ACTION OF DIETHIXIME, A NEW CHOLINESTERASE REACTIVATOR, ON THE CENTRAL NERVOUS SYSTEM

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UDC 615.355:577.152.311.042.5] .015.4:612.8

A new cholinesterase reactivator, p-bromobenzoylthiohydroximic acid S-diethylaminoethyl ester hydrochloride (diethixime, containing a tertiary nitrogen atom in its molecule), in a dose of 20 mg/kg (0.02 LD_{50}), unlike dipyroxime (containing a quaternary nitrogen atom), in a dose of 3 mg/kg (0.02 LD_{50}), was shown to have a central action on rats and rabbits poisoned with dimethyldichlorovinyl phosphate. This was shown by restoration of acetylcholinesterase activity in various parts of the rabbits' brains and normalization of the electroencephalogram and of the functional state of the spinal motoneurons of the rats.

KEY WORDS: cholinesterase reactivators; diethixime; dipyroxime; dimethyldichlorovinyl phosphate; monosynaptic potential.

The degree and character of the prophylactic and therapeutic action of the oximes depend on their structure. According to some workers [1, 2, 4] cholinesterase reactivators containing a quaternary nitrogen atom in their pyridine ring penetrate poorly through the blood–brain barrier (BBB) and, for that reason, they have a mainly peripheral action, so that they are not sufficiently effective in poisoning by some organophosphorus insecticides (OPI). The new cholinesterase reactivator diethixime (p-bromobenzoylthiohydroximic acid S-diethylaminoethyl ester hydrochloride), synthesized in the writers' institute [9, 10] and containing a tertiary nitrogen atom in its molecule, has strong therapeutic activity in animals poisoned with OPI [5, 6].

The object of this investigation was to study the central action of diethixime in animals poisoned with dimethyldichlorovinyl phosphate (DDVP). Comparative investigations were carried out with dipyroxime, the well-known cholinesterase reactivator.

EXPERIMENTAL METHOD

Experiments were carried out on 24 rabbits weighing 3-3.5 kg and 50 albino rats weighing 200-260 g. At least six animals were used in the experimental groups. The action of DDVP and the reactivators on the CNS was studied over a period of 3-120 min after administration in relation to acetylcholinesterase (ACE) activity in different parts of the brain, the functional state of the spinal motoneurons, and the EEG. DDVP was given by mouth to the animals in a dose of LD_{50} (10 mg/kg for rabbits, 40 mg/kg for rats). The reactivators, in therepeutic doses of 0.02 LD_{50} (20 mg/kg for diethixime and 3 mg/kg for dipyroxime) were injected intramuscularly 1-2 min after the poison. ACE activity in the various parts of the brain of the rabbits poisoned with DDVP and during treatment with the reactivators was determined colorimetrically [12] 90 min after administration of the substances. Animals with DDVP poisoning were decapitated in the agonal period. Motoneuron activity was determined in rats anesthetized with urethane (1 g/kg) with respect to the amplitude of the monosynaptic potential (MSP) recorded from the ventral root of segment L5-6 in response to stimulation of the corresponding dorsal root by preliminary and test stimuli separated by different time intervals. The intensity

Laboratory of General Toxicology and Laboratory of Experimental Therapy, All-Union Scientific-Research Institute of Hygiene and Toxicology of Pesticides, Polymers, and Plastics, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 29-32, January, 1977. Original article submitted May 3, 1976.

TABLE 1. Degree of Reactivation of ACE (in %) in Different Parts of the Brain of Rabbits Poisoned with DDVP (10 mg/kg), after Treatment with the Cholinesterase Reactivators Diethixime and Dipyroxime

Part of brain	Inhibition of ACE activity by DDVP, %	90 min after treatment with	
		diethixime (20 mg/kg)	dipyroxime (3 mg/kg)
Medulla	73	96	3.3
Corpora quadrigemina	65	86	28
Hippocampus and hypothalamus	68	88	25
Caudate nucleus	73	25	5

of stimulation was chosen so that only group 1 fibers were excited [11, 14]. The EEG was recorded by a unipolar method on a four-channel electroencephalograph from the two frontal and parietal lobes of the brain. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During the first 15-20 min after administration of DDVP to the rabbits they developed acute poisoning typical of OPI and characterized by excitation of muscarinic and nicotinic cholinergic systems. ACE was almost completely inhibited (by 65-73%) in the various parts of the brain where, under normal conditions, the activity of this enzyme is particularly high (Table 1). Treatment of the animals with diethixime was effective. The characteristic symptoms of poisoning also developed in this case but they were much less severe than in the untreated animals or in animals treated with dipyroxime. Diethixime restored ACE activity by the greatest degree in the medulla (96%), corpora quadrigemina (86%), and hippocampus and hypothalamus (88%), and by a lesser degree in the caudate nucleus of the corpus striatum (25%). When dipyroxime was used the reactivation of ACE in the hippocampus, hypothalamus, and superior colliculi was 25-28%, but in the medulla and the caudate nucleus it was only 3-5%. Compared with dipyroxime, diethixime clearly penetrated by a far greater degree through the BBB and restored ACE activity in the CNS. However, the different degrees of ACE reactivation in different parts of the brain could be explained by differences in the permeability of the barrier [3].

In control rats in response to preliminary stimulation of the dorsal root, an MSP was recorded from the ventral root, where it was due to the spread of excitation in axons of motoneurons (Fig. 1). In response to test stimulation at different time intervals after the preliminary stimulation, after an initial period of absence of MSP, caused by the refractory state of the afferent fibers, there was a prolonged (4-5 msec) phase of reinforcement of the test MSP. The degree of reinforcement, calculated as the ratio between the test MSP and the preliminary potential, was 300%. The reinforcement was followed by a phase of slight weakening of the potential (Fig. 1).

After poisoning of the rats with DDVP a considerable increase was observed in the amplitude of both the preliminary and the testing MSP. Meanwhile the degree of reinforcement (by 450%) and the duration of this phase (up to 15-17 msec) both increased significantly (Fig. 1). In the animals receiving DDVP and diethixime the preliminary and testing MSP and also the reinforcement 10-20 min later were almost indistinguishable from their values in the control rats. However, there was a rather later rise of the phase of reinforcement to the maximum (Fig. 1). Under the influence of DDVP and dipyroxime the changes in the testing MSP were similar to those observed during DDVP poisoning, although under these circumstances the reinforcement was greater (550%). As Fig. 1 shows, during the period of prolonged depression the changes in MSP in the control rats and after exposure to DDVP and DDVP with diethixime were similar, whereas after exposure to DDVP and dipyroxime the process was more marked. Since the first impulse reaching the motoneurons excited only some of them (10-20%) to an intensity sufficient to cause a discharge in their axons, but most of the cells usually remained in a state of local excitation [8, 13], the increase in the preliminary MSP observed under the influence of DDVP alone and of DDVP and dipyroxime could probably be due to an increase in the number of motoneurons in which spreading excitation was produced. Nevertheless the change in MSP to testing excitation was the overall expression of processes in motoneurons with both spreading and local excitation; unexcited motoneurons probably also participated in this response. DDVP clearly caused an increase in the monosynaptic

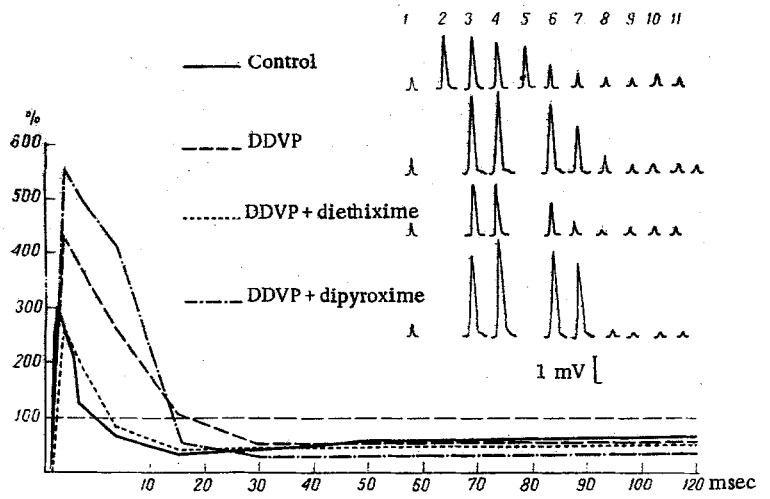

Fig. 1. MSP of rats during poisoning with DDVP and treatment with diethixime and dipyroxime. Top right of figure: preliminary MSP (1) and testing MSP (2-11), evoked 1.3, 1.7, 2.5, 3.3, 6.5, 15, 30, 50, 70, and 200 msec, respectively after preliminary impulse. Ordinate, ratio between amplitudes of testing MSP and preliminary MSP, taken as 100% (horizontal broken line); abscissa, time (in msec).

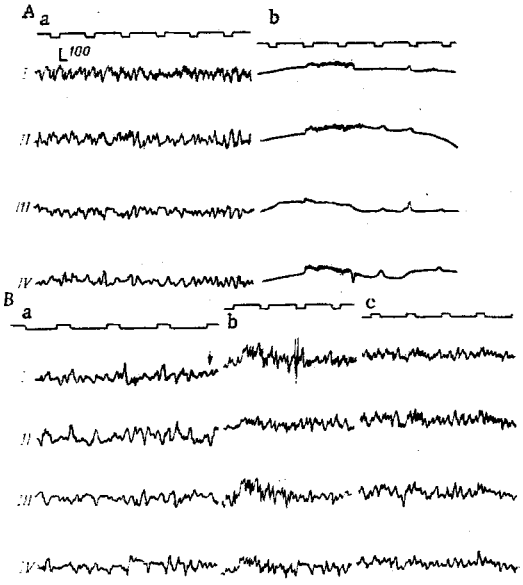

Fig. 2. EEG of rats after poisoning with DDVP and treatment with cholinesterase reactivator diethixime. I, II, III, IV) Derivations. A: a) Before administration of substances; b) 3 min after administration of DDVP. B: a) Before administration of substances; b, c) 20 and 40 min, respectively, after administration of DDVP and diethixime.

reflex response which may have been due to an increase in the excitability of the motoneurons. These changes were perhaps connected not only with ACE inhibition, but also with a disturbance of cholinergic structures, for example, in Renshaw cells, from which inhibitory influences to motoneurons originate [11, 15]. During the first 10-20 min the cholinesterase reactivator diethixime abolished the effect of DDVP, whereas dipyroxime did not abolish the action of the poison on the functional state of the motoneurons even after 90 min.

A study of the brain electrical activity showed that the EEG of the control animals was normal: The amplitude of the waves in all derivations was 93-100 μV and their frequency from 11 to 13 Hz (Fig. 2). The EEG as early as 3 min after administration of DDVP showed a marked generalized desynchronization response with

gradual depression of the potentials sometimes leading to their total disappearance. Changes in the EEG began much earlier than visible signs of poisoning appeared and they lasted for 60-90 min. Diethixime restored the brain electrical activity after 15-20 min. As a rule 40 min after administration of the preparation the EEG of the rats had returned almost to its initial values (Fig. 2). Treatment of the rats with dipyroxime did not lead to restoration of the brain potentials even 90 min after administration of the reactivator; the EEG was almost indistinguishable from that recorded in the poisoned animals, although in this case the rats did not die.

Considering that diethixime abolishes the neuromuscular block and the disturbances of cardiac activity and reactivates cholinesterase both at the periphery [7] and in the CNS, it can be concluded that it is a universal antidote for organophosphorus insecticides with a peripheral and central action.

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