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Bensultap decreases neuronal excitability in molluscan and mammalian central nervous system

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Abstract

Electrophysiological experiments were performed on *in vitro* neuronal preparations from terrestrial snail and rat brain slices, to determine the effect of the insecticide bensultap. Although bensultap has low toxicity in mammals, our results showed that bensultap altered the synaptic transmission in the vertebrate as well as in the invertebrate central nervous system. Bensultap caused a significant decrease of the ACh-induced current. The effect depended on the preapplication time and the concentration of the chemical. Bensultap also had an effect on the kinetic parameters of the ACh-induced current; the desensitization time was altered in a concentration-dependent manner. In the rat brain slice preparations, we observed an increase in the amplitude of the evoked responses after a 30 min treatment. There was no effect on paired-pulse depression, but LTP-induction was significantly inhibited by bensultap. The efficacy of synaptic transmission was modified by bensultap through effects on both input integration and output organization.

Keywords: Cholinergic receptors; Insecticide; Voltage clamp; In vitro slice experiments; *Helix pomatia*; Rat

1. Introduction

Environmental toxicity of pesticides depends on their stability and specificity, as well as on their accumulation in target and non-target organisms. To evaluate the risk of applying a chemical substance, the mechanism underlying the toxic effect has to be studied in different species (Fan et al., 1995).

To control insect pests, various compounds which disturb neuronal regulation, including those acting on the cholinergic system, were developed. Cholinesterase-inhibitor organophosphorous compounds (e.g., diazinon, chlorpyrifos) have been used since 1936. They cause overexcitation in the insect nervous system (Pope, 1999), that eventually leads to death. These compounds are however, highly toxic for other species, too (Ohayo-Mitoko et al., 1997; Blaquiere et al., 2000; Dam et al., 2000). Some of them are environmentally very stable, which meant that the risk of accumulation is very high, and as a result, permission to use them as pesticides has been restricted. Meanwhile, nicotinic acid (a toxin of *Nicotiana tabacum*) and its derivatives were introduced generally as effective pesticides. Nicotinic acid acts as an agonist at nicotinic acetylcholine (ACh) receptors, and also causes excessive excitation in insects, which have a large number of nACh receptors. Recently, derivatives of nicotinic acid, namely neonicotinoids, have been widely used to control different pests. These compounds decompose more readily and show larger specificity for insect transmitter receptors than the above mentioned pesticides. Some of them, such as imidacloprid, act as full nicotinergic agonists, while others, such as nereistoxin analogues antagonize the nicotinergic receptor activity. They influence the intensity of the sodium current through the ligand-gated cholinergic receptor (Matsuda et al., 2001).

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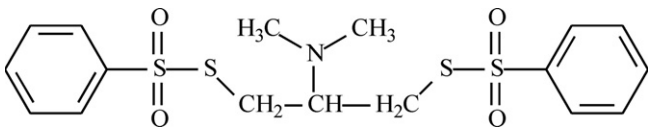

Fig. 1. Chemical structure of bensultap. Molecular weight: 431.6.

The basic structure of nicotinic cholinergic receptors is similar in all members of the animal kingdom (Walker et al., 1996). These ligand-gated ion-channels are composed of five subunits, each of which has four membrane-spanning regions. The subunits forming a functional receptor usually belong to different subclasses; thus, the receptors may possess very different characteristics depending on their subunit composition (Itier and Bertrand, 2001).

Bensultap *S*,*S*′-2-dimethylaminotrimethylene di(benzenethiosulfonate) (Fig. 1), a nereistoxin analogue, was introduced in 1993. It is used against the Colorado beetle and some other insect pests (Civelek and Weintraub, 2003).

There is evidence that high-dose *in vivo* bensultap treatment causes changes in the synaptic function, sleep–wake pattern and general behavior in rats (Szegedi et al., 2005). Although this study and other official data (The British Crop Protection Council (1987)) indicate that the toxicity of bensultap is low in mammals, it is important to characterize its effect in detail at moderate and high toxicity levels, such as may occur after accidental exposures. Bensultap is able to cross the blood–brain barrier (Dóczi et al., 1999); therefore, it is reasonable to investigate its direct neuronal effects. *In vitro* preparations are suitable for analysis of these direct neuronal effects. Besides, mammals investigations should involve other non-target animals, such as molluscs.

In the above cited investigations, bensultap was applied systematically and its effects were measured after being absorbed from the intestine. The goal of the present study was to determine the direct neuronal effects, i.e., changes in membrane characteristics and synaptic efficacy, following bensultap application. Electrophysiological experiments were performed on the isolated circum-esophageal ganglion ring prepared from terrestrial snail and on rat neocortical slices. The insecticide was added directly into the perfusion medium.

2. Materials and methods

2.1. Voltage-clamp experiments on identified mollusc neurons

2.1.1. Saline and chemicals

The composition of standard HEPES-buffered saline (HBS) was (in mM): NaCl 80, KCl 1.7, $CaCl_2$ 4, $MgCl_2$ 1.5, HEPES 5 dissolved in tridistilled water; pH 7.70 set by 2.5 mM NaOH. Bensultap-containing solution was freshly prepared from Bancol (Takeda Chemical, Japan) dissolved in HBS, to give a final concentration of 0.1–50 mg/l of bensultap. Each inorganic compound was purchased from SIGMA Co. The extracellular application of bensultap-containing solutions to the neurons and the change of the bathing solution (3 ml) were carried out by a peristaltic micropump (1.5 ml/min, Masterflex C/L,USA).

2.2. Animals and preparation

The conventional two-electrode voltage-clamp study was carried out on identified neurons of the mollusc *Helix pomatia*. The preparation of ganglia and isolation of identified neurons were described in our previous papers (Salánki et al., 1994). Animals were kept under a 12–12 h light–dark cycle at 22–25 °C, and fed *ad libitum*. The CNS was dissected and incubated in 0.1% Protease solution (SIGMA) in normal HBS for 5 min. After incubation, the CNS was rinsed twice and pinned down in a dish containing HBS. The sheath of connective tissue covering of the right cerebral ganglion was removed using scissors. The ganglia were allowed to recover at 4 °C for 2 h, and then stored at room temperature for 30 min. To test the effect of bensultap, the giant right parietal neurons (Sakharov and Salánki, 1969) were used. The neurons were identified according to their position, appearance, and electrical properties. All experiments were performed at room temperature (21 ± 1 °C). The perfusate in the bath was either HBS or HBS containing bensultap. ACh (SIGMA) was applied by microperfusion for periods of about 10–16 s. To ensure recovery from desensitization, an agonist was applied at 5 min intervals. At the beginning of the experiments 4–5 control responses were recorded (15–20 min). Neurons showing a marked decrease of the amplitude were discarded. The average amplitude of the consecutive control samples was counted for further analysis.

2.3. Voltage-clamp recordings

The conventional two-electrode voltage-clamp technique was employed (Salánki et al., 1994) to measure ACh-activated currents. A DAGAN 8500 amplifier (DAGAN Co, USA) was used to record the currents. Microelectrodes were made from borosilicate glass (Clark Co, England), and pulled on a gravity puller (NARISHIGE PC-10, Japan.) The resistance of the intracellular current microelectrode filled with 2.5 M KCl was 3.5–4 MΩ. The voltage recording electrode contained 2.25 M KAcetate + 25 mM KCl and had a resistance of 6–8 MΩ. The voltage and current signals were monitored on a Tektronix oscilloscope. For control of the experiment and data acquisition an AD/ DA converter (DIGIDATA1200, Axon Co, USA) was connected to a personal computer, on which a voltage-clamp software (WinWcp) developed by J. Dempster was run.

2.4. Data analysis

Data are presented as mean and standard deviation, and *n* refers to the number of preparations in all cases. The sta-