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Synthesis of dopamine from L-3,4-dihydroxyphenylalanine by human amniotic epithelial cells

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Abstract

In this study, the ability of human amniotic epithelial cells to synthesize dopamine from L-3,4-dihydroxyphenylalanine (L-DOPA) was examined. Dopamine synthesis was significantly increased time and L-DOPA concentration dependently, suggesting the presence of an aromatic L-amino acid decarboxylase enzyme. This was confirmed by the decrease in dopamine synthesis in the presence of an aromatic L-amino acid decarboxylase inhibitor, benserazide. These findings suggest that human amniotic epithelial cells have the capacity to take up and convert L-DOPA into dopamine.

Keywords: Amniotic epithelial cell; Aromatic L-amino acid decarboxylase; Dopamine

Previous results from this laboratory showed the use of human amniotic epithelial cells for transplantation therapy of lysosomal storage diseases (Sakuragawa et al., 1992) and that these cells are immunologically naive (Sakuragawa et al., 1995) and express neuronal and glial cell markers (Sakuragawa et al., 1996). Also, these cells possess choline acetyltransferase activity and synthesize and release acetylcholine (Sakuragawa et al., 1997). Moreover, these cells can synthesize catecholamines from L-tyrosine (Elwan and Sakuragawa, 1997). The present study examined the ability of these cells to take up and convert 3,4-dihydroxyphenylalanine (L-DOPA) into dopamine.

Human amniotic epithelial cells were prepared and cultured in RPMI-1640 medium as previously described (Elwan and Sakuragawa, 1997). Cultured cells were passaged and plated in replicates of three 60-mm dishes at a density of $\sim 2 \times 10^6$ cells/dish. After 72 h, the culture medium was aspirated off and the cells were washed twice with ice-cold filter-sterilized HEPES-buffered solution containing (in mM) NaCl, 115; KCl, 5.4; $CaCl_2$, 1.3; $MgCl_2$, 0.8; NaH_2PO_4, 1; glucose, 5.5; ascorbic acid, 1 and HEPES, 15, pH = 7.2. For concentration-dependent synthesis of dopamine, cells were incubated for 1 h in the above buffer in the presence of pyridoxal phosphate (50 μM) and L-DOPA (0–500 μM). Time course studies were performed by incubating the cells with 50 μM L-DOPA and 50 μM pyridoxal phosphate in either HEPES-buffer for 15, 30, 45, 60, 120 and 180 min or in serum-free RPMI-1640 medium for 3, 6, 12 and 24 h. Another set of dishes was incubated with benserazide (50 μM) 1 h before and during the incubation with L-DOPA. At the end of the incubation time, cells were collected and extracted for catecholamine assay using high-pressure liquid chromatography with electrochemical detection (Elwan et al., 1998).

Assay of catecholamines showed the presence of noradrenaline, dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid (data not shown); this both confirms and is supported by our previous results (Elwan and Sakuragawa, 1997). Baseline values for dopamine were between 0.98 ± 0.14 to 1.32 ± 0.18 ng/mg protein. Incubation of cells with L-DOPA produced a concentration-dependent increase in dopamine levels (Fig. 1A). This synthesis appears also to be time-dependent in cells incubated in either HEPES buffer (Fig. 1B) or serum-free RPMI-1640 medium (Fig. 1C). The present results suggest the ability of these cells to take up and decarboxylate L-DOPA. Similar findings have been reported for other cells possessing an aromatic L-amino acid decarboxylase enzyme (Dawson and Philips, 1990). The presence of measurable dopamine levels in cells cultured in HEPES buffer or in serum-free RPMI-1640 medium argues against the possibility an accumulation of dopamine from the body or from

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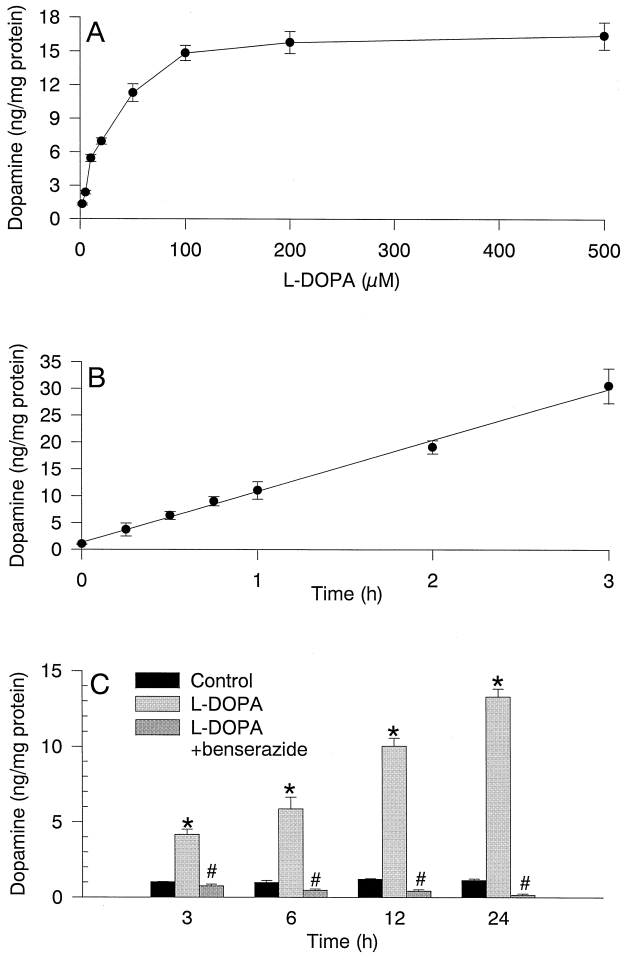

Fig. 1. Dopamine levels in the extract of human amniotic epithelial cells incubated in HEPES buffer with various concentrations of L-DOPA (A), or with 50 μM L-DOPA for increasing time intervals (B). Panel (C) depicts dopamine levels in cells incubated in serum-free RPMI-1640 medium enriched with 50 μM L-DOPA in the absence or presence of benserazide. Data expressed as means ± S.E.M. for three different dishes assayed in duplicate. * Significantly different from corresponding control. # Significantly different from L-DOPA-treated cells (one-way analysis of variance followed by Student's *t*-test).

serum included in the culture medium and suggests active synthesis of dopamine. This is confirmed by the present findings of time- and concentration-dependent synthesis of dopamine from L-DOPA and the inhibition of this synthesis by benserazide. Interestingly, however, cells incubated in HEPES buffer (Fig. 1B) produced more dopamine (30.55 ± 3.24 ng/mg protein after 3 h) than did cells incubated in serum-free RPMI-1640 medium (13.30 ± 0.53 ng/mg protein after 24 h, Fig. 1C) even though the same amount of L-DOPA was added to both media. The inhibition of L-DOPA uptake by extracellular amino acids included in the RPMI-1640 medium is a possible explanation for this observation (Tsai and Lee, 1996).

To the best of our knowledge, these results are the first to demonstrate that human amniotic epithelial cells can take up and decarboxylate L-DOPA with subsequent synthesis of dopamine. The present results make it likely that these cells could serve as a model to study aspects of catecholaminergic activity and be considered as possible candidates for allotransplantation therapy of Parkinson's disease.

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References

Dawson, R., Philips, M.I., 1990. Dopamine synthesis and release in LLC-PK1 cells. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 189, 423–426.

Elwan, M.A., Sakuragawa, N., 1997. Evidence for synthesis and release of catecholamines by human amniotic epithelial cells. NeuroReport 8, 3435–3438.

Elwan, M.A., Thangavel, R., Ono, F., Sakuragawa, N., 1998. Synthesis and release of catecholamines by cultured monkey amniotic epithelial cells. J. Neurosci. Res., in press.

Sakuragawa, N., Yoshikawa, H., Sasaki, M., 1992. Amniotic tissue transplantation: clinical and biochemical evaluation for some lysosomal storage diseases. Brain Dev. 14, 7–14.

Sakuragawa, N., Tohyama, J., Yamamoto, H., 1995. Immunostaining of human amniotic epithelial cells: possible use as a transgene carrier in gene therapy of inborn errors of metabolism. Cell Transplant 4, 343–346.

Sakuragawa, N., Thangavel, R., Mizuguchi, M., Hirasawa, M., Kamo, I., 1996. Expression of markers for both neuronal and glial cells in human amniotic epithelial cells. Neurosci. Lett. 209, 9–12.

Sakuragawa, N., Misawa, H., Ohsugi, K., Kakishita, K., Ishii, T., Thangavel, R., Tohyama, J., Elwan, M., Yokoyama, Y., Okuda, O., Arai, H., Ogino, I., Sato, K., 1997. Evidence for active acetylcholine metabolism in human amniotic epithelial cells: applicable to intracerebral allografting for neurologic disease. Neurosci. Lett. 232, 53–56.

Tsai, M.J., Lee, E.H.Y., 1996. Characterization of L-DOPA transport in cultured rat and mouse astrocytes. J. Neurosci. Res. 43, 490–495.