Short Communications

Dysplasia of subcommissural organ in congenital hydrocephalus spontaneously occurring in CWS/Idr rats

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Summary. The subcommissural organ (SCO) of the congenital hydrocephalus spontaneously occurring in CWS/Idr rats was severely reduced in size and displaced at some distance from the anterior end of the cerebral aqueduct. The cerebral aqueduct of the hydrocephalic rats was open throughout its total length during postnatal days 1–20, though it was somewhat narrower at its middle region than in the normal brain.
Key words. Congenital hydrocephalus; subcommissural organ; cerebral aqueduct; CWS/Idr rat.

CWS/Idr rats have been established and maintained by brother-sister mating in our institute (Idr) as a mutant having an autosomal recessive gene for congenital cataract. The animals originated in the descendants of heterozygotes derived from outcrossing spontaneously-occurring cataractous JCL:SD with normal inbred WKA/Hok rats [1]. Recently, congenital hydrocephalus has spontaneously occurred in this rat strain, at an incidence of about 5.8%, or 164 of 2805 living pups, from March 1980 to October 1987.

Although congenital hydrocephalus has been shown to occur in many mammalian species including human beings, surprisingly little is known about its pathogenetic mechanism. Recently, we have shown that the subcommissural organ (SCO) is maldeveloped in the congenital hydrocephalus induced in rats by prenatal X-irradiation [2], and that the SCO is completely absent in the congenital hydrocephalus spontaneously occurring in MT/HokIdr mice [3]. These studies have led us to examine the morphological aspects of the SCO in the congenital hydrocephalus occurring in CWS/Idr rats. The histological examination of the cerebral aqueduct is also employed, since aqueductal stenosis is widely considered to be a main cause of congenital hydrocephalus.

Materials and methods. Injection studies. Five hydrocephalic rats on postnatal days 10 and 20, and also 3 normal littermates on postnatal day 10, were anesthetized by phenobarbital, and a 1% Chicago Sky Blue solution in 0.9% saline was injected into their lateral ventricles. After about 5 min, their brains were excised, bisected coronally, and immersed in 95% ethanol. The brains were fixed in 95% ethanol for 24 h, dissected coronally with razor blades, and examined for the presence of dyes in the cerebral aqueduct [2].

Histological studies. A total of 8 normal and 16 hydrocephalic rats on postnatal days 1, 5, 10 and 20 was anesthetized by ethyl ether, and killed by cardiac perfusion with 10% formalin after the blood vessels had been flushed out with 0.9% saline. Perfusion with formalin was performed for 10 min with a flow rate of 9.5 ml/min. After completion of perfusion, the brains were fixed with Bodian II solution for 24 h, dehydrated with an ethanol series, and embedded in paraffin wax. Coronal and sagittal sections 10–12 µm thick were made with a rotating microtome and stained with hematoxylin and eosin.

To examine the secretory activity of the SCO, 5 normal and 8 hydrocephalic rats on postnatal day 10 were sacrificed by the same method. After perfusion with formalin, the brains were fixed with Bouin's solution for 5 days, and embedded in paraffin wax. Sagittal sections 12 µm thick from these materials were stained with the periodic acid Schiff reagent (PAS) [4].

Results. After injection of tracer dye into lateral ventricles, the caudal portion of the periaqueductal tissue of the normal brain was densely stained, whereas that of the hydrocephalic brain was only slightly stained (fig. 1), suggesting that the cerebrospinal fluid may not flow well in the cerebral aqueduct of the hydrocephalic brain.

Serial coronal sections of midbrains of normal and hydrocephalic rats on postnatal days 1, 5, 10 and 20 revealed that the cerebral aqueduct was open throughout its total length in both animals. However, the cerebral aqueduct in the middle region of the normal brain was somewhat expanded laterally in its central to slightly upper portion, whereas no such expansion was found in the hydrocephalic brain (figs 2a and 2b).

Figure 1. Caudal portions of the midbrains of normal (left) and hydrocephalic (right) 10-day-old CWS/Idr rats after injection of Chicago Sky Blue into the lateral ventricles. The periaqueductal tissue of the normal brain is densely stained, whereas that in the hydrocephalic brain is only slightly stained.

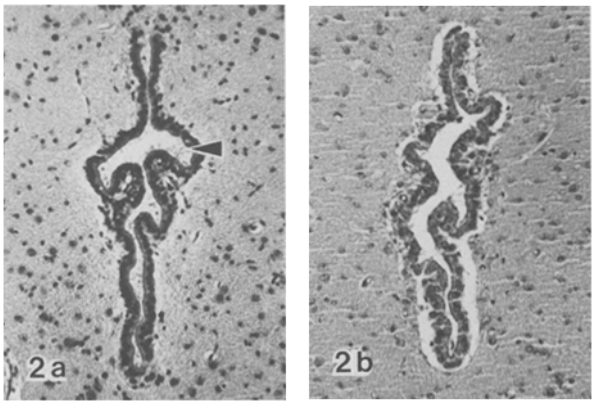

Figure 2. Coronal sections of the middle regions of cerebral aqueducts in normal (*a*) and hydrocephalic (*b*) 20-day-old rats. The cerebral aqueduct in the normal brain is somewhat expanded laterally towards its upper part (arrowhead), whereas no such expansion is observed in that of the hydrocephalic brain. × 120.

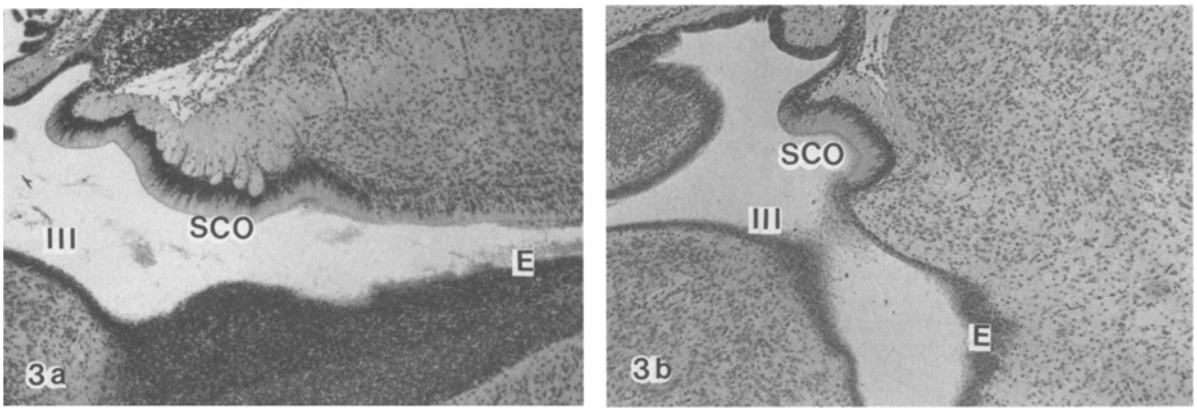

Figure 3. Sagittal sections showing a well-developed subcommissural organ (SCO) in the normal brain (*a*) and a dysplastic SCO in the hydrocephalic brain (*b*) of 1-day-old rats. III, third ventricle; E, anterior end of cerebral aqueduct. ×45.

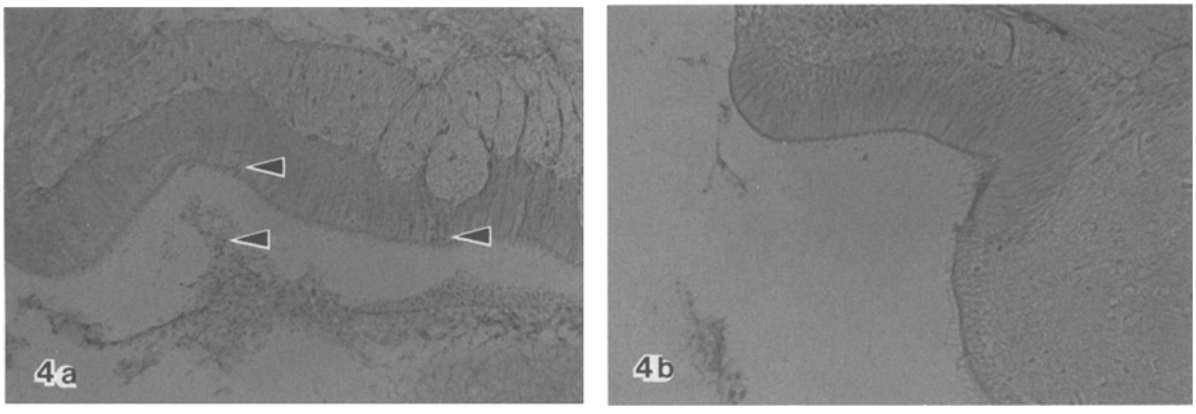

Figure 4. PAS-staining of the subcommissural organ in the normal (*a*) and hydrocephalic (*b*) brains of 10-day-old rats. Numerous PAS-positive granules are found in the SCO ependymal cells and also in association with the amorphous materials floating in the cerebral aqueduct of the normal brain (arrowheads), as opposed to far smaller amounts in the hydrocephalic brain. ×160.

Sagittal sections of the midbrain of a normal rat on postnatal day 1 showed that the SCO, consisting of an elongated, pseudostratified ependymal cell layer and a hypendymal layer containing glial cells, nerve fibers and vascular elements, was developed so as to extend from the roof of the caudal end of the third ventricle to the roof of the anterior end of the cerebral aqueduct (fig. 3a). In the hydrocephalic brain, however, the SCO was reduced to about one-third of the normal size, and developed only at the roof of the caudal end of the third ventricle, at some distance from the anterior end of the cerebral aqueduct (fig. 3b).

The same dysplastic SCO was also found in the hydrocephalic brains on postnatal days 5, 10 and 20 (data not shown), the fact indicating that neither reclamation nor degeneration of the dysplastic SCO occurs during the postnatal life of affected rats.

After PAS staining, numerous PAS-positive granules were present in the cytoplasm of the SCO ependymal cells of normal brain, particularly in the apical part of the cells. Numerous PAS-positive granules were also observed to associate with the amorphous materials floating near the apical surface of the SCO in the cerebrospinal fluid. A thread-like structure, densely stained with PAS and probably a Reissner's fiber, was seen in these amorphous materials (fig. 4a). In the hydrocephalic brain, however, the amount of PAS-positive granules in the SCO ependymal cells was greatly reduced, and the amorphous material floating in the cerebrospinal fluid was also decreased (fig. 4b).

Discussion. Although many investigators have considered stenosis of the cerebral aqueduct as a main cause of congenital hydrocephalus, the precise mechanism of aqueductal stenosis remains unclear. A number of investigators have recently regarded aqueductal stenosis as the secondary result of the compression exerted by the expanding hydrocephalic ventricles, since the cerebral aqueducts of the congenital hydrocephalic brains obtained from some animal models and also from some human autopsy materials were open throughout their total length when examined on an early postnatal day [3, 5]. In the present study, the cerebral aqueduct of the congenital hydrocephalus occurring in CWS/Idr rats was open throughout its total length during postnatal day 1 and day 20, although it was somewhat narrower in its middle region as compared with the normal one. This fact indicates that stenosis of the cerebral aqueduct may not be a main cause of congenital hydrocephalus in CWS/Idr rats.

The SCO of the congenital hydrocephalus in CWS/Idr rats was severely reduced in size and displaced at some distance from the anterior end of the cerebral aqueduct. Its secretory activity also declined as compared with that of the normal SCO. These observations are very similar to those made in the case of the congenital hydrocephalus experimentally induced in rats by prenatal X-irradiation [2]. In a pathogenetic study of X-ray-induced congenital hydrocephalus, it was shown that the anomaly of the SCO is not the result of degeneration of a pre-existing SCO, brought about by the hydrocephalic state, but a defect preceding the onset of cere-

bral ventricle enlargement[2]. In the present study, the embryonic development of the SCO in affected rats could not be investigated, since the incidence of congenital hydrocephalus in CWS/Idr rats is very low, and cranial enlargement in affected rats is not clearly recognizable in prenatal life. However, dysplastic SCO was always observed in hydrocephalic CWS/Idr rats during postnatal day 1 and day 20, indicating that the hydrocephalic state in CWS/Idr rats may not induce degeneration of pre-existing dysplastic SCO. Thus, dysplasia of SCO in congenital hydrocephalic CWS/Idr rats may be a primary defect and closely related to the cause of congenital hydrocephalus.

Numerous reports have been published in the literature on the functional significance of the SCO and its secretory product, Reissner's fiber, but none have been confirmed by direct evidence[3]. The present results and also our previous work showing SCO dysplasia in congenital hydrocephalus[2,3] suggest that the SCO and Reissner's fiber may be involved in the regulation of the intraventricular flow of cerebrospinal fluid. The precise mechanism awaits further elucidation.

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Selective effects of PHA on rat brush border hydrolases along the crypt-villus axis

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Summary. The mechanism of the toxicity of lectin from *Phaseolus vulgaris* seeds has been investigated on rat enterocytes. Cell isolation procedures showed a selectivity in the loss of brush border hydrolases; this indicated that the microvilli blebbing was not the only mechanism of action of lectins on rat enterocytes.
Key words. PHA; lectins; *Phaseolus vulgaris;* intestinal hydrolases; enterocytes; brush border membrane.

The toxicity of orally ingested lectins is primed by damage to the small intestine. Many studies have been devoted to the analysis of this cellular toxicity[1-4]. They concluded essentially that there is a microvillar fragility induced by lectins; this appeared as blebbing of brush border membranes leading to a decrease in hydrolase activities. The objective of the present study was to investigate this mechanism as a function of the age of enterocytes, i.e. along the crypt-villus axis.

Material and methods. Phaseolus vulgaris bean agglutinin (PHA) was purified from seeds (var. Lingot blanc) by affinity chromatography on fetuin-Sepharose CL-4B[5]. Growing male Sprague-Dawley rats, weighing 82 ± 1 g, were divided into 3 equal groups of 6 animals, according to the average body weight. They were kept in individual metabolic cages at constant temperature and given water ad libitum. The control diet of which the detailed composition was previously reported[6] contained 67% carbohydrates, 10% protein and 4% fat. The experiment was conducted as published elsewhere[7]. Group 1 was fed the diet ad libitum; group 2 received the same diet plus 0.25% pure PHA; group 3 was pair-fed with group 2, but received the control diet. All diets were isonitrogenous. Methyl-^{3}H-thymidine was used as an indicator for cell proliferation. For this purpose 50 µCi/rat of methyl-^{3}H-thymidine (77 Ci/mmol, NEN, Boston) were injected i.p. 1 h before sacrifice. After 17 days, rats were killed by cervical fracture. The entire duodenum, extending to the ligament of Treitz, was removed, washed with ice-cold saline, and everted. Duodenal samples were pooled for each group. Enterocytes were isolated by a sequential cell release technique according to Weiser[8] and modified by Raul et al.[9]. Samples were incubated in series successively for 10 min at 37 °C in phosphate-buffered saline (no Ca^{++}, Mg^{++}), EDTA 1.5 mM, dithiothreitol 0.5 mM, under agitation in a water bath shaker. After each incubation, the medium containing the released enterocytes was spun at 900 × g for 10 min at 4 °C. The pelleted cells were resuspended in cold NaCl 0.15 M, homogenized in a Dounce apparatus and assayed for alkaline phosphatase according to Bessey et al.[10]. Disaccharidases were measured by the method of Dahlqvist et al.[11] and aminopeptidase according to Maroux et al.[12] using L-alanine-p-nitroanilide as substrate. Specific activities were calculated as µmol of substrate hydrolyzed per min at 37 °C; nevertheless, they were plotted as arbitrary units, in order to avoid a graph littered with many ordinates. Proteins were estimated according to Lowry et al.[13]. For radioactivity determination, one aliquot of each cell fraction was applied to a 2.5 cm glass-fiber filter (GF/C, Watman). The filters were extensively rinsed with 5% (v/v) trichloroacetic acid, then dried. The radioactivity of each filter was determined in a liquid scintillation spectrometer (Tri-Carb, Packard).

Results. The figure shows the gradient of specific activity of enzymes along the crypt-villus axis of the duodenum of rats fed either the control diet or the PHA diet. The 100% of cells isolated corresponds to the sum of the fractions expressed as protein.

Group 1, which was fed the control diet, displayed the usual gradient of enzyme activity. In the group fed the PHA diet (fig., b), a different pattern of activity for each enzyme, particularly for maltase and to a lesser extent for aminopeptidase, was observed at the top of the villus and in the midvillus area. On the other hand ^{3}H-thymidine incorporation increased in the crypt cell compartment when compared to the group 1 (multiplying factor: 1.6). A more complex phenomenon was observed for pair-fed animals (fig., c) where enzyme activities are clearly higher than those of the control