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Sulfation of catecholamines, hypertensive and hypotensive drugs by monkey brain cortex enzymes

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Catecholamines are inactivated in the central nervous system by many enzymatic reactions including deamination, *O*-methylation and *O*-sulfation. Sulfation is the main biochemical pathway of catecholamines inactivation *in vivo* [1, 2]. The reaction plays an important role in blood pressure regulation as well as in the treatment of blood pressure disorders [3, 4]. The enzyme which transfers the sulfate group to catechol or phenolic compounds is phenol sulfotransferase (PST[1], EC 2.8.2.1). PST is widely distributed throughout the human brain showing the highest activity in cortex [5].

In our previous study we established that two different forms of PST are present in monkey (*Rhesus macaque*) brain cortex [6]. These forms can be separated on ion exchange cellulose. Both forms are active with *p*-nitrophenol, but only one with catecholamines. Because of the differences noted in the substrate specificity of the two forms it was of interest to investigate their ability to sulfate some hypo- and hypertensive drugs, as well as catecholamines derivatives.

The aim of this work was to study substrate specificity and affinity of either PST form towards catecholamine metabolites, catecholamine related compounds (adrenergics) and minoxidil.

The cerebral cortex of the monkey *Rhesus macaque* was homogenized in 3 vol. of 10 mM sodium phosphate buffer, pH 6.5, containing 1 mM dithiothreitol and 0.25 M sucrose, and centrifuged at 15000 × *g* for 15 min. The resulting supernatant was centrifuged at 100000 × *g* for 60 min, dialyzed against 10 mM sodium phosphate buffer, pH 7.4, with 1 mM dithiothreitol and placed on a DEAE-cellulose column (20 × 2 cm), stabilized with the same buffer. The cationic PST I was eluted with the buffer, and anionic PST II with a linear gradient of KCl as described previously [6]. The separated forms were further purified on Sephadex G-100 column (20 × 1 cm). PST activity was assayed as

Table 1
Isolation of monkey brain cortex PST

Procedure	Specific activity (pmole/mg per min)	
	1 mM *p*-Nitrophenol	50 μM Dopamine
15000 × *g* Supernatant	3.0 ± 0.5	0.50 ± 0.04
100000 × *g* Supernatant	5.3 ± 1.2	0.85 ± 0.04
DEAE-Cellulose chromatography PST I	11.2 ± 1.5	0
DEAE-Cellulose chromatography PST II	6.2 ± 0.5	0.52 ± 0.08

[1]Abbreviation: PST, phenol sulfotransferase

described by Barańczyk-Kuźma [7] using 1 mM *p*-nitrophenol or 50 μM dopamine as sulfate acceptor substrates (Table 1). The amount of ^{35}S-labeled products was determined by the method of Foldes & Meek [8]. Protein was determined by the method of Lowry *et al.* [9]. The specific activity of PST was expressed in pmoles of the product formed per mg of protein per minute, at 37°C. The effect of the compounds studied on PST activity was estimated after 15 min preincubation at 37°C of the enzyme with the appropriate concentration of the compound and 10 mM sodium phosphate buffer, pH 6.5.

A previous study demonstrated that cationic PST I did not utilize endogenous catecholamines [6]. Similarly, here we observed no activity with these compounds over a concentration range of 50 to 500 μM, either at pH 6.5 or 8.0 (Fig. 1A), but the specific activity of PST I with 1 mM *p*-nitrophenol was about twice as high as that of PST II (Table 1). Both PST forms were active with catecholamine derivatives (Fig. 1).They used α-methyldopa, a methylated precursor of catecholamines that is widely used as an antihypertensive drug, as well as normetanephrine and metanephrine, the α-methyldopa metabolites (Fig. 1A). Both enzymes showed the highest activity with MOPEG, the major metabolite of norepinephrine in mammalian central nervous system. 6-Hydroxydopamine, a neurotoxin which destroys catecholaminergic nerve terminals was also sulfated by monkey brain sulfotransferases (Fig. 1). Among the α-adrenergics studied, only phenylephrine could apparently be sulfated by both PST forms, whereas only PST II was active towards other adrenergics: octopamine, methoxamine and metaraminol (Table 2). Related drugs that lack a single free benzyl hydroxy group, methoxamine, mephentermine, ephedrine and pseudoephedrine were not substrates for either PST I or PST II over a concentration range of 0.05 - 1 mM (Table 2). They did not inhibit either the activity of these enzymes at concentrations up to 1 mM.

The apparent K_m's of PST I and PST II for *p*-nitrophenol were similar, 0.2 and 0.1 mM, respectively. In contrast to the results with *p*-nitrophenol the two enzyme forms demonstrated differentiated affinities for the methylated catecholamine derivatives, hypertensive and hypotensive drugs (Table 3). Both enzymes showed similar, although low affinity for α-methyldopa with K_m of 1.25 mM, but the affinity of PST II for α-methyldopa precursor, normetanephrine was more than 10 times higher. This may suggest, that the brain PST can influence α-methyldopa therapeutic treatment. PST II showed also much higher affinity for metaraminol than for its more therapeutically active isomer phenylephrine (Table 3).

Minoxidil, a pyrimidine *N*-oxide used as a hypotensive drug [10], was also sulfated in monkey brain. Otherwise than with the other compounds studied, sulfation converted mi-

Table 2
PST activity against exogenous substrates

Substrate \ Concentration	PST I		PST II	
	Activity (pmole/mg per min) at substrate concentration of:			
	50 μM	500 μM	50 μM	500 μM
p-Nitrophenol	3.2	9.4	1.5	5.4
Minoxidil	0.077	0.12	0.032	0.41
Phenylephrine	0.047	0.30	0.14	0.49
Octopamine	0	0	0.10	0.36
Methoxamine	0	0	0	0
Metaraminol	0	0	0.29	0.59
Mephentermine	0	0	0	0
Ephedrine	0	0	0	0
Pseudoephedrine	0	0	0	0

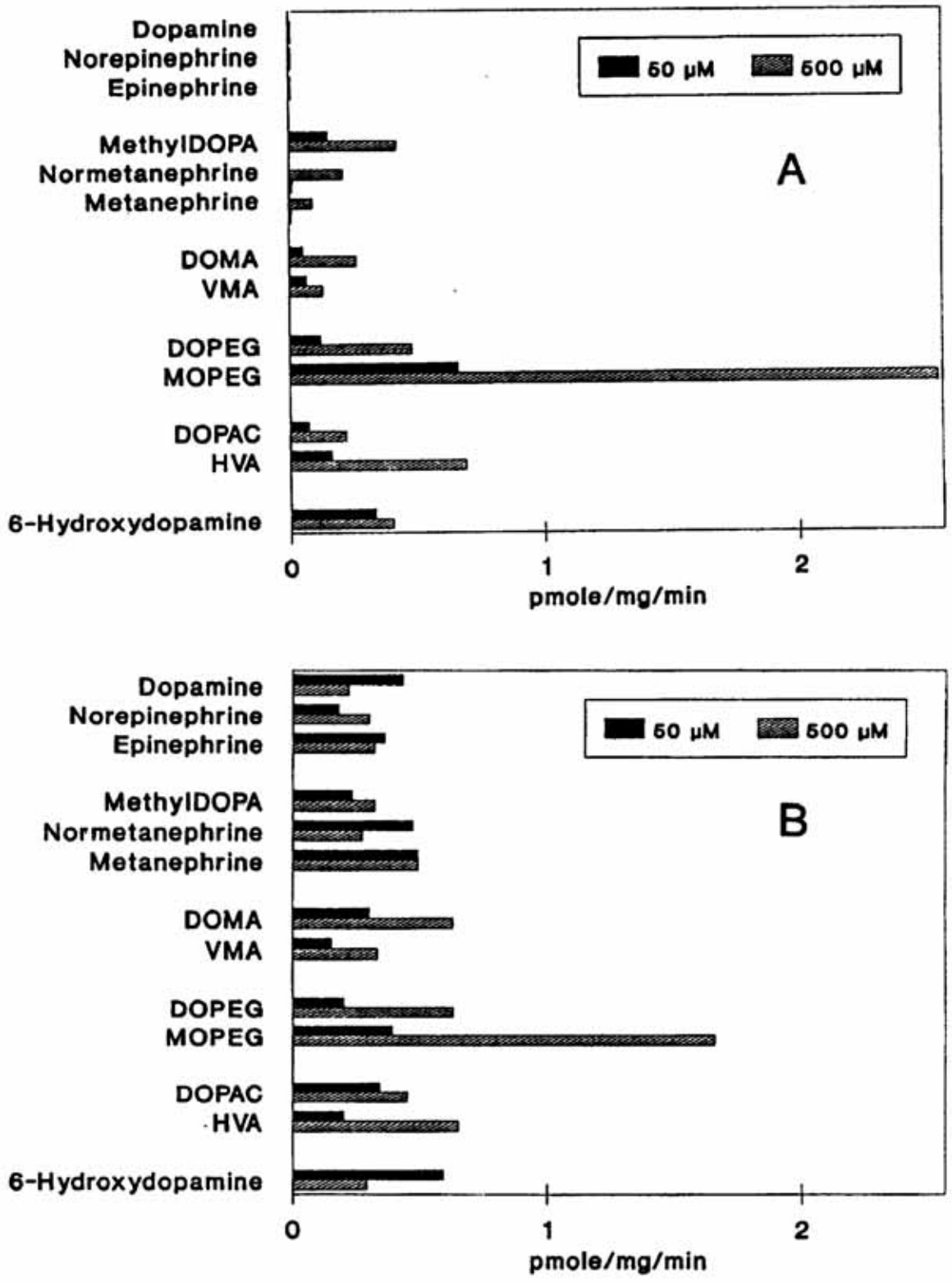

Fig. 1. Substrate specificity of phenol sulfotransferase from monkey brain cortex.
PST I (A) and PST II (B) activity was assayed using either 50 or 500 µM concentrations of the indicated substrates. DOMA, 3,4-dihydroxymandelic acid; VMA, 3-methoxy-4-hydroxymandelic acid; DOPEG, 3,4-dihydroxyphenylethylene glycol; MOPEG, 3-methoxy-4-hydroxyphenylethylene glycol; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid

Table 3
Substrate affinity of monkey brain cortex PST

Substrate	K_m (mM)	
	PST I	PST II
p-Nitrophenol	0.20	0.10
Dopamine	–	2.40×10^{-3}
Norepinephrine	–	0.10
Epinephrine	–	1.43×10^{-2}
Normetanephrine	7.80	1.00×10^{-2}
Metanephrine	13.30	7.14×10^{-3}
MethylDOPA	1.25	1.25
6-Hydroxydopamine	1.67×10^{-2}	1.17×10^{-2}
Minoxidil	3.30	1.04
Phenylephrine	5.00	0.62
Octopamine	–	0.50
Metaraminol	–	4.20×10^{-2}

noxidil into a biologically active form [11]. In monkey brain, both PST forms could use minoxidil as a substrate (Fig. 1B), whereas in human liver there is a special form, Mx-ST responsible for its sulfation [12]. The affinity of monkey brain PSTs towards minoxidil was lower than that of the human liver enzyme. The substrate specificity and sulfate conjugation contributions to monkey brain cortex metabolism of xenobiotics appear similar to those observed with human brain [5, 13] suggesting that this tissue may be useful in studies on the metabolism of hypertensive and hypotensive agents.

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