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MODULATORY EFFECTS OF NEUROTOXIC INSECTICIDES ON THE PERIPHERAL AND CENTRAL GABA-ERGIC ACTIONS

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On the terminal part of the guinea-pig ileum GABA produces contraction, whereas on the preterminal it produces an initial short-lasting contraction, followed by a prolonged relaxation. The increasing range of concentrations of GABA produces a concentration-dependent decrease in contractions and an increase in contractions of the preterminal ileum. Both contractions and relaxations can be blocked by atropine, indicating the cholinergic nature of the responses. These effects are due to the action on $GABA_A$ receptors.

Depending on the duration of the incubation period (3 and 60 sec) GABA produced either potentiation or depression of the contractile effects of acetylcholine on the ileum.

All the three neurotoxic insecticides (lindan, malathion, permethrine) affect the contractile effects of acetylcholine on the ileum. Lindan and permetrine antagonized the contractile effects of acetylcholine, whereas malathion produced a potentiation. Malathion significantly depressed the contractile effects of the electrical field stimulation of the ileum. This effect is probably realized through the local release of GABA.

Both lindan and permethrine were found to decrease the duration of the barbiturate sleeping time, whereas malathion significantly prolonged its duration. The action of lindan and permethrine is presumably realized by blocking or interfering with the function of $GABA_A$ receptors. Malathion is an anticholinesterase, thus producing an accumulation of acetylcholine at the critical sites, consequently producing a prolongation of the barbiturate sleeping time.

In conclusion, neurotoxic insecticides (lindan, permethrine, malathion) affect both central and peripheral GABA-ergic systems. They can produce either depression or stimulation of these systems. They also highly significantly modulate the activity of the cholinergic system in the isolated guinea-pig ileum.

Key words: malathion, lindan, permethrine, enteric nervous system, barbiturate sleeping time.

INTRODUCTION

It has been already published that neurotoxic insecticides (lindan, malathion, permethrine) depress the central cholinergically-mediated hypertension (CCMH) in anaesthetized rats (Stanković *et al*., 2004). Evidently, all three neurotoxic insecticides can inhibit transmitter interaction which is the basis of CCMH and which takes place in the central nervous system of the rat.

The neurotoxic insecticides affect the functions of the nervous system. Thus, malathion and other organophosphates accumulate acetylcholine to toxic levels (Plaa, 2004). This produces an alteration in neurologic and cognitive functions, including physiological symptoms of variable duration (Ecobichon, 2000). There is some indication that the action of these substances might be connected with neurologic complexes in Gulf War veterans (Haley *et al*.,1999). These substances might also be connected with organophosphate-induced delayed polyneuropathy (Lotti, 1991, 2002).

The major site of toxic actions of permethrine (and other similar substances) is the central nervous system (Plaa, 2004). The symptoms include excitation, convulsions and tetanic paralysis. Sodium, calcium and chloride channels are considered to be the main targets, thus creating serious health problems (Soderlund *et al*., 2002).

Lindan (hexachlorocyclohexane) is also neurotoxic, particularly in infants, children and pregnant women (Franz, 1996).

GABA and GABA-ergic system, together with chloride channels, represent the fundamental inhibitory system in the central nervous system (Nicoll, 2004). Besides, GABA is present in the enteric nervous system and may have some presynaptic effects (Katzung, 2004). It was therefore of interest to investigate and to compare the central and enteric effects of neurotoxic insecticides on the central and peripheral GABA-ergic systems.

MATERIAL AND METHODS

In vitro experiments were done on the isolated guinea-pig preterminal and terminal ileum. This was done because of the difference in reactivity and in responses to drugs between these two parts of the gut. The terminal ileum was cut only a few milimeters from the ileo-coecal valve, whereas preterminal ileum was cut at least 10 cm from the valve.

The isolated ileum was arranged in an isolated organ bath, mantaining the temperature of the Tyrode's solution at 35^{o} C. Contractions of the ileum were recorded on an Ugo Basile recording system.

In vivo experiments were done on Wistar male rats, weighing 150-200 g. Before the experiment, the animals were kept under standard laboratory conditions and allowed ad libitum pelleted food and water. Lindan, malathion and permethrine were injected intraperitoneally, whereas thiopenton-sodium was injected into the tail vein.

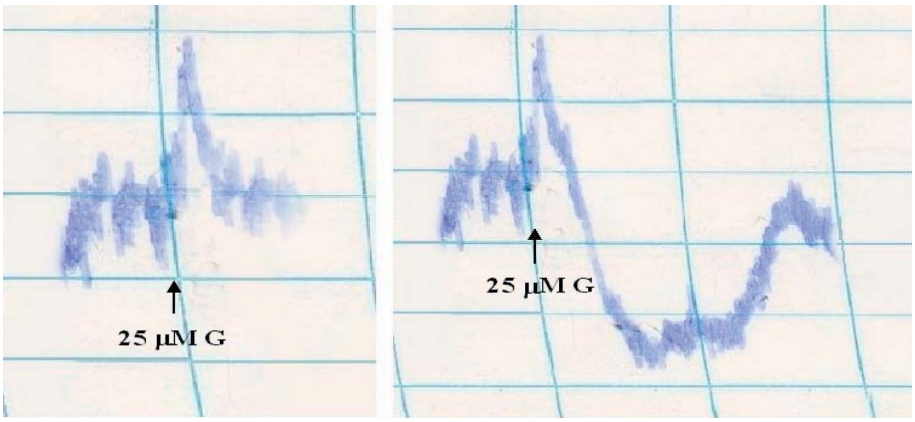
The following drugs were used: malathion (Cheminova Agro, Denmark), permethrine (Wellcome, London), lindan (Zorka, Šabac), GABA (gamma-

aminobutyric acid, (Sigma), acetylcholine (Serva, Feinbiochemica Heidelberg) and atropine sulphate.

Statistical analysis was made using a computer programme (Microsoft Excel Version 2000).

RESULTS

A difference was found in reaction to GABA between preterminal and terminal parts of the ileum. Thus, a regular contraction was recorded after addition of 25 μM GABA on the terminal ileum, whereas the same concentration of GABA produced a biphasic effect on preterminal part of the gut, consisting of an initial contraction followed by a prolonged relaxation (Fig. 1).



a) b)

Figure 1. Spontaneous activity and contractions of the isolated terminal (a) and preterminal (b) guinea-pig ileum. At the arrow (25 μM) GABA was added into bath. Contractions lasted about one minute, whereas relaxation lasted several minutes

The increasing range of concentrations of GABA (10, 25 and 50 μM) produced a concentration dependent decrease in contractions and an increase in relaxation, as shown in Fig.2. This dose-dependence had linear characteristics and was observed on the preterminal ileum (Fig. 2).

The contractile effects of acetylcholine and GABA on the isolated preterminal and terminal ileum can be easily blocked by atropine (0.01 μg/ml).

GABA in concentrations of 25 and 50 μM was found to potentiate the contractile effects produced by increasing the concentrations of acetylcholine. GABA was added into the bath 3 sec before addition of acetylcholine. The regression lines, which show the potentiating effect of GABA on the response to acetylcholine are shown in Fig. 3.

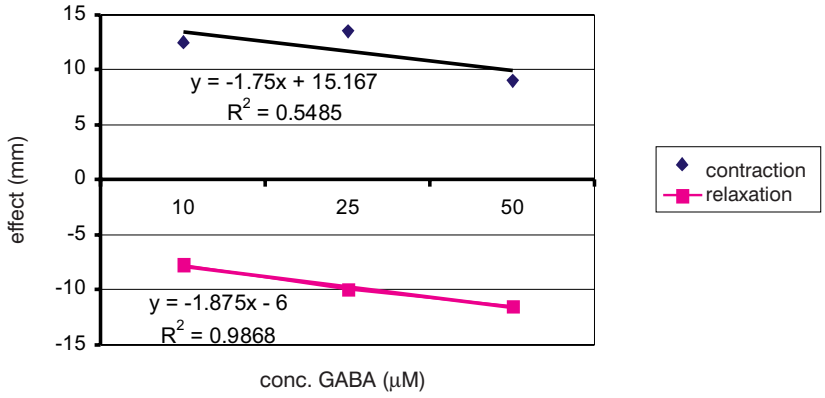


Figure 2. The effect of increasing concentractions of GABA on contractions and relaxations of the isolated preterminal ileum. Observe linear concentration-dependence

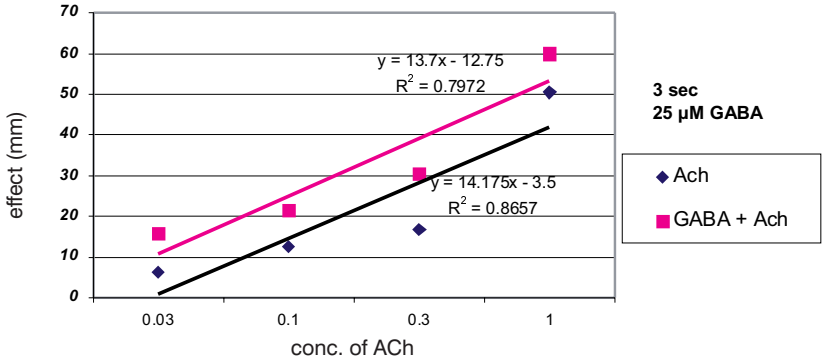


Figure 3. The regression lines showing the potentiating effect of 25 μM GABA on the responses of the isolated preterminal ileum to increasing concentrations of acetylcholine, added 3 sec after GABA

Interestingly, if the same concentrations of GABA were kept in the bath for 3 min before addition of increasing concentrations of acetylcholine, then the responses to acetylcholine were decreased.

Lindan by itself, in concentrations from 5 to 30 μM, produced relaxations in both preterminal and terminal isolated ileum. At the same time, lindan antagonized the contractile effect of GABA. The lowest concentration of lindan (5 μM) produced also a concentration-dependent inhibition of the contractile response to acetylcholine, as shown in Fig. 4.

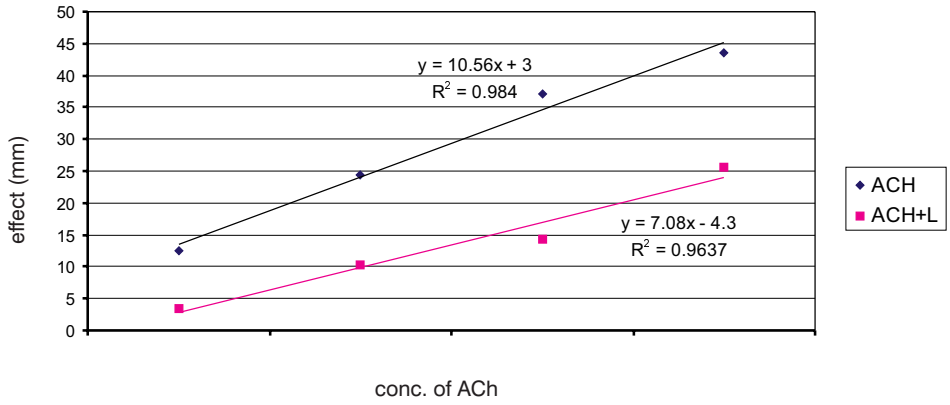


Figure 4. The effect of lindan (5 μM) on contractions of the preterminal ileum produced by increasing concentrations of acetylcholine

Permethrine (90 μM) antagonized the contractile effects of acetylcholine on the isolated ileum. This inhibitory effect of permethrine persists also in the presence of GABA (25 μM), as shown in Fig. 5. This means that, in the applied concentration, GABA did not affect the action of permethrine on the contractile effects of acetylcholine in the ileum.

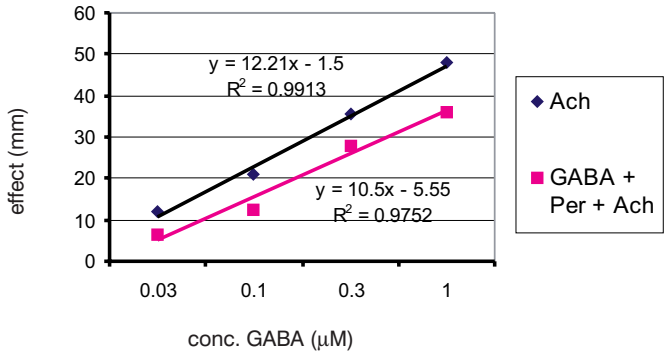


Figure 5. The effects of GABA and permethrine on the contractile effects of increasing doses of acetylcholine on the isolated preterminal ileum

Malathion is an organophosphate anticholinesterase and it was found to potentiate the contractile effects of acetylcholine on the ileum. On the other hand, malathion (20 μg/ml) significantly antagonized the contractile effects of the electrical field stimulation of the ileum, as shown in Fig. 6. Contrary to malathion, physostigmine (20 μg/ml), which is an anticholinesterase of the reversible type, produced potentiation of the contractile effects of electrical field stimulation of the ileum.

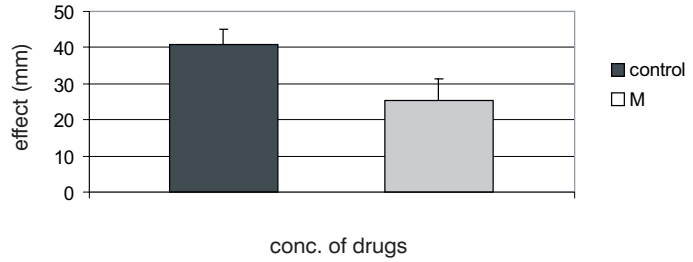


Figure 6. Inhibitory effect of malation (20 μg/ml) on the contractile responses to electrical field stimulation. Black column: control responses to electrical field stimulation. Gray column: responses to the same stimulation in the presence of malathion (20 μg/ml)

The barbiturate sleeping time is taken here as the classical indicator of the central GABA-ergic activity. Thus, increasing doses of thiopenton (15, 20, 25 and 30 mg/kg, intravenously) produced a dose-dependent duration of the barbiturate sleeping time. The neurotoxic insecticides have been shown to affect the barbiturate sleeping time. For example, lindan (0,1 mg/kg intraperitoneally) and permethrine (1 mg/kg intraperitoneally) significantly decrease the duration of the barbiturate sleeping time, whereas malathion (5 mg/kg intraperitoneally) significantly prolongs this time (Stanković *et al*, 2004, in press).

DISCUSSION

The present experiments show that GABA acts differently on various parts of the isolated guinea-pig ileum. On the terminal part of the ileum it regularly produces contraction, wheres on the preterminal ileum it produces an initial shortlasting contraction, followed by a prolonged relaxation. The increasing range of concentrations of GABA produced a concentration-dependent decrease in contractions and an increase in relaxation of the preterminal ileum. These findings indicate a difference in distribution of receptors implicated in GABA effects on various parts of the ileum.

Both types of responses, contraction and relaxation, can be completely blocked by atropine, indicating the cholinergic nature of the responses. Therefore, it is possible that the contractile effects of GABA on the ileum are due to

liberation of acetylcholine from its depot, as already described by Kleinrok and Kilbinger (1983). The mechanism of this liberation is described in details by Cherubini (1984). These effects are realized through the action on $GABA_A$ receptors, located on cholinergic motoneurones which inervate the longitudinal muscle of the ileum (Tanaka, 1985).

GABA was found to produce both potentiation and depression of the contractile effects of acetylcholine on the isolated ileum, depending on the duration of its action. If GABA was kept in the bath only 3 sec. before addition of increasing concentrations of acetylcholine, then a clear potentiation of the effects of acetylcholine was observed. If GABA was kept in the bath for 60 sec and more, then depression of responses to acetylcholine was found. The different actions of GABA on the responses to acetylcholine in the ileum can be explained by its double action on the gut. If incubation in the organ bath is too short (3 sec), then it produces only the release of acetylcholine from the depot. After longer incubation (60 sec) GABA probably activates $GABA_A$ receptors, the consequences of which is a depression of responses to acetylcholine. The possible mechanism of this action has already been discussed by Tanaka (1985) and also by Kleinrok *et al*. (1983).

Malathion significantly depressed the contractile effects of the electrical field stimulation of the ileum. Begg *et al*. (2002) found that electrically evoked contractions of the myenteric plexus-longitudinal muscle were inhibited by exogenous GABA or by the addition of the GABA releasing agent ethylenediamine. Marcoli *et al*. (2000) also found that GABA inhibited the cholinergic twitch responses in the guinea-pig ileum. This effect takes place through the $GABA_B$ receptors.

Lindan by itself produced relaxation of both preterminal and terminal ileum. This effect probably depends on the antagonism of the locally liberated acetylcholine in the tissue.

All three neurotoxic insecticides (lindan, permethrine, malathion) affect the contractile effects of acetylcholine on the ileum. Both lindan and permethrine antagonized the contractile effects of acetylcholine, whereas malathion as an anticholinesterase produced a potentiation. This effect probably does not depend of added GABA into the bath, permethrine still produced an inhibition of the response to acetylcholine.

Barbiturates (and many other drugs) bind to molecular components of the $GABA_A$ receptor present in neuronal membranes in the central nervous system. This receptor, which functions as a chloride ion channel, is activated by inhibitory transmitter GABA (Trevor and Way, 2004). This makes possible to take the barbiturate sleeping time as a standard indicator of the central GABA-ergic activity. In this series of experiments both lindan and permethrine shortened the duration of the barbiturate sleeping time, whereas malathion significantly prolonged it. The hypnotic activity of pentobarbiton, possibly of thiopenton as well, involves $GABA_A$ receptor function (Chweh *et al*. , 1987). This receptor can be blocked by convulsive substances like picrotoxin and bicucculine (Nicoll, 2004). Lindan and permethrine significantly shorten the barbiturate sleeping time, presumably by blocking or interfering with the function of $GABA_A$ receptors, as

shown in our experiments. On the other hand, malathion, being an anticholinesterase, accumulates acetylcholine at the critical sites, thus producing prolongation of the barbiturate sleeping time.

In conclusion, neurotoxic insecticides (lindan, malathion, permethrine) affect both central and peripheral GABA-ergic systems. They can produce either depression or stimulation of these systems. They also highly significantly modulate the activity of the cholinergic system in the isolated ileum.

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MODULATORNI UTICAJI NEUROTOKSIČNIH INSEKTICIDA NA PERIFERNA I CENTRALNA GABA-ERGIČKA DEJSTVA

STANKOVIĆ JASMINA, VARAGIĆ V i MILOVANOVIĆ S

SADRŽAJ

Na izolovanom terminalnom ileumu zamorca, GABA prouzrokuje kontrakciju, dok na preterminalnom ileumu prouzrokuje inicijalnu kontrakciju posle koje nastaje relaksacija. Izlaganjem preterminalnog ileuma zamorca rastućim koncentracijama GABA dobija se dvofazna reakcija. Povećanjem koncentracije GABA efekat kontrakcije se dozno-zavisno smanjivao, dok se efekat relaksacije doznozavisno povećavao. Efekat GABA i na terminalnom i na preterminalnom ileumu se može blokirati atropinom što ukazuje na moguću vezu GABA sa holinergičkim sistemom.

U zavisnosti od vremena inkubacije (3 i 60 sec) GABA prouzrokuje potenciranje ili smanjenje kontrakcija ileuma izazvanih acetilholinom (ACh).

Sva tri neurotoksična insekticida (lindan, permetrin, malation) utiču na kontrakcije ileuma prouzrokovane acetilholinom. Lindan i permetrin antagonizuju kontrakcije izazvane ACh, dok ih malation potencira. Malation značajno smanjuje kontrakcije ileuma prouzrokovane "električno-poljnom" stimulacijom.

Lindan i permetrin skraćuju barbituratno vreme spavanja, dok malation signifikantno produžava vreme spavanja pacova. Lindan i permetrin verovatno deluju blokirajući $GABA_A$ receptore. Malation kao antiholinesterazna supstanca prouzrokuje nagomilavanje acetilholina i produžavanje barbituratnog vremena spavanja.

Neurotoksični insekticidi (lindan, permetrin i malation) deluju na centralni i periferni GABA-ergički sistem. Oni mogu prouzrokovati bilo depresiju ili stimulaciju GABA-ergičkog sistema. Takođe, ovi neurotoksični insekticidi mogu značajno modulirati aktivnost holinergičkog sistema u izolovanom ileumu zamorca.