Biochemical Pharmacology, Vol. 53, pp. 1577–1586, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(97)00017-8

COMMENTARY

Human Placental Cholinergic System

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ABSTRACT. The occurrence of acetylcholine (ACh)-like activity in human placenta, a tissue without innervation, has been known for more than 60 years. However, the non-neuronal functions of ACh in human placenta are not clearly understood. The components of the cholinergic system-ACh, choline acetyltransferase, acetylcholinesterase, butyrylcholinesterase, muscarinic receptors, and nicotinic receptors—in human placenta have been demonstrated by unequivocal methods. Primate placentae store and release ACh by mechanisms similar to those of nervous tissue. However, there are many gaps in our knowledge, which include: (a) endogenous quaternary ammonium compounds other than ACh in human placental extracts; (b) the specificity of placental enzymes; (c) the subtypes and structures of placental muscarinic and nicotinic receptors; and (d) the significance of placental α-bungarotoxin binding proteins, ACh receptor stimulation-cellular signaling by second messengers, and activation of immediate early target genes (C-*fos*, C-*jun*) encoding transcription factors. Several hypothetical non-neuronal functions of ACh in placenta have been postulated based upon available experimental evidence. These include: (a) regulation of blood flow and fluid volume in placental vessels; (b) opening and closing of trophoblastic channels; (c) induction of contractile properties to myofibroblasts; (d) facilitation of amino acid transport necessary for fetal growth across placenta; (e) release of placental hormones; and (f) modulation of the formation of myometrial and placental prostaglandins in human parturition. All of these roles are reasonable, and some of these roles may turn out to be linked to one another to influence or maintain placental function. BIOCHEM PHARMACOL 53;11:1577–1586, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. acetylcholine; human placenta; acetylcholinesterase; butyrylcholinesterase; choline acetyltransferase; muscarinic receptors; nicotinic receptors; trophoblast channels; myofibroblasts; prostaglandins; quantal release

A neurotransmitter role for ACh† has been known since the discoveries of Dale [1] and Loewi and coworker during 1914 to 1926. The occurrence of ACh-like activity in human placental extracts was demonstrated by bioassay methods in the late 1920s or early 1930s [3, 4]. Improvements in the bioassay techniques for ACh and its detection by GC-MS were achieved in subsequent years [5]. It is now established by GC-MS that human term placenta contains about 112 nmol ACh/g wet tissue, which is about 7-fold higher than that of the brain tissue [6]. Although several studies have contributed to our understanding of the role of ACh in non-nervous tissues, especially human placenta, there are many gaps in our knowledge that have to be clarified in future studies. Although the placentae of animals (monkey, cow, rabbit, rat, mouse) have been reported to contain ACh, human placental ACh has been investigated more thoroughly than that of any other species. Therefore, the primary focus of this commentary is to evaluate the role of ACh in human placenta. For details, reviews on the development of our knowledge on placental ACh, regulation of its synthesis and release, and its possible roles in non-neuronal tissues should be consulted [7–12]. This commentary is intended to stimulate thought and research in unsolved problems and functions of ACh in human placenta.

The nerves that release ACh from their terminals are referred to as cholinergic nerves, and the nervous system in which ACh is involved as a chemical transmitter as the cholinergic nervous system. In this commentary, the ACh system in the placenta is referred to as the placental cholinergic system. There are five components on the basis of which a cholinergic system in tissues has been delineated: (a) ACh; (b) ChAs that catalyze the transfer of the acetyl moiety from ACoA to choline; (c) sources of precursors, ACoA and choline; (d) cholinergic receptors, muscarinic and nicotinic, at which ACh binds to initiate the physiological responses; (e) cholinesterases, AChE, and PChE that hydrolyze ACh into choline and acetic acid; and (f) an uptake system for choline and/or ACh.

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† *Abbreviations:* ACh, acetylcholine; ChA, choline acetyltransferase; ACoA, acetylcoenzyme A; AChE, acetylcholinesterase; PChE, pseudocholinesterase or butyrylcholinesterase; HC-3, hemicholinium 3; BETA, (2-benzoyl)ethyltrimethylammonium; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PME, phosphatidyl-*N*-methylethanolamine; IEG, immediate early target genes; QNB, quinuclidinylbenzilate; BGT, α-bungarotoxin; BGT-BP, α-bungarotoxin binding proteins; 5-HT, 5-hydroxy-tryptamine; EDRF, endothelium-derived relaxing factor; α-NETA, 2-(α-naphthoyl)ethyltrimethylammonium; PG, prostaglandin; AIB, α- aminoisobutyric acid; and CaA, carnitine acetyltransferase.

COMPONENTS OF THE HUMAN PLACENTAL CHOLINERGIC SYSTEM

Human Placental ACh

Several aspects of ACh occurrence in human placenta have been established. About 95% of ACh-like activity in human placenta is in a bound form, with most of the ACh localized, possibly within vesicles, in the villus tissue. However, ACh vesicles are not yet isolated by subcellular fractionation of placenta because the vesicular membrane is more sensitive than the plasma membrane to tissue homogenization procedures [13, 14]. The concentrations of ACh in floating villi and the basal plate are about 3.2 and 2.1 times higher than that in the chorionic plate [6]. There is variation of ACh content with gestational age. The highest concentration is found at about 20–22 weeks of gestation [6].

ChA

ACh is synthesized from ACoA and choline. The reaction between ACoA and choline is catalyzed by ChA. A number of investigators have obtained preparations of ChA with different degrees of purification from human placenta [15–19]. It has been shown that brain ChA and placental ChA synthesize ACh by the same enzyme mechanism, namely the ordered Theorell-Chance mechanism with ACoA as the leading substrate and CoA as the obligatory product [18, 20]. Placental homogenates from several other species—guinea pig, dog, cat, mouse, horse, and cow—have been shown to synthesize ACh in the presence of exogenous ACoA [21]. These homogenates synthesized other products besides ACh that were not identified. It is also not known how ACh, which is synthesized by ChA, enters the storage pool of ACh in placenta.

Sources of ACoA and Choline

ATP-citrate lyase is a cytoplasmic enzyme that occurs in several tissues. It catalyzes the formation of ACoA in the presence of citrate, coenzyme A, ATP and Mg^{2+}. In general, moderately high specific activities of this enzyme are present in tissues that have a high capacity for acetyl group utilization. Human placenta has a high capacity for ACh synthesis, and should have a high capacity for ACoA formation. To evaluate whether citrate is a source of the acetyl group for the formation of ACh in placenta, Chaturvedi and Sastry [22, 23] analyzed placental homogenates for ATP citrate lyase. They reported an ATP citrate lyase activity in human placentae of 701 nmol of ACoA formed/mg protein/h. It is not known if there are other sources (e.g. cytoplasmic acetate thiokinase) for the formation of ACoA in placenta. Pyruvate and glucose have been shown to be good precursors for acetyl groups of mammalian brain ACh [24], but whether they serve as precursors for acetyl groups of placenta ACh has yet to be investigated.

Human placenta does not have a high capacity for the synthesis of choline *de novo*. The choline required for ACh synthesis in placenta is derived from two sources: a choline uptake system (choline taken up by placenta from maternal circulation) and PC. The importance of placental transfer of choline, a prominent constituent of lipid-soluble and several water-soluble metabolites, to meet placental and fetal needs is now established. Choline-containing phospholipids cannot readily cross the placenta [25], and the fetal liver appears to have only limited ability to synthesize the amine. Choline is produced in the maternal liver, the organ with the highest synthetic capability in the body [26], and the placenta is involved in the transport of this choline. When human term placental fragments are incubated *in vitro*, free choline is rapidly taken up against a concentration gradient from extracellular water into the intracellular water compartment [27]. This choline is rapidly incorporated into several esterified products among which ACh is the most prominent.

Welsch [27, 28] demonstrated a choline transport system in human placental tissue. This transport system has low affinity for choline and is not sodium dependent. HC-3, an inhibitor of the high affinity choline uptake system in nervous tissue, is about 20–100 times less effective in decreasing ACh synthesis in human placenta than in the brain [27]. Inhibition of ChA by BETA decreases ACh synthesis and lowers the levels of ACh in placenta [8, 29, 30]. Acetylation of choline by ChA seems to be more important than the choline uptake system in human placenta [31]. Although the placental choline uptake system has low affinity for choline, it seems to be a high capacity system that provides adequate amounts of choline for ACh synthesis.

Membrane PC may serve as a source for choline, which may be utilized for ACh synthesis. Secondary membrane PE is converted stepwise to PME and PC by the two phospholipid-*N*-methyltransferases (PE-*N*-methyltransferase and PME-*N*-methyltransferase) in the presence of *S*-adenosyl-L-methionine. These enzymes have been shown to occur in human placental membranes [8, 32]. Choline can be formed from PC in tissues by three different pathways [12]: (1) the phospholipase D pathway in which PC is hydrolyzed to give choline; (2) the base exchange enzyme pathway in which blood ethanolamine is exchanged for the choline moiety of PC forming PE; and (3) the phospholipase A pathway in which free choline is liberated. The liberated free choline may be used for ACh synthesis. However, these hypothetical pathways have yet to be demonstrated to occur in human placenta.

Release of ACh from Human Placenta and Interaction at Muscarinic and Nicotinic Receptors

There is ample experimental evidence that ACh is released into both the maternal and fetal circulations. Some authors have measured ACh released from isolated cultured placental villus [33–35] and fragments of term placenta [36]. In these preparations, there is no fetal circulation; therefore, the fetal capillaries are collapsed, and the syncytiotrophoblast layer is exposed to the culture medium. The release of ACh-like activities into both maternal and fetal circulations has been measured using perfused human placental cotyleda [37].

The release of ACh from the isolated human placental villus resembles that from the nerve in several respects [33–35]. Isolated human placental villi contain 167 nmol ACh/g wet tissue. When they are incubated in a medium containing Krebs-Ringer bicarbonate buffer (pH 7.2 to 7.4, Ca^{2+} 2.54 mM), ACh (35 pmol/g/min) is released spontaneously. The rate of release of ACh is enhanced (a) during depolarization with high concentrations of K^+ (17–63 mM), (b) increasing concentrations of Ca^{2+} (4.64–9.4 mM), and (c) nicotine [33]. ACh is not released in the absence of extracellular Ca^{2+}, and depolarizing concentrations of K^+ and nicotine do not increase the rate of release of ACh in the absence of extracellular Ca^{2+}. Accordingly, morphine and enkephalins inhibit ACh release in the nervous tissue by inhibiting Ca^{2+} influx. The kappa opiate receptor agonist, ethylketocyclazocine (100 μM) depresses the rate of spontaneous release of ACh by about 50% [35, 38, 39]. The kappa antagonist (−)-2-(3-furylmethyl)-noretazocine (1 mM) enhances the rate of ACh release by about 18-fold. Endogenous methionine enkephalin, β-endorphin, and other opiate agonists seem to play a role in down-regulating ACh release by a negative feedback mechanism [35, 39]; they may decrease Ca^{2+} influx into the trophoblast and, consequently, decrease ACh release [40].

Substance P increases ACh release from nervous tissue and up-regulates ACh release [41, 42]. Although both opioid peptides and substance P occur in human placenta [43, 44], the role of substance P in the regulation of placental ACh is not known. The release of ACh-like activity into maternal circulation has been measured using perfused human placenta [38]. However, the effects of substance P-like agonists or their antagonists on this release have yet to be measured.

ACh release into fetal vasculature has been measured in the perfused single placental cotyledon and whole placenta [37, 38, 45]. ACh is definitely released into the fetal circulation. No evidence is available on whether released ACh affects vascular smooth muscle of the villus region or its vasoconstrictor responses. There is also no evidence whether endothelial cells are present in the human placental chorionic and villus stem vasculatures. There is some evidence to indicate that ACh relaxes the human umbilical artery by an endothelium-independent process [46]. It is generally accepted, from ample experimental evidence, that ACh produces physiological responses by activating different types (muscarinic or nicotinic) of cholinergic receptors.

ACh is known to activate different subtypes of muscarinic and nicotinic receptors, to open ion channels, to alter cell levels of second messengers (cyclic AMP, inositol-1:4:5-triphosphate), and to induce IEG in nervous tissue. Although there is some suggestive evidence that these mechanisms operate in human trophoblast, there are as yet no definite studies. There are two types of evidence to indicate that muscarinic receptors exist in the syncytiotrophoblast: (a) nicotine-induced release of ACh from the maternal side of the trophoblast is blocked by atropine [33, 34]; (b) the synthetic muscarinic ligand [^{3}H]QNB has been used to identify two classes of saturable muscarinic cholinergic binding sites with dissociation constants of 80 pM and 30 nM on plasma membrane from human trophoblast. The high- and low-affinity sites bind 9 and 910 fmol QNB/mg protein, respectively [47]. Specific binding was antagonized by selective muscarinic agonists and antagonists. There are at least 4–5 subtypes of pharmacologically distinct muscarinic receptors [48–51]. Five subtypes of muscarinic receptors have been cloned [49, 51], all of which seem to be linked to G-proteins. They act directly on ion channels or are linked to different second messenger systems. Subtypes of muscarinic receptors on the trophoblastic membrane have not been identified yet, and muscarinic receptors on the trophoblast have yet to be cloned.

The presence of muscarinic receptors has also been demonstrated on the endothelial cells of chorionic plate veins [9]. When helical strips of chorionic plate veins, previously contracted with 5-HT, were challenged with ACh, the tension of the helical strips decreased, and they relaxed. When the endothelial layer was removed by rubbing, the relaxation effect disappeared. QNB binding studies and use of selective muscarinic receptor antagonists to antagonize its specific binding will provide further evidence for the presence of muscarinic receptors and their subtypes on endothelial cells.

Nicotinic cholinergic receptors of *Torpedo* and *Electrophorus* electric organs have been isolated using BGT, which binds specifically to nicotinic receptors. ACh and nicotine prevent the toxin from binding. The identification of nicotinic cholinergic receptors in the mammalian CNS is an enigma [49]. BGT binding cannot be easily displaced by ACh or by unlabeled ganglionic or neuromuscular antagonists. They are simply classified as BGT-BP.

Many of the critical membrane proteins that bind cholinergic ligands in the nervous system are encoded by genes that belong to the ligand-gated ion channel gene superfamily [50–52]. This includes three gene families encoding protein subunits: (1) skeletal muscle nicotinic ACh receptors (NAChRs); (2) neuronal NAChRs; and (3) neuronal BGT-BPs. The occurrence of a BGT binding component has been demonstrated in human placenta using ^{125}I-BGT [14]. It is likely that the iodinated toxin-binding macromolecule is a protein (BGT-BP) [14]. It has yet to be determined whether BGT-BP from human placenta belong to a ligand-gated ion channel gene family.

Cholinesterases (AChE; PChE)

The action of ACh as a chemical transmitter is terminated due to hydrolysis by AChE. There are several ways in which the function of ACh in the placenta may be terminated: (1) hydrolysis of ACh by placental cholinesterases; (2) release of ACh into maternal and fetal circulations, diffusion, and hydrolysis by maternal and fetal circulations; and (3) reuptake of released ACh by the placental trophoblast. The degradation products of ACh, choline and acetate, may be reutilized in the ACh synthetic pathway of the placenta.

The presence of cholinesterases in the human placenta has been indicated by a number of investigations [53–55]. The human placental enzyme has been characterized as AChE by Ord and Thompson [53] and by Koshakji *et al.* [55]. Acetyl-β-methylcholine is a specific substrate for placental AChE, which does not hydrolyze benzoylcholine to a significant degree. Fant and Harbison [47] prepared plasma membrane vesicles from human trophoblast. This plasma membrane is not contaminated with erythrocytes as determined by electron microscopic examination. This preparation has an AChE activity of 13.8 nmol acetyl-β-methylcholine hydrolyzed/mg protein/min. It has very low PChE activity (0.1 nmol butyrylcholine hydrolyzed/mg protein/min). Further evidence for the occurrence of AChE in the human trophoblast is obtained in studies using a cultured cell line of trophoblast. The cytotrophoblast of the human placental villus is a stem cell that gives rise to the syncytiotrophoblast by a process of differentiation. A cell line, Jar cells, was established by Pattillo *et al.* [56, 57]. Cultured Jar cells have been analyzed for AChE and characterized using selective inhibitors of AChE and PChE [58, 59]. These studies indicate that AChE occurs in Jar cells, and that its gene is expressed even in transformed trophoblast cells. All of these observations establish the occurrence of AChE in the human trophoblast.

The concentrations of AChE and PChE are considerably lower in the placenta than in the nervous system. In view of the vascularity of the placenta and the high concentration of cholinesterases in maternal and fetal bloods, there is only a minor physiological requirement for high concentrations of AChE in human placenta. ACh that escapes placental cholinesterases and diffuses into maternal and fetal compartments is hydrolyzed by maternal and fetal plasma cholinesterases, respectively.

Uptake of ACh by Human Placenta

A part of ACh released onto the surface of the syncytiotrophoblast, where it exerts its physiological action, may be taken up by the trophoblast. An ACh uptake system has been well characterized in brain tissue [60]. An accumulation of ACh against a concentration gradient has been described in human term placenta [61, 62]. This uptake system has a K_m of about 15 mM. The uptake of ACh by placental villus was competitively inhibited by choline (5×10^{-4} M), HC-3 (2×10^{-4} M), and morphine (10^{-3} M). In view of the large quantities of ACh released from microvilli, this uptake system may play a significant role in conserving and reutilizing ACh.

ROLE OF ACH IN PLACENTA

The chemical transmitter function of ACh is well established; however, human placenta is not innervated. The evolutionary appearance of ACh preceded that of the nervous system. The ACh system is fully formed in the placenta during maturation and development of the placenta and the fetus. The ACh concentration decreases in aged (or term) placenta and syncytial degeneration [63, 64]. It may be rewarding to consider the role of ACh as a local messenger in placenta. ACh is synthesized in the cytoplasm of the syncytiotrophoblast [13, 14] and released from placenta into both maternal and fetal circulations [34, 37, 65]. Therefore, the physiological roles postulated relate to endogenously released ACh. These roles relate to the actions of ACh in the placental vessels, syncytiotrophoblast and its maturation and development, and modification of uterine function during labor. ACh may also regulate trophoblastic channels and may impart contractile properties to myofibroblasts in the placenta. All roles are reasonable, but more concrete experimental evidence is needed.

Regulation of Blood Flow and Fluid Volume in Placental Vessels

There are at least three possible sites where ACh may interact and exert regulatory control on the blood flow and fluid volume in the placental vasculature: (a) vascular endothelial cells [9, 66], (b) transtrophoblastic channels [67–69], and (c) myofibroblasts in villus stroma [70, 71].

Intravascular injection or infusion of ACh produces vasodilation in experimental animals. Endogenously released ACh (82.4 ng/min/g at 37.5°) may cause vasodilation of placental vasculature and decrease the resistance to fetal blood flow. It is difficult to demonstrate the vasodilatory effects of ACh on human placental vasculature. The effects of exogenously injected ACh on perfused human placenta have been studied by several investigators (see review by Sastry and Sadavongvivad [7]). In these preparations, endogenous ACh is released into the perfusion medium, and the placental vasculature is already dilated, making it difficult to demonstrate the vasodilatory effects of exogenously injected ACh. Therefore, these investigations reported no effect for exogenous ACh, and only weak dilation or vasoconstriction, enhanced by physostigmine and abolished by atropine. The vasoconstrictive response, normally observed with high concentrations of ACh, may be due to release of vasoactive substances (e.g. catecholamines, 5-HT), or it may have causes as yet unexplained. *In vitro* preparations of placental vasculature may be more useful to observe the relaxation effect of ACh.

Endothelial cells are necessary for ACh to cause relaxation in isolated blood vessel preparations [66]. Muscarinic receptors that are activated by ACh to release EDRF are located on endothelial cells, and EDRF mediates the ACh-induced relaxation of vascular smooth muscle. When endothelial cells are intact, ACh relaxes strips of chorionic vein contracted by 5-HT. However, this effect is seen in the chorionic veins of only 18% of term placentae. No information is available on the status of vascular endothelial cells in placentae used in these investigations. The status of endothelial cells in the vasculature of human placentae subjected to the trauma of delivery has yet to be investigated. No correlation has been established between the endothelial muscarinic receptors and the relaxation of

placental blood vessels. Further investigations are necessary to obtain evidence on the ACh-induced relaxation of human placental blood vessels and its dependence on endothelial cells.

Trophoblastic Channels, Fluid Balance, and Osmotic Pressure

In human placenta, the fetal tissue (chorion) is directly in contact with the maternal blood. The membrane barrier separating the maternal and fetal bloods consists of three layers: syncytiotrophoblast, connective tissue, and vascular fetal endothelium. Transfer of substances, ions, and fluids between maternal and fetal circulations is considered to be regulated by transport systems and permeability characteristics of the two plasmalemmas. During the past 20 years, several investigators have provided evidence for the existence of water-filled routes, so-called pores or channels, in rabbit, guinea pig, and human placentae [67–69]. Functionally, these channels are considered as possible sites of transfer for water-soluble, lipid-insoluble molecules (molecular diameter up to 1.5 nm). The fetus may eliminate surplus water through these channels. Fetal urine is delivered into the amniotic fluid, which is still inside the fetoplacental unit. Excessive fetal hydration first causes an increase of fetal venous pressure followed by a decrease in osmotic pressure. Both situations have been proven experimentally to open narrow channels that are dilated, allowing fetomaternal fluid shifts to maintain water and osmotic balance. It is tempting to speculate that ACh regulates these channels (open or closed) by action at cholinergic receptors, and that its hydrolysis results in the loss of this regulation. Irreversible or slowly reversible ChA inhibitors, BETA, α-NETA, and related compounds [72–74], may be useful to study the nature of these channels and fluid shifts between maternal and fetal circulations. If the trophoblastic channels were to be regulated by ACh, inhibition of ChA synthesis should affect their function.

Human Placental Myofibroblasts and Their Contractile Properties

The mechanisms of blood flow through placental vasculature are not established. Besides maternal and fetal blood pressures, other driving forces may also be responsible for the propulsion of blood through the complex villus arterial and capillary systems and the intervillus space. A large number of villus stromal cells have been characterized and classified as myofibroblasts, which have elongated and branched cytoplasmic projections interwoven in a network surrounding capillaries and spanning the region between stroma and the subtrophoblastic basement membrane [70, 71]. This indicates that the fetal vascular system of placenta is wrapped in a sheath of connective tissue containing numerous contractile myofibroblasts. The contractile forces generated by myofibroblasts in villus stroma are capable of shortening the length of villi, which alters the volume of intervillus space. These forces also provide forward propulsion of fetal blood flow through the placenta. Therefore, this alteration contributes to intraplacental blood propulsion in the fetal and maternal blood compartments. This process may aid in the exchange of substrates between the two compartments and alterations (open/shut) of trophoblastic channels.

Stromal myofibroblasts have rough endoplasmic reticulum indicative of high cellular synthetic activity [75]. Myofibroblasts develop contractile force upon stimulation with several biological agents. Placentae contain both muscarinic and nicotinic cholinergic receptors. The connective tissue-myofibroblast region of human placenta also contains α-BGT binding proteins, which may be composed of ion channels. ACh may provide the stimulus for contraction of myofibroblasts. It has yet to be determined whether cholinergic receptors are present on myofibroblasts and how they respond to ACh.

The fetal vasculature provides hydraulic support to the villous tree, such that changes in the umbilical perfusion pressure can alter the disposition of the villi within the intervillous space [76]. When fetal blood pressure rises, the villi will move apart. The villous membrane will tend to straighten, possibly unmasking receptors and transport sites. The enlargement of the clefts between adjacent villi will have a secondary effect upon the maternal circulation, promoting more even perfusion of the intervillous space at higher overall flow rates. This mechanism might provide a physical means by which the maternal and fetal circulations can locally interact in the villous.

Human Placental Cholinergic System and Amino Acid Uptake

A number of investigations using human placental tissue indicate a coupling between ACh release and active uptake of amino acids from maternal blood. Atropine causes a reduction in the uptake of AIB by isolated placental villus [77] or placental fragments [78]. Four different types of ChA inhibitors depress the uptake of AIB by isolated placental villus [30, 77–79]. There is a positive correlation between the inhibition of ChA and the degree of depression of AIB uptake. Extracellular Ca^{2+} must be present for placental release of ACh. The uptake of AIB by placental villus is depressed 90% in Ca^{2+}-free medium [8]. Inhibition of ChA decreases the synthesis of ACh and its release as well as AIB uptake in placental villus or explants [8, 77–79].

Placentae from women with preeclampsia have higher levels of ACh than control placentae, but the output of ACh from preeclamptic placentae is reduced significantly [80–82]. The transport of AIB from the maternal side to the fetal side is reduced in the preeclamptic perfused placenta [82]. These observations suggest that fetal intrauterine growth retardation in preeclamptic women can be partially explained by reduced placental ACh release and reduced amino acid transport.

Clinical observations on drug abuse during pregnancy

and fetal growth give further evidence for a functional link between ACh and placental transfer of amino acids. High concentrations of nicotine decrease placental ACh release [83, 84]. Maternal smoking depresses placental transfer of amino acids [84–86]. Opioids decrease ACh release from placenta and depress amino acid uptake [43, 87, 88]. Cocaine decreases placental ACh release as well as amino acid uptake [87, 89]. Tobacco smoking and addiction to opioids or cocaine cause intrauterine growth retardation. All these observations suggest a link between ACh release and amino acid transport in placenta. However, other factors may also play a significant role in intrauterine fetal growth retardation.

Placental ACh and PGs in Human Parturition

There is considerable evidence that PGs are effectors of myometrial contractions and cervical changes that occur during labor [90]. ACh can directly stimulate human myometrium during pregnancy [91]. ACh modulates PG production in other tissues [92, 93]. ACh may activate phospholipase A_2-like enzymes and increase production of arachidonic acid, a precursor for PG production, from membrane phospholipids [94–96]. No significant decrease in either placental ACh content or output is observed after labor is induced by oxytocin [97]. Induction of labor with oxytocin may circumvent the normal mechanism of ACh release during labor. These observations suggest that ACh in concert with PGs may be involved in human parturition.

Release of Placental Hormones by ACh

Human placenta is a source of chorionic gonadotropins and steroid hormones. Since the development of the placental cholinergic system follows the development of the syncytiotrophoblast, it would be interesting to determine the release of steroid hormones by ACh. Although the cytotrophoblast, the source of gonadotropin in the placenta, is fully developed in the first 3 months of gestation, some cytotrophoblastic cells remain in full-term human placenta. It has been reported that ACh stimulates placental release of chorionic somatomammotropin *in vitro* [98]. Isolation of cytotrophoblasts from term placenta and conditions for their culture have been described by some authors, and they may be useful to study the effects of ACh on the release of chorionic gonadotropin [99, 100].

ACh increases the release of immunoreactive corticotropin-releasing factor from human term placental cell cultures in a dose-related manner, and its effect is reversed by the cholinergic receptor antagonists atropine and hexamethonium [101]. Norepinephrine is as effective as ACh for releasing corticotropin-releasing factor. The effect of norepinephrine is antagonized by the α-adrenergic receptor antagonists prazosin and yohimbine. Placental and plasma concentrations of corticotropin-releasing factor reach their highest levels at term pregnancy and parturition. However, the significance of the effect of ACh and norepinephrine on the release of this factor is not well understood. Norepinephrine may induce release of ACh or vice versa.

CONCLUSIONS AND SCOPE OF FUTURE STUDIES

The main objective of this commentary was to evaluate the existing knowledge, studies that are being actively pursued, and future investigations about the placental cholinergic system, so that the role or function of ACh could be understood, particularly in human placenta and generally in non-neuronal tissues. The placental cholinergic system is comprised of ACh and molecular mechanisms activated by ACh, its synthesis, and the termination of its action. From this commentary, it is evident that all components of the cholinergic system—ACh, ChA, AChE, muscarinic receptors, and nicotinic receptors—are present in human placenta. However, there are several gaps in information that should be addressed in future studies. Investigators who used bioassay techniques have reported higher levels of ACh in human term placenta than those who used GC-MS methods. For example, according to Chang [102], the ACh-like biological activity in term placental extracts is equivalent to about 180–230 nmol ACh/g wet tissue, and Sastry *et al.* [6] found about 112 nmol ACh/g wet tissue. Using GC methods, about 55% of the ACh-like activity in the placental extracts is ACh. Compounds that contribute to excess ACh-like activity have yet to be identified. Two types of compounds may contribute to this ACh-like biological activity: (a) quaternary compounds that are not esters but are detected by GC methods. These compounds have to be identified and their biological activities have to be determined; and (b) some labile choline esters that cannot be detected by GC methods. The possible occurrence of lactoylcholine in animal tissues and human placenta has been indicated by some studies [103, 104], which are based on the pharmacodynamics of choline esters and atropine-like agents, the naturally occurring choline esters, the specificity of cholinesterases [103–109], and the specificity of ChAs [105, 110, 111]. Lactoylcholine is hydrolyzed by both types of cholinesterases (AChE, PChE) at rates comparable to those for ACh [103–109]. In a coupled system containing acetylthiokinase, human placental ChA and the necessary cofactors, the rate of synthesis of L-lactoylcholine is comparable to that of ACh [105]. Therefore, the occurrence of lactoylcholine in tissues is possible, especially under anaerobic glycolysis or conditions in which lactate accumulates in tissues. Blood lactate concentrations are higher in intrauterine growth-retarded babies than in their corresponding gestational peers. There are no reports in choline esters in placentae of growth-retarded babies.

The ChAs in human placentae and animal placentae have yet to be characterized. For example, purified neuronal ChA is specific for choline and does not utilize carnitine as a substrate. Purified CaA from liver does not utilize choline as a substrate. However, there are reports that placental ChA may exhibit CaA-like activity [112] and possess

chemical characteristics different from those of neuronal ChA [113]. There may be different types of ChAs, specific and nonspecific, with respect to either choline or ACoA. Their locations and functions may be different in the cell. Specific ChA may be present in the cytoplasm of the syncytiotrophoblast and may be involved mainly in the synthesis of ACh. Nonspecific ChA (or nonspecific CaA) may be localized in the plasma membrane or mitochondrial membranes of the syncytiotrophoblast. This enzyme may be involved in the transport of acetyl or acyl groups across plasma and/or mitochondrial membranes. Recent developments in the synthesis of specific inhibitors for ChA and CaA may facilitate the role of these enzymes in placenta [114, 115].

The placental cholinesterases, AChE and PChE, may exhibit different chemical characteristics toward their substrates when compared with cholinesterases of neuronal tissues [116]. Similarly, the placental nicotinic cholinergic receptor and BGT-BP may exhibit different chemical and functional characteristics when compared with the neuronal nicotinic receptor and/or BGT-BP. The functions of transtrophoblastic channels and myofibroblasts of placenta may also be modified by ACh activation of placental cholinergic receptors. For example, the subtypes of nicotinic receptors in muscle, electric organ, chicken brain, and mammalian brain vary in the composition of their pentameric complexes. Purified nicotinic receptor has yet to be isolated from placenta. It will be rewarding because placenta is an easily available human source.

Muscarinic receptors from the cholinergic nervous system have been classified into five subtypes (M1–M5). They are glycoproteins, coupled to G-proteins, and act directly on ion channels or are linked to second messenger systems. However, nothing is known about the nature and structure of muscarinic receptors in placenta. Due to the interrelationships of various components of the placental cholinergic system, progress may occur on different components simultaneously. However, progress will depend upon the use of correct model systems and techniques. Although ACh has been known to activate several types of muscarinic receptors and bring about alterations in second messenger systems in the nervous system, very little progress has been made in our understanding of the ACh-initiated membrane transduction mechanisms in placenta. There are many gaps in our knowledge of the placental cholinergic system, its function, and its molecular mechanisms.

Several studies of the author discussed in the present commentary have been supported by the U.S. Department of Health and Human Services, National Institutes of Health grants awarded by the Institute of Child Health and Development, the National Institute on Drug Abuse, the National Institute of Neurological and Communicative Disorders and Stroke, The Council for Tobacco Research, Inc., and The Smokeless Tobacco Research Council at different periods of his professional career. The present investigations of the author are supported by The Smokeless Tobacco Research Council, The Council for Tobacco Research, U.S.A., Inc., and The Study Center for Anesthesia Toxicology, Vanderbilt University.

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