EFFECT OF THE NEW CHOLINESTERASE REACTIVATOR LA-54 ON SPONTANEOUS NEUROMUSCULAR SYNAPTIC ACTIVITY IN ACUTE DDVF POISONING

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In experiments on albino rats in vivo the amplitude and frequency of the miniature end-plate potentials were unchanged after intramuscular injection of the cholinesterase reactivator LA-54 (40 mg/kg). It is concluded from this fact that the reactivator has no direct action on the neuromuscular synapse. When the functions of presynaptic and postsynaptic structures are disturbed and also when acetylcholinesterase activity in the neuromuscular synapse is inhibited, as a result of the action of DDVF (LD_{50}), LA-54 completely abolishes these disturbances.

An important property of organophosphorus compounds (OPCs) is their ability to block neuromuscular conduction. In experiments on the isolated neuromuscular preparation and on the whole animal it has often been shown that inhibition of this process, as well as its restoration by the action of reactivators, does not necessarily take place through changes in acetylcholinesterase activity. It has been postulated that in such cases the OPCs have a direct action on the cholinergic receptor of the end-plate [2, 6, 7, 9]. More recently, however, evidence has been obtained which increasingly supports the general theory of action of OPCs which is based on their anticholinesterase properties. In this view the therapeutic effect of reactivators is entirely the result of the restoration of cholinesterase activity [3, 4].

These facts suggest that the inhibition of neuromuscular transmission in poisoning by OPCs and the restoration of conductance under the influence of reactivators are complex phenomena [1] and are probably not only due to the anticholinestase mechanism but also dependent on the functional state of other components of the neuromuscular junction.

By studying the action of the new cholinesterase reactivator LA-54, which is the hydrochloride of the S-diethylaminoethyl ester of p-bromobenzthiohydroximic acid, on the spontaneous activity of the neuromuscular synapse in poisoning with the organophosphorus insecticide DDVF it is possible to obtain more precise information about the role of presynaptic and postsynaptic structures in the restoration of neuromuscular conductance and also to detect any possible effect of the reactivator on their function.

EXPERIMENTAL METHOD

Experiments were carried out on neuromuscular synapses of the lateral muscle of the 8th-10 segments of the tail of albino rats. The functional state of the synapses was assessed from changes in the amplitude and frequency of the spontaneous miniature end-plate potentials (MEPPs). The MEPPs were recorded intracellularly by glass microelectrodes filled with 3 M KCl solution (resistance 5-20 MΩ). Recordings were obtained in the usual way [5]. Three groups of animals with six rats in each group were

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used. The MEPPs were recorded from not less than 30 muscle fibers from each animal. The animals of group 1 received LA-54 by intramuscular injection in a dose of 40 mg/kg, the animals of group 2 received DDVF (1 LD_{50}) by gastric tube, while the rats of group 3 received DDVF followed immediately by the reactivator (40 mg/kg). The MEPPs were recorded immediately after administration of the compounds and thereafter periodically for 90 min.

EXPERIMENTAL RESULTS

After injection of the cholinesterase reactivator LA-54 into the rats the frequency and amplitude of the MEPPs did not change significantly during the 90 min they were recorded.

Under the influence of DDVF there was a marked increase in the amplitude and frequency of the MEPPs within the first minutes. The amplitude rose on the average by 150% (from 0.23 ± 0.08 to 0.58 ± 0.025 mV) compared with its initial value, and the frequency rose by 50% (from 1 ± 0.08 to 1.5 ± 0.12/sec). After combined administration of the poison and reactivator to the animals no change in MEPP frequency took place during the 90 min of the experiment. However, the amplitude of the MEPPs remained a little increased (on the average by 39%) during the first 30 min, subsequently returning to its normal initial level. LA-54 thus completely abolished the increase in the frequency and amplitude of the MEPPs caused by DDVF.

There is now sufficiently convincing experimental evidence to show that the frequency of appearance of the MEPP corresponds to the number of quanta of acetylcholine liberated by the nerve ending while the amplitude of the MEPP depends on the number of molecules of mediator interacting with the cholinergic receptor. This process is determined by the rate of hydrolysis of the mediator by acetylcholinesterase [8, 10]. Accepting these views it can be taken that the increase in amplitude of the MEPPs observed in these experiments through the action of DDVF is connected with suppression of acetylcholinesterase activity and stabilization of acetylcholine. The reduction of this effect by LA-54 is evidence that the reactivator largely restores the enzyme activity soon after administration of the poison: the normal amplitude of the MEPP is restored, especially toward the end of the period of recording (90 min). The increase in the frequency of the MEPPs under the influence of DDVF indicates that the liberation of quanta of acetylcholine by the presynaptic membrane is facilitated in that case. The slowing of the MEPPs to the control level produced by administration of the reactivator together with DDVF is the result of normalization of the liberation of quanta of mediator by the nerve ending.

There is a definite difference between the action of the cholinesterase reactivator LA-54 and that of TMB-4 on the spontaneous activity of the neuromuscular synapse: LA-54 does not affect presynaptic and postsynaptic structures TMB-4 greatly inhibits the liberation of acetylcholine by motor nerve endings.

It can be concluded from the results of these recordings from the end plate that the cholinesterase reactivator LA-54 has no direct action on the function of the presynaptic and postsynaptic formations, nor does it change the acetylchloniesterase activity. If these functions of the nerve endings are disturbed and acetylcholinesterase activity inhibited by DDVF, the cholinesterase reactivator LA-54 completely abolishes those disturbances.

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