0022-3565/03/3063-914–924$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 306:914–924, 2003

Vol. 306, No. 3
51839/1083637
Printed in U.S.A.

Differential Actions of Fipronil and Dieldrin Insecticides on GABA-Gated Chloride Channels in Cockroach Neurons

XILONG ZHAO, VINCENT L. SALGADO, JAY Z. YEH, and TOSHIO NARAHASHI

Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois (X.Z., J.Z.Y., T.N.); and Bayer AG, Bayer CropScience, Global Biology Insecticides, Monheim, Germany (V.L.S.)

Received March 19, 2003; accepted May 15, 2003

ABSTRACT

Fipronil and dieldrin are known to inhibit GABA receptors in both mammals and insects. However, the mechanism of selective toxicity of these insecticides between mammals and insects remains to be seen. One possible mechanism is that insect GABA receptors are more sensitive than mammalian $GABA_A$ receptors to fipronil and dieldrin. We examined differential actions of fipronil and dieldrin on GABA-gated chloride channels in insects and compared them with the data on mammalian $GABA_A$ receptors. Neurons were acutely dissociated from the American cockroach thoracic ganglia, and currents evoked by GABA were recorded by the whole-cell patch-clamp technique. GABA-evoked currents were carried by chloride ions, blocked by picrotoxinin, but not by bicuculline. Fipronil inhibited GABA currents with an IC_{50} value of 28 nM, whereas dieldrin exhibited a dual action potentiation with an EC_{50} value of 4 nM followed by inhibition with an IC_{50} value of 16 nM. Fipronil and dieldrin acted on the resting receptor at comparable rates, whereas fipronil blocked the activated receptor 10 times faster than dieldrin. Fipronil inhibition was partially reversible, whereas dieldrin inhibition was irreversible. Fipronil was 59 times more potent on cockroach GABA receptors than on rat $GABA_A$ receptors. However, the potentiating and inhibitory potencies of dieldrin in cockroach GABA receptors were comparable with those in rat $GABA_A$ receptors. It was concluded that the higher toxicity of fipronil in insects than in mammals is due partially to the higher sensitivity of GABA receptors. The mechanism of dieldrin's selective toxicity must lie in factors other than the sensitivity of GABA receptors.

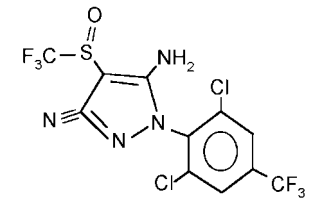
The selective toxicity between mammals and insects is one of the most important factors for the development of new insecticides. In fact, most insecticides on the market are more toxic to insects than to mammals. However, the mechanisms underlying high insecticidal activity and low mammalian toxicity of insecticides are not fully understood. Although the selective toxicity of certain insecticides (e.g., organophosphates) is due primarily to the differences in metabolism (O'Brien, 1967; Matsumura, 1985; Brooks, 1986; Mahajna et al., 1997; Hainzl et al., 1998), in many other cases it is related to higher insecticide sensitivities of target neuroreceptors and ion channels of insects than their mammalian counterparts as exemplified by pyrethroids (Song and Narahashi, 1996; Warmke et al., 1997).

GABA is a major inhibitory neurotransmitter in the nervous system of vertebrates as well as invertebrates (Osborne, 1996), and the GABA receptor is an important target of cyclodiene and hexachlorocyclohexane insecticides (Ghiasuddin and Matsumura, 1982; Narahashi, 2001). Dieldrin (Fig. 1), a cyclodiene, has been shown to exert a potent blocking action on both insect GABA receptors (Bermudez et al., 1991) and vertebrate $GABA_A$ receptors (Abalis et al., 1986; Nagata and Narahashi, 1994; Pomés et al., 1994). Fipronil (Fig. 1), a phenylpyrazole compound, was developed in the mid-1990s and became an excellent insecticide, mainly due to its high effectiveness against some of the dieldrin-resistant strains of insects and its low toxicity to mammals (Tingle et al., 2003). Although both fipronil and dieldrin are known to block GABA receptors (Millar et al., 1994; Hosie et al., 1995; Ikeda et al., 2001), the mechanisms of their high and selective toxicity against insects are not fully understood. Our hypothesis is that the differential sensitivities of the GABA receptors in insect and mammalian neurons are the basis of the selective toxicity of fipronil and dieldrin. Insect GABA receptors are distinctly different from mammalian $GABA_A$ receptors (Lees et al., 1987; Sattelle et al., 1991). Although both GABA receptors are blocked by picrotoxinin, bicuculline blocks the mammalian $GABA_A$ receptor but not the insect GABA receptor (Buckingham et al., 1994).

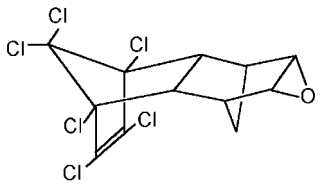
To explore the differential sensitivities of the GABA receptors between insects and mammals to insecticides, the actions of fipronil and dieldrin on GABA-induced currents were examined in cockroach thoracic ganglion neurons using the

This work was supported by a grant from the National Institutes of Health (NS 14143).
Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.103.051839.

ABBREVIATIONS: DRG, dorsal root ganglion; I-V, current-voltage.



Fipronil



Dieldrin

Fig. 1. Chemical structures of fipronil and dieldrin.

whole-cell patch-clamp technique. Both dieldrin and fipronil potently blocked the insect GABA-gated chloride channels at nanomolar concentrations. Compared with our previous studies with rat dorsal root ganglion (DRG) neurons (Nagata and Narahashi, 1994; Ikeda et al., 2001), the blocking action of fipronil on cockroach GABA receptors was much more potent than its action on mammalian $GABA_A$ receptors. In contrast, the action of dieldrin on insect GABA receptors was similar to its actions on mammalian $GABA_A$ receptors, exhibiting a dual action on GABA-induced chloride currents that manifested as an initial enhancement followed by a dramatic inhibition. It was concluded that the high sensitivity of insect GABA receptors to fipronil is one of the crucial mechanisms that underlie the selective toxicity between mammals and insects.

Materials and Methods

Cockroach Thoracic Ganglion Neuron Preparations. Adult American cockroaches (*Periplaneta americana*) were purchased from Carolina Biological Supply Company (Burlington, NC) and were maintained at 29°C under a 12-h light/dark cycle with free access to water and food (also supplied from Carolina Biological Supply Company). Isolation of neurons from cockroaches was performed at room temperature using enzymatic digestion and mechanical dissociation as described previously (Alix et al., 2002). Because the synaptic transmission across the thoracic ganglion of the cockroach was more susceptible to the action of dieldrin than that in the last abdominal ganglion (Wang et al., 1971), the thoracic ganglia were selected in the present study. Briefly, cockroaches were immobilized with pins dorsal side up on a dissection dish. The dorsal cuticle, gut, and some muscles were removed to allow access to the ventral nerve cord. Three thoracic ganglia were carefully dissected and placed in normal cockroach saline solution containing 200 mM NaCl, 3.1 mM KCl, 4 mM $MgCl_2$, 20 mM D-glucose, and 10 mM HEPES-acid, with pH adjusted to 7.4 with 1 mM NaOH. The ganglia were incubated for 30 min at room temperature (22–24°C) in the cockroach saline solution containing collagenase (type IA, 0.5 mg/ml; Sigma-Aldrich, St. Louis, MO) and hyaluronidase (type I-S, 1 mg/ml; Sigma-Aldrich). The ganglia were then rinsed twice in normal saline solution supplemented with 5 mM $CaCl_2$, fetal calf serum (5% by volume), and mechanically dissociated by gentle repeated trituration through a fire-polished Pasteur pipette. The dissociated neurons, suspended in the supplemented normal saline solution, were allowed to settle on coverslips coated with poly-L-lysine hydrobromide (mol. wt. >30,000; Sigma-Aldrich) in 55-mm tissue culture petri dishes. The neurons were incubated at 24°C for 12 to 24 h before recording GABA-induced currents.

Whole-Cell Current Recordings. Neurons were continuously perfused with the cockroach external solution containing 167 mM NaCl, 3.1 mM KCl, 33 mM D-gluconic acid, 5 mM $CaCl_2$, 4 mM $MgCl_2$, and 10 mM HEPES-acid (Alix et al., 2002). The pH was adjusted to 7.4 with 1 mM NaOH, and the osmolarity was 420 mOsM. Ionic currents were recorded using the whole-cell patch-clamp technique at room temperature (23°C). Pipette electrodes were made from 1.5-mm (o.d.) borosilicate glass capillary tubes and had a resistance of 2 to 3 MΩ when filled with the standard internal solution containing: 15 mM NaCl, 170 mM KCl, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM phosphocreatine diTris, 20 mM HEPES-acid, and 3 mM ATP-Mg^{2+} (Alix et al., 2002). The pH was adjusted to 7.4 with KOH, and the osmolarity was 420 mOsM. The membrane potential was clamped at −60 mV unless otherwise stated. The recording of whole-cell currents began 10 min after membrane rupture so that the cell interior milieu was adequately equilibrated with the pipette solution. Currents through the electrode were recorded with an Axopatch 200A amplifier (Axon Instruments, Inc., Union City, CA), filtered at 2 kHz, and stored by a PC-based data acquisition system that also provided preliminary data analysis. Data, when quantified, were expressed as the mean ± S.E.M.

Drug Application. Two methods of drug application were used. The fast application of test solution to the cell chamber through a U-tube was controlled by the computer-operated magnetic valve, which, when opened, allowed the test solution to bypass the chamber. When it was closed, the test solution was ejected through the hole of the U-tube that was located close to the cell. At the same time, another valve controlling the suction tube was opened, allowing the test solution to be sucked away quickly. The external solution surrounding the cell could be completely changed with a test solution within 30 ms. Test compound was coapplied with GABA. Alternatively, a test drug was added to the external solution that was continuously perfused through the recording chamber.

Chemicals. GABA (Sigma-Aldrich) was first dissolved in deionized water as the stock solution and then diluted with the cockroach external solution immediately before use. Dieldrin and fipronil (provided by Rhone-Poulenc Yuka Agro K.K., Akeno, Japan) were first dissolved in dimethyl sulfoxide to make stock solutions and then diluted with the external solution shortly before experiments. The final concentrations of dimethyl sulfoxide in test solutions were 0.1% (v/v) or less, which had no effect on the GABA-induced currents.

Analysis. Whole-cell currents were initially analyzed with the pClamp version 6.0.4 software to measure the current amplitudes and decay kinetics. The statistical analysis and the nonlinear regression analysis were carried out using the Sigmaplot 2001 software (SPSS Science, Chicago, IL). The dose-response relationship for GABA to activate the GABA receptor was evaluated by fitting the data to Hill equation: $I = I_{max}C_{50}{}^n/(C^n + C_{50}{}^n)$, where I is the current amplitude relative to the control maximum current, I_{max}; C is the chemical concentration; and n is the Hill coefficient. The dose-response relationship for insecticides to modulate the GABA response was evaluated with a similar Hill equation to estimate EC_{50} or IC_{50} values.

Results

Characteristics of GABA-Induced Chloride Currents. Pyriform cockroach neurons (20–80 μm in diameter) were selected to record the GABA-induced currents through-

out the experiment. However, there were 2 to 5% of the pyriform neurons tested that did not respond to GABA. With symmetric chloride concentrations between internal and external solutions, inward currents were induced by GABA at a holding potential of −60 mV.

The concentration-response relationship for GABA to activate GABA receptors was determined by applying various concentrations of GABA (1–3,000 μM) via a U-tube at an interval of 60 s (Fig. 2A). Little or no currents were discernible at GABA concentrations equal to or less than 3 μM. The current amplitude increased steeply as the GABA concentration was increased from 30 to 100 μM, reaching a maximum at 1000 μM. At low GABA concentrations, the current rose slowly and was maintained during application of GABA. For instance, the time constant of the rising phase of the current activated by 30 μM GABA was 265.8 ± 15.8 ms (n = 64). As the GABA concentration was increased, the initial rising phase of the GABA-induced current was accelerated, and the current amplitude reached a peak and was followed by a prominent decay phase.

The concentration-response relationship was constructed by plotting the peak amplitudes, normalized to the one induced by 1000 μM GABA, as a function of the GABA concentrations as shown in Fig. 2B. The concentration-response relationship was fitted to a sigmoid curve with an EC_{50} value of 52.9 ± 5.6 μM (n = 4) and a Hill coefficient of 2.11 ± 0.1 (n = 4), indicating that the activation of GABA receptor in cockroach neurons requires binding of two agonist molecules.

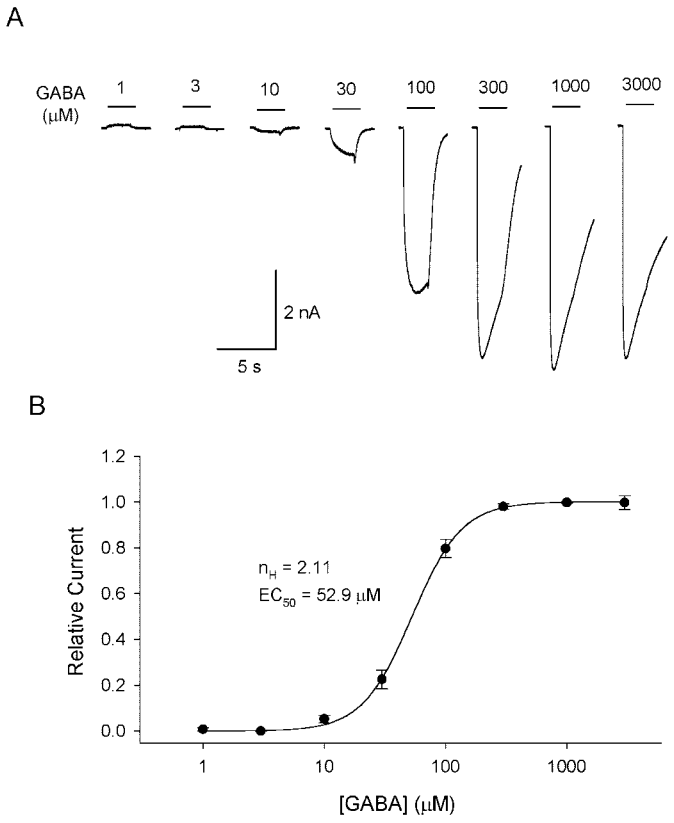


Fig. 2. GABA-induced currents in cockroach thoracic ganglion neurons and the concentration-response relationship. A, typical examples of current traces in response to various GABA concentrations at a holding potential of −60 mV. GABA was applied via the U-tube drug application system for 2 s. B, concentration-response relationship for GABA-induced currents. The peak amplitudes of currents were normalized to the current induced by 1000 μM GABA. The concentration-response curve was fitted to the sigmoid Hill equation with an EC_{50} value of 52.9 ± 5.6 μM and the Hill coefficient of 2.11 ± 0.1 (n = 4, mean ± S.E.M.).

To test whether GABA-induced currents are carried by chloride ions, currents were evoked by GABA at a holding potential ranging from −60 mV to +40 mV using internal and external solutions with symmetrical chloride concentrations. As shown in Fig. 3A, a large GABA-induced inward current was generated at −40 mV, the current decreased to near zero at 0 mV, and it became outward in direction at +40 mV. The current-voltage (I-V) relationship is shown in Fig. 3B. The inward currents were larger than the outward currents. The current reversed in polarity at a membrane potential of −2.8 mV (n = 10), which was very close to the calculated chloride equilibrium potential of −0.1 mV after correction of 3.1-mV junction potential. This result indicated that the GABA-induced current was carried by chloride ions as seen with mammalian $GABA_A$ receptors.

The GABA-induced chloride current in cockroach neurons, however, was not blocked by 10 μM bicuculline, an antagonist of the mammalian $GABA_A$ receptor (Fig. 4). In contrast, the GABA response was almost completely blocked by 10 μM picrotoxinin, a $GABA_A$ receptor channel blocker (Fig. 4). The GABA current suppressed by picrotoxinin was partially restored after washout with drug-free external solution.

Fipronil Blocks GABA Receptors in Both Resting and Activated States. Fipronil is known to block the $GABA_A$ receptor in rat DRG neurons with an IC_{50} value of 1.6 μM (Ikeda et al., 2001). It blocks both the resting receptors and activated receptors to the same extent. To examine whether fipronil exerted these two types of block in cockroach GABA receptors, protocols similar to those used by Ikeda et al. (2001) were used here. To examine the resting receptor block by fipronil, two protocols were used. In the first protocol, the effect of bath-applied fipronil on the GABA current was monitored during 2-s applications of 30 μM GABA pulses at an interval of 30 s at a holding potential of −60 mV (Fig. 5A). After several stable control current recordings had been established, 1 μM fipronil was continuously perfused into the bath and GABA and fipronil were coapplied every 30 s for 2 s via a U-tube. The current amplitude was gradually decreased during a 5-min treatment of fipronil (Fig. 5A). To examine whether the activation of the receptor by brief GABA test pulses was required for block caused by 1 μM fipronil, a second protocol was used (Fig. 5B). Fipronil was continuously perfused to the bath for 5 min during which time no GABA pulse was given and then GABA and fipronil were coapplied to examine the degree of block. This second protocol revealed that the GABA current was reduced to the same degree as that in the first protocol with repeated GABA stimulations during fipronil bath application. In both cases, no recovery of the GABA current was seen after 20-min washout (n = 3). The lack of use-dependent block indicated that fipronil was capable of blocking the resting GABA receptor without activation and that the first protocol using short GABA test pulses can be used for monitoring the time course of fipronil block of the resting GABA receptor.

To observe the rate of fipronil block of the activated GABA receptor, fipronil was coapplied with 100 μM GABA for 30 s at a holding potential of −60 mV via the U-tube perfusion system. As shown in Fig. 5C, in the absence of insecticide, the current evoked by 30-s application of 100 μM GABA decayed

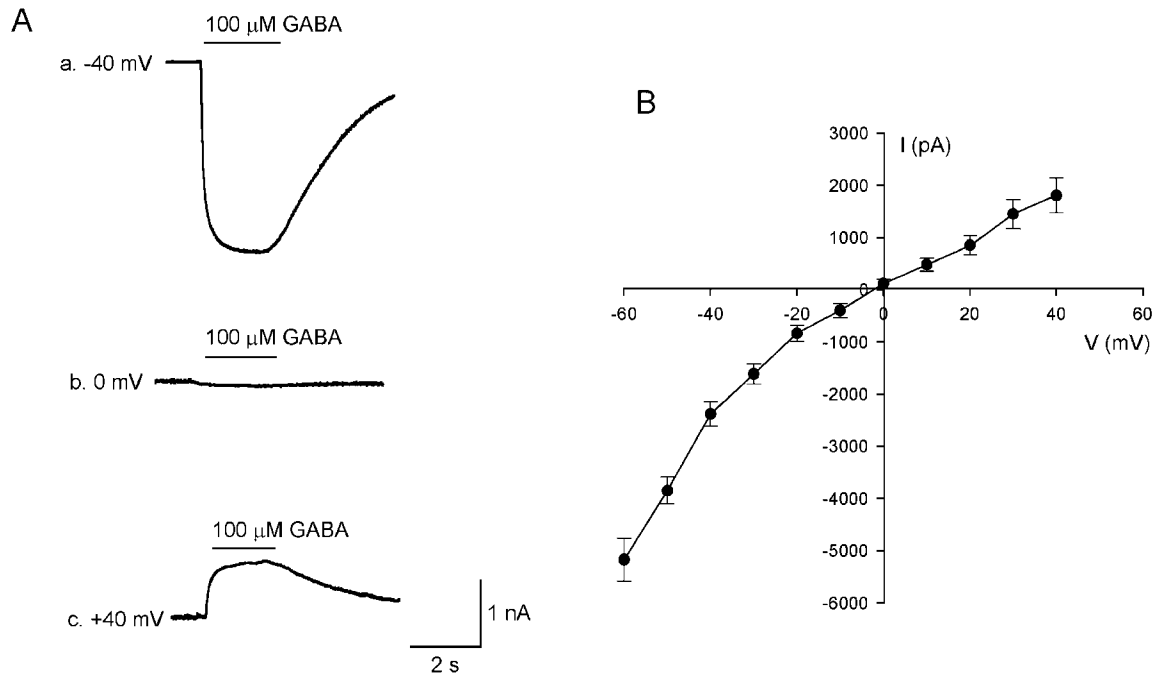


Fig. 3. I-V relationship for GABA-induced currents in cockroach thoracic ganglion neurons. A, currents were evoked by 2-s applications of 100 μM GABA via a U-tube using symmetrical chloride concentrations in internal and external solutions at membrane potentials of −40 mV (a), 0 mV (b), and +40 mV (c). B, I-V relationship of GABA-evoked currents. The reversal potential was estimated to be −2.8 mV (mean of 10 different cells), which was very close to the chloride equilibrium potential (−0.1 mV) after subtraction of 3.1-mV junction potential.

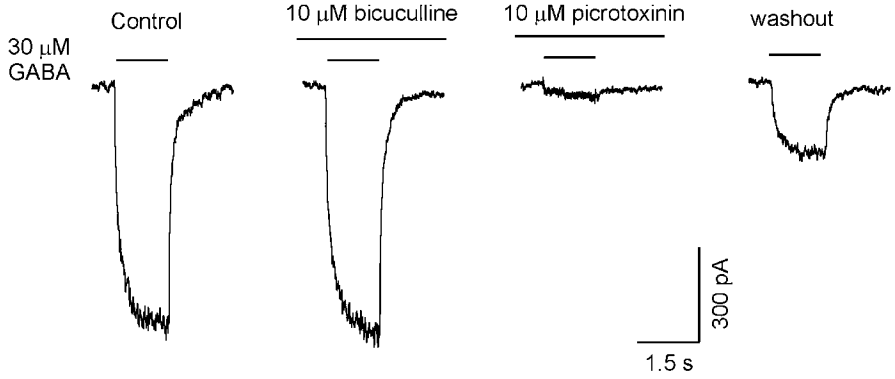


Fig. 4. Pharmacological properties of GABA receptor channels in cockroach ganglion neurons. Currents were induced by 1.5-s applications of 30 μM GABA via a U-tube at a holding potential of −60 mV. Bicuculline, a potent antagonist of mammalian $GABA_A$ receptor channels, was perfused through the bath starting 5 to 7 min before coapplication of GABA and bicuculline, and failed to block the GABA response. In contrast, picrotoxinin, a mammalian $GABA_A$ receptor channel blocker, inhibited the GABA response at 10 μM after 2- to 5-min bath perfusion. The block caused by picrotoxinin partially reversed after washout with drug-free external solution.

slowly, which is mostly likely due to desensitization of the receptors. After coapplication of 100 nM fipronil and 100 μM GABA, the GABA current showed the same rising phase and reached nearly the same peak as the control, but decayed with a faster time course to a very small steady-state current. These observations indicated that fipronil blocked the GABA receptor because it was activated by GABA. Receptor desensitization seems to play a small role in the acceleration of current decay in the presence of fipronil because the peak GABA current remained small during the second and third applications at a 2-min interval (Fig. 5C) and because desensitization in the absence of fipronil recovered within 2 min. The peak current during the second coapplication of fipronil and GABA was greater than the steady–state level of the first coapplication. This suggested that the receptor desensitization prevented fipronil block of the activated receptor. The use-dependent inhibitory action of fipronil on the peak current also indicated that fipronil molecules could not dissociate from the receptor with 2-min interpulse intervals. This was consistent with the result of washout experiment, in which recovery was very small even after a 10-min washout with fipronil-free solution. Coapplications of fipronil at a higher concentration of 1 μM induced a similar but more rapid decay and more extensive inhibitory action.

Comparison of the Kinetics of Fipronil Block of Resting and Activated GABA Receptors. The time course of the inhibitory actions of fipronil on the resting GABA receptors is illustrated in Fig. 6A. Inward chloride currents were induced by a 2-s application of 30 μM GABA at an interval of 30 s when the membrane potential was hold at −60 mV. After bath and U-tube application of fipronil, the time course of fipronil block was accelerated as the fipronil concentration

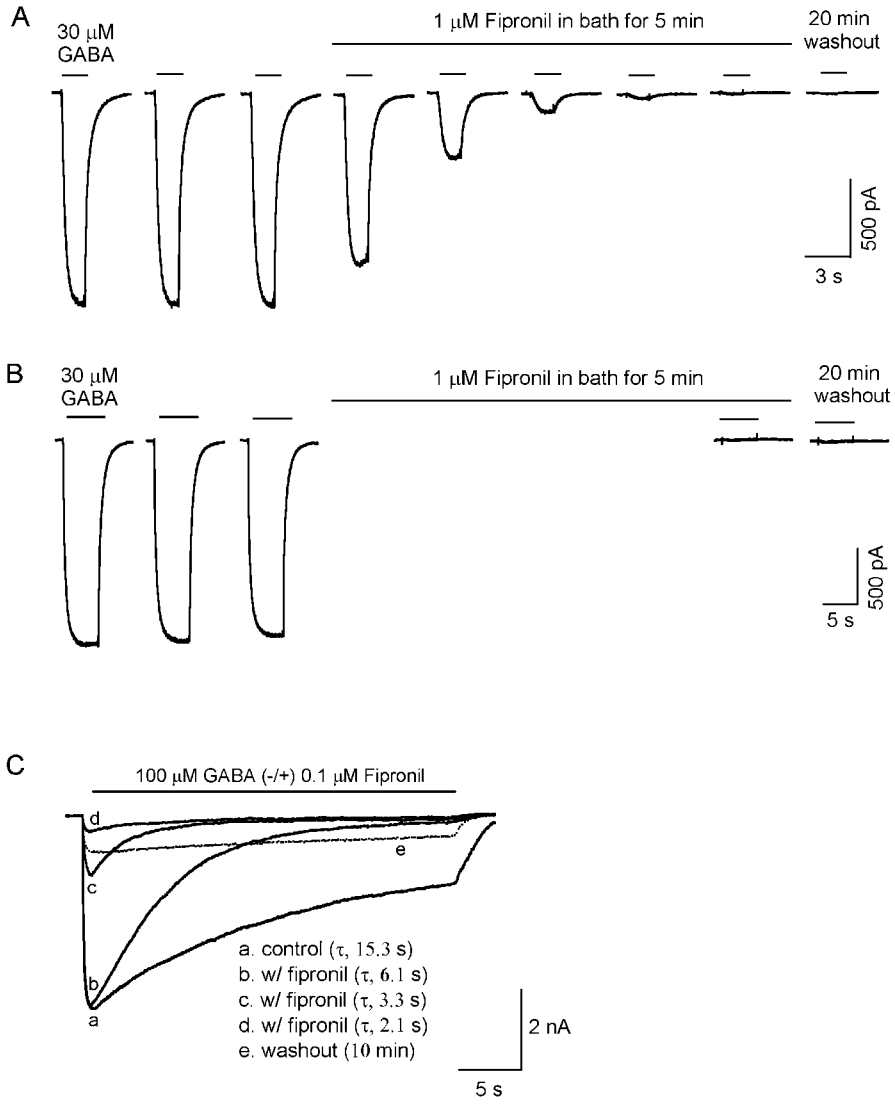


Fig. 5. Fipronil blocks cockroach GABA receptors in both resting and activated states. A, GABA (30 μM) was applied to a cockroach neuron for 2 s at a 30-s interval at a holding potential of −60 mV. Fipronil (1 μM) was perfused to the bath after several stable control recordings. The peak current amplitude was gradually decreased during 5-min treatment of fipronil. This time-dependent block was irreversible after 20-min washout with insecticide-free external solutions. B, a similar protocol to that of A except that, no GABA pulse was given during 5-min bath application of insecticide. GABA application after 5 min of fipronil perfusion revealed the same result as the above one with repeated GABA stimulations. Thus, no activation by GABA is required for fipronil block. C, currents induced by 30-s coapplication of 0.1 μM fipronil and 100 μM GABA, showing fipronil block of the activated receptor. a, control. b, c, and d, first, second, and third coapplications, respectively, at a 2-min interval. The peak amplitude of current was suppressed and the current decay was accelerated during these coapplications. e, partial recovery after a 10-min washout with fipronil-free solution.

was increased from 10 to 1000 nM. The time constants for the block were 249 ± 18 s (n = 5) in the presence of 30 nM fipronil, 267 ± 47 s at 100 nM (n = 3), 172 ± 13 s at 300 nM (n = 4), and 63 ± 7 s at 1,000 nM (n = 4).

To examine the blocking kinetics of fipronil on the activated GABA receptors, the GABA current induced by the first coapplication of fipronil with 100 μM GABA was normalized to the control current because the control current exhibited some decay (Fig. 5Ca). Figure 6B illustrates the time course of normalized currents evoked by 30-s 100 μM GABA pulses. The time constants estimated from the fit were 17.5 ± 2.9 s in 100 nM fipronil (n = 4) and 3.7 ± 0.8 s in 1000 nM fipronil (n = 4).

To compare the fipronil blocking kinetics of the resting receptor with those of the activated receptors, the reciprocals of their time constants of block are plotted as a function of fipronil concentrations as shown in Fig. 6C. Binding (k_{+1}) and unbinding (k_{-1}) rate constants were calculated from the relationship $1/\tau = [F]k_{+1} + k_{-1}$, where τ is the time constant of current decay and [F] is the fipronil concentration. The linear fit to the data of the resting receptor block gave a binding rate constant of 1.3×10^4 M^{-1} s^{-1} and an intercept gave an unbinding rate constant of 2.3×10^{-3} s^{-1}. This resulted in a K_d value of 179 nM. The linear fit to the data of the activated receptor gave a slope of 5.4×10^5 M^{-1} s^{-1} and an intercept of 5.2×10^{-2} s^{-1}. Assuming this additional decay in current caused by fipronil is due to an open channel blocking action, the value of 5.4×10^5 M^{-1} s^{-1} gives the binding rate constant, the value of 5.2×10^{-2} s^{-1} gives the unbinding rate constant, and the K_d value is 98 nM.

Figure 6D compares the dose-response relationships for block of the resting receptors to that of the activated receptors. For the resting receptor block, the current amplitudes recorded 7 min after fipronil application as shown in Fig. 6A

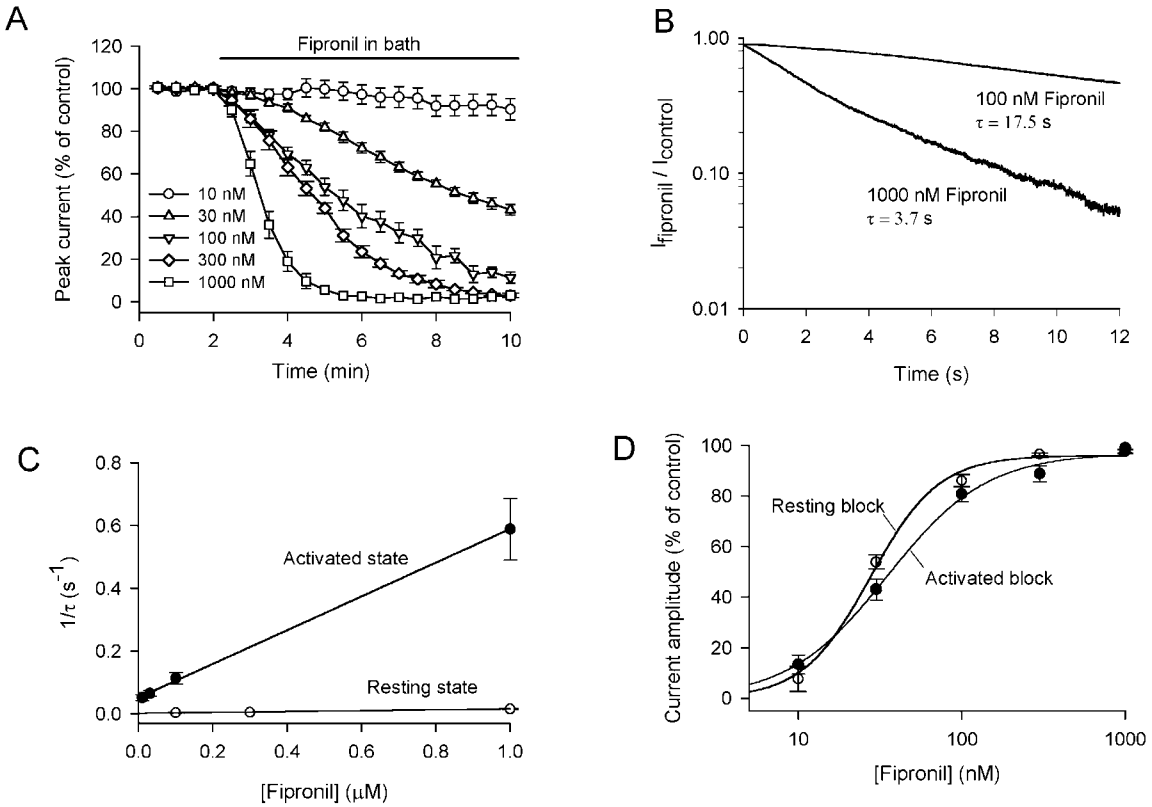


Fig. 6. A, time course of block of the resting GABA receptor caused by various concentrations of fipronil ranging from 10 to 1000 nM in cockroach neurons. Fipronil was applied to the bath and also coapplied with 30 μM GABA for 2 s via a U-tube. Current amplitudes were normalized to the control values prior to fipronil application. Time constants of the inhibition of current were 249 ± 18 s in 30 nM fipronil (n = 5), 267 ± 47 s in 100 nM fipronil (n = 3), 172 ± 13 s in 300 nM fipronil (n = 4), and 63 ± 7 s in 1000 nM fipronil (n = 4). Fipronil at 10 nM caused no measurable inhibition of current. B, time course of block of the activated GABA receptor caused by 100 and 1,000 nM fipronil in cockroach neurons. Fipronil and 100 μM GABA were coapplied via a U-tube, and the current induced by the first coapplication of fipronil and GABA (Fig. 5Cb) was normalized to the control current (Fig. 5Ca). The time constants for current decay were estimated to be 20.7 ± 2.8 s (n = 5), 16.1 ± 2.0 s (n = 5), 9.4 ± 1.4 s (n = 4), 7.8 ± 0.6 s (n = 5)n and 2.9 ± 0.3 s (n = 4) in 10, 30, 100, 300, and 1,000 nM fipronil, respectively. C, analysis of fipronil blocking kinetics of the resting and activated GABA receptors. The reciprocals of the time constants for current decay are plotted as a function of the fipronil concentration, and the binding (k_{+1}) and unbinding (k_{-1}) rate constants were calculated from the relationship $1/\tau = [F]k_{+1} + k_{-1}$, where τ is the time constant of current decay and [F] is the fipronil concentration. For the resting receptor block (○), $k_{+1} = 1.30 \times 10^4$ M^{-1} s^{-1}, $k_{-1} = 2.33 \times 10^{-3}$ s^{-1}, and K_d = 179 nM; for the activated receptor block (●), $k_{+1} = 5.4 \times 10^5$ M^{-1} s^{-1}, $k_{-1} = 5.2 \times 10^{-2}$ s^{-1}, and K_d = 98 nM. D, comparison of the dose-response relationships for fipronil block of the resting and activated GABA receptors. The fit to the dose-response data gave an IC_{50} value of 27.6 nM and a Hill coefficient of 2.09 for the resting receptor block (○), and an IC_{50} value of 35.0 nM and a Hill coefficient of 1.43 (n = 5) for the activated receptor block (●).

were normalized to the control and are plotted against the fipronil concentration. The data were fitted to a sigmoid Hill equation with an IC_{50} value of 28 nM and a Hill coefficient of 2.1 (n = 4). Because the equilibrium was not reached by 7 min at concentrations lower than 100 nM, the true IC_{50} value is somewhat lower than 28 nM.

For the block of the activated receptor, the steady-state block was calculated by comparing the current amplitude at the end of the 30-s pulse of GABA-fipronil coapplication (Fig. 5Cb) to the control current (Fig. 5Ca). The block amounted to 13.5 ± 3.7% (n = 5), 43.1 ± 4.2% (n = 5), 80.8 ± 3.1% (n = 4), 88.8 ± 3.2% (n = 5), and 98.9 ± 1.5% (n = 4), respectively, at 10, 30, 100, 300, and 1000 nM fipronil. When plotted on the same graph as that for the resting receptor block (Fig. 6D), these values are fitted to a sigmoid Hill equation with an IC_{50} value of 35 nM and a Hill coefficient of 1.4. Thus, fipronil has the same affinity for the resting and activated states of the GABA receptor, despite the different rates of blocking action on these two states.

Dual Action of Dieldrin on GABA-Induced Currents. Several protocols used to examine the fipronil action were also used to study the effects of dieldrin on cockroach GABA receptors. Bath and U-tube applications of 1 μM dieldrin exhibited a biphasic effect on the GABA current: the current was first potentiated and then inhibited (Fig. 7A). The current did not recover 20 min after washing with dieldrin-free external solutions. The time course of the dual action of dieldrin on GABA currents is illustrated in Fig. 7B. The potentiating action and inhibitory action seemed to have different dose dependencies as shown in Fig. 7C, in which the increase and the subsequent decrease of the peak current are plotted as a function of dieldrin concentration. The fit to the dose-potentiation data gave an EC_{50} value of 4.4 nM and a maximum potentiation to 40% of the control, whereas the fit to the dose-inhibition relationship gave an IC_{50} value of 15.5 nM and almost 100% maximum inhibition. Thus, it seems that dieldrin exerts the transient potentiation more potently than the delayed inhibition.

Experiments were conducted to test the use dependence of the blocking action of dieldrin using the same protocol as that for fipronil. GABA at 30 μM was applied for 2 s at an interval of 30 s at a holding potential of −60 mV. The current amplitude initially increased and was followed by a gradual decrease during 5-min treatment of dieldrin applied in U-tube

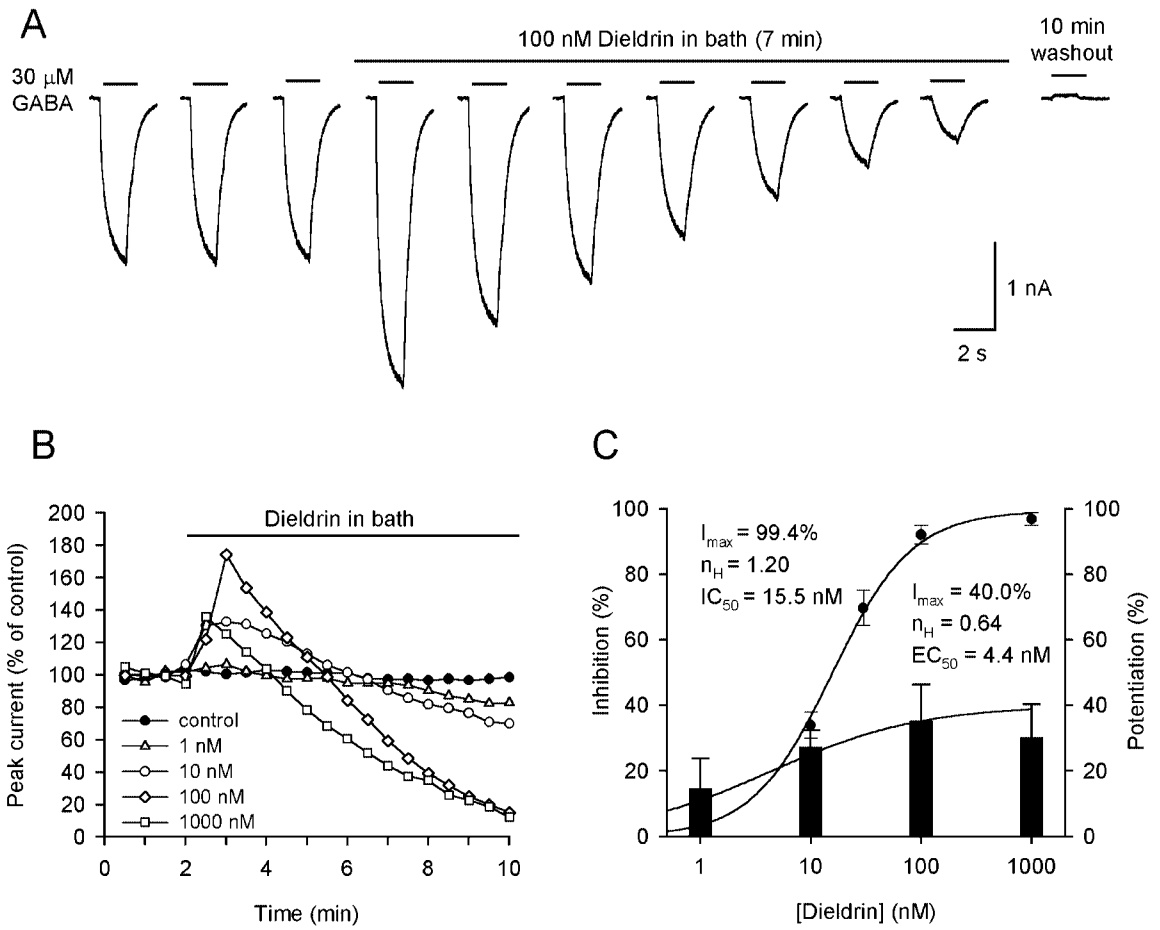


Fig. 7. Dual actions of dieldrin on cockroach GABA receptors. With the same recording protocol as that for fipronil, currents were induced by 1.5-s 30 μM GABA pulses at an interval of 30 s and at a holding potential of -60 mV. A, GABA-induced currents before, during and after bath and U-tube applications of 100 nM dieldrin. The currents were first potentiated and then inhibited during bath perfusion of dieldrin. The currents did not recover 10 min after washing with dieldrin-free solutions. B, time course of a dual action of dieldrin at various concentrations (1–1000 nM) on GABA-induced currents. The peak current amplitudes in dieldrin were normalized to the average peak current before dieldrin application. C, dose-response relationships for the enhancement and inhibition caused by dieldrin. The increases in peak current amplitude (black columns) caused by dieldrin at 1, 10, 100, and 1000 nM were 16.4 ± 6.9% (n = 4), 40.7 ± 14.4% (n = 6), 34.9 ± 11.4% (n = 5), and 30.0 ± 10.4% (n = 3), respectively. The potentiation curve was fitted to a sigmoid Hill equation with an EC_{50} value of 4.4 nM and a Hill coefficient of 0.64. The amplitudes of currents recorded 7 min after dieldrin treatment were normalized to the control and are plotted against the dieldrin concentration (●). The inhibitory dose-response curve was fitted to a sigmoid Hill equation with an IC_{50} value of 15.5 nM, and a Hill coefficient of 1.20.

and bath solutions (Fig. 8A). In another neuron, no GABA pulse via a U-tube was given during the 5-min bath application of dieldrin, and GABA-dieldrin coapplication via a U-tube was resumed after 5 min of dieldrin perfusion (Fig. 8B). The GABA current was reduced to the same degree in both protocols (n = 3). The lack of use-dependent block indicated that dieldrin blocked the GABA receptor in the resting state.

The kinetics of dieldrin block of the resting GABA receptor was estimated from the time course of the inhibitory action on the GABA-induced current. The reciprocals of the time constants are plotted as a function of dieldrin concentration in Fig. 9. The linear fit to the data of the resting receptor block gave a binding rate constant of 5.0×10^4 M^{-1} s^{-1} and an intercept gave an unbinding rate constant of 2.0×10^{-3} s^{-1}. The block of the activated GABA receptors by dieldrin could not be estimated from the current decay during coapplication of dieldrin with GABA, because the blocking action was complicated by the potentiating action. In a few experiments in which the potentiating action was not seen, the rate constants for blocking the activated receptor were estimated to be 187.9 ± 17.2 s (n = 10) and 161.3 ± 25.3 s (n = 3) in the presence of 100 and 1000 nM dieldrin, respectively. The reciprocals of these rate constants are plotted in Fig. 9 for comparison with those for the resting receptor block. The fit to the data of the activated receptor block gave a slope of 5.4×10^4 M^{-1} s^{-1} which represented the binding rate constant, and an intercept of 3.5×10^{-2} s^{-1} which represented the unbinding rate constant.

The potentiating action of dieldrin was dependent on GABA concentrations whereas the inhibitory action was not (Fig. 10A). With a near maximum inward chloride currents induced by high concentration of GABA (300 μM), no initial potentiating phase was observed after application of 100 nM dieldrin. The time course of the dieldrin inhibition of the current induced by 300 μM GABA was nearly identical to that of the inhibitory phase of the current induced by 30 μM GABA. Dieldrin at 100 nM inhibited the current induced by 300 μM GABA by 95.2 ± 1.5% (n = 5), which is not significantly different from its inhibition on the current induced by 30 μM GABA.

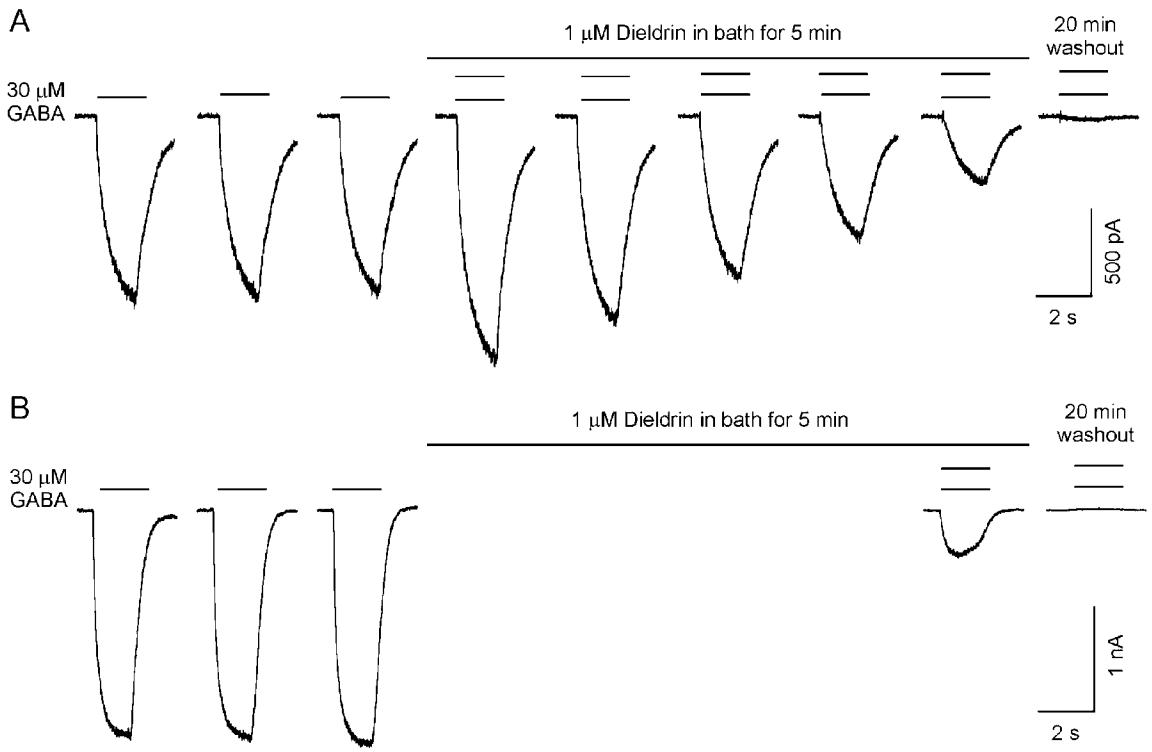


Fig. 8. Use-independent dieldrin inhibition of GABA-induced currents. A, GABA 30 μM was applied for 2 s at a 30-s interval at a holding potential of -60 mV. Dieldrin (1 μM) was perfused into the bath and via U-tube after several stable control recordings. The peak current amplitude was initially augmented and then gradually decreased to 33% of the control 5 min after treatment with dieldrin. This time-dependent inhibition was irreversible after 20-min washout with insecticide-free solution. B, to examine the possibility of the use dependence of dieldrin inhibition, no GABA pulse was given during 5-min bath application of dieldrin. GABA application after 5 min of dieldrin perfusion showed a similar inhibitory response (23% of control) to that caused by repeated GABA-dieldrin coapplication. Thus, no activation by GABA is required for dieldrin block.

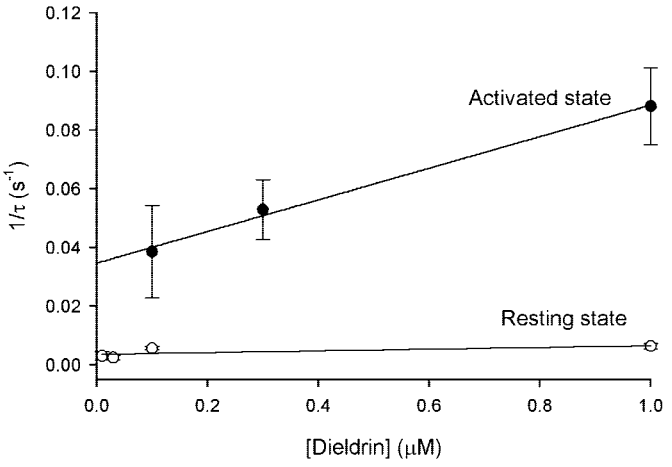


Fig. 9. Analysis of dieldrin blocking kinetics of the resting and activated GABA receptors. The experimental protocols and the method of analysis are similar to those for fipronil (Fig. 6). The binding and unbinding rate constants for the resting receptor block (○) were estimated to be 5.0×10^4 M^{-1} s^{-1} and 2.0×10^{-3} s^{-1}, respectively, with a resultant K_d of 40 nM. The rate constants for the activated receptor block (●) were estimated form experiments in which no potentiating effect was seen: the binding rate constant, 5.39×10^4 M^{-1} s^{-1}; the unbinding rate constant, 3.49×10^{-2} s^{-1}; and K_d, 647 nM.

In contrast to the inhibitory action, the potentiating action of dieldrin varied among the neurons tested. The potentiating action was observed in 64% of the neurons tested, whereas the inhibitory action was observed in all neurons tested. However, when the time course of current changes obtained from the cells exhibiting both enhancing and inhibitory responses was compared to that from the cells exhibiting inhibition alone, the two inhibitory curves overlapped with a similar time course (Fig. 10B).

Discussion

Characteristics of Cockroach GABA Receptors. The GABA-gated chloride channels are widely distributed on the soma and neuropile membranes in the cockroach nervous system (Sattelle, 1992). In the present study, the GABA-activated currents were recorded from neurons isolated from the thoracic ganglia of American cockroaches. GABA activated the receptors to generate an inward chloride current at -60 mV with an EC_{50} value of 53 μM and a Hill coefficient of 2.1, which suggested that at least two GABA molecules are required for activation of the receptor. The shape of dose-response relationship resembled that of a previous study (Alix et al., 2002), but our EC_{50} value cannot directly be compared with their value because pressure injection used to apply GABA to activate the receptor in their study makes it difficult to estimate the exact concentration of GABA. Although there are reports describing bicuculline antagonism against the insect GABAergic neuronal activity (Roberts et al., 1981; Waldrop et al., 1987), the present study demonstrated that the GABA receptors of isolated cockroach neurons are insensitive to bicuculline, a potent mammalian $GABA_A$ receptor antagonist, but are sensitive to the blocking action of picrotoxinin, a channel blocker of mammalian $GABA_A$ receptors. The results are in agreement with the reports from other laboratories (Lees et al., 1987; Sattelle et

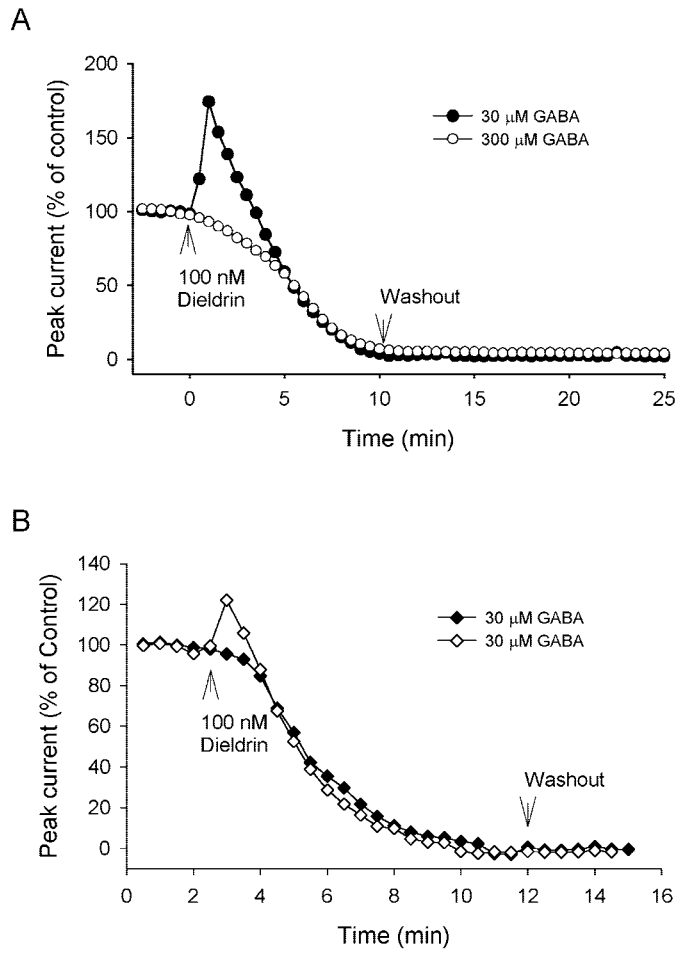


Fig. 10. The potentiating action of dieldrin on cockroach GABA currents depended on the degree of receptor activation and varied among the neurons tested, but inhibition was always observed. A, currents were evoked in a neuron by 30 μM GABA (filled circles) or 300 μM GABA (opened circles) for 2 s at an interval of 30 s at a holding potential of −60 mV. Dieldrin 100 nM was applied through bath and U-tube after several stable control recordings. The currents induced by 30 μM GABA were first enhanced and then inhibited by dieldrin, whereas the currents induced by 300 μM GABA were inhibited without initial enhancement. B, currents from two separate neurons were evoked by 30 μM GABA for 2 s at an interval of 30 s at a holding potential of −60 mV. In one neuron (opened diamonds), dieldrin induced an initial potentiation of currents followed by a slow inhibition. In another neuron (filled diamonds), dieldrin did not induce potentiation but a time-dependent inhibition.

al., 1988). As the critical target of insecticides such as cyclodienes, avermectins, fipronil, and spinosyns (Lummis, 1990; Gant et al., 1998; Watson, 2001), insect GABA receptors with pharmacological properties distinct from mammalian $GABA_A$ receptors (Sattelle et al., 1991) seem to be a basis for the selective toxicity of these insecticides in mammals and insects.

Fipronil Inhibition of GABA-Induced Current. The present study demonstrated that fipronil exerted a potent inhibitory action on GABA-induced chloride channel currents in cockroach thoracic ganglion neurons with an IC_{50} value of 28 nM. The rate at which fipronil interacted with the resting receptor was estimated from the onset of inhibition of the current activated by GABA applications. The binding rate constant of fipronil was estimated to be 1.3×10^4 M^{-1} s^{-1} and its unbinding rate constant 2.33×10^{-3} s^{-1}, both of which differ from the kinetics of interactions of fipronil with $GABA_A$ receptors in rat DRG neurons (Ikeda et al., 2001). The binding constant of fipronil in the cockroach GABA receptor is about 20 times greater than that in rat DRG $GABA_A$ receptors, whereas the unbinding rate constant is about 10 times smaller, resulting in a higher affinity of fipronil for the cockroach GABA receptor. These results corroborate those of the previous study (Gant et al., 1998), showing that fipronil binds more tightly to the insect GABA receptor than to the $GABA_A$ receptor in mammals.

Fipronil inhibits the GABA receptor without requiring channel opening (Fig. 5). The activation of the receptor, however, greatly facilitates fipronil block of the receptor as illustrated in Fig. 5C. For example, in the presence of 1000 nM fipronil, the time constant for the resting receptor block was 73 s, and the time constant for the activated receptor block was 1.7 s. The concentration dependencies illustrated in Fig. 6C indicate that fipronil binds to the activated receptor 41 times faster than to the resting receptor, whereas it unbinds from the activated receptor 23 times faster than from the resting receptor. Thus, the activation of the receptor facilitates fipronil to interact with its binding site. Consistent with its blocking action of the activated receptor is the observation that, at the single-channel level, fipronil reduced the duration of channel opening in S2 cell line expressing the wild-type *Rdl*(ac) *Drosophila melanogaster* homomer-forming ionotropic GABA receptor subunits (Grolleau and Sattelle, 2000).

The studies using recombinant dieldrin-sensitive and dieldrin-resistant *Rdl* receptors of *D. melanogaster* expressed in *Xenopus* oocytes (Buckingham et al., 1994; Hosie et al., 1995) revealed that fipronil at 1 to 100 μM blocked dieldrin-sensitive GABA-gated chloride channels by 30 to 95% and dieldrin-resistant GABA receptor channels by −25%. In these studies, the blocking action of fipronil on both types of GABA receptors is similar to that of picrotoxinin, indicating that fipronil and picrotoxinin may share a common mechanism in blocking GABA responses. In rat DRG neurons, however, the previous study shows that fipronil and picrotoxinin have their own binding sites without sharing a common binding site on $GABA_A$ receptors (Ikeda et al., 2001). They conclude that fipronil and picrotoxinin may act as allosteric modulators at different sites to block the $GABA_A$ receptors. This point remains to be examined in the cockroach GABA receptors.

Dual Actions of Dieldrin on GABA Receptors. The present study showed that dieldrin exerted a dual action on cockroach GABA receptor channels: an initial enhancement of the GABA-induced current was followed by an inhibition. The potentiating action of dieldrin was observed in 64% of the neurons tested, whereas the inhibitory action was seen in all of the neurons tested. In addition, the potentiating action was not observed at high GABA concentrations, whereas the inhibitory action was seen regardless of GABA concentrations. Furthermore, dieldrin exerted these two actions with different potencies and efficacies: it potentiated the current with an EC_{50} value of 4.4 nM and a maximum enhancement of 40% of the control, and it inhibited the current with an IC_{50} value of 15.5 nM, amounting to nearly 100% inhibition. In the other 40% of the neurons tested, dieldrin did not exert its potentiating action on the GABA-induced current, and its inhibitory action was similar to the inhibitory action observed in neurons in which the dual action was seen.

The dual action may be mediated at two different sites on the same GABA receptor or on two subtypes of GABA receptors. To further examine these two hypotheses, dieldrin action was studied in the $GABA_A$ receptors with known subunits expressed in human embryonic kidney cells (Nagata et al., 1994). They found that the dual action of dieldrin was observed in the GABA receptor with $\alpha1\beta2\gamma2$s or $\alpha6\beta2\gamma2$s combinations. However, only suppression was observed in the $\alpha1\beta2$ combination of $GABA_A$ receptors. These results indicate that γ subunit is necessary for the enhancing effect and that there is no subunit selectivity for the suppressive effect (Nagata et al., 1994). It is tempting to speculate that the dual action of dieldrin on cockroach GABA receptors may be related to the differences in subtypes of GABA receptors.

In dissociated cockroach neurons, multiple conductance states of 11, 17, and 19 pS were detected in GABA receptor single-channel currents (Shimahara et al., 1987; Malecot and Sattelle, 1990). The open-time distributions were fitted to two exponential functions and the close-time distributions were fitted to three exponential functions. Noise analysis demonstrated that lindane and dieldrin decreased the frequency of GABA chloride channel openings without changing the mean open time (Bermudez et al., 1991). Therefore, the dual action of dieldrin may also be related to different gating properties of the cockroach GABA-receptor chloride channels. Despite the uncertainty of the toxicological significance of the enhancement of GABA-induced currents by dieldrin, the dual action of dieldrin is worthy of further study to elucidate the mechanism of interactions of dieldrin with the GABA system.

Comparison of Blocking Kinetics between Fipronil and Dieldrin. Fipronil and dieldrin inhibited the resting GABA receptor in cockroach neurons at a comparable rate both in terms of binding and unbinding rate constants (Figs. 6C and 9). The slightly faster onset of fipronil block is due to its faster binding rate constants: 1.4×10^4 M^{-1} s^{-1} for fipronil and 5.0×10^3 M^{-1} s^{-1} for dieldrin. In contrast, fipronil interacted with the activated receptor at a rate at least 10 times faster than that for dieldrin. The binding rate constants were 5.4×10^5 M^{-1} s^{-1} for fipronil and 5.4×10^4 M^{-1} s^{-1} for dieldrin, and the unbinding rate constants were 5.2×10^{-3} s^{-1} for fipronil and 3.5×10^{-2} s^{-1} for dieldrin. It remains to be seen how these differences in blocking kinetics are reflected in their insecticidal activities.

Selective Toxicity of Insecticides. Both dieldrin and fipronil have selective toxicity between insects and mammals. For dieldrin, the oral LD_{50} in mammals was estimated to be 38 to 87 mg/kg b.wt. in rats (Allen et al., 1979). In comparison, the topical LD_{50} in American cockroaches was 1.3 to 1.5 mg/kg (Giannotti et al., 1959; O'Brien, 1967). For fipronil, the oral LD_{50} in rats was 91 mg/kg, and the LD_{50} values in insects were 0.07 mg/kg in corn rootworm (Scharf and Siegfried, 1999) and 0.13 mg/kg in house fly (Hainzl and Casida, 1996). With the topical application of dieldrin to cockroaches, convulsions developed followed by paralysis. The synaptic transmission across the metathoracic ganglion but not the last abdominal ganglion was markedly facilitated, suggesting that the synapse in the metathoracic ganglion was one of the important loci of the dieldrin action (Wang et al., 1971).

There are many biological and physiological differences between vertebrates and invertebrates, especially the enzymatic metabolic degradation of insecticides. Any of these differences might be a factor for the selective toxicity of compounds in insects and mammals. The primary goal of the present study was to compare the sensitivity of insect GABA receptors with that of mammalian $GABA_A$ receptors to dieldrin and fipronil.

Fipronil inhibited the cockroach GABA receptors with an IC_{50} value of 28 nM (present study) and the rat $GABA_A$ receptors with an IC_{50} value of 1660 nM (Ikeda et al., 2001). Thus, the cockroach GABA receptors are 59 times more sensitive to fipronil than the rat $GABA_A$ receptors. Therefore, the fipronil's higher blocking potency in the insect GABA receptors compared with that in the mammalian $GABA_A$ receptors accounts at least in part for the selective toxicity between insects and mammals. Because different combinations of GABA receptor subunits and/or different amino acid compositions confer differential sensitivity to fipronil, these differences may be partly responsible for the selectivity of fipronil block of GABA receptors between cockroach and rat neurons (Ratra and Casida, 2001).

In contrast, the mechanism of the selective toxicity of dieldrin must lie in factors other than the GABA receptor sensitivity, because dieldrin exerts both potentiating and inhibitory actions on cockroach and rat GABA receptors with comparable potencies. The EC_{50} values for potentiation are 4.4 and 5 nM in cockroach and rat receptors, respectively, and the IC_{50} values for inhibition are 15.5 and 92 nM in cockroach and rat receptors, respectively (present study; Nagata and Narahashi, 1994). Therefore, the inhibitory potency in cockroach is only 6 times higher than that in rat. The difference in metabolic degradation of dieldrin in insects and mammals is among the most likely causes of the selective toxicity. In addition, the blocking action of dieldrin on the glycine receptor (Vale et al., 2003) and invertebrate-specific glutamate-activated slow-desensitizing chloride channels (our unpublished data) may also account for the selective toxicity of dieldrin in insects.

Recently, our studies and other reports demonstrate that fipronil also modulates the glutamate-activated chloride channel, a unique ligand-gated anion channel present in insects but not in vertebrates (Raymond et al., 2000; Horoszok et al., 2001). Fipronil is much more potent than dieldrin in blocking the glutamate-gated chloride channels (X. Zhao, V. L. Salgado, J. Z. Yeh, and T. Narahashi, unpublished data). The modulation of the insect-specific ion channels may also play an important role in the selective toxicity between mammals and insects, and offers a unique approach to the development of newer insecticides.

Acknowledgments

We thank Sandra Guy for technical assistance and Julia Irizarry for secretarial assistance.

References

Abalis IM, Eldefrawi ME, and Eldefrawi AT (1986) Effects of insecticides on GABA-induced chloride influx into rat brain microsacs. *J Toxicol Environ Health* **18:**13–23.

Alix P, Grolleau F, and Hue B (2002) Ca^{2+}/calmodulin-dependent protein kinase regulates GABA-activated Cl^- current in cockroach dorsal unpaired median neurons. *J Neurophysiol* **87:**2972–2982.

Allen JR, Hargraves WA, Hsia MTS, and Lin FSD (1979) Comparative toxicology of chlorinated compounds on mammalian species. *Pharmacol Ther* **7:**513–547.

Bermudez I, Hawkins CA, Taylor AM, and Beadle DJ (1991) Actions of insecticides on the insect GABA receptor complex. *J Receptor Res* **11:**221–232.

Buckingham SD, Hoise AM, Roush RL, and Sattelle DB (1994) Action of agonists and

convulsants antagonists on a *Drosophila melanogaster* GABA receptor (Rdl) homo-oligomer expressed in *Xenopus oocytes*. *Neurosci Lett* **181:**137–140.
Brooks GT (1986) Insecticide metabolism and selective toxicity. *Xenobiotica* **16:**989–1002.
Gant DB, Chalmers AE, Wolff MA, Hoffman HB, and Bushey DF (1998) Fipronil: action at the GABA receptor. *Rev Toxicol* **2:**147–156.
Ghiasuddin SM and Matsumura F (1982) Inhibition of gamma-aminobutyric acid (GABA)-induced chloride uptake by gamma-BHC and heptachlor epoxide. *Comp Biochem Physiol* **73C:**141–144.
Giannotti O, Metcalf RL, and March RB (1959) The mode of action of aldrin and dieldrin in *Periplaneta americana* (L.). *Ann Entomol Soc Am* **119:**588–592.
Grolleau F and Sattelle DB (2000) Single channel analysis of the blocking actions of BIDN and fipronil on a *Drosophila melanogaster* GABA receptor (RDL) stably expressed in a *Drosophila* cell line. *Br J Pharmacol* **130:**1833–1842.
Hainzl D and Casida JE (1996) Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. *Proc Natl Acad Sci USA* **93:**12764–12767.
Hainzl D, Cole LM, and Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem Res Toxicol* **11:**1529–1535.
Horoszok L, Raymond V, Sattelle DB, and Wolstenholme AJ (2001) GLC-3: a novel fipronil and BIDN-sensitive, but picrotoxinin-insensitive, L-glutamate-gated chloride channel subunit from *Caenorhabditis elegans*. *Br J Pharmacol* **132:**1247–1254.
Hosie AM, Baylis HA, Buckingham SD, and Sattelle DB (1995) Actions of the insecticide fipronil, on dieldrin-sensitive and -resistant GABA receptors of *Drosophila melanogaster*. *Br J Pharmacol* **115:**909–912.
Ikeda T, Zhao X, Nagata K, Kono Y, Shono T, Yeh JZ, and Narahashi T (2001) Fipronil modulation of γ-aminobutyric $acid_A$ receptors in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* **296:**914–921.
Lees G, Beadle DJ, Neumann R, and Benson JA (1987) Responses to GABA by isolated insect neuronal somata: pharmacology and modulation by a benzodiazepines and a barbiturate. *Brain Res* **401:**267–278.
Lummis SCR (1990) GABA receptors in insects. *Comp Biochem Physiol* **95C:**1–8.
Mahajna M, Quistad GB, and Casida JE (1997) Acephate insecticide toxicity: safety conferred by inhibition of the bioactivating carboxyamidase by the metabolite methamidophos. *Chem Res Toxicol* **10:**64–69.
Malecot C and Sattelle DB (1990) Single channel recordings from insect neuronal GABA-activated chloride channels. *J Exp Biol* **151:**495–501.
Matsumura F (1985) *Toxicology of Insecticides*, 2nd ed, Plenum Press, New York.
Millar NS, Buckingham SD, and Sattelle DB (1994) Stable expression of a functional homo-oligomeric *Drosophila* GABA receptor in a *Drosophila* cell line. *Proc R Soc Lond B Biol Sci* **258:**307–314.
Nagata K, Hamilton BJ, Carter DB, and Narahashi T (1994) Selective effects dieldrin on the $GABA_A$ receptor-channel subunits expressed in human embryonic kidney cells. *Brain Res* **645:**19–26.
Nagata K and Narahashi T (1994) Dual action of the cyclodiene insecticide dieldrin on the γ-aminobutyric acid receptor-channel complex of rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* **269:**164–171.
Narahashi T (2001) Neurophysiological effects of insecticides, in *Handbook of Pesticide Toxicology*, 2nd ed (Krieger RI ed) vol 1, pp 335–351, Academic Press, Inc., San Diego, CA.
O'Brien RD (1967) *Insecticides: Action and Metabolism*, Academic Press, Inc., New York.
Osborne RH (1996) Insect neurotransmission: neurotransmitters and their receptors. *Pharmacol Ther* **69:**117–142.
Pomés A, Rodriguez-Farré E, and Suñol C (1994) Disruption of GABA-dependent chloride flux by cyclodienes and hexachlorocyclohexanes in primary cultures of cortical neurons. *J Pharmacol Exp Ther* **271:**1616–1623.
Ratra GS and Casida JE (2001) GABA receptor subunit composition relative to insecticide potency and selectivity. *Toxicol Lett* **122:**215–222.
Raymond V, Sattelle DB, and Lapied B (2000) Co-existence in DUM neurons of two GluCl channels that differ in their picrotoxin sensitivity. *Neuroreport* **11:**2695–2701.
Roberts CJ, Krogsgaard-Larsen P, and Walker RJ (1981) Studies of the action of GABA, muscimol and related compounds on *Periplaneta* and *Limulus* neurons. *Comp Biochem Physiol* **69C:**7–11.
Sattelle DB (1992) Receptors for L-glutamate and GABA in the nervous system of an insect (*Periplaneta americana*). *Comp Biochem Physiol* **103C:**429–438.
Sattelle DB, Lummis SCR, Wong JFH, and Rauh JJ (1991) Pharmacology of insect GABA receptors. *Neurochem Res* **16:**363–374.
Sattelle DB, Pinnock RD, Wafford KA, and David JA (1988) GABA receptors on the cell-body membrane of an identified insect motor neuron. *Proc R Soc Lond B Biol Sci* **232:**443–456.
Scharf M and Siegfried B (1999) Toxicity and neurophysiological effects of fipronil and fipronil sulfone on the western corn rootworm (Coleoptera: Chrysomelidae). *Arch Insect Biochem Physiol* **40:**150–156.
Shimahara T, Pichon Y, Lees G, Beadle VA, and Beadle DJ (1987) γ-Aminobutyric acid receptors on cultured cockroach brain neurons. *J Exp Biol* **131:**231–244.
Song J-H and Narahashi T (1996) Modulation of sodium channels of rat cerebellar Prukinje neurons by the pyrethroid tetramethrin. *J Pharmacol Exp Ther* **277:**445–453.
Tingle CC, Rother JA, Dewhurst CF, Lauer S, and King WJ (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. *Rev Environ Contam Toxicol* **176:**1–66.
Vale C, Fonfria E, Bujons J, Messeguer A, Rodriguez-Farre E, and Sunol C (2003) The organochlorine pesticides γ-hexachlorocyclohexane (lindane), α-endosulfan and dieldrin differentially interact with $GABA_A$ and glycine-gated chloride channels in primary cultures of cerebellar granule cells. *Neuroscience* **117:**397–403.
Waldrop B, Christensen TA, and Hildebrand JG (1987) GABA-mediated synaptic inhibition of projection neurons in the antennal lobes of the sphinx moth *Manduca sexta*. *J Comp Physiol* **161:**23–32.
Wang CM, Narahashi T, and Yamada M (1971) The neurotoxic action of dieldrin and its derivatives in the cockroach. *Pestic Biochem Physiol* **1:**84–91.
Warmke JW, Reenan RA, Wang P, Qian S, Arena JP, Wang J, Wunderler D, Liu K, Kaczorowski GJ, Van der Ploeg LH, et al. (1997) Functional expression of *Drosophila* para sodium channels. Modulation by the membrane protein TipE and toxin pharmacology. *J Gen Physiol* **110:**119–133.
Watson GB (2001) Actions of insecticidal spinosyns on γ-aminobutyric acid responses from small-diameter cockroach neurons. *Pestic Biochem Physiol* **71:**20–28.

Address correspondence to: Dr. Toshio Narahashi, Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611-3008. E-mail: narahashi@northwestern.edu