# Pyrethroid Receptor in the Insect $Na^+$ Channel: Alteration of Its Properties in Pyrethroid-Resistant Flies†

David Pauron,‡ Jacques Barhanin,§ Marcel Amichot,‡ Madeleine Pralavorio,‡ Jean-Baptiste Bergé,‡ and Michel Lazdunski*,§

*Centre de Biochimie, Centre National de la Recherche Scientifique, Parc Valrose, 06034 Nice Cedex, France, and Station de Nématologie et de Génétique Moléculaire des Invertébrés, INRA, B.P. 2078, 123 boulevard Francis Meilland, 06602 Antibes, France*

*Received July 21, 1988; Revised Manuscript Received October 11, 1988*

ABSTRACT: Resistance to insecticides is a major problem in agriculture. [$^3$H]Saxitoxin binding experiments have shown that pyrethroid-sensitive and pyrethroid-resistant flies have the same amount of $Na^+$ channel protein in their brain membranes ($K_d$ = 0.2–0.3 nM and $B_{max}$ = 185–215 fmol/mg of protein for both types of flies). Also, although flies are resistant to pyrethroids, they remain as sensitive to batrachotoxin, which is another type of $Na^+$ channel activators, as pyrethroid-sensitive flies. Pyrethroid binding sites have been characterized by use of the properties of pyrethroids to increase the specific [$^3$H]batrachotoxinin A 20$\alpha$-benzoate binding component. $K_{0.5}$ values for association of pyrethroids at the $Na^+$ channel of pyrethroid-sensitive flies are in the range of 0.15–0.25 $\mu$M. Conversely, pyrethroids do not produce a significant increase of [$^3$H]batrachotoxinin A 20$\alpha$-benzoate binding in pyrethroid-resistant flies even at high concentrations of the insecticide. It is concluded that linkage between pyrethroid and batrachotoxin binding sites is altered in the pyrethroid-resistant fly strains we studied. This alteration is probably due to a drastically decreased affinity of the $Na^+$ channel for pyrethroids.

Pyrethroids form one of the newest and most potent classes of insecticides (Casida et al., 1983). However, the intense spreading in the fields of these insecticides [as well as insecticides of other classes (Hutson & Roberts, Eds., 1985)] has led to the appearance of insecticide-resistant insects. This resistance has been particularly well studied in houseflies (Keiding, 1976).

The target of pyrethroid action is the voltage-sensitive $Na^+$ channel. This has been shown by electrophysiological studies on different types of neuronal cells or tissues (Vijverberg et al., 1982; Lund & Narahashi, 1983; Yamamoto et al., 1983; Sattelle & Yamamoto, 1988), by $^{22}Na^+$ flux studies using cultured neuronal cells (Jacques et al., 1980; Roche et al., 1985), and more recently by binding studies using mammalian brain membranes (Brown & Olsen, 1984; Lombet et al., 1988). Pyrethroids have been found to stimulate the binding of the now classical $Na^+$ channel toxin batrachotoxin (Albuquerque & Daly, 1976) to mammalian brain synaptosomes, and this action is synergistic with that of other $Na^+$ channel toxins such as brevetoxins and sea anemone toxins (Lombet et al., 1988).

Resistance to pyrethroids can of course be due to several factors: (i) a change in the number of binding sites for pyrethroids (Chang & Plapp, 1983); (ii) a decrease of affinity of pyrethroids for their specific receptors on the $Na^+$ channel protein or else a normal binding of pyrethroids which would not be expressed as in susceptible insects; (iii) a change of the accessibility of pyrethroids to the excitable system of the insect; (iv) an increased in vivo degradation of the pyrethroid. Of course the resistance could also be polyfactorial (metabolic and nonmetabolic). This seems to be the case in fly strains such as Learn-PyR (Scott & Georghiou, 1986).

This paper compares $Na^+$ channel properties in pyrethroid-sensitive and pyrethroid-resistant housefly strains and shows that, in these strains, the resistance may be due to a change in the affinity of the $Na^+$ channel protein for pyrethroids.

## Experimental Procedures

*Toxins and Drugs.* Tetrodotoxin was purchased from Sankyo Chemical Co. (Japan). [$^3$H]Saxitoxin ([$^3$H]STX)[1] (specific activity 2.33 TBq/mmol) and [$^3$H]batrachotoxinin A 20$\alpha$-benzoate ([$^3$H]BTX-B) (specific activity 1.6 TBq/mmol) were from Amersham and Du Pont de Nemours, France, respectively. The two pyrethroids deltamethrin and RU39568 were kindly provided by Dr. Michel Roche, Procida, France. Stock solutions (10 mM) were prepared in dimethyl sulfoxide (DMSO) and aliquots kept at −20 °C. Veratridine was from Sigma.

*Housefly Strains.* Strains used were OMS, line SRS, a susceptible strain of the World Health Organization, strain Super *kdr* (Sawicki, 1978), NAIDM insecticide susceptible, and Learn-PyR (Scott & Georghiou, 1986).

*Membrane Preparations.* Purified head membranes corresponding to the different housefly strains were prepared as described earlier (Pauron et al., 1985). Storage in liquid nitrogen did not induce any loss in binding properties. In vitro assays indicated that these membranes were unable to metabolize [$^{14}$C]deltamethrin and that they contained no mixed-function oxidase activity (data not shown).

*Binding Assays.* Direct [$^3$H]STX binding was measured as previously reported for the ethylenediamine derivative of tetrodotoxin [$^3$H]en-TTX (Pauron et al., 1985). Competition

† This work was supported by the Centre National de la Recherche Scientifique (ATP 1217) and by the Institut National de la Recherche Agronomique (AIP 4555).

* To whom correspondence should be addressed.
‡ Station de Nématologie et de Génétique Moléculaire des Invertébrés.
§ Centre de Biochimie.

[1] Abbreviations: STX, saxitoxin; TTX, tetrodotoxin; BTX, batrachotoxin; BTX-B, batrachotoxinin A 20$\alpha$-benzoate; DMSO, dimethyl sulfoxide; AChE, acetylcholinesterase.

0006-2960/89/0428-1673$01.50/0 

Table I[a]

| housefly strain | $kd_{50}$ of deltamethrin | $ED_{50}$ of BTX ($\mu$M) |
|---|---|---|
| OMS | 5.9 (±0.85) | 33 (±9.00) |
| NAIDM | 4.7 (±0.78) | 35 (± 7.20) |
| Super *kdr* | 20 (±7.40) | 28 (±12.00) |
| Learn-PyR | 43 (±8.87) | 37 (±8.60) |

[a] $kd_{50}$ of deltamethrin expressed in minutes and $ED_{50}$ of batrachotoxin were determined as indicated under Experimental Procedures.

experiments using [$^3$H]BTX-B were performed by incubating the tritiated ligand with membranes in the absence or presence of increasing pyrethroid concentrations for 2 h at 22–24 °C. The incubation medium was 140 mM choline chloride, 50 mM HEPES–Tris, pH 7.4, 5.4 mM KCl, and 0.8 mM $MgSO_4$. The bound fraction was measured after rapid filtration through GF/B filters (Whatmann) prewashed in 0.03% poly(ethylenimine) and then washed twice with 5 mL of a solution containing 140 mM NaCl, 20 mM HEPES–Tris at pH 7.4, and 3 mM $CaCl_2$. Nonspecific binding was determined in parallel incubations containing 100 $\mu$M veratridine. Control experiments have shown that 1% DMSO (the maximal amount in our incubations) had no effect on [$^3$H]BTX-B binding.

Bradford's technique (Bradford, 1976) was used to quantify protein concentrations with bovine serum albumin as a standard.

*In Vivo Toxicity Measurements.* Knock-down effect of deltamethrin was measured according to the tarsal contact method: flies of each strain were put in batches of four in vertical glass tubes treated with 10 mM deltamethrin in acetone. $kd_{50}$ corresponds to the mean value of time necessary for two of the four flies in each batch to fall in the bottom of the tube without being able to climb up the walls of the tube. For batrachotoxin toxicity, a paralysis assay was developed by analogy with paralysis assays developed for aconitin on larvae (Bloomquist & Miller, 1986). After they were anaesthetized with ether, flies were treated by topical application on the thorax with 0.5 $\mu$L of a sublethal dose of piperonyl butoxide. After a recovery period of 2–3 h, flies were slightly anaesthetized one at the time with $CO_2$ and injected in the ventral part of the thorax with 100 nL of various concentrations of batrachotoxin. The number of paralyzed flies was determined 10 min after injection. In order to characterize synergy between batrachotoxin and pyrethroids, OMS and Learn-PyR strains were at first injected with 100 nL of DMSO (control) or 0.3 $\mu$M deltamethrin in DMSO. Three hours later, flies were treated with 100 nL of 0.1 mM batrachotoxin. Numbering surviving flies 2 or 24 h later gave identical results.

*Measurements of Acetylcholinesterase Activity (AChE).* AChE specific activity was determined in triplicate according to the procedure of Ellman et al. (1961).

## Results and Discussion

The work described in this paper concerns the interaction of pyrethroids with the $Na^+$ channel protein in pyrethroid-sensitive and pyrethroid-resistant houseflies. In vivo, as shown in Table I, pyrethroids such as deltamethrin are much less toxic to resistant flies as compared to sensitive ones. The time necessary to observe the knock-down (*kd*) effect is about 4 times longer for Super *kdr* and about 10 times longer for Learn-PyR flies as for sensitive OMS and NAIDM flies. Conversely, the $ED_{50}$ for the paralysis effect induced by one of the other types of the $Na^+$ channel effectors, batrachotoxin, is similar for the four strains. This result is in good agreement with a recent report by Bloomquist and Soderlund (1988) that *kdr* and Super *kdr* strains are not resistant to veratridine, an alkaloid which belongs to the same class of $Na^+$ channel effectors as batrachotoxin (Catterall, 1986).

Table II[a]

| housefly strain | AChE activity [$\mu$mol min$^{-1}$ (mg of protein)$^{-1}$] | [$^3$H]STX binding | |
|---|---|---|---|
| | | $K_d$ (nM) | $B_{max}$ (fmol/mg of protein) |
| OMS | 1.91 (±0.1) | 0.30 (±0.04) | 185 (±30) |
| NAIDM | 2.07 (±0.1) | 0.28 (±0.03) | 215 (±10) |
| Super *kdr* | 2.03 (±0.1) | 0.25 (±0.03) | 205 (±20) |
| Learn-PyR | 1.88 (±0.1) | 0.20 (±0.05) | 200 (±25) |

[a] Acetylcholinesterase activity was measured in triplicate experiments as described by Ellman et al. (1961). [$^3$H]STX binding parameters were calculated from experiments presented in Figure 1. Standard deviations result from three independent experiments done in identical conditions.

Saxitoxin and tetrodotoxin form one of the most widely used class of voltage-gated $Na^+$ channel effectors (Ritchie & Rogart, 1977; Catterall, 1986; Kao & Levinson, 1986; Lazdunski et al., 1986). They block $Na^+$ influx through the channel by binding to the protein on a site which has clearly been demonstrated to be unrelated to the receptor site for pyrethroids (Jacques et al., 1980). Direct binding studies performed with tritiated derivatives of these toxins have provided a measure of the density of voltage-sensitive $Na^+$ channels in a variety of tissues from different animal species including insect neuronal membranes (Reed & Raftery, 1976; Weigele & Barchi, 1978; Chicheportiche et al., 1980; Rogart et al., 1983; Pauron et al., 1985; Kao & Levison, 1986).

Figure 1 shows [$^3$H]STX binding to the different brain membrane preparations from the different fly strains. Scatchard plots of the specific binding components (Figure 1, insets) are linear for all fly strains, which demonstrates that the tritiated toxin binds to a single class of receptor sites. Values for dissociation constants ($K_d$) and maximal binding capacities ($B_{max}$) are given in Table II. These values were unchanged in experiments done in the presence of 10 $\mu$M deltamethrin. Table II compares the levels of [$^3$H]STX binding sites and of AChE activity taken as an internal standard in the different membrane preparations. The [$^3$H]STX binding results lead to the conclusion that, in contrast with suggestions of other authors (Chang & Plapp, 1983), the *kd* resistance mechanism is not related to a change in $Na^+$ channel density. Similar conclusions were obtained from equilibrium binding studies of an iodinated derivative of $\gamma$ toxin from the venom of the scorpion *Tityus serrulatus serrulatus* (Barhanin et al., 1982) which also binds with high affinity to fly neuronal membranes (Pauron et al., 1985).

Since a change in the number of $Na^+$ channels cannot be invoked to explain the *kd* resistance phenomenon, we investigated the hypothesis of a modification of affinity of the binding site for pyrethroids in the $Na^+$ channel protein of resistant flies. Pyrethroids are very lipophilic. This property is an advantage for their use in agriculture, but it constitutes a difficulty for a direct pharmacological characterization of binding sites using [$^3$H]pyrethroids. Until now, no convincing direct binding experiments using radiolabeled derivatives of pyrethroids have been presented, and we have ourselves been unsuccessful with several different [$^3$H]pyrethroids. $Na^+$ influx studies have shown that pyrethroids stimulate $Na^+$ entry in mammalian neuronal cells in culture when used in the presence of other $Na^+$ channel effectors such as the lipophilic toxins batrachotoxin and veratridine or polypeptide toxins from sea anemone and scorpion venoms (Jacques et al., 1980; Roche et al., 1985). It has been shown that pyrethroids increase the binding of a tritiated derivative of batrachotoxin, [$^3$H]BTX-B,

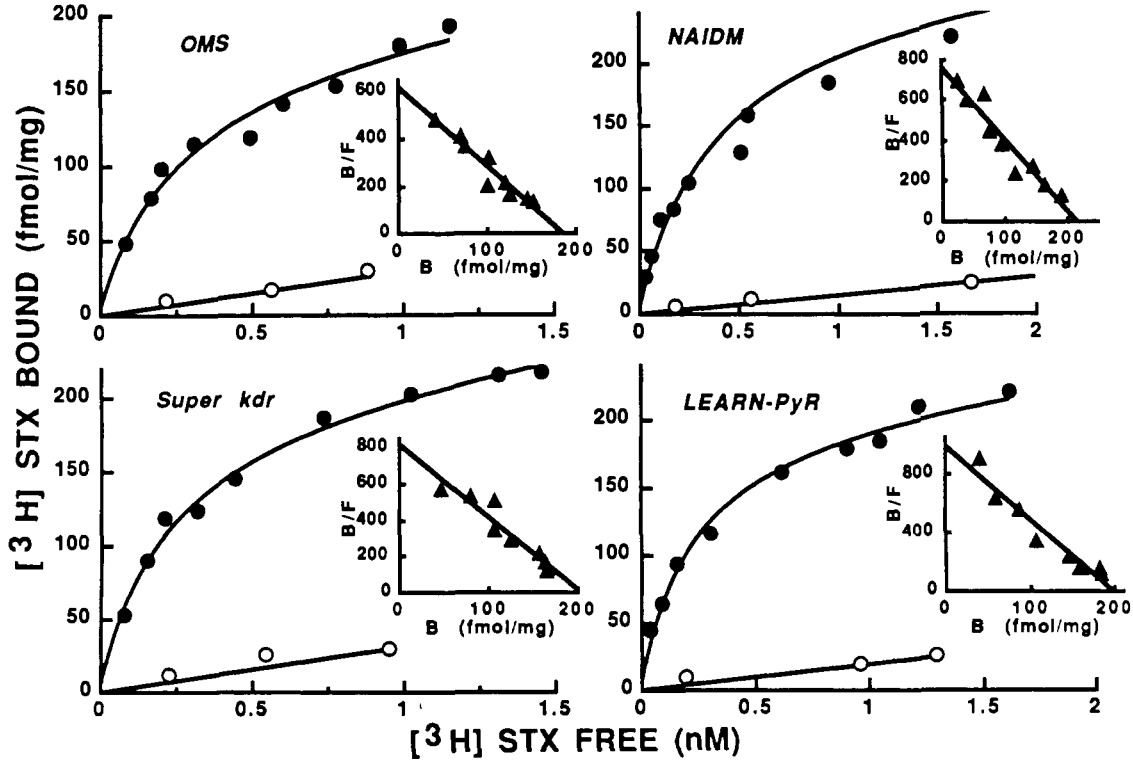


FIGURE 1: Direct binding of [$^3$H]STX to head membranes of various housefly strains. Membranes (0.81 mg of protein/mL for OMS, 0.96 mg of protein/mL for NAIDM, 0.7 mg of protein/mL for Super *kdr*, 0.48 mg of protein/mL for Learn-PyR) were incubated with increasing concentrations of [$^3$H]STX for 1 h at 22 °C. Specific binding is the difference between total binding (●) and nonspecific binding (○) measured in the presence of 1 μM TTX. Insets: Scatchard plots of the data. *B* is for [$^3$H]STX specific binding and *F* for unbound tritiated ligand. *B*/*F* ratio in mL/mg.

to mammalian synaptosomal membranes by increasing the affinity of this labeled toxin to its receptor site without modifying the maximal binding capacity (Lombet et al., 1988). Deltamethrin (10 μM) has no effect on binding of saxitoxin and γ toxin from *T. serrulatus serrulatus*, two other specific toxins for $Na^+$ channels, on binding of dendrotoxin I, a blocker of voltage-dependent $K^+$ channels, on glibenclamide binding, specific for ATP-sensitive $K^+$ channels, or on binding of (+)PN200-110 binding, a $Ca^{2+}$ channel antagonist (data not shown). Hence, potentiation of batrachotoxin binding by pyrethroids cannot be considered as due to a general unspecific effect on neuronal membranes.

This synergistic effect between pyrethroids and batrachotoxin is also directly observed on OMS flies. Lethality observed on flies treated with BTX is only 28.6% (16 over 56) in control conditions and reaches a value of 64.4% (38 over 59) for flies pretreated with a subtoxic dose of deltamethrin.

Figures 2 and 3 show the results of binding assays performed on flies with [$^3$H]BTX-B in the absence or in the presence of various concentrations of two α-cyanopyrethroids, deltamethrin and RU39568. With no insecticide present in the medium, specific binding of [$^3$H]BTX-B, which is the difference between total binding and nonspecific binding measured in the presence of 100 μM veratridine, is very low and similar for the four-fly preparations. Addition of the pyrethroids greatly enhanced the specific binding component of [$^3$H]BTX-B to head membrane preparations of OMS and NAIDM flies (Figures 2 and 3). This stimulation of binding is dependent upon the pyrethroid concentration in the incubation. It reaches a plateau value for pyrethroid concentrations of about 5 μM with a maximal binding component being about 4.5-fold higher than the corresponding component measured with [$^3$H]BTX-B alone. Both pyrethroids have the same efficacy, and the apparent affinity determined at the half-maximal stimulation

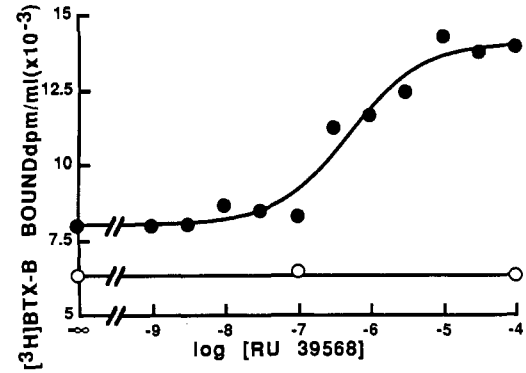


FIGURE 2: Effect of RU39568 on [$^3$H]BTX-B binding to OMS head membranes (1.1 mg of protein/mL). Total binding of [$^3$H]BTX-B was determined in the absence or in the presence of increasing concentrations of the pyrethroid RU39568 (●). Nonspecific binding values (○) were obtained in parallel incubations containing 100 μM veratridine. [$^3$H]BTX-B concentration was 5 nM. In these conditions, the amount of specifically bound toxin varied from 15 fmol/mg of protein in the absence of added drug to 71 fmol/mg of protein at maximal stimulation.

value, $K_{0.5}$, is 0.25 μM for OMS flies and 0.15 μM for NAIDM ones.

Because pyrethroid-modified $Na^+$ channels adopt long-opening modes, activation of only a small proportion of the total number of $Na^+$ channels will be sufficient to observe a depolarization or a repetitive activity (Ruigt, 1984; Chinn & Narahashi, 1986; De Weille et al., 1988) leading to toxicity (Lund, 1984). Therefore, it would not be surprising to observe toxic effects of pyrethroids on insects at concentrations which are 1 or 2 orders of magnitude lower than the $K_{0.5}$ values measured in this work.

In contrast with the situation in sensitive strains, the stimulatory effect of pyrethroids on [$^3$H]BTX-B binding is only

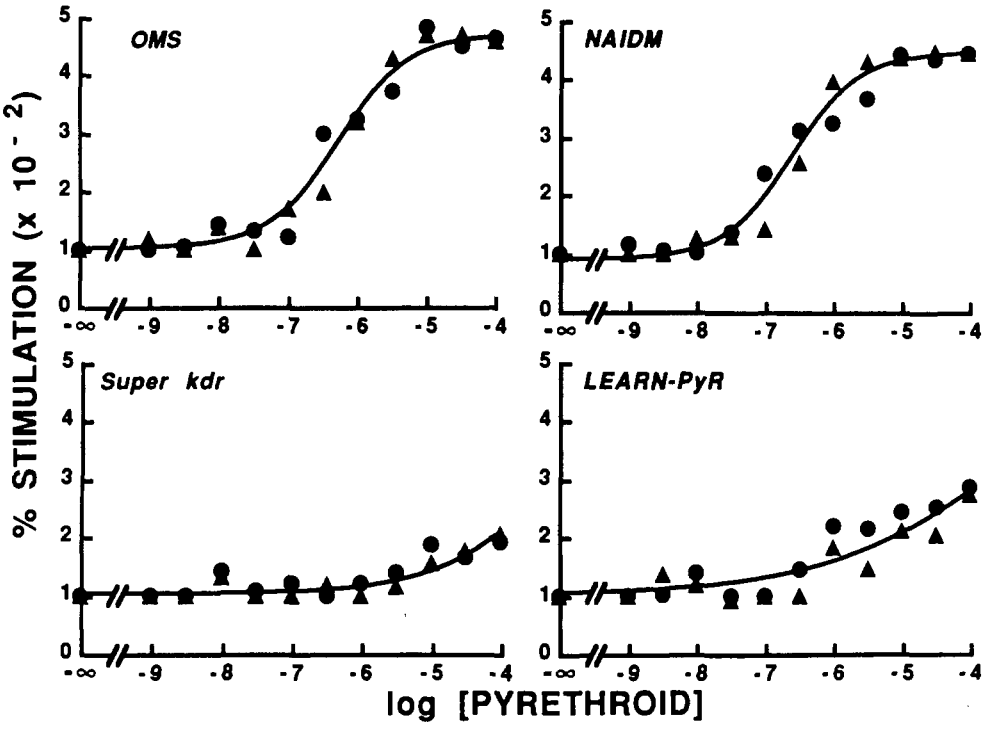


FIGURE 3: Effects of RU39568 and deltamethrin on [$^3$H]BTX-B binding to neuronal membranes from pyrethroid-sensitive and pyrethroid-resistant flies. Experiments were performed as described in the legend of Figure 2. Results are expressed in percentage of enhancement of [$^3$H]BTX-B specific binding in the presence of RU39568 (●) or deltamethrine (▲). 100% represents the value for [$^3$H]BTX-B specific binding measured in the absence of any insecticide which was found to be similar for the four strains (14–16 fmol/mg of protein at [$^3$H]BTX-B concentration of 5 nM).

partially observed when [$^3$H]BTX-B is assayed on membranes prepared with heads from Super *kdr* and Learn-PyR flies as shown on the lower part of Figure 3. Even at 100 $\mu$M concentrations of pyrethroids, the plateau value of binding activity observed for NAIDM and OMS flies is not reached with pyrethroid-resistant flies. Solubility problems prevented an investigation of the effects of higher concentrations of the pyrethroids on these membranes of mutant flies. Nonetheless, comparison of the data presented in Figure 3 clearly demonstrates that the synergistic effect of pyrethroids on [$^3$H]BTX-B binding is absent or at least drastically altered in mutant flies. The in vivo toxicity for batrachotoxin in resistant flies is not significantly affected by pyrethroid pretreatment under conditions in which synergy was seen with pyrethroid-sensitive flies. Lethality values are 23% (32 over 137) and 23% (33 over 140) for control and deltamethrin-treated flies, respectively. These observations support the idea that a modification of the pyrethroid binding site or a modification of its allosteric linkage to the batrachotoxin binding site on the $Na^+$ channel protein is responsible for the knock-down resistance mechanism in the mutants investigated in this work.

Pyrethroids are also known to be much more toxic to insects than to mammals (Casida et al., 1983). Among the many possible reasons for a higher toxicity in insects are the easier access of these insecticides to the insect excitable system and a possibly better affinity of pyrethroids for the insect versus the mammalian $Na^+$ channel. Apparent affinities found for different pyrethroids using the same type of biochemical assay used here with rat neuronal membranes (Lombet et al., 1988) are about 2 orders of magnitude lower than those found for pyrethroid-sensitive insects ($K_{0.5}$ = 20 $\mu$M for rat membranes against $K_{0.5}$ = 0.15–0.25 $\mu$M for flies membranes in measurements in the sole presence of batrachotoxin and pyrethroids). Relatively high apparent affinities ($K_{0.5} \sim 0.2$ $\mu$M) can be found for the interaction of pyrethroids with rat brain membranes. However, such relatively high affinities can only be obtained in the presence of sea anemone toxins and brevetoxin, which then potentiate the effects of pyrethroids of [$^3$H]BTX-B binding (Lombet et al., 1988). Neither sea anemone toxins nor scorpion toxins (*Androctonus australis* or *Leiurus quinquestriatus* toxins) have any potentiating effect on the pyrethroid ability to increase [$^3$H]BTX-B binding to insect membranes although these polypeptide toxins are active on the insect $Na^+$ channel (Pauron et al., 1985; Gordon et al., 1984). Similarly, brevetoxin potentiates pyrethroid effects on [$^3$H]BTX-B binding on mammalian neuronal membranes but not on insect neuronal membranes (not shown).

In conclusion, this paper (i) shows that, as for the mammalian $Na^+$ channel, there is a synergy between pyrethroid and batrachotoxin binding and (ii) demonstrates that pyrethroid-resistant flies of the *kdr* type have a $Na^+$ channel which is unaltered with respect to saxitoxin binding but which is altered at the pyrethroid binding site. These results are confirmed by in vivo experiments where synergy for toxicity is observed in sensitive flies and is absent in resistant ones. Cloning the $Na^+$ channel cDNA in sensitive and resistant flies is now under way and should permit identification of the structural alterations which are responsible for the resistance. This task is facilitated by a recent report of a *Drosophila melanogaster* $Na^+$ channel sequence which has been shown to be highly homologous to the $Na^+$ channel sequence in rat brain (Salkoff et al., 1987).

ACKNOWLEDGMENTS

We are grateful to Dr. Michel Roche (Procida, France) for the gift of the OMS strain and the pyrethroids, to Dr. G. P. Georghiou (University of California, Riverside) for kindly providing NAIDM and Learn-PyR strains, and to Dr. J. W. Daly for supplying batrachotoxin. Thanks are due to M.-M. Larroque, P. Loncle, and C. Roulinat-Bettelheim for skillful technical assistance.

**Registry No.** RU39568, 98444-62-3; TTX, 4368-28-9; STX, 35523-89-8; BTX-B, 78870-19-6; deltamethrin, 52918-63-5.

## REFERENCES


Albuquerque, E. X., & Daly, J. W. (1976) in *Receptors and Recognition* (Cuatrecasas, P., Ed.) Vol. 1, pp 299–336, Chapman and Hall, London.

Barhanin, J., Giglio, J., Lēopold, P., Schmid, A., Sampaio, S. V., & Lazdunski, M. (1982) *J. Biol. Chem. 257*, 12553–12558.

Bloomquist, J. R., & Miller, T. A. (1986) *NeuroToxicology 7*, 214–224.

Bloomquist, J. R., & Soderlund, D. M. (1988) *Mol. Pharmacol. 33*, 543–550.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.

Brown, G. B., & Olsen, R. W. (1984) *Soc. Neurosci. Abstr. 10*, 865.

Casida, J. E., Gammon, D. W., Glickman, A. M., & Lawrence, L. J. (1983) *Annu. Rev. Pharmacol. Toxicol. 23*, 413–438.

Catterall, W. A. (1986) *Annu. Rev. Biochem. 55*, 953–985.

Chang, C. P., & Plapp, F. W. (1983) *Pestic. Biochem. Physiol. 20*, 86–91.

Chicheportiche, R., Balerna, M., Lombet, A., & Lazdunski, M. (1980) *Eur. J. Biochem. 104*, 617–625.

Chinn, K., & Narahashi, T. (1986) *J. Physiol. (London) 380*, 191–207.

De Weille, J. R., Vijverberg, H. P. M., & Narahashi, T. (1988) *Brain Res. 445*, 1–11.

Ellman, G. L., Courtney, K. D., Andress, V., & Featherstone, R. W. (1961) *Biochem. Pharmacol. 7*, 88–95.

Gordon, D., Jover, E., Couraud, F., & Zlotkin, E. (1984) *Biochim. Biophys. Acta 778*, 349–358.

Hutson, D. H., & Roberts, T. R., Eds. (1985) Progress in pesticide biochemistry and toxicology, in *Insecticides*, Vol. 5, Wiley, New York.

Jacques, Y., Romey, G., Cavey, M.-T., Kartalovski, B., & Lazdunski, M. (1980) *Biochim. Biophys. Acta 600*, 882–897.

Kao, C. Y., & Levinson, S. R., Eds. (1986) Tetrodotoxin, saxitoxin and the molecular biology of the sodium channel, *Ann. N.Y. Acad. Sci. 479*.

Keiding, J. (1976) *Pestic. Sci. 7*, 283–291.

Lazdunski, M., Frelin, C., Barhanin, J., Lombet, A., Meiri, H., Pauron, D., Romey, G., Schmid, A., Schweitz, H., Vigne, P., & Vijverberg, H. P. M. (1986) *Ann. N.Y. Acad. Sci. 479*, 204–220.

Lombet, A., Mourre, C., & Lazdunski, M. (1988) *Brain Res. 459*, 44–53.

Lund, A. E. (1984) *Pestic. Biochem. Physiol. 22*, 161–168.

Lund, A. E., & Narahashi, T. (1983) *Pestic. Biochem. Physiol. 20*, 203–216.

Pauron, D., Barhanin, J., & Lazdunski, M. (1985) *Biochem. Biophys. Res. Commun. 131*, 1226–1233.

Reed, J. K., & Raftery, M. A. (1976) *Biochemistry 15*, 944–953.

Ritchie, J. M., & Rogart, R. B. (1977) *Rev. Physiol. Biochem. Pharmacol. 79*, 1–50.

Roche, M., Frelin, C., Bouneau, P., & Meinard, C. (1985) *Pestic. Biochem. Physiol. 24*, 306–316.

Rogart, R. B., Regan, L. J., Dziekan, L. C., & Galper, J. B. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1106–1110.

Ruigt, G. S. F. (1984) in *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (Kerkut, G. A., & Gilbert, L. I., Eds.) Vol. 12, pp 183–263, Pergamon, Oxford.

Salkoff, L., Butler, A., Wei, A., Scavarda, N., Giffen, K., Ifune, C., Goodman, R., & Mandel, G. (1987) *Science 237*, 744–748.

Sattelle, D. B., & Yamamoto, D. (1988) *Adv. Insect Physiol. 20*, 147–213.

Sawicki, R. M. (1978) *Nature 275*, 443–444.

Scott, J. G., & Georghiou, G. P. (1986) *Biochem. Genet. 24*, 25–37.

Vijverberg, H. P. M., Van Der Zalm, J. M., & Van Den Bercken, J. (1982) *Nature 295*, 601–603.

Weigele, J. B., & Barchi, R. L. (1978) *FEBS Lett. 91*, 310–314.

Yamamoto, D., Quandt, F. N., & Narahashi, T. (1983) *Brain Res. 274*, 344–349.