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Concerted action of the cytosolic sulfotransferase, SULT1A3, and catechol-*O*-methyltransferase in the metabolism of dopamine in SK-N-MC human neuroblastoma cells

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ABSTRACT

Conjugation reactions catalyzed by the cytosolic sulfotransferase, SULT1A3, or catechol-*O*-methyltransferase (COMT) are known to be involved in the regulation and homeostasis of dopamine and other monoamine neurotransmitters. Whether different conjugation reactions may act in a concerted manner, however, remains unclear. The current study aimed to investigate the concerted action of SULT1A3 and COMT in dopamine metabolism. Analysis of the medium of SK-N-MC cells, metabolically labeled with [^{35}S]sulfate in the presence of dopamine, revealed the generation and release of predominantly [^{35}S]sulfated 3-methyldopamine and, to a lesser extent [^{35}S]sulfated dopamine. Addition to the labeling medium of tropolone, a COMT inhibitor, enhanced the production of [^{35}S]sulfated dopamine, with a concomitant decrease of [^{35}S]sulfated 3-methyldopamine. Enzymatic assays using the eleven known human cytosolic SULTs revealed SULT1A3 as the major enzyme responsible for the sulfation of both dopamine and 3-methyldopamine. Kinetic analysis showed that the catalytic efficiency of SULT1A3 with 3-methyldopamine was 1.6 times than that with dopamine. Using subcellular fractions prepared from SK-N-MC cells, the majority of COMT dopamine-methylating activity was found to be present in the cytosol. Collectively, these results imply a concerted action of sulfation and methylation in the irreversible inactivation and disposal of excess dopamine in SK-N-MC cells.

1. Introduction

Homeostasis of dopamine as a major catecholamine neurotransmitter is critical for the normal functioning of the nervous system (Palkovits and Brownstein, 1983; Tyce et al., 1986; McGinty, 1999; Goldstein et al., 2003). Abnormal levels of dopamine have been associated with neurologic disorders such as schizophrenia and Parkinson's disease (Jones et al., 1995; Stokes et al., 1999; Huttunen et al., 2008). Numerous studies have demonstrated the involvement of conjugation reactions in the regulation and homeostasis of dopamine (Tyce et al., 1986; Mulder and Jakoby, 1990; Coughtrie, 1998; Eisenhofer et al., 1999; Mannisto and Kaakkola, 1999; Goldstein et al., 2003). Among them, sulfation and methylation are two most thoroughly documented conjugation pathways for the biotransformation/disposal of dopamine and other catecholamine neurotransmitters.

Sulfation is generally known as a major Phase II metabolic pathway for the detoxification of drugs and xenobiotics (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994). Increasingly, it has been shown to be involved in the biotransformation and homeostasis of endogenous steroid/thyroid hormones and catecholamine neurotransmitters (Falany, 1997; Weinshilboum et al., 1997; Strott, 2002). The cytosolic sulfotransferases (SULTs) catalyze the transfer of a sulfuryl group from the active sulfate, 3′-phosphoadenosine-5′-phosphosulfate (PAPS), to an acceptor substrate compound containing hydroxyl or amino group (Lipmann, 1958). Sulfation of target molecules by SULT enzymes generally leads to the inactivation of their biological activity and/or the increase in their water-solubility, thereby facilitating their removal from the body (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994). Of the

Abbreviations: SULT, sulfotransferase; COMT, catechol-*O*-methyltransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; AdoMet, *S*-adenosyl-L-methionine.

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eleven known human cytosolic SULTs, SULT1A3 (previously called the monoamine (M)-form phenol sulfotransferase or catecholamine-preferring phenol sulfotransferase) plays an important role in the homeostasis of dopamine and other monoamine neurotransmitters (Falany, 1997; Weinshilboum et al., 1997; Strott, 2002). Catechol-*O*-methyltransferase (COMT), which is also known as a classical Phase II enzyme, catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to one of the catecholic hydroxyl group in an acceptor compound (Mannisto and Kaakkola, 1999). This reaction is highly selective to target molecules such as catecholamine neurotransmitters and catecholestrogens that contain two vicinal hydroxyl groups on an aromatic ring (Mannisto and Kaakkola, 1999). Previous reports have revealed that one single COMT gene encodes both a soluble COMT and a membrane-bound COMT (Salminen et al., 1990; Lundstrom et al., 1995; Ulmanen et al., 1997). Both these two forms of COMT catalyze specifically the 3-*O*-methylation of dopamine, thereby regulating its level and activity (Lotta et al., 1995; Mannisto and Kaakkola, 1999). Previous studies have demonstrated that SULT1A3 and COMT are expressed in glial cells that provide support and nutrition, as well as participate in signal transmission in the nervous system (Yu and Walz, 1985; Richard et al., 2001; Hong et al., 1998).

We report in this communication a possible concerted action of methylation and sulfation in the metabolism of dopamine in SK-N-MC cells as a model for dopaminergic neurons. A systematic analysis of the sulfating activities of the eleven known human cytosolic SULTs revealed SULT1A3 as the major enzyme responsible for the sulfation of dopamine and 3-methyldopamine. Kinetic constants of SULT1A3 toward these two latter substrates were determined. The subcellular distribution of COMT dopamine-methylating activity was examined using soluble and membrane fractions prepared from SK-N-MC cells.

2. Materials and methods

2.1. Materials

Dopamine, 3-methoxytyramine hydrochloride (3-methyldopamine hydrochloride), L-3,4-dihydroxyphenylalanine (L-dopa), adenosine 5′-triphosphate (ATP), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), 3-(*N*-morpholino)propanesulfonic acid (Mops), Trizma base, dithiothreitol (DTT), dimethyl sulfoxide (DMSO), pargyline hydrochloride, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), *S*-(5′-adenosyl)-L-methionine (AdoMet) chloride, ninhydrin, catechol-*O*-methyltransferase from porcine liver, minimum essential medium (MEM) were products of Sigma Chemical Co. (St. Louis, MO). 3-Methoxy-L-tyrosine dihydrate (3-methyl-L-dopa), carrier-free sodium [^{35}S]sulfate, and Ecolume scintillation cocktail were obtained from MP Biomedicals, Inc. (Irvine, CA). *S*-[methyl-^{14}C]-AdoMet was purchased from PerkinElmer, LAS Inc. (Boston, MA). Cellulose thin-layer chromatography (TLC) plates were from EMD Chem. Inc. (Gibbstown, NJ). Complete Mini protease inhibitor cocktail was from Roche Diagnostics GmbH (Mannheim, Germany); and Ultrafree-MC 5000 NMWL filter units were products of Millipore (Bedford, MA). Fetal bovine serum was from Biomeda (Foster City, CA). SK-N-MC human neuroblastoma cell line (ATCC HTB-10) was obtained from American Type Culture Collection (Manassas, VA). All other chemicals were of the highest grade commercially available.

2.2. Metabolic labeling of cultured SK-N-MC cells

SK-N-MC cells were routinely maintained, under a 5% CO_2 atmosphere at 37 °C, in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), penicillin G (30 μg/ml), and streptomycin sulfate (50 μg/ml). Confluent cells grown in individual wells of a 24-well culture plate, pre-incubated in sulfate-free (prepared by omitting streptomycin sulfate and replacing magnesium sulfate with magnesium chloride) MEM without FBS for 4 h, were labeled with 0.25 ml aliquots of the same medium containing [^{35}S]sulfate (0.3 mCi/ml) plus 50 μM dopamine or 50 μM 3-methyldopamine. In parallel experiments, cells grown in individual wells were pre-incubated for 4 h in sulfate-free MEM containing varying concentrations (ranging from 0 to 500 μM) of tropolone, an inhibitor of COMT. Thereafter, the cells were labeled with the same medium containing [^{35}S]sulfate plus 50 μM dopamine and the same concentrations of tropolone. At the end of a 21-h labeling period, the media were collected, spin-filtered to remove high-molecular weight [^{35}S]sulfated macromolecules, and subjected to the two-dimensional thin-layer analysis for [^{35}S]sulfated dopamine or 3-methyldopamine based on the procedures described below in Section 2.4.

2.3. Preparation of purified human cytosolic SULTs

Recombinant human P-form (SULT1A1 and SULT1A2) and M-form (SULT1A3) phenol SULTs, thyroid hormone SULT (SULT1B2), two SULT1Cs (designated #1 and #2), estrogen SULT (SULT1E1), dehydroepiandrosterone (DHEA) SULT (SULT2A1), two SULT2B1s (designated a and b), and a neuronal SULT (SULT4A1), expressed using pGEX-2TK or pET23c prokaryotic expression system, were prepared as previously described (Sakakibara et al., 1998a,b, 2002; Suiko et al., 2000; Pai et al., 2002).

2.4. SULT assay

The sulfating activity of the recombinant human cytosolic SULTs was assayed using PAP[^{35}S] as the sulfate group donor. The standard assay mixture, in a final volume of 25 μl, contained 50 mM of Mops buffer at pH 7.0, 1 mM DTT, and 14 μM PAP[^{35}S]. Stock solutions of the substrates, prepared in water, were used in the enzymatic assay. The substrate, at 10 times the final concentration (50 μM) in the assay mixture, was added after Mops buffer and PAP[^{35}S]. A control with water alone was also prepared. The reaction was started by the addition of the SULT enzyme (0.5 μg), allowed to proceed for 3 min at 37 °C, and stopped by placing the thin-walled tube containing the assay mixture on a heating block, pre-heated to 100 °C, for 2 min. The precipitates were cleared by centrifugation at 13,000 × *g* for 1 min, and the supernatant was subjected to the analysis of [^{35}S]sulfated product using the previously established TLC procedure with *n*-butanol/isopropanol/88% formic acid/water (1:1:3:1; by volume) as the solvent system (Liu and Lipmann, 1984). For two-dimensional thin-layer analysis, the thin-layer electrophoresis (TLE), with 7.8% acetic acid/2.5% formic acid (pH 1.9) as the electrophoresis buffer, was carried out first, followed in the second dimension by the above-mentioned TLC (Liu and Lipmann, 1984). Each experiment was performed in triplicate, together with a control without enzyme. The results obtained were calculated and expressed in nanomoles of sulfated product formed/min/mg purified enzyme.

2.5. Preparation of crude homogenate and soluble and membrane fractions of SK-N-MC cells

The crude homogenate as well as soluble and membrane fractions of SK-N-MC cells were prepared by an established procedure (Vieira-Coelho and Soares-da-Silva, 1999) with minor modifications. Briefly, confluent SK-N-MC cells grown in ten 100 mm tissue culture dishes were scraped off into Hanks' balanced salt solution. Upon centrifugation at 1000 × *g*, the