Tissue-specific inhibition of embryonic neural cell proliferation by rat brain extract

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Summary. The effect of rat brain tissue extract on the proliferative activity of chicken embryonic neural tube and other tissues was studied. Only tissue-specific inhibitory action was found to be similar to substances of the chalone group.

The existence of a feedback regulating mechanism mediated by chalones has been proved in more than 20 tissues and cell systems of vertebrates[1,2]. Chalones are very small proteins and peptides produced in all tissues with mitotic activity, intervening in the course of the cell cycle, controlling the size of cell populations and probably taking part in the differentiation process[3,4]. Some highly differentiated and functionally specialized cells (for example heart muscle cells) lose their ability for mitotic division in the adult organism. Kriek et al.[5] have recently demonstrated that a chalon-like type of regulation exists in this tissue as well. A similar situation is found in the CNS. Here, besides ganglion cells which definitely lose their mitotic ability in the postnatal period, there are also various glial cells capable of proliferation in the adult organism under some circumstances (e.g. during inflammation, or irradiation, or following a trauma). All these cells (except for the microglia) are derived from one basic type found during the embryonic period in the inner ependymal zone of the medullary tube[6,7]. This allows the use of the embryonic neural tube as a suitable testing system when trying to find out whether the cell division in the CNS is also blocked by tissue-specific and species-nonspecific inhibitors.

Materials and methods. 4-6-day-old and 14-day-old chicken embryos were used in the present study. The neural tube of younger embryos is completely closed and has the character of a continually proliferating cell population. Cell division takes place here only in the ependymal layer acting as the matrix zone and the cells are not yet differentiated into glioblasts and neuroblasts. The tissues of older embryos were used as a controlling system to test the possible nonspecific effect. Brains and livers of adult male rats, Wistar strain, removed under sterile conditions, served for the preparation of the neural and liver extracts[8]. The tissues were homogenized (ratio to sterile distilled water 1:3), the homogenate was filtered through gauze and centrifuged in Janetzki K 24 (15 min, 250 rps, 4 °C) and Janetzki VAC 60 (120 min, 600 rps, 4 °C, overloading 120,000 × g) centrifuges. The obtained supernatant was tested. The protein quantity was determined by Lowry's method[9] using bovine serum albumin as the standard. Just before application it was diluted with saline solution. Individual doses (in 5×10^{-4} l of liquid) were injected with 5×10^{-5} g of colchicine (Fluka) into the amniotic cavities of the chicken embryos. Whole embryos or pieces of tissues were fixed in Carnoy's fluid after 4 h of incubation at 37 °C. They were subjected to the routine histological technique of embedding in paraffin and mitotic activity was evaluated in sections stained with Feulgen's reaction using the dithionite-leucofuchsin reagent[10]. The statistical significance of the differences in mitotic activity between the individually tested groups was determined by the t- and U-tests.

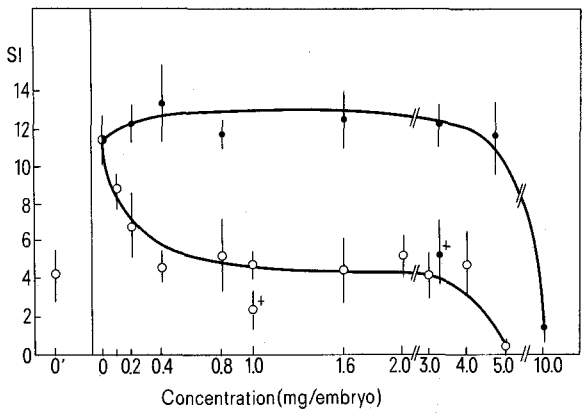

Figure 1. Effect of neural (○——○) and liver (●——●) extracts on mitotic activity of neural tube. The number of cells in the M phase of the cell cycle per unit lenght of the inner circumference of the neural tube is determined. The value obtained gives the so-called surface index (SI) suitable for the comparision of proliferative activity in the same tissues[11]. O', control group without colchicine; O, group with colchicine. Points with + / express the SI values in control groups without colchicine. With single points is shown SD × 2.

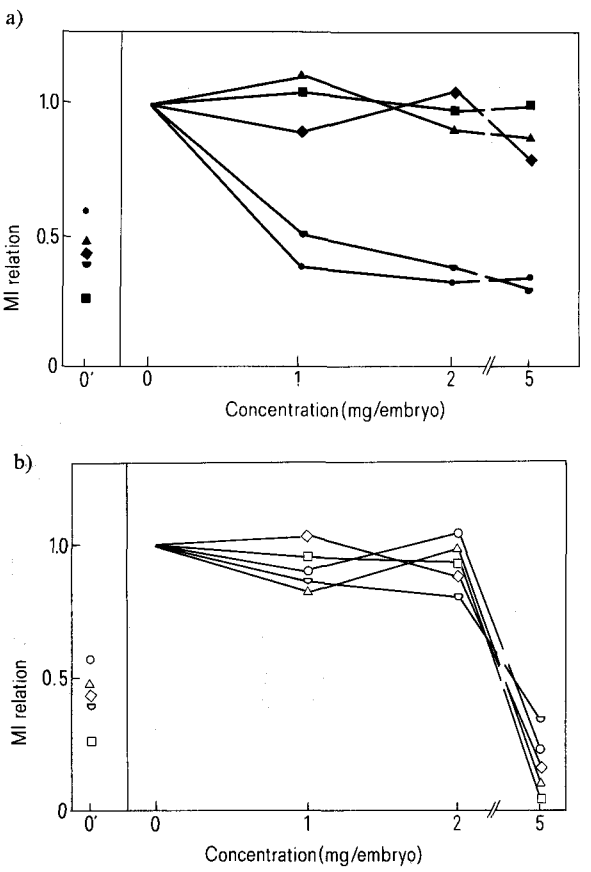

Figure 2. Effect of neural (a) and liver (b) extracts on the MI of hepatocytes (○, ●) and mesenchymal cells (□, ■) in 4-6-day-old chicken embryos and on the MI of the bronchial epithelium (△, ▲), intestinal epithelium (◇, ◆) and hepatocytes (⌓, ⌣) in 14-day-old chicken embryos. Only MI-values of hepatocytes with 5 mg/embryo concentration show a significant difference from others.

Results and discussion. The effect of neural and liver extracts on the mitoses of the neural tube is shown in figure 1. Mitotic activity is stabilized at approximately ½ of the original value from the concentration of about 5×10^{-4} g of proteins of brain extract per embryo. Its level is not changed by the 4×10^{-3} concentration of proteins, but it drops rapidly afterwards. With this concentration the nonspecific toxic effect is likely to predominate; this is, among other things, indicated by the virtually complete mitotic inhibition found in other tissues (fig. 2,a). The liver extract does not show any inhibitory action with lower concentrations, its toxic effect being shown first with the dose of 1×10^{-2} g protein per embryo. Figures 2,a and b demonstrate that the inhibitory effect of the brain extract is not evident with low doses in the other studied tissues and that only hepatocytes are inhibited by liver extract.

Substances with regulating effects have been isolated from the neural tissue before. Inhibition of the development of homologous neural structures by brain extracts has been described by Török and Törö[12]. On the other hand, Tiedemann[13] reported the isolation of the neural inducing factor. The question whether the neural tissue is subject to chalone regulation is far from answered yet. It is not for example clear what the effect of high protein doses would be after longer time, or what possible morphogenic or teratogenic action the extract might have. Without considering all these aspects, the specific toxicity of the extract cannot be excluded. In spite of this, the facts obtained so far indicate the possible effect of tissue-specific and species-nonspecific inhibitors. The inhibitory influence could also be mediated by the inactivation of the so-called nerve growth factor[14] stimulating the multiplication of the embryonic nerve cells.

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Identification of type I collagen fibrils in human dentine. Electron microscope immunotyping[1]

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Summary. Using immunoperoxidase labeling with anticollagen antibodies and electron microscopy, the collagen fibrils constituting the matrix of human dentine were identified as type I collagen. Thus, the unique odontoblastic origin of dentinal collagen was confirmed.

Pulp and dentine are the 2 major components of the tooth. The former is a soft connective tissue consisting of ground substance, collagen, reticulin fibrils and various cells. The latter represents the mineralized organic matrix composed mainly of collagen fibrils and elaborated probably by the odontoblasts throughout the life of the tooth. The existence of intermolecular cross-links, generating a high degree of insolubility, has made the biochemical analysis of the dentine collagen a difficult task[2]. However, a few investigators[3-5] identified dentinal collagen as type I collagen, with traces of type I trimer (3α1(I) chains), respectively, in bovine or rat teeth. Using immunological techniques and the light microscope, Lesot and Ruch[6] and Thesleff et al.[7] confirmed the presence of type I collagen in the dentinal matrix of mouse tooth germs. Sauk et al.[8] found that type III collagen does exist in the dentine of patients affected by osteogenesis imperfecta. However, there is very little information concerning the quality of human dentinal collagen. So, in the present paper, its identification is described, using immunoperoxidase labeling and the electron microscope.

Material and methods. Ten partially developed, unerupted wisdom teeth were removed from children for orthodontic reasons and immediately cracked open in a vice. For standard electron microscopy, the dentinal fragments were placed in 2% glutaraldehyde, 0.1 M cacodylate solution (pH: 7.4), washed in 0.2 M cacodylate-sucrose buffer (pH: 7.4) and progressively demineralized in HNO_3 (from 10 to 2%) for 2 weeks at room temperature. The specimens were then washed again, routinely postfixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Thin sections were then contrasted in uranyl acetate followed by lead citrate and examined under the electron microscope.

For indirect immunostaining with antibodies using the peroxidase procedure, the fractured dentine fragments were fixed in a paraformaldehyde-cacodylate-sucrose solution as described previously[9]. After washing, the samples were progressively demineralized in HNO_3 (from 10 to 2%) for 2 weeks at room temperature, washed again, embedded in tissue teck II O.C.T. medium and quickly frozen.

Cryostat sections (10 µm) were incubated in monospecific antitype I and III collagen antibodies overnight at 4 °C,