Published in final edited form as:

Exp Mol Pathol. 2008 October ; 85(2): 117–121. doi:10.1016/j.yexmp.2008.06.003.

Different inflammatory reactions to vitamin D₃ among the lateral, third and fourth ventricular choroid plexuses of the rat

Seymour Levine*, Arthur Saltzman, and Stephen D. Ginsberg

Nathan S. Kline Institute for Psychiatric Research, Orangeburg, New York 10962, USA

Abstract

The four choroid plexuses in the brain ventricles are not identical, but differences among them have rarely been studied. The present work concerns the inflammatory and hemorrhagic choroid plexitis produced in Lewis rats by a single gavage of cholecalciferol (vitamin D₃) or related steroids with vitamin D activity. Plexitis was very severe in the fourth ventricular plexus, somewhat less severe in the lateral ventricular plexuses, and almost absent in the third ventricular plexus. These findings were compared to the scanty data from the literature on differences among the plexuses.

Keywords

Choroid plexus; Cholecalciferol; Vitamin D₃; Inflammation; Calcification

Introduction

The brain has choroid plexuses in each lateral ventricle, the third ventricle and the fourth ventricle. The extensive literature on choroid plexuses attests to their importance for production of cerebrospinal fluid and maintenance of homeostasis in the brain: (reviewed by Milhorat, [1976]; Netsky and Shuangshoti [1975]; and Johanson [2003]). Quay [1966] studied differences in metabolism of the several choroid plexuses and he had the following complaint: “Unfortunately, published results are sometimes not accompanied by mention of the particular ventricular sources of the choroid plexuses studied. Even when the anatomical sources are mentioned there is no evidence of comparisons having been made of choroid plexuses from different regions. The tacit but unstated and untested assumption prevails that the choroid plexuses of different ventricles are metabolically similar or equivalent”. With only a few exceptions, the current literature still implies that all four plexuses are essentially identical. Nevertheless, differences in development and histology of the several plexuses have been described in fetal rabbits and fetal humans [Tennyson 1975, Tennyson and Pappas 1964]. Also functional differences among the several choroid plexuses have been

© 2008 Published by Elsevier Inc.

*Corresponding author: Phone: 845-398-5541; fax: 845-398-5531 levines@NKLRFMH.Org (S. Levine).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

described [Harbut and Johanson 1986, Walters et al. 1992]. In the present work, knowledge of the heterogeneity of choroid plexuses of rats has been extended. Toward this end, the inflammatory response of the four choroid plexuses to a vitamin D₃ preparation has been studied histologically.

Materials and Methods

Lewis rats of both sexes were bred and maintained in this Institute in plastic cages with corncob bedding. Tap water and food pellets (Laboratory Rodent Diet 5001, PMI Nutrition International, Brentwood, MO) were freely available. Rats were used when 100-200 grams in weight. They were given cholecalciferol (vitamin D₃), or other steroids dissolved at 4mg/ml in vegetable oil, at a dose of 1ml/100g by gastric intubation. Three or four days later the rats were euthanized in carbon dioxide and serum was obtained for chemical analysis in a Hitachi Model 747-100 chemical analyzer. For comparison with cholecalciferol, groups of 4 rats were given ergocalciferol (vitamin D₂, 25 hydroxy cholecalciferol), or dihydrotachysterol ("Hytakerol"), a highly potent synthetic material with activity similar to parathormone. Other groups of 4 rats were given chemically related sterols which did not affect calcium and phosphate metabolism: 7-dehydrocholesterol (precursor of cholecalciferol) and ergosterol (precursor of ergocalciferol). Control rats were given vegetable oil without any steroid.

Statistical analysis: two sample t tests compared the serum calcium, phosphorus and urea nitrogen levels between the cholecalciferol treated and control rats.

Brains were fixed in Bouin's fluid or phosphate buffered 10% formalin (4% formaldehyde). Brain in coronal sections and viscera were embedded in paraffin and sections were cut at 5 microns. Sections were stained by hematoxylin and eosin. Formalin fixed tissues were also stained by the Von Kossa procedure for carbonates and phosphates (usually accepted as representing calcium deposits).

The lesions were scored according to the extent and severity of the hemorrhage, edema and inflammation. Plexuses with severe lesions over all or most of its extent were scored 4+; severe lesions that involved about half the plexus were 3+, and severe lesions over only a small area were 2+. Lesions that had only mild edema, very few neutrophils and no hemorrhage were scored 1+.

Results

The normal choroid plexus consisted of papillary processes (villi) covered by cuboidal or columnar epithelium and containing capillaries. The base of the plexus, from which the papillary processes arose, contained arterioles, venules, and a few fibroblasts.

After the treatment with cholecalciferol, many areas of plexus were hemorrhagic and/or were inflamed as indicated by exudation of fibrin-rich edema fluid and infiltration by polymorphonuclear neutrophils (Fig. 1). In most areas, the epithelium that covered the villi was intact but it was severely attenuated or ulcerated when the underlying stroma was

densely packed with neutrophils or was very edematous. Such areas frequently had blood, fibrin and edema fluids in the ventricular lumens as well as in the plexus.

The most severe and widespread lesions were found in the fourth ventricular plexuses (Table 1). The lesions in the lateral ventricular plexuses were usually less severe but in 6 of the 35 rats they were equal or slightly more severe than the lesions in the fourth ventricular plexuses. The third ventricular plexuses were devoid of lesions in all experiments except for 3 rats that had minimal inflammation (1+) in this plexus. The brain parenchyma had no inflammation.

Von Kossa stains of formalin-fixed brains revealed only minor calcification in a few small areas of the choroid plexus inflammation. The calcium granules in these areas were often deposited in a linear manner along capillaries or encircling capillaries. The bulk of the inflamed or hemorrhagic areas had no evidence of calcium deposition, in contrast to heavy calcification of heart, kidney and brown fat caused by cholecalciferol in the same rats. Plexuses with little or no inflammation had no calcium deposits. Ependyma and brain parenchyma had no calcium deposits.

Dihydratichysterol in doses of 1 or 4 mg/100g, and ergocalciferol in dose of 4mg/100g, produced choroid plexitis identical to that produced by cholecalciferol at 4mg/100g. In contrast, the steroids without vitamin D activity (7-dehydrocholesterol and ergosterol) were ineffective despite doses of 4 or 16 mg/100 g. Control rats gavaged only with vegetable oil had no plexitis.

Serum levels of calcium were elevated in cholecalciferol treated rats, average 17.4 mg/dL, compared to control rats, average 12.1 mg/dL (Table 2). Serum phosphorus in cholecalciferol treated rats was also increased but to a lesser degree: average 13.2 mg/dL compared to normal rats, average 11.3 mg/dL. Urea nitrogen was usually elevated but variable (Table 2). This reflects the variable degree of calcific deposits in renal cortex. Ca, P and urea values were all significantly different between treated and control groups, $p < .0001$, when each was tested by a two group t test with 45 degrees of freedom (Table 2).

Discussion

Vitamin D is known to increase the calcium content of the blood and also the brain, the cerebrospinal fluid and the choroid plexuses (Murphy 1988). Excessive vitamin D has caused calcification of the choroid plexus of cats and other species (Morita 1995), but inflammation and hemorrhage were not mentioned. Hans Selye [1962] in his monograph on Calciphylaxis, made several references to the choroid plexuses of rats. He observed calcification of the blood vessels of the third ventricular plexus 6 to 15 days after administration of dihydratichysterol orally and chromic or chromous chloride intravenously (see page 100, Plate V A and figure 69). He made no mention of inflammation and none is visible in his photomicrographs. The choroid plexuses of the lateral and fourth ventricular plexuses were not mentioned or illustrated. The absence of inflammation in Selye's rats may be due to his use of the third ventricle plexus and to a longer survival. He also gave a metallic salt intravenously (we did not inject a metallic salt). Nevertheless, the inflammation

in the choroid plexuses of our rats was caused by or related to disturbance of calcium-phosphorus metabolism as the lesions were produced only by the steroids known to have vitamin D – like activity.

The other difference with Selye's results concerns the paucity of calcium disclosed by Von Kossa stains in the choroid plexuses of our rats despite the massive calcification of heart and kidney. However, this does not exclude an essential and primary effect of calcium because the Von Kossa stain may not reveal low concentrations of this mineral. More studies of calcium in the choroid plexus are needed; choroid plexus is not even mentioned in the excellent review of calcification processes by Anderson (1980).

Inflammation of the choroid plexuses has been recorded in lymphocytic choriomeningitis and other infectious diseases (extensively reviewed by Netsky and Shuangshoti, [1975]) and other instances have been added subsequently [Levine 1987, Levine et al. 1987, Quan et al. 1999] but comparisons of the various plexuses have not been recorded.

In 1973, we reported that the anti-mitotic drug, cyclophosphamide, produced choroid plexitis in rats [Levine and Sowinski 1973]. The lesions were characterized by severe edema and hemorrhage with relatively minor leukocytic infiltration. The lesions were most severe in the fourth ventricular plexus and least severe in the third ventricular plexus, a distribution similar to the cholecalciferol lesions reported herein. (The extraneural lesions caused by cyclophosphamide were completely different in distribution and character from the extraneural lesions after cholecalciferol). The paucity of leukocytes in the choroid plexuses can be explained by the toxic effect of cyclophosphamide on leukocytes.

The coincidence of distribution patterns in plexus lesions caused by these two quite different agents suggested that there might be some underlying guiding influence related to plexus structure or function that determined distribution. Innervation might be such a guiding influence. However, the number of sympathetic and cholinergic nerve fibers in the ventricular choroid plexuses of rats and other species followed the pattern: third > lateral > fourth [Lindvall et al. 1977, 1979], which is the reverse of the cholecalciferol and cyclophosphamide lesions.

Scanning electron microscopy revealed an interesting morphologic difference between the normal rat's fourth and lateral ventricular plexuses, namely bleb-covered choroidal epithelial cells were much more common in the fourth ventricle [Collins and Morriss 1975]. Transmission electron microscopy did not reveal any ultrastructural differences among the lateral, third and fourth ventricular choroid plexuses of the young pig [Davis et al. 1973] but we are not aware of a similar study on the rat.

The rat's plexuses were studied for content of transferrin and its messenger RNA; the lateral and third ventricular plexuses were richly endowed but the fourth ventricle lacked this important entity [Bloch et al. 1987]. This distribution is completely out of synchrony with our observations of plexitis. It is interesting that differences among the plexuses are already present in the newborn rat [Strazielle and Gherzi-Egea 1997].

Wet and dry weight of the rat's choroid plexuses (absolute or relative to brain weight) as well as rate of increased weight growth, were greatest for the fourth ventricle plexus and least for the third ventricle plexus and intermediate for lateral ventricle plexuses [Quay 1972]. The fourth ventricular plexus was also the heaviest after feeding rats a low K, low Na diet [Quay 1972]. These data parallel our observations on plexitis.

Different abilities in the several plexuses for uptake of cholecalciferol may have been important for our observations, especially because binding sites for 1, 25-dihydroxyvitamin D3 have been described in mouse and calf choroid plexus [Walters et al. 1992]. However, no pertinent data are available except that Suzuki et al. studied uptake of cimetidine by lateral and fourth ventricular plexuses and found no differences in drug transport [Suzuki et al 1986]. The third ventricular plexus was not examined. However, Suzuki et al. (1974) found the third ventricular plexus response to reserpine was less than the other plexuses.

The literature cited above has revealed differences among the choroid plexuses with respect to weight and surface ultrastructure that parallel and might be related to the distribution of cholecalciferol lesions. The vascular patterns in the normal lateral ventricular plexus of dogs, rabbits and humans have been studied [Gomez and Potts, 1982; Millen and Woollam, 1953] but there is no such study on rats nor any comparison of the vascularity of the several plexuses despite the obvious importance of blood supply for development of lesions. Nevertheless, the histology of the rat choroid plexus suggests that vascularity may be important. The third ventricular plexus differs from the others because it is smaller, its villi are shorter and all of them are relatively close to the large veins in the tela choroidea that forms the roof of the third ventricle (Fig. 1). (The tela choroidea is a double fold of pia mater between the brain tissue above (fornix) and the epithelium in the roof of the third ventricle below; it contains the choroid and internal cerebral veins). In the other ventricles, the villi and the vessels they contain are greatly elongated and most of each villus is at a considerable distance from the tela choroidea and its vessels. This suggests the possibility that the usual congested appearance of the veins in the third ventricular tela choroidea indicates low or slow blood flow which might be related to the virtual absence of lesions in the third ventricular choroid plexus.

In many species the choroid plexuses contain mast cells which could influence an inflammatory process, but the rat's choroid plexuses do not contain mast cells or they are very scanty [Dropp 1972, Kiernan 1976].

In summary, three different experimental lesions have been reported. Both the cyclophosphamide plexitis and the cholecalciferol lesions described herein are hemorrhagic and inflammatory but differ in cell types and etiology. Selye's choroid plexus injury takes the form of calcification with no inflammation described so far, but it is caused by the same vitamin D derivatives that we used in the present work. The overlap in histopathology and etiology of these three forms of choroid plexus injury should be studied further to resolve the discrepancies, but especially to expand investigation of differences among the four choroid plexuses contained in every brain.

Acknowledgement

We thank Morris Meisner and Joseph Wanderling for statistical analysis.

References

- Anderson HC. Pathology Annual. 1980; 15:45–75. [PubMed: 6256707]
- Bloch B, Popovici T, Chouham S, Levin MJ, Tuil D, Kahn A. Transferrin gene expression in choroid plexus of the adult rat brain. Brain Res. Bull. 1987; 18:573–576. [PubMed: 3300864]
- Collins P, Morriss GM. Changes in the surface features of choroid plexus of the rat following the administration of acetazolamide and other drugs which affect CSF secretion. J. Anat. 1975; 120:571–579. [PubMed: 1240096]
- Davis DA, Lloyd BJ Jr, Milhorat TH. A comparative ultrastructural study of the choroid plexuses of the immature pig. Anat. Rec. 1973; 176:443–454. [PubMed: 4723406]
- Dropp JJ. Mast cells in the central nervous system of several rodents. Anat. Rec. 1972; 174:227–237. [PubMed: 4627786]
- Gomez DG, Potts DG. The lateral, third and fourth ventricle choroid plexus of the dog: a structural and ultrastructural study. Ann. Neurol. 1982; 10:333–340. [PubMed: 7316486]
- Harbut RE, Johanson CE. Third ventricle choroid plexus function and its response to acute perturbations in plasma chemistry. Brain Res. 1986; 374:137–146. [PubMed: 3719323]
- Johanson, CE. The choroid plexus–CSF nexus; Gateway to the brain.. In: Conn, PM., editor. Neuroscience in Medicine. 2nd ed.. Humana Press; Totawa, NJ: 2003. p. 165–195.
- Kiernan JA. A comparative survey of the mast cells of the mammalian brain. J. Anat. 1976; 121:303–311. [PubMed: 819404]
- Levine S. Choroid plexus: target for systemic disease and pathway to the brain (editorial). Lab. Invest. 1987; 56:231–233. [PubMed: 2950275]
- Levine S, Fish D, Magnarelli LA, Anderson JF. Choroid pleinitis in white-tailed deer (*Odocoileus virginianus*) in southern New York State. Vet. Pathol. 1987; 24:207–210. [PubMed: 3603961]
- Levine S, Sowinski R. Choroid pleinitis produced in rats by cyclophosphamide. J. Neuropathol. Exp. Neurol. 1973; 32:365–370. [PubMed: 4724824]
- Lindvall M. Fluorescence histochemical study on regional differences in the sympathetic nerve supply of the choroid plexus from various laboratory animals. Cell Tiss. Res. 1979; 198:261–267.
- Lindvall M, Edvinsson L, Owman C. Histochemical study on regional differences in the choroid plexus from various laboratory animals. Exp. Neurol. 1977; 55:152–159. [PubMed: 403083]
- Milhorat TH. Structure and function of the choroid plexus and other sites of cerebrospinal fluid formation. Int. Rev. Cytology. 1976; 47:225–288.
- Millen JW, Woollam DHM. Vascular patterns in the choroid plexus. J. Anat. 1953; 87:114–123. [PubMed: 13044723]
- Morita T, Awakura T, Shimada A, Umemura T, Nagai T, Haruna A. Vitamin D toxicosis in cats: Natural outbreak and experimental study. J. Vet. Med. Sci. 1995; 57:831–837. [PubMed: 8593288]
- Murphy VA, Smith QR, Rapoport SI. Regulation of brain and cerebrospinal fluid calcium by brain barrier membranes following vitamin D-related chronic hypo- and hypercalcemia in rats. J. Neurochem. 1988; 51:1777–1782. [PubMed: 2846785]
- Netsky, MG.; Shuangshoti, S. The choroid plexus in health and disease. University Press of Virginia; Charlottesville: 1975.
- Quan N, Mhlana JD, Whiteside MB, McCoy AN, Kristensson K, Herkenham M. Chronic overexpression of proinflammatory cytokines and histopathology in the brain of rats infected with *Trypanosoma brucei*. J. Comp. Neurol. 1999; 414:114–130. [PubMed: 10494082]
- Quay WB. Regional and quantitative differences in the postweaning development of choroid plexuses in the rat brain. Brain Res. 1972; 36:37–45. [PubMed: 5008383]
- Quay WB. Regional differences in metabolism and composition of choroid plexuses. Brain Res. 1966; 2:378–389. [PubMed: 5968205]

- Quay WB. Regional differences in the postweaning growth by choroid plexuses as affected by dietary salt deficiencies. *Amer. J. Anat.* 1972; 134:59–69. [PubMed: 5031976]
- Selye, H. *Calciphylaxis*. The University of Chicago Press; Chicago: 1962.
- Strazielle, N.; Ghersi-Egea, J-F. Drug metabolism in newborn rat choroid plexus from lateral, third and fourth ventricle.. In: van Zutphen, LFM.; Balls, M., editors. *Animal Alternatives, Welfare and Ethics*. Elsevier Science B.V.; 1997. p. 895-901.
- Strazielle N, Ghersi-Egea J. Choroid plexus in the central nervous system: Biology and physiopathology. *J. Neuropathol. Exp. Neurol.* 2000; 59:561–574. [PubMed: 10901227]
- Suzuki Y, Sawada Y, Sugiyama Y, Iga T, Hanano M. Comparative uptake of cimetidine by rat choroid plexuses between the lateral and the fourth ventricles. *J. Pharmacobio-Dyn.* 1986; 9:327–329. [PubMed: 3723323]
- Suzuki Y, Ito T. Reserpine-induced glycogen accumulation in the epithelial cells of the mouse choroid plexus. *Brain Res.* 1974; 70:113–122. [PubMed: 4822040]
- Tennyson, V. Ultrastructural characteristics of the telencephalic and myelencephalic choroid plexuses in fetus of man and rabbit, and a comparison with the adult choroid plexus in rabbit.. In: Netsky, MG.; Shuangshoti, S., editors. *The choroid plexus in health and disease*. University Press of Virginia; Charlottesville: 1975. p. 36-71.
- Tennyson VM, Pappas GD. Fine structure of the developing telencephalic and myelencephalic choroid plexus in the rabbit. *J. Comp. Neurol.* 1964; 123:379–411. [PubMed: 14244077]
- Walters MR, Fischette CT, Fetzer C, May B, Riggle PC, Tibaldo-Bongiorno M, Christakos S. Specific 1, 25 dihydroxyvitamin D3 binding sites in choroid plexus. *Eur. J. Pharmacol.* 1992; 213:309–311. [PubMed: 1325917]

Fig. 1.

Choroid plexuses from lateral , third and fourth ventricles, stained with hematoxylin and eosin , and photographed at the same magnification.

A. Normal fourth ventricular choroid plexus between cerebellum (above) and medulla (below). The triangular shaped ventricle contains about 20 villi cut in cross section or longitudinally.

B. Fourth ventricular choroid plexitis. The villi in the center are enlarged by edema (pale areas). The contiguous villi on either side are enlarged, thickened and agglutinated by very

severe inflammation, hemorrhage, necrosis and ulceration so that individual villi cannot be discerned. The enlargement of the fourth ventricle and its choroid plexus is caused by inflammation and hemorrhage, not by optical magnification which is identical in all the illustrations.

C. Normal lateral ventricular choroid plexus, between septum and callosal radiation – corpus striatum.

D. Lateral ventricular choroid plexitis, not quite as severe as fourth ventricle in B.

E. Normal third ventricular choroid plexus.

F. Third ventricular choroid plexus with no inflammation despite threatment with cholecalciferol. The plexuses in lateral and fourth ventricles of this rat had severe inflammation. (Measuring bar is 200 microns).

Fig. 2.

Choroid plexuses from third and fourth ventricles, all photographed at the same magnification.

A. Normal third ventricular choroid plexus at higher magnification than Fig. 1. B, C, D.

Fourth ventricular choroid plexitis at higher magnification than Fig. 1.

B. All the villi contain many leukocytes, especially the mitten-shaped villus in the left upper corner. Note the copious fibrin rich edema fluid that surrounds all the villi. In the right lower corner, there is a normal cerebellar folium.

C. Inflammation and hemorrhage enlarges all the villi and some are ulcerated (left upper corner), with leukocytes and edema fluid beginning to exude. Ependyma in left lower corner is normal.

D. None of the villi are ulcerated but all are filled with leukocytes (especially villus in left upper area) or edema fluid. Measuring bar is 200 microns.

Table 1

Histologic scores of inflammation in the various choroid plexuses of 35 rats treated with cholecalciferol^a

<u>Distribution of choroid plexitis (incidence in each plexus)</u>			
<u>Severity of choroid plexitis</u>	<u>In fourth ventricle</u>	<u>In lateral ventricle(s)</u>	<u>In third ventricle</u>
4+	13	1	0
3+	8	4	0
2+	8	12	0
1+	0	7	3
0	6	11	32

^a In all 35 rats the slides had plexuses from fourth and third ventricles and at least one of the two lateral ventricles.

Table 2

Serum calcium, phosphorus and urea nitrogen in cholecalciferol treated rats

<u>Treatment</u>	<u>N</u>	<u>Calcium</u>	<u>Phosphorus</u>	<u>Urea N</u>
Cholecalciferol	20	17.4 ± 1.4	13.2 ± 1.3	46.7 ± 24.5
Controls	27	12.1 ± 1.4	11.3 ± 1.5	19.8 ± 4.7

Average serum levels are in mg/dL ± standard deviation. Differences between treated and control values are all significant (p < .0001, tested by two group t test with 45 degrees of freedom).