PENETRATION OF ANTICHOLINESTERASE COMPOUNDS INTO THE NERVOUS SYSTEM OF THE ASIATIC LOCUST

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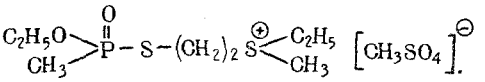
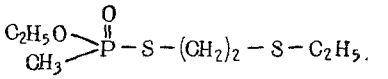
The present investigation was carried out to determine the character of the localization of cholinesterase in the nervous system of the locust,and to study the mechanism of action of certain anticholinesterase compounds (neostigmine, eserine, and N. N. Godovikov's preparations GD-42 and GD-7) in relation to their structure. The preparations GD-42 and GD-7 are particularly interesting. They are closely similar in their chemical structure but differ in the presence or absence of a charge on the sulfur atom:



Methylsulfomethylate of the O-ethyl-S-β-mercaptoethyl ester of methylphosphinic acid (GD-42) and:



O-ethyl-S-β-mercaptoethyl ester of methylphosphinic acid (GD-7).

All the experiments were carried out on the imago of the Asiatic locust Locusta migratoria L.

EXPERIMENTAL METHOD AND RESULTS

To determine the localization of cholinesterase, Gerebtzoff's modification of the Koelle–Friedewald histochemical method was used [1].

In the intact insects the cholinesterase activity was localized to the region of the neuropile, where all the synaptic contacts occur (see figure, a). Activity of the enzyme was also found along the course of the nerve fibers and in the nuclei of the glial and sheath cells. No enzyme activity was detected in the body of the nerve cells, in agreement with the findings of other authors [4, 6].

In the next experiments the histochemical method of determining the localization of cholinesterase was used to assess the action of anticholinesterase compounds.

The test preparations were injected with a microsyringe into the body cavity of the locust. After injection of each one of the anticholinesterase compounds, the motor activity of the locust was disturbed. The order in which these disturbances developed generally speaking coincided with the stages described by Jochum [3] in insects poisoned with organophosphorus compounds.

In the course of the investigation it was found that the lethal doses for the completely ionized compounds were much higher (2 mg/g for neostigmine, 0.014 mg/g for GD-42) than for the slightly ionized compound at the pH of physiological saline and hemolymph (eserine–0.01 mg/g) or the un-ionized compound (GD-7, 0.00011 mg/g).

The charged compounds were less toxic to the locust than the uncharged. Since the anticholinesterase preparation GD-42, which is charged in vitro, possesses an affinity for cholinesterase almost 3500 times greater than that of GD-7, the suggestion was made that the two compounds penetrate to an unequal degree through the sheath covering the nervous system of the locust. To study this problem, equal quantities by weight of the preparations GD-7 and GD-42 were injected into two groups of locusts in known lethal doses. The insects began to have convulsions 30 sec after injection of the preparation GD-7, and

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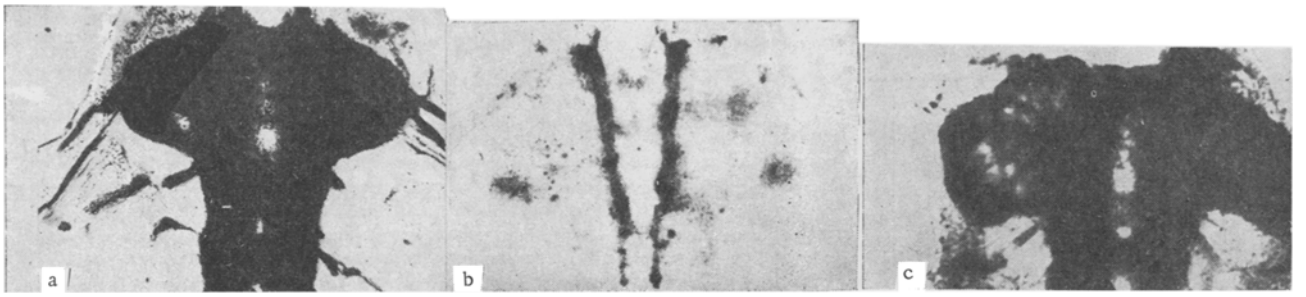

Localization of cholinesterase activity in sections of the metathoracic ganglion of the Asiatic locust. a) Normal; b) 10 min after injection of 20 μg of preparation GD-7; c) 10 min after injection of 20 μg of preparation GD-42. The substrate used was acetylthiocholine iodide.

they were completely immobilized after 10 min. Meanwhile, the behavior of the locust receiving preparation GD-42 was indistinguishable from the behavior of the insects of the control group receiving equivalent doses of physiological saline. Death of the insects took place much later after injection of the preparation GD-42 than after injection of GD-7 (30-60 min, or sometimes later still).

When the histochemical reaction was performed on sections of the metathoracic ganglion of a locust poisoned with GD-7, cholinesterase activity was practically absent and the sections remained colorless (see figure, b). In the ganglion of a locust poisoned with preparation GD-42, after 10 min the enzyme activity was indistinguishable from normal (see figure, c).

Similar results were obtained in the series of experiments with eserine and neostigmine. The un-ionized compounds thus penetrated much more readily through the ganglion sheath to the synaptic zones and inhibited the cholinesterase activity in the abdominal nerve chain, as a result of which the insect died.

It may be concluded from the results of these experiments that the selective barriers of the central nervous system of insects play an essential role in the mechanism of action of anticholinesterase compounds. The physiological significance of the sheaths in the insect nervous system is particularly high, for they are an important link in the chain of metabolic processes between the hemolymph and the elements of the abdominal nerve chain [2, 5].

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