Actions of amino-acids on the isolated hemisected spinal cord of the toad

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Commentary by

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This paper was based largely on the dedicated efforts of Jeff Watkins and John Phillis, the experimentation forming portion of the latter's Ph.D thesis submitted in 1960. Early in 1958 we embarked on an investigation of the nature of neurotransmitters in the mammalian spinal cord, using mulibarrel micropipettes and microelectrophoretic techniques which were developed in 1957 in studies with Rosamund Eccles of the excitation by acetylcholine of Renshaw cells in the cat spinal cord *in vivo*. The decision to concentrate initially on the actions of compounds known to exist in mammalian central nervous tissue, and the demonstration that 4-aminobutyric acid (GABA) depressed the firing of spinal interneurones whereas L-aspartic and L-glutamic acid were excitants, led to an expanding interest in the structure-activity relationships of these and related naturally occurring and synthetic amino acids, and in their mode of action as possible synaptic transmitters.

Microelectrophoretic procedures offer considerable advantages for directly demonstrating *in vivo* the inhibition or excitation of anatomically and physiologically identified central neurones, as well as for determining the membrane conductance alterations underlying these actions and comparing them with those generated synaptically. These advantages, however, are offset to a considerable extent by the technical difficulties of such investigations (including the animal preparation and the lack of precise knowledge about extracellular amino acid concentrations) and the relatively limited number of neurones and compounds which could be studied in any one preparation. Given the large and ever increasing number of amino acids and related compounds which required study, the need arose for a simpler, more rapidly prepared *in vitro* vertebrate spinal cord preparation which could be used for screening purposes.

As a consequence the isolated hemisected spinal cord of the Queensland toad, *Bufo marinus*, was chosen. This tissue could be perfused with solutions of known concentrations of amino acids, and the depression and excitation of motoneurones could be inferred by recording spontaneous and dorsal root-evoked responses from ventral roots. Comparisons could be made readily with the effects of the reference amino acids, GABA and L-glutamic acid. Such a preparation, however, provided no information about the site and mode of action of particular test substances.

The tests were carried out in parallel with a continuing microelectrophoretic study of amino acid actions on cat spinal neurones, the results of which were published in 1959 and 1960 in Nature, the Journal of Physiology and the Journal of Neurochemistry. A particularly noteworthy finding in the toad was the high potency of N-methyl-D-aspartic acid, which had been synthesised by Jeff Watkins. This observation was subsequently confirmed in the cat. In general, results using the toad cord were consistent with those found in the cat, although some differences still remain to be explained. Overall, however, these investigations led to the development of numerous synthetic agonists, and later to the recognition of specific antagonists, of amino acid inhibition and excitation, thus contributing at a relatively early stage to our current understanding of inhibitory and excitatory amino acids as the major neurotransmitters in the mammalian central nervous system.

Subsequent studies in Canberra have been concerned predominantly with the inhibitory roles of glycine and GABA, the latter at A and B receptor subtypes. In the United Kingdom Jeff Watkins, pursuing his interests in excitatory amino acid receptors and actions, continues to make very significant contributions to the development of spe-

cific agonists and antagonists, enabling different subtypes of receptor to be identified and their roles in synaptic excitation to be determined. As a consequence of these early and later investigations, and of those carried out elsewhere, considerable interest has been generated in the pharmaceutical industry world-wide in the design of agents with which to selectively modify the synthesis, release, action or inactivation of particular amino acid neurotransmitters for therapeutic benefit.

References

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