EXCITOTOXINS

Edited by
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Proceedings of an International Symposium held at The Wenner-Gren Center, Stockholm, August 26–27, 1982

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PREFACE

This book is the proceedings of an International Wenner-Gren Center Foundation Symposium on "Excitotoxins" held at the Wenner-Gren Center in Stockholm on August 26 and 27, 1982. We are particularly happy that so many of the leading scientists in this field have been able to participate in this symposium. Since the book on "Kainic Acid" appeared in 1978 edited by Dr. McGeers and Dr. John Olney there has been an explosive interest in the research on neuroexcitatory and toxic amino acids. We therefore felt the time was right to bring the leading experts in this field together by organising a symposium on "Excitotoxins". In this way we hoped to have a penetrating and friendly discussion on the mechanisms underlying the neuroexcitatory and neurotoxic properties of excitotoxins and their relationship to the glutamate and aspartate neuron systems of the brain.

In Sweden we have previously had a symposium on "6–hydroxydopamine as a denervation tool in catecholamine research" held in Göteborg, Sweden, July 17–19, 1975 and organized by Drs. Gösta Jonsson, Torbjörn Malmfors and Charlotte Sachs. This symposium illustrated the considerable interest Swedish neuroscientists have had on highly specific neurotoxins, such as 6–hydroxydopamine, 5,6–dihydroxytryptamine and 5,7–dihydroxytryptamine; neurotoxins, which can produce damage to a certain type of transmitter-identified neuron. However, the neurotoxins, kainic acid and ibotenic acid represent another type of an invaluable tool in the experimental studies on brain function.

With the help of these powerful neurotoxins you can analyse the neuronal networks in the brain in a new way, since in a given area it is possible to specifically lesion the postsynaptic components without lesioning the presynaptic component. Thus, axons of passage and afferent inputs into the area, in which the neurotoxins have been injected, are spared, while the nerve cell bodies and the dendrites degenerate. Another important aspect to consider is that the excitotoxins, when injected into the mammallian brain, provide animal models of human pathology, such as Huntington's disease and temporal lobe epilepsy and possibly also presenile and senile dementias. In this way the excitotoxins also give indications as to the possible etiology of neurodegenerative diseases in man. Thus, it seems possible that a deranged metabolism in brain can lead to the formation of endogenous excitotoxins related to glutamate and aspartate.

In order to develop drugs which can prevent nerve cell degeneration in the brain it will become of paramount importance to better understand the molecular mechanism of action of kainic acid and of ibotenic acid and how the binding sites for these excitotoxins relate to the various classes of receptors for excitatory amino acids. Obviously, the development of potent antagonists or modulators of the kainate and ibotenate binding sites could represent a new possible type of treatment of neurodegenerative diseases and of epilepsia. Finally, another aspect to consider is that the research on excitotoxins may lead to new ideas in the field of human surgery. Thus, it may be speculated that some excitotoxins (bound to specific receptor agonists or antagonists) can specifically bind to certain target cells where they may exert their neuroexcitatory and neurotoxic actions.

This year the secretary of the Wenner-Gren Foundation, Professor Y. Zotterman died after working for the Foundation for many years. We will always remember him as a wonderful person and an outstanding sensory physiologist who was in love with the

neurosciences. He enthusiastically initiated and supported Wenner-Gren Symposia including the present one. We are very grateful to Mrs. Gun Hultgren at the symposium secretariat for her excellent assistance.

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Session I

EXCITOTOXIC AMINO ACIDS: LOCALIZATION, CHEMISTRY, PHYSIOLOGY, PHARMACOLOGY AND BIOCHEMISTRY

Chairman: U. S. von Euler

IDENTIFICATION OF EXCITATORY AMINO ACID PATHWAYS IN THE MAMMALIAN NERVOUS SYSTEM

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INTRODUCTION

Although the short-lasting excitatory action of glutamate on neurons was demonstrated more than 20 years ago (Curtis and Watkins, 1960), it has proved difficult to confirm its role as a neurotransmitter in the mammalian CNS. Unlike other putative neurotransmitters, glutamate fullfills a multitude of tasks in the brain. It is involved in energy metabolism and fatty acid synthesis, incorporated into proteins and peptides and is even a precursor for the inhibitory neurotransmitter GABA. In addition its action as a neuroexcitant has been regarded as universal and non-specific.

In the last 5 years, however, several new methods (Table 1) have been applied to define and characterize specific glutamate pathways in the mammalian brain (for discussion: Fonnum and Malthe-Sørenssen, 1981). One should not forget, however, that evidence for glutamate as a transmitter at the neuromuscular junction of invertebrates, e.g. the locust, started to accumulate 15 years ago (Usherwood, et al, 1968).

Table 1.

BIOCHEMICAL METHODS FOR IDENTIFICATION OF GLUTAMERGIC TERMINALS:

1. HA UPTAKE STUDIES (AUTORADIOGRAPHY & BIOCHEMISTRY)
2. AMINO ACID LEVEL
3. ANTEROGRADE OR RETROGRADE AXONAL TRANSPORT
4. ELECTRICAL OR K^+ STIMULATED RELEASE
5. IMMUNOHISTOCHEMISTRY
6. GLUTAMATE ENZYMES

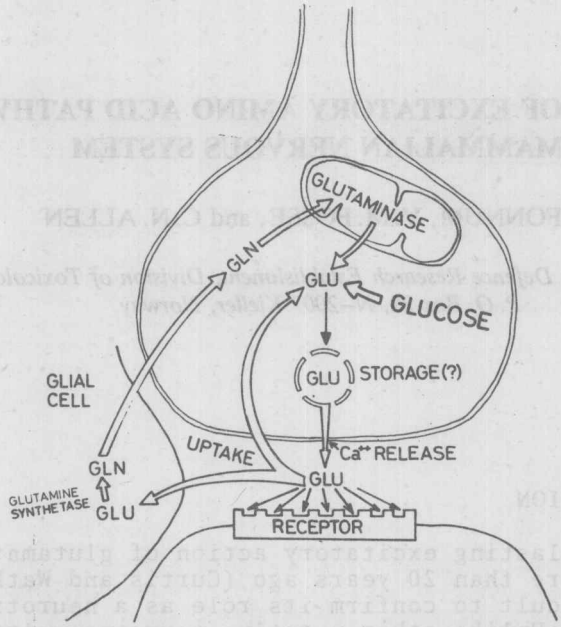

Fig. 1. Schematic drawing of a glutamergic synapse.

The most commonly used method to identify a glutamergic pathway is the high affinity uptake of L-glutamate or D-aspartate, measured either in a sucrose homogenate or autoradiographically in a brain slice (Table 1). The uptake system does not distinguish between D- and L-Asp or L-Glu, whereas D-Glu is a good inhibitor (Balcar and Johnston, 1972, 1973; Davies and Johnston, 1976). This allows us to use the metabolically stable D-Asp as a false transmitter.

THE VISUAL SYSTEM

The pyramidal cells in layer 6 of visual cortex projects ipsilaterally to the lateral geniculate body (Gilbert and Kelly, 1975). Ablation of visual cortex was accompanied by a severe reduction in high affinity uptake of both D-Asp and L-Glu in the ipsilateral lateral geniculate body (Table 2). Similarly there was a substantial reduction in L-Glu levels, but not in that of other amino acids including Asp, demonstrating that a large glutamate pool was lost when the corticogeniculate terminals degenerate (Table 2). Further, injection of the false transmitter ^{3}H D-Asp into the lateral geniculate body leads to labelling of cells in layer 6 of visual cortex (Baughman and Gilbert, 1980). This is an example of retrograde

transport of the transmitter from the terminal region to the cell bodies (Cuenod, et al, 1981). Also release experiments confirmed that glutamate was the excitatory transmitter of the cortico-geniculate pathway. Depolarization of geniculate slices with K^+ lead to a Ca^{++} dependent release of both endogenous glutamate and exogenously added ^3H D-Asp. The release of both compounds were reduced after lesion of the cortical input (Table 2, Fig. 2). Therefore, the corticogeniculate pathway fullfills the 4 most commonly used criteria to demonstrate that Glu is a transmitter.

Table 2

THE EFFECT OF VISUAL CORTEX ABLATION ON TRANSMITTER PARAMETERS IN LATERAL GENICULATE BODY.

PARAMETER	% OF NORMAL
HA [^{3}H]· D-Asp uptake	30
HA [^{3}H]· L-Glu uptake	40
HA [^{3}H]· GABA uptake	95
Glu level	72
Asp level	95
Gln level	108
GABA level	100
K^+-evoked release of [^{3}H]-D-Asp	51
K^+-evoked release of L-Glu	62

Pyramidal cells in the visual cortex also project to several other areas in brain such as superior colliculus and pulvinar (Carpenter, 1976; Lund, 1978). Visual cortex ablation was accompanied by a 40-50% decrease in uptake of D-Asp and L-Glu, and a significant reduction in the Glu level in superior colliculus (Lund Karlsen and Fonnum, 1978). In this case, however, there was no retrograde transport to visual cortex after injection of D-Asp in superior colliculus (Baughman and Gilbert, 1980). After visual cortex ablation the reduction in uptake in superior colliculus is only 10% of that in lateral geniculate body. The possible glutamergic pathway could therefore be too diffuse to allow detection by this method. This is a discrepancy which warrants further investigation. Recently we have obtained evidence based on reduction in HA-uptake and release of endogenous Glu (Figure 2) that also the cortico-pulvinar pathway uses Glu as its transmitter (Fosse and Fonnum, to be published). Complete transection of the total cortical input to the pontine nuclei reduced the uptake of D-Asp and L-Glu by more than

60%, showing that Glu/Asp are strong candidates also in this case (Storm-Mathisen, pers. communication. A major part of this input is probably derived from the visual cortex (Carpenter, 1976).

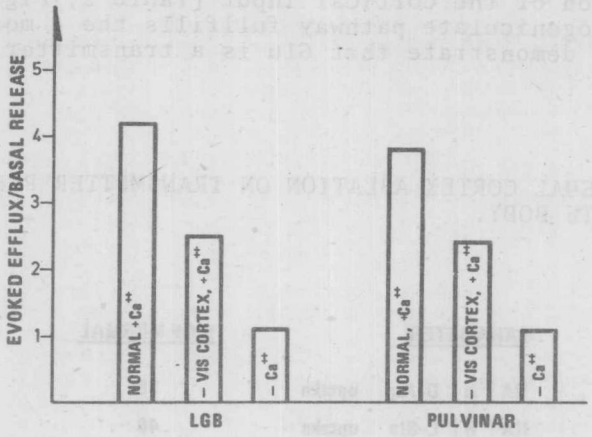

Fig. 2. The effect of visual cortex ablation on K^+-evoked release of endogenous L-glutamate from LGB and pulvinar.

Although Glu is considered as a major transmitter in the pigeon optic nerve (Cuenod, et al, 1981), the evidence is not as strong for the retinotectal projection of mammalian species. Applications of the techniques outlined in Table 1 have shown that at the most only 5-15% of the retinal ganglion cells use Glu or Asp as their transmitter in the rat.

There is good electrophysiological evidence that Glu is the transmitter of the photoreceptor cells and bipolar cells in the retina (Redburn, 1981). The most compelling (histochemical) evidence has been obtained by autoradiogrphy after uptake into the goldfish retina. From these studies Glu may be considered as the transmitter of the rods whereas Glu and Asp may function as the transmitter of the red and green sensitive cones (Marc and Lam, 1982). The immunohistochemical localization of aspartate transaminase in the cone cells and terminals of the guinea pig retina has been taken as evidence for Asp/Glu as a transmitter of these cells (Altschuler, et al, 1982). As will be discussed below, there is not a close correlation between the level of aspartate transaminase and the density of Glu/Asp terminals.