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FORUM MINIREVIEW

The Non-neuronal Cholinergic System

Non-neuronal Neurotransmitters and Neurotrophic Factors in Amniotic Epithelial Cells: Expression and Function in Humans and Monkey

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ABSTRACT—Human amniotic epithelial cells (HAEC) are formed from epiblasts on the 8th day after fertilization. Because they lack major histocompatibility complex (MHC) antigen, human amniotic tissue transplantation has been used for allotranplantation to treat patients with lysosomal diseases. We have provided evidence that HAEC have multiple functions such as synthesis and release of acetylcholine (ACh) and catecholamine (CA) as well as expressing mRNA coding for dopamine receptors and dopamine (DA) transporter (DAT). On the other hand, we showed that monkey amniotic epithelial cells (MAEC) synthesize and release CA and posses DA receptors and DAT. Detection of muscarinic actylcholine receptors indicates the presence of an autocrine mechanism in HAEC. Recently, we found that HAEC have neurotrophic function in conditioned medium from HAEC, indicating the presence of a novel neurotrohpic factor that is synthesized and released from HAEC. The amniotic membrane may have a significant role in supplying neurotrophic factors as well as neurotransmitters to the amniotic fluid, suggesting an important function in the early stages of neural development of the embryo. This review will focus on the neuropharmacological aspects of HAEC and MAEC in relation to the physiology of amniotic membrane.

Keywords: Acetylcholine, Amniotic epithelial cell (human and monkey), Catecholamine, Neurotransmitter, Neurotrophic factor

The amniotic epithelial cells are formed from the epiblast at the 8th day of fertilization. At the end of the 3rd month, the amnion and chorion are fused to form the amniochorionic membrane. The amniotic membrane is composed of five layers: an amniotic epithelial cell layer, a basement membrane, a compact layer, a mesenchymal cell layer and a spongy layer (1). Because of these embryological characteristics, the amniotic epithelial cells may have the potential to differentiate into various organs, including heart, liver and brain. We have found that human amniotic epithelial cells (HAEC) express both neuronal and oligodendrocyte markers (2, 3) Recently, we provided evidence for the synthesis and release of acetylcholine (ACh) (4), catecholamnies (CA) (5, 6), and neurotrophic factors (7) by HAEC and monkey amniotic epithelial cells (MAEC).

Non-neuronal ACh has attracted attention since a widespread expression of the cholinergic system were demonstrated in mesothelial, endothelial and circulating blood cell, etc. (8). Non-neuronal dopamine (DA) has been found in the gastrointestinal system (9) and kidney (10). In addition, neurotrophins are expressed both in the mesenchyme and the epithelium of developing skin (11). The production of these neurotrasmitters in the human amniotic membrane must be of relevance to fetal development like the other biological substances such as neutrotrophic factors.

Acetylcholine metabolism

We investigated the presence of choline acetyltransferase (ChAT) and ACh in HAEC using different experimental approaches (4). Cultured HAEC and its tissue showed strong immunoreactivtiy against ChAT antibody

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(Fig. 1). ChAT activity in primary cells was 24.9 ± 8.5 pmol · mg protein^{-1} · h^{-1}. Using HPLC with electrochemical detection, ACh was detected in both cell incubation media and cell pellets, indicating that these cells synthesize and release ACh in a time-dependent manner. Additional confirmation of this hypothesis was gained from RT-PCR and Western blot analyses that revealed the expression of ChAT-mRNA and ChAT protein, respectively, in HAEC. RT-PCR analysis showed HAEC expressed the mRNA for muscarinic receptor subtypes m1, m2, m4, m5, whereas expression of mRNA for m3, m4, m5 was confirmed in human amniotic epithelial tissue (N. Sakuragawa et al., unpublished data). In addition, we provided evidence for the presence of ACh in the amniotic fluid and amniotic fluid cells (12). These data indicate that human amniotic membrane play an important role in regulating transport of ACh across the maternal-fetal interface via the autocrine/paracrine system.

Catecholamine metabolism

Using HPLC with electrochemical detection (HPLC-DEC), we detected norepinephrine (NE), DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the extracts of cultured HAEC. The capacity of HAEC to synthesize CA was tested by supplementing the incubation medium with L-tyrosine (CA precursor) and tetrahydrobiopterin (tyrosine hydroxylase cofactor). This treatment significantly increased the production of catechoalamines, suggesting CA synthesis by HAEC. In contrast, pharmacological inhibition of tyrosine hydroxylase (TH) by α-methyl-*p*-tyrosine significantly reduced CA levels, further confirming CA synthesis by HAEC. In addition, HAEC showed the ability to release CA into the culture medium both spontaneously and in response to high concentration of potassium (5).

The presence of TH was further confirmed by RT-PCR and Western blotting studies which revealed that HAEC express TH-mRNA and TH protein, respectively. Furthermore, immunocytochemical staining provided further evidence for the expression of TH by cultured HAEC (13) and human amniotic epithelial tissue (Fig. 1).

We also tested the ability of HAEC to take up and decarboxylase L-3,4-dihydroxyphenylalanine (L-DOPA) with subsequent synthesis of DA. We found that DA synthesis is significantly increased in a time- and L-DOPA concentration-dependent fashion, suggesting the presence of aromatic L-amino acid decarboxylase (AADC) enzyme. This was confirmed by the decreased DA synthesis in the presence of AADC inhibitor, benserazide (14).

Based on theses findings of synthesis and release of CA including DA by HAEC, we therefore suggest that these cells may be a possible candidate for transplantation therapy of neurodegenerative diseases like Parkinson's disease and also may serve as a model to study the aspects of catecholaminergic activity in human cells. In fact, in a recent study we found that transplantation of HAEC alleviate Parkinson-like symptoms in a rat model of the disease (13).

Catecholamines in MAEC

The promising findings of CA in HAEC prompted us to examine the presence of CA in MAEC (6). Immunocytochemical staining revealed the presence of TH, AADC, dopamine-β-hydroxylase (DBH) and DA immunoreactivities, suggesting the capability of these cells to synthesize CA. Similar to HAEC studies, HPLC-ECD analyses indicated the presence of NE, DA, and DOPAC in the cell extracts of cultured MAEC. Incubation of MAEC for various time intervals in medium supplemented with L-tyrosine and tetrahydrobiopterin significantly increased the production of CA, thus confirming active synthesis of CA by MAEC, and increasing the incubation time increases this synthesis. In contrast, pharmacological inhibition of TH by α-methyl-*p*-tyrosine significantly reduced CA production, further confirming CA synthesis by MAEC. Catecholamines were also released into the cell incubation media both spontaneously and in response to depolarization with high concentration of potassium.

We tested the presence of DA receptors in MAEC (15) and we found that these cells express D_1 receptor mRNA that has a 99% homology with human D_1 receptors. Radioligand saturation binding studies revealed that ^{3}H-SCH-23390 binds with high affinity to a D_1-like site. Competition experiments using a variety of dopaminergic agents verified the specific binding to these receptor sites and showed that the rank order of potency of these compounds in competing with ^{3}H-SCH-23390 for binding sites to be consistent with the pharmacology of the dopaminergic D_1 receptors.

Also, we found that MAEC express dopamine D_2 receptor mRNA that has a 98% homology with human dopamine D_2 receptor (16). Radioligand saturation binding studies showed a ^{3}H-YM-09151-2 high affinity binding site. Competition experiments with a variety of displacing drugs demonstrated that D_2, but not D_1 antagonists, potently compete with ^{3}H-YM-09151-2 for the binding sites with a rank order of potency that is consistent with the pharmacology of the dopamine D_2 receptors.

Furthermore, we investigated the presence of DA transporter (DAT) in MAEC using radioligand binding experiments (17). Saturation studies showed a ^{3}H-mazindol high affinity binding site. Competition studies showed that selective DAT inhibitors are the only potent displacers of ^{3}H-mazindol binding and that the rank order of potency of the competing drugs is consistent with the pharmacology of DAT.

Therefore, we suggest that MAEC may serve as a model

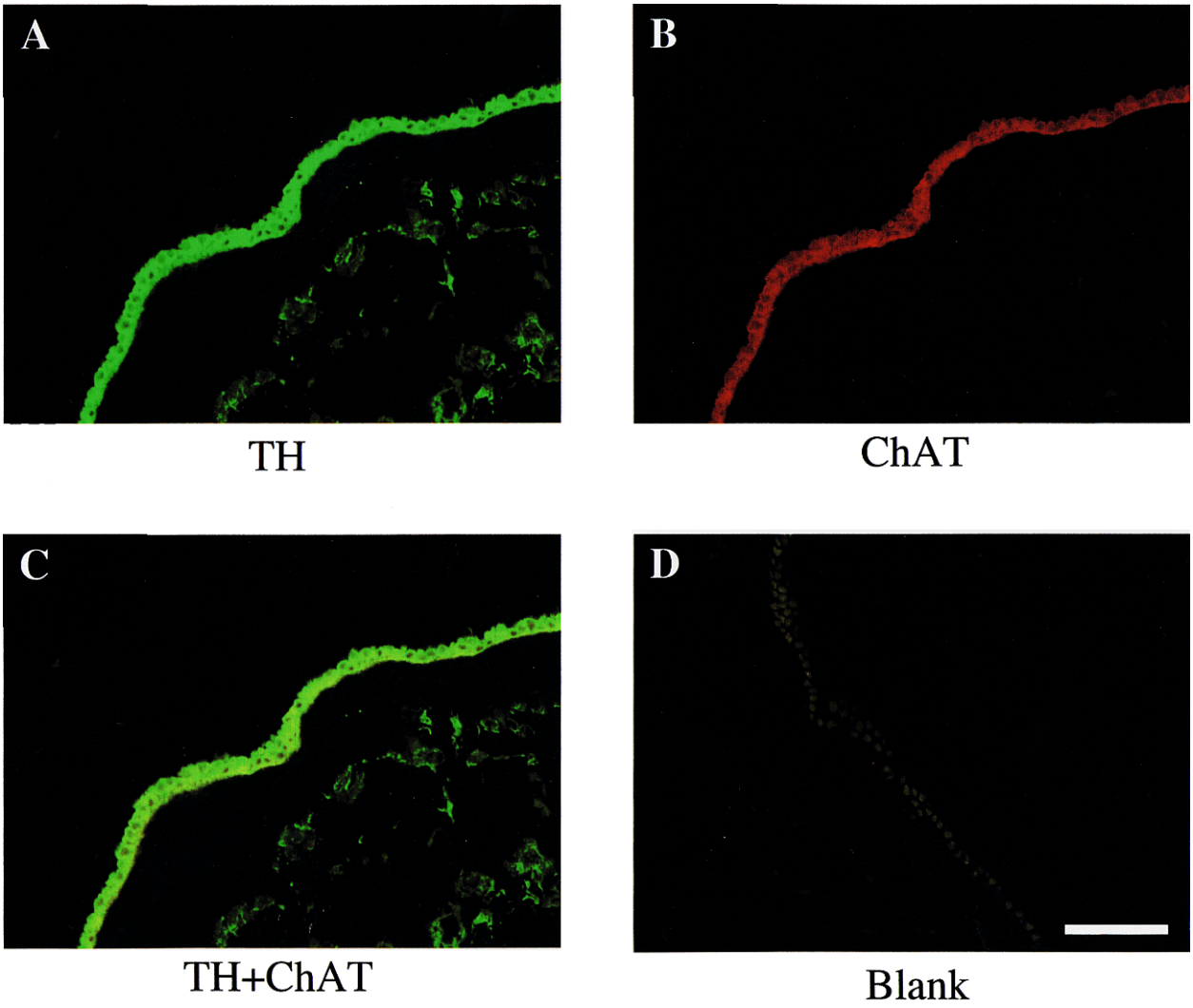

Fig. 1. Immunoreactivity to the polyclonal antibody (Ab) of tyrosine hydroxylase (TH) and monoclonal Ab of choline acetyltransferase (ChAT) in a cryostat-section of human amniotic epithelial tissue. Strongly positive cells to the TH Ab were present in the amniotic membrane and the mesenchym layer (A). Contrarily, ChAT-positive cells were present only in the amniotic membrane (B). Double staining showed the co-expression of ChAT and TH in the amniotic membrane (C). Scale bar, 50 μm.

to study the aspects of catecholaminergic activity in primate cells and may be a possible candidate for allotrasplantation therapy of monkey model of Parkinson's disease. Also, these cells may provide a potential primate cell model to study dopamine D_1 and D_2 receptors and DAT, which can be used to explore new drugs acting on these sites without the need for transplantation or cloning procedures using non-primate cells.

Neutotrophic factors (7)

We showed the neurotrophic function of a conditioned medium from HAEC using cultured cortical neurons of E18 rats. Extensive analyses with various techniques such as immunostaining, RT-PCR and enzyme immunoassay demonstrated that HAEC synthesize and release brain-derived neurotrophic factor (BDNF), neurotropin-3 (NT-3) and nerve growth factor (NGF). Other neurotrophic factors were not detected in a cultured medium of HAEC by enzyme immunoassay. Various neurotrophic factors or growth factors did not show neurotrophic effects on E18 rat neuron except for epidermal growth factor (EGF). Since EGF was not detected in the conditioned medium of HAEC, these data indicate the presence of a novel neurotrophic factor that is synthesized and released from HAEC.

Conclusion

The amniotic membrane is a very interesting tissue in terms of the pharmacological aspects of non-neuronal neurotrasmitters and neutrotrophins for which we provided evidence for synthesis and release into amniotic fluid. During

the early stages of neural development, the amniotic membrane lines the amniotic cavity and comes in direct contact with the neuroepithelium. The amniotic membrane may have a significant role in supplying neurotrophic factors as well as neurotransmitters, suggesting an important function in the early stages of neural development of the embryo.

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