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Short communication

Uptake of dopamine by cultured monkey amniotic epithelial cells

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Abstract

In this study, the ability of monkey amniotic epithelial (MAE) cells to take up dopamine was tested by incubating the cells in buffer containing unlabeled dopamine under different experimental conditions followed by assaying dopamine content using high performance liquid chromatography with electrochemical detection. Results showed the capability of MAE cells to take up dopamine in a time- and concentration-dependent fashion, and also this uptake is sodium-dependent. Further, selective dopamine transporter blockers inhibited dopamine uptake with rank order of potency that is consistent with the pharmacology of the dopamine transporter. These results suggest that MAE cells may be potential model to study dopamine uptake and release, and to explore new drugs affecting these processes.

Keywords: Amniotic epithelial cell; Dopamine transporter; Dopamine uptake

1. Introduction

The major mechanism of controlling intercellular dopamine concentration is re-uptake by dopamine transporter. Aside from its physiological functions, dopamine transporter is a target for psychostimulants like cocaine and amphetamine (Usdin et al., 1991; Giros et al., 1992; Eshleman et al., 1994), antidepressants (Tatsumi et al., 1997), as well as some neurotoxins including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Alexander et al., 1992; Gnanalingham et al., 1995; Mackenzie et al., 1997) and 6-hydroxydopamine (Dubocovich and Zahniser, 1985). Moreover, studies of postmortem human brain indicate that there is great loss of dopamine transporter in parkinsonian patients (Niznik et al., 1991).

Previous studies from this laboratory have demonstrated that monkey amniotic epithelial (MAE) cells possess the catecholamine-synthesizing enzymes and are able to synthesize and release dopamine (Elwan et al., 1998). Also, we discovered that MAE cells natively possess dopamine receptor binding sites of both D_1 (Elwan et al., 1999b) and D_2 types (Elwan et al., 1999a). More recently, we reported that these cells are having [^{3}H]mazindol binding sites that are displaying the pharmacological profile of dopamine transporter (Elwan and Sakuragawa, 2000). To this end, we thought it might be of interest to study the ability of MAE cells to take up dopamine.

2. Materials and methods

2.1. Preparation of cells

Monkey amniotic epithelial membrane was peeled from the chorion of an emergency Cesarean section placenta of cynomolgus monkey and was used to obtain MAE cells according to the previously described procedure for human amniotic epithelial cells (Elwan and Sakuragawa, 1997). The isolated amniotic membrane was dissected out into small pieces and was then treated with 0.125% trypsin for 20 min. The isolated cells were collected by centrifugation and were cultured under humidified atmosphere of 5% CO_2 in air at 37 °C in RPMI-1640 medium enriched with 10% fetal bovine serum, penicillin 100 U/ml, streptomycin 100 μg/ml, and glutamine 0.3 mg/ml (Elwan et al., 1998). 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;

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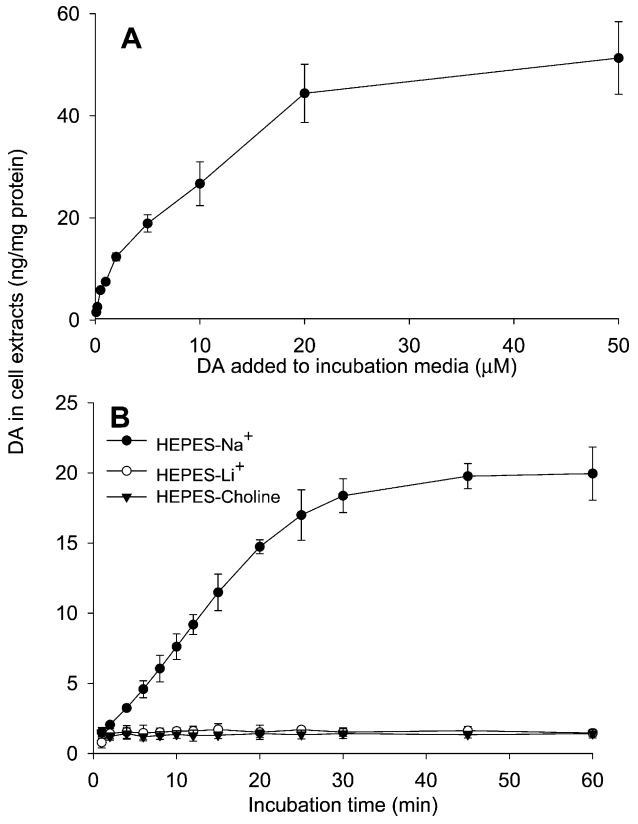

Fig. 1. Dopamine (DA) levels in the extract of monkey amniotic epithelial cells incubated for 10 min in HEPES-buffer with various concentrations of unlabeled dopamine (A), or with 1 μM dopamine for increasing time intervals in the presence of sodium, lithium or choline (B). Data expressed as means ± S.E.M. for three different dishes assayed in duplicates.

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. The incubation medium was also supplemented with pargyline and 3-phenylpropargylamine (10 μM each) to inhibit monoamine oxidase and dopamine-β-hydroxylase activities, respectively.

2.2. Dopamine uptake

For concentration-dependent dopamine uptake, cells were incubated at 37 °C for 10 min in 3 ml of the above HEPES-buffer in the presence of increasing concentrations of unlabeled dopamine (0–50 μM).

Time course studies were performed by incubating the cells under the above-mentioned conditions for various time intervals (1–60 min) with 1 μM dopamine in HEPES-buffer. To test sodium dependency of dopamine uptake, two other sets of experiments were carried out by incubating MAE cells for different time intervals with 1 μM dopamine in HEPES-buffer containing lithium or choline with corresponding omission of sodium.

To investigate the effect of uptake blockers, a set of dishes was incubated for 10 min with 1 μM DA in HEPES-buffer containing increasing concentrations (0–50,000 nM) of one of the monoamine uptake blockers.

At the end of the incubation time, cells were collected and extracted for dopamine assay using high performance liquid chromatography with electrochemical detection (Elwan et al., 1998). Protein concentration was determined according to Lowery's method using bovine serum albumin as standard (Lowry et al., 1951).

3. Results

Incubation of MAE cells in HEPES-buffer supplemented with unlabeled dopamine resulted in increased dopamine concentration in the cell extracts. This increased cellular dopamine concentration was dependent on both the incubation time and on the concentration of the unlabeled dopamine added to the incubation medium (Fig. 1A,B). Replacement of sodium in the HEPES-buffer with either lithium or choline resulted in decreased dopamine concentration in the extracts of MAE cells (Fig. 1B).

Incubation of cells with unlabelled dopamine in the presence of monoamine transporter blockers resulted in decreased dopamine concentration in cell extracts (Fig. 2). The potent dopamine uptake blockers were GBR 12935 (1-[2-diphenylmethoxy]ethyl-4-(3-phenylpropyl)-piperazine), mazindol, nomifensine and amfonilic acid; the IC_{50} (nM) were 24.1 ± 4.7, 28.2 ± 4.7, 28.3 ± 7.3, and 30.5 ± 6.7, respectively. Benztropine and imipramine displayed relatively low potency in inhibiting dopamine uptake; the IC_{50} (nM) were 1183.5 ± 380.3 and 1663.1 ± 546.8, respectively.

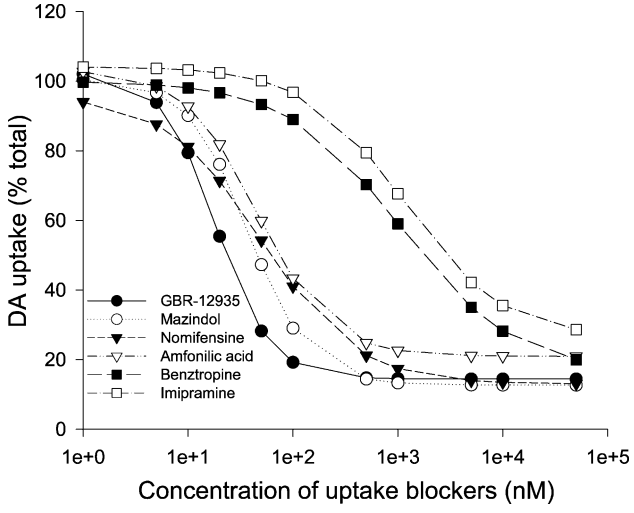

Fig. 2. Representative experiments showing the inhibition of dopamine (DA) uptake in the presence of uptake blockers. Cells were incubated for 10 min with 1 μM dopamine in HEPES-buffer containing increasing concentrations of one of the monoamine uptake blockers. Experiments were done in triplicates.

4. Discussion

In the present study, incubation of MAE cells in medium supplemented with unlabeled dopamine resulted in increased dopamine concentration in the cell extracts, suggesting an ability of dopamine uptake. It appears that this dopamine uptake is dependent on incubation time and on the concentration of dopamine added to the incubation medium. Similar findings have been reported for the uptake of either unlabeled dopamine or [^{3}H]dopamine by genetically modified cells expressing the dopamine transporter (Giros et al., 1992; Eshleman et al., 1994; Gu et al., 1994). These findings both confirm and are supported by our previous results indicating the presence of [^{3}H]mazindol binding sites in MAE cells (Elwan and Sakuragawa, 2000). Moreover, similar to previous reports (Giros et al., 1992; Eshleman et al., 1994; Gu et al., 1994), replacement of sodium with either lithium or choline resulted in decreased cellular dopamine concentration, thus indicating that dopamine uptake by MAE cells is a sodium-dependent process.

Further characterization of the uptake was carried out by testing a series of compounds for their ability to inhibit the uptake of a fixed concentration (1 μM) of dopamine (Fig. 2). Compounds tested dose-dependently inhibited dopamine uptake in a uniphasic manner with a rank order of potency that is clearly suggestive of the dopamine transporter. Drugs known to possess selective dopamine transporter blocker activity such as GBR 12935, mazindol, nomifensine and amfonilic acid displayed the highest potency in inhibiting dopamine uptake, while imipramine (a preferential noradrenaline uptake blocker) displayed the lowest potency. This rank order of potency of the tested drugs in inhibiting dopamine uptake by MAE cells is consistent with the previously reported data obtained from dopamine uptake (Usdin et al., 1991; Giros et al., 1992; Eshleman et al., 1994, 1995; Pristupa et al., 1994; Pifl et al., 1996) and dopamine transporter radioligand binding studies (Pristupa et al., 1994; Eshleman et al., 1995; Pifl et al., 1996; Tatsumi et al., 1997; Elwan and Sakuragawa, 2000).

A widely used strategy to study dopamine transporter is by genetic modification of cells to express this transporter (Usdin et al., 1991; Giros et al., 1992; Eshleman et al., 1994; Pristupa et al., 1994; Pifl et al., 1996; Tatsumi et al., 1997). It has been reported, however, that the type of transfected cells as well as the level and type of expression could influence the pharmacological profile of dopamine transporter (Pristupa et al., 1994; Pifl et al., 1996). There also may be some differences between native and cloned dopamine transporter. For instance, in a study using genetically modified cells expressing human dopamine transporter, [^{3}H] GBR-12935 failed to display the expected pharmacological profile; a surprising result considering the binding profile of the same ligand to the native dopamine transporter in human caudate membranes (Pristupa et al., 1994).

Based on the present findings and on our observations that MAE cells represent a homogenous population of non-tumor fetal primate cells that can easily be isolated in large quantities in a purified and functional state and maintained in primary culture with easy manipulation, it is therefore suggested that MAE cells could represent a source of primate dopamine transporter without transformation or cloning procedures using non-primate cells. Despite these, however, more extensive studies are needed to further characterize the process of dopamine uptake by these cells before being considered as a valuable primate model to study dopamine uptake and release and to explore new drugs affecting these processes.

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