EDITORIAL

THE METABOLIC ACTIVITY OF THE ADULT RABBIT CHOROID PLEXUS: AN ENZYMATIC HISTOCHEMICAL APPROACH

Shehab H. Mehenna Al-Dulaymi[1], Hani T. Azzawi[2]

1. Faculty of Medicine , University of Gezira , Sudan
2. Faculty of Medicine , University of Muta, Jordon.

Abstract:

Objective: To investigate the metabolic activity of the adult rabbit choroid plexus, using succinate dehydrogenase, phosphorylase and α-naphthylacetate esterase as histochemical markers of, the aerobic, glycolytic, and lipolytic pathways, respectively.
Methods: Coverslip-mounted choroid plexus sections of adult rabbits were stained histochemically for the above enzymes. To characterize the esterase isoform(s), sections were incubated with various esterase modifiers before identification of the esterase activity. Sections of liver and kidney (controls) were simultaneously treated as for choroid plexus sections.
Results: Strong reactivity of the choroidal epithelium for both succinate dehydrogenase and esterase was readily detectable, while phosphorylase activity was virtually absent. In contrast to the *B-isoform* of esterase characteristically dominated the controls, the choroidal esterase activity was attributed mainly to *C-isoform*.
Conclusion: The results suggest that the energy required for CSF formation by the adult choroid plexus is derived almost exclusively from aerobic oxidation, including fat metabolism. The high esterase activity in the choroid plexus , and in particular the unique pattern of the choroidal esterase *versus* the esterase of the controls, were interpreted to offer a potential target for future inhibitors of the energy of fat metabolism and thereby for CSF reduction.

Keywords: Choroid plexus metabolism, Histochemistry, Esterase, Phosphorylase, Succinate dehydrogenase, Rabbit.

EDITORIAL

ملخص:

النشاط الايضي للضفيرة المشيمية في الأرنب البالغ : دراسة كيميانسجية انظيمية

الهدف: دراسة النشاط الايضي للضفيرة المشيمية في الأرنب البالغ من خلال دراسة الفعالية الكيميانسيجية لكل من نازعة هيدروجين السكسنات وفسفورلاز الالفا (1،4) كلوكان واستراز اسيتات الالفا نفثول كوسمة للأكسدة الهوائية والأكسدة اللاهوائية وتحلل ألشحوم بالتتابع . **الطرق :** تم تلوين كيميانسجي لمقاطع مجهرية للضفيرة المشيمية لإظهار فعالية الانظيمات المذكورة أعلاه . لغرض تمييز نوع الفعالية الاستزازية عوملت المقاطع بمثبطات الفعالية الاستزازية التقليدية مباشرة قبل التلوين الكيميانسجي لاستراز اسيتات الالفا نفثول . وقد استخدمت مقاطع من الكبد والكلية للتحكم وعوملت بالطريقة ذاتها التي تم فيها معاملة مقاطع الضفيرة. **النتائج:** ظهر أن لنازعة هيدروجين السكسنات والاستراز فعالية عالية في الظهاره المشيمية بخلاف الفسفورلاز التي كانت فعاليته معدومة تقريباً. وخلافاً للنوع (ب) استراز الذي كان متغلباً في مقاطع التحكم ,فان نوع الفعالية الاستزازية في الظهارة المشيمية يمكن عزوه إلى نوع (ج) .**الاستنتاج:** تشير النتائج إلى أن العمليات الايضية في الضفيرة المشيمية للأرنب البالغ تعتمد حصراً على الأكسدة الهوائية بما في ذلك العمليات الايضية الناتجة من أكسدة الشحوم داخل خلايا النسيج . كما أن خاصية الفعالية الاستزازية للضفيرة مقارنةً بنسيج التحكم قد ترشدنا إلى استعمال مثبطات الفعالية الاستزازية للسيطرة على إفراز السائل المخي النخاعي .

Introduction:

It has been well established that the choroid plexus is the primary site of cerebrospinal fluid (CSF) production, and that the secretion of this fluid is under an active rather than passive mechanism[1,2]. This implies an expenditure of metabolic energy. In the present work, the source of this energy was investigated histochemically.

Literature review on the metabolic activity of the mammalian choroid plexuses revealed a wealth of, data; the bulk of which were derived from histological [3-7], physiological[2,5,8,9], biochemical[1,2,10-13], and the more sophisticated immunohistoch- emical studies[3,14-17]

From enzymatic histochemical view, however, the accessible information on the choroid plexus metabolism were unfortunately somewhat sparse and fragmentary. Using enzyme histochemical methods, intense staining for the mitochondrial enzymes, namely succinate dehydrogenase[18] and cytochrome oxidase[19] was readily demonstrated in the adult rat choroid plexus epithelium. The activity of the carbonic anhydrase, one of the key enzymes responsible for the secretion of CSF, was detected histochemically in the adult rabbit choroid plexus epithelium[20], and was expressed in human fetal choroid plexus as early as the 9th week of gestation[15]. Ibrahim and Castellani[21] reported a negative histochemical reaction for phosphorylase in the adult rat choroid plexus. The histochemical activity of Na^+, K^+ - ATPase was found to be localized predominantly to the plasma membranes and intracellular organelles of the adult choroidal epithelium[13]. Apart from these limited data, a relatively thorough and integrated enzymatic histochemical work on the metabolism of mammalian choroid plexus has not been previously reported. In particular, the histochemical identification in the choroid plexus of the nonspecific carboxylesterases (EC 3.1.1) and their possible role in the metabolism of this tissue have not yet been considered in the literature.

It is quite known that the energy-rich adenosine triphosphate (ATP) may be generated *in vivo* via either aerobic(oxidative) pathway or anaerobic(glycolytic) pathway or both. However , different tissues vary in their potential ability to follow any or both of these pathways for energy production. For instance, in the mature erythrocytes, the energy required for transcellular transport is derived exclusively from anaerobic glycolysis[22,23]. By contrast, the immature erythrocytes

obtain their energy via the oxidative metabolism. The fovea of the retina and the cornea rely mainly on anaerobic glycolysis[22] . In skeletal muscle cells, the energy required for the contraction-relaxation cycle can be generated via the two pathways; the anaerobic glycolysis will operate under circumstances when the oxidative metabolism cannot keep up with the energy needs (e.g. exercising muscle)[22,23]. By general consent in the domain of histochemistry, any of the mitochondrial enzymes, such as succinate

EDITORIAL

dehydrogenase[23], is a reliable *indicator of aerobic respiration*. That phosphorylase is a likely candidate *marker of anaerobic glycolysis* is suggested by the ultrastructural and histochemical studies on glycogen of mammalian tissues, including the choroid plexuses at different stages of development[5-7] and following altitude exposure[3]. In contrast to the postnatal choroid plexus which lacked glycogen, the prenatal plexus, normally growing in a relatively hypoxic environment, harboured abundant glycogen.. As glycogen is the natural substrate for phosphorylase[23], it may be conceivable that the activity of this enzyme can be upregulated to parallel the cellular glycogen synthesis under the hypoxic environment characteristically prevalent during prenatal life. On the contrary, the activity of phosphorylase is conceivably downregulated when aerobic respiration prevails as in the postnatal life. According to this conceit, an *in vitro* phosphorylase activity in a tissue is supposed to reflect, at least in part, the potential capacity of the tissue for *de novo* synthesis of ATP via anaerobic glycolysis. Based on the aforementioned, the present histochemical study was undertaken to elucidate the preferred metabolic pathway(s) of the choroid plexus by which the energy may be derived for CSF secretion. To fulfill this objective, succinate dehydrogenase (SDH) and α-1,4, glucan phosphorylase were chosen as histochemical markers of, respectively, the aerobic and the glycolytic pathways in the adult rabbit choroid plexus.

Although glucose is the fuel substrate that can be readily used by all tissues as a source of metabolic energy, the degree of utilization of fatty acids as subsidiary substrates for energy production varies considerably from tissue to tissue[22,23]. For instance, cardiac and skeletal muscles, under ordinary conditions, depend heavily on fatty acids as a major energy source. By contrast, the contribution of fatty acid metabolism to the total energy requirement of erythrocytes is almost nil. Similarly, the nervous tissue apparently oxidizes fatty acids to a minimal degree if at all. The involvement of the mammalian choroid plexus in fat metabolism is suggested by only few studies. The activity of carnitine palmitoyl-transferase in the rabbit choroid plexus, measured by biochemical assay[11], was relatively somewhat higher than that of the skeletal muscle, the tissue known to rely heavily on fatty acids as substrates for energy production. Other biochemical study[12] demonstrated the fatty acid desaturase, delta-6 desaturase, in the rat choroid plexus. Using immunocytochemical methods, the enzyme fatty acid amide hydrolase, which catalyses hydrolysis of the sleep-inducing lipid, oleamide, was readily expressed by the rat choroid plexus[16] Despite the long time since the discovery of the nonspecific carboxylesterases (EC 3.1.1) and the concept of their putative role in fat metabolism[24-30], the literature is apparently devoid of information on the histochemical activity of these enzymes in mammalian choroid plexuses. On the other hand, a fund of knowledge, derived from several biochemical studies on various mammalian tissues, tends to favor the view that this enzyme family,namely carboxylesterases, can be an eligible *marker of lipolysis*. An intriguing observation from these studies was that the activity of the esterases could be significantly influenced by variation in the amount of dietary fat[24-27] or by exposure to lipophilic agents[31-35]. Anyhow, the link between this enzyme family and the energy production from endogenous lipid metabolism should, at the present time, remain an extrapolation. At the very least, the above studies have provided a rationale for the inference that an *in vitro* esterase activity in a tissue incubated with a standard fatty acid ester, such as α-naphthylacetate, presumably reflects the potential capacity of the tissue for hydrolytic cleavage of the acyl groups of fatty acid esters. As a result, free fatty acids are released and can then be made more readily available as fuel for energy production by β -oxidation. Based on this inference, the present study was planned to histochemically investigate the esterase activity of the rabbit choroid plexus using "α -naphthylacetate", as a substrate for these lipolytic enzymes. Further
extension of the histochemical work was to characterize esterase isoform(s) in this tissue.

Materials and Methods:

Two years old New Zealand rabbits, four males and four females, each weighed 2000 grams, were sacrified by exsanguination after light ether anaesthesia. Craniotomy and laparotomy were simultaneously performed on each animal. 0.5 cm blocks of renal cortex, liver and quadriceps femoris were excised as control tissues. The choroid plexuses of the lateral ventricles were stripped out. One plexus was immediately incorporated into an incision already made in a control tissue block. This unfixed composite

EDITORIAL

tissue block was then mounted with a drop of 15% gum acacia (BDH, Anala R) on a piece of cork and quenched in liquid nitrogen. The other choroid plexus together with a block of control tissue were fixed[36] for one hour at 0-4 °C in a freshly prepared 2% paraformaldehyde (BDH, Lab Reagent) in 0.067 M phosphate buffer (NaH2PO4, Na2HPO4, BDH, Anala R) pH 7.2, followed by washing into two changes of the same buffer, 30 minutes each. The fixed choroid plexus was then incorporated into an incision made in the control tissue block, and the whole fixed composite block was mounted on a piece of cork and quenched in liquid nitrogen.

From the frozen tissue blocks, already prepared, six micrometers thick sections were cut by Slee cryostat at –20 °C, mounted on coverslips and used for histochemical staining of the activities of the following enzymes.

I- Succinate dehydrogenase (EC 1.3.99.1) was demonstrated on unfixed frozen sections using nitro-blue tetrazolium as an electron acceptor and sodium succinate as a substrate[37] .

II- α-(1,4) Glucan phosphorylase (EC 2.4.1.1.) was demonstrated on unfixed frozen sections using glucose-1-phosphate as a substrate and dextran as a glycosyl acceptor[38].

III- α-Naphthylacetate esterase (EC 3.1.1.) was demonstrated on fixed frozen sections using α-naphthylacetate as a substrate and hexazotized neufuchsin as a coupling agent[39].

Esterase Modifiers:

For identification of the esterase isoform(s) in the tissues, the following enzyme modifiers were used[31,39,40] :

1. Eserine (Fluka AG, Buchs SG) as an inhibitor of acetylcholinesterase activity
2. PCMB (p-chloromercuric benzoate) (Fluka AG, Buchs SG) as an inhibitor of A-esterase activity
3. E600 (diethyl p-nitrophenyl phosphate) (Koch-Light) as an inhibitor of both acetylcholinesterase and B-esterase activities.

Based on the use of these modifiers, tissue sections were divided into five groups, each of which was treated with one or more of the modifiers prior to histochemical demonstration of the α -naphthylacetate esterase activity as follows:

First group was treated for one hour in 10^{-5} M eserine in 0.2 M phosphate buffer pH 7, then washed for one hour in the same buffer prior to demonstration of esterase activity.
Second group was treated for one hour in 10^{-3} PCMB in 0.1 M glycine-NaCL-NaOH buffer pH 7.5, then washed for one hour in the same buffer before demonstration of esterase activity
Third group was treated for one hour in 10^{-5} M E600 in 0.2 M phosphate buffer pH 5.3, washed for one hour in the same buffer and incubated for demonstration of esterase activity.
Fourth group was treated first with PCMB, then followed by eserine prior to demonstration of esterase activity.
Fifth group was treated first with PCMB, then followed by E600 prior to demonstration of esterase activity

Results:

I. Succinate dehydrogenase (SDH)

The choroidal epithelial cells showed strong bluish cytoplasmic staining reaction whose intensity was subjectively almost comparable to that of the epithelium of the renal cortical tubules (control) (Fig. 1).

II. α -1,4-Glucan phosphorylase

Within an incubation period of one hour, no demonstrable activity was detected in the choroid plexus. Comparatively, sections of quadriceps femoris (control) exhibited intense bluish cytoplasmic staining as early as 15 minutes of incubation (Fig. 2). When the incubation period of the medium, in which the choroid plexus had been incubated, was extended to three hours, mild phosphorylase activity was just discernible in only few epithelial cells and few capillaries of the connective tissue core (Fig. 3).

III. α –Naphthylacetate esterase (α-NAE)

Almost homogeneous distribution of intense dark red precipitate of α-naphthyl acetate esterase activity was

EDITORIAL

observed throughout the choroidal epithelial cytoplasm with maximum intensity in the supranuclear regions (Fig.4). Subjectively , the intensity of the staining was almost comparable to that of the epithelium of the renal cortical tubules (control) (Fig.4).

Esterase Modifiers:

None of the choroid plexus sections, treated with the three esterase modifiers before demonstration of α – naphthylacetate esterase activity, exhibited significant inhibition of this activity (Figs 5 & 6; Table 1).

In the control tissue sections, pretreated with eserine alone or with PCMB alone or with combination of both modifiers, the demonstrable esterase activity was, like the choroid plexus, resistant to inhibition by these two modifiers (Fig. 5). However, unlike the resistance of the choroid plexus esterase to inhibition by the three modifiers, the esterase activity of the control tissues, pretreated with E600 or with PCMB-E600 combination, was markedly inhibited (Fig. 6; Table 1). Subjectively, the degree of inhibition of the control tissue esterase by either E600 or PCMB-E600 combination was almost similar.

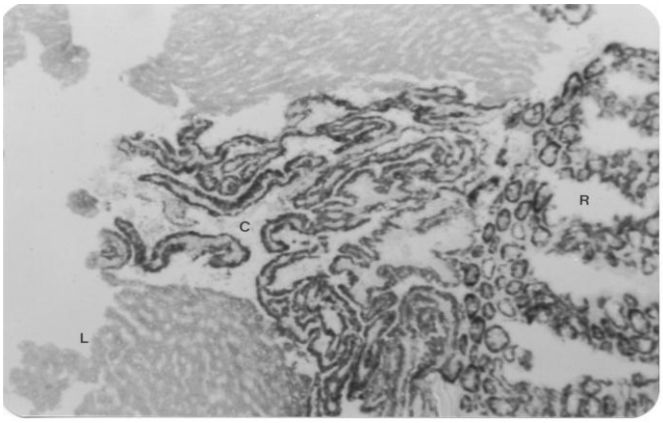
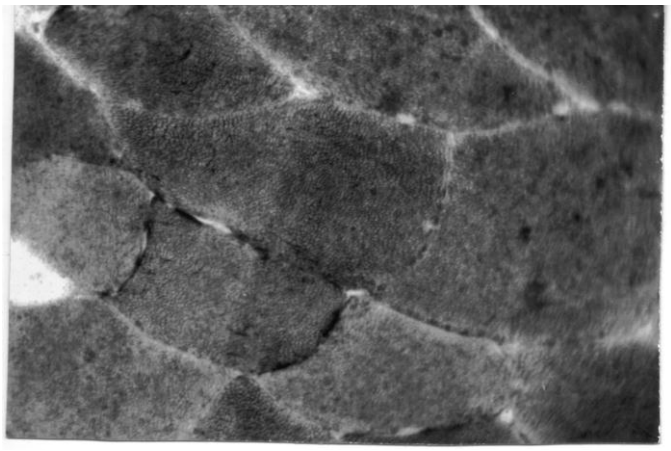


Fig. 1: Intense SDH activity in choroid plexus (C), renal cortex (R) and liver (L) (X160).



EDITORIAL

Fig. 2: Intense phosphorylase activity in quadriceps femoris (X630).

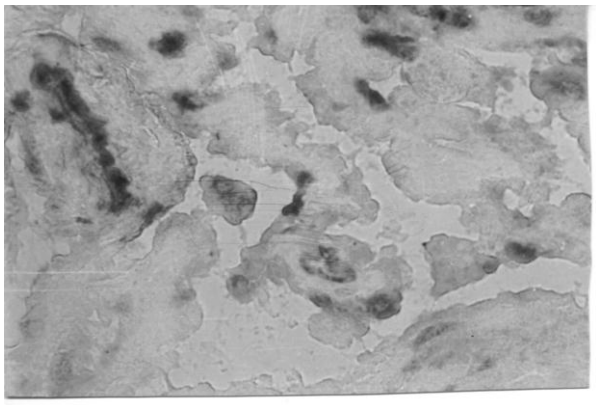


Fig. 3: Only few phosphorylase-reactive cells in choroid plexus (X630).

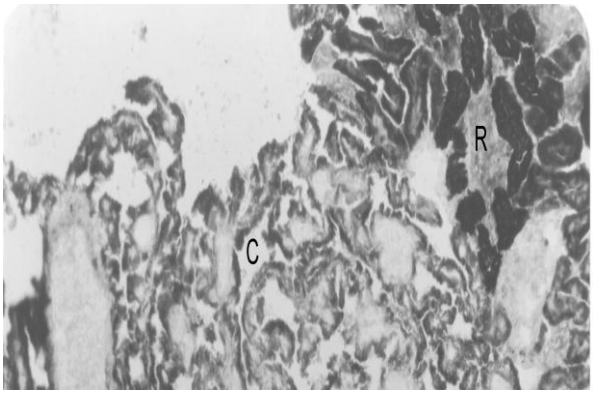
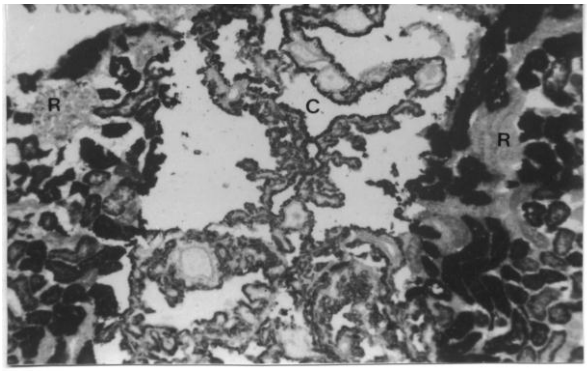


Fig. 4: Intense α-NAE activity in choroid plexus (C) and renal cortex (R) before treatment with modifiers (X160).



EDITORIAL

Fig. 5: Intense α-NAE activity in choroid plexus (C) and renal cortex (R) after treatment with eserine or PCMB (compare with Fig. 4) (X160).

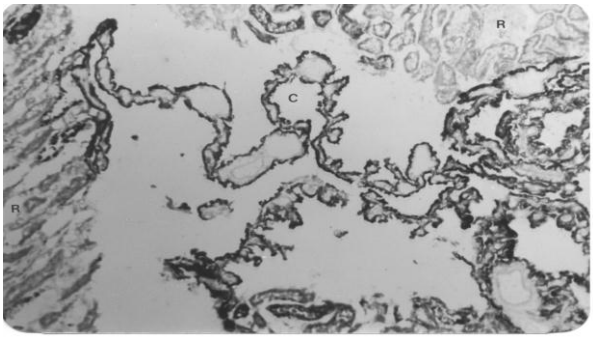


Fig.6: Intense α-NAE activity in choroid plexus (C) after treatment with E600. The adjacent renal tubules (R) show dramatic inhibition of α-NAE activity (compare with Fig. 4) (X160).

Table 1: Subjective assessment of α-naphthylacetate esterase activity in the rabbit choroid plexus *versus* control tissues before and after treatment with the modifiers

Tissue	Activity without modifier	Activity after treatment with				
		Eserine	PCMB	PCMB + serine	E600	PcmB + E600
Choroidal epithelium	+++	+++	++±	++±	++±	++±
Renal cortical tubular epithelium	+++	++±	++±	++±	±	±
Hepatocytes	+++	++±	++±	++±	±	±

++± = Very intense activity
++± = Intense activity
± = Mild residual activity

Discussion

The negligible reaction for phosphorylase in the adult rabbit choroid plexus (Fig.3) suggests that anaerobic glycolysis was not involved in the energy production by the postnatal plexus. The intense activity of succinate dehydrogenase in the adult choroidal epithelium (Fig.1) was quite compatible with the voluminous mitochondrial content of this epithelium[4]. Overall, the absence in the adult choroid plexus of both glycogen[6,7] and phosphorylase coupled with a high succinate dehydrogenase lend support to the *conclusion that the energy required for CSF secretion by the postnatal plexus was derived almost exclusively from the aerobic oxidation.*

Despite the broad range of physiological functions assigned to nonspecific carboxylesterases, their precise mechanism of action still await further studies. Perhaps, the most assertive function of these enzymes is their potential involvement in the metabolism of chemicals containing ester or amide bonds,such as drugs and pesticides[31-35]. The hydrolysis of these bonds by carboxylesterases is generally considered a housekeeping detoxification reaction, resulting in loss of

biological activity of the chemicals. Thus, the occurrence of esterases in the choroidal epithelium(Fig.4), the interface between the blood and CSF, might confer a blood-CSF barrier function to these enzymes,

EDITORIAL

protecting the central nervous system from ester or amide compounds.

With reference to the premise already extrapolated from the early studies[24-35] that esterases could be *marker of lipolysis*, the enrichment of the choroidal epithelium in these enzymes might be interpreted to mean the virtual dependence of this epithelium on fatty acids as substrates for energy production. By their putative lipolytic action , presumably on cytosolic fat depot , the esterases might be assumed to increase the intracytoplasmic availability of free fatty acids as fuel for energy yield via β-oxidation. According to this extrapolation, the esterase recruitment by the choroidal epithelium would attest in favor of the *conclusion that, at least ,a considerable fraction of the total energy of aerobic oxidation in the choroid plexus could presumably be attributed to fat metabolism.*

The eserine-and PCMB- resistant α-naphthylacetate esterase activity in the control tissues(liver and kidney) (Fig5; Table1) suggests that neither acetylcholinesterase nor A-esterase were virtually present in these tissues[31,39,40]. That, at least, a major portion of the α-naphthylacetate esterase activity of the control tissues could be accounted for by *B-esterase*[31,39,40] was inferred from the substantial inhibition of this activity by E600 (Fig 6; Table 1). This finding was in accordance with the observations[41]that *B-esterase,* the isoform of esterase inhibited by serine hydrolase inhibitors [29,31], is widely distributed in different mammalian tissues with the highest activity being expressed by the liver and moderate activity by the kidneys. In contrast to the control tissues, the marked resistance of the choroidal esterase to the three conventional modifiers (Figs 5 & 6; Table 1) tends to justify the *conclusion that the bulk of the total α-naphthylacetate esterase activity of the choroidal epithelium could be ascribable to C-esterase*[39,40].

Recommendations:

1. That a portion of the energy of choroid plexus metabolism could presumably be assignable to fat oxidation inevitably provides a rationale for a hitherito unraised question: should inhibition of the esterase activity become feasible, is it possible to deplete this portion of cellular energy and thereby to attain CSF reduction? Future research is invited to shed light on this issue.

2. Unlike the *B-isoform* of esterase characteristically dominating the control tissues of the present study and of other mammalian tissues[41], the bulk of the esterase activity of the choroid plexus belonged to *C-isoform*. Whether the choroidal C-isoform of the rabbit was exclusively rabbit-specific or an esterase pattern common to the choroid plexuses of other mammalian species can only be verified by future research. Due to the lack of tissue specificity of the available inhibitors of Na^+, K^+-ATPase[9,10], the enzyme involved in energy transformation, the need for a metabolic inhibitor acting selectively on choroid plexus is strongly justifiable. Thus, the common expression in various mammals of an esterase pattern identical to that encountered in rabbit choroid plexus may offer a promising opportunity for future development of a metabolic inhibitor specifically targeting the choroid plexus.

References:

1. Keep RF, Xiang J, Ulanski LJ, Brosius FC, Betz AL. Choroid plexus ion transporter expression and cerebrospinal fluid secretion. Acta Neurochir Suppl Wien 1997; 70: 279-281.
2. Speake T, Whitwell C, Kajita H, Majid A, Brown PD. Mechanisms of CSF secretion by the choroid plexus. Microsc Res Tech 2001; 52: 49-59.
3. Wang D, Kaur C. Choroid plexus epithelial cells in adult rats show structural alteration but not apoptosis following an exposure to hypobaric hypoxia. Neurosci Lett 2001; 297: 77-80.
4. Cornford EM, Varesi JB, Hyman S, Damian RT, Raleigh MJ. Mitochondrial content of choroid plexus epithelium. Exp Brain Res 1997; 116: 399-405.
5. Dziegielewska KM, EK J, Habgood MD, Saunders NR. Development of the choroid plexus. Microsc Res Tech 2001; 52: 5-20.
6. Tennyson VM, Pappas GD. The fine structure of the choroid plexus: Adult and developmental stages. In: Progress in Brain Research. Brain Barrier Systems: Lajtha A, Ford DH

EDITORIAL

(Eds.).Amsterdam: Elsevier, 1967; 29: 63-85.
7. Shuangshoti S, Netsky MG. Human choroid plexus: Morphologic and histochemical alterations with age. Am J Anat 1970; 128: 73-96.
8. Segal MB. Transport of nutrients across the choroid plexus. Microsc Res 2001; 52: 38-48.
9. Lindvall-Axelsson M, Owman C. Actions of sex steroids and corticosteroids on rabbit choroid plexus as shown in transport capacity and rate of cerebrospinal fluid formation Neurol Res 1990; 12: 181-186.
10. Fisone G, Synder GL, Fryckstedt J, Caplan MJ, Aperia A, Greengard P. Na^+, K^+ - ATPase in the choroid plexus. Regulation by serotonin/protein kinase C pathway. J. Biol Chem 1995; 270: 2427-2430.
11. Kim CS, Hoppel CL. Carnitine palmitoyltransferase activity in the rabbit choroid plexus: its possible function in fatty acid metabolism and transport. Neurosci Lett 1992; 140: 13-15.
12. Bourre JM, Dinh L, Boithias C, Dumont O, Piciotti M, Cunnane S. Possible role of the choroid plexus in the supply of brain tissue with polyunsaturated fatty acids. Neurosci Lett 1997; 224: 1-4.
13. Kvitnitskaia RT, Shkapenko AL. Acomparative ultracytochemical and biochemical study of the ATPases of the choroid plexus in aging. Tsitologiia 1992; 34: 81-87.
14. Parkkila S, Parkkila AK, Rajaniemi H, Shah GN, Grubb JH, Waheed A, Sly WS. Expression of membrane-associated carbonic anhydrase XIV on neurons and axons in mouse and human brain. Proc Natl Acad Sci USA .2001; 98: 1918-1923.
15. Catala M. Carbonic anhydrase activity during development of the choroid plexus in the human fetus. Childs Nerv Syst 1997; 13: 364-368.
16. Egertova M, Cravatt BF, Elphick MR. Fatty acid amide hydrolase expression in rat choroid plexus: possible role in regulation of the sleep- inducing action of oleamide. Neurosci Lett 2000; 282: 13-16.
17. Eakin TJ, Antonelli MC, Malchiodi EL, Baskin DG, Stahl WL. Localization of the plasma membrane Ca(2+) ATPase isoform PMCA3 in rat cerebellum, choroid plexus and hippocampus. Brain Res Mol Brain Res 1995; 29: 71-80.
18. Schachenmayr W. Uber die Ertwicklungvan Ependym und Plexus chorioideus der Ratte. Z. Zellforsch 1967; 77: 25-63.
19. Kim CS, Roe CR, Ambrose WW. L-Carnitine prevents mitochondiral damage induced by octanoic acid in the rat choroid plexus. Brain Res 1990; 536: 335-338.
20. Al-Dulaymi HS. A modification in Hansson's technique for histochemical demonstration of carbonic anhydrase activity in tissue sections. The Yemeni J Med Sciences 2001; 1: 84-89.
21. Ibrahim MZM, Castellani P. Demonstration of phosphorylase in the rat brain. Histochemie 1968; 16: 9-14.
22. Roskoski R. Biochemistry, Ist ed, Philadelphia, WB Saunders Company 1996, pp. 150 & 500.
23. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Biochemistry, 25th ed, A Lange Medical book. Appleton & Lange, California 2000; pp. 137, 199 & 763.
24. Van-Lith HA, Meijer GW, Van-Zutphen LFM, Beynen AC. Plasma esterase-1 (ES-1) activity is increased in rats fed high-fat diets. Lipids 1989; 24: 86-88.
25. Van-Lith HA, Meijer GW, Van-der Wouw MJA, Den-Bieman M, Van-Tintelen G, Van-Zutphen LFM, Beynen AC. Influence of amount of dietary fat and protein on esterase-1 (ES-1) activities of plasma and small intestine in rats. Br J Nutr 1992; 67: 379-390.
26. Alexon SE, Finlay TH, Hellman U, Svensson LT, Diczfalusy U, EggertseG. Molecular cloning and identification of a rat serum carboxylesterase expressed in the liver. J Biol Chem 1994 ;269 :17118-17124.
27. Ellinghaus P , Seedorf U, Assmann G. Cloning and sequencing of a novel murine liver carboxylesterase cDNA. Biochim Biophys Acta 1998; 1397:175-179.
28. Harrisson EH. Lipases and carboxylesterases: possible roles in the hepatic metabolism of retinol.

EDITORIAL

Ann Rev Nutr 1998 ;18 :259-276.

29. Diczfalusy MA , Bjorkhem I, Einarsson K, Alexon SEH. Formation of fatty acid ethyl esters in rat liver microsomes. Evidence for a key role for acyl-CoA : ethanol O-acyltransferase .Eur J Biochem 1999; 259:404-411.
30. Sanghani SP, Davis WI , Dumaual NG, Mahrenholz A, Bosron WF Identification of microsomal rat liver carboxylesterases and their activity with retinyl palmitate. Eur J Biochem 2002;269:4387-4398
31. Johnsen H, Odden E, Lie O, Johnsen BA , Fonnum NG .Metabolism of T-2 toxin by rat liver carboxylesterase. Biochem Pharmacol 1986;35:1469- 1473
32. Tang J , Chambers JE. Detoxication of paraoxon by rat liver homogenate and serum carboxylesterases and esterases. J Biochem Mol Toxicol 1999;13:261-268
33. Kudo S, Umehara K ,Hosokawa M, Miyamoto G ,Chiba K, Satoh T. Phenacetin deacetylase activity in human liver microsomes:distribution, kinetics ,and chemical inhibition and stimulation .J Pharmacol Exper Ther 2000;294:80-87
34. Alexon SEH, Diczfalusy M , Halldin M, Swedmark S. Involvement of liver carboxylesterases in the in vitro metabolism of lidocaine. Drug Metab Dispos 2002;30:643-647
35. Xie M ,Yang D,Wu M ,Xue B, Yan B. Mouse liver and kidney carboxylesterase (M-LK) rapidly hydrolyzes antitumour prodrug irinotecan and the N-terminal three quarter sequence determines substrate specificity. Drug Metab Dispos 2003;31:21-27.
36. Pease DC. Histological Techniques for Electron Microscopy. 2nd ed., Academic Press, N.Y. & London, 1964: 14-81.
37. Nachlas M.M., Tsou, K.C., De Souza, E., Cheng, C.S., Seligman A.M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. J. Histochem. Cytochem. 1957; 5: 420-436.
38. Meijer AEFH. Improved histochemical method for the demonstration of the activity of α-glucan phosphorylase.I. The use of glycosyl acceptor dextran. Histochemie 1968; 12: 244-252.
39. Pearse AGE. Histochemistry Theoretical and Applied. 3rd ed. Churchill Livingstone Edinburgh & London 1972; 2: 761-807.
40. Al-Khalisi MH, AL-Khafaj FA, Al-Azzawi HT. The distribution of non- specific esterases in the laminae of the grey matter of the rat' s spinal cord under normal circumstances and during pain.J Fac Med Baghdad 1999;41:528-532
41. Satoh T, Hosokawa M. The mammalian carboxylesterases:from molecules to functions. Annu Rev Pharmacol Toxicol 1998;38:257-188