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INSECT OLFACTION: A HYPOTHESIS ABOUT THE ROLE OF LIQIUD- CRYSTALLINE PORE TUBULES IN OLFACTORY SENSILLA AS CONDUCTORS OF INFORMATION ON THE NATURE OF SIGNALING MOLECULES

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New facts concerned with the previously formulated hypothesis about the role of liquid-crystalline pore tubules in insect olfactory sensilla are considered in the article. Together with the pores they form stimuli conduction system and function not as conductors of odorant molecules, but information about chemical nature of the latter [Chaika, 1991, 1997, 2013].

Pore tubules are extracellular structures extending from the pores in the cuticle of olfactory sensilla of insects and other arthropods [Steinbrecht, 1997]. In the course of ontogenesis pore tubules are formed in the cavity of olfactory hairs before the dendrites of receptor cells enter it [Ernst, 1969]. Pore tubules do not branch, their diameter equals to 10-15 nm and the length can measure up to 350 nm (fig. 1, a-e). It is considered that the presence of pore-tubule system in insects with thick cuticular cover solves the problem of effective access of odorant molecules to the dendrites of receptor cells.

In relation to the chemical nature of pore tubules it is suggested that they are composed of lipids [Hawke, Farley, 1971]. We used different methods for detection of lipids at the ultrastructural level to test this hypothesis: impregnation with osmium, fixation of the material with glutaraldehyde that bind lipids badly, and lipid extraction. While studying the section through of cuticular parts of olfactory sensilla it is found that pore tubules are well detected at fixation of the material in 2% osmium tetroxide solution as ethylene lipid groups react with it. Pore tubules almost completely disappeared after glutaraldehyde treatment of the material. Similar result was also observed after treatment of the material in methanol-chloroform mixture by Napolitano (Geyer, 1973). These data confirms the lipid composition of pore tubules in olfactory sensilla.

Relying on similarity of pore tubules of olfactory sensilla and pore channels of cuticle it has been suggested that the pore tubules are composed of lipids which are in the mesomorphous state or in other words have liquid-crystalline structure [Locke, 1965].

The lipid composition of pore tubules answered the question about the difficulty of diffusion of lipophilic molecules of many pheromones through lipophobe intrasensillar (liquor) fluid in sensilla cavity, which contains the dendrites of neurons. The liquid-crystal structure of pore tubules best of all matched to explain the mechanism of rapid penetration of odorant molecules to the membrane of a receptor as the liquid-crystal substances are characterized by plasticity, high adsorbing activity, easy substitutability of molecules [Brown, Walken, 1982].

Thus, before the 90-s of the last century the pore tubules were regarded as just conduction structure for odorant molecules. However even by that time it was obvious that the process of penetration of odorant molecules to the receptor membrane of dendrites is much more complex.

First, it failed to detect direct contact of pore tubules with the membrane of dendrite of receptor cell in many insects. Secondly, many insects do not have any pore tubules in the olfactory sensilla, for instance in some trichoid single-walled and in all double-walled sensilla. Moreover the question of the mechanisms of the molecule transport to the membrane of dendrite of receptor cell still provoked a discussion. According to the hypothesis of passive transport, odorant molecules penetrate by diffusion through pores and pore tubules after its adsorption on the surface of cuticular part and then reach the receptors on the membrane of peripheral branch of receptor cell [Kaissling, 1974]. Besides, the possibility of flow of molecules occurred on the cuticle of a hair into pores by lipoprotein layer covering the cuticle was admitted. However, some experimental data evidence against this hypothesis. Odorant molecules which do not got in the pores at once, are unlikely to have any signaling value, because otherwise it is difficult to explain the rapidity of insect reaction to an olfactory stimulus [Skirkevicius, 1986] . Moreover the admission of the signaling value of the molecules adsorbed by the surface of antenna cuticle complicates the explanation of the mechanism of finding the odor source by insects, because in this case an insect must perceive substance even if it – insect – will be out of the odor track. In the disfavour of the hypothesis of passive diffusion evidences the fact concerning significant divergence of the time of actual formation of receptor potential in neurons of silkworm sensilla and estimated time according to the diffusion coefficient of the bombikol pheromone [Steinbrecht, Kasang, 1972].

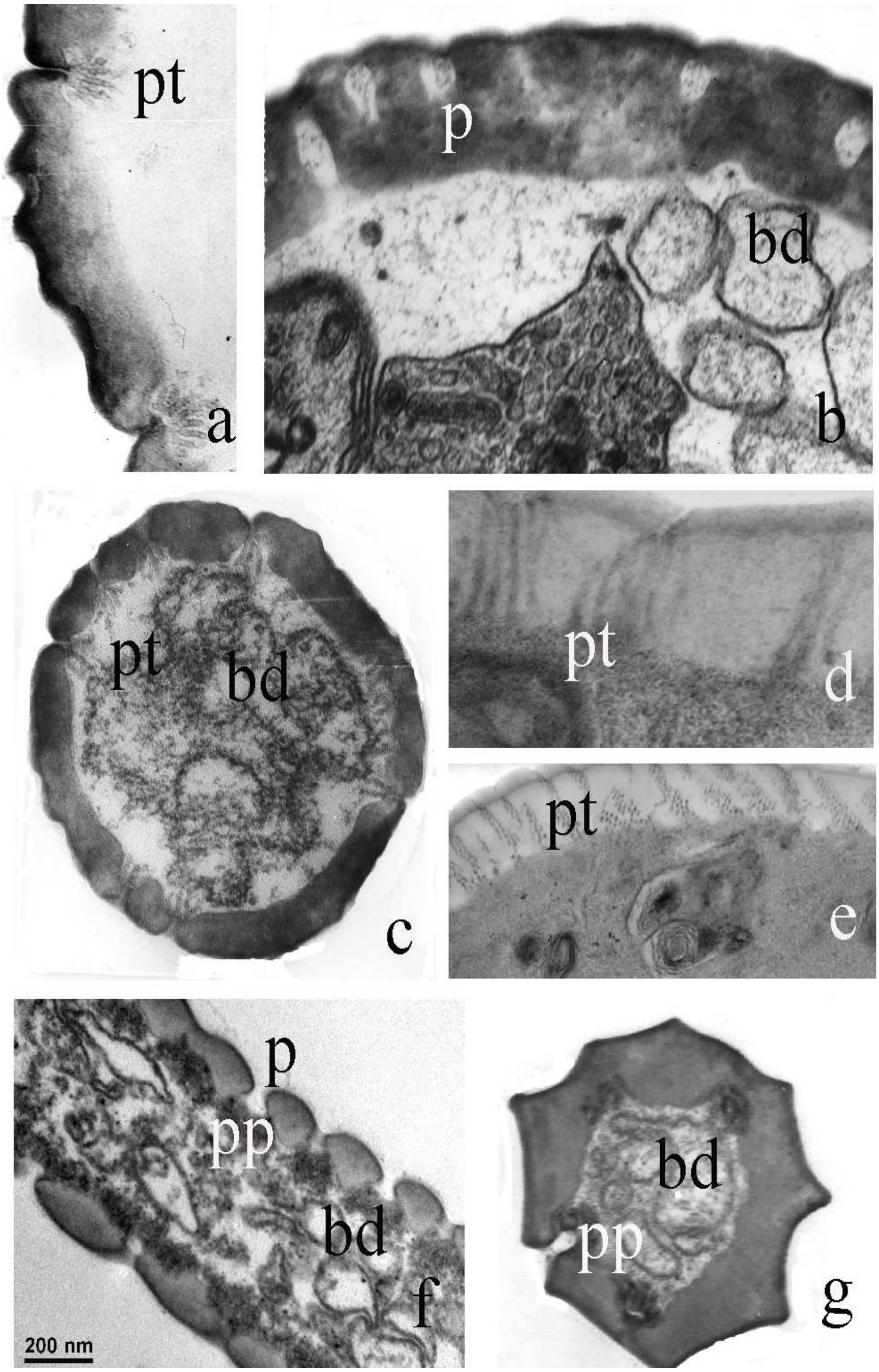

Fig. 1. Section through of cuticular parts of olfactory antennal sensilla: a – knee peg of *Phlebotomus papatasii*, x 60 000; b – sensilla trichodea of biting midges *Culicoides fascipennis*, x 60 000; c – sensilla basiconica of gnats *Boophthora erythrocephala*, x 28 000; d, e – sensory dome of larvae *Sarcophaga* sp., x 80 000 (d), x 40 000 (e); f – sensilla auricularia of *Ephestia kuhniella*, g – sensilla basiconica in olfactory pit of flea *Ceratophyllus sciurorum*, x 50 000. bd – branches of dendrites, p – pores, pp – porous plaque with pore tubules, pt – pore tubules.

Therefore some authors have developed the hypothesis of active transport of odorant molecules to the loci of the membrane of receptor cell [Norris, 1977]. According to this hypothesis, odorant molecules, which have got into the cavity of cuticular part of sensillum through a pore, bind with protein molecules and due to them are actively transported to the membrane of receptor cell. The first fact that has confirmed the perspectiveness of development of this hypothesis was the detection of a protein in the antenna of *Antheraea polyphemus* butterfly, which did not possess inactivating properties in accordance to pheromone molecules, but was able to bind with them [Vogt, Riddiford, 1981].

In recent years due to the wide use of molecular biology methods, odorant-binding proteins and odorant-inactivating enzymes were isolated from the fluid of cavities of the olfactory sensilla of insects (lepidopterans, *Drosophila* fly, mosquitoes, honeybee), and olfactory receptors were identified in the membrane of dendrites of receptor cells [Fan et al., 2011; Leil, 2013].

What function do pore tubules perform in the modern scheme of odorant perception mechanism? After all the conduction function for long time regarded as basic to pore tubules is performed by special proteins. To answer this question the hypothesis about the role of pore tubules as a dispenser of odorant molecules inside sensillum was formulated [Steinbrecht, 1997]. Following the uncertainty around the role of pore tubules in the olfactory process the latter often are just simply mentioned alongside with pores or not recorded in the schemes of the process of reception at all.

Nevertheless it is hardly possible to ignore the role of pore tubules in the process of reception of odorants. First of all the question is not withdrawn yet about how odorant molecules get to the receptor membrane of a dendrite in sensilla where the contact of the pore tubules with membrane receptor is observed? The problem is complicated by the fact that pore tubules are hollow, and their core filled with amorphous material. If odorant molecules get into liquor fluid avoiding pore tubules, what function the latter perform? In some insects pore tubules are located so tightly that almost totally block free communication of pore with the cavity of cuticular part (Fig. 1, f, g).

According to the liquid-crystal state of pore tubules we have expressed an original hypothesis that the pore tubules in olfactory sensilla may serve as not a conductor of molecules themselves, but information only about chemical nature of the latter [Chaika, 1991, 1997, 2013]. In this case we are talking about those sensilla, where pore tubules are in contact with the membrane of the dendrite. It is known that getting of even a millionth dose of any substance on liquid-crystal cholesteric changes the lead of the helix of liquid crystal, which are found in experiments on change of colour of the reflected light [Brown, Walken, 1982]. Recently liquid crystals are widely used in biological sensors [Woltman et al., 2007; Smalyukh1, 2010]. If pore tubules liquid-crystal structures are indeed, then upon a contact with the membrane of dendrite of receptor cell, the latter can instantly get the information about the molecules got in the pores of the olfactory sensilla. This is principally important for detecting vital signals, e.g. sex pheromone molecules. Besides, insects with pheromone communication systems (lepidopterans, bugs, hymenopterans, some dipterans) have olfactory sensilla with pore tubules.

The present hypothesis to a certain degree contradicts the central dogma of sensory reception, according to which the information carrier must certainly get on the membrane of exteroceptor, however it provides the interaction of stimulus with receptor membrane of neuron, although this interaction is performed indirectly – through pore tubules. This process is similar to the perception of signal stimulus by secondary sensory cells in some sensory organs vertebrata.

As for the possibility itself of such an unusual way to get information through the membrane of receptor cells in some olfactory sensilla, it is required to point out principal differences between the two types of chemical reception – gustatory and olfactory. In taste receptors, for example, due to their structure the direct contact of molecules of a substance with membrane of a receptor always exist. As A.V. Minor (1983) noted, taste receptors perceive metabolic stimuli carrying the information on the validity of food substrate, whereas the olfactory receptors perceive just signal stimuli. Regarding the differentiation of olfactory stimuli into pheromone and general, it should be pointed out significant autonomy of transmission of information about the pheromones signal in the central nervous system, which includes macroglomerular complex in deutocerebrum and special neurons which associate this complex with mushroom bodies of protocerebrum. It is appropriate to admit the presence of independent input channels for pheromone signals, too. Nothing but pore tubules can be such channels in the peripheral part of olfactory system.

The necessity of detailed study of pore-tubule system of olfactory sensilla of insects and other arthropods is quite obvious in the context of existing new data on the mechanism of reception of olfactory stimuli reception. Knowledge of molecular mechanisms of reception of stimuli of various modality is necessary as well for construction of highly sensitive biosensor devices (Varfolomeev et al, 2000), including devices operating in real time mode detecting a variety of chemical compounds in atmosphere (Portable..., 2012). It is hoped that new studies must confirm or disprove our formulated hypothesis that liquid-crystal pore tubules are not conductors of vital molecules of signaling compounds, but only information about their chemical nature.

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