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Sodium channel polypeptides in central nervous systems of various insects identified with site directed antibodies

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Immunoprecipitation, radiophosphorylation and SDS-PAGE autoradiography enable the characterization of sodium channel polypeptides in the central nervous system of insects belonging to four phylogenetically distinct orders: grasshoppers, cockroaches, flies and moth larvae. It has been shown that the insect sodium channels: (1) Are recognized by the previously described (Gordon et al. (1988) Biochemistry 27, 7032–7038) site directed antibodies corresponding to a highly conserved segment linking the homologous domains III and IV in the vertebrate sodium channel α subunits. (2) Serve as substrates for phosphorylation by cAMP-dependent protein kinase. (3) Are devoid of disulfide linkage to smaller subunits unlike sodium channels in vertebrate brain. (4) Are glycoproteins as shown in the grasshopper by the decrease of apparent molecular weight following endoglycosidase F treatment and specific binding to the lectins concanavalin A and wheat germ agglutinin. (5) Reveal a diversity with regard to their (a) apparent molecular masses which range from 240 to 280 kDa and (b) V8 proteinase digestion phosphopeptides indicating either differences in the positioning of the enzymatic cleavage and/or phosphorylation sites. These results provide the first evidence for structural diversity of sodium channel subtypes among various insect orders and are compared to their mammalian counterparts.

Introduction

The voltage-sensitive sodium channels are integral membrane proteins responsible for the generation of action potentials in excitable cells Sodium channels isolated in functional form contain a large α subunit with an apparent M_r of 260000 [1] In the rat brain, α subunits are associated noncovalently with a β1 subunit (M_r 36000) and are disulfide linked to a β2 subunit with an M_r of 33000, which can be removed upon reduction without loss of functional activity [2,3] The purified eel electroplax sodium channel is functionally active as a single α subunit [4]

Sodium channel polypeptides have been purified from various vertebrate excitable tissues and the primary structures of the eel electroplax sodium channel and three distinct sodium channel α subunits from rat brain have been elucidated by cloning and sequence analysis of the complementary DNAs (cDNA) [5–7] These sequence determinations reveal homology among the various vertebrate channels In each case, the cDNA encode a large polypeptide of about 2000 amino acids containing four conserved repeated domains A high degree of conservation is also present in a short segment, linking homology domains III and IV Site directed antibodies raised against a synthetic peptide (SP19) which corresponds to the above conserved segment (anti-SP19 antibodies) were recently shown to identify sodium channel α subunits in a wide range of excitable tissues, such as rat brain, muscle and heart, and eel brain and electroplax [8]

Purification of sodium channel polypeptides from the insect central nervous system (CNS) have not been reported, but the structures of two putative sodium channels from *Drosophila*, which have recently been deduced from the genomic DNA sequence [9–11], are similar to the vertebrate sodium channels in amino acid sequence However, certain structural differences between the insect and vertebrate sodium channels are suggested by the existence of insect selective neurotoxins derived from scorpion venom which affect sodium conductance exclusively in insect neuronal preparations [12,13] In the present study, the anti-SP19 antibodies were used as tools for the identification and biochemical examination of sodium channels in central nervous system (CNS) of insects representing four phylogeneti-

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cally distinct orders, and their comparison to their mammalian counterparts

Materials and Methods

Materials The chemicals listed below were obtained from the following sources The catalytic subunit of cAMP-dependent protein kinase from Sigma (U S A), [γ-^{32}P]ATP (3000 Ci/mmol) from New England Nuclear (NEN, U S A), [11-^{3}H]saxitoxin (63 Ci/mmol) from Amersham Laboratories (U K), wheat germ lectin (WGA) from *Triticum vulgaris* immobilized on Sepharose 6MB and concanavalin A-Sepharose 4B from Sigma (U S A), endoglycosidase F (*Flavobacterium meningosepticum*) (Endo F) from NENZYMES (NEN, U S A), Protein A-Sepharose CL-4B from Sigma (U S A), *Staphylococcus aureus* V8 proteinase type XVII-B from Sigma (U S A)

Synthetic peptide SP19 and anti-SP19 antibodies The synthetic peptide SP19 (KTEEQKKYYNAMKKLGSKK) and the anti-SP19 antibodies were previously described [8], and were kindly provided by Dr William A Catterall

Preparation of insect neuronal membranes All dissections and preparations of insect neuronal tissues were performed in buffer E of the following composition 0 25 M mannitol, 10 mM EDTA, 5 mM Hepes (adjusted to pH 7 4 with Tris), phenylmethylsulfonyl fluoride (50 μg/ml), 1 μM pepstatin A, 1 mM iodoacetamide and 1 mM 1,10-phenanthroline The following animals and their respective nervous tissues were dissected and employed grasshopper *Locusta migratoria* brains, subesophageal ganglia and ventral nerve cords, cockroach *Periplaneta americana* ventral nerve cords, blowfly *Sarcophaga falculata* whole heads, larvae of 5th instar of the moth *Spodoptera littoralis* brains and ventral nerve cords and rat whole brains The dissected tissues were treated according to a previously described procedure [14] Briefly, the dissected nervous tissues were homogenized on ice in buffer E, debris were removed by centrifugation at 800 $\times g$ and the membranes collected at 27000 $\times g$ (P_2) The P_2 pellet was suspended in buffer E and adjusted to 12 5% Ficoll After centrifugation at 10000 $\times g$ for 75 min the floating pellicle (P_2L) representing the enriched synaptosomal fraction was collected Following treatment by hypotonic medium (5 mM Tris-HCl (pH 7 4), 1 mM EDTA and the above proteinase inhibitors) the membranes were collected, resuspended in buffer E and stored at $-80\,^{\circ}C$ until used In the case of the nervous tissue from the moth larvae, after the homogenization the trachea were removed by filtration through a nylon mesh The membranes were collected by centrifugation at 27000 $\times g$ and washed once by buffer E No further fractionation was done Rat brain crude synaptosomal membrane fraction (P_3) was prepared according to Ref 15 The P_3 membranes were lysed by the hypotonic medium as described above and stored at $-80\,^{\circ}C$

Solubilization, immunoprecipitation and phosphorylation of sodium channels Concentration of sodium channels in the various membrane preparations was determined by specific binding of saxitoxin ([^{3}H]STX) at 20 nM on ice using the rapid filtration assay on GF/F filters (Whatman) as described in Ref 15 Samples of membrane preparations, corresponding to 100–300 fmoles of STX binding sites, were diluted in a solubilization medium composed of 50 mM choline chloride, 10 mM EDTA, 10 mM EGTA, 50 mM potassium phosphate (pH7 4), 3% Triton X-100 containing the proteinase inhibitors phenylmethylsulfonyl fluoride (50 μg/ml), iodoacetamide (1 mM), pepstatin A (1 μM) and 1,10-phenantroline (1 mM) After incubation for 1 h on ice, residual membranes were sedimented at 8000 $\times g$ for 20 min The supernatant was incubated for 16 h with 8 μl of anti-SP19 antibodies at 4 °C The antigen-antibody complexes were isolated by adsorption to 10 mg of protein A-Sepharose and centrifugation at 8000 $\times g$ for 1 min The pellets were washed twice with phosphorylation buffer as previously described [16] The immunoprecipitated sodium channels were radiolabelled by phosphorylation with 1 μg of catalytic subunit of the cAMP-dependent protein kinase (reconstituted in 5 mM dithiothreitol) and 10 μCi of [γ-^{32}P]ATP for 1 min at 36 °C according to Refs 16 and 17

SDS gel electrophoresis (SDS-PAGE) The pellets after phosphorylation were suspended in a sample buffer composed of 3% SDS, 30 mM Tris (adjusted to pH 6 8 with HCl), 2 mM EDTA, 5% sucrose, 5% 2-mercaptoethanol and boiled for 2–3 min In the case where the effect of reduction of disulfide bonds was studied (Fig 2), reduction was done in the presence of 15 mM 2-mercaptoethanol All samples were incubated with 60 mM iodoacetamide and boiled again for 1 min The proteins were resolved by electrophoresis through a stacking gel of 3% acrylamide and separating gel of 3–10%, as previously described [16,18] Radioactive bands were visualized by autoradiography

Results

Immunoprecipitation by anti-SP19 antibodies and phosphorylation of various insect neuronal sodium channels

The predicted intracellular loop connecting the third and fourth homologous domains contains a long cytoplasmic segment of identical amino acid sequence in the three sodium channels from rat brain, eel electroplax and the two putative *Drosophila* sodium channels The anti-SP19 antibodies, which recognize this segment, were shown to efficiently immunoprecipitate purified rat brain sodium channels and to identify sodium channels in various vertebrate excitable tissues as well as in insect

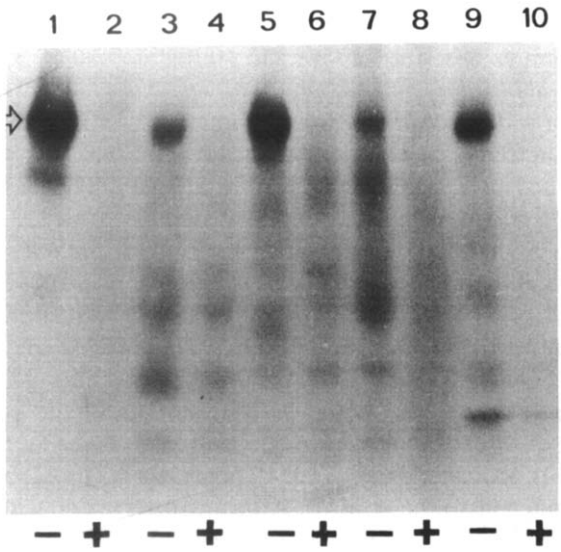

Fig 1 Identification of sodium channel α subunits by immunoprecipitation and phosphorylation Lysed P_3 membranes from rat brain (lanes 1, 2), lysed P_2L membranes from grasshopper central nervous tissue (lanes 3, 4), lysed P_2L membranes from cockroach ventral nerve cord (lanes 5, 6), lysed P_2L membranes from fly heads (lanes 7, 8) and total membrane fraction from moth central nervous tissue (lanes 9, 10) were prepared (see Materials and Methods), and sodium channels were solubilized and immunoprecipitated with anti-SP19 antibodies in the presence (+) or absence (−) of 15 μg of the synthetic peptide SP19 After radioactive labeling by reaction with the catalytic subunit of cAMP-dependent protein kinase and [γ-^{32}P]ATP, the phosphorylated sodium channels were resolved by SDS-PAGE after reduction of disulfide bonds, as described under Methods 100–300 fmoles of sodium channels, as assessed by saxitoxin binding assay of the corresponding membrane preparations, were used in each lane The arrow marks the migration position of the α subunit from rat brain (M_r = 260000)

(*Schistocerca americana*) neuronal preparation [8] All these sodium channels have been shown to be substrates for the cAMP-dependent protein kinase except the sodium channel from skeletal muscle [8]

In order to identify and examine the phosphorylation of sodium channels in various insect central nervous tissues, sodium channels, measured as saxitoxin binding sites, were solubilized with Triton X-100 and immunoprecipitated with anti-SP19 antibodies The immunoprecipitates were then incubated with [γ-^{32}P]ATP and catalytic subunit of the cAMP-dependent protein kinase under conditions suitable for phosphorylation of sodium channels from vertebrate tissues, and the phosphorylated polypeptides were analyzed by SDS-PAGE As illustrated in Fig 1, sodium channels from grasshopper (lane 3), cockroach (lane 5), fly (lane 7) and moth (lane 9) CNS were recognized by the anti-SP19 antibodies and phosphorylated by cAMP-dependent protein kinase

As shown in Fig 1, immunoprecipitation of sodium channel α subunits from rat brain (lane 1) and all insect sodium channels (lanes 3, 5, 7 and 9) was prevented by prior incubation of the antibodies with excess SP19 peptide, confirming their identification (lanes 2, 4, 6, 8 and 10, respectively) These results indicate that the segment recognized by the anti-SP19 antibodies is conserved in all the above insect sodium channels However, their apparent molecular weights show some variations (see Table I and Fig 2)

Are α subunits of insect sodium channels disulfide linked with smaller (β2) subunits?

When rat brain sodium channels are immunoprecipitated, phosphorylated and analyzed by SDS-PAGE, the phosphorylated α subunits reveal a decrease in the apparent molecular weight following reduction of disulfide bonds from M_r 290000 (Fig 2, lane 9) to M_r 260000 (Fig 2, lane 10), characteristic of the free α subunits [1] This change in the apparent molecular weight was shown to be a direct consequence of the release of the β_2 subunit [2] Thus a decrease of the apparent molecular weight of α subunits upon reduction provides evidence for the presence of disulfide-linked β_2 subunits in sodium channels also from other biological sources [8]

With this background the data presented in Fig 2 clearly indicate that, in contrast to the rat brain, the

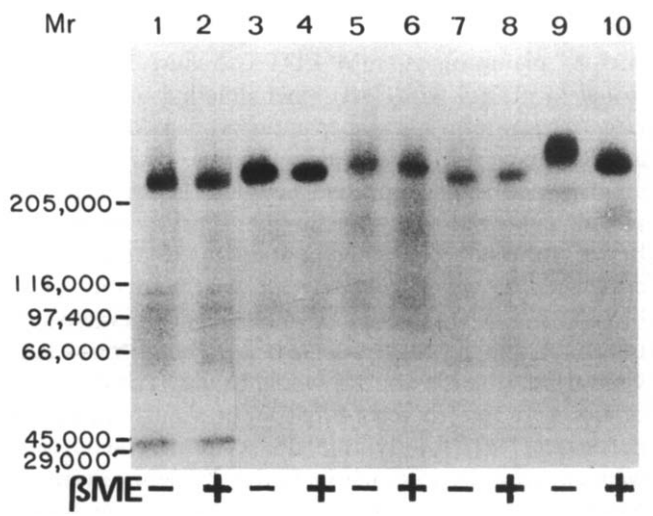

Fig 2 Immunoprecipitation and phosphorylation of sodium channels, with and without reduction, derived from the CNS of various insects Sodium channels were solubilized from membrane preparations (as in Fig 1), immunoprecipitated with SP19 antibodies, radioactively labeled by phosphorylation with cAMP-dependent protein kinase and [γ-^{32}P]ATP, resolved by SDS-PAGE with (lanes 2, 4, 6, 8 and 10) or without (lanes 1, 3, 5, 7 and 9) reduction of disulfide bonds with 15 mM 2-mercaptoethanol (βME) and visualized by autoradiography as described under Materials and Methods As shown, there was no significant change in the apparent molecular weight of the sodium channel α subunits in the CNS of grasshopper (lanes 1, 2), cockroach (lanes 3, 4), fly heads (lanes 5, 6) or moth (lanes 7, 8) following reduction, in contrast to the rat brain (lanes 9, 10) sodium channels The bars on the left indicate the migration positions of molecular weight markers From top to bottom myosin (M_r = 205000), β-galactosidase (M_r = 116000), phosphorylase B (M_r = 97400), bovine albumin (M_r = 66000), egg albumin (M_r = 45000) and carbonic anhydrase (M_r = 29000)

TABLE I

Apparent molecular weight of sodium channel α subunits in insect CNS

The data represent mean $M_r \pm$ S E ($n = 3$)

The insect		Apparent molecular weight
species	order	
Grasshopper *Locusta migratoria* *Schistocerca americana*	Orthoptera (Exopterygota)	247200 ± 2500 280000 [a]
Cockroach *Periplaneta americana*	Dictyoptera (Exopterygota)	255600 ± 2800
Fly (head) *Sarcophaga falculata*	Diptera (Endopterygota)	260000 ± 1500
Moth *Spodoptera littoralis*	Lepidoptera (Endopterygota)	238800 ± 2800

[a] From Gordon et al [8]

various insect CNS sodium channels are devoid of disulfide linked smaller subunits, as previously shown in *Schistocerca gregaria* locust [8]

The molecular weight analysis of the insect sodium channels in Fig 2 reveal variations in their apparent molecular weight in the range of 240000–260000 as specified in Table I

Analysis of limited proteolytic maps of sodium channels from insects

Molecular weight differences among the various insect sodium channel α subunits suggest that they are distinct polypeptides To provide further evidence for their chemical variability partial proteolytic maps were obtained from the phosphorylated α subunits resolved by SDS-PAGE The various sodium channels were solubilized, immunoprecipitated, phosphorylated and separated by a SDS gel (as in Figs 1 and 2) The excised ^{32}P-labeled α subunit bands were subjected to proteolytic cleavage using the V8 proteinase in a second gel, according to the method of Cleveland et al [19] (see legend to Fig 3) Various amounts of the proteinase, several digestion duration and separating conditions were examined Fig 3 presents proteolytic products separated under conditions of complete cleavage indicated by the absence of uncleaved phosphorylated polypeptides at the starting point of the SDS gel as well as absence of size changes in the low molecular weight proteolytic products As illustrated in Fig 3 the various insect α subunits treated by the V8 proteinase resulted in phosphopeptides of different number or mobility typical for each insect sodium channel source These differences were also observed when incomplete cleavage was applied (data not shown) The results suggest that the various insect sodium channels may present structural variations due to either different V8 proteinase cleavage sites (aspartic and glutamic acid residues) and/or different positions or number of phosphorylation sites

Glycosylation of insect sodium channels

15–30% of the apparent mass of purified sodium channel α subunits from vertebrate brains, skeletal muscle and eel electroplax consist of N-linked carbohydrate, half of which is sialic acid [20–23] Glycosidases reduce the apparent molecular weight of these α subunits, providing a method to examine the extent of their glycosylation [8,20] Little is known about the structure of insect glycoproteins except that N-linked oligosaccarides from insect cells lack sialic acid [24]

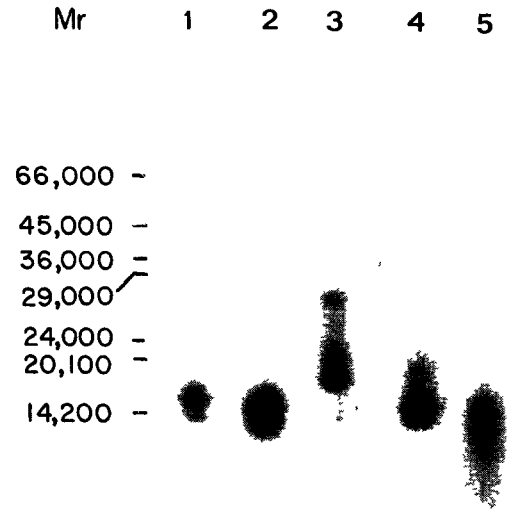

Fig 3 Proteolytic maps of the insect sodium channel α subunits treated by the *S aureus* V8 proteinase ^{32}P-labeled sodium channel α subunits were identified by autoradiography after immunoprecipitation, radiophosphorylation and separation on a 3–10% polyacrylamide SDS-PAGE as described under Materials and Methods and in the legend to fig 1 The α subunit gel slices were excised from the gel and equilibrated in 0 125 M Tris-HCl (pH 6 8), 0 1% SDS, 1 mM EDTA, 20% glycerol, placed in the sample wells of a second gel with a stacking gel of 3% and a separating gel of 8–15% polyacrylamide The electrophoresis was done as described in Cleveland et al [19] The digestion was allowed to continue for 30 min using 25 units of the V8 proteinase The separated cleavage products were identified by autoradiography The cockroach sodium channel (lane 1) yielded two major polypeptides with apparent molecular weights of 15800 and 13500 The grasshopper digested sodium channel (lane 2) yielded two similar peptides with apparent molecular weights of 14800 and 13000 The fly sodium channel (lane 3) resulted in a set of peptides of different molecular weight, the major being 30700, 20170 and 17800, clearly distinct from the others The moth channel (lane 4) resulted in not clearly resolved peptides, the higher being about 20000 and the lower in the range of 16700–14600 The rat brain sodium channel α subunits (lane 5) used for comparative purposes, resulted in small molecular weight peptides which were not clearly resolved under any of our conditions The bars on the left correspond to the migration positions of the following molecular weight markers (from top to bottom) Bovine albumin ($M_r = 66000$), egg albumin ($M_r = 45000$), glyceraldehyde-3-phosphate dehydrogenase ($M_r = 36000$), carbonic anhydrase ($M_r = 29000$), trypsinogen ($M_r = 24000$), trypsin inhibitor ($M_r = 20100$) and α-lactalbumin ($M_r = 14200$)

TABLE II

Binding of saxitoxin receptors (STXR) from grasshopper CNS to lectins immobilized on Sepharose

	STXR (fmol)	Fraction from total bound STXR (%)	Recovery of specifically bound (%)	STXR protein (mg)	Measured binding activity (pmol STX/mg protein)
Specifically bound STXR to WGA [a]	544	53	100	1 27	0 43
Eluted STXR by 250 mM GlcNAc [a]	182	18	33 5	0 16	1 15
Specifically bound STXR to ConA [b]	1068	57	100	– [c]	– [c]
Eluted STXR by 100 mM α-methyl-mannoside	335	19	32 8	– [c]	– [c]

[a] Neuronal membranes from *Locusta migratoria* CNS were solubilized in 1% cholate, 0 2% Triton X-100, 0 2% phosphatidylcholine in 130 mM choline chloride, 1 mM EDTA, 20 mM Hepes-Tris (pH 7 4) in the presence of proteinase inhibitor cocktail (Buffer A) The solubilized membranes were centrifuged for 30 min at 132000 × *g* and the supernatant was mixed with 0 4 ml WGA-Sepharose beads equilibrated in buffer A The solubilized receptors were allowed to bind the lectin for 4 h at 4°C, and the beads were washed eight times with 0 5 ml of buffer A Elution of bound material was performed by 250 mM GlcNAc in buffer A Aliquots were taken at each step for determination of [^{3}H]STX binding and protein contents Protein determination was performed using the method of Ref 41 The bound STX receptor to the pre-equilibrated WGA-Sepharose with 250 mM GlcNAc and in the presence of 250 mM GlcNAc throughout is defined as non specific binding to WGA-Sepharose and was determined in a parallel assay The specific binding to the lectin is defined as the unbound STX receptors in the presence of GlcNAc The estimation of the unbound or eluted STX receptors was performed in the presence of 20 nM [^{3}H]STX with or without 2 μM Tetrodotoxin for 1 h on ice and terminated by rapid filtration according to Bruns et al [42]

[b] STX receptor binding to ConA-Sepharose was measured under the same conditions as described above in (a), except that ConA-Sepharose was used (instead of WGA-Sepharose) and buffer A was supplemented with 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 2 mg/ml hemoglobin to improve specific binding to the ConA The non specific binding was measured in the presence of 100 mM α-methylmannoside Prior to the [^{3}H]STX binding assay, the unbound or eluted material was supplemented with 5 mM EDTA and 5 mM EGTA in order to chelate divalent cations which may interfere with the saxitoxin binding

[c] In the experiments with ConA-Sepharose, it was impossible to quantitate the solubilized membrane proteins due to the presence of hemoglobin (see b))

In order to examine the nature of the glycosylation of the insect sodium channel polypeptides, we measured the shift in apparent molecular weight of the α subunit after enzymatic deglycosylation, using endoglycosidase F (Endo F) Endo F cleaves glycans of both the high mannose and the complex type, linked through asparagine to the protein backbone [25] The treatment with Endo F resulted in a molecular weight decrease of nearly 11% of the grasshopper sodium channel polypeptide mass (Fig 4, lane 1) It is possible that other carbohydrate moieties are attached through O-linked bonds which are not cleaved by the Endo F No acid treatment, that hydrolyzed the O-linked as well as the N-linked carbohydrates [26] was used, since this treatment was claimed to result in no recovery of the α-subunit polypeptide from rat brain [20]

In order to further establish the presence of carbohydrate moieties in the grasshopper sodium channel, we examined lectin binding properties to the solubilized native sodium channels Sodium channels from grasshopper CNS were solubilized by treatment with ionic and nonionic detergents and were detected in solubilized form by high affinity binding of saxitoxin (STX) (Table II) The solubilized sodium channels were applied to wheat germ agglutinin (WGA) or concanavalin A (ConA) immobilized on Sepharose beads and the bound material was eluted by *N*-acetylglucosamine (GlcNAc) or α-methylmannoside, respectively Binding to the lectins and recoveries of eluted STX receptors were comparable for the two lectins studies (see Table IIa, b) Specific binding to the lectins was about 55% of the total amount of STX receptor bound in the absence of sugars (Table II), indicating that the insect sodium channels form relatively high hydrophobic interactions with the lectins and Sepharose Material eluted from the WGA-Sepharose revealed an increase of about three times in its saxitoxin specific binding capacity (Table II)

The data presented in Table II suggest that the grasshopper sodium channels are indeed glycoproteins Since the WGA minimal requirements for binding are both *N*,*N*′-diacetylchitobiose core and β-*N*-acetylglucosamine attached to a β-linked mannose, we suggest that at least part of the high mannose carbohydrate moieties of the insect sodium channels possess *N*-acetylglucoseamine in a terminal position

Discussion

Comparison with vertebrate sodium channels

Our results suggest that the conserved sequence recognized by the anti-SP19 antibodies in sodium channel α subunits in a wide range of vertebrate excitable tissues [8] is also highly conserved in various insects This high degree of conservation provides further evidence for the suggested function of this segment in the inactivation of sodium channels [27,28]

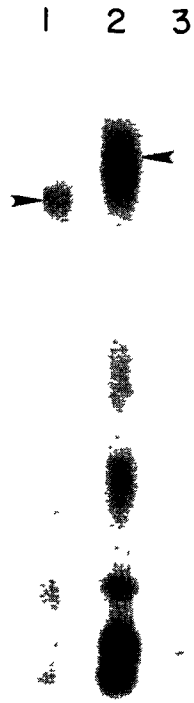

Fig 4 Effect of endoglycosidase F (Endo F) on ^{32}P-labeled sodium channel α subunits from grasshopper The sodium channels were immunoprecipitated by anti-SP19 antibodies and radiolabeled by phosphorylation with catalytic subunit of cAMP-dependent protein kinase and [γ-^{32}P]ATP as described under Materials and Methods Removal of carbohydrate using Endo F was carried out under the conditions described by Elder and Alexander [25] The labeled immunoprecipitated sodium channels were adjusted to 0 1% SDS, 1% Triton X-100, 50 mM EDTA, 1% 2-mercaptoethanol and 100 mM sodium phosphate (pH 6 1) and incubated for 4 h at 37 °C with 1 unit of endoglycosidase F (lane 1) The digestion was terminated by addition of concentrated sample buffer to achieve 3% SDS in the samples and boiled for 3 min (see Materials and Methods) A control sample was treated in the same way but without addition of Endo F (lane 2) The SP19 peptide was added to one sample during incubation together with the anti-SP19 antibodies in order to identify the sodium channel polypeptide (lane 3) Following glycosidase digestion, the samples were analyzed by SDS-PAGE in 3–10% acrylamide and autoradiography Arrows indicate the sodium channel α subunit The shift in apparent molecular weight of the grasshopper sodium channel is by about 27000

Like all sodium channel α subunits in vertebrate CNS the present insect channels also serve as substrates for the cAMP-dependent protein kinase It was recently proposed that the cAMP-dependent phosphorylation modifies the functional properties of vertebrate Na^+ channels [29,30] It is noteworthy, that the phosphorylation sites in the rat brain sodium channel α subunits [31] are allocated on a segment which is highly variable in other known vertebrate sodium channels [5,32,8] None of the phosphorylation sites which have been identified in the rat brain sodium channel [31] was revealed in the amino acid sequence of the putative *Drosophila* sodium channel from the *para* locus [11] It is thus expected that the phosphorylation sites we have detected in the various insect sodium channels differ from those of vertebrates

The recent amino acid sequence of *Drosophila* sodium channel reveals six possible N-linked glycosylation sites located in presumptive extracellular domains [11] Our results, obtained with the grasshopper neuronal preparation, indicate that the insect sodium channels are, indeed, glycoproteins It is possible, however, that the extent of glycosylation may vary in different insect species and this may account in part for the variability demonstrated in the insect sodium channel polypeptides examined so far (Table I) Glycosylation of mammalian sodium channels was shown to play an important role in the synthesis of functional sodium channels [33,34] and their assembly and insertion into the cell surface [35] It is noteworthy, however, that in contrast to various vertebrate sodium channel preparations, neuroaminidase failed to reduce the apparent molecular weight of an insect sodium channel polypeptide [8], confirming the absence of sialic acid in insect cells [24].

Unlike the sodium channels in brains of various classes of vertebrates, namely mammalian, avian, reptilian, amphibian and bony fish [36], the sodium channels in the various insect orders studied are not disulfide linked to small β2 subunits (Fig 2, and Ref 8) These results are consistent with the primary functional role of the α subunit, revealed by the findings that α-subunit mRNAs can direct the synthesis of functional sodium channels in *Xenopus* oocytes [37,38,32]

Diversity of insect sodium channels

The various insect CNS sodium channels reveal some diversity in their apparent molecular weights and the limited proteolytic phosphopeptide maps The latter may indicate differences either in the positions of the cleavage and/or phosphorylation sites of the various insect sodium channels Analysis of the cDNAs from the *para* locus in *Drosophila* suggests that a minimum of three, and possibly more, different forms of sodium channels could be expressed from the *para* locus by alternative splicing [11] Our data suggest that various insects, belonging to different orders, may preferentially express different sodium channel polypeptides as their major sodium channel form In addition to the differences outlined above, a clear pharmacological distinction between the insect and vertebrate sodium channels has been demonstrated in studies with the insect selective neurotoxins derived from scorpion venoms [12] These toxins were shown to bind and affect sodium conductance exclusively in insect neuronal membranes [39] and are devoid of any toxicity to mammals [40] The clarification of the structural basis of the insect sodium channel directed selectivity of the above insect toxins requires further study

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References

1 Catterall, W A (1986) Annu Rev Biochem 55, 953–985
2 Messner, D J and Catterall, W A (1986) J Biol Chem 261, 211–215
3 Messner, D J, Feller, D J, Scheuer, T and Catterall, W A (1986) J Biol Chem 261, 14882–14890
4 Recio-Pinto, E, Duch, D S, Levinson, S R and Urban, B W (1987) J Gen Physiol 90, 375–395
5 Noda, M, Shimizu, S, Tanabe, T, Takai, T, Kayano, T, Ikeda, T, Takahashi, H, Nakayama, H, Kanaoka, Y, Minamina, N, Kangawa, K, Matsuo, H, Raftery, M, Hisose, T, Inayama, S, Hayashida, H, Miyata, T and Numa, S (1984) Nature (London) 312, 121–127
6 Noda, M, Ikeda, T, Kayano, T, Suzuki, H, Takeshima, H, Kurasaki, M, Takahashi, H and Numa, S (1986) Nature (London) 320, 188–192
7 Kayano, T, Noda, M, Flockerzi, V, Takahashi, H and Numa, S (1988) FEBS Lett 228, 187–194
8 Gordon, D, Merrick, D, Wollner, D A and Catterall, W A (1988) Biochemistry 27, 7032–7038
9 Salkoff, L, Butler, A, Wei, A, Scavada, N, Giffen, K, Ifune, C, Goodman, R and Mandell, G (1987) Science (Washington, D C) 237, 744–749
10 Ramaswami, M and Tanouye, M A (1989) Proc Natl Acad Sci USA 86, 2079–2082
11 Loughney, K, Kreber, R and Ganetzky, B (1989) Cell 58, 1143–1154
12 Zlotkin, E (1988) in Comparative Invertebrate Neurochemistry (Lunt, G G and Olsen, R W, eds), pp 256–324, Croom Helm Publ, London
13 Gordon, D, Jover, E, Couraud, F and Zlotkin, E (1984) Biochim Biophys Acta 778, 349–358
14 Zlotkin, E and Gordon, D (1985) in Neurochemistry Techniques in Insect Research (Miller, T A and Breer, H, eds), Springer Series in Experimental Entomology, pp 243–295, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo
15 Catterall, W A, Morrow, C and Hartshorne, R (1979) J Biol Chem 254, 11379–11387
16 Schmidt, J, Rossie, S and Catterall, W A (1985) Proc Natl Acad Sci USA 82, 4847–4851
17 Gordon, D, Merrick, D, Auld, V, Dunn, R, Goldin, A L, Davidson, N and Catterall, W A (1987) Proc Natl Acad Sci USA 84, 8682–8686
18 Maizel, J V (1971) Methods Virol 51, 179–224
19 Cleveland D W, Fischer, S G, Kirschner, M W and Laemmli, U K (1977) J Biol Chem 252, 1102–1106
20 Messner, D J and Catterall, W A (1985) J Biol Chem 260, 211–215
21 Miller, J A, Agenw, W S and Levinson, S R (1983) Biochemistry 22, 462–470
22 Grishin, E V, Kovalenko, V A, Pashkov, V N and Shamotienko, O G (1984) Biol Membr 1, 858–867
23 Roberts, R H and Barchi, R L (1987) J Biol Chem 262, 2298–2303
24 Hsieh, P and Robbins, P W (1984) J Biol Chem 259, 2375–2382
25 Elder, J H and Alexander, S (1982) Proc Natl Acad Sci USA 79, 4540–4544
26 Edge, A S B, Faltynek, C R, Hof, L, Reichert, L E and Weber, P (1981) Anal Biochem 118, 131–137
27 Vassilev, P M, Scheuer, T and Catterall, W A (1988) Science 241, 1658–1661
28 Stuhmer, W, Conti, F, Suzuki, H, Wang, X, Noda, M, Yahagi, N, Kubo, H and Numa, S (1989) Nature 339, 597–603
29 Coombs, T, Schewer, S Rossie, S and Catterall, W A (1988) Biophys J 53, 542a
30 Catterall, W A (1988) Science 242, 50–61
31 Rossie, S, Gordon, D and Catterall, W A (1987) J Biol Chem 36, 17530–17535
32 Noda, M, Ikeda, I, Suzuki, T, Takeshima, H, Takahashi, T, Kuno, M and Numa, S (1986) Nature (London) 322, 826–828
33 Bar Sagi, D and Prives, J (1983) J Cell Physiol 114, 77–81
34 Waechter, C J, Schmidt, J and Catterall, W A (1983) J Biol Chem 258, 5117–5123
35 Schmidt, J W and Catterall, W A (1986) Cell 46, 437–445
36 Wollner, D A, Messner, D J and Catterall, W A (1987) J Biol Chem 262, 14709–14715
37 Sumikawa, K, Parker, I and Miledi, R (1984) Proc Natl Acad Sci USA 81, 7994–7998
38 Goldin, A L, Snutch, T, Lubbert, H, Dowsett, A, Marshall, J, Auld, V, Downey, W, Fritz, L C, Lester, H A, Dunn, R, Catterall, W A and Davidson, N (1986) Proc Natl Acad Sci USA 83, 7505–7507
39 Pelhate, M and Zlotkin, E (1982) J Exp Biol 97, 66–77
40 Dianous, S, Hoarau, F and Rochat, H (1987) Toxicon 25, 411–417
41 Peterson, G L (1977) Anal Biochem 83, 346–356
42 Bruns, R F, Lawson-Wendling, K and Pugsley, T A (1983) Anal Biochem 132, 74–81