A Comparative Study of the Regional Distribution and Structural Attachment of Cerebral Glutamine Synthetase

The intracellular distribution of glutamine synthetase has recently been examined in cortical tissue of rat brain[1] and in rat liver[2] and, in both instances, it was found to be predominantly microsomal. In cerebral tissue, however, it was necessary to use water[3] in lieu of 0.25 *M* sucrose as the homogenizing medium in order to achieve maximal retention of the enzyme in the microsomal pellet. These studies, as well as those of Wu[2] on the hepatic enzyme, have revealed that glutamine synthetase is membrane-associated and, more precisely, that its attachment to the outer surface of the microsomal membrane system may be easily disrupted with resulting solubilization of the enzyme. The differences in the nature of the structural attachment to the microsomal membranes of rat cerebral cortex of glutamine synthetase and of ATP-ase and esterase (substrate: *o*-nitrophenyl acetate)[4] have been recently examined and the results form part of a separate communication[5].

In pursuance of this work, we have determined the degree of attachment to particulate structures of glutamine synthetase in various regions of the brain of the rabbit and the cat and have briefly examined, in the latter species, the effect on the localization of the enzyme, of seizure-inducing doses[6] of intraventricular sodium.

Cats were killed with an intraperitoneal injection of pentobarbital sodium 125 mg/kg. The brain was excised at 4°C and weighed portions of cerebral cortex, cerebellar cortex, hippocampus, thalamus, and hypothalamus placed in 1 ml of ice-cold water. The homogenization, the centrifugation of a suitably diluted homogenate (about 1:30, w/v) and the determination of enzyme activity in the post-mitochondrial supernatant (Fractions PS), the microsomal suspension (Fraction P) and the high-speed soluble supernatant (Fraction S) were carried out, as previously described[1,5]. Except for the manner in which the animals were sacrificed (air injection into the ear vein and sacrifice within 10 min for the rabbit; decapitation of the pentobarbital anesthetized rat) all procedures were identical to those used for the cats. A unit of glutamine synthetase activity refers to the number of μmoles of glutamohydroxamate formed/30 min. Specific activity is in units/mg of protein.

The results of determinations of enzyme activity and enzyme attachment are seen in Table I. Highest overall activity was found in the rabbit brain and, within the brain regions of this species, activity was rather uniformly elevated. In agreement with the observation that cerebral glutamine synthetase activity of mammals is generally higher than the corresponding cerebellar activity[7], this was found to be true for the cat as well, although it was somewhat less apparent in the rabbit[7].

Of particular interest, however, are the differences found to exist between the structural attachment of glutamine synthetase in the cerebral cortex of the rat, where, upon fractionation, about 70% of the activity present in the post-mitochondrial supernatant[8] was found to be associated with the microsomal pellet, and the corresponding attachment in the rabbit (12% microsomal) and the cat (50% microsomal). Since all of the examined brain regions of the cat and the rabbit exhibited a uniformly low degree of structural attachment to microsomal structure, the present findings are taken to denote and, perhaps, to be due to, differences in the presumably species-specific structural composition of the microsomal membranes[9–11].

Studies on the cerebral[5] and the hepatic[2] microsomal glutamine synthetase of the rat have shown that NaCl ($> 0.10\,M$) causes a release of enzymic activity from the microsomal pellet into the supernatant fluid. In an attempt to correlate these *in vitro* findings with a possible *in vivo* role of Na^+ in the regulation of the intracellular location of glutamine synthetase, cats were anesthetized (35 mg/kg, Na pentobarbital), 15 min later a Collison cannula[12] was implanted in the lateral ventricle and 0.2 ml of a 3% (w/v) NaCl was injected. Cannulated controls received an equal volume of 0.9% NaCl (w/v). The animals were killed in the cold room (4°) by decapitation exactly 2 min later, the cerebral cortex excised and the 3 subcellular fractions, described above, were isolated. In these experiments, homogenates which were prepared from the brains of two animals and were made up in cold water to three different dilutions were centrifuged. This was done to minimize the effect of dilution on the enzyme activity and on its partitioning between Fractions P and S.

Table I. Glutamine synthetase: regional distribution and structural attachment in the brain of the rat, the rabbit, and the cat

Species	Rat (6)		Rabbit (3)		Cat (4)	
Brain region	Sum, fractions[a] P + S	% Activity in fraction S (P + S = 100)	Sum, fractions[a] P + S	% Activity in fraction S (P + S = 100)	Sum, fractions[a] P + S	% Activity in fraction S (P + S = 100)
Cerebral cortex	53.0	29.5	89.4	88.8	42.9	49.2
Cerebellar cortex	–	–	78.3	74.3	25.5	69.0
Hippocampus	–	–	94.6	81.4	29.8	65.7
Thalamus	–	–	72.7	86.4	24.4	65.5
Hypothalamus	–	–	84.2	75.7	20.4	68.0

[a] Units/g.

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The results of a typical experiment (Table II) indicate that the intraventricular injection of hypertonic NaCl failed to influence the degree of association of glutamine synthetase with the microsomal components of cat cerebral cortex over and above the levels noted in the control animals injected with 0.9% NaCl. Yet, by comparison with values obtained from non-injected controls (Table I), it would appear that an appreciable release of microsomal glutamine synthetase occurred as a result of the intraventricular administration of a non-convulsant dose of NaCl[13,14].

Table II. Effect of intraventricular administration of the NaCl on the structural attachment of glutamine synthetase of cat cerebral cortex

NaCl administered	Homogenate dilution		
	% Activity in fraction P[a]		
%	1:10	1:30	1:50
0	–	50.8[b]	–
0.9	14.0	23.6	24.4
3.0	18.5	18.2	23.0

[a] Activity in fraction PS = 100%. [b] Calculated from value in Table I.

Résumé. Des différences spécifiques de localisation régionale et d'attachement aux structures microsomiales de la glutamine-synthetase cérébrale sont décrites. L'administration intraventriculaire de doses non-convulsivantes de NaCl provoque chez le chat des modifications dans la fixation particulaire de l'enzyme aux structures corticales.

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Über die Herkunft der auf der Basalplatte liegenden, den intervillösen Raum begrenzenden Zellen bei der Geburtsplacenta des Menschen

In seiner zusammenfassenden Arbeit über die Anatomie der menschlichen Placenta schreibt Stieve[1] (p. 133): «Dagegen findet man in den letzten Monaten der Schwangerschaft sehr häufig Stellen, an denen die Basalplatte gegen den intervillösen Raum zu von einer gleichmässig zusammenhängenden Lage abgeplatteter Zellen überzogen wird. Diese Gebilde lassen keine Zellgrenzen erkennen, es handelt sich offenkundig um eine besondere Form des Syncytiums». Diese Zellage (vgl. Figur 1) wird von den Anhängern der Trophoblastschale als letzter Rest des serotinalen (basalen) Ektoderms[2] oder der Trophoblastschale[3] aufgefasst.

In Untersuchungen über die Ausgestaltung der Placenta hat Ludwig[4] in Übereinstimmung mit Heinz[5] festgestellt, dass die Decidua basalis mit Ausnahme derjenigen Stellen, an welchen Haftzotten verankert sind, ohne irgendeine Bekleidung an den intervillösen Raum anstösst. Auf dieser Oberfläche schlägt sich allmählich aus dem intervillösen Raum eine Fibrinschicht nieder, die zuerst von Rohr[6] beschrieben worden ist. Eigentümlicherweise findet sich die Lage von abgeplatteten Zellen immer über dem Rohrschen Fibrin gegen den intervillösen Raum hin (Figur 1).

Man nimmt heute an, dass die Arteriosklerose mit einer Abscheidung von Fibrin auf der geschädigten Gefässwand beginnt[7]. Sekundär werden diese wandständigen kleinen Thromben von den anstossenden Endothelzellen überwachsen[7]. Es stellt sich daher die Frage, ob die abgeplatteten Zellen auf der Basalplatte der Geburtsplacenta wirklich als Überreste der Trophoblastschale[1–3] anzusehen sind oder ob sie, ähnlich wie bei der Arteriosklerose, sekundär aus den mütterlichen Uteroplacentargefässen über das Rohrsche Fibrin hinüberwachsen.

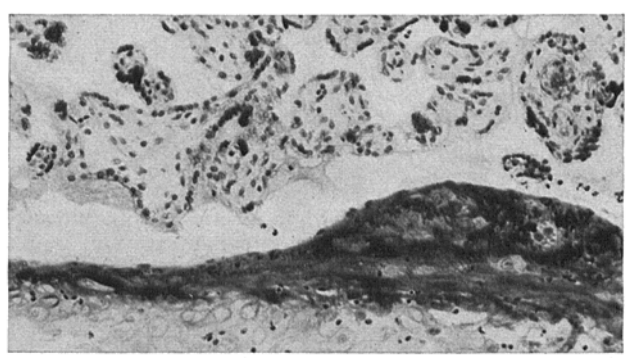

Fig. 1. Basalplatte der Placenta eines männlichen Neugeborenen. Fixation: Davidson. Färbung: Thionin nach Klinger-Ludwig[8]. Das Rohrsche Fibrin ist dunkel angefärbt. Darüber liegt als Grenzschicht gegen den intervillösen Raum die Endothellage. Vergr. 130 ×.

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