Pesticide Biochemistry and Physiology 148 (2018) 144–150

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

The immediate influence of deltamethrin on ion transport through rabbit skin. An *in vitro* study

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ARTICLE INFO

Keywords:
Pyrethroide
Sodium transport
Dermal absorption

ABSTRACT

Deltamethrin can be absorbed into the respiratory tract, the gastrointestinal tract and through the skin. The study was designed to assess the effect of deltamethrin on electrophysiological parameters of rabbit's skin, studied *in vitro,* to identify the mechanism of action and effects of short-term dermal exposure to deltamethrin. The objective of the study was to investigate changes in electrophysiological parameters after exposure to 0.01 M deltamethrin under unchanged conditions, in the presence of amiloride (sodium transport blocker) and bumetanide (chloride transport blocker).

Exposure to deltamethrin reduced the electrophysiological reaction of examined tissue in unchanged conditions and during the sodium reabsorption phase but did not influence the chloride ion secretion phase. The presented data show that the pyrethroide affects transepithelial ion transport in the external layers of the skin. The inhibition of chloride and sodium ions enabled evaluation of the impact of the pesticide on dermal transport.

1. Introduction

Pyrethroids are widely used in agriculture, industry, medicine, as well as households. Antimalarial programs recommend mosquito nets be soaked with deltamethrin [1–5]. The widespread use of this insecticide is associated with its high activity against invertebrates, medium activity against amphibians, and relatively low toxicity to mammals [1,3,6]. The influence of xenobiotics on different species is helpful in understanding the regulatory mechanisms in the skin, regarding different sensitivity of animal tissues to the substances used [6]. The neurotoxic activity of deltamethrin is connected with the prolonged opening of voltage-gated sodium channels and influence on the function of chloride and calcium channels. The prolonged opening of sodium channels results in membrane depolarization, repetitive discharges and synaptic disturbances leading to hyperexcitatory symptoms of poisoning [8,9,12].

Deltamethrin can be absorbed through the respiratory tract, the gastrointestinal tract and through the skin transcellularly and intracellularly [8,9,13–19]. The main route of poisoning in humans is the respiratory tract and through skin, despite low absorption [8,13]. Especially the face can be exposed to the action of pesticides [8,18], however, depending on the purpose of using insecticides, potential skin exposure varies in different parts of the body [20]. Additional factors contributing to poisoning include skin injuries, warmth, insolation, perspiration and hydratation [6,8,13,18,21,23]. Furthermore, mosquito nets impregnated with deltamethrin may be the source of temporary as well as long-term exposure to insecticides [1–3,5,15,18].

Even temporary contact of rat skin with deltamethrin solutions results in active penetration into the tissue [2,14,22,23]. In humans, after a single short-term exposure, deltamethrin is detected in the urine

Abbreviations: RH, Ringer solution; A, Amiloride solution; B, Bumetanide solution; Delta-RH, Deltamethrin solution in RH; Delta-A, Deltamethrin solution in Amiloride; Delta-B, Deltamethrin solution in Bumetanide; PD, Transepithelial potential difference; dPD, Change of the transepithelial electrical potential difference during stimulation; PDmin, Minimal transepithelial potential difference during stimulation; PDmax, Maximal transepithelial potential difference during stimulation; R, Transepithelial electrical resistance

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https://doi.org/10.1016/j.pestbp.2018.04.011
Received 15 October 2017; Received in revised form 9 March 2018; Accepted 23 April 2018
Available online 25 April 2018
0048-3575/

within 12 h [8,22,24] and its metabolites remain detectable up to 48 h [8]. Young children and pregnant women are more susceptible to pyrethroids through altered metabolism by toxic substances [14,17].

Localized effects of deltamethrin exposure on mammals' skin may lead to irritation, tingling, itching, burning sensation, pain, redness, and rashes. Paraesthesia can occur as a consequence of the prolonged opening of sodium channels [8,9,13,25]. Transepithelial ion transport processes generate and maintain an electric field on the epithelial surface which is generally recognized as caused by sodium ion reabsorption and chloride ion secretion [26,27].

The current study was designed to assess the effect of deltamethrin exposure on transepithelial ion transport through the mammalian skin surface using rabbit skin, studied *in vitro*. The objective of the study was to investigate changes in electrophysiological parameters after pesticide exposure under three conditions: unchanged, inhibited sodium reabsorption and inhibited chloride secretion. Amiloride was used as a selective blocker of the sodium channel, which allowed inhibition of sodium ion reabsorption, and thus a predominance of chloride ion secretion was obtained [21,27–31]. To achieve inhibition of chloride ion transport and a predominance of sodium ion reabsorption, bumetanide was used [27,29,30]. The results enabled the identification of the mechanism of action and effects on ion channels following immediate dermal exposure to deltamethrin.

2. Material and methods

In the experiments, an Ussing apparatus was used for the measurements of electrophysiological parameters in isolated epithelial tissue.

2.1. Animals

The specimens were excised from adult, albino, New Zealand rabbits of both sexes, weighing between 3.5 and 4.0 kg, three to four months old. The animals were maintained on a 12/12 light/night cycle, with food and water available *ad libitum*. The rabbits were asphyxiated with a high concentration of isoflurane and CO_2 (approx. 60% of the inhaled air). Hairs were shaved mechanically. Afterwards, the skin of the abdomen was severed and the membranous part, muscles, fat, and vessels were discarded. The remaining part of skin was subdivided into 2 cm^2 pieces, which were submerged and incubated in the appropriate solution, according to the experimental protocol (see Table 1). Rabbits' skin prepared this way incorporates keratinocytes (95%), corneocytes, fibroblasts, immunocompetent cells, hair follicles, and nerve fiber endings [32]. The Local Committee for Ethical Animal Experiments approved the experiments (no. 16/2012, June 21, 2012).

2.2. Chemicals and solutions

The following chemicals and solutions were used in the study:

- Deltamethrin – [(*S*)-Cyano-(3-phenoxyphenyl)-methyl]-(1*R*,3*R*)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate; 505.2 g/mol (Sigma-Aldrich), dissolved and diluted (0.01 mM) in RH (Delta-RH), A (Delta-A), and B solutions (Delta-B).
- Amiloride hydrochloride hydrate (A) – amidynoamid acid, 3,5-diamino-6-chloro-2-carboxylic acid; 266.09 g/mol (Sigma-Aldrich), used as an inhibitor of transepithelial transport of sodium ions (0.1 mM), dissolved and diluted in RH.
- Bumetanide (B) – 3-butylamino-4-phenoxy-5-sulfamoylbenzoic acid; 364.42 g/mol (Sigma-Aldrich), used as an inhibitor of transepithelial transport of chloride ions (0.1 mM), dissolved in DMSO (dimethyl sulfoxide, Sigma-Aldrich) and diluted in RH, final concentration DMSO 0.1%.
- Hepes – 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid, 238.30 g/mol (Sigma-Aldrich).
- RH – Ringer solution (K^+ 4.0 mM; Na^+ 147.2 mM; Ca^{2+} 2.2 mM; Mg^{2+} 2.6 mM; Cl^- 160.8 mM; Hepes 10.0 mM), which was adjusted to pH 7.4 under the control of a pH-meter; basic solution with isoosmotic properties.

Mineral compounds (KCl, NaCl, $CaCl_2$, $MgCl_2$) were purchased from POCH, Poland.

2.3. Experimental procedure

To estimate immediate and prolonged effects of deltamethrin on sodium channels, skin fragments were divided into three groups:

1. Undisturbed ion transport (RH) – fragments incubated in Ringer fluid which does not affect ion transport ($N = 46$)
2. Inhibited chloride transport (B) – fragments incubated in Ringer fluid with the chloride transport blocker: bumetanide ($N = 49$)
3. Inhibited sodium transport (A) – fragments incubated in Ringer fluid with the sodium transport blocker: amiloride ($N = 40$)

For each group, electrophysiological parameters during pre-treatment (30 min), short-term deltamethrin treatment (15 s), and post-treatment – mechanical stimulation (15 s) were assessed according to the procedure presented in Table 1.

The experiments consisted of measuring the following parameters:

1. Transepithelial potential difference:
   - mean (PD), electrical potential difference in stationary conditions (just before stimulation, mV)
   - minimal (PDmin) and maximal (PDmax) transepithelial electrical potential difference (mV),
2. Transepithelial electrical resistance (R, Ω/cm^2).

The electric potential difference (PD) was recorded continuously. Electrical resistance of tissue (R) was determined by applying a current

Table 1
The measurements' order and fluids applied for each group.

Group	Pre- incubation fluid (30 min)	Electrophysiological measurements: PD, PDmax, PDmin, R		
		Incubation fluid (30 min)	Chemical stimulation fluid (15 s)	Pre- and post-treatment fluid (15 s)
Undisturbed ion transport (U) $N = 46$	RH	RH	Delta-RH	RH
Inhibited sodium transport (A) $N = 40$	A	A	Delta-A	A
Inhibited chloride transport (B) $N = 49$	B	B	Delta-B	B

N - number of specimens; RH - Ringer solution; A - amiloride (0.1 mM) solution in RH; B - bumetanide (0.1 mM) solution in RH; Delta-RH - deltamethrin solution 0.01 mM concentration in RH; Delta-A - deltamethrin 0.01 mM concentration in A; Delta-B - deltamethrin 0.01 mM concentration in B;