Substrate-dependent regulation of MAO-A in rat mesangial cells: involvement of dopamine D2-like receptors

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Submitted 3 March 2002; accepted in final form 28 August 2002

THE BIOGENIC AMINE DOPAMINE plays a key role in the regulation of a variety of physiological functions in the central nervous system (27) and in peripheral organs (13, 33). Dopamine effects depend on the occupancy of specific G-coupled, seven-transmembrane domain receptors, which were divided into two subfamilies, and on the subsequent generation or inhibition of intracellular messengers (20). To date, the two families of dopamine receptors are designated as D1-like and D2-like. The D1-like family is composed of two distinct receptors (D1 and D5). The D2-like family includes three distinct receptors (D2a–D2d). The amount of dopamine available for receptor stimulation is one of the factors affecting the extent of the cell effects elicited by this amine. The tissue concentration of dopamine depends, in part, on the activity of the amine-synthesizing or -degrading enzymes. The mitochondrial enzyme monoamine oxidases (MAOs) represent one of the major metabolic pathways for biogenic amine degradation. These enzymes are subdivided into two major forms, A and B, encoded by two distinct genes with an identical intron/exon structure (28) and differentiated on the basis of substrate specificity and inhibition by synthetic compounds (37).

MAO-A and -B are widely distributed in renal cortex and medulla. Most of the studies on renal MAOs concerned the proximal tubule. In this segment of the nephron, MAO-A is the predominant isoform, and it is involved in regulation of the amount of dopamine and serotonin synthesized and released by the epithelial tubular cells (8, 30). We have recently shown that hydrogen peroxide, which is produced by MAO during dopamine degradation, may also mediate the receptor-independent effect of dopamine. Indeed, we demonstrated that dopamine, independently of receptor activation, induces sequential ERK activation and renal epithelial cell proliferation that are fully dependent on hydrogen peroxide generation by MAOs (34, 35).

MAO-A is also the predominant isoenzyme expressed in rat mesangial cells (24). These cells, which are major constituents of renal glomerulus, play a critical role in the regulation of glomerular filtration rate (26) and participate in the development of functional and morphological glomerular abnormalities in inflammatory processes (9). In these cells, MAO-A regulates the extracellular concentrations and the proliferative effects of serotonin, a biogenic amine involved in the development of glomerulonephritis (24).

If the key role of MAOs in regulation of tissue concentrations and receptor-mediated effects of dopamine has been largely demonstrated (22, 28, 36, 37), the existence of a back-regulation of MAO activity and expression by dopamine has not been investigated. In contrast, some studies suggested that dopamine may regulate enzymes involved in catecholamine synthesis. Indeed, it has been shown that dopamine agonists...
decrease tyrosine hydroxylase expression in rat melanotrophs (21) and nigrostriatal dopaminergic neurons (11) and rat brain DOPA-decarboxylase (7). In addition, it has been reported that cAMP and PKA, which are intracellular second messengers of dopamine receptors (27), regulate the expression of tyrosine hydroxylase (10, 17), dopamine β-hydroxylase and phenylethanolamine N-methyltransferase (10), three catecholamine-synthesizing enzymes. Although there are not direct demonstrations that dopamine could also regulate expression and/or function of MAO, such a possibility is supported by the following observations: 1) L-DOPA administration, which increases dopamine synthesis, also increases MAO activity (6, 18); 2) a concomitant modification of brain dopamine content and MAO activity is observed during ontogenesis (31) and aging (23, 37); and 3) human MAO-A and MAO-B promoters possess a putative cAMP responsive element (39, 40) suggesting a potential gene transcriptional regulation by receptors coupled to adenylyl cyclase.

On the basis of these observations, we investigated, in the present study, whether dopamine receptor activation could mediate changes in MAO expression and function in proximal tubule and glomerular mesangial cells of rat kidney that contain MAO-A (24) as well as D₁- and D₂-like dopamine receptors (1, 2, 12, 29).

Our results show for the first time that D₂-like receptors are responsible for the transcriptional regulation of MAO-A expression and the consequent increase in the enzyme activity. This effect was observed in mesangial cells but not in proximal tubule cells, indicating that dopamine-dependent MAO-A regulation may be cell specific.

**EXPERIMENTAL PROCEDURES**

**Materials.** RPMI 1640 (10×) and fetal calf serum were purchased from Gibco BRL (Life Technologies, Cergy Pontoise, France). Bromocriptine mesylate and domperidone were obtained from RBI (Illkirch, France). Dopamine, H89 dihydrochloride, and pargyline were purchased from Sigma (St. Louis, MO). SKF-38393, sulpiride, and quinpirole were purchased from Tocris. [14C]serotonin chased from Sigma (St. Louis, MO). SKF-38393, sulpiride, Dopamine, H89 dihydrochloride, and pargyline were purchased from Sigma (St. Louis, MO). SKF-38393, sulpiride, and quinpirole were purchased from Tocris. [14C]serotonin was obtained from DuPont NEN Life Science Products (Boston, MA). Rabbit β-ATPase antibody was a generous gift from Dr. J. Lunardi (Grenoble, France).

**Cultured mesangial cells.** All animal experiments were carried out according to the protocols of the Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996; authorization 00577, 1989, Paris, France). Rat mesangial cells were cultured from isolated renal glomeruli of male Sprague-Dawley rats (150 g body wt; Harlan-Gannat) as previously described (24). In brief, glomeruli were isolated by a conventional sieving method and used for primary culture in RPMI 1640 medium supplemented with 100 U/ml of penicillin/streptomycin, 2 mM glutamine, 1 mM sodium pyruvate, and 15% fetal bovine serum. Outgrowing cells were subcultured and maintained in the same medium supplemented with 15% SVF. The cells between passages 4 and 10 were used for experiments.

**Rat proximal tubule cell culture.** Rat proximal tubule epithelial cells were isolated from Sprague-Dawley rats (40 g), as previously described (35). Briefly, kidneys were removed aseptically, decapsulated, and minced coarsely in HBSS supplemented with 10 mM HEPES and 5 mM Na-glucose, pH 7.4. Cortex was separated from medulla and incubated in HBSS supplemented with 0.48 U/ml collagenase and 0.1% BSA in a flask under gentle stirring for 40 min at 37°C in a 5% CO₂ atmosphere. To separate homogeneous populations of nephron segments, the mixture of tubules was suspended in 42% Percoll that was made isotonic with 10X concentrated Krebs-Henseleit buffer (1.18 M NaCl, 47 mM KCl, 100 mM HEPES, 200 mM cyclamic acid, 1.26 mM MgSO₄, 11.4 mM KH₂PO₄, and 50 mM glucose) and was centrifuged (17,000 rpm, 30 min, 4°C). The F4 layer, composed of proximal tubules, was suspended in culture medium (DMEM/Ham’s F-12 medium supplemented with 25 mM HEPES, 25 mM NaHCO₃, 4 mM glutamate, 20 mM sodium selenite, 10 ml/l of a 100X nonessential amino acid mixture, 50 U/ml penicillin, 50 µg/ml streptomycin, 10 µg/ml insulin, 5 mM transferrin, 0.1 nM dexamethasone, 10 ng/ml EGF, and 5 µg/ml triodothyronine) and plated on 1, 3, and 6-well plates, 100-mm Petri dishes and six multiwell plates, respectively, that had been coated with collagen Type I from calf skin. Fetal calf serum (5%) was added in the culture medium until the first change (2 days after seeding). The experiments were performed at day 5.

**RNA isolation and RT-PCR.** Total RNA was extracted from confluent mesangial cell culture by using the acid guanidium thiocyanate-phenol-chloroform method as described by Chomczynski and Sacchi (5).

First-strand cDNA was synthesized from 0.5 µg of total RNA by reverse transcription for 60 min at 42°C in a final volume of 20 µl of RT buffer with 100 U of Superscript II (Gibco BRL/Life Technologies) 0.25 µg oligo(dT) 12–18, 0.5 mM dNTPs, 5 mM DTT, and 32 U RNase inhibitor. First strand cDNA (5 µl) was then used to amplify MAO-A and GAPDH fragments by PCR. Reaction mix containing PCR buffer with 1.5 mM MgCl₂, 0.2 mM dNTPs, 60 nM of primers, 2 U Taq polymerase, and reverse transcription reaction was denatured at 93°C for 2 min 30 s, and consequently MAO-A was amplified by 30 cycles with a DNA thermal cycler (TRIO-Thermoblock, Biometra, Göttingen, Germany). To evaluate the PCR products comparatively, we amplified at the same time and for 20 cycles the GAPDH product. A cycle was composed of a denaturation step at 95°C for 1 min, a primers annealing step at 56°C, and an extension step at 72°C for 1 min. The final extension step was prolonged to 10 min.

The absence of contaminants was routinely checked by RT-PCR assays of negative control samples in which the Superscript was omitted.

**Primers used.** Primers for MAO-A were defined by bases 1537–1556 5’-GTGGGCTCTCTCTGCTTTGT-3’ (forward) and 2037–2016 5’-AGTGCCAACGGTAGTGTATCA-3’ (reverse); and for GAPDH by bases 510–523 5’-AATGCATC-GTCATT-GTC-CC-CAACACCA-A-3’ (forward) and 980–960 5’-GTCACT-GAGAGCATTGCGACG-3’ (reverse) (3). The expected size of the amplification product was 500 and 470 bp for MAO-A and GAPDH, respectively.

**MAO activity.** Mesangial cells were harvested by scraping in sodium phosphate buffer (50 mM, pH 7.5) supplemented by protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 µg/ml bacitracin, 2 µg/ml soybean trypsin inhibitor (Sigma)). Crude extracts of proteins (10–20 µg) were incubated at 37°C for 20 min in sodium phosphate buffer with 10–400 µM of [14C]serotonin (AS: 52.3 Ci/mmol; NEN Life Science Products). The reaction was ended by the addition of...
0.1 ml of HCl, 4 N at 4°C. The reaction product was extracted (efficiency 92%) with 1 ml of ethyl acetate/toluene (vol/vol), and the radioactivity contained in the organic phase was counted in a liquid scintillation spectrometer at 97% efficiency. Nonspecific MAO-A activity was defined by activity remaining in the presence of 10^{-5} M pargyline, an MAO inhibitor. Proteins were measured according to the Lowry method (Bio-Rad) using bovine albumin as standard.

Western blot analysis. Crude protein extracts were solubilized in loading buffer (60 mM Tris-HCl, pH 6.8, containing 2% SDS, 10% glycerol, 1% β-mercaptoethanol, and 0.05% bromophenol blue) in a boiling water bath for 5 min. Total proteins (50 μg) were loaded onto 10% SDS-PAGE gel. Resolved proteins were electrophoretically transferred to a polyvinylidene difluoride membrane (NEN Life Science Products) with 1% BSA (Amersham Pharmacia Biotech, Buckinghamshire, UK) dissolved in wash buffer (phosphate buffer saline, pH 7.5, and 0.1% Tween 20). The membranes were blocked overnight at 4°C with 1% BSA (Amer- 
°branes were incubated with peroxi-
dase-labeled anti-rabbit IgG for 40 min (TEBU, Santa Cruz Biotechnology, Santa Cruz, CA). Bound antibodies were de-
tected with enhanced chemiluminescence (Amersham Pharmacia Biotech) and exposure to Amersham Hyperfilm.

**cAMP production.** The cells were incubated with D_{1}- or D_{2}-like dopaminergic receptor agonists for 15 min or pre-
treated with forskolin for 10 min before bromocriptine addi-
tion and then washed twice with PBS and scraped. The cAMP contents were measured by using the cAMP [125I] assay system (dual range) with magnetic separation (BIO-
TRAK, Amersham Pharmacia Biotech). The sensitivity of the assay was 2.7 pmol/ml. The cAMP values obtained were normalized with total protein contents in each dish as deter-
mined by the Lowry method.

**Statistical analysis.** MAO steady-state kinetic parameters were evaluated by using nonlinear square curve-fitting pro-
cedures (Prism GraphPad, San Diego, CA). Results are ex-
pressed as means ± SE. The statistical significance of differ-
ence between two experimental groups was evaluated by Student’s t-test. A P value <0.05 was considered significant.

**RESULTS**

**Dopamine increased MAO-A activity in rat mesan-
gial cells via D_{2}-like dopamine receptors.** MAO-A is the predominant isoform expressed in rat proximal tubule and mesangial cells (24). To define whether dopamine receptor stimulation is able to regulate MAO-A activity, we first incubated proximal tubule and mesangial cells with 10 μM dopamine, 10 μM SKF-38393 (D_{1}-like agonist), and 5 μM bromocriptine (D_{2}-like agonist). As shown in Fig. 1A, incubation of proximal tubule cells with dopamine, SKF-38393, or bromocriptine for 48 h did not modify MAO-A activity. In contrast, MAO-A activity was increased after incubation of mesangial cells with bromocriptine or quinpirole for 48 h (Fig. 1, A and B). No modifications of the enzyme activity were observed at incubation times shorter than 24 h, which-
ever D_{2}-like agonists were used. A significant increase in MAO-A activity was also observed by using the endogenous receptor agonist dopamine. In contrast, the enzyme activity was unaffected by cell treatment

with the D_{1}-like agonist SKF-38393 (Fig. 1, A and B). The effect of 1 μM bromocriptine was fully prevented by the preincubation of mesangial cells with the D_{2}-like receptor antagonists sulpiride and domperidone,
indicating that D2-like receptors are the dopamine receptor subtypes responsible for regulation of MAO-A activity (Fig. 1C). As shown in Fig. 2, the effect of bromocriptine was dose (A) and time (B) dependent, reaching the maximum between 1 and 10 μM bromocriptine and 48 h, respectively.

To determine whether the increase in MAO-A activity by bromocriptine was related to a change in the amount of MAO-A and/or to a modification of its affinity for the substrate, we performed velocity vs. substrate concentration experiments in treated and untreated mesangial cells. As shown in Fig. 3A, incubation of mesangial cells with bromocriptine for 48 h increased the $V_{\text{max}}$ of $[^{14}\text{C}]$5HT oxidation (control cells: $V_{\text{max}} 99 \pm 12 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ of protein}$; bromocriptine-treated cells: $V_{\text{max}} 215 \pm 15 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \text{ of protein}$, $n = 3$, $p < 0.005$) without significant modification of the $K_m$ (control cells: $K_m 55 \pm 12 \mu\text{M}$; bromocriptine-treated cells: $K_m 68 \pm 7 \mu\text{M}$). These results suggest that the augmentation of MAO-A by bromocriptine is related to the increase in the number of the enzyme molecules. This possibility was further supported by Western blot analysis showing that the intensity of the immunoreactive band corresponding to MAO-A was stronger in lysates from bromocriptine than in those from untreated cells (Fig. 3B). As we have reported above for the regulation of the enzyme

Fig. 3. Effect of bromocriptine on MAO-A activity and protein expression in mesangial cells. A: enzyme assays were performed in cell lysates from mesangial cells treated or not treated with 5 μM bromocriptine for 48 h. $V_{\text{max}}$ and $K_m$ of MAO-A for $[^{14}\text{C}]$5HT oxidation were determined for control cells and bromocriptine-treated cells. Values are representative of 3 independent experiments. B: Western blot analyses were performed on cell lysates from mesangial cells untreated or treated with bromocriptine (5 μM) or dopamine (10 μM) for 48 h. Crude extract (50 μg) was loaded onto gels for electrophoresis and processed as described in EXPERIMENTAL PROCEDURES. The histogram represents the ratio of optical densities corresponding to MAO-A and β-ATPase bands. The figure is representative of 4 independent experiments. $*P < 0.05$ compared with control values.
activity (Fig. 1), the effect of bromocriptine was mimicked by the endogenous amine dopamine.

**D2-like dopamine receptors induced MAO-A mRNA expression.** To determine whether modifications in MAO-A activity by bromocriptine were related to changes of mRNA turnover, we measured the expression of MAO-A mRNA by semiquantitative RT-PCR. Mesangial cell incubation with bromocriptine (5 μM) for 24 h led to a significant increase in the intensity of the RT-PCR product corresponding to MAO-A (Fig. 4). This effect was not maintained at 48-h incubation, when the mRNA level of MAO-A returned to the control value. To investigate whether the effect of bromocriptine involved a modification of mRNA synthesis and/or degradation, we determined the influence of the transcriptional inhibitor actinomycin D. As shown in Fig. 5, incubation of mesangial cells with actinomycin D did not modify the intensity of the MAO-A band at times up to 24 h. This indicates that as previously reported for the GADPH mRNA (15), which we used here as the reference RT-PCR product, MAO-A mRNA belongs to the “long half-life” mRNAs. At all times tested, bromocriptine, in the presence of actinomycin D, did not affect the expression of MAO-A RT-PCR product, indicating that this D2-like receptor agonist does not modify mRNA degradation. Compared with the results presented in Fig. 4, the data showed in Fig. 5 reveal that actinomycin D prevented the increase in MAO-A expression by bromocriptine observed at 24 h. These results indicate that bromocriptine increases MAO-A expression by a transcriptional mechanism. This possibility is further supported by the demonstration that actinomycin D also prevented the bromocriptine-dependent increase in MAO-A activity (Fig. 5B).

**Stimulation of MAO-A activity by bromocriptine was linked to an inhibition of adenylyl cyclase activity.** In mesangial cells, previous studies have reported a stimulation of cAMP generation by D1-like dopamine receptors, whereas the second messengers mediating the
effects of D2-like receptors have not been described. In a first series of experiments, we investigated the role of D2-like receptors on cAMP generation, the most widespread signal transduction pathway linked to these receptors (32). Incubation of mesangial cells with bromocriptine strongly inhibited forskolin-stimulated generation of cAMP, indicating that as previously reported for others tissues, D2-like receptors are linked to an inhibition of cAMP production (Fig. 6). To determine whether a decrease in intracellular cAMP is involved in the pathway mediating the D2-like receptor-dependent MAO-A regulation, we tested the effect of the PKA inhibitor H89 on MAO-A activity. As shown in Fig. 7, H89 increased MAO-A activity to an extent similar to that observed with bromocriptine. In contrast, 8-bromo-cAMP, a cell-permeable and stable analog of endogenously generated cAMP, suppressed the bromocriptine-stimulated mesangial cell MAO-A activity. These results suggest that inhibition of cAMP generation and a consequent decrease in PKA activity are involved in the regulation of MAO-A expression by D2-like receptors.

DISCUSSION

MAO expression and activity are regulated in various situations, including development (3, 14, 16), aging (25), and pathologies (28, 37). In some of these cases, changes in MAO activity depend on the hormonal environment. Several studies have identified steroid hormones as factors contributing to the hormonal MAO regulation (4, 19, 38). As far as we know, to date, the potential role of hormones acting on seven-transmembrane domain receptors, and particularly of catecholamine receptors, in regulation of MAO expression has not been investigated.

In the present study, we show that an MAO substrate, dopamine, can upregulate MAO-A in rat mesangial cells. These cells contain D1-like receptors positively linked to adenylate cyclase (29), and we have shown that they also express D2-like receptors responsible for inhibition of cAMP generation. Our results indicate that D2- but not D1-like receptors are responsible for MAO-A regulation. Indeed, we showed that MAO-A activity was increased by the D2-like receptor agonists bromocriptine and quinpirole and this effect was fully prevented by the D2-like receptor antagonists sulpiride and domperidone. The fact that bromocriptine effect was mimicked by quinpirole, a preferential D3 agonist, suggests that dopamine-mediated regulation of MAO-A expression involved this D2-like receptor subtype. This hypothesis is supported by radioligand binding and autoradiography studies, which have shown the expression of D3 receptors in glomerular mesangial cells (2). In contrast, MAO-A activity was unaffected by the D1-like receptor agonist SKF-38393. According to the intracellular messenger linked to two dopamine receptor subtypes, we found that the effect of D2-like receptor stimulation was mimicked by the PKA inhibitor H89 but not by 8-bromo-cAMP, an activator of the cAMP/PKA pathway. The effect of D2-like receptor stimulation on MAO-A activity was undetectable after 24 h and reached the maximum after 48 h, suggesting that MAO-A regulation may require an ex novo enzyme synthesis. This possibility was confirmed by enzyme assays and Western blots that showed a concomitant increase in V_{max} of [14C]5HT oxidation and in the detection of the MAO-A immunoreactive band. The increase in MAO-A activity was preceded by a signifi-
cant augmentation of the MAO-A mRNA that was maximal after 24 h and returned to the control values after 48 h. The effects on MAO-A mRNA and activity were fully prevented by actinomycin D, indicating that D2-like receptor stimulation increases the transcription of the MAO-A gene. It is interesting to note that regulation of MAO-A expression was not observed in proximal tubules that as mesangial cells express D1- and D2-like receptors. This suggests that MAO-A regulation by D2-like receptors is not a generalized phenomenon but could be rather dependent on the cell type. Although the expression of D2-like receptors in glomerular mesangial cells has been provided by radioligand binding and autoradiography studies (2), their intracellular messengers as well as their functional properties were still not defined. Our recent studies have shown that these receptors are not involved in mesangial cell contraction or proliferation (Pizzinat N, Marchal-Victorion S, Maurel A, and Parini A, unpublished observations). In contrast, the demonstration that D2-like receptors regulate MAO-A expression supplies the first evidence for a functional activity of these receptors in mesangial cells. It is conceivable that in vivo, long-term regulation of MAO-A expression by dopamine may participate in the control of biogenic amine availability and effects in glomeruli as well as in the regulation of the amount of hydrogen peroxide generated by MAO-A during substrate degradation. If this is the first demonstration that dopamine D2-like receptors are involved in regulation of an enzyme responsible for catecholamine degradation, another report showed that these receptors modulate the expression of the catecholamine-synthesizing enzyme tyrosine hydroxylase (21). That study, performed in rat melanotroph, demonstrated that D2-like receptor stimulation induced an effect on tyrosine hydroxylase expression that was opposite to that observed for MAO-A in mesangial cells. However, even if opposite, the effects of D2-like receptors on MAO-A and tyrosine hydroxylase expression share some common features, particularly the requirement of long incubation times and the ex novo enzyme synthesis. In addition, regulation of both MAO-A and tyrosine hydroxylase seems to depend on the increase in the basal level of intracellular cAMP. Indeed, we found that the increase in MAO-A expression by bromocriptine was mimicked by the PKA inhibitor H89 and was prevented by the PKA activator 8-BrcAMP. Concerning tyrosine hydroxylase, it has been shown that as observed after D2-like receptor stimulation in rat melanotroph (21), H89 induced a decrease in the enzyme activity in bovine chromaffin cells (10). Interestingly, the decrease in basal intracellular cAMP also reduced the expression of two other catecholamine-synthesizing enzymes, dopamine b-hydroxylase and phenylethanolamine N-methyltransferase (10). Taken together, these results suggest that basal cAMP levels may contribute to maintain the equilibrium between the expression of catecholamine-synthesizing and -degrading enzymes and, consequently, the normal tissue levels of catecholamines. It is conceivable that tonic stimulation of dopaminergic D2-like receptors may be one of the factors controlling the steady-state intracellular cAMP concentration and the basal activity of the catecholamine synthetic and metabolic pathway.

In conclusion, this work revealed a previously unknown mechanism of regulation of MAO-A expression by one of its substrates, dopamine. These results could be the first step for the further characterization of the relationships between dopamine, their synthesizing and degrading systems not only in kidney but also in other organs in which this amine plays a physiological or pathological role.

The authors wish to thank Jacqueline Mechi and Marie Helène Seguelas for assistance with the cell culture. S. Marchal-Victorion is supported by a fellowship from Servier Laboratories (Courbevoie-France).

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