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SHORT COMMUNICATIONS

Distribution of phenolsulphotransferase and monoamine oxidase in the common marmoset

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Phenolsulphotransferase (PST) catalyses the sulphoconjugation of a wide range of phenols, including the phenolic monoamines, and this process serves both to detoxify them and facilitate their excretion [1]. In man, the enzyme exists in two forms which have been called M and P: PST M acts specifically on monoamines such as tyramine and dopamine, and PST P on other, mainly exogenous phenols, including phenol itself at μM concentrations [2]. The P form is selectively inhibited by 2,6-dichloro-4-nitrophenol (DCNP). The two forms also have different physical properties, the M form being more thermolabile [2, 3]. They also show a somewhat different distribution in human tissues [2], and are under separate control [4] but, in general, activity of both is high in the small intestine, very low in the brain and intermediate in platelets. A recent study, which did not distinguish the two forms, also showed high activity in human liver and intestine, and lower but detectable levels in lungs and kidneys [5].

The nature and distribution of the two forms of the enzyme in certain other species, including the rat, appear to be very different [6, 7, 8] but have not so far, to our knowledge, been investigated in any primate other than man. In this study, we have examined the distribution of PST in the common marmoset, to determine whether this animal is a better model for man than the rat. The distribution of the two forms of monoamine oxidase (MAO), which has been more intensively studied, has been examined in parallel.

Materials and methods

Four common marmosets (330–360 g, 2–3 years old, 2 males, 2 females) were killed by decapitation under deep anaesthesia with sodium pentobarbitone (50 mg/kg body weight). Brain, lung, liver, stomach, small intestine and kidney were excised immediately and stored at −20° for 8 months until assay. Before assay, the intraluminal content of stomach and small intestine were washed out with buffer, and all tissues were homogenized (10% w/v) in 100 mM sodium phosphate buffer, pH 7.4, using an Ultra–Turrax homogenizer. Assays were run on the whole homogenate.

Activity of PST was estimated by a modification [9] of the radioenzymatic method of Foldes and Meek [10] using radioactive [^{35}S]3′-phosphoadenosine-5′-phosphosulphate (PAPS) (New England Nuclear Corp., Boston, MA) as sulphate donor. Tyramine and phenol were employed as sulphate acceptor for PST M and PST P, respectively. The incubation mixture (150 μl) consisted of 10 mM potassium phosphate buffer (pH 7.4) containing 0.66 μM [^{35}S]PAPS and 40 μM tyramine or 10 μM phenol (all final concentrations) and 20 μl tissue homogenate. They were incubated for 100 min at 37°. The reaction was stopped by the addition of barium acetate (200 μl, 0.1 M) and unreacted PAPS was removed by two successive precipitations with barium hydroxide (200 μl, 0.1 M) and zinc sulphate (200 μl 0.1 M). All tissue homogenates were assayed in duplicate using a blank with no acceptor substrate for each sample. For studies using DCNP, the inhibitor was added immediately prior to the assay incubation. Inhibitor blanks were obtained at each DCNP concentration in the absence of phenolic substrate.

MAO activity was assayed by the method of Lewinsohn *et al.* [11]. [^{14}C]5-hydroxytryptamine (5-HT) and [^{14}C]phenylethylamine (PEA) (Amersham International, Amersham, U.K.) were used as substrates for MAO A and MAO B, respectively. The incubation mixture (140 μl) consisted of 0.1 M sodium phosphate buffer (pH 7.4), containing 500 μM 5-HT or 20 μM PEA (final concentrations), and 10 μL of 2% w/v tissue homogenate and they were incubated for 30 min at 37°. The reaction was terminated by adding 100 μl of 2 M citric acid and the product extracted into 3 ml of toluene–ethylacetate (50:50 v/v) for 5-HT or into 3 ml toluene for PEA. All assays were performed in duplicate. Protein was assayed by the method of Lowry *et al.* [12].

Results and discussion

Figure 1 shows the inhibitory effect of DCNP on marmoset liver PST activity, using phenol and tyramine as substrates. DCNP selectively inhibited sulphoconjugation of phenol, with an IC_{50} of 6.3×10^{-5} M, whereas no effect was observed on tyramine sulphoconjugation at 10^{-4} M.

Table 1 shows the specific activity of PST towards tyramine (PST M) and phenol (PST P) in the different tissues studied. With tyramine, the small intestine was most active followed by liver, whilst the brain showed the lowest activity. With phenol, liver was most active followed by lung, and again activity was extremely low in the brain. Specific activity with tyramine was greater than that with phenol in all tissues studied. However, the ratio of activities towards these two substrates differed considerably from tissue to tissue.

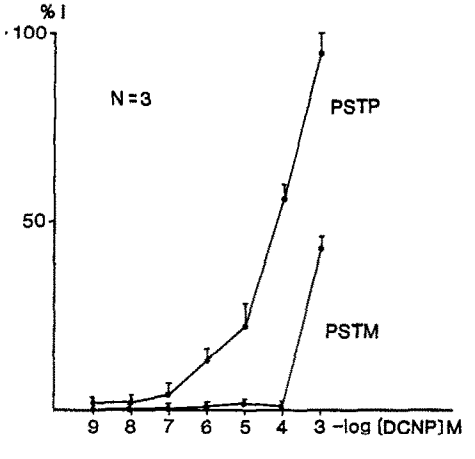

Fig. 1. Inhibition by DCNP of marmoset liver PST activity using either tyramine (PST M) or phenol (PST P) as substrate. Assay conditions were described in Materials and Methods. The results represent mean ± SE using 10% (w/v) liver homogenates from 3 marmosets.

Table 1. Distribution of phenolsulphotransferase (PST) M & P and monoamine oxidase (MAO) A & B activities in different tissues of the marmoset ($n = 4$) (mean ± SE)

	PST M (pmoles/10 min/mg protein)	PST P	PST M/PST P activity ratio
Cerebral cortex	1.0 ± 0.1	0.13 ± 0.02	7.7
Lung	11.5 ± 2.2	3.7 ± 0.2	3.1
Liver	166 ± 11.6	10.7 ± 2.7	15.5
Stomach	4.6 ± 1.0	0.5 ± 0.1	9.2
Small intestine	221 ± 27.5	2.7 ± 0.2	82.1
Kidney	2.2 ± 0.3	1.3 ± 0.3	1.7
	MAO A (nmoles/30 min/ng protein)	MAO B	MAO A/MAO B activity ratio
Cerebral cortex	1.8 ± 0.1	6.0 ± 0.3	0.3
Lung	0.1 ± 0.002	0.02 ± 0.003	5.0
Liver	231 ± 16.7	10.8 ± 0.1	21.5
Stomach	17.2 ± 2.9	1.4 ± 0.1	12.3
Small intestine	32.2 ± 5.6	6.1 ± 1.3	5.3
Kidney	78.1 ± 9.0	9.5 ± 0.6	8.2

All assays were as described in Materials and Methods, and are given as the mean ± SE values from duplicate assays.

Table 1 also shows the specific activity of MAO A and MAO B in different tissues. Here also there was a marked variation of both activities from tissue to tissue. Specific activity of MAO A was highest in the liver followed by kidney, small intestine, stomach, cerebral cortex and lung. Specific activity of MAO B was also highest in the liver followed by kidney, cerebral cortex, small intestine, stomach and lung. MAO A activity was 5.0 to 21.5 times higher than MAO B in all tissues except cerebral cortex in which MAO B activity was 3.3 times higher than MAO A.

To determine the relative importance of PST and MAO in the metabolism of monoamines, the ratio of MAO A/PST M and MAO B/PST M was calculated for different tissues. The former ratio, was highest in the kidney, at 12,000 and lowest in the lung, at 3. The latter was highest in the cerebral cortex with a ratio of about 2,000 and lowest in the lung at 0.7.

The data presented in both Fig. 1 and Table 1 suggest that the marmoset, like man, possesses at least two forms of PST, under separate control in the different tissues, with different substrate specificity and inhibitor sensitivity. Insofar as one form conjugates low concentrations of phenol and is sensitive to inhibition by DCNP and another acts on tyramine and is more resistant to DCNP, they correspond to the human P and M forms [2] so that we shall employ the nomenclature for this species also.

Table 1 shows that the brain had very low levels of both forms of PST. Thus the activity of PST in the marmoset, as in man [13], appears to be predominantly peripheral. In all tissues, the M form predominated, with particularly high activity in small intestine, liver and lung. The stomach had considerably lower levels of both forms than the small intestine; the low activity of the kidney suggests that any sulphoconjugates found in the urine originate from other tissue sites.

Both forms of MAO are also likely to have a role in metabolising exogenous monoamines, as high activity values were observed in liver and intestine. However, there are clear differences from the distribution of PST. The lung had relatively low MAO activity whereas the kidney was relatively rich in both forms. The most important difference, however, was in the brain, where MAO clearly does have a function. As in other primates [14], MAO B predominated, being the only tissue richer in B than A.

In most tissues, MAO had a much higher specific activity than PST, as in man [15], ranges being in nmoles substrate metabolised per mg protein per min for the former and pmoles for the latter.

Thus, in general, this pilot study suggests that the marmoset is a good model for studying human sulphoconjugation. In both species levels are highest in liver and intestine, followed by lung and kidney [5]. As in man, PST exists in two forms, the phenol-conjugating form, PST P, being more sensitive to DCNP. As in man also, both forms of PST are present predominantly outside the brain, and are considerably less active than MAO.

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Effects of diazepam on regional brain homovanillic acid following phencyclidine or Δ^9-tetrahydrocannabinol

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Some drugs which can produce or exacerbate psychotic states in humans preferentially activate dopaminergic neuronal pathways in rat brain. Under certain conditions, phencyclidine (PCP) and Δ^9-tetrahydrocannabinol (THC) increase homovanillic acid (HVA) in limbic and cortical regions at doses that do not affect caudate HVA [1, 2]. Mild footshock stress in rats produces a similar selective activation of dopaminergic pathways, an effect which can be antagonized by diazepam [3–6]. In this report, we describe the effects of diazepam upon the regional increase in HVA produced by PCP and THC.

Materials and methods

Male Sprague–Dawley rats (Charles River, 250 mg) were used. Rats were injected with diazepam (2 or 5 mg/kg intraperitoneally) at 90 min and either PCP (5 mg/kg) or THC (20 mg/kg) at 60 min prior to killing the animals. Control animals received saline or diazepam alone. Brain samples for prefrontal cortex, olfactory tubercle, and caudate were obtained as previously described and frozen at −60° until assayed for HVA by gas chromatography–mass spectrometry using deuterated internal standards [7]. PCP and THC were provided through the courtesy of the Research Technology Branch, Division of Research, NIDA. PCP was dissolved in aequeous solution. THC was prepared for injection using 0.1 ml Tween 80, dried under nitrogen, and resuspended in saline. Injectable diazepam was purchased from commercial sources. The effects of diazepam upon THC- and PCP-induced regional increases in HVA were assessed with a one-way ANOVA and the Bonferonni *post hoc* test [8].

Results and Discussion

PCP produced a significant increase in HVA in olfactory tubercle and prefrontal cortex but not in caudate (Table 1). When animals were pretreated with diazepam, the effect of PCP in olfactory tubercle and prefrontal cortex was partially antagonized. Thus, following PCP, HVA in the olfactory tubercle and prefrontal cortex of diazepam-treated animals was significantly lower than the HVA in these regions from animals who received PCP alone but still significantly higher than in the saline-treated or diazepam-treated animals. Diazepam alone decreased HVA in the

Table 1. Effect of PCP upon regional brain HVA following diazepam pretreatment

	HVA (ng/g)		
	Caudate	Olfactory tubercle	Prefrontal cortex
Saline	982 ± 35	425 ± 29	65 ± 4
Diazepam	820 ± 42*	351 ± 12	56 ± 3
PCP	914 ± 49	643 ± 43†	129 ± 7†
Diazepam + PCP	814 ± 35*	507 ± 34‡	83 ± 6§

Each value is the mean ± SE of seven to eight animals. Diazepam (2 mg/kg, i.p.) was administered at 90 min and PCP (5 mg/kg, i.p.) at 60 min prior to killing the animals.

* $P < 0.01$ compared to saline. Not significantly different from PCP.

† $P < 0.01$ compared to saline or diazepam.

‡ $P < 0.01$ compared to PCP, $P < 0.05$ compared to saline and diazepam.

§ $P < 0.001$ compared to PCP, $P < 0.05$ compared to saline and diazepam.