Choline Acetyltransferase of Human Placenta During the First Trimester of Pregnancy

The presence in human placenta of high concentrations of choline acetyltransferase (acetyl-CoA-choline O-acetyltransferase, EC 2.3.1.6., ChAc), the enzyme which catalyzes the biosynthesis of acetylcholine (ACh) from acetylcoenzyme A (AcCoA) and choline has been well documented[1-3]. Remarkable variations in specific activity and total tissue content in dependency of length of gestation were reported for this enzyme by measurement of ChAc in acetone dried tissue powders derived from placentae between 11 weeks of pregnancy and term[2,4]. The functions of ACh in placenta which is not innervated are still unknown. The compound was first proposed to induce uterine contractions during delivery[5], but more recently it has been speculated that ACh fulfills as yet undefined functions in active transport and permeability regulation[4,6]. In view of the potential importance of ACh for placental function it was of interest to learn more about the development of the cholinergic system in this organ. This communication reports detection of ChAc in placenta specimens as early as 6 weeks after conception. Chorionic villous tissue was obtained from vacuum uterine evacuation procedures[7]. The vacuum aspirate was collected in small cloth bags from which pieces of chorionic membrane with chrionic villi attached were removed and washed in icecold Krebs-Henseleit solution. Chorionic villi were subsequently scraped off the membrane. Initially ChAc determinations were performed on such specimens without histological control. However, variable results and sometimes lack of enzyme activity prompted us to routinely use a small piece of tissue thought to be representative for the composition of the whole sample for histological examination. The problem of collecting pure chrionic villi by teasing them away from membrane fragments of the chorionic sac applied primarily to the early specimens (6–9 weeks), because the amount of villous tissue is small and the chorionic sac usually torn into many small fragments by vacuum suction. The material was blotted on absorbent paper, wet weight determined and then homogenized in hand operated Tenbrook[8] glass homogenizers to yield a 1:10 (w/v) suspension in distilled water. This homogenate was further diluted to a final concentration of 1:100 (w/v). ChAc was determined using 1-^{14}C-AcCoA[9] with an ion exchange method[10] and the incubation medium described elsewhere[11]. Reaction tubes of duplicate samples contained the equivalent of 0.5 mg of tissue wet weight in a total volume of 100 µl and were incubated 10 to 20 min. at 37 °C. The following control experiments were performed to ensure that the synthesis of ^{14}C-ACh formation by placental ChAc: 1. Omission of choline from the incubation medium. 2. Use of specific inhibitors of ChAc which have become available more recently only such as: a) Chloroacetyl-choline (ClACh) synthesized in our laboratory[12], b) bromoacetylcholine (BrACh)[12,13] and c) 4-(7-naphthylvinyl) pyridine (NVP)[14,15]. Protein was determined with the Biuret method[16].

The earliest placenta specimens deriving from pregnancies of 6–7 weeks duration had significant amounts of radioactivity in the fraction into which authentic 1-^{14}C-ACh or any 1-^{14}C-ACh synthesized during the preceeding reaction would elute, suggesting presence of ChAc. Boiling of the homogenate prior to incubation reduced the amount of ^{14}C in those samples to background levels of the radiometric method (0.4% of total radioactivity, which was 0.1 µCi per tube). When choline was omitted from tubes containing immature placenta homogenate radioactivity in the ACh fraction was also reduced to background levels. These observations allowed the conclusions that the 1-^{14}C-acetyl transfer was a) catalyzed by heat labile enzymes and b) probably due to ChAc because when

[1] R. S. COMLINE, J. Physiol., Lond. *105*, 6P (1946).
[2] C. O. HEBB and D. RATKOVIC, J. Physiol., Lond. *163*, 307 (1962).
[3] D. MORRIS, Biochem. J. *98*, 754 (1966).
[4] G. BULL, C. O. HEBB and D. RATKOVIC, Nature, Lond. *190*, 1202 (1961).
[5] H. C. CHANG and A. WONG WONG CHIN, J. Physiol. Lond. *7*, 151 (1933).
[6] G. B. KOELLE, Neurosci. Res. Progr. Bull. *5*, 44 (1967).

[7] The author gratefully acknowledges the assistance of the professional staff of Access Center and E. W. Sparrow Hospital in Lansing, Michigan, in securing placenta specimens.

[8] Kontes Corporation, Vineland, New Jersey.
[9] New England Nuclear Corporation, Boston, Mass., specific activity about 50 mCi/mmole. This was diluted with unlabeled Ac CoA from Pabst Laboratories, Milwaukee, Wisc., to result in a final specific activity of about 1.5 mCi/mmole.

[10] B. K. SCHRIER and L. SHUSTER, J. Neurochem. *14*, 977 (1967).
[11] F. WELSCH, Am. J. Obstet. Gynec., in press. (1974).
[12] C. Y. CHIOU and B. V. R. SASTRY, Biochem. Pharmac. *17*, 805 (1968).

[13] Bromoacetylcholine was a gift from Dr. B. V. R. SASTRY, Department of Pharmacology Vanderbilt University School of Medicine, Nashville, Tenn.

[14] C. J. CAVALLITO, H. L. WHITE and H. S. YUN, in *Drugs and cholinergic Mechanisms in the CNS* (Eds. E. HEILBRONN and A. WINTER; Försvarets Forskningsanstalt, Stockholm 1970), p. 97.

[15] Commercially available from Calbiochem, La Jolla, California.

[16] A. G. GORNALL, C. S. BARWILL and M. M. DAVID, J. biol. Chem. *177*, 751 (1949).

Table I. Effects of inhibitors of ChAc on enzyme activity of immature human placenta

Drug	Drug concentration (*M*)	ACh synthesized moles $\times 10^{-6}$/g fresh tissue $\times$ h^{-1}		Inhibition (%)	
		Expt No. 1	Expt No. 2	Expt No. 1	Expt No. 2
None (Control)	0	16.28	18.81	0	0
Br ACh	5×10^{-6}	1.25	0	92	100
Cl ACh	5×10^{-6}	1.96	0	88	100
None (Control)	0	13.80	22.35	0	0
NVP	2.5×10^{-4}	3.51	6.14	75	73
NVP	2.5×10^{-5}	11.72	16.81	15	25

Values represent mean of duplicate determinations. ChAc derived from placentae 6–9 weeks gestational age. Enzyme activity is expressed in moles of ACh synthesized per gram fresh tissue. Br ACh, Cl ACh and NVP were handled with the precautions indicated in the text.

choline was omitted no 1-^{14}C-ACh was formed. Further identification that the enzyme was ChAc was obtained by inhibitor studies. Cl Ach and BrACh were prepared just prior to transfer into incubation tubes because of their instability in aqueous solution[17]. At a concentration of 5×10^{-6} *M* both halogenated ACh derivatives inhibited the enzymatic 1-^{14}C-acetyl transfer by about 90% and 100% in two placenta homogenates (Table I). NVP solutions were handled in a darkroom only[18]. This drug inhibited the enzyme of immature placentae by 75% at 2.5×10^{-4} *M* or 15 and 25% at 2.5×10^{-5} *M* (Table I). The results obtained with these three specific ChAc inhibitors strongly supported the conclusion that the early placentae examined contained this enzyme at 6–9 weeks of gestation. ChAc activity at this stage of pregnancy was already higher than in term placenta (Table II) and gradually increased. All values shown after 12 weeks are single determinations from elective abortions (13 and 14 weeks) or premature deliveries (27–37 weeks). Elective terminations of pregnancy under the 1973 United States Supreme Court ruling are preferably performed between 7–10 weeks which made such specimens more readily available.

Table II. ChAc activity of human placenta at various lengths of pregnancy

Length of pregnancy (weeks)	ACh synthesized	
	Moles $\times 10^{-6}$/g fresh tissue $\times$ h^{-1}	Moles $\times 10^{-9}$/mg protein $\times$ h^{-1}
6–7	17.34 ± 2.31 (5)	245 ± 54 (5)
8	16.64 ± 3.16 (9)	291 ± 37 (9)
9	20.47 ± 3.67 (5)	388 ± 40 (5)
10	44.70 ± 14.93 (3)	643 ± 67 (3)
11	44.53 ± 6.89 (3)	672 ± 52 (3)
12	37.88 ± 2.88 (2)	751 ± 17 (2)
13	34.40	882
14	55.59	794
27	24.10	199
32	32.50	262
36	20.33	289
37	12.64	177
41–42 (term)	7.85 ± 1.04 (6)	60.0 ± 8.02 (4)

Values represent mean ± S.E. of the mean. The number of placentae is indicated in brackets. All determinations from pregnancies longer than 12 weeks are single observations with exception of the term placentae. ChAc activity is expressed in moles of ACh synthesized per gram fresh tissue or mg protein as specified.

The results allow the speculation that ChAc is present in human placenta as soon as placentation begins. The development of ChAc activity during the second and third trimester resembled previous observations[4] and enzyme activity seemed quite comparable when calculated back to a fresh weight basis in both immature and term placenta[2–4]. There was a rapid decline of ChAc towards the normal end of pregnancy, the activity being lower in the term placenta than at any previous point of measurement (Table II).

The ChAc of early placental villous tissue was easily released upon homogenization. The addition of butanol[19] or ether, which greatly increased the enzyme activity in brain tissue[20, 21] did not result in formation of more 1-^{14}C-ACh, an observation which is in agreement with observations on term placenta[2].

The presence of ChAc with high specific activity in immature placenta suggests a function of ACh throughout pregnancy rather than a role during the delivery process only[5]. The highest enzyme activity was observed long before term (Table II and refs.[3, 4]). These findings support previous speculations[4, 6] that ACh (which was detected in significant concentrations by pyrolysis gas chromatography in 8-9-week-old placentae, unpublished observations) might have some function in permeability and transport regulation. The present results would suggest that such as yet undefined cholinergic modulation of placenta functions could become active at a very early stage of fetal development.

Zusammenfassung. Cholinacetyltransferase (ChAc) wurde mit radiometrischer Methode im Homogenat unreifer menschlicher Plazenta bestimmt. Das Enzym war bereits nach 6–7 Wochen Gravidität eindeutig messbar und hatte höhere spezifische Aktivität als dasjenige der reifen Plazenta. Die Ergebnisse lassen vermuten, dass ChAc schon mit Beginn der Plazentation auftritt und unterstützen früher geäusserte Vermutungen, dass ACh in der menschlichen Plazenta bisher noch nicht genauer definierte Aufgaben in der Regulation von Membranpermeabilität und aktivem Transport haben könnte.

F. Welsch[22]

Department of Pharmacology, B 403 Life Sciences, Michigan State University, East Lansing (Michigan 48824, USA), 29 October 1973.

[17] D. Morris and D. S. Grewaal, Eur. J. Biochem. *22*, 563 (1971).
[18] H. L. White and C. J. Cavallito, Biochim. biophys. Acta *206*, 242 (1970).
[19] V. Glover and D. P. I. Green, J. Neurochem. *19*, 2465 (1972).
[20] G. Bull, C. O. Hebb and D. Ratkovic, J. Neurochem. *17*, 1505 (1970).
[21] G. Bull and B. Oderfeld-Nowak, J. Neurochem. *18*, 935 (1971).
[22] Supported by USPHS grant No. HD 07091-02. The technical assistance of Mrs. Cynthia Knight is gratefully acknowledged.

The Role of Thyroxine in the Maintenance of a Normal Glycogenolytic Response to Splanchnic Nerve Stimulation in Adrenalectomized Calves

Stimulation of splanchnic sympathetic efferent fibres at physiological frequencies causes rapid mobilization of liver glycogen in the calf. Three separate pathways mediate this response: direct activation of the hepatic sympathetic innervation[1], release of glucagon from the pancreatic islets[2] and the release of catecholamines from the adrenal medullae[3]. The experiments described here were devised to elucidate the extent to which thyroxine might influence the first two of these mechanisms.

Five pedigree Jersey calves were thyroidectomized between 7 and 15 days of age and maintained on a milk diet. 3 weeks later the animals were anaesthetized with