Effects of concanavalin A on developing ganglion cells in the retina of chick embryos

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SUMMARY

The administration of concanavalin A (Con A) (50–200 µg/egg) to chick embryos between the third and the seventh day of incubation has the following effects on the retina:

1. Con A causes the degeneration of a large number of ganglion cells and consequently the layer that should be formed by these cells is not present or is constituted only by a small number of ganglion cells.

2. The lectin seems to be effective only when it is administered during the postmitotic phase of the ganglion cells.

3. The degenerated cells are phagocytosed by the Müller cells in a manner similar to that occurring during the natural cell death in normal retinal development.

4. The differentiation of other retinal elements (photoreceptors, bipolar, amacrine and Müller cells) is not affected by the lectin administration.

5. The administration of Con A in later stages of development, even at ten times higher dosages (2000 µg/egg), fails to affect retinal neurogenesis.

It is suggested that Con A binding to receptor sites of the cell membrane affects the distribution or mobility of surface components producing an alteration in the mechanism by which the developing cells regulate positional information during retinal neurogenesis.

INTRODUCTION

The neurogenesis of the nervous system includes a number of events distinguishable as cell proliferation, migration, cytodifferentiation and the establishment of specific connexions (Cowan, 1979).

All these processes occur during periods of development characteristic for each of the different regions of the nervous system. Thus the neurogenetic sequence begins in the medulla earlier than in the retina and the development of the cerebellum in the chick embryo takes place in the later stages of incubation and continues through the first days after hatching. The major events of cell proliferation occur in the chick retina during the first week of incubation. The postmitotic ganglion cells reach their final position or layer beneath the vitreal surface on the seventh to eighth day of incubation and the same time the inner plexiform layer becomes discernable (Meller, 1968; Kahn, 1973, 1974; Meller &
Con A effects on developing chick retina

Tetzlaff, 1976). During these processes the developing retinal cells are organized in columns and this fact suggested that the cell-to-cell contacts in a given column facilitate the transfer of the necessary positional information for correct migration so that the cells can reach their destination at the right time (Meller, 1979a). Cell migration, cell recognition and the formation of specific contacts are probably dependent upon some properties of the cell membrane (Roseman, 1970; Roth, McGuire & Roseman, 1971; Roth, 1973). Some authors (Brunngraber, 1969; Barondes, 1970; Gombos et al., 1978) have proposed that glycoprotein molecules situated on the cell membrane act as information molecules in contact phenomena that take place in the developing nervous system especially at the time of migration and synaptogenesis. In addition, the localization of such recognition molecules changes during development preparing the cell membrane for specific processes of cell recognition. Plant lectins, such as Con A, binding to sugar molecules of the cell membrane caused aggregation of embryonic cells depending on their stage of differentiation (Moscona, 1971; 1974). Various types of lectins show a binding specificity of cells of different brain regions (Hatten & Sidman, 1978). Furthermore, the linkage of lectins to the cell membrane produces alterations in the migration of cells as described by Moran (1974a, b) in the developing neural crest, cessation of morphogenetic movements in amphibian embryos (Boucaut et al. 1979) and the inhibition of the closure of the neural tube (O’Dell, Tencer, Monroy & Brachet, 1974; Lee, 1976).

The present work studies the effects of the lectin Con A on the development of the chick retina. Con A causes degeneration of ganglion cells in an early stage of morphological differentiation when it has been administered during the postmitotic period. Differentiation processes in other retinal cells, however, are not affected.

MATERIALS AND METHODS

Concanavalin A (Sigma, München) (50–200, and 2000 μg/egg in Hanks) was injected into the amnionic cavity of 3- to 15-day-old chick embryos. After various incubation times, the embryos were fixed either in a glutaraldehyde/paraformaldehyde or a 1 % osmium tetroxide solution for electron microscopy or in Bouin’s fixative for light microscopy. Embryos older than 14 days were perfused through the heart. Paraffin embedding and routine electron microscopical techniques (Alcohol dehydration, Epon embedding) were used. A total of ca. 600 eggs were injected for this investigation. Less than 50 % of the

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Fig. 1. (a) Semithin section of the retina of a 14-day-old embryo (× 1000). (b, c, d) Retina of a 14-day-old embryo that received a dose of 200 μg Con A on the seventh day of incubation. Observe the malformation of the ganglion cell layer and the necrotic cells in the bipolar layer (× 1000). (e) Oil immersion of the vitreal portion of the retina of a 14-day-old embryo, treated as in b, c, d. Observe the phagocytosed material in the Müller cell feet (× 2000).
Con A effects on developing chick retina

Embryos injected with Con A in saline solution survived. Lethality was independent of the dose employed and the stage of development at the moment of injection. Only 30% of the injected surviving embryos treated in the first week of incubation exhibited Con A effects in the retina. No effects on the retina were observed in the surviving embryos injected in the second (8–15) week of incubation. No macroscopical malformations were observed.

RESULTS

The most important events of cell proliferation and migration in the chick retina occur during the first week of incubation. The formation of the inner plexiform layer takes place between the seventh and the eighth days as a consequence of the migration of the ganglion cells. Synaptic development begins on the 14th day and continues until hatching. The morphological differentiation of receptors, bipolar, amacrine and Müller cells takes place during the final third of incubation (for details see Meller, 1964; Meller & Breipohl, 1965; Meller, 1968; Meller & Tetzlaff, 1976).

The effects of Concanavalin A

Figure 1b shows an overview of a portion of a retina of a 14-day-old chick embryo that was treated on the seventh day of incubation with a dose of 200 µg Con A. A comparison with the control (Fig. 1a) clearly demonstrates the following effects:

(I) Abundant cell degeneration localized in the basal portion of the bipolar cell layer. Numerous pycnotic nuclei are situated between the bipolar cells (Figs. 1b, c).

(II) Ganglion cells are not present in the habitual position and normal number. Examination of the whole retina in serial sections shows that this is true for most of it, although single or groups of ganglion cells are visible in their habitual position. The finding of isolated emigrated ganglion cells seems to be fortuitous. They are found in irregularly distributed areas.

(III) The development of bipolar cells seems to be unaffected. Photoreceptor cells (Fig. 1d) developed inner segments characteristic of this stage.

(IV) In further stages of development prior to hatching (Fig. 2), the necrotic material can only be observed in the basal prolongations of the Müller cells or in the extracellular spaces near the vitreal surface (Fig. 1e). Ganglion cell layer and fiber layer are missing (Fig. 2).

The electron microscopical investigation confirms the main aspects of these light microscopical observations. Figs. 3 and 4 show overviews of the retina in

Fig. 2. (a) Paraffin section of a retina of an 18-day-old embryo, control (×1200). (b) Semithin section of a retina of an 18-day-old embryo injected on the third day of incubation with 200 µg Con A. The ganglion cell layer is missing (×1200).
Con A effects on developing chick retina

the stages of 12 and 17 days of incubation, respectively. Both embryos were injected on the seventh day of incubation with a dose of 200 μg Con A. One can observe the absence of a ganglion layer. A considerable number of pycnotic nuclei, fragments of cell cytoplasm of degenerated cells and osmophilic bodies of unidentifiable material are present at the 12-day stage (5 days after injection) between the bipolar cells (Fig. 5a). Figures 5(b), (c) demonstrate the necrotic material phagocytosed by the prolongations of the Müller cells and accumulated in their basal feet. The development of the outer plexiform layer, especially of the synapses between receptor and bipolar cells is similar to that in controls, whereas the synaptic organization of the inner plexiform layer is obviously altered by the absence of most of the ganglion cells. These alterations will be the subject of a separate study. Con A did not affect the ganglion cells, even at a ten times higher dose (2000 μg/ml), when it was administered after the 8th day of incubation.

DISCUSSION

Con A administration causes the degeneration of developing ganglion cells in the retina and consequently the malformation of the ganglion cell layer with a high degree of cell loss. As a result the fibre layer is also poorly developed.

This effect is brought about when the lectin is administered at a determined stage of development, in this case the postmitotic period in the differentiation of these cells. Histoautoradiographic (Kahn, 1974) and morphological data (Meller & Tetzlaff, 1976) show that the transition from the proliferation phase to the postmitotic phase occurs in the ganglion cells between the third and the fifth day of incubation and the inner plexiform layer, a consequence of ganglion cell migration, becomes discernible between the seventh and the eighth day of incubation. Only during this limited period of time does Con A cause the degeneration of developing ganglion cells. The fact that the retinae of only 30 % of the surviving embryos are affected cannot yet be explained. A precise understanding of the mode of Con A incorporation in the living embryo requires further investigation. Inhibiting effects of Con A on morphogenesis have been reported in early stages of neurogenesis. Moran (1974a, b) described an inhibition of gastrulation and neurulation in amphibian embryos depending on the concentration of the lectin. Such inhibition of neuronal differentiation was also found after Con A administration by Lamon & Duprat (1976) although other cells such as myoblasts, epithelial cells and melanophores are less affected by the lectin. Con A blocked neural tube formation in explanted chick embryos but somite and heart development and blood island formation were usually unaffected (Lee, 1976). Similar results by Boucaut et al. (1979) in amphibian

Fig. 3. Electron microscopical overview of the retina of a 12-day-old embryo injected on the seventh day of incubation with a dose of 200 μg Con A (× 1600).
Fig. 4. Electron microscopical overview of the retina of a 17-day-old embryo injected on the seventh day of incubation with a dose of 200 μg Con A (×1200).
Con A effects on developing chick retina

Embryos were interpreted as a consequence of a masking by the lectin of the glycoproteins related with cell recognition and migratory activity. In all of these experiments, the apparently higher degree of sensitivity of the developing nervous structures to the action of the lectin still remains inexplicable. Kleinschuster & Moscona (1972) postulated the existence of different receptor sites for lectins during development. The receptor sites might be masked during maturation with a trypsin-sensitive material making them less accessible for interaction with Con A. In this case the changes in the membrane surface would be correlated with the developmental process. Subsequently Martinozzi & Moscona (1975) suggested that the most essential feature distinguishing embryonic cells from adult ones is not the masking of receptors but changes in the fluidity of the cell membrane affecting the mobility and topographical distribution of the receptors. Krach, Green, Nicolson & Oppenheimer (1974) who worked with agglutination assays, proposed that receptor binding sites change during development and maturation and their different topography would affect the social behaviour of the cells during organogenesis. Evidence of different responses of diverse cells of the neural crest to lectin, depending on the stage of differentiation, were also detected by Sieber-Blum & Cohen (1978). Some authors (Pfenninger & Maylié-Pfenninger, 1975; Pfenninger & Rees, 1976; Vaughn, Henrickson & Wood, 1976) described changes in the glycoprotein composition of the cell membrane in developing neuroblasts and in the portion of the cell membrane in the receptor cells that forms synaptic contacts (McLaughlin & Wood, 1977) and during brain development (Margolis & Gomez, 1974). Changes in the glycoprotein patterns were also detected with other lectins such as wheat germ agglutinin (WGA) and ricin (RCA) during the development of the chick retina between the seventh and thirteenth day of incubation (Mintz & Glasser, 1978). Despite these investigations, the question of whether Con A receptor sites are directly responsible for cell adhesion remains open (Steinberg & Gepner, 1973). In this connexion Zanetta et al. (1978) demonstrated an affinity of the parallel fibres to Con A during the major events of cerebellar development and suggested that the ‘recognition molecules’ contribute to form a preliminary type of contact between complementary neuronal surfaces. The presence of temporary gap junctions during the early stages of retinal neurogenesis has been interpreted as the morphological basis of a mechanism whereby the developing cells reorganize or regulate positional information during migration (Sheffield & Fischman, 1970; Dixon & Cronly-Dillon, 1972; Jacobson, 1976; Fujisawa, Morioka, Watanabe & Nakamura, 1976). Possibly the uncoupling of these contacts between the developing ganglion cells under the influence of Con A leads to the degeneration of the cells. This remains to be verified in vivo because, as demonstrated in vitro, Con A partially inhibits the formation of specific contacts such as gap or tight junctions (Meller, 1979b). From the data provided by the studies with lectins and the present results it can be said that the macromolecular characteristics of the cell
membrane, especially the glycoproteins in the membrane play a decisive role during the neurogenesis.

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REFERENCES


Fig. 5. (a) Basal portion of the bipolar cell layer of a 14-day-old embryo injected on the seventh day of incubation with 200 µg Con A. Observe the pycnotic nuclei and the degenerated cell material (x 4500). (b) Basal portion of the retina of a 14-day-old embryo injected on the seventh day of incubation with 200 µg Con A (x 6000). (c) Basal portion of the retina of a 17-day-old embryo injected on the seventh day of incubation with 200 µg Con A. Observe the phagocytosed material in the Müller cell prolongations (x 18000).


Con A effects on developing chick retina


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