The peripheral nervous system of mutants of early neurogenesis in Drosophila melanogaster

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Summary. Mutations previously known to affect early neurogenesis in Drosophila melanogaster have been found also to affect the development of the peripheral nervous system. Anti-HRP antibody staining has shown that larval epidermal sensilla of homozygous mutant embryos occur in increased numbers, which depend on the allele considered. This increase is apparently due to the development into sensory organs of cells which in the wild-type would have developed as non-sensory epidermis. Therefore, the neurogenic loci seem to act on the peripheral nervous system. Different regions of the ectodermal germ layer are distinguished with respect to their neurogenic abilities.

Key words: Peripheral nervous system – Neurogenesis – Mutants – Drosophila

Introduction

The segregation of neural progenitor cells from the undifferentiated ectoderm is one of the first steps in the development of the Drosophila nervous system. The progenitor cells of the central nervous system (CNS), the so-called neuroblasts (Wheeler 1891, 1893), leave the ectoderm soon after gastrulation; most, or all, neuroblasts segregate before having mitotically divided after formation of the cellular blastoderm (Hartenstein and Campos-Ortega 1984; Technau and Campos-Ortega 1985). In contrast, the progenitor cells of larval epidermal sensory organs, and thus of the peripheral nervous system (PNS), become distinguishable later in embryogenesis, after germ-band shortening, when the remaining epidermal cells are close to terminating their divisions (Hartenstein and Campos-Ortega 1985; Campos-Ortega and Hartenstein 1985a). It has been shown that for a number of insect species the entire set of cells that form a given sensillum (trichogen cell, tormogen cell, sensory neuron(s), glia cell) arise from related mitoses; these cells group together to form a primordial sensory organ, and cytodifferentiation of sensory neuron and supporting cells follows (see comprehensive review of Bate 1978).

Neuroblasts of the ventral cord have been shown to originate from the ventral neurogenic region (Hartenstein and Campos-Ortega 1984, 1985; Technau and Campos-Ortega 1985) where they occur mixed with progenitors of the ventral epidermis and its annexes (e.g. sensilla, salivary glands) in a proportion of approximately one neuroblast to four epidermal, and other, progenitor cells. Some sensory organs originate from the dorsal epidermal anlage, from which no neuroblast for the CNS arises (Campos-Ortega 1983; Hartenstein and Campos-Ortega 1984; Technau and Campos-Ortega 1985). A number of neurogenic mutations have been described, the main defect of which consists in a misrouting of presumptive epidermal progenitors into the neurogenic pathway of development (Lehmann et al. 1983). These mutations define seven complementation groups which have, at least, one feature in common: when the function of any of these genes is completely lost all cells of the ventral neurogenic region, instead of only every fourth, develop as neuroblasts (Jimenez and Campos-Ortega 1982; Hartenstein and Campos-Ortega 1984). Apparently, during normal development these genes provide a sort of genetic switch, which decides how many cells of the neurogenic ectoderm are going to enter the neurogenic pathway. Further study of the phenotype of mutations in neurogenic genes has shown that, besides at early neurogenesis, their function is also required for the development of several different types of imaginal disc cells, for example bristles and ommatidia of the compound eye, where these genes may participate in the decision between neural and epidermal lineages (Dietrich and Campos-Ortega 1984).

In the first descriptions of the embryonic phenotype of the neurogenic mutants under discussion, the embryonic PNS was not studied in great detail (Poulson 1937; Lehmann et al. 1983). It was concluded from a light microscopic reconstruction of the chordotonal organs of homoyzogotes for a few alleles that, numerically, this type of sensilla was not very grossly affected. However, neural specific staining techniques applied to the study of these mutants, i.e. anti-HRP antibody (Jan and Jan 1982), have shown us that neurogenic mutations indeed increase the number of all types of embryonic sensilla at the expense of other epidermal cells. Therefore, the neurogenic loci seem to act on the development of the larval and imaginal PNS in a similar way to that in which they act on central neurogenesis. Since the wild-type larval PNS consists of a relatively small number of different sensory organs and nerves, which are located at well-defined positions and, therefore, clearly identifiable (Hertweck 1931; Campos-Ortega and Hartenstein 1985a), it seems better suited for a more subtle assessment of the action of the neurogenic loci on neurogenesis.
than the CNS itself. In the present report, we deal with several aspects of PNS development based on the phenotype of neurogenic mutations.

Materials and methods

Embryos of Drosophila melanogaster, wild-type (Oregon R) and homozygous for various neurogenic mutant alleles of the genes Notch (N), almondex (amx), master mind (mam), big brain (bib), neutralised (neu), Delta (Di) and Enhancer of split (E(sp)) (see Table 1), were stained with the antibody against horseradish peroxidase (anti-HRP), which exhibits a conspicuous affinity for the membranes of both mature and immature neural cells (Jan and Jan 1982). Visualisation of the binding sites was obtained by an HRP-coupled anti-immunoglobulin reacted with the substrate diamino benzidine (DAB).

Staged embryos were dechorionised and davetellinised according to the method of Zalokar and Erk (1977). 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) (pH 7.2) was used as fixative. After devetellinisation, embryos were postfixed for 1 h in the same fixative and washed for 1 h in several changes of 0.1 M PBS buffer containing 0.3% Triton X-100. Anti-HRP (Sigma) was diluted 1:3000 in 0.1 M PBS containing 10% goat serum and 0.1% Triton X-100. After 1 h preincubation in this solution without antibody, embryos were incubated overnight at room temperature in the antibody-containing solution; consecutively the embryos were washed in several changes of 0.1 M PBS over 2 h. This was followed by incubation for 5 h at room temperature in affinity-purified, HRP-coupled goat anti-rabbit IgG (Sigma), diluted at 1:50 in 0.1 M PBS containing 10% goat serum and 0.3% Triton X-100. After several washes, first in 0.1 M PBS and then in 0.1 M phosphate buffer (pH 7.3), embryos were reacted in DAB (Sigma) diluted at 0.01% in 0.1 M phosphate buffer containing 0.002% of 33% hydrogen peroxide. The reaction was interrupted after 1–5 min by thinning out the substrate with 0.1 M phosphate buffer. The embryos were dehydrated in ethanol (50%, 70%, 90%, 96%, 5 min each; 100%, 15 min) and propylene oxide (15 min) and left for at least 5 h in a mixture of propylene oxide and Epon. Finally the embryos were individually mounted on glass slides with a needle, oriented and covered with Epon and a coverslip. Polymerisation of the Epon followed overnight at 60°C.

Temperature shift experiments with the temperature sensitive allele $D^{pB37}$ were performed as follows. Flies were allowed to lay eggs on agar plates, which had been brought to a temperature of either 18°C (permissive temperature) or 29°C (restrictive temperature). After 2.5 h development at either of these temperatures, eggs were collected and dechorionised. The embryonic stage of individual embryos was determined by microscopic examination (see Campos-Ortega and Hartenstein 1985a, for a table of normal development), and the embryos were then allowed to continue development on the surface of destilled water previously brought to a temperature of 18°C or 29°C. Once the adequate stage had been determined by microscopic inspection (segregation of most central neuroblasts completed, stage 10 in Campos-Ortega and Hartenstein 1985a), embryos were shifted down or up by transferring them to water of the corresponding temperature. Further development continued until maturation of sensilla was completed. Embryos were then fixed and labelled with anti-HRP, as described above.

The study of the anti-HRP-stained embryos was performed at 1250-fold magnification. Qualitative and quantitative abnormalities of identified sensilla were recorded in standardised graphs of the PNS (see Fig. 1). At least 8–10 individuals homozygous for each neurogenic allele included in this report were carefully studied.

Results

The peripheral nervous system of the wild-type Drosophila embryo

In the Drosophila embryo the anti-HRP antibody binds to the whole of the sensory neurons, and to parts of several, if not all, trichogen cells, i.e. anti-HRP stainings show in any sensillum at least a soma with a clearly distinguishable dendrite directed toward the outside, in most cases surrounded by a conspicuous sheath and an axon projecting toward the CNS. In the following, we summarise the major aspects of the composition of the larval PNS in Drosophila after labelling with anti-HRP (cf. Campos-Ortega and Hartenstein 1985a; Fig. 1). Most sensilla which were defined as such by HRP labelling could be assigned to distinct cuticular specialisations detectable in preparations of the larval cuticle; furthermore, most sensilla have been characterised by scanning and transmission electron microscopy (Kankel et al. 1980; Campos-Ortega 1982; Singh and Singh 1984; Campos-Ortega and Hartenstein 1985a; Hartenstein, unpublished observations).

Three types of sensilla are present in any of the thoracic and abdominal segments of the Drosophila larva: (i) trichoid sensilla, (ii) campaniform sensilla and (iii) chordotonal organs. Trichoid sensilla and campaniform sensilla are innervated by a single sensory neuron each. Chordotonal organs comprise one, three or five scolopidia and, therefore, sensory neurons. A fourth type of sensillum, to be classified as basiconical sensillum, only occurs in the three thoracic (T1–3) and last two abdominal (A8–9) segments. In T2–3 these special sensilla were called “black organs” by Lohs-Schardin et al. (1979) and “black dots” by Campos-Ortega and Hartenstein (1985a), being innervated by the dendrites of two or three sensory neurons each; basiconical sensilla in A8 and A9 form part of the so-called sensory cones (Hertweck 1931) and exhibit a single dendrite each.

The gnathal segments and the procephalon also contain distinct groups of sensilla. Except for some chordotonal organs, gnathal sensilla are arranged to form complex sensory organs: the hypophysis and labial complex of the labial segment (Hertweck 1931); the terminal and ventral organs of maxillar and mandibular segments; the dorsal organ of the procephalic lobe; the epiphysis and dorsal pharyngeal organ of the labrum. On the basis of their cellular architecture terminal, dorsal and ventral organs (which together form the antennal-maxillary complex), and the labial complex, can be considered as arrays of poly-innervated basiconical and campaniform sensilla (Hertweck 1931; Kankel et al. 1979; Singh and Singh 1984; Campos-Ortega and Hartenstein 1985a). The cellular organisation of hypophysis, epiphysis and dorsal pharyngeal organ has not been investigated sufficiently to allow their unequivocal classification.