Action of Insecticidal *N*-Alkylamides at Site 2 of the Voltage-Sensitive Sodium Channel

James A. Ottea, Gregory T. Payne, and David M. Soderlund*

Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456

Nine synthetic *N*-alkylamides were examined as inhibitors of the specific binding of [^{3}H]-batrachotoxinin A 20α-benzoate ([^{3}H]BTX-B) to sodium channels and as activators of sodium uptake in mouse brain synaptoneurosomes. In the presence of scorpion (*Leiurus quinquestriatus*) venom, the six insecticidal analogues were active as both inhibitors of [^{3}H]BTX-B binding and stimulators of sodium uptake. These findings are consistent with an action of these compounds at the alkaloid activator recognition site (site 2) of the voltage-sensitive sodium channel. The three noninsecticidal *N*-alkylamides also inhibited [^{3}H]BTX-B binding but were ineffective as activators of sodium uptake. Concentration–response studies revealed that some of the insecticidal amides also enhanced sodium uptake through a second, high-affinity interaction that does not involve site 2, but this secondary effect does not appear to be correlated with insecticidal activity. The activities of *N*-alkylamides as sodium channel activators were influenced by the length of the alkenyl chain and the location of unsaturation within the molecule. These results further define the actions of *N*-alkylamides on sodium channels and illustrate the significance of the multiple binding domains of the sodium channel as target sites for insect control agents.

INTRODUCTION

Naturally occurring *N*-alkylamides exhibit varying degrees of biological activity (Jacobson, 1971; Su, 1985). Structure–toxicity evaluations have led to the development of synthetic *N*-alkylamides with increased insecticidal activity that show promise as insect control agents (Crombie and Denman, 1984; Elliott, 1985; Miyakado et al., 1985a,b; Black et al., 1986; Elliott et al., 1987a–e), especially for use against populations of insects in which pyrethroid resistance has arisen (Elliott et al., 1986).

Electrophysiological studies with insect nerve preparations indicate that *N*-alkylamides disrupt the normal functioning of nerve membrane sodium channels (Blade et al., 1985; Lees and Burt, 1988). Recognition sites exist on voltage-sensitive sodium channels for a variety of neurotoxic agents (Catterall, 1988) including the channel blockers tetrodotoxin and saxitoxin (site 1), the alkaloids batrochotoxin and veratridine (site 2), polypeptide toxins isolated from scorpion and sea anemone venoms (sites 3 and 4), and the brevetoxins and ciguatoxins (site 5). Additional sites on the sodium channel are proposed for the pyrethroid insecticides and DDT (Jacques et al., 1980; Ghiasuddin and Soderlund, 1985; Bloomquist and Soderlund, 1988; Brown et al., 1988; Lombet et al., 1988; Payne and Soderlund, 1989), the pumiliotoxins (Gusovsky et al., 1988), and a polypeptide toxin from *Goniopora* corals (Gonoi et al., 1986). Results from biochemical assays show that a representative synthetic *N*-alkylamide, BTG 502, exerts its effect at the activator recognition site (site 2) of the sodium channel (Ottea et al., 1989), a domain distinct from that involved in the action of DDT and the pyrethroids.

In the present study, radioligand binding and ion flux assays were employed to define further the actions of a series of *N*-alkylamides on sodium channels. All of the analogues tested were inhibitors of the specific binding of [^{3}H]batrachotoxinin A 20α-benzoate (BTX-B). However, only the *N*-alkylamides with significant insecticidal activity were able to stimulate the uptake of sodium into mouse brain synaptoneurosomes. These results are consistent with the action of these compounds at site 2 of the sodium channel.

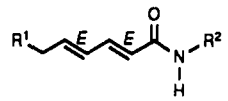
MATERIALS AND METHODS

Chemicals. The *N*-alkylamides used in this study (A–I, Table I) were generously provided by N. Janes (Rothamsted Experimental Station, Harpenden, England). In bioassays with house flies (*Musca domestica* L.) and mustard beetles (*Phaedon cochleariae* Fab.), the insecticidal potencies of A–F were 1–8% of that measured for bioresmethrin, whereas G–I were nontoxic (Elliott, 1985; Elliott et al., 1987a–e; N. F. Janes, personal communication). Veratridine (VTD) and scorpion venom (*Leiurus quinquestriatus*; ScV) were purchased from Sigma Chemical Co. (St. Louis, MO). Carrier-free ^{22}NaCl (1.053 Ci/mg) was obtained from Amersham Corp. (Arlington Heights, IL) and [^{3}H]BTX-B (42.7 Ci/mmol) was purchased from Du Pont NEN Research Products (Boston, MA). Pumiliotoxin B (PTX-B) was a gift from J. Daly (National Institute of Arthritis, Metabolism and Digestive Disease, Bethesda, MD).

Preparation of Synaptoneurosomes. Synaptoneurosomes were prepared by using the method of Brown (1986) with slight modifications (Ottea et al., 1989). Brains from male ICR mice (Blue Spruce Farms, Altamont, NY) were removed following cervical dislocation, rinsed in homogenization buffer, and blotted dry on filter paper. The homogenization buffer used in sodium flux experiments contained (millimolar) choline chloride (130), HEPES (30), glucose (5.5), $MgCl_2 \cdot 6H_2O$ (3), KCN (10), and ouabain (3). For measurements of the specific binding of [^{3}H]BTX-B, the homogenization buffer was composed of (millimolar) choline chloride (130), HEPES (50), glucose (5.5), $MgCl_2 \cdot 6H_2O$ (0.8), and KCl (5.4). The pH of the homogenization buffers was adjusted to 7.4 with Tris base. The brains were dissected free of white matter, minced in homogenization buffer, and homogenized by hand with 4–5 strokes of a Dounce homogenizer. Following dilution to a final concentration of 1

0021-8561/90/1438-1724$02.50/0

Table I. Structures of N-Alkylamides and Their Effects on [^{3}H]BTX-B Binding and Sodium Uptake[a]



designation	R^1	R^2	[^{3}H]BTX-B binding[b] K_i, μM	[^{3}H]BTX-B binding[b] Hill slope	sodium uptake[c] $K_{0.5}$, μM	sodium uptake[c] E_{max}, nmol
BTG 502[d]	5-bromonaphth-2-yl	$CH(CH_3)CH(CH_3)_2$	1.43	0.83	1.7	1.17
A	C_6H_5	$CH_2CH(CH_3)_2$	45.8	1.04	13.7 (0.03)[e]	0.43
B	C_6H_5	$CH(CH_3)CH(CH_3)_2$	50.1	0.75	16.0 (0.06)	0.35
C	3,5-difluorophenyl	$CH_2C(CH_3)_3$	13.3	1.46	6.58	0.78
D	dibenzofuran-3-yl	$CH_2C(CH_3)_3$	9.22	1.08	1.41 (0.02)	1.10
E	$CH_3(CH_2)_5$	$CH_2CH(CH_3)_2$	21.6	1.07	1.99	0.37
F	$C_6H_5(CH_2)_6$	$CH_2CH(CH_3)_2$	244	0.28*	ND[f]	ND
G	$C_6H_5CH_2$	$CH_2CH(CH_3)_2$	15.5	0.87	–[g]	–
H	(3E,5E)-N-(1,2-dimethylpropyl)-6-phenylhexa-3,5-dienamide		>60	ND	–	–
I	(2E,4E)-N-(2-methylpropyl)-5-phenylpenta-2,4-dienamide		>60	ND	–	–

[a] Data represent mean values from results of at least four triplicate experiments using freshly prepared synaptoneurosomes for each assay. [b] Data from inhibition experiments were analyzed by least-squares regression. The only Hill value statistically different from unity was that measured for F (*, Student's t-test, $p < 0.05$). [c] Values from uptake studies were analyzed by the method of Wilkinson (1961). [d] Data for BTG 502 are from Ottea et al. (1989). [e] For compounds producing biphasic response curves, $K_{0.5}$ values for the high-affinity component of sodium uptake, estimated by visual inspection of dose–response plots, are shown in parentheses. [f] Value not determined. [g] Stimulation of sodium uptake was not statistically significant.

brain/7.5 mL of homogenization buffer, the homogenate was centrifuged at 1000g for 15 min. The resulting pellet was gently resuspended in 3.5 (for $^{22}Na^+$ uptake) or 6 mL (for [^{3}H]-BTX-B binding) of homogenization buffer containing 1 mg/mL BSA, filtered through three layers of nylon mesh, and used immediately for assays.

Sodium Uptake Assays. The uptake of $^{22}Na^+$ into synaptoneurosomes was measured by the method of Tamkun and Catterall (1981) as modified by Bloomquist and Soderlund (1988). N-Alkylamides (0–120 μM) and ScV (25 μg in homogenization buffer) were added directly to synaptoneurosomal membranes (100 μL; ca. 300 μg of protein), whereas PTX-B (10 μM) was allowed to evaporate just prior to the addition of membranes. Preliminary experiments showed that the amount of ethanol used to deliver the N-alkylamides and PTX-B in these assays (0.4–1 μL) had no adverse effect on the levels of uptake measured. Sodium uptake was initiated by the addition of 100 μL of sodium flux buffer containing 150 nCi of $^{22}Na^+$. Following incubation at 37 °C for 15 s, uptake was terminated by the addition of 3 mL of ice-cold washing buffer (Bloomquist and Soderlund, 1988) and rapid vacuum filtration. The filters were rinsed with an additional 6 mL of washing buffer, and levels of sodium influx into the synaptoneurosomes were quantified by scintillation counting of the filters using a 4:1 mixture of Betafluor (National Diagnostics, Manville, NJ) and ethylene glycol monomethyl ether as the scintillant. Data points represent results of three to six triplicate experiments using freshly prepared synaptoneurosomes for each assay.

BTX-B Binding Assays. Inhibition of the specific binding of [^{3}H]BTX-B was measured by the method of Catterall et al. (1981) as modified by Payne and Soderlund (1989). For the measurement of total binding, incubation mixtures contained [^{3}H]BTX-B (20 nM; 136.6 nCi/inc), synaptoneurosomes (ca. 160 μg of protein), N-alkylamides (0–120 μM final concentrations in 0.8 μL of EtOH), and ScV (30 μg/inc) in a total volume of 160 μL of homogenization buffer containing 1 mg/mL BSA. The mixtures were incubated at 37 °C for 45 min after which time 120-μL aliquots were applied to Whatman GF/C filters and rinsed immediately (2 × 5 mL) under vacuum with ice-cold washing buffer (Catterall et al., 1981). Radioactivity remaining on the filters was quantitated by liquid scintillation spectrometry using Liquiscint (National Diagnostics) as the scintillant. Specific binding was calculated as the amount of total binding displaced by 500 μM VTD. The data presented are means from four triplicate assays using freshly prepared synaptoneurosomes for each assay.

RESULTS

Initial Screen of N-Alkylamides for Activity on Sodium Channels. The insecticidal activities of the

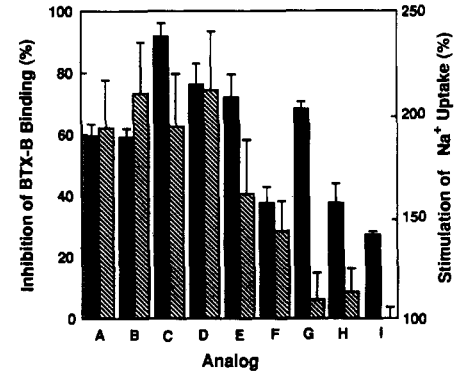


Figure 1. Inhibition of [^{3}H]BTX-B binding (solid bars) and enhancement of sodium uptake (hatched bars) by N-alkylamides (60 μM) in the presence of ScV (25 μg). Bars represent mean activity (±SE) based on at least four triplicate experiments. Values for sodium uptake are corrected for activity in the presence of ScV alone.

N-alkylamides used in this study are reported elsewhere (Elliott, 1985; Elliott et al., 1987a–e). In general, A–F possess some degree of toxicity to house flies or mustard beetles, whereas G–I are nontoxic. In the presence of a saturating concentration of ScV (30 μg), both toxic and nontoxic compounds inhibited the specific binding of [^{3}H]-BTX-B (Figure 1). At the concentration used in these assays (60 μM), the inhibition produced by C was nearly complete (91.6%). Levels of inhibition in the presence of the other insecticidal analogues were within the range 37.5% (F)–76.3% (D). In assays with the noninsecticidal analogues, inhibition by G (75.2%) was comparable to that measured with the insecticidal analogues D and E, while H and I were relatively poor inhibitors.

Whereas all of the compounds inhibited the binding of [^{3}H]BTX-B, only the insecticidal analogues (A–F) were able to stimulate the influx of sodium significantly in the presence of a saturating concentration of ScV (Figure 1). In addition, the stimulation of uptake produced by a subsaturating concentration of BTG 502 (10 μM) was inhibited 54% by G (75 μM), suggesting that the analogues which were inactive as stimulators of uptake acted as antagonists (data not shown). The greatest values for stimulation of sodium uptake (approaching or exceeding 200% of control values) were measured in incubations with A–D (60 μM). In the absence of ScV, no statistically significant

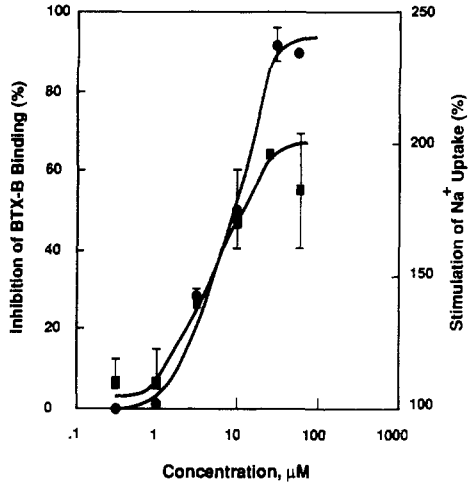


Figure 2. Concentration-dependent inhibition of [^{3}H]BTX-B binding (●) and activation of sodium uptake (■) by C in the presence of ScV (25 μg). Points represent mean activity (±SE) based on at least four triplicate experiments. Values for sodium uptake are corrected for activity in the presence of ScV alone.

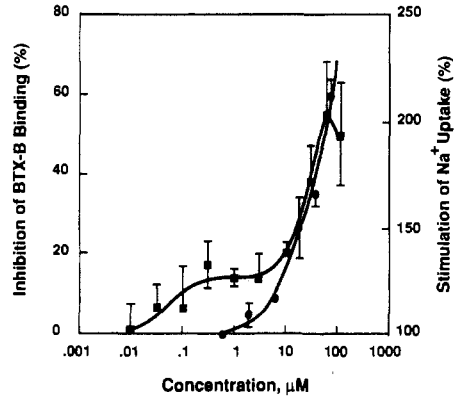


Figure 3. Concentration-dependent inhibition of [^{3}H]BTX-B binding (●) and activation of sodium uptake (■) by A in the presence of ScV (25 μg). Points represent mean activity (±SE) based on at least four triplicate experiments. Values for sodium uptake are corrected for activity in the presence of ScV alone.

stimulation of uptake was detected with any of the analogues tested (data not shown).

Effects of *N*-Alkylamides on [^{3}H]BTX-B Binding. At the highest concentration tested in dose–response experiments (75 μM), all of the compounds produced incomplete inhibition of the specific binding of [^{3}H]-BTX-B (cf. C and A in Figures 2 and 3, respectively). The dibenzofuranyl-substituted analogue, D, was the most potent inhibitor of binding with a K_i of 9.22 μM (Table I) and produced maximal levels of inhibition (76.4%) at 30 μM (data not shown). F was the least effective inhibitor of [^{3}H]BTX-B binding with an estimated K_i of 244 μM. The mean K_i value measured in assays with the non-toxic compound, G (15.5 μM), was similar to that measured for the insecticidal analogues C and E. The Hill slopes measured for A–E and G (Table I) were not significantly different from 1 (Student's t-test, $p < 0.05$). In contrast, the Hill slope estimated for F was 0.28.

Effects of *N*-Alkylamides on Sodium Uptake. Two distinct patterns of stimulation were measured in dose–response experiments for the analogues that enhanced sodium uptake. The relationship between increasing concentration and enhancement of sodium uptake was monophasic in tests with C (Figure 2) and E (data not shown) and was qualitatively similar to that measured previously for BTG 502 (Ottea et al., 1989). In contrast, biphasic patterns of stimulation were seen in experiments with compounds A (Figure 3), B, and D (data not shown). Concentration–response parameters for the action of *N*-alkylamides as stimulators of sodium uptake are summarized in Table I. In assays with most of these analogues, maximal levels of sodium uptake were detected at concentrations that were subsaturating with respect to the inhibition of [^{3}H]BTX-B binding (Figure 3). Thus, the analogue concentrations producing half-maximal levels of enhancement ($K_{0.5}$) were generally less than the corresponding binding affinity constants for inhibition (K_i). In the present study, D was the most potent ($K_{0.5}$ = 1.41 μM) and efficacious (E_{max} = 1.10 nmol/assay) activator of sodium uptake. Compound F, the analogue least potent as an inhibitor of [^{3}H]BTX-B binding, was also the least active stimulator of sodium flux. The low levels of stimulation in the presence of this analogue precluded the estimation of values for $K_{0.5}$ and E_{max}. No significant enhancement of sodium uptake was detectable in tests with G–I.

In tests with analogues producing biphasic uptake curves, inhibition of [^{3}H]BTX-B binding was measurable only at concentrations corresponding to the lower affinity component for the stimulation of sodium uptake (Figure 3). Because PTX-B stimulates sodium uptake in the presence of ScV via an action independent of site 2 (Gusovsky et al., 1988), the possible actions of the analogues at the PTX-B recognition site were explored by assessing the effects of an *N*-alkylamide exhibiting biphasic stimulation of sodium uptake on sodium uptake stimulated by PTX-B. In these experiments, A at 0.3 or 75 μM did not inhibit PTX-B (10 μM) dependent uptake, and the level of uptake measured in assays with combinations of A and PTX-B was equivalent to the sum of that produced by the two compounds individually (data not shown).

DISCUSSION

The *N*-alkylamide BTG 502 was shown previously to represent a novel chemical class of neurotoxins that act at site 2 of the sodium channel (Ottea et al., 1989). Results presented here from experiments using a series of structural analogues of BTG 502 provide further insight into the mechanism of action for these compounds. The principal actions of the *N*-alkylamides, as measured in [^{3}H]-BTX-B binding and sodium uptake assays, were similar to those measured previously with BTG 502 (Ottea et al., 1989) and are consistent with an action of these compounds at site 2 of the sodium channel. All of the analogues inhibited the specific binding of [^{3}H]BTX-B, a radioligand that specifically labels site 2 (Catterall et al., 1981). For compounds A–E, inhibition of binding occurred within the same range of analogue concentrations producing enhancement of sodium uptake (Figures 2 and 3). In addition, the Hill slopes calculated from the inhibition data (Table I) were not statistically different from unity (except in the case of F; see below). The inhibition of [^{3}H]-BTX-B binding by G was not associated with stimulation of sodium uptake, a finding that implicates this compound as an antagonist at site 2.

Site 2 does not appear to be the only domain of the sodium channel affected by the *N*-alkylamides. The existence of multiple sites on the sodium channel for the action of *N*-alkylamides is also suggested by the biphasic patterns of activity measured in sodium uptake assays with A, B, and D and the lack of significant inhibition of [^{3}H]BTX-B binding at concentrations of these *N*-alkylamides that describe the high-affinity component of the stimulation of sodium uptake. In these experiments,

stimulation of uptake was detected only in the presence of ScV and was completely blocked by tetrodotoxin. Therefore, the high-affinity component of sodium uptake resulted from the interaction between these compounds and the sodium channel at a domain other than site 2, rather than from an indirect effect on vesicle depolarization leading to a stimulation of sodium influx. A similar effect on sodium uptake that is independent of site 2 has been described for the alkaloid PTX-B, a neurotoxin that binds to a unique domain on sodium channels (Gusovsky et al., 1988). However, our preliminary results indicate that the stimulation of sodium uptake by *N*-alkylamides at concentrations too low to affect site 2 does not appear to be associated with an interaction at the PTX-B site. Therefore, the high-affinity component of sodium channel activation measured with some *N*-alkylamides involves an unknown binding domain on the sodium channel. The potential for exploitation of this binding site for insect control is unknown; however, for the *N*-alkylamides tested here, the stimulation of sodium uptake by this mechanism does not appear to be correlated with insecticidal activity.

The actions of compound F in these assays were different from those measured for the insecticidal analogues A–E. Compound F stimulated low levels of sodium uptake (Figure 1) and weakly inhibited [^{3}H]BTX-B binding, but the Hill slope of the latter interaction was substantially less than 1.0 (Table I). These data suggest that the interaction of F with the sodium channel may involve either negative cooperativity or multiple binding domains that are directly or allosterically detected in [^{3}H]BTX-B binding assays (Weiland and Molinoff, 1981). These findings therefore may implicate an effect of this compound at a sodium channel binding domain other than site 2.

Our studies also permit the identification of some features of chemical structure that define the activity of the *N*-alkylamides as both sodium channel toxins and insecticides. The 2,4-dienamides A–F exhibited insecticidal activity and stimulated the uptake of radiosodium. In contrast, H, the 3,5-dienamide otherwise identical in structure to B, was neither toxic to insects nor active as an enhancer of sodium uptake. These findings confirm the critical role in this series of compounds for the position of unsaturation in effecting toxicity (Elliott et al., 1987b) and imply that this requirement reflects the specificity of the target site. In addition, alterations in the length of the alkylene chain in *N*-(2-methylpropyl)-2,4-dienamides was found to influence the toxicity of these compounds to house flies and mustard beetles (Elliott et al., 1987d). Results from sodium flux and [^{3}H]BTX-B binding assays showed that increasing the length of the chain by two carbons (compare A and G, Table I) effectively separates the binding of *N*-alkylamides to site 2 from the subsequent transduction event that alters sodium uptake. Thus, G apparently binds to site 2 but functions only as an antagonist of other activators and is not effective as an insecticide. This finding illustrates the need for functional assays as well as binding assays to establish the mechanisms of action of new compounds. Finally, methyl substitution at the α position of the amide moiety does not affect the activity of *N*-alkylamides as sodium channel toxins (see A and B, Table I) or as insecticides (Elliott et al., 1987b).

In summary, the results of assays to determine the biochemical effects of *N*-alkylamides confirm and extend previous findings with BTG 502 in mouse brain preparations (Ottea et al., 1989) and electrophysiological studies with insect nerve preparations (Blade et al., 1985; Lees and Burt, 1988) and implicate site 2 of the sodium channel as the site of action for these compounds. Because the *N*-alkylamides appear to affect a sodium channel domain distinct from that of the pyrethroids and DDT, the development of such compounds represents an attractive strategy for the control of insect populations in which pyrethroid resistance has arisen.

ACKNOWLEDGMENT

These studies were supported by grants from the National Institutes of Health (ES02160) and the National Science Foundation (PCM-8400099/Biological Instrumentation). We thank Pamela Adams for her excellent technical support.

LITERATURE CITED

Black, M. H.; Blade, R. J.; Moss, M. D. V.; Nicholson, R. A. Novel Insecticidal Unsaturated Lipid Amides. *Pestic. Sci. Biotechnol., Proc. Int. Congr. Pestic. Chem., 6th, 1986*; Greenhalgh, R., Roberts, T. R., Eds.; Blackwell: Oxford, U.K., 1987; pp 1B–21.

Blade, R. J.; Burt, P. E.; Hart, R. J.; Moss, M. D. V. The Action of Insecticidal Isobutylamide Compounds on the Insect Nervous System. *Pestic. Sci.* **1985**, *16*, 554.

Bloomquist, J. R.; Soderlund, D. M. Pyrethroid Insecticides and DDT Modify Alkaloid-Dependent Sodium Channel Activation and its Enhancement by Sea Anemone Toxin. *Mol. Pharmacol.* **1988**, *33*, 543–550.

Brown, G. B. ^{3}H-Batrachotoxinin-A Benzoate Binding to Voltage-Sensitive Sodium Channels: Inhibition by the Channel Blockers Tetrodotoxin and Saxitoxin. *J. Neurosci.* **1986**, *6*, 2064–2070.

Brown, G. B.; Gaupp, J. E.; Olsen, R. W. Pyrethroid Insecticides: Stereospecific Allosteric Interaction with the Batrachotoxinin-A Benzoate Binding Site of Mammalian Voltage-Sensitive Sodium Channels. *Mol. Pharmacol.* **1988**, *34*, 54–59.

Catterall, W. A. Structure and Function of Voltage-Sensitive Ion Channels. *Science* **1988**, *242*, 50–61.

Catterall, W. A.; Morrow, C. S.; Daly, J. W.; Brown, G. B. Binding of Batrachotoxinin A 20-α-Benzoate to a Receptor Site Associated With Sodium Channels in Synaptic Nerve Ending Particles. *J. Biol. Chem.* **1981**, *256*, 8922–8927.

Crombie, L.; Denman, R. Insecticidal Amides. Synthesis of Natural 2(*E*),4(*E*),10(*E*)-Pipericide, its 2(*E*),4(*E*),10(*Z*)--Stereomer, and Related Isobutylamides. *Tetrahedron Lett.* **1984**, *25*, 4267–4270.

Elliott, M. Lipophilic Insect Control Agents. In *Recent Advances in the Chemistry of Insect Control*; Janes, N. F., Ed.; Royal Society of Chemistry: London, 1985; pp 73–102.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A.; Sawicki, R. M. Insecticidal Amides with Selective Potency Against a Resistant (*Super-kdr*) Strain of Houseflies (*Musca domestica* L.). *Agric. Biol. Chem.* **1986**, *50*, 1347–1349.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Khambay, B. P. S.; Sawicki, R. M. Selectivity and Resistance to Non-Ester Pyrethroids and *N*-Alkylamides in Houseflies (*Musca domestica* L.). In *Combating Resistance to Xenobiotics-Biological and Chemical Approaches*; Ford, M. G., Holloman, D. W., Khambay, B. P. S., Sawicki, R. M., Eds.; Ellis Horwood, Ltd.: Chichester, England, 1987a; pp 306–313.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A. Synthesis and Insecticidal Activity of Lipophilic Amides. Part 1: Introductory Survey, and Discovery of an Active Synthetic Compound. *Pestic. Sci.* **1987b**, *18*, 191–201.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A. Synthesis and Insecticidal Activity of Lipophilic Amides. Part 3: Influence of Chain Length and Terminal Group in *N*-(2-Methylpropyl)-2,4-Dienamides. *Pestic. Sci.* **1987c**, *18*, 211–221.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A. Synthesis and Insecticidal Activity of Lipophilic Amides. Part 5: Influence on Activity of Varying the Substituent on Nitrogen. *Pestic. Sci.* **1987d**, *18*, 229–238.

Elliott, M.; Farnham, A. W.; Janes, N. F.; Johnson, D. M.; Pulman, D. A. Synthesis and Insecticidal Activity of Lipophilic Amides. Part 6: 6-(Disubstituted-phenyl)hexa-2,4-dienamides. *Pestic. Sci.* **1987e**, *18*, 239–244.

Ghiasuddin, S. M.; Soderlund, D. M. Pyrethroid Insecticides: Potent, Stereospecific Enhancers of Mouse Brain Sodium Channel Activation. *Pestic. Biochem. Physiol.* **1985**, *24*, 200–206.

Gonoi, T.; Ashida, K.; Feller, D.; Schmidt, J.; Fujiwara, M.; Catterall, W. A. Mechanism of Action of a Polypeptide Neurotoxin from the Coral *Goniopora* on Sodium Channels in Mouse Brain Neuroblastoma Cells. *Mol. Pharmacol.* **1986**, *29*, 347–354.

Gusovsky, F.; Rossignol, D. P.; McNeal, E. T.; Daly, J. W. Pumiliotoxin B Binds to a Site on the Voltage-Dependent Sodium Channel that is Allosterically Coupled to Other Binding Sites. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1272–1276.

Jacobson, M. The Unsaturated Isobutylamides. In *Naturally Occurring Insecticides*; Jacobson, M., Crosby, D. G., Eds.; Dekker: New York, 1971; pp 137–176.

Jacques, Y.; Romey, G.; Cavey, M. T.; Kartalovski, B.; Lazdunski, M. Interaction of Pyrethroids with the Na^+ Channel in Mammalian Neuronal Cells in Culture. *Biochim. Biophys. Acta* **1980**, *600*, 882–897.

Lees, G.; Burt, P. E. Neurotoxic Actions of a Lipid Amide on the Cockroach Nerve Cord and on Locust Somata Maintained in Short-term Culture: A Novel Preparation for the Study of Na^+ Channel Pharmacology. *Pestic. Sci.* **1988**, *24*, 189–191.

Lombet, A.; Mourre, C.; Lazdunski, M. Interaction of Insecticides of the Pyrethroid Family with Specific Sites on the Voltage-dependent Sodium Channel from Mammalian Brain. *Brain Res.* **1988**, *459*, 44–53.

Miyakado, M.; Nakayama, I.; Inoue, A.; Hatakoshi, M.; Ohno, N. Chemistry and Insecticidal Activities of *Piperaceae* Amides and Their Synthetic Analogues. *J. Pestic. Sci.* **1985a**, *10*, 11–17.

Miyakado, M.; Nakayama, I.; Inoue, A.; Hatakoshi, M.; Ohno, N. Insecticidal Activities of Phenoxy Analogues of Dihydropipericide. *J. Pestic. Sci.* **1985b**, *10*, 25–30.

Ottea, J. A.; Payne, G. T.; Bloomquist, J. R.; Soderlund, D. M. Activation of Sodium Channels and Inhibition of [^{3}H]-Batrachotoxinin A-20-α-Benzoate Binding by an *N*-Alkylamide Neurotoxin. *Mol. Pharmacol.* **1989**, *36*, 280–284.

Payne, G. T.; Soderlund, D. M. Allosteric Enhancement by DDT of the Binding of [^{3}H]Batrachotoxinin A-20-α-benzoate to Sodium Channels. *Pestic. Biochem. Physiol.* **1989**, *33*, 276–282.

Satelle, D. B.; Yamamoto, D. Molecular Targets of Pyrethroid Insecticides. *Adv. Insect Physiol.* **1988**, *20*, 147–213.

Soderlund, D. M.; Bloomquist, J. R. Neurotoxic Actions of Pyrethroid Insecticides. *Annu. Rev. Entomol.* **1989**, *34*, 77–96.

Soderlund, D. M.; Bloomquist, J. R.; Payne, G. T.; Ottea, J. A. Pharmacological Characterization of Insecticide-Binding Domains of the Voltage-Sensitive Sodium Channel. In *Insecticide Action: From Molecule to Organism*; Narahashi, T., Chambers, J., Eds.; Plenum: New York, 1989; pp 85–97.

Su, H. C. F. *N*-Isobutylamides. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*; Kerkut, G. A., Gilbert, L. I., Eds.; Pergamon: New York, 1985; Vol. 12, pp 273–289.

Tamkun, M. M.; Catterall, W. A. Ion Flux Studies of Voltage-Sensitive Sodium Channels in Synaptic Nerve-Ending Particles. *Mol. Pharmacol.* **1981**, *19*, 78–86.

Weiland, G. A.; Molinoff, P. B. Quantitative Analysis of Drug-Receptor Interactions: I. Determination of Kinetic and Equilibrium Properties. *Life Sci.* **1981**, 313–330.

Wilkinson, G. N. Statistical Estimations in Enzyme Kinetics. *Biochem. J.* **1961**, *80*, 324–332.

Received for review November 20, 1989. Accepted March 20, 1990.

Registry No. A, 99083-23-5; B, 105952-38-3; C, 127883-00-5; D, 127883-01-6; E, 24738-51-0; F, 108331-93-7; G, 10585-96-3; H, 109347-33-3; I, 66110-12-1.