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INHIBITION AND ACTIVATION OF ENZYMATIC TRANSFER OF THE METHYL GROUP FROM 5-METHYLTETRAHYDROFOLATE TO TRYPTAMINE BY CATECHOLAMINE METABOLITES AND ANALOGUES

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Abstract—The enzymatic transfer of the methyl group from 5-methyltetrahydrofolate to tryptamine was inhibited *in vitro* by the catecholamine metabolites 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylethanol and 3,4-dihydroxyphenylglycol and to a lesser extent, by the serotonin metabolites 5-hydroxyindoleacetic acid and 5-methoxyindoleacetic acid. The tyrosine metabolite 2-hydroxyphenylacetic acid and the noradrenaline metabolite 3,4-dihydroxymandelic acid, however, increased the enzymatic activity up to 3-fold. A regulatory action of monoamine metabolites on methyl group transfer to tryptamine is suggested.

The occurrence of an enzyme transferring the methyl group of 5-methyltetrahydrofolate (5-MTHF) to arylalkylamines in mammalian brain is now well established [1–4]. This enzyme has been reported to lead to *N*-methylated derivatives, epinine being the product when dopamine is the substrate [1, 4], and *N*-methyltryptamine when tryptamine is the substrate [2, 3]. Very recently, the products from this enzymatic reaction have been shown to differ chromatographically from the expected *N*-methylated derivates [5]. Other groups reported the formation of cyclized products, e.g. 2-methyl-1,2,3,4-tetrahydro-β-carboline from *N*-methyltryptamine, with the ^{14}C originating from 5-MTHF probably forming the bridge between the *N* atom and the C-2 of the indole ring [6, 7].

On the other hand, *N*-methyltryptamine and *N,N*-dimethyltryptamine are formed from tryptamine *in vivo* in pargyline-pretreated rats [8, 9]. Also, the methyl group transfer using 5-MTHF occurs at a much higher rate *in vitro* than with S-adenosylmethionine [2, 4]. It is possible therefore that the 5-MTHF-mediated reaction leads to *N*-methylated derivatives *in vivo*, and that the cyclization occurring *in vitro* is due to a subsequent reaction which may not occur *in vivo*.

It has been suggested that enzymatic *N*-methylation might be involved in an indoleamine inactivation process [10] probably analogous to *O*-methylation of catecholamines. On the other hand, *N*-methylated monoamines have been implicated in the etiology of psychotic disorders [1, 11–13], and have also been found in the urine of schizophrenics [14, 15].

Looking for a possible interaction between neuroleptic drugs and this enzyme system, we found recently [16] that 3,4-dihydroxyphenylacetic acid (DOPAC) and to a lesser extent homovanillic acid (HVA), two dopamine metabolites, were able to act as inhibitors of this transferase in conditions that might be fulfilled under neuroleptic treatment.

The purpose of this work is to show the specificity of the inhibitory action of DOPAC and to compare it with the effects of other catecholamine metabolites and related substances.

MATERIALS AND METHODS

Tryptamine hydrochloride was bought from Merck AG, Darmstadt, W. Germany. *N*-Methyltryptamine, 3,4-dihydroxyphenylacetic acid, 2-, 3- and 4-hydroxyphenylacetic acid, mandelic acid, 3- and 4-hydroxymandelic acid, 5-hydroxyindoleacetic acid and 5-methoxyindoleacetic acid were obtained from Fluka AG, Buchs, Switzerland. DL-3,4-Dihydroxymandelic acid was obtained from Calbiochem, and vanilmandelic acid, 3,4-dihydroxyphenylethanol, 3-methoxy-4-hydroxyphenylethanol and DL-3,4-dihydroxyphenylglycol were purchased from Labkemi AB, Göteborg, Sweden.

1,2,3,4-Tetrahydro-β-carboline and 2-methyl-1,2,3,4-tetrahydro-β-carboline were synthesized for us by Dr. A. Storni in our Chemical Department. *N*(5)-Methyl-[^{14}C]tetrahydrofolate (sp. act. 60 mCi/mmole) was bought from the Radiochemical Centre, Amersham, U.K. Instagel® was obtained from Packard Instruments Co. Inc., Warrenville, U.S.A.

The enzyme was prepared according to the procedure outlined by Hsu and Mandell [2] and Laduron [1] with minor modifications [16]. The assay procedure was essentially as published by Hsu and Mandell [2], with two modifications. The reaction mixture contained 10^{-3} M tryptamine, $3{\cdot}16 \times 10^{-5}$ M 5-MTHF[^{14}C] (0·95 μCi/assay), K-phosphate buffer pH 6·5 (phosphate concn 0·15 M), $1{\cdot}25 \times 10^{-5}$ M mercaptoethanol, 5×10^{-4} M EDTA and enzyme preparation containing 0·5 mg protein per assay. The protein concentration was measured according to the

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method of Warburg [17]. The final volume of the incubation mixture was 0·5 ml. In the experiments in which both DOPAC and 3,4-dihydroxymandelic acid (DOMA) were added to the incubation medium (Fig. 8) the final volume was 0·6 ml. Correspondingly, the concentrations of buffer, enzyme and substrate were $\frac{5}{6}$ of those in all the other experiments. The final step in the isolation of the reaction product was changed. Instead of evaporating the toluene–isoamylalcohol extract to dryness and dissolving the residue in scintillation fluid, 5 ml of the 7 ml of the organic phase were back-extracted into 1 ml of 0·2 M HCl; 0·2 ml of this acidic extract were counted with 10 ml of Insta-gel®. Blanks were obtained using boiled enzyme. The products of the reaction with tryptamine and *N*-methyltryptamine as substrates were chromatographed on Silicagel thin-layer plates (Polygram SIL-N-HR, Macherey-Nagel and Co., Düren, W. Germany) using the following solvents: (a) isopropanol–NH_3 25% H_2O (85:5:10); (b) butanol–acetic acid–H_2O (40:10:10) (plates were run twice in these two solvent systems); (c) chloroform–methanol–NH_3 10% (60:35:5). In the recovery experiment, ^{3}H-tryptamine (10^{-3} M, 0·1 μCi) was carried through the extraction procedure. All the solutions used for the assays were adjusted to pH 6·5 before use.

RESULTS

Recovery of the assay procedure. To get an estimate of the recovery of the isolation procedure, [^{3}H]tryptamine was used in the recovery experiment under the assumption that its partition coefficient is comparable to that of the reaction product. Under these conditions, the recovery of the method was 95 ± 2·8% (n = 4).

Identification of the reaction product. All three chromatographic systems used separated *N*-methyltryptamine, 1,2,3,4-tetrahydro-β-carboline, *N,N*-dimethyltryptamine and 2-methyl-1,2,3,4-tetrahydro-β-carboline from each other. The radioactive products of the reactions with tryptamine or *N*-methyltryptamine as substrates ran isographic with 1,2,3,4-tetrahydro-β-carboline and 2-methyl-1,2,3,4-tetrahydro-β-carboline respectively. No radioactivity could be detected in any other region of the chromatogram.

Blanks. Table 1 shows that blanks containing tryptamine and inhibitor or activator obtained with boiled enzyme are identical. Those containing intact enzyme and neither substrate nor inhibitor nor activator are about 30 per cent lower. If intact enzyme is incubated with DOPAC, the resulting "blanks" are 25 per cent lower than without DOPAC, and with DOMA they are 65 per cent higher.

Effect of mercaptoethanol on methyl transfer to tryptamine. The effect of $1{\cdot}25 \times 10^{-5}$ M mercaptoethanol on methyl transfer was investigated. There was no difference between the reaction rate in the samples incubated with (23·6 ± 0·9 pmole/hr/mg protein, n = 6) or without mercaptoethanol (22·8 ± 0·9 pmole/hr/mg protein, n = 6). The antioxidant was therefore used in all the experiments presented below to prevent oxidation of catechols and related substances.

Kinetic pattern of the inhibition of methyl transfer to tryptamine by DOPAC. The inhibition of methyl transfer to tryptamine by DOPAC is competitive with tryptamine, which is obvious from the double reciprocal plot (Fig. 1). The corresponding plot when the concentration of 5-MTHF, the methyl donor, was varied, is no longer linear. It is evident from Fig. 2, however, that the inhibition is not competitive.

Influence of hydroxyphenylacetic acids and mandelic acids. To investigate the specificity of the inhibition of the reaction by DOPAC, structurally related substances were tested. From Fig. 3 it can be seen that 3-hydroxyphenylacetic acid (3-OPAC) and 4-hydroxyphenylacetic acid (4-OPAC) were without influence up to a concentration of 5×10^{-2} M. 2-Hydroxyphenylacetic acid (2-OPAC) activated the reaction in a concentration-dependent manner up to a factor of about 4 at the highest concentration of 5×10^{-2} M. DOPAC, which was used in this experiment for comparison, inhibited methyl transfer in a concentration-dependent manner as previously reported [16]. The radioactive product from the 2-OPAC-stimulated reaction was chromatographed on thin-layer plates. Radioactivity was located exclusively in the spot corresponding to tetrahydrocarboline. Mandelic acid and 4-hydroxymandelic acid (4-OMA) were also without effect (Fig. 4).

3,4-Dihydroxymandelic acid (3,4-DOMA), the noradrenaline metabolite corresponding to DOPAC, activated the enzyme at concentrations above 10^{-3} M, reaching a plateau between 5×10^{-3} M and 2×10^{-2} M and falling to about control level at 5×10^{-2} M. The effect of 3-hydroxymandelic acid (3-OMA) was borderline; it always showed a slight stimulatory effect, though not usually as high as in the experiment shown in Fig. 4.

Effect of other catecholamine metabolites. Vanilmandelic acid (VMA, a noradrenaline or adrenaline metabolite) (Fig. 5) showed no inhibition up to a concentration of 5×10^{-2} M. DOPAC was run for comparison and showed the usual inhibition (see Fig. 3 and Ref. 16).

Table 1. Comparison of different procedures to obtain blank values

	Tryptamine 10^{-3} M		Tryptamine 10^{-3} M + DOMA 5×10^{-3} M	Tryptamine 10^{-3} M + DOPAC	DOMA 5×10^{-3} M	DOPAC 2×10^{-2} M
Intact enzyme (counts/min)	2918	1100	4806	1886	1810	828
Boiled enzyme (counts/min)	1574		1451	1589		

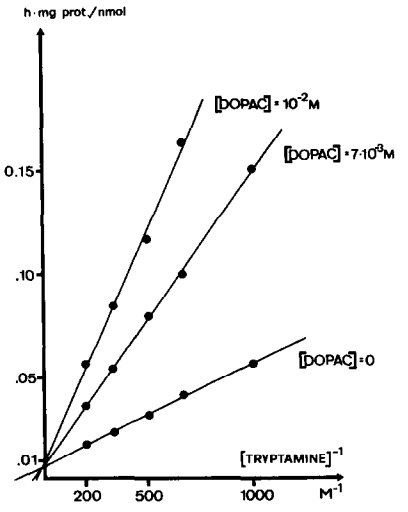

Fig. 1. Double reciprocal plot of reaction velocity vs substrate concentration with different DOPAC concentrations. 5-MTHF, $3{\cdot}16 \times 10^{-5}$ M

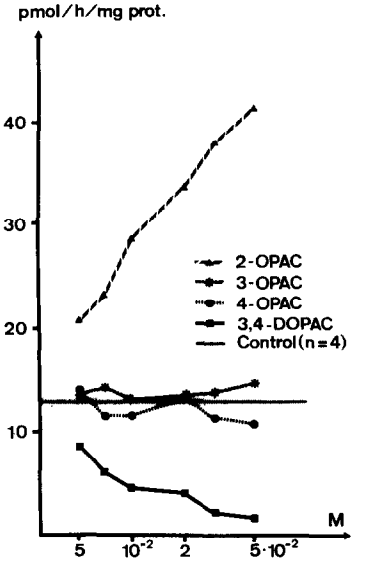

Fig. 3. Influence of hydroxylated phenylacetic acids on methyl transfer. Tryptamine, 10^{-3} M; 5-MTHF, $3{\cdot}16 \times 10^{-5}$ M. Shaded area represents standard error of the mean.

The dopamine metabolite 3,4-dihydroxyphenylethanol (DHPE) and the corresponding noradrenaline deamination product, 3,4-dihydroxyphenylglycol (DHPG) also inhibited the reaction to a somewhat lesser extent than DOPAC (Fig. 6). 3-Methoxy-4-hydroxyphenylethanol (MHPE, dopamine metabolite) showed a borderline activation, which was reproducible in several experiments.

Effect of serotonin metabolites. 5-Hydroxyindoleacetic acid (5-HIAA) and 5-methoxyindoleacetic acid also showed some degree of inhibition (Fig. 7), which, however, was still less than that of DHPG or DHPE.

Antagonistic effects of DOMA and DOPAC on enzymatic activity. The inhibition of the enzyme by a concentration of DOPAC ($4{\cdot}16 \times 10^{-3}$ M) which reduces the reaction rate to about 30 per cent of the control can be gradually antagonized by increasing concentrations of DOMA, as illustrated in the upper part of Fig. 8. Similarly activation by DOMA ($8{\cdot}33 \times 10^{-3}$ M), which increases the enzymatic conversion by a factor of about 2·5 can be counterbalanced by increasing DOPAC concentrations (Fig. 8, lower part).

DISCUSSION

Recently Hsu and Mandell [18] showed a partial inhibition of tryptamine methylation by mercaptoethanol at a concentration of $7{\cdot}5 \times 10^{-6}$ M. In our experiments, mercaptoethanol at a concentration of $1{\cdot}25 \times 10^{-5}$ M gave no enzyme inhibition. This discrepancy may be explained by different experimental conditions.

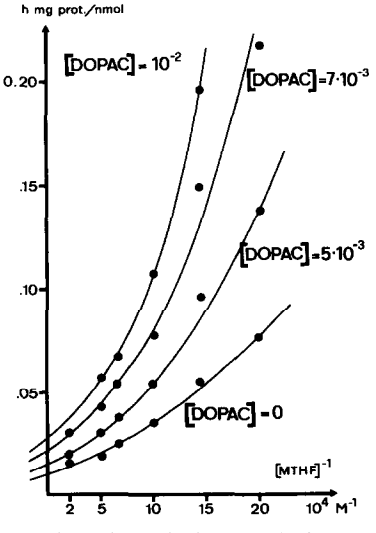

Fig. 2. Double reciprocal plot of velocity vs cosubstrate concentration with different DOPAC concentrations. Tryptamine, 5×10^{-3} M.

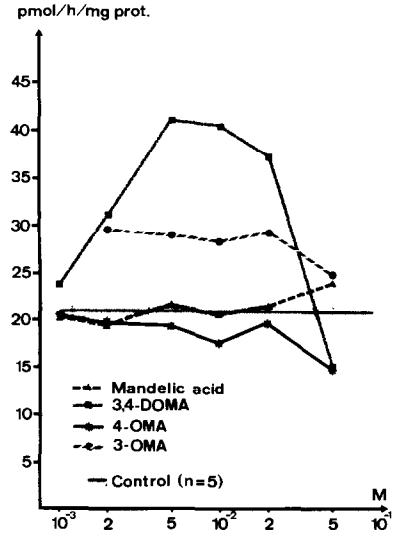

Fig. 4. Influence of mandelic acid and hydroxylated mandelic acids on methyl transfer.

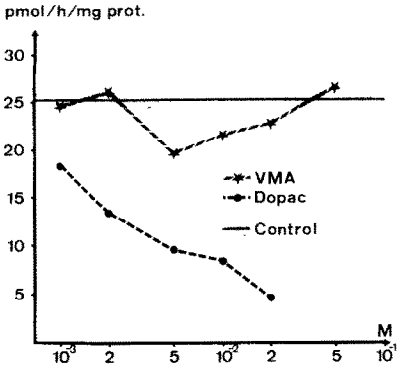

Fig. 5. Influence of vanilmandelic acid on methyl transfer.

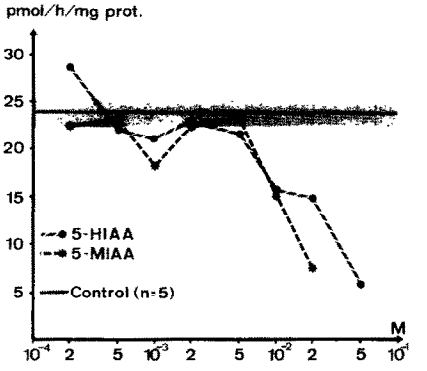

Fig. 7. Effect of indoleacetic acids.

The nature of the recently reported inhibition of tryptamine *N*-methylation by DOPAC was analyzed by investigating the effect of related substances. The fact that neither 3-OPAC nor 4-OPAC nor the corresponding mandelic acids nor mandelic acid itself showed any signs of inhibition of the methyl group transfer reaction suggests that the inhibitory effect of DOPAC is specific. This is supported by the kinetic data, i.e. that the inhibition is competitive with the substrate tryptamine, but not with the methyl donor 5-MTHF. Knowing that catecholamines are good substrates of the enzyme [1, 19], it is possible that the inhibitory action of DOPAC is due to a displacement of substrate at the active site(s). This is supported by the fact that other related substances, such as HVA, DHPG, DHPE, 5-HIAA and 5-MIAA also inhibited the reaction by varying degrees.

A new feature of the enzyme is the possibility of activation by substances formed endogenously in mammals. 2-OPAC, a tyrosine metabolite, is excreted in an amount of about 1 mg daily in healthy humans which is greatly increased in phenylketonuria [20]. The substance has, however, not been detected in brain tissue so far.

DOMA, a deamination product of noradrenaline, is excreted at a rate of 98 μg/24 hr by humans [21]. The occurrence of substantial amounts in normal nervous tissue has not been demonstrated so far [22]. This subject has, however, not been studied extensively. Laduron *et al.* [23] have pointed out that this enzyme probably possesses more than two active sites showing first negative, then positive cooperativity with increasing substrate concentrations. An alternative which cannot be ruled out to date would be the existence of isoenzymes. In either case, the possibility of activation of the enzyme system by relatively simple substances suggests the existence of other sites on the enzyme.

The effect of one of the most prominent noradrenaline metabolites, 3-methoxy-4-hydroxyphenylglycol (MHPG), has not yet been tested because this substance is only available as a piperazine salt, and substances related to piperazines have been claimed to be inhibitors of the tryptamine *N*-methylating enzyme [24]. The preparation of a suitable form of MHPG is under way.

Of course, in view of the opposite effect of the corresponding metabolites of noradrenaline and dopamine, i.e. DOMA and DOPAC, on enzyme activity, it is tempting to speculate on a regulatory mechanism depending on the balance between the activity of the

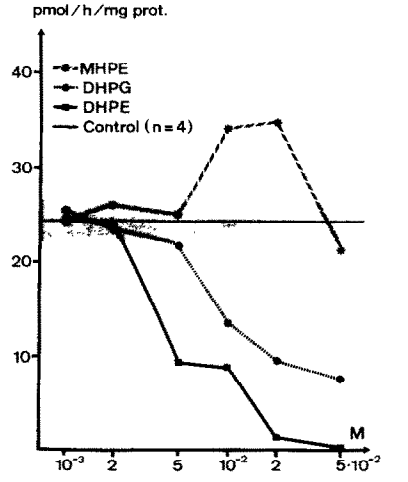

Fig. 6. Effect of alcoholic catecholamine metabolites.

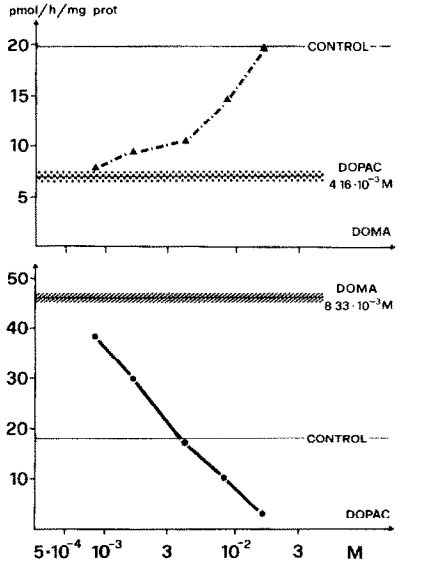

Fig. 8. Upper part: antagonism by DOMA of enzyme inhibition by DOPAC. Lower part: antagonism by DOPAC of enzyme activation by DOMA.

noradrenergic and dopaminergic systems. As is evident from Fig. 8, this regulatory mechanism operates perfectly well *in vitro*. Activation by DOMA can be counterbalanced by DOPAC and inhibition by the latter can be reversed by the former. It is of interest to note in this context that competition is in this case not between inhibitor and cofactor as with feedback inhibition of tyrosine hydroxylase by catecholamines [25] or tryptophan hydroxylase inhibition by dopamine [10], but between inhibitor and substrate.

Another interesting point is revealed in Table 1. DOMA incubated alone with intact enzyme gave somewhat more counts/min than intact enzyme without DOMA. DOPAC in turn slightly diminished the number of cpm, i.e. both compounds act in the same direction as they do in the presence of tryptamine. This cannot be due to any derivation of DOMA or DOPAC, because the extraction procedure is specific for amines and does not extract acids or neutral compounds. One would suspect, therefore, a contamination of some material used in the assay, probably an amine which can be converted by the enzyme. Contamination of the (dialyzed) enzyme preparation is as improbable as that of both DOPAC and DOMA. The most likely explanation is an impurity in the 5-MTHF, which is supplied as a 92 per cent pure material.

Thus, we have seen that the enzyme formerly known as "aromatic amine-*N*-methyltransferase" can be influenced by catecholamine metabolites and related substances. However, it must be kept in mind that the described interactions have been demonstrated in an *in vitro* system only. To validate such a hypothesis, the demonstration of a similar situation *in vivo* is indispensable.

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