Central Non-NMDA Receptors of a Honey Bee with Hereditary Kynurenine Deficiency

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 4, pp. 458-460, April, 2003
Original article submitted November 5, 2002

Pharmacological characteristics of non-NMDA receptors involved in associative learning were studied in wild honey bees (normal) and carriers of *snow laranja* mutation (kynurenine deficiency) by pharmacological analysis and behavioral criteria. The effects of systemic injections of non-NMDA receptor agonists (AMPA, kainic, quisqualic, and domoic acids), AMPA receptor antagonist (NS257-HCl), and AMPA receptor modulator (cyclothiaside) on retention of conditioned reflexes in short-term memory (1 min after the end of learning) were studied. The pharmacological characteristics of non-NMDA receptors were changed in *snow laranja* mutants with kynurenine deficiency.

Key Words: *honey bee; kynurenines; snow laranja mutation; non-NMDA receptors; short-term memory*

L-Glutamic acid, the main excitatory transmitter in CNS of mammals, plays the key role in neuroplastic and neurodegenerative processes [1,6, 10,12]. Physiological effect of the excitatory amino acids is mediated through ionotropic (subtypes NMDA and non-NMDA) and metabotropic glutamate receptors. Detection of endogenous factors modulating the state of excitatory amino acid receptors is of both theoretical and practical importance. The products of tryptophan metabolism kynurenine and kynurenic acid are endogenous ligands of excitatory amino acid receptors in mammals [1,11] and insects, and hence, can act as endogenous modulators of glutamate receptors. Polyfunctional role of kynurenines in the regulation of CNS function in health and disease is well known [1,8,11]. Insects can serve as an excellent model for the studies of the role of kynurenines in the regulation of the functional state of excitatory amino acid receptors due to natural mutations blocking successive stages of the kynurenine pathway of tryptophane metabolism (KPTM). NMDA and non-NMDA ionotropic glutamate receptors were detected in the cerebral ganglion of insects [9]. The involvement of these receptors in associative learning in insects was demonstrated [4,5]. Changed sensitivity of the main sites of NMDA receptors under conditions of kynurenine deficiency was demonstrated on genetic models (*snow laranja* mutants) [3].

We compared the pharmacological characteristics of central non-NMDA receptors in bees with normal kynurenine content and in *snow laranja* mutants with kynurenine deficiency.

MATERIALS AND METHODS

Experiments were carried out on wild bees (15-30 days) (*Apis mellifera carnica Polm*) with normal kynurenine content and *snow laranja* mutants with kynurenine deficiency caused by mutation in gene controlling activity of tryptophane oxygenase (the first enzyme of KPTM). Pharmacological characteristics of non-NMDA receptors involved in retention of associations in the short-term memory were studied in bees

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0007-4888/03/1354-0390$25.00

with normal genotype and homo- and heterozygotes by the *snow laranja* mutation [4]. The behavioral criterion was used: the number of immobilized bees responding by conditioned reaction (trunk stretching) to a conditioned signal (clove odor) 1 min after a single combination of the conditioned stimulus and unconditioned food support (50% sucrose solution). For standardization of the baseline alimentary excitability the bees were isolated from the family 3 h before the experiment. Before learning the effects of the studied substances on the sensory and alimentary excitability were studied. The effects of non-NMDA receptor ag-

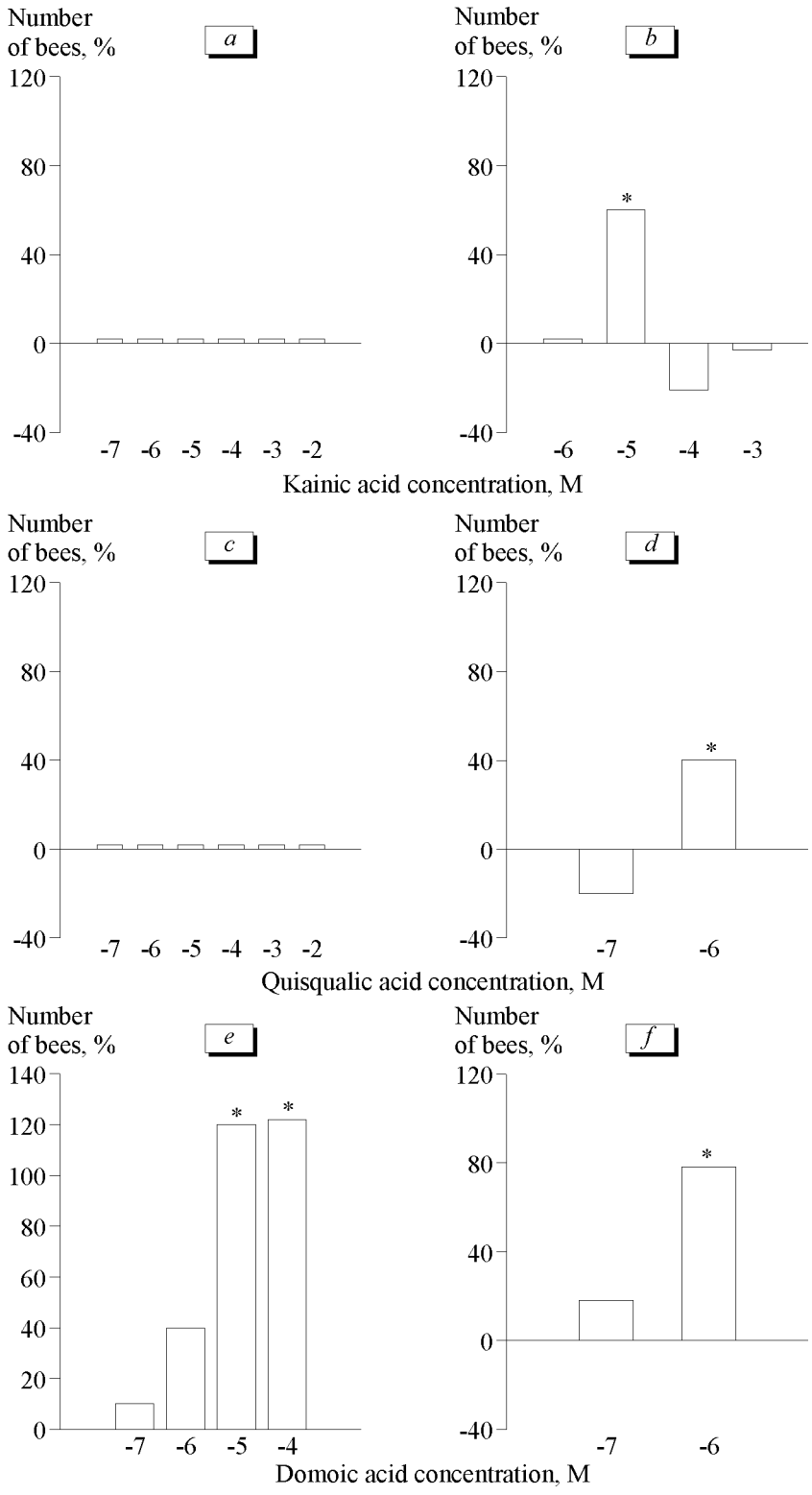

Fig. 1. Effects of kainic (*a*, *b*), quisqualic (*c*, *d*), and domoic acids (*e*, *f*) on retention of conditioned reflex in short-term memory of bees with normal genotype (*a*, *c*, *e*) and *snow laranja* mutants (*b*, *d*, *f*). Abscissa: concentrations of the test substances (M): 2 (10^{-2}), 3 (10^{-3}), *etc.*; ordinate: changes in the number of bees retaining the conditioned reflex in comparison with the control (point "0"). *$p<0.05$ compared to the control.

onists (AMPA, quisqualic, kainic, and domoic acids, Sigma), AMPA receptor modulator (cyclothiaside, Sigma), and AMPA receptor competitive antagonist NS257-HCl (RBI) on the retention of conditioned reflexes in the short-term memory were studied. The test compounds were injected (2 μl) under the second abdominal sternite to immobilized cooled bees 20 min before learning. The bees injected with the same volume of normal saline served as controls. Each experimental series was performed on 36-60 bees.

The results were statistically processed using Statgrafic plus software Student's *t* test and Mann—Whitney *U* test; the method was described in detail previously [5].

RESULTS

Injection of all test substances did not modify the sensory and alimentary excitability of bees of different genotypes. Injections of kainic and quisqualic acids in a wide range of concentrations (10^{-7}-10^{-2} M) had no effect on the formation of short-term memory in wild type bees, but significantly increased the number of mutant bees responding by conditioned reaction (trunk stretching in response to odor stimulus) 1 min after single learning session (Fig. 1, *a-d*). The sensitivity of the receptor to domoic acid in *snow laranja* mutants was by one order of magnitude higher (10^{-6} M; Fig. 1, *e-f*) than in normal insects (10^{-5} M). Active threshold concentrations of AMPA (10^{-5} M), cyclothiaside (10^{-5} M), and NS257-HCl antagonist (10^{-5} M) in homozygotes did not differ from those in wild type insects. Similar changes in the sensitivity of non-NMDA receptors to all test substances were observed in heterozygotes.

These changes in pharmacological characteristics of non-NMDA receptors under conditions of hereditary kynurenine deficiency can be caused by changes in the expression of receptor protein and in the subunit composition of the receptor. Previous experiments showed that the decrease of kynurenic acid content in patients with Huntington chorea [7] was paralleled by changes in the subunit composition of NMDA receptor. Another cause can be a delay in attaining the definite level of receptor functioning in the ontogeny previously observed in mutants [2]. Our findings can be used in the studies of the mechanisms of neurological diseases and mental deficiency under conditions of disordered kynurenine metabolism.

The study was supported by the Russian Foundation for Basic Research (grant No. 00-04-49312).

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