

DUPLICATION OF THE ANTERIOR-PIT COMPARTMENT IN ADULT REGENERATES OF HALLER'S ORGAN OF THE TICK *HYALOMMA ANATOLICUM* (ACARI: IXODIDAE) UNDER INFLUENCE OF RETINOIC ACID

ДУПЛИКАЦИЯ ПРЕДКАПСУЛЯРНОГО ОТДЕЛА В ИМАГИНАЛЬНЫХ РЕГЕНЕРАТАХ ОРГАНА ГАЛЛЕРА У КЛЕЩА *HYALOMMA ANATOLICUM* (ACARI: IXODIDAE) ПОД ВЛИЯНИЕМ РЕТИНОВОЙ КИСЛОТЫ

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ABSTRACT

Treatment of engorged *Hyalomma anatolicum* nymphs (after amputation of their left foreleg) with olive oil solution of retinoic acid (10^{-4} M) has resulted in a specific teratogenic effect (duplication of the anterior-pit compartment in adult regenerates of Haller's organ) that corresponds to similar effect produced by this retinoid (duplication of axial structures in limb regenerates) in vertebrate animals.

РЕЗЮМЕ

При обработке напитавшихся нимф клеща *Hyalomma anatolicum* (после ампутации у них передней ноги) масляным раствором ретиноевой кислоты (в концентрации 10^{-4} M) у части особей наблюдался специфический тератогенный эффект (удвоение предкапсулярного отдела в имагинальных регенератах органа Галлера). Сходный эффект (дубликация осевых структур в регенератах конечностей), вызываемый этим ретиноидом, известен у позвоночных животных.

INTRODUCTION

Retinoic acid (RA) and other retinoids represent vitamin A metabolites of high biological activity. They are known to modify proliferation and differentiation of animal cells *in vitro*, and to induce teratogenesis during animal development *in vivo*. One of the most unique morphogenetic effects of them is the modification of spatial patterns in differentiation of animal limb regenerates [Stocum, Maden, 1990]. This effect of RA and other retinoids is represented usually by formation of additional axial structures in regenerating appendages [Maden, 1996].

Unfortunately, investigations of RA and its action were undertaken mainly on vertebrate ani-

mals. Arthropods in this regard are studied much worse, though retinoids belong to the same group of sesquiterpenoid compounds, as juvenile hormones (JH) controlling morphogenetic and reproductive events in insects [Laufer, 1988; Gilbert et al., 2000]. It is worth to mention that functional similarity of these compounds was revealed by Czech insect physiologist K. Slama [1962] in experiments with an impact of vitamin A on larvae of the bug *Pyrrhocoris apterus*, and afterwards was confirmed by other Czech students on the RA action to larvae of the bug *Dysdercus cingulatus* and the beetle *Tenebrio molitor* [Nemec et al., 1993].

It is revealed now, that cellular mechanisms of development control in all arthropods, both in insects [Riddiford et al., 1999], crustaceans [Chung et al., 1998] and chelicerates, particularly in ixodid ticks [Guo et al., 1998; Palmer et al., 1999], involve nuclear retinoid receptors (RAR and RXR). It is shown also, that RA in crustaceans affects the early stages of limb regeneration due to its role in the RXR transcription [Chung et al., 1998].

Until the last time arachnids remained a group of arthropods not studied in regard to morphogenetic action of RA and other retinoids. It was shown only recently that RA by its physiologic and morphogenetic effect in the tick *Ixodes ricinus* is similar to insect JH analogs, and its juvenilizing effect is well expressed in results of regeneration of Haller's sensory organ that is located on their foreleg tarsi [Belozarov, 2003]. Additional SEM studies revealed the occurrence of supplementary capsular compartments in nymphal Haller's organ regenerates of this prostriate tick affected by RA [Belozarov, 2002].

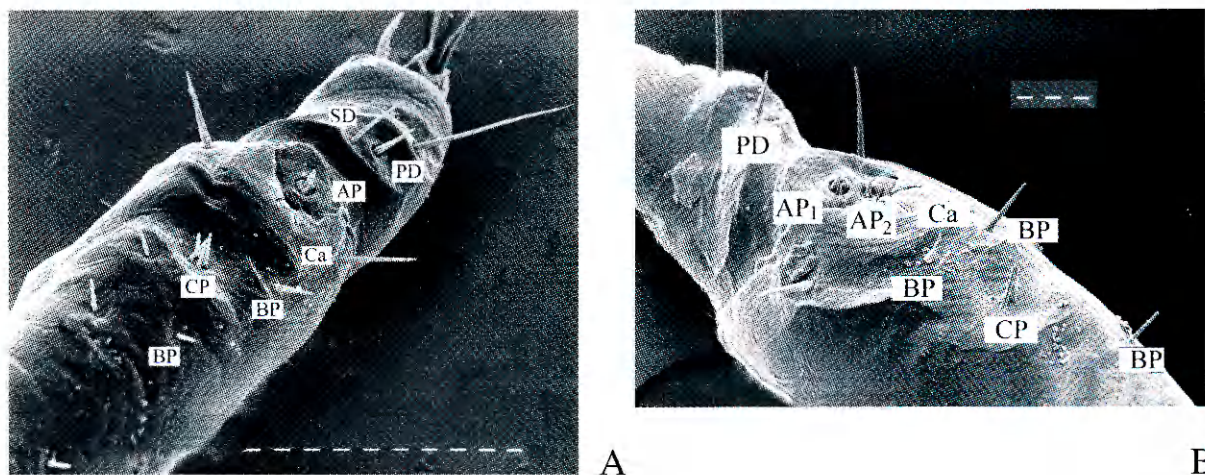


Fig. 1. Normal Haller's organ of *Hyalomma anatolicum* adult male (A) and Haller's organ regenerate of *H. anatolicum* adult female (B). *SP* and *PD* — smooth and porous sensilla of distal compartment; *AP*, *AP₁* and *AP₂* — anterior-pit compartment; *Ca* — capsular orifice; *CP* and *BP* — central and bordering postcapsular sensilla. Bars = 10 μ m.

Рис.1. Нормальный орган Галлера самца *Hyalomma anatolicum* (A) и регенерат органа Галлера самки *H. anatolicum* (B). *SD* и *PD* — гладкая и пористая сенсиллы дистального отдела; *AP*, *AP₁* и *AP₂* — предкапсулярные отделы; *Ca* — капсулярное отверстие; *CP* и *BP* — центральные и краевые сенсиллы посткапсулярного отдела. Масштаб — 10 мкм.

The paper presents new data on specific morphogenetic effect of RA to regenerative events in metastriate ixodid ticks, resulted in the development of additional structures in Haller's organ regenerates along its axes, that confirms the similarity of these chelicerate arthropods with other animals (even vertebrates) in regard to morphogenetic action of RA.

MATERIALS AND METHODS

The work was conducted with nymphs of *Hyalomma anatolicum* Koch (aged 3–4 month) from its laboratory colony established from engorged females collected in Turkmenia. Nymphs after feeding on mice were used mainly to study their respiration dynamics [Belozero, 1998]. The rest of engorged diapausing nymphs, maintained both before and after feeding under 25°C and short-day conditions (LD 12:12), was used to study morphogenetic effect of RA and methoprene (an insect JH analog). In two weeks after engorgement they were operated (amputation of left foreleg at the level of femur), and just after operation they were treated by an olive oil solution of RA (Sigma Corp., USA) or methoprene (Zoecon Co, USA) in concentration of 10^{-4} and 10^{-5} M respectively. Six RA treated nymphs and six methoprene treated nymphs were transferred into long-day regimen (LD 20:04) enabling termination of diapause. In two months they molted to adult stage (2 females and 2 males after RA treatment, while 2 females and 3 males after methoprene treatment). Four groups of adult ticks

were used as controls: after amputation of left foreleg and olive oil treatment (n=4), without amputation, but with treatment by olive oil (n=5) or by oil solution of methoprene (n=5), and with no treatment (n=14). In one week after molting they were fixed in 70% ethyl alcohol. Left and right foreleg tarsi in test and control ticks were examined later by scanning electron microscopy with SEM 501B PHILIPS at the Laboratory of Electron Microscopy, Faculty of Geology, St. Petersburg University. Tick preparations for microscopy were prepared through air-drying and gold-coating of tick specimens on stabs in a sputter coater SC-500 EMSCOPE. Some data concerning effect of methoprene on Haller's organ regeneration in *H. anatolicum* cited in this paper, were partly published already [Belozero, 1999].

RESULTS

Haller's organs are the main organs of distant reception in ixodoid ticks [Sonenshine, 1991; Balashov, 1998]. An important feature of these paired complex sensory organs, peculiar for all ixodoid ticks and located on their foreleg tarsi, is a high stability and species-specificity of their structure, in regard to the number, type and topography of external sensory elements represented by different trichoid and basiconical sensilla. These features (together with very limited number of sensilla) make Haller's organ to be a convenient experimental model with easily recognized changes in its structure [Belozero, 2001]

Table

Sensillar sets in external compartments of adult Haller's organ on the left foreleg of *Hyalomma anatolicum*

Таблица

Набор сенсилл в наружных отделах органа Галлера (левая нога) у взрослых клещей *Hyalomma anatolicum*

Variants	n	Distal compartment		Anterior-pit compartment		Postcapsular compartment	
		Sensillar set	% of ticks	Sensillar set	% of ticks	Sensillar set	% of ticks
1. Control ticks (without amputation and treatment)	14	Norm (1 PD+1 SD)	100	Norm (1P+2G+2T+1C)	100	Norm (4CP+4 BP)	100
2. Control ticks (without amputation, but with oil treatment)	5	Norm	100	Norm	100	Norm	100
3. Control ticks (without amputation, but with methoprene treatment)	5	Norm	100	Norm	100	Norm	100
4. Regenerate (with oil treatment)	4	Norm Norm - 1 SD	50 50	Norm Norm + 1P Norm + 1G	50 25 25	Norm Norm - 1CP	50 50
5. Regenerate (with methoprene treatment)	5	Norm Norm - 1 SD	40 60	Norm Norm - 1T	80 20	Norm Norm + 1CP Norm - 2CP Norm - 2 CP - 1BP	40 20 20 20
6. Regenerate (with retinoic acid treatment)	4	Norm Norm - 1 SD	25 75	Norm Duplication APC	50 50	Norm Norm + 1CP Norm - 1CP - 1BP	50 25 25

Notes: Norm — normal sensillar sets (shown in parentheses for variant 1). An addition or loss of some sensilla are shown by marks + or -. Designations for sensilla are given in the text.

Haller's organ in *H. anatolicum*, as well as in all ixodoid ticks, consists of the capsule and three external compartments (distal, anterior pit and postcapsular) with peculiar sets of sensilla (Fig. 1, A). In adult *H. anatolicum* organ (control variants 1–2 in the Table) there are normally two sensilla (one porous *PD* and one smooth *SD*) in the distal compartment, six sensilla (one porous *P*, two grooved *G*, two thin *T* and one conical *C*) in the anterior-pit compartment, and eight sensilla (the compact group of four central sensilla *CP* surrounded by four bordering sensilla *BP*) in the postcapsular area. In regard to the number of capsular sensilla, *H. anatolicum* is similar, apparently, to the related tick *H. asiaticum*, that has five porous sensilla in its capsule [Leonovich, 1978; Belozerov, Leonovich, 1995]. The mentioned sets of sensilla are identical in adult males and females of *H. anatolicum* (due to the absence of sex dimorphism in Haller's organ structure in all ixodoid ticks), while the organ of the left (or right) foreleg represents a mirror image of the organ on the contralateral foreleg.

Regeneration of Haller's organ in *H. anatolicum* adults (after foreleg amputation at the preceding nymphal stage) is accompanied by regular modifications in its structure (variant 4 in the Table), similar with those described in *H. asiaticum* [Belozerov, Leonovich, 1995]. They are characterized by a loss of smooth sensilla *SD* in the distal compartment, and by a decrease in the number of central sensilla (up to 3 *CP*) in postcapsular area, as well as by an increase in the number of porous (up to 2*P*) and grooved sensilla (up to 3*G*) in the anterior-pit compartment of some regenerates.

An impact of methoprene on regenerative events in *H. anatolicum* (variant 5 in the Table) resulted in a weak, but specific juvenilizing effect, expressed in preventing multiplication of porous and grooved sensilla in the anterior-pit compartment [Belozerov, 1999], and in an increase of variability in postcapsular sensillar sets. The normal development of Haller's organ on the left foreleg in control ticks (variant 3 in the Table), as well as on the right, non-amputated foreleg in test

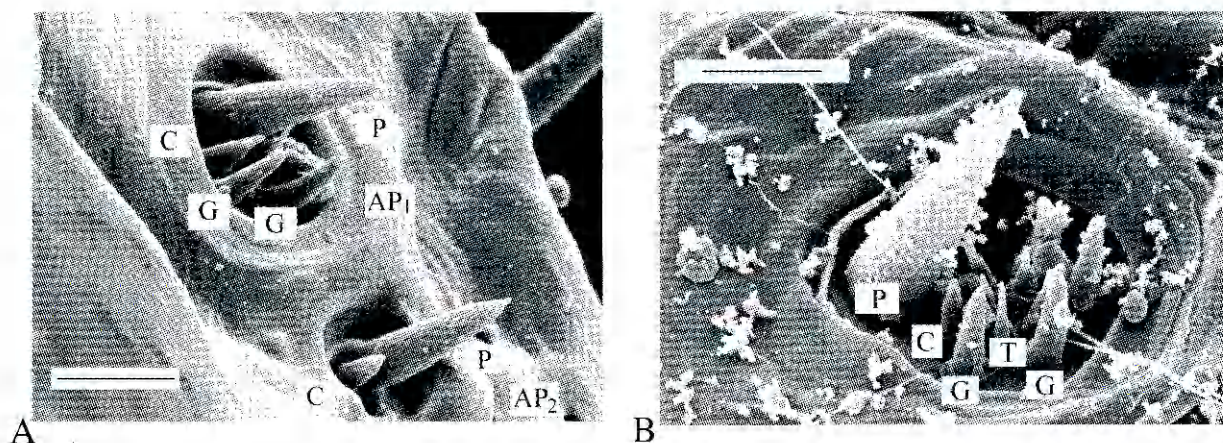


Fig. 2. Duplication of anterior-pit compartment in regenerated Haller's organ of adult *Hyalomma anatolicum* ticks under influence of retinoic acid. A — enlarged image of duplicated anterior-pit compartment in *H. anatolicum* female (see Fig. 1B). The anterior duplicate (AP_1) with the complete set of sensilla — one porous (P), two grooved (G), two thin (T) and one conical (C), while the posterior duplicate (AP_2) possess only two sensilla — one porous (P) and one conical (C). B — duplicated anterior-pit compartment in regenerated Haller's organ of *H. anatolicum* male. Bars = 10 μ m.

Рис.2. Дупликация предкапсулярного отдела в регенератах органа Галлера у взрослого клеща *Hyalomma anatolicum* под влиянием ретиноевой кислоты. А — увеличенное изображение дубликатов предкапсулярного отдела у самки (см. рис. 1В). Передний (AP_1) дубликат отдела с полным набором сенсилл — одной пористой (P), двумя бороздчатыми (G), двумя тонкими (T) и одной конической (C), а задний (AP_2) дубликат только с двумя сенсиллами — одной пористой (P) и одной конической (C). В — дупликация предкапсулярного отдела в регенерате органа Галлера у самца. Масштаб — 10 мкм.

specimens, were not influenced by methoprene in *H. anatolicum*.

An impact of the RA oil solution on operated *H. anatolicum* nymphs (just after amputation of their left foreleg) induced no specific modifications in the structure of distal compartment, though postcapsular sensillar sets demonstrated an increased variability (variant 6 in the Table), similar to that in the methoprene affected regenerates. Haller's organ regenerates in two test ticks revealed no regenerative changes in their anterior-pit compartments. However, two other test ticks (one female and one male) showed very peculiar morphogenetic response to RA during Haller's organ regeneration expressed in duplication of the whole anterior-pit compartment (Fig.1,B), but not in duplication or multiplication of its sensilla.

The Haller's organ regenerate in adult female was characterized here by proximodistal duplication of the anterior-pit compartment (with an occurrence of two topographically separated duplicates). In regard to their sensillar sets these duplicates (Fig.2, A) were not identical: the perfect complex of sensilla was observed only in the distal duplicate, while the proximal one was underdeveloped and possessed two sensilla only (porous P and conical C), though by their size, form and structure they did not differ from the same sensilla of the distal duplicate. Other external compartments revealed quite common re-

generative changes (the retention of single porous sensillum PD in the distal compartment, and the loss of two sensilla, one central PC and one bordering PB , in the postcapsular area).

In its turn, duplication of the anterior-pit compartment in the male Haller's organ was different by its character. Being anteroposterior, it concerned the main body of this compartment represented by the complex of grooved G , thin T and conical C sensilla, but did not concern the porous P -sensillum. The anterior-pit compartment was presented, therefore, by one common, non-divided pit with single porous sensillum and two mirror-symmetrical sets of other sensilla (Fig. 2, B). The distal compartment had here, as well as in female, only one porous sensillum PD , while the postcapsular compartment had the perfect, non-changed sensillar set ($4CP + 4BP$).

DISCUSSION

In metastriate ixodid ticks (particularly in *Hyalomma* ticks) changes in Haller's organ external structure determined by reparative regeneration are expressed usually much weaker, than in more primitive prostriate ticks represented by *Ixodes* ticks [Belozеров, 2001]. This is obviously revealed under juvenilizing effect of JH analogs on Haller's organ regeneration, and this effect is much more pronounced in *I. ricinus*, than in *H. anatolicum*

[Belozero, 1999, 2001]. Investigations of the RA influence on events of normal and reparative morphogenesis in *I. ricinus* have shown that prostriate ixodids are also characterized by high sensitivity to this retinoid compound that is similar to JH analogs by its juvenilizing effect [Belozero, 2003]. However, juvenilizing efficiency of retinoids, though rather weak, can be combined in metastriate ticks with their strong and specific morphogenetic response to these compounds (during regenerating events), being expressed in duplication of axial structures. Similar response to RA is well known in different vertebrate animals [Maden, 1996; Tsonis, 1996].

Literature on teratology of ixodoid ticks [Pavlovsky, 1939; Schulze, 1950; Pervomajsky, 1954; Campana-Rouget, 1959; Buczek, 1994; Guglielmo et al., 1999] contains numerous descriptions of duplication in body parts and appendages (e.g. limb axial dividing, i.e. schizomely) discovered in tick specimens from ixodological collections. The most of them are discovered in representatives of the family Argasidae and in metastriate ticks from the family Ixodidae. In prostriate ixodid ticks (*Ixodes*) only two cases of body parts duplication were described in *I. ricinus* nymphs [Campana-Rouget, 1959; Siuda, 1991]. The duplication of some parts in Haller's organ (the capsule, in particular) is known also only in metastriate ixodids and argasids — *Aponomma lucasi* and *Boophilus decoloratus* [Schulze, 1941], *Hyalomma steineri* [Schulze, 1950; Campana-Rouget, 1959] and *Argas reflexus* [Buczek, 1994]. All the mentioned malformations in field collected ticks are of unknown etiology, while schizomely in argasid ticks, as shown experimentally, can be evoked as a result of either regeneration of amputated legs [Campana-Rouget, 1939; Obenchain, Oliver, 1972], or irradiation by ultraviolet rays [Pavlovsky, Skrynnik, 1957].

Though the duplication of legs or body parts resulted after surgery operation is unknown in prostriate ixodid ticks, but the duplication of the capsule in Haller's organ regenerates of *I. rubicundus* nymphs and adult ticks is known to be quite usual phenomenon, as well regular as regenerative modification in sensillar sets of the organ [Belozero et al., 1997]. Similar capsular duplications were revealed recently in *I. persulcatus* larvae and *I. ricinus* nymphs, under effect of RA in the latter case [Belozero, 2002]. However, as shown above in this paper, Haller's organ regeneration affected by RA in the metastriate tick, *H. anatolicum*, results in duplication of the precapsular (anterior-pit) compartment, but not of the capsule, as in prostriate

ticks. This is an additional evidence of difference between metastriate and prostriate ticks in regard to peculiarities of their developmental events and of RA role in mechanisms controlling their normal development and teratogenesis. The difference between these two groups of ixodid ticks deserves, of course, more attention and deeper experimental research.

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