Effect of Chlorophos (Dipterex, Trichlorphon) on High-Threshold Potassium and Calcium Channels of the Neuronal Membrane

Yu. V. Bukanova and E. I. Solntseva

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The effect of chlorophos (dipterex, trichlorphon) on high-threshold potassium and calcium currents is studied on isolated snail neurons using the patch-clamp technique. Chlorophos (10-1000 μmol/liter) is found to reversibly lower the peak amplitude of a high-threshold potassium current by 30% on average and exerts two independent effects on a high-threshold calcium current: reversible lowering of the peak amplitude by 35% on average and, in 30% of cases, reversible inhibition of its activation, inactivation, and deactivation. This effect is abolished by adding diltiazem (a calcium channel antagonist) in a concentration of 100 μmol/liter to the medium.

Key Words: *insecticides; chlorophos; potassium channels; calcium channels; snail neurons*

The mechanisms by which organic pesticides exert their neurotoxic effect are currently being studied by neuropharmacologists. It has been demonstrated that critical targets of organic pesticides in nervous tissue are ion channels of the surface cell membrane [1]. Close attention in this respect has been paid to the pyrethroids and organochlorines. Pesticides of these classes are able to lower current amplitude through voltage-dependent calcium [12,14,15], potassium [12-14,16], and chlorine [10] channels and modulate sodium channel gating in a complex fashion by inhibiting activation and deactivation of these channels [1,8,14,17].

At the same time, the effect of organophosphorus pesticides (dichlorvos, chlorophos, malathion, etc.) on ion channels remains unclear. These compounds are effective inhibitors of acetylcholinesterase, which is thought to be the chief mechanism of their neurotoxic action [4,6,7,11,18]. However, some neurotropic effects of organophosphorus compounds cannot be attributed to the above mechanism, for instance, chlorophos-induced depolarization of the presynaptic terminal [7] or lowered rate of impulse transmission [5,7]. These changes may be due to the action of the pesticide on the ionic conductivity of the neuronal membrane.

The aim of the present study was to investigate the effect of chlorophos on the voltage-dependent potassium and calcium channels of the common snail.

MATERIALS AND METHODS

The experiments were carried out on isolated nonidentified neurons of the snail *Helix pomatia* using the patch-clamp technique. Two microelectrodes filled with potassium citrate (2 mol/liter) were introduced into the cell to fix the potential and to record the transmembrane currents. The solution used for recording the potassium currents contained (in mmol/liter): 100 NaCl, 4 KCl, 5 $CaCl_2$, 4 $MgCl_2$, and 5 tris-(oxymethyl)-aminomethane, and that for recording the calcium currents 4 KCl, 10 $CaCl_2$, 4 $MgCl_2$, 5 tris-(oxymethyl)-aminomethane, and 95 tetraethylammo-

Laboratory of Functional Synaptology; Research Institute of the Brain, Russian Academy of Medical Sciences, Moscow (Presented by Yu. A. Romanov, Member of the Russian Academy of Medical Sciences)

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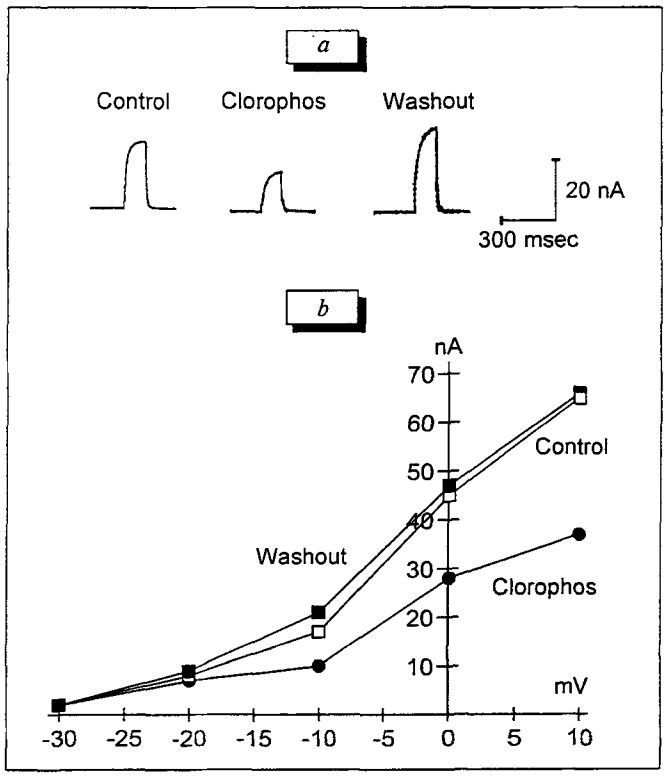

Fig. 1. Chlorophos-induced inhibition of I_K. *a*) I_K in the control, in the presence of chlorophos (50 μmol/liter), and after washout with the control solution; *b*) volt-ampere characteristics of peak I_K in the control, in the presence of chlorophos, and after washout with the control solution.

nium bromide. The pH of these solutions was adjusted to 7.6 with tris and HCl. Diltiazem (DT, Sigma) and chlorophos (trichlorphon, technical grade) were used in the experiments. The chlorophos solutions were prepared no more than 2 days before the experiment and the pH was adjusted to 7.6 with KOH. DT was diluted in the control solution similarly to chlorophos. The substances were added to the medium when the flow was stopped. The experiments were carried out on a Nihon Kohden standard device for microelectrode studies.

RESULTS

The effect of chlorophos on high-threshold potassium currents (I_K) was studied on 9 cells. The holding voltage (V_h) was set at -60 mV, and I_K was recorded during depolarization pulses (V_t) increasing the membrane potential to -30±10 mV with a step of 10 mV. Pulses of 150 msec duration were delivered at 30-60-sec intervals. Chlorophos was added to the medium in a concentration range of 1-500 μmol/liter, the threshold concentration being 10 μmol/liter. In all the studied cells chlorophos inhibited I_K by 30% on average. No clear dose dependence of the effect was noted. The effect took 1-3 min to develop and was completely reversed by a 20-30-min washout with the control solution. The substance did not markedly affect the rate of activation and inactivation of the potassium current. Figure 1 illustrates a typical effect of chlorophos on I_K recorded on one of the tested cells. Leakage currents were measured by delivering a hyperpolarizing pulse which lowered the membrane potential from -60 to -90 mV. For construction of the volt-ampere characteristics, I_K was calculated by subtracting the corresponding leakage current amplitude from the total recorded amplitude.

In order to elucidate whether the effect of chlorophos is specific for high-threshold I_K, we also studied the effect of this substance on a low-threshold fast potassium current (I_A). I_A was recorded after hyperpolarization of the membrane from V_h=-60 mV to -120 mV produced by conditioning 150-msec pulses. Chlorophos was found to have no marked effect on I_A, suggesting that its effect is specific for high-threshold I_K.

The effect of chlorophos on a high-threshold calcium current (I_{Ca}) was studied on 14 cells. The agent was added to the medium in a concentration range of 1-1000 μmol/liter. In 9 of the 14 cells chlorophos lowered the amplitude of I_{Ca} by 35% on average, the threshold concentration being 10 μmol/liter. No marked dose-effect dependence was noted. The effect was completely reversed by washing the cell with the control solution for 20-30 min. In 5 of the 14 cells no marked blocking effect was observed. Figure 2 demonstrates a typical blocking effect of the substance on I_{Ca} recorded in one cell.

In 4 of the 14 cells chlorophos in a concentration of 50-100 μmol/liter markedly inhibited activation and inactivation of I_{Ca}. Such a modulation of the gating mechanism of I_{Ca} developed upon the addition of the agent and disappeared after washing with the control solution much more slowly than the above-described chlorophos-induced blockade of I_{Ca}. The latency of chlorophos-induced I_{Ca} modulation and washing time were 5-25 min and 50-60 min, respectively.

The same cells were used for a study of the interaction between chlorophos and DT, a classic calcium channel antagonist. In the control solution DT (100 μmol/liter) inhibited I_{Ca} by 30% on average and sharply accelerated its decay (Fig. 3, *a*). The effect developed after a short latent period (1 min) and rapidly disappeared the cell was washed with the control solution. In the study of the effect of chlorophos and DT, the preparations were added to the experimental medium in the following order: chlorophos (50-100 μmol/liter) followed 30 min later (when the chlorophos-induced inhibition of I_{Ca} activation and inactivation attained the maximum) by DT in a concentration of 100 μmol/liter. I_{Ca} was recorded over the subsequent 5-10 min in the presence of both sub-

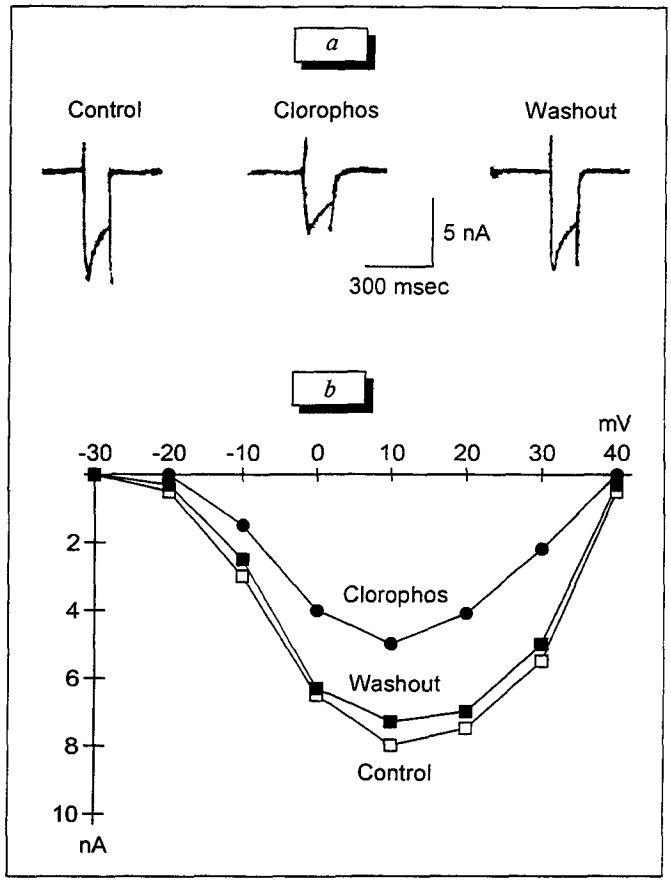

Fig. 2. Chlorophos-induced inhibition of I_{Ca}. *a*) I_{Ca} in the control, in the presence of chlorophos (10 μmol/liter), and after washout with the control solution; *b*) volt-ampere characteristics of peak I_{Ca} in the control, in the presence of chlorophos, and after washout with the control solution.

stances and then the cell was washed with the control solution for 60 min. This experimental protocol revealed that DT abolished the chlorophos-induced inhibition of I_{Ca} activation and inactivation. Figure 3, *b* presents a record of I_{Ca} obtained in one particular experiment, from left to right: I_{Ca} in the control solution, 30 min after the application of chlorophos (100 μmol/liter), 5 min after the addition of DT (100 μmol/liter) to the chlorophos-containing medium, and after 5- and 60-min washing with the control solution.

These data provide new insight into the mechanisms of neurotoxic action of organophosphorus insecticides. Until now the toxic effect of chlorophos and other insecticides had been thought to be mediated through inhibition of acetylcholinesterase [4,6,7, 11,18], although some authorities have also described other biochemical changes in the nervous tissue, namely, inhibition of the neuropathic target esterase [3,6,9], alkaline phosphatase, lactate dehydrogenase, and glutamate dehydrogenase and activation of aspartate transaminase [6], modulated activity of Na,K-ATPase [6,7], and disturbances in catecholamine and 5-hydroxytryptamine metabolism [6].

This rapid effect of chlorophos on ion channels of isolated neurons can hardly be attributed to the above-mentioned alterations in metabolic processes in the nervous tissue. Like other types of insecticides, chlo-

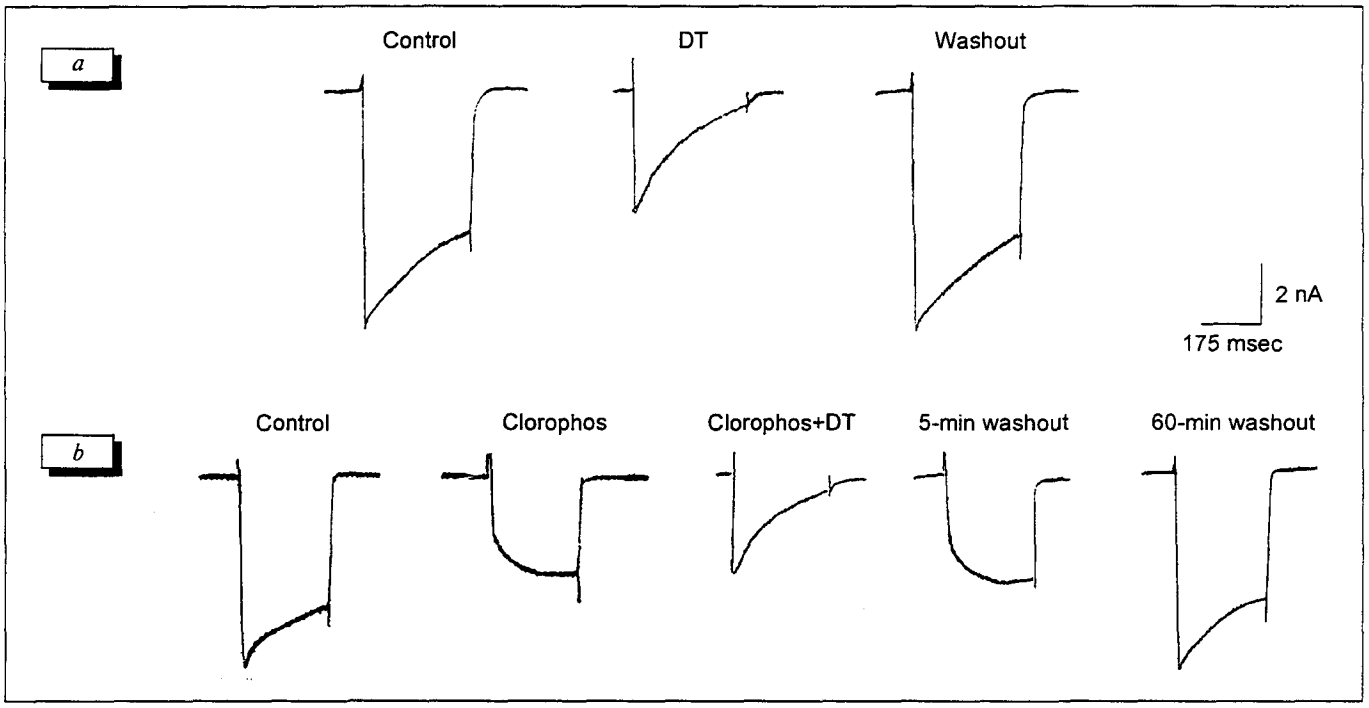

Fig. 3. Modulation of the gating mechanisms of the calcium channel by chlorophos and DT. *a*) I_{Ca} in the control, in the presence of DT (100 μmol/liter), and after washout; *b*) I_{Ca} in the control, in the presence of chlorophos (100 μmol/liter), in the presence of both agents (100 μmol/liter each), and 5 and 60 min after washout with the control solution.

rophos probably exhibits a membranotoxic effect and thereby modulates the functioning of ion channels.

In the present study we have described three effects of chlorophos on voltage-dependent ion channels of the neuronal membrane: first, a partial block of I_K conductance, second, a partial block of I_{Ca} conductance, and third, attenuation of the gating function of the Ca^{2+} current. The first and second effects were described earlier for other types of insecticides, chlordecone [12] and tetramethrin [15] in experiments on cultured nerve cells. However, there are no published data on insecticide-induced slowing of the gating function of the Ca^{2+} current. It is interesting to note that this effect is similar to the slowing of the gating function of the Na^+ channel in neuroblastoma cells [1,15,17] and the giant axon of the squid [8] induced by such insecticides as pyrethroids and DDT analogs.

Our experiments showed that the calcium channel antagonist DT abolishes the chlorophos-induced modulation of the gating mechanism of calcium channels. This suggests an allosteric interaction between chlorophos and DT binding sites on the gate structures of the Ca^{2+} channel, so that binding of DT suppresses the effect of chlorophos.

The physiological importance of the chlorophos-induced modulations in the functioning of ion channels may lie in changes in the intracellular Ca^{2+} content. The agent evidently has a dual effect on this process. On the one hand, it inhibits Ca^{2+} entry due to a partial blockade of Ca^{2+} channels. On the other hand, it may enhance Ca^{2+} entry due, first, to inhibition of the inactivation of the Ca^{2+} channels and, second, to partial blockade of the K^+ channels. The Ca^{2+} level in an individual cell presumably depends on which of these processes prevails.

It may be assumed that the observed effect of chlorophos on ion channels contributes to the development of its neurotoxic effect, since the concentrations inducing modulation in channel functioning are equal to the mean toxic doses for warmblooded animals (100-1000 mg/kg, i.e., 300-3000 μmol/liter [4,18]).

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REFERENCES

1. A. S. Kontush, *Biol. Nauki*, № 6, 5-19 (1991).
2. V. B. Prozorovskii and N. V. Savateev, *Nonanticholinesterase Mechanisms of Action of Anticholinesterase Preparations* [in Russian], Leningrad (1976).
3. N. N. Tkachenko, *A Study of the Delayed Neurotoxic Effect of Some Organophosphorus Insecticides* [in Russian], Abstract of PhD. Thesis, Kiev (1989).
4. *Trichlorfon: UN Program on the Environment* [in Russian], Moscow (1983).
5. A. N. Urazaev and M. Ya. Tremasov, in: *Intensification of Reproduction and Prevention of Sterility in Agricultural Animals* [in Russian], Vol. 90, Kazan (1989), pp. 145-148.
6. *Organophosphorus Insecticides: A General Introduction, International Program of Chemical Safety* [in Russian], Moscow (1990).
7. S. I. Fudel'-Osipova, Yu. S. Kagan, S. D. Kovtun, and U. A. Kuz'minskaya, *Physiological and Biochemical Mechanism of Action of Pesticides* [in Russian], Kiev (1981).
8. L. D. Brown and T. Narahashi, *Brain Res.*, **584**, № 1-2, 71-76 (1992).
9. J. L. De Bleecker and J. L. De Reyck, *Clin. Neurol. Neurosurg.*, **94**, № 2, 93-103 (1992).
10. P. J. Forshaw, T. Lister, and D. E. Ray, *Neuropharmacology*, **32**, № 2, 105-111 (1993).
11. T. R. Fukuto, *Environ. Health Perspect.*, **87**, 245-254 (1990).
12. K. Inoue, K. Nakazawa, T. Obama, *et al.*, *Pharmacol. Toxicol.*, **67**, № 5, 444-446 (1990).
13. L. D. Leake, J. A. Dean, and M. G. Ford, in: *Neuropharmacology and Pesticide Action*. Eds. M. G. Ford and E. Horwood (1986), pp. 244-266.
14. T. Narahashi, in: *Neurotoxicology of Insecticides and Pheromones*, New York-London (1979), pp. 211-243.
15. T. Narahashi, in: *Neuropharmacology and Pesticide Action*. Eds. M. G. Ford and E. Horwood (1986), pp. 36-60.
16. M. Omatsu, K. Murayama, H. Kitasato, *et al.*, *Pestic. Biochem. Physiol.*, **30**, 125-135 (1988).
17. M. Oortgiesen, R. G. van Kleef, and H. P. Vijverberg, *Brain Res.*, **518**, № 1-2, 11-18 (1990).
18. *Trichlorfon, Health and Safety Guide, Health Safety Guide*, № 6, 1-40 (1991).