influence on the origin and development of the JCSMR (see Fenner and Curtis, 2001). In 1953 the early JCSMR departments were housed in "temporary" buildings, which are still in use by the ANU, and the "permanent" building, now (2005) in the course of demolition and replacement, was occupied early in 1957.

Eccles had moved from Dunedin in September 1952 and commenced research in Canberra in March 1953 (see Curtis and Andersen, 2001). I wrote to him in August 1953 and received a very encouraging letter and a number of recent reprints. We arranged to meet in Melbourne in November when he was to lecture about his recent intracellular studies of the ionic basis of synaptic transmission in the mammalian spinal cord. At this time I had begun reading his 1953 monograph *The Neuro-Physiological Basis of Mind,* which had introduced me to an entirely new literature dealing with membranes, ionic conductances underlying resting, and action and synaptic potentials, all subsequent to my lectures in physiology some 6 years earlier.

I was fascinated by the lecture, which included much unpublished material, and after a short discussion of my background and interest in the pharmacology of central synaptic transmission I was virtually assured of a 3-year Research Scholarship to commence in his laboratory in February 1954. My appointment was confirmed soon after this by the ANU, which also provided a house for me and my family about 2 km from the University.

My wife and I, and our 16 month old son, Christopher Mark, arrived in Canberra on February 16. Our daughter Belinda Jane was born later that year in October. We had some difficulty in accepting that this rather small town was destined to be the nation's capital. February 16, 1954 is a very significant date in the history of science in Australia, because on that day Queen Elizabeth II in person founded the Australian Academy of Science by presenting its Charter to a number of Petitioners, which included 10 Fellows of the Royal Society of London then resident in Australia. This was the second time that a reigning monarch had presented a Charter to such a body since 1662, when King Charles II had presented his Charter to the Royal Society of London. Eccles (FRS 1941) was one of the petitioners, served on the Academy's inaugural Council and was its second President (1957–1961). I was later elected to the Academy in 1965 and was its eleventh President (1986–1990).

JCSMR Department of Physiology

In 1951 Eccles' outstanding achievement in Dunedin had been the intracellular recording of resting, synaptic and action potentials from spinal motoneurons *in vivo,* in collaboration with Jack Coombs and Laurence Brock. These results led him to discard his electrical hypotheses of central excitatory and inhibitory synaptic transmission, and to accept that these processes were chemical in nature. Another significant finding in Dunedin was that the convulsant alkaloid strychnine blocked the short latency and duration "direct" inhibition of spinal motoneurons. His aim in Canberra was to extend his research to provide further understanding of synaptic transmission in the mammalian CNS.

The Department's temporary (1953–1957) laboratories in a long prefabricated "hut" included three electrically shielded rooms, an electronics/mechanical workshop, a photographic darkroom, and an excellent range of specialized equipment. Apart from oscilloscopes, few items of appropriate apparatus were then available commercially. In Dunedin Jack Coombs, a brilliant physicist, had designed an innovative, versatile, reliable, and readily operated electronic stimulating and recording unit (the ESRU), and a number of these had been manufactured in New Zealand for the new Department in Canberra.

Coombs accompanied Eccles to Canberra and had also designed stable and low noise amplifiers, "cathode-follower" input stages necessary for recording through high-resistance electrolyte-filled microelectrodes and many other items of equipment. All of these initially depended on thermionic "valves," and radio-B "dry" and "lead-acid" batteries. Oscilloscope traces were photographed on still or moving 35-mm film using Grass kymograph cameras, and averaging was performed by superimposition of many photographed traces. Many hours were spent in measuring filmed

responses and in calculating results using logarithmic tables or slide rules, and later a hand-cranked calculator.

Eccles had appointed an excellent and very helpful technical staff, including Arthur Chapman, who had previously been his laboratory assistant in Oxford, Sydney, and Dunedin, and Gerry Winsbury, an innovative and skilled engineer. Much of the mechanical equipment, including sturdy animal frames, micromanipulators, and microelectrode "pulling" machines, was designed in the Department, and was made in the Department or by members of the JCSMR main workshop.

The major contributions of Eccles to CNS neurophysiology in Oxford, Sydney, and Dunedin had depended on the use of cats. Accordingly, arrangements were made in Canberra to provide an adequate supply of these animals for the continuation of his and his colleagues' research.

Introduction to Research

My introduction to neurophysiological research in 1954 was to observe the Coombs, Eccles, and Paul Fatt team completing their major study, which had begun a year earlier, of the biophysical properties of the membrane of spinal motoneurons and the nature of the ionic events underlying excitatory and inhibitory post synaptic potentials (EPSPs and IPSPs). I thus acquired knowledge of the animal preparation, including the dissection of hind limb peripheral nerves, lumbar laminectomies, the procedures necessary to maintain anesthetized cats for 24 hours or longer, and the preparation and filling of single- and double-barrel microelectrodes, then pulled by Fatt by hand over a small flame.

Eccles, Fatt, and K. Koketsu were also completing a study of the cholinergic excitation by impulses in motor axon collaterals of ventral horn interneurons that monosynaptically inhibited motoneurons, a recurrent inhibition blocked by strychnine. They named these interneurons "Renshaw" cells in honor of Birdsey Renshaw (1911–1948) who had earlier at the Rockefeller Institute in New York recorded their action potentials extracellularly. In addition Eccles, Fatt and Sven Landgren were engaged in a study of "direct" short latency and duration inhibition in the lumbar cord, which proved to be disynaptic. "Direct" IPSPs were shown to be blocked by intravenous (IV) strychnine whereas monosynaptic EPSPs were not affected.

There was indeed a wealth of research activity, ready access to experienced investigators, particularly Eccles, for advice and many techniques with which to become familiar. Furthermore, the developing JCSMR library was located in the same building, with continuous access to an accumulating range of current and back-numbers of all major neurophysiological journals. Friday afternoon seminars dealt with new results, drafts of papers in preparation, and critical analyses of other investigators' publications.

Hours of experimentation were long, often exceeding 24 hours, generally twice weekly. I had no other means of transport than a bicycle, but I rapidly became accustomed to very early morning arrival at the Department and a return home in the early hours of the next day, despite frequent and severe winter frosts in Canberra.

My first direct hands-on involvement in research was with Coombs and Landgren in mid-1954 in the extracellular recording and plotting of field potentials in lumbar spinal segments generated at areas of synaptic excitation by impulses in different groups of muscle and cutaneous afferent fibers. These fiber groups were characterized by their electrical thresholds and conduction velocities. A requirement for straight single-barrel microelectrodes led to the manufacture of a primitive spring loaded puller and the use of an electrically heated coil to melt the glass. This ultimately led to my involvement in the development of a considerably more sophisticated gravity and solenoid driven puller capable of dealing with multibarrel micropipettes (see Curtis, 1964).

Tetanus Toxin and Strychnine

In 1905 Sherrington had noted that the effects of tetanus toxin on spinal reflexes were similar to those of strychnine and proposed that both converted inhibition into excitation. In late 1954, as my introduction to neuropharmacology, I joined Eccles and Vernon Brooks in a study of the effects of tetanus toxin on a number of short latency and duration inhibitions of monosynaptic spinal reflexes by single volleys in a variety of muscle and cutaneous afferent fibers. Recurrent inhibition mediated by Renshaw cells was also examined. The toxin was injected either into a peripheral muscle nerve or directly into the spinal ventral horn in order to initially localize its action so that reflexes and inhibitions on the contralateral side could initially be used as controls. These experiments usually extended for 24 hours or longer. Like strychnine, the toxin enhanced polysynaptic but not monosynaptic spinal reflexes and diminished and eventually abolished all of the inhibitions studied. Because the toxin appeared to have no effect on field potentials generated by interneurons on the "direct" and recurrent inhibitory pathways, it thus appeared to act either pre- or postsynaptically at spinal inhibitory synapses. This study resulted in my first coauthorship of a letter to *Nature* and a paper in the *Journal of Physiology*.

Subsequently in 1955–1956 I showed that IV strychnine also blocked all of the short latency and duration inhibitions that had been shown to be reduced by tetanus toxin, as well as the inhibition of lumbar extensor muscle monosynaptic reflexes produced by stimulating ipsilateral forelimb cutaneous afferent fibers. Another type of "descending" inhibition, the reduction of lumbar monosynaptic reflexes by electrical stimulation of the

anterior lobe of the cerebellum, was also blocked by tetanus toxin locally injected into the lumbar spinal cord.

Many years later (see later), whereas strychnine was shown to be a selective postsynaptic antagonist at glycine-mediated (glycinergic) inhibitory synapses, tetanus toxin was shown to reduce transmitter release at both glycinergic and GABA-mediated (gabergic) central inhibitory synapses.

Another neuropharmacological study early in 1955 with Eccles and Rosamond Eccles involved the effects of intra-aortic administered drugs on lumbar dorsal-ventral root monosynaptic reflexes, all branches of the aorta below the renal arteries except the lumbar arteries to the spinal cord having been ligated. The transient reduction of reflexes by nicotine and acetylcholine (ACh), the enhancement of the effect of ACh by IV physostigmine, and the reduction of the actions of both ACh and nicotine by either IV dihydro-B-erythroidine or strychnine indicated that the inhibition was the consequence of the direct excitation of Renshaw cells.

Motoneurons

I was fortunate early in 1956 to join Eccles and Coombs in an analysis of orthodromic, antidromic, and directly excited action potentials recorded intrasomatically from motoneurons in the lumbar spinal cord of the cat and an analysis of the factors determining the generation of an impulse propagating into motor axons. Measurements were also made of the electrical properties of the motoneuron membrane as a means for estimating the time course of transmitter action underlying EPSPs and IPSPs. This collaboration with Eccles was later extended to a study of the amplitude of EPSPs, as a measure of transmitter release, during and after repetitive stimulation of the monosynaptic pathway.

Another project at this time was collaboration with Eccles and Anders Lundberg in recording action and synaptic potentials intracellularly from neurones in Clarke's column, neurones of origin of the dorsal spinocerebellar tract. Both this, and a similar study early in 1975 with Kres Krnjevic and Ricardo Miledi of the shortest latency inhibition of sacral motoneurons by impulses in low threshold contralateral dorsal root fibers, indicated the involvement of an inhibitory interneuron in these types of inhibition.

I submitted my Ph.D. thesis (Synaptic Transmitters in the Spinal Cord) in July 1956 and was admitted to the degree in December, which was awarded in May 1957. In the meantime, in July 1956 I had been appointed a Research Fellow (nontenured) in the Department of Physiology. Promotion to the tenured position of Fellow followed in December 1957.

Neuropharmacology

In January 1957 the Department of Physiology moved into the new JCSMR building. The additional space enabled the expansion of the staff and an opportunity for an increased interest in CNS neuropharmacology and neurochemistry. There were six large laboratories, each with an air-conditioned shielded room, studies for each member of the academic staff and research students, mechanical and electronic workshops, a dark room, and a room for histology. Since 1953, despite the somewhat remote location of Canberra, the reputation of Eccles and the high quality and originality of the research being carried out continued to attract numerous distinguished scientists from abroad. By 1966 when Eccles moved from Canberra to the United States, 74 investigators from 14 countries had collaborated in research and published with him.

Microelectrophoresis

Now with my own laboratory, I was able to direct my research toward the pharmacological aspects of central excitatory and inhibitory synaptic transmission. Identification of the transmitter of a particular pathway would enable the rational development of therapeutic agents with specific effects at its synapses, where defects of transmission resulted in human neurological disorders. Additionally, knowledge of transmitters could also provide an explanation of the effects of known drugs and toxic agents on the nervous system.

As an initial step in developing appropriate techniques I chose to make a further study of the cholinergic excitatory synapse between motor axon collaterals and spinal Renshaw cells. This had been investigated in 1953 by Eccles, Fatt, and Koketsu and their findings, as well as the results I had obtained with Eccles and Rosamond Eccles in 1955, suggested that a blood–brain barrier prevented the access of some systemically administered compounds, including ACh, to central synapses.

Additionally, when compounds are administered systemically or topically, difficulties arise in ascribing the effects on central neurons *in vivo* solely to actions at specific central synapses under investigation. Hence, there was a need to combine extra- or intracellular recording from single anatomically and physiologically defined neurones with the administration of compounds of pharmacological interest directly and in a controlled fashion in their immediate environment. Pressure injection was not suitable because of leakage from micropipettes and the displacement of neurones at the injection site.

Local administration was clearly desirable, and the technique used earlier by William Nastuk, and by Bernard Katz and Jose del Castillo, to investigate the chemical sensitivity of the end-plate of skeletal muscle fibers

in vitro was considered to be particularly appropriate. For most compounds of interest a suitable salt can be chosen which dissociates in aqueous solution and for which the complementary anion or cation is pharmacologically inactive. Consequently the diffusional efflux (leakage) of the active ion from the orifice of a glass micropipette containing the solution can be reduced by passing a suitably directed electrical (retaining) current through the pipette, and the active ion ejected by reversing the direction of the current flow (see Curtis 1964).

In these earlier *in vitro* studies, intracellular recording microelectrodes and extracellular micropipettes were attached to separate micromanipulators. Because such a procedure is impracticable when dealing with single neurons located within CNS tissue *in vivo,* recording microelectrodes and drug-containing pipettes need to be attached to one micromanipulator. Consequently multibarrel "microelectrodes" were designed and made in Canberra. Extracellular neuronal action potentials were recorded using the central barrel of five- or seven-barrel assemblies. The blanks were manufactured by the JCSMR workshop's skilled glass blowers, and drawn out to the desired shape and length in a vertical "puller." The tips were broken and ground back to an overall diameter of 4–8 microns. Later, central barrels were also used as stimulating electrodes to excite nerve fibers and unmyelinated axon terminations (see later).

For recording intracellular potentials, pencil or coaxial electrodes were initially used, with a single- or double-recording electrode protruding 60–100 microns through the 8- to 10-micron diameter orifice of the single drug-containing barrel. These proved to be unsatisfactory, and in 1967 separate single- or double-barrel glass recording microelectrodes were cemented along the side and parallel to five- or six-barrel assemblies, projecting beyond their tips by 40–100 microns.

Although the term "micro*iontop*horesis" has been used for this technique of drug administration, the delivery of nonionized drugs in aqueous solution containing sodium and chloride ions can also be controlled by electrical currents. Hence electro*osmosis* may also be involved, particularly when, as became apparent, dilute solutions of ionized compounds in 165 mM sodium chloride enabled better control of diffusional leakage by retaining currents. Hence the term "micro*electrop*horesis" seemed to be preferable to describe this microelectrophoretic (ME) procedure of drug administration.

The major significance of the introduction in Canberra of multibarrel microelectrodes was the ability to examine the effects of as many as six different agents on a single neuron or axon. It thus became possible to mimic, enhance, and antagonize the actions of synaptically released transmitters, thereby providing information relevant to transmitter identification. The major limitation of the ME technique is the inability to quantify extracellular drug concentrations, which would be highest close

to the site of administration and lower further away depending on distance, diffusion in the complex extracellular space, and inactivating processes in CNS tissues such as enzymes and cellular uptake (see Curtis 1964).

Until 1962 extracellular action potentials of single neurons were photographed on moving film for later calculation of firing frequencies and durations of action. All of the ancillary equipment for switching, timing, measuring, and recording retaining and ejecting currents was designed and made in the Department. By late 1962 rate meters enabled the continuous plotting on ink recorders of firing frequencies of single neurons, and later window discriminators were designed to ensure that, of the action potentials monitored on an oscilloscope, those of only one neuron were counted. Computerized equipment also became available, initially in 1966 with a grant from the Wellcome Trust, for the on-line production of peristimulus histograms of neuronal action potentials and for the averaging of intra- and extracellularly recorded potentials and ventral root recorded reflexes.

Acetylcholine and Renshaw Cells

In 1957 Rosamond Eccles and I confirmed the cholinergic nature of the synaptic excitation of lumbar Renshaw cells by impulses in motor axon collaterals: all were excited by ME ACh, nicotine, and decamethonium and synaptic and ACh excitation were enhanced and prolonged by Prostigmine and edrophonium and reduced by dihydro-beta-erythroidine, tubocurarine, and procaine. As at the neuromuscular junction the receptors appeared to be essentially of a nicotinic type. Some of these experiments were carried out while Bernard Katz was visiting Canberra.

In 1960 a study with John Phillis and Jeff Watkins (see later) indicated that apart from the excitation of Renshaw cells by ACh at motor axon collateral synapses, ACh, adrenaline, noradrenaline, 5-hydroxy-tryptamine (5HT), ATP, and histamine were unlikely to function as transmitters influencing spinal neurons. The polysynaptic excitation of Renshaw cells by impulses in dorsal root fibers, however, appeared not to be cholinergic. This latter finding was later confirmed in a more detailed study with Ronald Ryall from 1962. Both nicotinic and muscarinic subtypes of receptor were detected on Renshaw cells, both of which were involved in the excitation of these neurones by non-physiological maximal ventral root stimulation.

In another ME investigation of Renshaw cells in 1964, David Quastel and I found that hemicholinium-3 (HC-3) slowly increased the latency and decreased the number of responses of Renshaw cells to continued 5 per second ventral root stimulation. Because the excitation of these cells and of spinal interneurons by dorsal root volleys was not affected, these observations were consistent with a reduced release of ACh from the cholinergic terminals of motor axon collaterals as a consequence of a reduced synthesis of ACh. In 1975, with Bob Craig and David Lodge, this study was repeated

using Renshaw cells, which were excited by volleys in two ventral roots. HC-3 appeared to also have a direct effect on ACh release at motor axon terminals independent of repetitive activity.

Interest in ACh as a transmitter elsewhere in the CNS involved investigations in the brainstem with Kiyomi Koizumi in Brooklyn (see later), in the lateral geniculate nucleus in Canberra with Ross Davis, in the ventrobasal thalamus with Per Andersen, in the cerebellar cortex with Michael Crawford, Paul Voorhoeve, and Victor Wilson, and in the cerebral cortex with Michael Crawford. The excitation of neurons in these regions by ACh involved receptors of a muscarinic subtype, and no evidence could be obtained for an association of these with major excitatory synaptic pathways.

Amino Acids

In January 1958 Jeff Watkins, a chemist with considerable experience in the extraction and characterization of organic compounds from plants and sponges, joined the Department of Physiology as a Research Fellow. Rather than undertaking the extraction and characterization of possible transmitters from mammalian brain and determining their actions on central neurons *in vivo,* the decision was taken to test some of the numerous compounds already reported in the neurochemical literature, to be present in the mammalian CNS. Thus, we collaborated in examining the central effects of a range of naturally occurring and synthetic amino acids, a project which subsequently resulted in the recognition of amino acids as major transmitters in the mammalian CNS.

Our close collaboration continued to 1965 when Watkins moved to England. From then, while retaining an interest in excitatory amino acids (EAAs), my research was largely centered on the inhibitory amino acids, glycine and GABA. He continued to be involved in the discovery of EAA agonists and antagonists, the recognition and classification of multiple receptors for these excitants and the establishment of the role of L-glutamic acid (L-GLUT) as a major central transmitter (Watkins, 2000). His outstanding contributions in Bristol led in 1988 to his election to Fellowship of the Royal Society of London and the award of the Thudichum Medal by the Biochemical Society in 2000.

GABA had been found in mammals to be uniquely present in central nervous tissues. This amino acid had been reported to have an inhibitory effect in crustacea, and when administered topically to the mammalian cerebral cortex to depress potentials generated by synaptic activation of cortical neurons. Consequently, in March 1958, together with John Phillis, I began a ME examination of the effect of this, and a number of related naturally occurring and synthetic neutral amino acids, on spinal Renshaw cells, inter- and motoneurons *in vivo.*

Glycine, beta-alanine, and GABA ejected as cations all reversibly depressed the excitability of these neurons, and increased the membrane conductance of motoneurons. Using coaxial electrodes (see previous discussion) to record intracellularly from motoneurons, however, we were unable to detect any increase in membrane potential by beta-alanine and GABA as occurs during synaptic inhibition. Furthermore, IV strychnine, in doses that effectively reduced short latency postsynaptic spinal inhibitions, failed to modify the depression of cell excitability by GABA or beta-alanine. (Unfortunately the effect on the action of glycine was not tested.) Hence at that time we considered that GABA was unlikely to be an inhibitory transmitter in the spinal cord.

In view of the relatively large amounts of L-GLUT in CNS tissue, and its metabolic relationship to GABA, we started in April 1958 an examination of the effects of L-GLUT and related acidic amino acids on spinal neurons. We were not aware at that time of Takashi Hayashi's reports of the convulsant actions of sodium aspartate and glutamate following direct injection into the motor cortex of dogs. Aspartate (ASP), GLUT, and cysteate (CYST) anions excited spinal neurons, and depolarization of the membrane of motoneurons, as occurs during synaptic excitation, was detected with coaxial electrodes.

As had been found with the excitation of Renshaw cells by ACh, extracellular enzymic inactivation was then regarded as important in determining the time course of the recovery following synaptic release or the local administration of central transmitters. The recovery of the excitation of interneurons by L- and D-isomers of these three EAAs, however, had similar time courses. In addition, some specific and nonspecific inhibitors of enzymes likely to be associated with the degradation of the L-isomers did not prolong their effects. Thus, we considered that EAAs were not excitatory transmitters, but we remained interested in finding structurally related agonists and antagonists in order to determine the nature of EAA receptors. An investigation of the excitatory action of calcium complexing agents of the versene group excluded the possibility that EAA excitation was the consequence of sequestration of extracellular calcium ions.

The continuing study of the effects of a large number of natural and synthetic amino acids structurally related to GABA and L-GLUT, in both the cat spinal cord *in-vivo* and the hemisected spinal cord of the toad *in vitro,* provided information about the structural features of amino acid receptor sites. One amino group and at least one acidic group were essential for activity, and the close structural relationship between depressant and excitant amino acids led to the proposal that the former interacted with a two-point receptor and the latter with a three-point receptor (Curtis and Watkins 1960).

These findings led to the synthesis by Watkins of numerous analogues of GABA and L-GLUT. Noteworthy were the higher potencies

of 3-amino-l-propanesulphonic acid (3APS) than of GABA, and of D-homocysteic and N-methyl-D-aspartic (NMDA) acids than of D- and L-GLUT. NMDA later became a key compound in the classification of EAA receptors. Commercially available DL-homocysteic acid (DLH) was subsequently used in ME studies to maintain constant rates of neuronal firing when studying synaptic inhibition.

In 1961 Watkins and I had examined the effects of amino acids applied in solution on potentials recorded from the surface of the parietal cerebral cortex of cats. Excitant amino acids produced spreading depression whereas GABA and 3APS depressed potentials evoked by transcallosal stimulation. Crawford and I extended this study in 1963 to single cortical neurons, and found no essential differences between the sensitivities of these and spinal neurons to ME depressant and excitant amino acids. In subsequent investigations in Canberra and elsewhere, amino acids having inhibitory or excitatory effects on spinal neurons had the same effects on neurons throughout the cat CNS (see Curtis and Johnston, 1974).

Travel Abroad

In view of the comparative isolation of Australia and delays in receiving journals from Europe and the United States, the ANU had made liberal arrangements for research staff, particularly for those with tenure, to spend periods abroad visiting research institutions, participating in research, and attending conferences in order to keep abreast of major developments in their particular fields of research.

In May 1959 I was invited to a symposium entitled "Inhibition in the Nervous System and Gamma-Aminobutyric Acid" held at the City of Hope Medical Center at Duarte, California and sponsored by the U.S. Air Force Office of Scientific Research. This was my first venture abroad. Gene Roberts was a very welcoming host, and I was able to meet a number of distinguished physiologists and biochemists, including G. Bishop, T. Bullock, K. Elliott, M. Fuortes, J. Folchi-Pi, R. Gerard, H. Grundfest, T. Hayashi, H. Jasper, D. Tower, A. van Harreveld, H. Waelsch, H. Weil-Malherbe, and C. Wiersma. This was also my first meeting with Hugh McLennan, a later collaborator in Canberra in 1970 and 1978. My paper dealt with the effects of GABA, L-GLUT, and related amino acids on spinal neurons, and my negative conclusions related to transmitter functions were unfortunately based on a faulty technique and incorrect assumptions.

I also flew to New York to visit Chandler Brooks, Chairman of the Department of Physiology at the Downstate Medical Center, Brooklyn, who had previously offered me an appointment as a Visiting Professor for 12 months from September that year, and to Washington D.C. to visit Kay Frank and his colleagues at the National Institutes of Health, Bethesda.

Brooklyn

I had been awarded a Fulbright Travel Grant, and on September 22, 1959 my family and I flew to San Francisco enroute to New York. Chandler Brooks had spent a year (1946–1948) in Dunedin with Eccles, investigating excitation and inhibition in the cat spinal cord at a time when Eccles strongly believed that central synaptic transmission was an electrical process. Brooks had wide interests in endocrinology, neurophysiology, and, more recently, cardiac physiology. He and his wife Nelle were extremely hospitable hosts, and had arranged for us to rent an apartment near the Medical Center.

Kiyomi Koizumi and I examined the effects of ME amino acids, ACh, tubocurarine, 5-HT, and noradrenaline on unidentified neurons in the brain stem of decerebrate cats. The resulting paper was published in the *Journal of Neurophysiology,* despite the comment of a referee, a distinguished neurophysiologist, that the ME technique had a very limited future. I also became involved in lectures to medical students and a postgraduate course in neuropharmacology. While in Brooklyn I had the opportunity to visit and give lectures at a number of centers and to meet distinguished neuroscientists at the Rockefeller Institute including D. P. C. Lloyd, R. Lorente de No, H. K. Hartline, and Victor Wilson who later collaborated with me in Canberra.

My family and I became accustomed to living in an apartment in busy Flatbush. The children attended a public school close by, and Christopher, then aged 7, was regarded by his teacher as somewhat backward in understanding spoken English until Lauri explained that we also had problems with the Brooklyn version of "English." Subsequently he had no further language problems. A subway station close by provided an opportunity to spend weekends exploring the numerous wonders of Manhattan and enjoying concerts at Carnegie Hall and operas at the "Met." The purchase of a car early in 1960 enabled us to explore Long Island, Staten Island, Princeton, Washington, and parts of Pennsylvania, and later to travel to Woods Hole (see later) and New England.

In April 1960 I attended the American Physiological Society meeting in Chicago, and in May I was invited to present a paper at the International Symposium on Nervous Inhibition held at the Friday Harbour Laboratories of the University of Washington. Again this provided an opportunity to meet many distinguished neuroscientists from the United States and abroad, including T. H. Bullock, J. Dudel, C. Eyzaguirre, R. Granit, O. F. Hutter, S. W. Kuffler, W. K. Livingston, D. M. Maynard, H. Patton, D. P. Purpura, T. C. Ruch, J. Szentagothai, A. L. Tow, and J. W. Woodbury.

This was followed by an invitation to present a paper at the Fourth International Neurochemical Symposium which was held in the Villa Monastero at Varenna on Lake Como from June 12–17. Listening to papers

presented by many distinguished neurochemists, I realized that there was a need to apply biochemical techniques at a cellular and synaptic terminal level in the mammalian CNS. Because facilities for this type of complex investigation were unlikely to become available in Canberra, I decided to concentrate on using ME techniques in order to determine if a particular compound present in the mammalian CNS required further investigation as a possible transmitter.

From late June 1960 we spent three summer months at Woods Hole. I had received a U.S. Fellowship in Neuromuscular Physiology to visit Kuffler's group at the Marine Biological Laboratories, and the excellent library provided an opportunity to complete several papers for publication. We were able to rent a Cape Cod cottage in Devil's Lane, close to the Nobska Light and gradually became accustomed to the sound of its fog horn. We met many U.S. biologists and their families, and became aware of their concern about the implications of the nightly passage of the USSR Sputnik across their skies. Visits to New Bedford, Plymouth, Boston, and Mount Washington in early fall, provided an understanding of the beginnings of the European occupation of the North American continent.

Canberra

We returned to Canberra in mid-September 1960. Although I had been impressed by the facilities and funds available in the United States for the development of new techniques for investigating the mammalian CNS, we decided that our family lifestyle in Canberra, and the future opportunities available at the ANU, including the possibility of establishing a Department of Pharmacology within the JCSMR, were preferable.

While I was in the United States, the Australian Physiological Society had been established at the initiative of W. V. Macfarlane, and the first meeting was held in Sydney in May 1960. (In 1967 the word "Pharmacology" was included in the name, to be dropped in 2003.) I joined late in 1960 and served as a member of the Council (1961–1964, 1967–1971, 1975–1979), as Editor of the Proceedings of the Society (1971–1972), as President (1992–1995), and since then I have been an Honorary Member.

Back in my own laboratory, Watkins and I resumed our study of the effects of amino acids on spinal neurons, and I completed an earlier investigation of the effect of ME strychnine on the IPSPs of cat spinal motoneurons. These IPSPs were reduced by strychnine hydrochloride in intravenous doses of 0.1–0.2 mg/kg, and thus by extracellular concentrations of the order of 0.5 microM. In 1958 and 1959 I had used co-axial microelectrodes. The outer barrels contained 90 mM strychnine hydrochloride, and the diffusion of strychnine from the relatively large (8–10 micron) orifice had not been controlled by the retaining currents used. When dilute

solutions, 1–2 mM strychnine hydrochloride in 165 mM NaCI, were used the reversible reduction of IPSPs was readily observed.

As a consequence, in subsequent studies depending on intracellular recording from neurons, parallel electrodes were used. A separate but multibarrel assembly attached to the recording microelectrode enabled the controlled retention and ejection extracellularly of as many as six compounds, each barrel having an orifice of 1–2 microns diameter. Care was also taken to select appropriate concentrations of each of the compounds to be used and to determine that diffusional leakage was adequately controlled.

Thalamic Neurones

Systemically administered lysergic acid diethylamide (LSD-25) and some related compounds, many of which were antagonists of the action of 5HT on smooth muscle, had been reported to reduce transmission at optic nerve synapses on neurones in the cat dorsal lateral geniculate nucleus (LGN). Although 5HT had not been detected in this area of the nervous system, this raised the possibility that the transmitter at optic nerve terminals could be a related compound. The blood–brain barrier, however, is relatively impermeable to 5HT. Hence, in 1961, together with Ross Davis who had earlier investigated this synapse with P. O. Bishop in Sydney, we examined the effects of ME indoles related to 5HT, and derivatives of lysergic acid and of phenylethylamine, on the excitation of LGN neurons by impulses in optic nerve fibers.

Many of these substances depressed transmission, particularly 4-, 5- and 7-HT, psilocin, bufotenine, LSD-25, ergometrine, methylergometrine, dopamine, and mescaline, but did not influence antidromic excitation of LGN neurons by volleys in the optic radiation or excitation by ME L-GLUT. The active depressants thus either blocked the access of the optic nerve transmitter to subsynaptic receptors or reduced its release. Reduced transmitter release was unlikely to result from the activation of postsynaptic receptors at axo-axonic synapses on optic nerve terminals because these terminals appear to be presynaptic at axo-axonic synapses within the LGN (Szentagothai, 1968).

None of these depressants effective in the LGN influenced synaptic excitation of spinal interneurons and Renshaw cells, and, in a subsequent study in 1962–1963 with Per Andersen 4-HT, 5-HT, and dopamine did not reduce the excitation of ventrobasal thalamic relay neurons by impulses in medial lemniscus fibers, although the spontaneous “spindle” activity of some neurons was reversibly reduced.

Conferences Abroad

In October 1962 I presented a paper at the First Asian and Oceanian Neurological Congress held in Tokyo, and on the way to England visited the

Instituto Superiore di Sanita in Rome where Dr. D. Bovet and his colleagues had synthesized and provided me with a number of compounds having strychnine-like actions. In England I lectured at the Institute of Animal Physiology at Babraham and the Department of Experimental Neuropharmacology of the University of Birmingham, and visited the Departments of Physiology and Pharmacology in Oxford. Arriving in Boston on October 22, to visit Steve Kuffler and his colleagues at the Harvard Medical School, I immediately became aware of the critical situation that had arisen between the United States and the USSR as a consequence of the latter's installation of missiles in Cuba. President Kennedy's dramatic televised ultimatum to the USSR, and the advice of colleagues in Boston and New York, led me to abandon my intended visit to New York to present a paper by invitation at a meeting of the Manfred Sakel Foundation and to return to Australia with minimal delay. The subsequent removal of the missiles by the USSR avoided further action by the United States.

In November/December 1964 I had been invited to present a paper at a conference "Nerve as a Tissue" held at the Lankenau Hospital in Philadelphia. Prior to this event I visited and lectured at the Department of Anatomy, chaired by John Szentagothai, of the Medical University in Budapest, the Max-Planck Institute for Psychiatry in Munich, and the Department of Anatomy of the University of Oslo. Following the meeting in Philadelphia I attended a Neurosciences Research Program study session at MIT in Boston, and lectured at the Department of Pharmacology of the University of Pennsylvania, the Department of Physiology of the Johns Hopkins School of Medicine in Baltimore, and the National Institute of Mental Health in Washington DC.

Continuing our interest in excitant amino acids, Watkins had obtained a sample of beta-N-oxalyl-L-alpha beta-diaminopropionic acid (OXDAPRO) from P. S. Sarma, and a number of synthetic oxalyl derivatives of amino acids from his colleague D. R. Rao, in India. There was speculation that one or more amino acids, including OXDAPRO, present in *Lathyrus sativus* could be toxic factors responsible for human neurolathyrism in situations of famine and nutritional deficiencies. OXDAPRO was a potent excitant of cat spinal and cortical neurons, only slightly less effective than NMDA in ME studies. This finding, in 1965 with Watkins and Tim Biscoe, was early but indirect evidence of the later recognized neurotoxicity of EAAs. OXDAPRO did not penetrate the adult blood–brain barrier, but possibly did under extreme nutritional stress.

Chair of Pharmacology

The year 1965 ushered in a period of considerable change in my research group. In April I was elected to Fellowship of the Australian Academy of Science, and a year later was appointed to a personal Chair of Pharmacology within the Department of Physiology. Watkins moved to England in

April 1965, and Graham Johnston, a graduate of the Universities of Sydney and Cambridge, and then involved in postdoctoral research at the University of California Berkeley, accepted an appointment to the Department in September. As had been the case with Watkins, he was an experienced organic chemist, and we collaborated closely on the central neuropharmacology of amino acids until he moved to the University of Sydney in 1980 as Professor of Pharmacology.

John Eccles had shared the 1963 Nobel Prize in Physiology or Medicine with A. L. Hodgkin and A. F. Huxley, and in August 1966 he resigned from his Chair in Canberra to take up an appointment in Chicago. I was Acting Head of the Department until September 1967 when P. O. Bishop, a distinguished visual neurophysiologist from the University of Sydney, took up his appointment as Head of the Department of Physiology. In 1968 the title of my Chair was changed to Neuropharmacology, and my colleagues and I were recognized as an independent research group within the Department. Earlier, in 1966, I had received a 2-year grant from the National Institute of Neurological Diseases and Blindness to equip a second electrically shielded neurophysiological laboratory.

In April 1965 I attended a Work Session of the Neurosciences Research Program in Brookline, and also visited and lectured at the Harvard Neurological Unit of the Boston City Hospital, the Rockefeller Institute in New York, The National Institute of Medical Research at Mill Hill, the National Hospital for Nervous Diseases, Queen Square, the Department of Biophysics, University College London, and the Departments of Pharmacology in Oxford and Cambridge. I was also able to visit SpringerVerlag in Heidelberg to check the page proofs of a book *Studies in Physiology,* containing essays by previous colleagues of John Eccles in honor of his 1963 Nobel Prize, which I had edited with Archie McIntyre who had been a colleague of Eccles in New Zealand.

Glycine and Strychnine

In September 1966 I was invited to present a paper in Athens at the Twelfth International Meeting of Neuropsychology and to lecture at the Department of Neurology of Athens University. Later that month I presented by invitation a paper "Pharmacology and Neurochemistry of Mammalian Central Inhibitory Processes" at the Fourth International Meeting of Neurobiologists in Stockholm dealing with the "Structure and Function of Inhibitory Neuronal Mechanisms." Here I heard for the first time the report by Bob Werman and Morris Aprison that the concentration of glycine in the ventral grey matter of the cat spinal cord was higher than that of any other amino acid and that this amino acid was associated with some interneurons. Furthermore, ME glycine hyperpolarized cat spinal motoneurons, increased the membrane conductance, and the equilibrium

potential of the hyperpolarization appeared to be identical to that of short latency IPSPs, which result from an increased permeability to chloride ions.

This very strong indication that glycine could be an inhibitory transmitter in the mammalian spinal cord was indeed a very significant breakthrough, which subsequently should have been recognized internationally as a major advance in the understanding of mammalian central neurotransmission. I was very aware that we had never examined the effect of ME strychnine on the depression of neuronal excitability by glycine and that this was yet to be examined elsewhere. After visits to Goteborg, Oslo, Amsterdam, Babraham, London, and Vancouver, I returned to Canberra in October.

With Johnston and Leo Hosli, and using parallel electrodes to combine intracellular recording with extracellular ME amino acid administration, the Aprison and Werman glycine results were soon confirmed. We used a range of small anions injected intracellularly to demonstrate the similarity between the membrane permeability change underlying IPSPs and hyperpolarization by glycine. Additionally, however, we found that the effect of ME GABA on motoneurons was identical to that of glycine.

Both glycine and GABA are present in the mammalian spinal cord, and the difference in their pharmacological effects was clearly demonstrated by the selective reduction by ME or IV strychnine of both the inhibitory effect of glycine and short latency IPSPs recorded from motoneurons. The depressant effects of some other alpha and beta amino acids were also blocked by strychnine: L-alpha-alanine, beta-alanine, serine, and taurine. A number of alkaloids and synthetic compounds having the same effect as strychnine on short latency and duration spinal postsynaptic inhibitions were also selective glycine antagonists, including brucine, diaboline, thebaine, laudanosine, dendrobine, morphine, and 4-hydroxy-strychnine (see Curtis and Johnston, 1974b). Later in the 1980s a number of bicyclic 5-isoxazolol derivatives and a steroid derivative (RU5135) were added to this list.

Thus, glycine was the first amino acid to be generally recognized as an inhibitory transmitter in the mammalian CNS, and a number of "glycinergic" spinal neurons were identified, including Renshaw cells and group 1A interneurons of the "direct" inhibitory pathway. We had observed that in comparison with GABA, glycine was a weak depressant of postcruciate cerebral cortical neurons, an effect also blocked by strychnine. Together with Paul Voorhoeve and Victor Wilson we had found in 1963 that the recurrent inhibition of cerebral Betz cells evoked by pyramidal tract stimulation, and the basket cell inhibition of cerebellar Purkinje cells evoked by local cortical stimulation, were not affected by IV strychnine in doses that considerably reduced short latency and duration inhibitions of spinal motoneurons. Later in 1965 Biscoe and I found that neither the recurrent inhibition of Betz cells following a pyramidal tract volley nor the

inhibition produced by exciting nearby inhibitory interneurons with DLH were reduced by ME strychnine. Thus, glycine appeared to be of minor significance as an inhibitory transmitter in these supraspinal regions, in which strychnine-insensitive inhibitions we later found to be mediated by GABA.

Amino Acid Uptake

Johnston and his colleagues had been investigating glycine metabolism and uptake in the spinal cord *in vitro,* and it seemed very probable that the actions of two mercurial compounds which enhanced and prolonged the depression of spinal neuron firing by glycine, generally without affecting the action of GABA, was related to the inhibition of membrane transport processes rather than to enzyme inhibition. There was at this time increasing *in vitro* neurochemical evidence for the presence in CNS tissues of specific and high affinity, sodium-dependent uptake processes for particular amino acids, including those of interest as central transmitters.

Such transport, essential for maintaining intracellular intermediary metabolism, including transmitter synthesis, came to be regarded as more important than extracellular enzymic inactivation for the rapid removal of synaptically (and ME) released amino acid transmitters. Differences were apparent between postsynaptic receptors and membrane sites associated with transport, and we were interested in finding specific antagonists of the *in vivo* transport of individual transmitter amino acids that had no effects on postsynaptic receptors (see Curtis and Johnston, 1974a).

Tetanus Toxin Revisited

Another convulsant agent of neuropharmacological interest is tetanus toxin, shown earlier to block the types of spinal postsynaptic inhibition by now recognized as mediated by glycine. In 1968 Chet de Groat and I found that when injected into the spinal cord this toxin suppressed the synaptic inhibition of lumbar Renshaw cells by volleys in hind paw afferent fibers but did not diminish the depressant actions of ME glycine and GABA. Johnston had not detected any significant reduction by tetanus toxin of spinal levels of these amino acids, although there was a marked increase in the level of L-ASP. Consequently we proposed that tetanus toxin interfered with the synaptic release of glycine.

A later study in 1971 with Dominik Felix, Christopher Game, and Roy McCulloch of the effect of tetanus toxin on bicuculline-sensitive GABA-mediated "presynaptic" inhibition of spinal reflexes, and basket cell postsynaptic inhibition of cerebellar Purkinje cells, indicated that tetanus toxin also reduced the synaptic release of GABA.

Colorado Springs

In 1968 I spent three short periods abroad. In February, at the invitation of the U.S. National Institute of Neurological Diseases and Stroke (NINDS), I went to a meeting held in Colorado Springs to plan a symposium to be held in November on the topic "Basic Mechanisms of the Epilepsies." Late in September I visited the CIBA Company in Basel, gave an invited paper at a Mechanisms of Synaptic Transmission Symposium held at Einsiedeln under the auspices of the Swiss Society of the Natural Sciences and later visited Jeff Watkins, then a member of the staff of the MRC Neuropsychiatric Research Unit at Carshalton in England.

I returned to Colorado Springs in November, and presented a paper in which I reviewed the then current evidence for the involvement of ACh, norepinephrine, dopamine, 5-HT, L-ASP, L-GLUT, glycine, GABA, and some other compounds present in the mammalian CNS as synaptic transmitters. This 3-day event was an excellent opportunity to meet many basic and clinical neuroscientists internationally recognized for their expertise. On my return journey I spent 2 days with John Eccles who had by then moved to Buffalo.

Excitatory Amino Acids

In Canberra, Johnston and I continued to investigate depressant and particularly EAA analogues, the latter as a means for establishing L-ASP and L-GLUT as transmitters. He had become very interested in the possibility of making use of L-GLUT and GABA analogues of restricted conformation as agonists and antagonists of amino acid receptors. This followed our finding in 1968 with Arthur Duggan and Chet de Groat that ibotenic acid excited and muscimol inhibited spinal neurons, the latter effect at strychnine-insensitive receptors. These two isoxazoles, isolated from mushrooms of the genus *Amanita,* are structurally similar to L-GLUT and GABA. Their opposite effects were consistent with earlier findings with Watkins regarding the structural requirements for excitatory and inhibitory amino acid actions, and that alpha-decarboxylation of an EAA produced an inhibitory amino acid.

The inhibitory action of muscimol was later shown to be blocked by the GABA antagonist bicuculline. In 1978 David Lodge, Hugh McLennan and I observed that the excitation of spinal neurons by ibotenic acid was followed by a prolonged depression that was blocked by bicuculline. Hence ibotenic acid was presumably converted *in-vivo* to muscimol or a related compound. As a consequence of the effects of ibotenic acid and muscimol, Johnston began collecting and synthesizing further related compounds.

In addition to our interests in EAA receptors and selective antagonists we continued to investigate the mechanism of EAA depolarization and its

relation to synaptic excitation. In our earlier studies (1958–1962) using coaxial electrodes with single- or double-barrel intracellular microelectrodes, ME NMDA, L-ASP, L-GLUT, and L-CYST depolarized motoneurons, and the reversal potential of this depolarization was consistently observed to be at a lower membrane potential than that of monosynaptic EPSPs.

A number of factors, however, need to be taken into consideration when interpreting observations of this type as indicating a difference between the ionic events underlying ME amino acid and synaptic depolarization. Given the complex morphology of motoneurons, the probable intrasomatic location of intracellular microelectrodes, and the distribution of excitatory synapses on somas and dendrites, it is probable that perisomatic concentrations of EM amino acids were higher than those at distal dendrites, as were changes in membrane potential produced by passing current through the intracellular microelectrode.

In a later study (1968–1971) using parallel micropipettes, depolarization of motoneurons by ME DLH was not affected by increasing the intracellular chloride concentration, which readily converted hyperpolarization by glycine into depolarization, or by EM tetrodotoxin in a concentration adequate to suppress neurone action potentials. Hence, when taken into consideration with the then accumulating neurochemical evidence related to the CNS distribution of EAAs, the ionic basis of amino acid excitation was considered to be probably identical to that accounting for EPSPs, an increase in membrane permeability to sodium and potassium ions.

By 1969 we had not been able to find selective antagonists of excitation by either L-ASP or L-GLUT or of the cellular uptake of these EAAs. The different distributions of these amino acids in dorsal and ventral roots and in the spinal grey matter, however, suggested an association of L-ASP with intraspinal excitatory interneurons and of L-GLUT with primary afferent fibers. Consistent with this proposition, from 1970 Duggan found that interneurons excited monosynaptically by afferent impulses were more sensitive to EM L-GLUT than to L-ASP whereas Renshaw cells, which respond polysynaptically but not monosynaptically to such impulses, were more sensitive to L-ASP than to L-GLUT.

Later, in 1974 with Game and McCulloch, Johnston and I found a greater difference in the sensitivity of these two types of neurons to kainic acid (KAIN), a conformationally restricted analogue of L-GLUT, and NMDA, considered to be too small a molecule to interact with L-GLUT receptors.

Oxford

In May 1969 my wife and I traveled via Vancouver, Washington D.C., and New York to Oxford where Charles and Cynthia Philips had arranged for us to rent a flat overlooking Oriel Square until late August. In addition

to enjoying Oxford and its environs, which we explored by bicycle, this enabled me to complete the writing of several papers and to visit and lecture at a number of University Departments in Oxford, London (three Special University Lectures at the Middlesex Hospital Medical School), Birmingham, Paris (a lecture at the College de France), Basel (a main lecture at the Fourth International Congress of Pharmacology), Zurich, Freiburg, Heidelberg, Frankfurt, and Oslo. We returned to Canberra in mid-September via Milan (a paper at the Second International Meeting on Neurochemistry), Pisa, Rome, Athens, and Madras.

GABA and Bicuculline

Reports in the late 1950s that picrotoxin blocked the action of GABA at invertebrate inhibitory synapses, and the observations in the early 1960s by Eccles and his colleagues of the reduction of the "presynaptic" inhibition of monosynaptic reflexes by this convulsant but not by strychnine (see later) suggested that in addition to glycine GABA was also a transmitter in the mammalian spinal cord.

I had not been able to demonstrate, however, that IV or ME picrotoxin, and picrotoxinin later prepared by Johnston, influenced the effect of GABA on spinal neurons. Because these convulsants are poorly soluble and not ionized in water, local concentrations achieved by ME administration were presumably too low, as also were those concentrations achieved after IV administration (1–2 mg/kg), to significantly reduce the effects on neurons of relatively high and localized concentrations of ME GABA. In a series of papers published in 1970, Masao Ito and his colleagues reported that IV picrotoxin blocked the synaptic inhibition of Deiters' neurons in the cat lateral vestibular nucleus by impulses in Purkinje cell axons, thus supporting strong neurochemical evidence for GABA as the inhibitory transmitter of these axons.

A study by Johnston and several colleagues of the structure of strychnine, some related alkaloids and synthetic compounds, all of which were selective glycine antagonists, provided an understanding of how these interfered with the action of this particular amino acid at receptors on central neurons. As a consequence, he was then able to propose the possible structure of selective GABA antagonists, and suggested that a number of convulsant isoquinoline alkaloids be tested. Of particular interest was bicuculline, a phthalide isoquinoline alkaloid isolated from *Dicentra cucullaria* and *Corydalis* species, and found in the 1930s to be a potent convulsant when administered IV to rabbits (see Curtis and Johnston, 1974b).

Beginning in March 1970, and in a study with a number of colleagues including Johnston, Duggan, Dominik Felix, and McLennan, we found that ME bicuculline, ejected from a solution of the hydrochloride, reversibly blocked the inhibitory action of ME GABA but not of glycine on

spinal interneurons and Renshaw cells, cerebral pyramidal cells, cerebellar Purkinje cells, hippocampal pyramidal cells, Deiters' neurones, thalamocortical relay neurones in the ventrobasal thalamus and lateral geniculate nucleus, and olfactory bulb mitral cells. Furthermore, IV bicuculline hydrochloride (0.2–0.5 mg/kg) reduced presynaptic inhibition of spinal reflexes and the accompanying primary afferent depolarization (PAD). In addition, bicuculline reduced the strychnine-insensitive postsynaptic inhibitions of Deiters' cells, Purkinje cells, pyramidal cells in the cerebral and hippocampal cortices, and thalamocortical relay cells.

Our first paper reporting bicuculline as a GABA antagonist was a letter to *Nature,* received on May 19 and published on June 27, 1970. These observations provided strong support for increasing neurochemical evidence of the association of GABA with particular inhibitory pathways in the mammalian CNS. In addition, McLennan found that bicuculline blocked the inhibitory effect of GABA and the picrotoxin-sensitive synaptic inhibition of stretch receptor neurones of the Australian freshwater crayfish *Eustacus armatus.*

In contrast with strychnine, the central effects of IV and ME bicuculline were of relatively short duration. The low aqueous solubility, and rapid conversion of bicuculline in neutral solution to inactive bicucine, led to Johnston preparing bicuculline methochloride (BMC), a more soluble, stable and potent GABA antagonist for ME studies. As expected from its quaternary structure, BMC did not readily penetrate the mammalian blood–brain barrier. Other selective GABA antagonists found in the initial and later studies included (+) but not (−) bicuculline, corlumine, bicucine methyl ester, coriamyrtin, and benzyl penicillin. Bicucine and bicuculline diol were inactive, and N-methyl bicuculline was a glycine antagonist (see Curtis and Johnston, 1974a,b).

In 1983, Richard Malik and I, in collaboration with S. J. Enna and his colleagues in the United States, found that securinine and dihydrosecurinine, indolizidine alkaloids from the shrub *Securineqa suffructicosa,* were selective antagonists of mammalian CNS GABA receptors. In the same year, in collaboration with the French investigators C. G. Wermuth and K. Biziere, Bruce Gynther and I found that 2-(carboxy-3′-propyl)-3-amino-6-paramethoxyphenylpyridazinium bromide, a pyridazinyl derivative of GABA, was also a selective GABA antagonist in the cat spinal cord.

Taken in conjunction with our earlier observations of the antagonism between strychnine and glycine, these results with selective GABA antagonists confirmed our earlier classification of inhibitory amino acids as either glycine-like (glycine, L-alpha-alanine, beta-alanine, and taurine) or GABA-like (GABA, beta-hydroxy GABA, muscimol, and 3-APS). Antagonism by bicuculline was subsequently the basis for identifying GABA-A subtype receptors and inhibitory processes, as distinct from receptors later defined

as GABA-B, which were insensitive to bicuculline and activated by baclofen (see later).

Bicuculline: A Postscript

In 1982 we learned of an earlier but unfortunately unpublished investigation of the convulsive activity of bicuculline. In the early 1960s Dr. K. Tsou in the Department of Pharmacology of the Shanghai Institute of Materia Medica had obtained from colleagues a sample of an alkaloid isolated from *Corydalis humosa* Migo, which was later identified as bicuculline. He found this to be an extremely potent convulsant when applied to the cerebral cortex, and studied its interaction with similarly applied GABA. The alkaloid also reduced strychnine-insensitive "presynaptic" inhibition of spinal reflexes, and he wondered about its possible use in studying other central inhibitions, including those possibly mediated by GABA. He had no facilities, however, for further investigations at a cellular level.

These results were presented at a Congress of the Chinese Society for Physiological Sciences in the summer of 1964. Subsequently, a full-length paper submitted to *Acta Physiologia Sinica* in 1965 was never published, since in 1966, with the beginning of the Cultural Revolution, the publication of all Chinese scientific journals was suspended. Dr. Tsou's research ceased, and he was assigned initially to screen antifertiliy drugs, then to take care of animals and eventually to be a farm laborer. He was aware of our 1970 *Nature* article, but was unable to return to his own research until 1976. In that year he and his colleagues were able to publish a paper about the CNS effects of bicuculline as a possible GABA antagonist in a new Chinese language journal *Diseases of the Nervous System.*

In Canberra we became aware in 1982 of his observations and subsequent problems from Zhi-Qi Zhao, a visiting scientist from Shanghai. Tsou's attendance at a meeting elsewhere in China prevented my meeting him in Shanghai in September 1984, but later that year Johnston was able to meet him and to discuss their mutual interests in bicuculline and GABA.

Amino Acid Analogues, Agonists, and Antagonists

Johnston and Philip Beart had continued to synthesize isoxazole and amino acid analogues of glycine, GABA, and L-GLUT having restricted conformation. The finding that 4-aminotetrolic acid activated GABA-A receptors on spinal neurons indicated that GABA, a flexible molecule, acted in an extended rather than in a folded conformation. This was consistent with the structural similarities of bicuculline and muscimol. Supporting evidence came later using *trans*- and *cis*-aminocrotonic acids, the former an

extended and the latter a folded analogue of GABA, and a series of cyclopropane, cyclobutane, cyclopentene, and cyclopentane restricted analogues of GABA.

Together with Duggan and Felix, Johnston and I continued to test the effects of a large number of L-ASP and L-GLUT analogues, and drugs known to depress the excitability of central neurons, particularly to find specific antagonists of either EAA. Tests were carried out on spinal, thalamic, and cerebral cortical neurons. Renshaw cells were particularly useful, because further investigation was not warranted if a potential EAA antagonist also reduced excitation by ACh. Compounds warranting further interest included L-glutamic acid diethyl ester (GDEE), L-methionine-DL-sulphoximine, 2-methoxy-aporphine, and 1-hydroxy-3-amino-pyrrolidone-2, but all reduced excitation by both EAAs.

In 1973 we found kainic acid (KAIN), a conformationally restricted analogue of L-GLUT, to be a more potent excitant of spinal neurones than NMDA, DLH, and ibotenic acid. An analysis of molecular models suggested that KAIN and ibotenic acid, and L-GLUT in a partially extended conformation, interact with the same receptors. L-ASP would not be able to interact with these sites, although L-GLUT in a partially folded conformation would be able to interact with L-ASP-preferring receptor sites. The possibility of finding selective antagonists for each of these two EAAs present in the mammalian spinal cord, based on our experience with strychnine and bicuculline as selective antagonists of glycine and GABA, respectively, thus seemed to be remote.

In the 1970s there was increasing evidence, mainly from the investigations of John Olney and his colleagues, that EAAS were also neurotoxic. KAIN proved useful experimentally as an excitotoxin for producing localized lesions in the CNS, because histological evidence suggested that only neurons and not axons were destroyed. Malik and I provided physiological evidence for this in 1984: ME KAIN, in concentrations that destroyed lumbar motoneurons *in vivo,* did not affect nearby primary afferent fibers, their unmyelinated synaptic terminations, or axo-axonic GABA-releasing synaptic terminals upon them.

Australian Neuroscience Society

In July 1971 I attended the Third International Meeting of the International Society of Neurochemistry in Budapest, the XXV International Congress of Physiological Sciences in Munich, and contributed to a Satellite Symposium on Physiology and Pharmacology of Synapses in Basel. I also lectured at the University of Vienna, and, as a member since 1959, attended a meeting in Oxford of The Physiological Society. Lectures were given at the National Hospital for Nervous Diseases in London and the National University in Athens.

In Budapest I had an opportunity to discuss with Lawrie Austin, a neurochemist from Monash University in Melbourne, the idea of setting up an Australian Neuroscience Society. The purpose was to stimulate discussions and cooperative interactions between basic scientists and clinicians in view of the increasing interests in basic neuroscience by neurologists and neurosurgeons in Australia. Later that year a large majority of those we had identified as possibly being interested clearly favored this proposal, and a 1-day meeting was held at Monash University in November 1972. As the majority of attendees favored an informal structure, further 1-day meetings were held annually until February 1980 when a more formal structure was established with officers and representatives from each Australian state and New Zealand. A constitution was adopted in 1981, and thereafter the Society has continued to grow and flourish.

Conferences Abroad

In November 1971 I participated in another NINDS symposium in Colorado Springs concerned with Experimental Models of Epilepsy. I was also able to visit Winnipeg, St. Louis, New York, Vancouver, and Duarte. The visit to John Olney at the Department of Psychiatry at Washington University, St. Louis, was important in view of his increasing interest in EAAs as neurotoxins.

In November 1972 I was invited to visit and lecture at the Merck Frosst Laboratories at Pointe Claire, Quebec, and also visited The Rockefeller University New York, the School of Medicine of the University of Pittsburgh, the Health Sciences Centre of the University of Western Ontario, and John Eccles and his colleagues in Buffalo.

Department of Pharmacology

The establishment of a Department of Pharmacology within the JCSMR had been considered for many years, but it was not until the retirement of Adrien Albert in 1972 that the top of four floors of laboratory space occupied by his Department of Medical Chemistry became available. For some years he had provided space for Watkins and then Johnston on this floor, at the same level as my laboratories in the Department of Physiology. In January 1973 the University Council established a Department of Pharmacology within the JCSMR, and in March I was appointed Foundation Head and Professor.

Several years elapsed before building operations enabled us to occupy three large electrophysiological/pharmacological laboratories, each with an electrically shielded room, several chemical laboratories, and electronic and mechanical workshops. From 1957 I had continued to use the stimulating portion of the ESRU, which had been designed early in the 1950s by Coombs

and based on the use of thermionic tubes. In 1985, the need for additional timing facilities led to the design and construction by Alex Saeck of a more compact and entirely solid state stimulating unit that I used until 1995.

The availability of more laboratory space enabled new lines of research to be developed, including those dealing with peptide transmitters, opioids and transmission in nociceptive pathways (Arthur Duggan), nerve growth factors controlling the development of neurons (Ian Hendry), and autonomic neurotransmission (Caryl Hill). In 1985 Duggan and Hendry developed an antibody-coated microprobe technique for detecting the sites of release in the CNS of neuropeptides.

Baclofen

In late 1972 Johnston and I became interested in a lipophilic derivative of GABA, baclofen (4-amino-3-(4-chlorophenyl)-butyric acid). This had been synthesized in 1962 by H. Keberle at Ciba in Basel as better able to pass the blood–brain barrier than GABA. Monosynaptic and polysynaptic spinal reflexes of cats were reduced by IV baclofen, and oral baclofen alleviated spinal spasticity in humans in the absence of clinically significant effects on supraspinal centers and the peripheral autonomic nervous system.

In our initial 1973 investigation with Game and McCulloch, ME baclofen depressed the spontaneous and EAA-induced firing of spinal interneurons, cortical pyramidal tract neurons and cerebellar Purkinje cells, but had little effect on Renshaw cells. This depression, of longer duration than that produced by GABA, was not blocked by bicuculline or strychnine. We also observed a prolonged reduction by ME baclofen of intracellularly recorded EPSPs and IPSPs from motoneurons, indicative of an increased membrane conductance.

Following suggestions by others that baclofen had a presynaptic action reducing transmitter release at the terminals of spinal primary afferent fibers, in 1979 Lodge, Joel Bornstein, Martin Peet, and I reinvestigated the spinal effects of baclofen. Monosynaptic spinal reflexes and dorsal root potentials evoked by afferent volleys were considerably reduced by IV baclofen (2–3 mg/kg). Two effects of ME (−)-baclofen became apparent, the (+) isomer being inactive. The monosynaptic, but not polysynaptic, excitation of spinal interneurons by impulses in low threshold muscle and cutaneous afferent fibers was reversibly reduced. Baclofen, however, had little or no effect on the cholinergic monosynaptic excitation of Renshaw cells by ventral root impulses and the polysynaptic excitation of these cells by primary afferent impulses.

This selective presynaptic reduction in transmitter release was apparent even when the background firing rate of the cells was maintained at the control level by ME EAAs. The reduction in sensitivity to EAAs indicated a

postsynaptic depressant effect of baclofen, which generally required higher extracellular concentration than those influencing transmitter release.

By that time I had developed techniques for investigating the physiology and pharmacology of primary afferent unmyelinated terminations in the ventral horn (see later). Without altering the electrical excitability of these structures, or their depolarization by ME GABA, (−)-baclofen reduced the synaptic GABA-mediated and bicuculline-sensitive depolarization (PAD) produced by tetanic volleys in flexor muscle group 1a afferent fibers. Later the same results were obtained with muscle group 1A and 1B primary afferent terminations in Clarke's column of the cat spinal cord.

Meanwhile, Norman Bowery and his colleagues had shown that (−)-baclofen and GABA reduced transmitter release in the peripheral autonomic superior cervical ganglion, an effect not blocked by bicuculline. The possibility of a novel receptor for GABA, activated by baclofen, was proposed, and in 1983 they reported the detection on rat brain crude synaptic membranes of two distinct binding sites for radiolabeled GABA: GABA-A sites blocked by bicuculline and GABA-B sites which also bound (−)-baclofen but were not blocked by bicuculline. Selective baclofen antagonists, which would enable the physiological significance of GABA-B binding sites to be determined, were not available.

A number of *in vitro* studies had shown that (−)-baclofen and GABA reduced the influx of calcium ions through voltage-activated channels of dorsal root ganglion cells and decreased the duration of the action potentials of these neurons. In an investigation that began in 1987, with Gynther, David Beattie, and Gary Lacey, we found that ME baclofen also reduced the duration of the action potentials of intraspinal primary afferent unmyelinated terminations *in vivo*. This effect, blocked by baclofen antagonists (see later), was most likely produced by a reduction of the relatively small and late calcium influx associated with transmitter release rather than by an enhanced influx of potassium ions. Baclofen, however, did not reduce the duration of the action potentials of motor axon collateral unmyelinated cholinergic terminations.

Our observations were consistent with the reduction by low concentrations of (−)-baclofen of the calcium-dependent release of excitatory transmitter from the intraspinal terminals of primary afferent fibers, and of GABA at axo-axonic synapses upon these terminals (see later). The selectivity of baclofen for reducing transmitter release from primary afferent fibers was subsequently confirmed in 1984 when Malik and I found that IV baclofen had little or no effect on the monosynaptic excitation of interneurons in the spinal intermediate nucleus by impulses in rubrospinal, corticospinal, and descending propriospinal fibers. Additionally, IV baclofen in doses as high as 6 mg/kg did not modify excitatory or inhibitory transmission in the cat cerebellar cortex, a region rich in GABA-B binding sites, for which physiological significance remains to be established.

Our findings thus suggested that the mechanism of transmitter release at central excitatory synapses of axons of peripheral dorsal root ganglia differed from that of neurons having cell bodies *within* the CNS. This may reflect the presence of both GABA-A and GABA-B receptors only on primary afferent terminations and possible differences in intracellular processes related to transmitter release and the voltage activation of calcium channels.

Baclofen Antagonists

On the basis of structural considerations, and the central effects of a large number of GABA analogues, including 3-aminopropylphosphonic acid (Curtis and Watkins, 1965), in 1986 David Kerr, Rolf Prager, and their colleagues in Adelaide prepared phaclofen, (3-amino-2-(4-chlorophenyl)-propylphosphonic acid). This phosphonic analogue of baclofen reversibly blocked the depression by baclofen of cholinergic twitch responses of guinea pig gut *in vitro,* and in the cat spinal cord ME phaclofen blocked the reduction of primary afferent transmitter release by ME (–)-baclofen. Phaclofen was subsequently used to demonstrate GABA-B receptor-mediated effects in a number of *in vitro* CNS preparations, but this compound did not penetrate the cat blood–brain barrier.

Later, in 1988, we found that 2-hydroxy-saclofen (3-amino-2(4-chlorophenyl)-2-hydroxy-propyl-sulphonic acid), also synthesized by the Adelaide group, reduced the presynaptic effect of (–)-baclofen on primary afferent transmitter release. The postsynaptic depression of cell excitability by baclofen was also reduced, but not that by GABA. In 1987 P. Berthelot and his colleagues in Lille had reported that 3-(5-methoxybenzo [b]furan-2-yl)-GABA inhibited baclofen binding to rat brain membranes, and we subsequently showed this to be a baclofen antagonist, blocking pre- and postsynaptic effects in the spinal cord. We also demonstrated that three thienyl-GABA derivatives, shown in Lille to block baclofen binding to rat brain synaptic membranes, were baclofen agonists in the spinal cord.

In this latter study, Lacey and I were able to use one of the synthetic phosphinic acid analogues of GABA, which were selective GABA-B antagonists. These, together with a number of related GABA-B agonists, had been generously provided by Helmut Bittiger, Wolfgang Froestl, and Stuart Mickel and their colleagues at Ciba-Geigy AG in Basel (CGP series, see Froestl et al., 1996). From mid-1992 Lacey and I compared the effects of a range of CGP GABA-B agonists with those of (–)-baclofen in the cat and rat spinal cord *in vivo* and examined the selectivity of a number of antagonists. Our findings essentially confirmed published results of other *in vivo* and *in vitro* studies regarding agonist and antagonist activity (see Lacey and Curtis, 1994).

In particular, ME 3-aminopropyl-methyl-phosphinic acid had effects identical to those of (–)-baclofen on spinal neurones and synaptic terminals but was effective at considerably lower concentrations. All of the antagonists tested, which were effective in much lower concentrations than those required in our earlier tests of phaclofen and 2-hydroxy-saclofen, selectively blocked all spinal effects of baclofen and no convincing evidence was obtained of any antagonism of the effects of GABA on neurones and afferent terminations. Those antagonists reported to pass the blood-brain barrier in rats did so in cats, and we chose two for a further study of the possible role of GABA-B receptors in spinal "presynaptic" inhibition, my last neuropharmacological study (see later).

An unexpected finding was that 3-aminophosphinic acid, established as a potent baclofen agonist on the basis of binding data and *in vitro* pharmacological studies using rat supraspinal CNS tissues, was neither a baclofen agonist nor an antagonist in the cat spinal cord *in vivo*. The heterogeneity of GABA-B receptors was further demonstrated by the finding with Kerr and his colleagues in Adelaide that the nitropropane analogue of GABA, a GABA-B antagonist in the guinea pig gut preparation *in vitro*, was neither a GABA-B antagonist nor agonist in the rat isolated neocortex and was a GABA-A agonist in the cat spinal cord *in vivo*.

The Copenhagen Connection

In February 1974, Povl Krogsgaard-Larsen from the Royal Danish School of Pharmacy (now the Danish University of Pharmaceutical Sciences) joined the Department as an Honorary Fellow. This first of four visits (later visits in 1977, 1981, 1987) began a highly successful and productive collaboration which continued for 18 years. He and his colleagues had become interested in the structural features of excitatory and inhibitory amino acids, and were providing us with a large number of new and novel compounds synthesized in Copenhagen to add to those already being prepared by Johnston and his colleagues. Conformationally restricted analogues of GABA and L-GLUT, including chiral analogues of fully established stereochemical conformation, were of particular interest, especially isoxazoles related to muscimol and ibotenic acid. There was a very close collaboration between Krogsgaard-Larsen and Johnston with his considerably smaller number of chemical colleagues in Canberra.

Compounds related to GABA were tested *in vivo* in the cat spinal cord by ME techniques and by *in vitro* ligand binding techniques as possible agonists or antagonists at GABA-A and glycine receptors, in addition to being tested as antagonists of sodium-dependent uptake of GABA by rat brain slices and as inhibitors of enzymes associated with GABA metabolism. Excitants related to L-GLUT were compared with ME L-GLUT, NMDA, and ibotenic, quisqualic, and kainic acids. Use was also made of an increasing

number of antagonists of EAA excitation, which we and others were investigating to assess the degree of specificity towards central "aspartate-" or "glutamate-preferring" receptors.

Krogsgaard-Larsen and his colleagues introduced too many new compounds for studying the nature of central amino acid receptors to enable the inclusion of details of all of our findings in Canberra. These were reported in 27 papers coauthored by colleagues in Canberra and Copenhagen from 1975 to 1993. Some compounds were potent GABA-A agonists, including isoguvacine, 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol (THIP), dihydro- and thio-muscimol, and piperidine-4-sulphonic acid (P4S), a cyclic analogue of 3APS and poorly taken up by spinal tissue. Others, such as nipecotic acid (piperidine-3-carboxylic acid), blocked the cellular uptake of GABA *in vitro,* enhanced the effect of GABA on spinal neurones but blocked that of glycine. Some isoxazole derivatives were also glycine antagonists. Confirmation was also obtained that the conformation of GABA to activate GABA-A receptors differed from that associated with the carrier responsible for the *in vitro* cellular uptake of this amino acid.

The therapeutic potential of THIP was recognized in 1977–1978, and this compound is now (2005) in advanced phase III clinical trials as an analgesic and hypnotic. The lipophilic derivative of nipecotic acid, Tiagabine, is marketed as an antiepileptic.

A number of ibotenic acid derivatives were potent excitants, including 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), which later was a key compound in the classification of L-GLUT receptors, and 4-bromo-homoibotenic acid. Using GDEE and alpha-aminoadipic acid (aAA) as antagonists of glutamate- and aspartate-preferring receptors, respectively (see later), excitation by AMPA and 4-bromo-homoibotenic acid were reduced by GDEE. In contrast, aAA was an antagonist of excitation by ibotenic acid and NMDA.

Royal Society of London

In 1974 I was elected to Fellowship of the Royal Society of London, and during a period of study leave from late June to late October I signed the Charter Book at Carlton House Terrace during a meeting of the Society's Council. My wife, daughter, and I spent 2 months living in Cambridge at Churchill College, and 1 was able to visit colleagues having similar research interests in Cambridge, Oxford, London, and Bristol, to attend meetings of The Physiological Society and the British Pharmacological Society, and to attend the IX Congress of the International College of Neuropsychopharmacology in Paris and a NATO Advanced Study Institute concerned with Metabolic Compartmentation in Relation to Structure and Function of the Brain in Oxford.

Returning to Canberra via Copenhagen, Munich, Basel, and Zurich, at which I lectured about our research in Canberra, I also contributed to a Symposium on Neurotransmitters at the 26th International Congress of Physiological Sciences in Delhi. This was followed by the presentation of the opening Review Lecture at a Satellite Symposium on Drugs and Central Synaptic Transmission held at the Indian Central Drug Research Institute in Lucknow.

GABA Uptake

From 1975, continuing our interest in the influence of cellular uptake on the action of amino acids on neurons, we investigated the effects on the depression by EM GABA and other inhibitory amino acids on the firing of spinal, cerebellar and cerebral cortical neurons by a number of inhibitors of the *in vitro* uptake of GABA. These included in 1975 (+)-2-4-diaminobutyric acid (DABA), (−)-nipecotic acid, and 2,2-dimethyl-beta-alanine, and in 1976 arecaidine and guvacine, constituents of the betel nut *Areca catechu.* Although there was some degree of selectivity in the enhancement of the effects of GABA *in vivo,* this was not high. Furthermore, we were not able to demonstrate any prolongation of the GABA-mediated basket cell inhibition of cerebellar Purkinje cells by EM nipecotic acid or DABA. It is possible, however, that these inhibitors are also taken up by tissue elements during ME administration and hence do not gain access to many GABA-ergic synapses.

An unexpected finding in 1975 with Johnston and Lodge was the enhancement of the action of muscimol by nipecotic acid and DABA. Subsequent *in-vitro* studies confirmed the presence of a relatively inefficient sodium-dependent transport process for muscimol in rat cortical slices.

Benzodiazepines and Barbiturates

Our interest in these clinically used agents stemmed from reports of the enhancement of spinal GABA-mediated “presynaptic” inhibition of spinal reflexes by pentobarbitone and by diazepam. We found in 1976 that IV diazepam also prolonged basket cell GABA-mediated inhibition of cerebellar Purkinje cells but did not influence the effect of EM GABA on these and spinal neurons.

In 1977 Lodge and I reported that in cats anesthetized with alpha-chloralose and urethane or barbiturates, ME pentobarbitone enhanced the depression of the firing of spinal interneurons by GABA more than that by glycine. Furthermore, in decerebrate cats we found IV pentobarbitone enhanced and prolonged the inhibitory action of GABA, but not that of glycine, on spinal neurons. Relevant *in vitro* studies suggested that barbiturates enhance the binding of GABA to postsynaptic receptors.

Later, in 1982, John Leah, Malik, and I reported that midazolam, a benzodiazepine effective as an anesthetic in humans at IV doses of the order of 0.3 mg/kg, was relatively less effective in cats. In animals anesthetized with pentobarbitone sodium, however, IV midazolam in doses as low as 0.1 mg/kg enhanced and prolonged "presynaptic" inhibition of reflexes. Furthermore, ME midazolam enhanced the inhibitory effect of GABA, but not of glycine, on spinal neurons and the depolarization of primary afferent terminations by ME GABA and P4S. These effects, which were blocked by a benzodiazepine antagonist, R021-3981, were thus consistent with an enhancing action of benzodiazepines at or near central GABA-A receptors.

GABA and Inhibition in the Spinal Cord

In 1976 I began an investigation of the role of GABA as an inhibitory transmitter in the cat lumbar spinal cord, which continued until 1995. In 1960, Eccles and numerous colleagues began a comprehensive investigation of the mechanism and organization of the prolonged inhibition of extensor muscle monosynaptic spinal reflexes by impulses in flexor muscle afferent fibers. Unlike relatively short duration "direct" inhibition of motoneurons by impulses in low threshold afferent fibers of muscles of opposing function, they were unable to record intracellular IPSPs from motoneurons. Monosynaptic EPSPs were reduced in magnitude but not in time course, and hence there appeared to be no associated increase in motoneuron membrane conductance (Eccles, 1964).

This inhibition of monosynaptic reflexes was accompanied by central depolarization of the extensor group 1A primary afferent fibers. This PAD was detected by recording dorsal root potentials or by determining the electrical excitability of afferent fibers within the cord. Accordingly, the inhibitory process was considered to be essentially presynaptic in nature. Eccles postulated that a synaptically generated increase in ion conductance, and the consequent depolarization of primary afferent boutons, would reduce the amplitude of terminal action potentials and thus diminish transmitter release. PAD was also detected in primary afferent fibers of cutaneous origin and to accompany the inhibition of impulse transmission through the dorsal column nuclei.

In 1962 George Gray had provided morphological evidence for axo-axonic synapses on spinal primary afferent boutons, and subsequent investigators found that the presynaptic elements of these synapses were also in contact with motoneurons in a triad arrangement. Later GABA and the GABA synthesizing enzyme glutamic acid decarboxylase were detected in the presynaptic elements of many spinal axo-axonic synapses.

Eccles, with Robert Schmidt and Bill Willis, found in 1961 that both the presynaptic inhibition of spinal reflexes and PAD were enhanced by IV barbiturates and chloralose. The inhibition and PAD were usually increased

by strychnine in doses that considerably reduced "direct" inhibition but were reduced by picrotoxin, which did not decrease "direct" inhibition. Additionally, the effects of GABA and 3APS applied topically to the surface of the spinal cord on PAD were interpreted in terms of a depolarizing action of these amino acids on primary afferent terminals. Because picrotoxin blocked both inhibitory synaptic transmission and the effect of GABA in a number of crustacean preparations, these pharmacological findings were regarded as strong evidence that GABA was the depolarizing transmitter generating PAD and "presynaptically" reducing transmitter release from primary afferent boutons in the cat spinal cord. A role for GABA as a "postsynaptic" transmitter hyperpolarizing spinal neurons appeared to have been excluded.

At that time we had not yet demonstrated that GABA hyperpolarized motoneurons and had not been able to show that picrotoxinin was a GABA antagonist in the cat CNS. It was not until 1970 that we demonstrated bicuculline to be a specific GABA antagonist, which reduced "presynaptic" inhibition of spinal reflexes, PAD, and the hyperpolarization of motoneurons by GABA.

In late 1962 Ryall and I had attempted to provide direct evidence that GABA depolarized the intraspinal terminals of muscle and cutaneous primary afferent fibers. Brief current pulses (max 12 microamps) passed through the central barrel of five-barrel microelectrodes were used to excite fibers near ventral horn motoneurons or dorsal horn interneurons excited monosynaptically by afferent volleys. In retrospect our technique of measuring changes in the amplitude of summed potentials recorded peripherally of impulses in a number of fibers before, during, and after ME GABA was quite inadequate to be interpreted in terms of changes in terminal excitability. Our results suggested that GABA decreased rather than increased fiber excitability, an effect later shown to reflect changes in the composition of the extracellular fluid rather than in the excitability of afferent boutons (see later).

In addition to finding that GABA hyperpolarized motoneurons, in the 1970s we were obtaining evidence that the polysynaptic inhibition of dorsal horn interneurons by impulses in cutaneous afferent fibers had two components, early glycinergic followed by GABA-ergic. Based on the selective effects of ME strychnine and bicuculline, the same result was observed when cutaneous afferent volleys were used to inhibit the firing of Renshaw cells and ventral horn group 1A inhibitory interneurons by volleys in high threshold cutaneous afferent fibers. In the case of Renshaw cells, both components of this afferent inhibition were blocked by tetanus toxin.

Because the firing of neurons in these three studies had been maintained by ME DLH, the synaptic and ME GABA depression of excitability was surely largely the consequence of the activation of neuronal postsynaptic receptors. Furthermore, by then there were several reports of the

detection of increases in motoneuron membrane potential and conductance associated with "presynaptic" inhibition, suggesting that a postsynaptic component of predominantly dendritic origin was also involved in this type of inhibition of motoneurons.

Primary Afferent Terminations

My return in 1976 to a study of the effect of GABA on primary afferent terminals was stimulated by two reports that ME GABA enhanced the excitability of these structures in the dorsal and ventral horn of the mammalian spinal cord. Furthermore, GABA had been reported to depolarize mammalian dorsal root and autonomic ganglia *in vitro* at nonsynaptic GABA-A receptors.

Lodge, Stephen Brand, and I recorded monophasic action potentials of single group 1A afferent fibers within an extensor muscle peripheral nerve in response to just-threshold 0.3 msec cathodal pulses of less than 2 microamps passed through the central barrel of seven barrel microelectrodes. With the tip of the electrode within the extensor muscle motonucleus, and by controlling the pulse amplitude manually, we found terminals, the thresholds of which were reduced by brief tetanic (4 at 320 Hz) stimulation of low threshold afferent fibers of flexor muscles 50 msec earlier. These were conditions known to depolarize primary afferent fibers (PAD) and "presynaptically" inhibit extensor reflexes. ME GABA and EAAs reversibly reduced terminal thresholds, but only PAD and the effect of GABA were reduced by ME BMC. Glycine had no consistent effects on terminal excitability.

This technique was subsequently refined in order to continuously monitor the threshold of a terminal. The frequency of single action potentials recorded peripherally, and identified by shape, amplitude, and latency, controlled a feedback circuit to regulate the amplitude of 10 Hz stimulating pulses (0.2–0.3 msec, <1 microamp) to maintain a 50% firing index. The multiplying factor in this circuit was plotted on a multichannel paper recorder, together with stimulating pulse amplitude, latency of response, percentage changes in termination threshold, microelectrophoretic currents, and other parameters including time, arterial blood pressure, and end-tidal carbon dioxide levels. There was also a need for continuous oscilloscopic monitoring of the recorded action potentials.

From early 1978 we used a simple electrophysiological test to assess whether the central fibre stimulated was myelinated or unmyelinated. The anodal blocking factor (BF) of a nerve fiber is the reciprocal of the ratio between the amplitudes of the threshold stimulating current and the higher current which blocks the transmission of impulses by hyperpolarizing the fiber adjacent to the site of excitation. Measurements of this factor for

myelinated fibers in the dorsal horn and dorsal column, and our experience with low threshold ventral horn afferent structures depolarized by GABA and P4S at BMC-sensitive GABA receptors, led to our accepting an upper figure of four as an indication that the structure stimulated was unmyelinated. Thereafter, unmyelinated preterminal axons and terminal boutons were referred to as "terminations."

In the late 1980s we also made use of the time course of the recovery of the central excitability of afferent myelinated fibers and terminations following a centrally generated or an orthodromic impulse as an indication of action potential durations and rates of repolarization. As expected, GABA-sensitive terminations had faster rates of repolarization and briefer action potentials (APs) than did myelinated fibers.

Termination GABA Receptors

Lodge, Bornstein and I used a number of GABA-A agonists to demonstrate that termination GABA receptors were similar or even identical to those on spinal neurons. In a more detailed study with Lodge of primary afferent fibers and ventral horn terminations, ME GABA and BMC had no effect on either the PAD or the excitability of myelinated fibers. BMC readily blocked PAD and depolarization by GABA of terminations but not depolarization by ME potassium or L-GLUT (see later), and occasionally increased termination thresholds, presumably by decreasing background PAD.

During depolarization by GABA, and other GABA-A analogues including 3AP, P4S, and muscimol, synaptically generated PAD, measured as a percentage change in threshold, was decreased. As this did not occur when terminations were depolarized by ME potassium or L-GLUT, activation of termination GABA-A receptors appeared to increase the membrane conductance, presumably to chloride ions. Such an increase had been established *in vitro* to account for the depolarization by GABA of dorsal root ganglion cells, cells that have a higher intracellular chloride concentration than neurons located within the CNS. That the equilibrium potential for ion fluxes generating PAD was at a more depolarized level than the resting membrane potential was supported later by demonstrating that PAD and depolarization by ME GABA and P4S were all enhanced during the hyperpolarization of a termination that followed prolonged tetanic excitation of its peripheral nerve.

The prolongation of termination but not fiber APs by ME tetraethylammonium (TEA) and 4-aminopyridine (4AP) indicated that voltage-activated potassium channels contributed to the repolarization of termination excitable membranes. Depolarization of terminations by GABA-A agonists and during PAD also prolonged termination APs, changes which did not occur during equivalent depolarization by an EAA (see later). Thus an increase in termination membrane conductance could be

the major factor in the reduction of transmitter release during activation of presynaptic GABA-A receptors, rather than mere depolarization.

In 1985 with Gynther and Malik, GABA-A receptors were also shown to present on groups 1A and 1B muscle afferent terminations in the lumbar intermediate nucleus and in Clarke's column. I was unable, however, to find any BMC-sensitive effects of GABA agonists or any evidence of synaptically generated PAD on the lumbar spinal terminations of the vestibulospinal tract (with Wilson and Malik), the rubrospinal tract (with Malik), and motor axon collaterals (with Gynther). In contrast with dorsal root ganglion cells which are depolarized by GABA at nonsynaptic receptors, the neurons of origin of all of these three types of termination, in the lateral vestibular nucleus, the red nucleus, and spinal motoneurons, respectively, are all hyperpolarized by ME GABA at receptors related to BMC-sensitive postsynaptic inhibitions.

Fundamental differences thus became apparent between the central spinal terminations of neurones located *within* the CNS and those with *peripheral* cell bodies. In addition to differences in embryological origin, there are differences in intracellular ion content and pharmacological properties and possibly also in the process of transmitter release at axon terminals. This latter difference was suggested by the differing effect of (−)-baclofen on transmitter release from the axonal terminations of these two types of neurons (see previous discussion). Another difference between central and peripheral neurons was the finding with Gynther that a number of divalent metal cations, including cadmium, magnesium, and calcium, reduced the depolarization of primary afferent terminations by ME GABA and P4S, and synaptic PAD, yet did not influence the depression of spinal neurone firing by these GABA-A agonists. Hence there also may be differences in the manner by which GABA-A recognition sites activate chloride ionophores of central and peripheral neurons.

Nonsynaptic Amino Acid Effects on Afferent Fibers

When relatively large ME currents were used to eject GABA, the initial reduction in termination thresholds was often not sustained and was occasionally followed by an increase in threshold that either was not affected or was enhanced by BMC. In a later more extensive study with Lodge, Peet, Bornstein, and Leah, we examined the effects on afferent myelinated fibers and terminations of a large number of GABA-like and unrelated amino acids about which direct or indirect information was available concerning their sodium-dependent uptake by mammalian brain preparations *in vitro*.

Our findings indicated that observed increases in termination and fiber thresholds most likely resulted from alterations in the ionic constitution of the extracellular fluid near the tip of the stimulating microelectrode

as a consequence of the ouabain-sensitive cotransport of amino acids and sodium ions into neurons and glia. GABA-A agonists, which were poor substrates for such uptake processes, such as muscimol, P4S and 3APS, reduced but did not increase termination thresholds and had no effect on myelinated fibers.

Lodge, Max Headley, and I reinvestigated an earlier observation that EAAs, including L-GLUT, L-ASP, QUIS, KAIN, and NMDA, reduced the threshold of muscle and cutaneous afferent terminations. In addition myelinated fibers stimulated in spinal grey matter were also depolarized but, unlike ME potassium, these EAAs had no effect on myelinated afferent fibers stimulated in the dorsal columns. The insensitivity of depolarization by EAAs to ME BMC, and its reduction by selective EAA antagonists, suggested that this also was a consequence of alterations in the extracellular medium, in these conditions resulting from the depolarization and firing of neurons.

GABA-A and GABA-B Inhibition of Spinal Reflexes

By the early 1990s there were a number of reports that, under *in vitro* conditions, relatively short latency and duration biculline-sensitive postsynaptic hyperpolarizations of neurons within supraspinal regions of the mammalian CNS were accompanied by a longer latency and duration GABA-B receptor-mediated hyperpolarization. Accordingly, from 1992 until 1995, Lacey and I compared the effects of IV bicuculline and several CGP baclofen antagonists on the "presynaptic" inhibition of lumbar spinal reflexes in cats anesthetized with pentobarbitone sodium (Curtis and Lacey, 1998).

Two forms were studied of this inhibition of monosynaptic extensor reflexes, recorded from transected ventral roots, by twice-threshold tetanic stimulation of flexor muscle afferent fibers: that following a brief tetanus of four volleys at 333 Hz, as in the 1960s investigations by Eccles and his colleagues of "presynaptic" inhibition and that during and after continuous 333 Hz stimulation for 10 to 60 seconds.

On the basis of the effects of IV administered selective GABA-A and -B antagonists, two components of brief tetanus inhibition were detected: early GABA-A associated with PAD and a later GABA-B component of more prolonged duration. Inhibition during and after continuous tetanic stimulation of flexor afferent fibers, however, appeared to be predominantly associated with GABA-B receptors. Although GABA hyperpolarized neurons and depolarized primary afferent terminations at postsynaptic receptors in the mammalian spinal cord, we were unable to determine the relative roles in the inhibition of spinal reflexes of GABA-A and GABA-B receptors at either of these sites. The presence of GABA-B receptors on

spinal and other neurons would account for our earlier observed depression of neuronal excitability by ME baclofen.

Conferences Abroad

In the meantime, to return to 1976, in March, at the invitation of the Houston Neurological Society and the Department of Neurobiology and Anatomy of the University of Texas at Houston I was one of two invited overseas speakers at a Neurological Symposium concerned with the Basic and Clinical Aspects of Neurotransmitter Function. I also gave lectures at the Department of Neurobiology and Anatomy, to the Houston Chapter of the American Neuroscience Society, at the University of Texas Medical Branch at Galveston, and at the University of Pittsburgh.

In July 1977 my wife and I, as guests of the USSR Academy of Sciences, visited research institutes in Moscow, Leningrad, and Kiev over a period of 2 weeks during which I delivered a number of lectures. In Moscow I had the opportunity to meet Dr. Y. S. Sverdlov whose paper in 1975 with S. N. Kozhechkin reporting the depolarization of ventral horn afferent fibers by ME GABA led me resume my interest in reinvestigating the role of this amino acid in spinal "presynaptic" inhibition. Unfortunately Sverdlov was no longer involved in neurophysiological research. In Kiev at the Bogomoletz Institute of Physiology I renewed my acquaintance with Platon Kostyuk, who had spent 6 months in Canberra in 1961 working with Eccles, and in Leningrad at the Sechenov Institute I met Alexander Shapovalov who was later to visit Canberra.

Before returning to Canberra I attended the XXVII International Congress of Physiological Sciences in Paris, contributed to a Satellite Symposium held in Cambridge concerned with microelectrophoretic methods for investigating central transmitters, and, as an invited speaker and a member of the Faculty, participated in a NATO Advanced Study Institute held in Norway dealing with amino acids as chemical transmitters.

EAA Antagonists

In 1978 we resumed our interests in EAA antagonists following published reports that D-alpha-aminoadipic acid (DaAA) acted preferentially at spinal aspartate-preferring receptors, as did GDEE at glutamate-preferring receptors. Lodge, Headley, and I confirmed this for DaAA, which antagonized excitation of interneurons and Renshaw cells by NMDA and L-ASP to a greater extent than that by L-GLUT. Additionally, DaAA reversibly reduced the polysynaptic noncholinergic excitation of Renshaw cells, which earlier we had considered could be mediated by L-ASP.

Subsequently, these findings were confirmed with McLennan and Lodge in Canberra, using DLaAA, which Watkins and I had earlier found to be

a weak excitant. Excitation by NMDA and ibotenic acid was most readily reduced by DLaAA and least by GDEE; that by L-GLUT and quisqualic acids (QUIS) were most reduced by GDEE and least by DLaAA; that by L-CYST, L-ASP, and D- and L-homocysteic acids were reduced to some extent by both, and excitation by kainic acid (KAIN) was relatively insensitive to both antagonists. Additionally both DLaAA and particularly GDEE reduced the excitation of Renshaw cells by ACh. Later we found that D-5-aminohex-2-enedioic acid, a conformationally restricted analogue of DaAA prepared by Rob Allen, also reduced excitation by NMDA to a greater extent than that by L-GLUT.

These results raised questions as to whether either D-aAA or GDEE were sufficiently selective for distinguishing between L-GLUT and L-ASP as synaptically released central excitatory transmitters *in vivo*. Furthermore, KAIN, structurally a L-GLUT analogue, appeared not to activate glutamate-preferring receptors.

By this time there was increasing acceptance that EAAs chemically related to L-ASP and L-GLUT interacted with a number of different types of receptor. In the early 1980s at least three EAA receptors had been proposed: NMDA, selectively antagonized by D-aAA and 2-amino-5-phosphonovaleric acid (2AP5); QUIS, selectively antagonized by GDEE; and KAIN for which no antagonist had been found. Some receptors were activated by more than one class of EAA. The simple classification of aspartate- and glutamate-preferring receptors clearly required revision to take into account new information from *in vivo* and *in vitro* studies of the effects of an expanding range of EAA agonists and antagonists of various degrees of specificity.

We in Canberra were very familiar with the readily demonstrable high degree of specificity of ME strychnine and bicuculline as glycine and GABA antagonists, respectively, at inhibitory synapses in the mammalian CNS. Accordingly, we considered that a similar high degree of specificity was required of antagonists for identifying *in vivo* the EAA transmitter at particular central excitatory synapses, for which L-ASP and L-GLUT were likely candidates.

In 1982 Peet, Leah, and I compared the effects of a number of compounds, reported by others to be EAA antagonists, on the excitation of lumbar spinal interneurons by ME NMDA, QUIS, and KAIN with those on mono- and polysynaptic excitation by volleys in cutaneous and muscle afferent fibers. Antagonists which reduced excitation by NMDA and L-ASP depressed polysynaptic excitation, thus supporting the role of L-ASP as the transmitter of excitatory spinal interneurons. We were unable, however, to find any selective antagonism of the monosynaptic excitation of spinal interneurons by impulses in primary afferent fibers by antagonists somewhat more selective for QUIS receptors, evidence that would support the transmitter role of L-GLUT.

EAA Uptake Inhibitors

Early in the 1970s Johnston and V. J. Balcar had screened a large number of amino acid analogues and other compounds as inhibitors of the high affinity uptake of L-GLUT and L-ASP by rat brain slices. Threo-3-hydroxy-DL-aspartic acid, which we had found some years earlier to excite spinal interneurons, was a potent inhibitor, and the subsequent preparation of the two isomers indicated that the L-isomer was approximately twice as effective as the D. In *in vivo* tests on spinal neurons, in subthreshold concentrations for excitation, both isomers enhanced excitation by L-GLUT, L-ASP, and QUIS and had much less effect on excitation by NMDA, KAIN, and ACh. Similar *in vivo* results were found using dihydrokainic acid, a very weak excitant, which was twice as effective as KAIN as an inhibitor of the high affinity uptake of L-GLUT and L-ASP *in vitro*.

These *in vivo* and *in vitro* observations strongly supported the importance of cellular uptake in determining the duration of action of ME excitant amino acids present in the mammalian CNS. Because L-GLUT and L-ASP, however, appeared to share the same transport process, the use of uptake inhibitors *in vivo* seemed to be unlikely to be useful in distinguishing between CNS pathways using either as an excitatory transmitter.

Further EAA Investigations

Following Johnston's move to the Chair of Pharmacology at Sydney University in January 1980, and the need to provide more support for other lines of research within my Department in the JCSMR within funding of decreasing value, I decided to reduce my interests in EAAs. A major factor that led to this decision was the expanding complexity of the field of EAA research, including the ever increasing number of individual EAA binding sites revealed by *in vitro* investigations (see Watkins, 2000), the physiological significance of many of which had yet to be established. Additionally, numerous large research groups abroad, particularly those associated with pharmaceutical companies, were becoming interested in specific EAA antagonists active *in vivo,* and hence considered to be of potential clinical and financial benefit.

To return to 1978, when in May I presented the opening lecture "Gabergic Transmission in the Mammalian Central Nervous System" at an Alfred Benzon Symposium in Copenhagen, which had been organized by Povl Krogsgaard-Larsen and his colleagues. This covered virtually all aspects of current research related to GABA, including the possibility of designing useful drugs for the management of GABA-related neurological disorders. I was also able to visit Sandoz Ltd. in Basel and to present a paper "Problems

in the Evaluation of Glutamate as a Central Nervous System Transmitter" at a Symposium organized by the Mario Negri Institute in Milan. In July 1978 I made a brief visit to Paris to attend the VIIth International Pharmacological Congress.

Multiple Sclerosis

From 1978 I also became involved with the National Multiple Sclerosis Society of Australia as Chairman of its Scientific Research Advisory Board, which advised the Society on the allocation of research grants. This was the consequence of my earlier experience as a member of a number of NHMRC grant-allocating committees, and the fact that I was not directly involved in MS-related research, rather than, like the other members of the Board, from my having an intimate knowledge of this disorder. I continued as Chairman until 1983 and was reappointed from 1993 until 1996.

Animal Experimentation

In view of my experience in using fully anesthetized or decerebrate cats in neurophysiological and pharmacological investigations I was invited in 1979 by the NHMRC and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) to chair a committee to revise the NHMRC booklet *Code of Practice for the Control of Experiments in Animals.* This had been prepared in 1969 by a committee chaired by Professor A. K. McIntyre. The 1979 version was subsequently revised a number of times, and the 1983 edition included the requirement for research institutions or organizations to establish Animal Experimentation Ethics Committees. The 7th edition, *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* was published in 2004.

Conferences Abroad

In March 1982 I attended the IBRO Congress held in Lausanne and gave lectures at the Max-Planck Institute for Biophysical Chemistry in Gottingen, the Institute of Physiology at the University of Aarhus, the MRC Clinical Pharmacology Unit in Oxford, the Departments of Physiology at the University of Bristol and University College London, and the Department of Psychiatry of the Athens University Medical School.

In July 1984 I briefly visited Krogsgaard-Larsen in Copenhagen, participated in a Symposium "GABA Mechanisms and Their Clinical Significance" at Cambridge, and attended the IXth International Congress of Pharmacology in London and a Satellite Symposium "Electrophysiology of the In Vitro Synapse" in St Andrews. At the Faculty of Medicine of the University of Tokyo I gave a lecture "The Micropharmacology of

Spinal Terminals" and then with my wife spent 2 weeks as guests of the Academia Sinica visiting the Shanghai Brain Research Institute, where we met the distinguished Chinese physiologist H-T. Chang, and the Institute of Biophysics in Beijing.

In April 1987 I attended a symposium "Excitatory Amino Acids in Health and Disease" held at the Royal Institution in London, and in August delivered a plenary lecture "Amino Acid Neurotransmitters" at the Xth International Congress of Pharmacology in Sydney, my one and only "performance" on the vast stage of the Concert Hall of the Sydney Opera House.

In August 1988 I had been invited to a Swedish-Australian Scientific Symposium "Neural and Hormonal Mechanisms in Circulatory Homeostasis" held at Wenner Gren Centre in Stockholm. This enabled me to visit and lecture at the Department of Physiology of the University of Goteborg and to meet Anders Lundberg with whom I had collaborated in Canberra in 1957, the Nobel Institute for Neurophysiology of the Karolinska Institute in Stockholm and Krogsgaard-Larsen's Department in Copenhagen. At the invitation of the President of the USSR Academy of Sciences, my wife and I then spent 2 weeks visiting and lecturing at research Institutes in Moscow and Kiev. It was a great pleasure to see Platon Kostyuk in Kiev and to meet Dimitri Kharkevich and his colleagues in Moscow.

We returned home via Oslo to visit Per Andersen and London to attend a Royal Society Dinner. I also participated, in my then role as President of the Australian Academy of Science, with Sir George Porter, President of the Royal Society, in unveiling a memorial plaque to Sir Joseph Banks at the new Banks Building at the Royal Botanic Gardens at Kew. This plaque was jointly sponsored by the Royal Society and the Academy. Earlier in April 1988, Australia's Bicentennial Year, Sir George had unveiled a bust of Banks at the Australian National Botanic Gardens in Canberra.

Australian Academy of Science

I had been elected to Fellowship of the Australian Academy in 1965, and had been a member of the Council (1974–1977) and a Vice-President (1975–1977). I also served on a number of Standing, Sectional, and National Committees, and, as recipient of the Academy's Burnet Medal, delivered the Burnet Lecture in 1983. This, the Academy's major award for research in the biological sciences, honors the contributions to science in Australia of Sir Macfarlane Burnet OM, KBE, MD, FAA, FRS, Nobel Laureate, the Academy's fourth President (1965–1969).

In May 1986 I was elected as President for a 4-year term. Fortunately the location of the Academy very close to the JCSMR considerably reduced the impact that this appointment could have had on my

research program. I am currently a member of the Academy's House Committee and Chairman of the Editorial Board of "Historical Records of Australian Science."

JCSMR Administration

In 1988, following a major ANU review of the School's research activities and structure, the departmental organization was replaced by four divisions, and from January 1989 I was invited to be Head of the Division of Neuroscience. This incorporated my Pharmacology (later Neuropharmacology) Group, the Physiology Group (Peter Gage), the Experimental Neurology Group (Steven Redman), and the Visual Neurosciences Unit (Bill Levick).

This appointment, however, was very brief as in March 1989 Robert Porter, who had been Director of the School and Howard Florey Professor of Medical Research since March 1980, resigned to take up the position of Dean of the Faculty of Medicine at Monash University in Melbourne. I was invited by the ANU Council to accept the Directorship and the Howard Florey Professorship from March 1989 until my retirement in December 1992. For the first year of this appointment I was also President of the Australian Academy of Science.

In 1989 the JCSMR had 73 full-time research staff, 56 PhD students, 125 technical support staff, and 66 administrative, general, and animal support staff. The total annual expenditure was A$14.6 million, 90% of which was derived from the School's allocation of the University's block grant from the Federal Department of Employment, Education, and Training. Members of the research staff were not able to apply for research grants awarded by the major Government grant-giving bodies, the NHMRC and the Australian Research Council (ARC), except in a few areas of research specified as being of special interest.

This appointment was in fact a "caretaker" role, because in 1987 the Federal Labor Government had announced its intention to bring about radical changes in the Australian tertiary education sector. An external committee was appointed by the Government in January 1990 to review the standing, role and funding of the ANU Institute of Advanced Studies (IAS) in the Australian higher education and research system. This included consideration of the then seven individual Research Schools: Biological Sciences, Chemistry, Earth Sciences, Pacific Studies, Physical Sciences, Social Sciences, and the JCSMR. Each had been reviewed in the past by committees, appointed by the University Council, and consisting of experts in the appropriate disciplines from Australia and abroad. The research activities and structure of the JCSMR had been reviewed in 1978 and 1988, the second led to the abolition of the departmental structure and the creation of four divisions.

Only one of the eight IAS Reviewers had experience in biomedical research, and although their recommendations, received by the Government in October 1990, could be regarded as generally favorable for the Institute, those specifically related to the JCSMR were far from welcome. Some research activities were suggested as being more appropriate for transfer to Biological Sciences or Chemistry, some to be discontinued and then the School to be "detached" from the ANU as an institute with its own governing body and management, funded for research by the NHMRC but affiliated with the ANU for academic purposes.

These recommendations were not acceptable to the University Council, and to the large majority of the JCSMR staff who strongly asserted that as one of the four Foundation Research Schools of the ANU the JCSMR should remain a formal component of the IAS. As Director I had spent considerable time in preparing a submission for this review. Then there was the need to respond to the report as it affected the JCSMR, and to participate in numerous discussions and negotiations with NHMRC representatives about future funding and administrative arrangements for the JCSMR. The NHMRC was then within the Department of Health, Housing, and Community Services (DHHCS), and in March 1991 funds considered appropriate for the JCSMR were transferred from the Department of Higher Education to DHHCS for a period of 5 years.

The details of the resultant complex bureaucratic saga are not relevant to this autobiography (see Fenner and Curtis, 2001). The School's precarious situation was referred to a Standing Committee of the parliamentary Senate, and in the meantime the School continued to receive its block grant from DHHCS. By this means, rather than transferring funding for the School directly to the NHMRC, the Government had accepted that the School should remain part of the University, which would then be responsible for its overhead costs. In August 1992 a liaison Committee was established with representatives of the ANU and DHHCS to consider policy and administrative issues related to the JCSMR. This arrangement continued until December 1996 when, following a change in government, funding for the School was restored as part of the ANU block grant from the Department of Education.

It is of historical interest that by the late 1990s, with the decreasing value of the government block grant to the IAS, there was an increasing need for the JCSMR to obtain additional funding from commercial interests and nongovernment research granting bodies. By 1998 approximately 78% of the JCSMR expenditure (A$19 million) was covered by the block grant. In 2001, however, the School's research staff gained access to the NHMRC and ARC for project grants. This access, increasingly staged over 3 years, was accompanied by a reducing block grant. The school's success in obtaining project and other grants resulted in a considerable increase

in total annual expenditure, approximately A$36 million in 2004, of which only 57% was derived from the government block grant.

Retirement

In the meantime, well aware that the JCSMR required a long-term director, and more than anxious to return to hands-on research rather than to continue dealing with ministers, reviewers, and administrators, I resigned as Director in March 1992 and returned to the Division of Neuroscience. During the rest of that year until my retirement on December 31, and then for 3 years as a University Fellow, with an ANU grant for research expenses and travel, and with the financial support of the JCSMR, I was able to complete several research projects with the assistance of Gary Lacey. My last experiment was on December 21, 1995, in the laboratory that I had occupied since February 1975, and for which I had designed, and even constructed, much of the special items of equipment.

In 1987, in recognition of my contributions to medical science, I had been invited to become a Fellow of the Royal Australasian College of Physicians. My contributions to medical sciences, particularly in the fields of research and administration, were also recognized in January 1992 by my appointment as a Companion in the Order of Australia, Australia's highest award for eminent achievement. Later in December 2002 I received a Centenary Medal for "Service to Australian society and science in neurophysiology."

With financial assistance from the Academy of Science, the JCSMR and the Department of Pharmacology of the University of Sydney, Graham Johnston and Steven Redman organized a 1-day symposium on Neurotransmission at the School on February 3, 1993 to mark the occasion of my retirement. I was indeed honored by the presence of many of my previous colleagues from Australia and abroad who attended, including Jeff Watkins, Graham Johnston, Povl Krogsgaard-Larsen, Victor Wilson, Hugh McLennan, Per Andersen, Dominik Felix, Bruce Gynther, and David Kerr.

Conferences Abroad

In late May 1990 I had presented a paper "GABA and Presynaptic Inhibition" at a symposium in Vancouver on the occasion of Hugh McLennan's retirement. My wife and I then briefly visited the Rockefeller University in New York, and then spent 18 days in the UK visiting London, Oxford, and Bristol. Then to Zurich to present a paper "Micropharmacology of Presynaptic Terminals" at the Brain Research Institute before delivering the opening lecture "Early Excitement About Amino Acids" at a meeting

held at Flims. Finally, I delivered a Plenary Lecture dealing with amino acid transmitters at the IUPHAR Congress in Amsterdam.

In September 1992 I attended the Second International Conference on GABA-B Receptors in Interlaken. I returned to Europe in May 1993 to present a paper at a gathering of ex-colleagues of John Eccles, held at the Max-Planck Institute for Brain Research in Frankfurt, to celebrate his 90th birthday and his outstanding contributions to neuroscience. I was also able to visit Ciba-Geigy AG in Basel to discuss our results with a series of baclofen agonists and antagonists, which had been provided earlier by Helmut Bittiger and his colleagues.

My final science-related period abroad was from October 1 to mid-November 1994 and included Vancouver, Ottawa, lectures in Edinburgh, Goteborg, and Tampere, a Plenary Lecture at the FAOPS Congress in Shanghai, and lectures at the Institute of Biophysics in Beijing and the University of Hong Kong.

Post-1995

From December 1995 until late 1998 I occupied a small office in the JCSMR Division of Neuroscience to complete a number of manuscripts, and then decided to work from home, visiting the school occasionally as a Visiting Fellow. With a small word processor I learned to type and prepared a number of obituaries of John Eccles who died on May 2, 1997 at the age of 94. Per Andersen and I authored his biographical memoirs, which were published in December 2001 by the Academy of Science and the Royal Society. I was also invited by Frank Fenner, who in 1948 had been the Foundation Professor of Microbiology, subsequently Director of the School from 1967 to 1973, and a Visiting Fellow since 1979, to join him in preparing a book *The John Curtin School of Medical Research. The First Fifty Years, 1948-1998.* This was published locally in August 2001 by Brolga Press, the manager of which was Robert Kirk, a former Head of the School's Department of Human Genetics. Our book, of 565 pages, included a detailed and illustrated history of the school and its staff, together with 85 invited essays covering highlights of many of the school's research achievements.

From 1998 until 2001, largely because of my experience on grant-allocating committees, I was a member of the Panel of Advisors of the National Institute of Forensic Science. I now enjoy a busy retirement, totally free of any involvement in laboratory research. I remain deeply concerned, however, that although the relevance of the increasing number of *in vitro* biophysical and molecular biological discoveries to the complex operation of the mammalian CNS and its disorders clearly requires *in vivo* confirmation, very few neuroscientists are currently receiving training in techniques appropriate to research using whole animals. I am practically free of administrative responsibilities, apart from those associated with

several Standing Committees of the Academy of Science, and now can enjoy adequate time for nonscientific reading, listening to music at home and at concerts in Canberra, visiting relatives and friends, gardening, wombling, learning new techniques for turning wood, and making the appropriate tools.

I was extremely fortunate in having had John Eccles as my scientific mentor, which led to my holding full-time research positions in the JCSMR from 1954 until 1995. Jack Coombs had an important influence on my ability to design and make electronic equipment, and collaboration with Rosamond Eccles in 1955 and 1957 resulted in the choice of Renshaw cells with which to initiate ME techniques. I was also particularly fortunate in having Jeff Watkins and later Graham Johnston as collaborators, because, without their expertise as organic chemists and their acquisition of a considerable understanding of the mammalian CNS, neuropharmacology and neurochemistry may not have been developed as research disciplines in the school.

I have been extremely fortunate, living in a somewhat remote region of the world, to have had numerous opportunities to travel abroad and to meet so many scientists with interests in the nervous system in the UK, United States, Canada, Denmark, Norway, Sweden, Finland, Holland, France, Germany, Switzerland, Italy, Hungary, Russia, Ukraine, India, China, and Japan.

My interests in the CNS were broadened by collaboration with David Kerr and his colleagues in Adelaide, and with a number of distinguished neuroscientists from abroad, including Anders Lundberg, Per Andersen, Paul Voorhoeve, Victor Wilson, Tim Biscoe, Hugh McLennan, and Povl Krogsggard-Larsen. I am also grateful to the many Research and Postdoctoral Fellows, and PhD Scholars, who contributed so much in the JCSMR Departments of Physiology and Pharmacology and the Division of Neuroscience. Many later occupied senior academic or administrative positions in Australia and abroad, including John Phillis, Ron Ryall, Michael Crawford, Chet de Groat, Arthur Duggan, Dominik Felix, Christopher Game, Roy McCulloch, David Lodge, Max Headley, Joel Bornstein, Martin Peet, John Leah, Richard Malik, Bruce Gynther, David Beattie, and Gary Lacey.

My research would not have been possible without skilled technical assistance from numerous members of the school's and departmental support staff, particularly Gerry Allen, Bob Ayling, Pam Blower, Arthur Chapman, Geoff Cohen, Arlene Daday, Lionel Davies, Don Geary, Laurel Graham, Barbar McLachlan, Beth McNaughton, Bryan Maher, Margaret Rodda, Alex Saeck, Pat Searle, Helen Taylor, Les Tranter, Toyne Van Arkel, Helena Walsh, Les Wells, and Gerry Winsbury.

Finally, without the support of the Australian National University, John Eccles and particularly that of my wife Lauri and our children,

who survived our move to Canberra in 1954, I would not have been in a position to have been invited to prepare this autobiography.

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