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online data

SUPPLEMENTARY ONLINE DATA

Identification of a cluster of residues in transmembrane segment 6 of domain III of the cockroach sodium channel essential for the action of pyrethroid insecticides

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Table S1 Effects of alanine substitution of residues in IIIS6 on the voltage dependence of activation and inactivation

Results are means ± S.D. for *n* oocytes. An unpaired Student's *t* test was used to evaluate the significance of changes in mean values. Asterisks indicate significant differences from the wild-type channel as determined by Student's *t* test ($P < 0.05$).

	Activation		Inactivation		
Sodium channel type	$V_{0.5}$ (mV)	k (mV)	$V_{0.5}$ (mV)	k (mV)	n
Wild-type	−28.23 ± 0.54	5.09 ± 0.94	−48.57 ± 0.13	5.02 ± 0.18	30
V1510A	−24.15 ± 0.38	5.31 ± 0.66	−48.50 ± 1.13	4.95 ± 0.28	9
F1511I	−28.17 ± 1.07	4.74 ± 0.94	−45.15 ± 0.15	5.02 ± 0.07	9
F1512A	−28.94 ± 1.42	4.69 ± 0.64	−46.62 ± 0.46	4.94 ± 0.35	10
I1513A	−23.61 ± 1.28*	5.25 ± 0.33	−49.49 ± 1.23	4.92 ± 0.51	6
I1514A	−36.40 ± 2.07*	6.10 ± 1.60	−54.22 ± 0.95*	4.76 ± 0.27	12
F1515A	−27.49 ± 1.42	5.08 ± 0.64	−45.39 ± 1.09	5.03 ± 0.15	12
G1516A	−17.07 ± 2.07*	5.23 ± 0.55	−51.15 ± 1.01	4.54 ± 0.38	30
S1517P	−16.98 ± 0.70*	3.79 ± 0.21	−44.46 ± 0.55	5.43 ± 0.60	6
F1518A	−30.18 ± 1.83	4.56 ± 0.06	−49.16 ± 0.76	4.70 ± 0.16	10
T1520A	−31.26 ± 0.45	4.24 ± 0.55	−47.13 ± 1.01	5.02 ± 0.22	6
L1521A	−19.60 ± 1.54*	6.22 ± 0.52	−54.02 ± 0.91*	4.52 ± 0.32	19
N1522A	−20.29 ± 0.91*	7.17 ± 0.40	−59.19 ± 0.43*	4.56 ± 0.22	15
L1523A	−27.68 ± 2.16	5.17 ± 0.48	−52.89 ± 0.83	4.07 ± 0.28	7
F1524A	−36.98 ± 1.61*	3.38 ± 0.37	−41.80 ± 0.83*	5.77 ± 0.64	8
I1525A	−22.97 ± 2.27*	4.51 ± 1.52	−42.49 ± 0.39*	5.57 ± 0.18	7
G1526A	−20.43 ± 0.53*	7.71 ± 0.30	−57.84 ± 2.28*	5.83 ± 0.12	14
V1527A	−30.09 ± 0.78	5.27 ± 0.63	−55.88 ± 2.05*	5.35 ± 0.61	6
I1528A	−38.89 ± 1.88*	3.34 ± 0.32	−47.91 ± 0.93	4.84 ± 0.47	12
I1529A	−25.40 ± 1.84	5.03 ± 0.42	−49.80 ± 0.90	4.65 ± 0.23	11
D1530A	−22.54 ± 1.79*	6.52 ± 0.70	−54.13 ± 1.08*	4.61 ± 0.18	9

Table S2 Time constants of the decay of pyrethroid-induced tail currents

Type-II pyrethroid (deltamethrin, cypermethrin and fluvalinate) -induced tail currents were fitted with bi-exponential functions for the wild-type, G1516A and I1514A channels, but mono-exponential function for F1518A and N1522A channels. Type-I pyrethroid (permethrin, bioresmethrin, fenfluthrin and bioallethrin) -induced tail currents were fitted with single-exponential function for the wild-type and mutant channels. Substitutions F1518A and N1522A almost completely abolished the action of type I pyrethroids, therefore fitting of tail current decay was not possible (–). Results are means ± S.D. Asterisks show that the time constant of decay of pyrethroid-induced tail currents in channels was significantly different from the parental channel $BgNa_v1$-1A ($P < 0.05$; Student's *t* test).

	Wild-type		G1516A		F1518A	N1522A	I1514A	
Pyrethroid	τ_1 (s)	τ_2 (s)	τ_1 (s)	τ_2 (s)	τ (s)	τ (s)	τ_1 (s)	τ_2 (s)
Deltamethrin	1.6 ± 0.4	0.36 ± 0.08	1.3 ± 0.3	0.32 ± 0.06	0.30 ± 0.06*	0.29 ± 0.03*	1.8 ± 0.2	0.43 ± 0.08
Cypermethirn	1.7 ± 0.1	0.29 ± 0.05	1.2 ± 0.4	0.26 ± 0.01	0.37 ± 0.07*	0.35 ± 0.06*	1.9 ± 0.4	0.36 ± 0.11
Fluvalinate	1.3 ± 0.5	0.27 ± 0.06	1.1 ± 0.5	0.25 ± 0.02	0.20 ± 0.03*	0.28 ± 0.12*	1.4 ± 0.1	0.31 ± 0.08
Permethrin	0.48 ± 0.17		0.20 ± 0.08*		–	–	0.35 ± 0.12	
Biorethmethrin	0.19 ± 0.07		0.25 ± 0.07		–	–	0.17 ± 0.04	
Fenfluthrin	0.21 ± 0.06		0.25 ± 0.07		–	–	0.26 ± 0.03	
Bioallethrin	0.23 ± 0.07		0.22 ± 0.05		–	–	0.24 ± 0.06	

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Table S3 Effects of amino acid substitutions I1514, G1516, F1518 and N1522 on the voltage-dependence of activation and inactivation

Results are means ± S.D. for *n* oocytes. An unpaired Student's *t* test was used to evaluate the significance of changes in mean values. Asterisks indicate significant differences from the wild-type channel as determined by Student's *t* test ($P < 0.05$).

	Activation		Inactivation		
Sodium channel type	$V_{0.5}$ (mV)	*k* (mV)	$V_{0.5}$ (mV)	*k* (mV)	*n*
Wild-type	−28.23 ± 0.54	5.09 ± 0.94	−48.57 ± 0.13	5.02 ± 0.18	30
I1514A	−36.40 ± 2.07*	6.10 ± 1.60	−54.22 ± 0.95*	4.76 ± 0.27	12
I1514V	−32.40 ± 2.93*	4.77 ± 1.14	−50.03 ± 1.26	4.69 ± 0.22	5
I1514F	−33.16 ± 2.77*	6.47 ± 0.63	−53.43 ± 0.89*	4.60 ± 0.12	6
I1514D	−33.48 ± 2.54*	5.16 ± 1.73	−54.28 ± 0.52*	4.59 ± 0.21	4
I1514C	−37.34 ± 1.89*	5.89 ± 1.34	−51.92 ± 1.25	4.45 ± 0.18	7
G1516A	−17.07 ± 2.07*	5.23 ± 0.55	−51.15 ± 1.01	4.54 ± 0.38	30
G1516D	−33.54 ± 1.79*	3.88 ± 0.94	−43.67 ± 1.49*	4.73 ± 0.16	25
G1516N	−27.45 ± 1.67	4.27 ± 0.59	−42.50 ± 1.32*	5.07 ± 0.47	20
F1518A	−30.18 ± 1.83	4.56 ± 0.06	−49.16 ± 0.76	4.70 ± 0.16	10
F1518W	−42.28 ± 1.50*	3.27 ± 0.10	−51.70 ± 0.42	4.78 ± 0.29	6
F1518C	−27.79 ± 2.21	5.14 ± 0.57	−47.36 ± 1.27	5.00 ± 0.27	12
F1518D	−29.12 ± 0.83	5.98 ± 0.40	−48.58 ± 1.52	4.90 ± 0.19	9
N1522A	−20.29 ± 0.91*	7.17 ± 0.40	−59.19 ± 0.43*	4.56 ± 0.22	15
N1522G	−25.79 ± 2.16	5.68 ± 0.82	−50.58 ± 1.32	4.45 ± 0.32	17
N1522D	−29.81 ± 1.42	5.08 ± 1.00	−50.03 ± 0.64	5.00 ± 0.15	12
N1522K	−37.63 ± 2.66*	4.45 ± 0.59	−51.86 ± 1.21	4.94 ± 0.23	13

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