Short communication

Effects of Dineopentyl and Dipinacoline Ethers of Glutamic Acid on Neuromuscular Transmission in Locust (*Locusta migratoria*)

S. V. Ivlev[1], E. F. Lipskaja[2], Yu. E. Mandelshtam[1] and L. I. Deiko[3]

1 *I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, M. Thorez Ave.* 44, 194223 *Leningrad, USSR*
2 *Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska* 5, 833 06 *Bratislava, Czechoslovakia*
3 *Department of Organic Chemistry, A. I. Gercen Leningrad State Pedagogical Institute, Embankment of Moyka River* 48, 191186 *Leningrad, USSR*

The problem of the relationship between structure of dicarboxylic acid derivatives and their capacity to act on glutamate receptors has been extensively studied in various animal species (Piggot et al. 1975; Walker and James 1980; Puil 1981; Mandelshtam 1983). A number of substances with anticonvulsive properties (Meldrum et al. 1983; Colton and Colton 1985) and other preparations with pharmacological potential were derived from these studies (Simon et al. 1984; Hadberg et al. 1986; Baudry and Lynch 1984).

L-glutamic acid is the excitatory transmitter of neuromuscular transmission in insects (Usherwood and Machili 1968). The study of the specific aspects of glutamate reception in neuromuscular synapses therefore is of interest from the viewpoint of comparative neuropharmacology as well as for the development of new drugs with effects targeted on the locomotor activity of this animal species. Glutamate acid receptors are classified in three categories according to the agonist type they are capable of reacting with: N-methyl-D-aspartate, kainate and quisqualate (Nistri and Constanti 1979). Insect muscle fibres contain quisqualate type receptors since they are activated by quisqualic acid while being insensitive to N-methyl-D-aspartate, and can be blocked by diethyl ether of glutamic acid (Usherwood 1981). Previous works on neuromuscular synapses of the locust reported that the capacity to block synaptic transmission is directly proportional to both the length and ramification of alkyl radicals of dicarboxylic acid ethers. Symmetric ethers were shown to be more active than the respective γ-ethers of glutamic and/or aspartic acid (Aleksandrov and Baranov 1984; Aleksandrov and Speranski 1985). In the present work the effects of symmetric

Reprint requests to Dr. E. F. Lipskaja

ethers of glutamic acid (Glu), with long alkyl radicals (dineopentyl, DNPE, and dipinacoline, DPE), on neuromuscular transmission in the locust were studied.

Experiments were performed on thergocoxal muscle (muscle No 120) of the metathorax of the locust (*Locusta migratoria migratorioides R.F.*). The muscle isolated together with the nerve was placed in a perfusion chamber. Changes in membrane potential (MP) following the addition of l-glutamate (Glu), excitatory postsynaptic potentials (EPSPs), and miniature excitatory postsynaptic potentials (MEPSPs) were studied both under normal conditions and following the application of various concentrations of DNPE and DPE. The ethers were synthetized in Department of Organic Chemistry, A. I. Gercen Leningrad State Pedagogical Institute.

Intracellular potentials were recorded with glass microelectrodes filled with 2 mol/l potassium citrate. The resistance of the microelectrodes ranged between 15—20 MOhm. In studying EPSPs a series of 5 rectangular stimuli (1 ms each) were applied at a frequency of 100 Hz on the nerve using a suction electrode. Glu was administered by the method of microsuperfusion (Gapon et al. 1978) from a glass micropipette with a tip diameter of 100—200 μm. The solution contained (in mmol/l) : NaCl 135.0, KCl 3.0, $CaCl_2$ 2.0, $MgCl_2$ 1.6, KH_2PO_4 3.0, Na_2HPO_4 3.5, $KHCO_3$ 4, pH = 6.8 at room temperature. To abolish muscle contractions in response to nerve stimulation and to preserve neuromuscular transmission in functional state a high-magnesium (25 mmol/l), low-calcium (1.4 mmol/l) solution was used. The ethers studied were dissolved in this latter solution which was then used to perfuse the chamber with the nerve-muscle preparation. EPSPs, responses to Glu, and MEPSPs were periodically recorded during continuous perfusion. The values obtained were used to construct dose--response curves for glutamate responses in dependence on ether concentrations, and EC_{50} values (the drug concentration which decreases by 50 per cent the amplitude of glutamate potentials) were determined. The EC_{50} values were used to compare the blocking activities of the ethers studied.

The resting membrane potential of muscle fibres ranged between 55—70 mV and was not affected by the ethers. DNPE and DPE (2×10^{-5}—3×10^{-5} mol/l) decreased depolarization responses of muscle fibre membrane to Glu, while concentrations of 1×10^{-4} mol/l of the same compounds entirely blocked these responses. The effect was fully reversible: the responses restored after 10—15 min of washout with physiological saline. The values of EC_{50} for DNPE and DPE were 4×10^{-5} mol/l and 5×10^{-5} mol/l respectively (Fig. 1 *C*, *D*). It is evident that the ethers studied affected only the steepness of the dose-response curve constructed for the amplitudes of depolarization responses to various concentrations of Glu. This suggests that the blocking effect of the ethers on glutamate receptor could be of noncompetitive nature (Fig. 2).

EPSPs were affected by the ethers in a more complex way. Ether concentra-

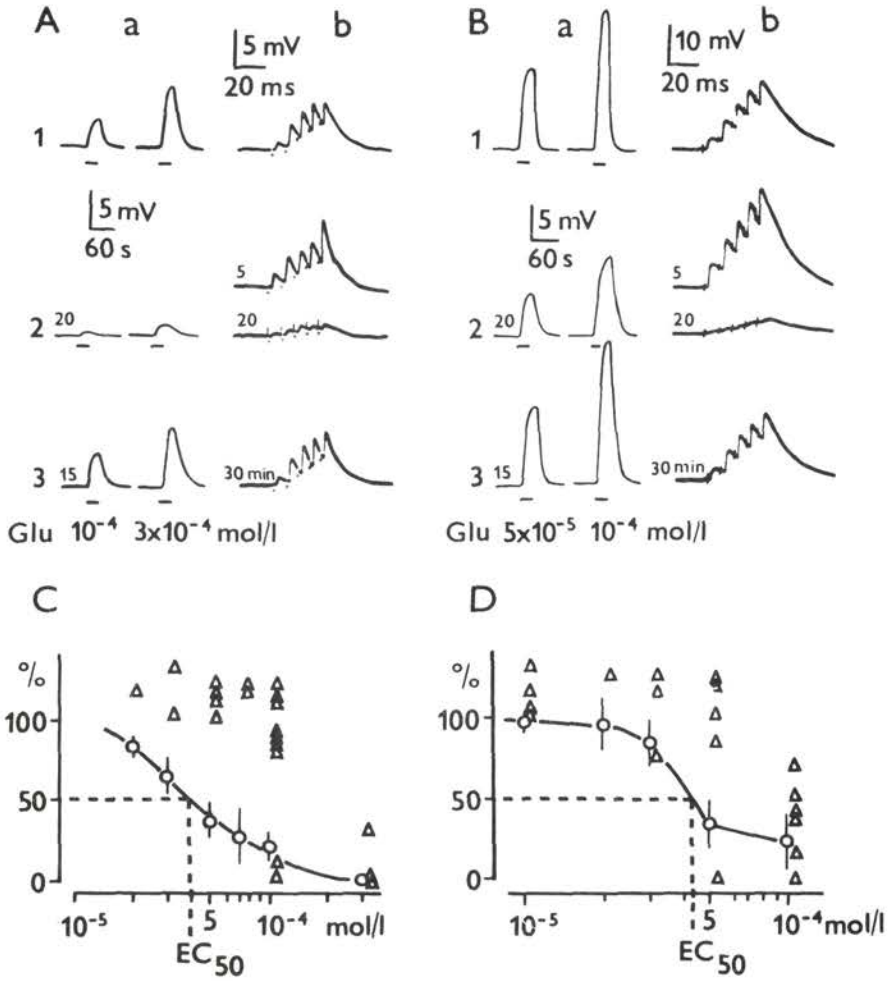

Fig. 1. Effects of dineopentyl (DNPE) (*A, C*) and dipinacoline (DPE) (*B, D*) ethers of glutamic acid (Glu) on glutamate response (a) and EPSPs (b) of locust muscle fibres. 1: depolarization responses to glutamate and EPSPs in physiological saline, 2: in the presence of ethers, 3: following washout. *C, D*: graphical determination of EC_{50} values for glutamate responses (o) and changes in EPSPs amplitudes (Δ) after 20 min of ether action. Abscissa: logarithm of ether concentration. Ordinate: percentage of the glutamate response amplitude. Membrane depolarization in physiological saline was taken for 100%. Glu concentrations: 3×10^{-4} mol/l (*C*) and 1×10^{-4} mol/l (*D*).

tric Glu ethers to block glutamate responses of locust muscle fibres is dependent on length and ramification of alkyl radicals as well as on the hydrophobicity of the compound (Aleksandrov and Baranov 1984)). The EC_{50} values obtained in our experiments for glutamate response-blocking effects of DNPE and DPE were one order of magnitude smaller than the respective values for the most active of all Glu ethers studied so far.

With regard to our results it is interesting to note that alcohols were reported to decrease sodium conductance of squid axon membrane. Alcohols which are highly hydrophobic (pentylalcohol, octylalcohol, etc.) are also surface active agents. Haydon and Urban (1983) explained the mechanism of action of alcohols by their being incorporated into membranes where they modify the environment of sodium channels thus disturbing the functioning of these channels. DNPE and DPE can also be considered surface active compounds as they contain hydrophobic groups in their molecules. It may be supposed that the mechanism of their action on neuromuscular synapses of the locust is similar to that of alcohols on squid axon membrane. If this assumption proves true, both DNPE and DPE could be expected to block sodium channels in both nerve and muscle cells.

A comparison of the effects of DNPE and DPE with those of symmetric Glu ethers studied previously (Aleksandrov and Baranov 1984; Aleksandrov and Speranski 1985) showed that the blocking effect of these drugs on neuromuscular transmission in insects increases with the increasing length of alkyl radicals. However, undesired, "side" effects appear as the degree of hydrophobicity is increased. In conclusion, the blocking effect of diethyl ether of glutamic acid is determined mainly by its interaction with glutamate receptors; however, the mechanism of the blocking action of DNPE and DPE is more complex and may be mediated, among others, by the action of these compounds on sodium conductance of excitable membranes.

References

Aleksandrov V. G., Baranov G. M. (1984): The effects of L-glutamic acid derivatives on neuromuscular synapses of the thergocoxal muscle of the locust *Locusta migratoria*. Zh. Evol. Biokhimii Fiziol. **20**, 422—425 (in Russian)

Aleksandrov V. G., Speranski E. M. (1985): The effect of glutamic and aspartic acids and their esters on fast and slow fibres of thergocoxal muscle of the locust *Locusta migratoria*. Zh. Evol. Biokhimii Fiziol. **21**, 203—206 (in Russian)

Baudry M., Lynch C. (1984): Glutamate Receptor Regulation and the Substrates of Memory. In: Neurobiology of Learning and Memory (Eds. G. Lynch et al.), pp. 431—447, Guilford Press, N. Y., London

Colton C. A., Colton J. S. (1985): Blockade of hyperbaric oxygen induced seizures of excitatory amino acid antagonists. Can. J. Pharmacol. **63**, 519—521

Gapon S. A., Kachman A. N., Frolova E. V. (1978): Studies of the chemoreceptive membrane in identified neuron of the gastropod *Planorbarius corneus* by different methods of drug application. Zh. Evol. Biokhimii Fiziol. **14**, 259—265 (in Russian)

Hadberg H., Anderson P., Butcher S., Sandberg M., Lehmann A., Hamberger A. (1986): Blockade of N-methyl-D-aspartate-sensitive acidium amino acid receptors inhibits ischemia induced by accumulation of catabolites in the rat striatum. Neurosci. Lett. **68**, 311—316

Haydon D. A., Urban B. W. (1983): The action of alcohols and other non-ionic surface active substances on the sodium current of squid giant axon. J. Physiol. (London) **341**, 411—427

Mandelshtam Yu. E. (1983): Insect Neuron and Muscle. Nauka, Leningrad (in Russian)

Meldrum B. S., Wardley-Smit B., Halsey M., Rostein J. C. (1983): 2-Aminophosphonoheptanoic acid protects against the high-pressure neurological syndrome. Eur. J. Pharmacol. **87**, 101—104

Nistri A., Constanti A. (1979): Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. Prog. Neurobiol. **13**, 150—235

Piggot S. M., Kerkut G. A., Walker R. J. (1975): Structure-activity studies on glutamate receptor sites of three identifiable neurones in the sub-oesophageal ganglia of *Helix aspersa*. Comp. Biochem. Physiol. **51C**, 91—100

Puil E. (1981): S-glutamate: Its interaction with spinal neurons. Brain Res. **228**, 229—322

Simon R. P., Swan J. H., Griffths T., Meldrum B. (1984): Blockade of N-methyl-D-aspartate receptors may protect against ischemia damage in the brain. Science **226**, 850—852

Usherwood P. N. R. (1981): Glutamate Synapses and Receptors on Insect Muscle. In: Glutamate as Neurotransmitter (Eds. G. Di Chiara and G. L. Gessa), pp. 183—193, Raven Press, N. Y.

Usherwood P. N. R., Machili P. (1968): Pharmacological properties of excitatory neuromuscular synapses in locust. J. Exp. Biol. **49**, 341—361

Walker R. J., James V. A. (1980): The central nervous system of *Limulus polyphemus*: Physiological and pharmacological studies. Comp. Biochem. Physiol. **66c**, 121—124

Final version accepted October 10, 1988