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TIME-DEPENDENT INHIBITION OF PLATELET AGGREGATION BY DOPAMINE AND 3-METHOXYTYRAMINE

IMPORTANT ROLES OF THEIR METABOLITES

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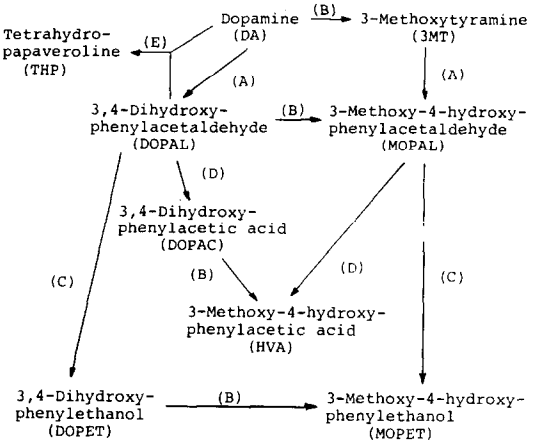
Abstract—The effects of dopamine (DA) and 3-methoxytyramine (3MT) on arachidonic acid (AA)-, collagen-, and adenosine 5'-diphosphate (ADP)-induced rabbit platelet aggregation were studied in relation to their metabolites *in vitro*. DA and 3MT did not inhibit AA- and collagen-induced platelet aggregation immediately after addition of a low concentration, but inhibition did occur, in a time-dependent and concentration-dependent manner, when DA and 3MT were incubated in platelet-rich plasma (PRP). The concentration of DA and 3MT decreased time-dependently, and the main metabolites of DA and 3MT were 3,4-dihydroxyphenylacetaldehyde (DOPAL) and 3-methoxy-4-hydroxyphenylacetaldehyde (MOPAL), respectively, as determined by high performance liquid chromatography. These aldehydes were found to have potent inhibitory activities nearly equal to those of 3,4-dihydroxyphenylethanol (DOPET) and 3-methoxy-4-hydroxyphenylethanol (MOPET) on both AA- and collagen-induced platelet aggregation. DOPET and MOPET were detected only in small concentrations. Taking into account both the anti-platelet potency and the concentrations detected in PRP of DOPAL and MOPAL, it is concluded that the time-dependent inhibition of the platelet aggregation by DA and 3MT may be due to the mediation of their corresponding main metabolites, DOPAL and MOPAL, respectively, while the concentration-dependent inhibition by DA and 3MT seems to be largely due to an innate activity.

The platelet has been used as a model system for the study of dopamine (DA) utilization in the central nervous system [1–4]. Although the effects of DA on human or rabbit platelet function have been reported [5, 6], little is known of the relationship between DA and its metabolites on platelet function.

We examined the effects of DA and 3-methoxytyramine (3MT) on arachidonic acid (AA)-, collagen-, and adenosine 5'-diphosphate (ADP)-induced rabbit platelet aggregation in relation to their metabolites *in vitro*. Among the DA metabolites, 3,4-dihydroxyphenylethanol (DOPET) and 3-methoxy-4-hydroxyphenylethanol (MOPET) reportedly have potent inhibitory activities on AA- and collagen-induced platelet aggregation [7]. Thus, these active metabolites may contribute to the inhibition of aggregation when DA or 3MT is incubated in platelet-rich plasma (PRP) and metabolized. These compounds are minor metabolites in tissues or body fluid [8–12]. DA and 3MT are possibly metabolized in PRP by their corresponding enzyme systems (Fig. 1); however, the metabolism of these compounds and the amount of their metabolites formed in PRP have not been determined.

We designed a method with which the following metabolites of DA [3,4-dihydroxyphenylacetaldehyde (DOPAL), DOPET, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenyl-



(A) Monoamine oxidase (MAO; EC 1.4.3.4)
(B) Catechol-O-methyltransferase (COMT; EC 2.1.1.6)
(C) Aldehyde reductase (EC 1.1.1.2)
Alcohol dehydrogenase (EC 1.1.1.1)
(D) NAD-dependent aldehyde dehydrogenase (EC 1.2.1.3)
(E) Condensation

Fig. 1. Possible metabolic pathways of dopamine.

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acetaldehyde (MOPAL), MOPET and homovanillic acid (HVA)] can be analyzed by high performance liquid chromatography (HPLC). The above presumed phenomena was investigated with regard to inhibition of platelet aggregation by DA or 3MT; the inhibitory effects of these DA metabolites on platelet aggregation were also given attention.

MATERIALS AND METHODS

Chemicals and reagents. All chemicals and reagents used were of analytical grade. AA (Grade I, Sigma Chemical Co., St. Louis, MO) was used as sodium salt. DOPET, MOPET, and *p*-hydroxyphenylethanol (*p*-HPET) were prepared by reduction of their analogous acids with $LiAlH_4$ [13]. DOPAL and MOPAL were synthesized from *l*-epinephrine [14] and DL-methanephrine [15] respectively. Tetrahydropapaveroline (THP) hydrobromide was synthesized from papaverine [16, 17]. Other materials used were: DA hydrochloride (Nakarai Chemicals Ltd., Kyoto); 3MT hydrochloride, DOPAC, indomethacin, and adenosine 5'-diphosphate (ADP) sodium salt type I (Sigma Chemical Co.); aspirin (Sanko Seiyaku Kogyo Co., Tokyo); HVA (Tokyo Kasei Kogyo Co., Tokyo); and collagen (from equine tendon, Hormon Chemie, Munchen).

Preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP). Blood was collected into a plastic tube containing 0.1 vol. of sodium citrate solution (3.8%, w/v), from the carotid artery of rabbits of either sex (2.5 to 3.5 kg). The citrated blood was centrifuged at 150 *g* for 20 min. PRP was carefully separated from the bottom layer, using a plastic pipette, and kept at room temperature in a siliconized glass tube. PPP was prepared by centrifugation of PRP at 1500 *g* for 10 min. The platelet count in the PRP was adjusted to 3.0–3.5 $\times 10^5$ platelets/μl by appropriate dilution with autologous PPP. This platelet count was determined by a Coulter Counter model Z-B (Coulter Electronic Inc. Hialeah, FL). All experiments were performed within 1–3 hr after blood collection.

Platelet aggregation experiments. Aggregation experiments were performed at 37° by a modification of the method of Born [18] using a dual sample aggregation meter (SIENCO, model DP-247E, Colorado). The light transmission was adjusted to 0 and 100% with PRP and PPP respectively. PRP (440 μl) in a siliconized glass cuvette was preincubated with a solution of each test sample or vehicle for 3–30 min, while stirring at 1200 rpm with a siliconized bar. Platelet aggregation was then initiated by the addition of an aggregating agent (40 μl) (100 μM AA, 15 μg/ml collagen, or 10 μM ADP, final concentration each). Comparative experiments were performed on the same batch of platelets, to determine the potency of each test sample in inhibiting aggregation, assessed by using the aggregation response 4 min after the addition of the aggregating agent, relative to the control response with addition of the vehicle (saline or ethanol).

Incubation of DA or 3MT in PRP and preparation of samples. The incubation experiments, done to determine recovery of the amines added and the concentration of DA metabolites after various incubation periods, were carried out in the aggregometer, under the same conditions as platelet aggregation experiments. The PRP samples (440 μl each) incubated with DA or 3MT (200–5000 μM) at 37° for 3–60 min were rapidly cooled to 0–4° in an ice bath and frozen. In the case of determination of DA, 3MT, and THP concentrations, the samples were further lyophilized, successively, and pooled at −80° for subsequent analysis. For the quantification of DA metabolites, the incubated samples were treated as described below, successively and without lyophilization.

Procedure for determinization of DA, 3MT, or THP concentration in PRP. All experiments were carried out in a cold room. The lyophilized samples were dissolved by adding dilute sodium hydroxide (pH 8.7, 960 μl). The dissolved DA and THP samples were applied to aluminium oxide columns (200 mg, active w-200 neutral, Wolem Pharma GmbH & Co., Eschwege). The dissolved 3MT sample was applied to a column (packed volume 1.5 ml) of Amberlite CG-50 (type 2, 200–400 mesh, Rohm & Hass Co., Philadelphia) equilibrated previously with 0.1 M phosphate buffer (pH 6.1). All these columns were washed with cold distilled water (1.44 ml), and DA and THP samples were further washed successively with 480 μl of cold 0.2 N hydrochloric acid (HCl). The DA or THP adsorbed on the aluminum column was eluted with 1.92 ml of cold 0.2 N HCl, and the 3MT on the column of Amberlite CG-50 was eluted with 1.68 ml of cold 0.5 N HCl. Each eluate was lyophilized and the so-obtained sample was dissolved in 1.44 ml water. A 10-μl aliquot of the solution was directly injected into the HPLC system and analyzed. The concentrations in the incubated PRP samples were determined from calibration curves constructed for extracted PRP standards by measuring the peak heights at four to six different concentrations. The recoveries of DA, 3MT, and THP at time 0 (no incubation) were 90.2 ± 2.2, 102 ± 2. and 63.8 ± 4.1% respectively (mean ± S.E.M. of triplicate determinations).

Procedure for determination of the concentrations of DA metabolites in PRP. To the incubated samples were added 6 N HCl (115 μl), water (960 μl), and *p*-HPET (5.305 μg) as an internal standard; the preparations were left for 15 min at 4° and then applied to Amberlite XAD-4 columns (0.45 × 10 cm, Rohm & Hass Co.). The resin was prepared previously by a grinding procedure similar to that described by Takahashi *et al.* [19], and particles of 60–100 mesh were used. The charged columns were successively washed with 20 ml of 0.1 M formic acid, 1 ml of water, and 1.4 ml of 50% (v/v) methanol, and were eluted with 2.6 ml of 100% methanol. The eluates were concentrated to a small volume (175–190 μl) at 20–23° under a stream of oxygen-free nitrogen, and methanol was added up to 240 μl. A 12-μl aliquot of the solution was injected directly into the HPLC system and analyzed. Duplicate samples of PRP "spiked" with appropriate amounts of DOPAL, MOPAL, DOPET, MOPET, DOPAC, HVA, and *p*-HPET were prepared as above, and the recovery was determined. Recovery (mean ± S.E.M.):108 ± 3% for DOPAL, 105 ± 2% for MOPAL, 104 ± 2%

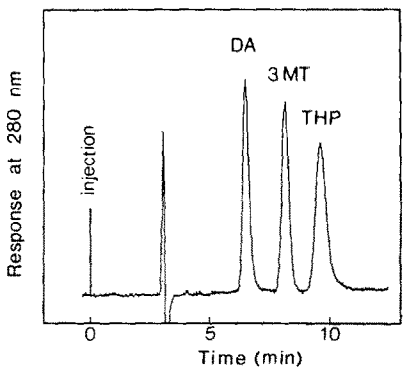

Fig. 2. High performance liquid chromatogram of standard DA, 3MT, and THP. Injected amount: DA (263 ng), 3MT (282 ng), and THP (255 ng). Conditions: see Materials and Methods.

for DOPET, 102 ± 2% for MOPET, 106 ± 3% for DOPAC, 104 ± 3% for HVA, and 100 ± 1% for *p*-HPET. Sensitivity: 2–4 ng for all metabolites. Calibration curves were constructed by adding known amounts of DA metabolites and an internal standard to control PRP, and then analysis was carried out. The peak height ratio of DA metabolites to the internal standard was plotted against the amount of DA metabolites added, and used to calculate the amount of DA metabolites in the incubated PRP samples.

HPLC system. HPLC was performed on an ALC/GPC 204 (Waters Associates, Inc., Milford, MA) machine equipped with an ultraviolet detector at 280 nm. A μBondapak C_{18} column (4 mm i.d. × 30 cm, Waters Associates, Inc.) was used for all analyses. An isocratic mode was employed, and the mobile phase for analysis of DA, 3MT, or THP was a mixture of methanol–water (30:70, v/v) containing 0.005 M heptane sulfonic acid (pH 3.3, PIC B7, Waters Associates, Inc.) (Fig. 2); for DA metabolites, a mixture of methanol–water (5:95, v/v) containing the same reagent as above was used (Fig. 3). These solvent systems were eluted at the flow rate of 1 ml/min at 24°.

RESULTS

Inhibitory effects of various concentrations of DA and 3MT on platelet aggregation. Figure 4 compares the effects of various concentrations of DA and 3MT and illustrates the differing inhibitory potencies on AA- and collagen-induced platelet aggregation. These amines inhibited both aggregations, in a concentration-dependent manner, though a high concentration was required to exhibit inhibitory effects in the case of a 3-min preincubation. 3MT was more effective than DA on both AA- and collagen-induced aggregation.

Time-dependent inhibition by DA and 3MT. When the incubation time of PRP with each amine (200 μM final concentration) was increased up to 30 min before the addition of the aggregating agents, AA- and collagen-induced platelet aggregations were time-dependently inhibited (Fig. 5). DA and 3MT inhibited 50% of AA-induced aggregation with incubation for 6–9 min, respectively, while DA and 3MT inhibited 50% of the collagen-induced aggregation with incubation for 20–30 min, respectively. With a short preincubation, DA and 3MT inhibited more effectively the AA-induced than the collagen-induced aggregation. 3MT exerted its inhibitory effect earlier than DA in the case of both aggregations.

Potentiating action of DA on ADP-induced platelet aggregation. It has been reported [5, 6] that DA accelerates rabbit and human platelet aggregation induced by ADP. In the present experiments, the potentiating action of DA on 10 μM ADP-induced rabbit platelet aggregation was also observed, but

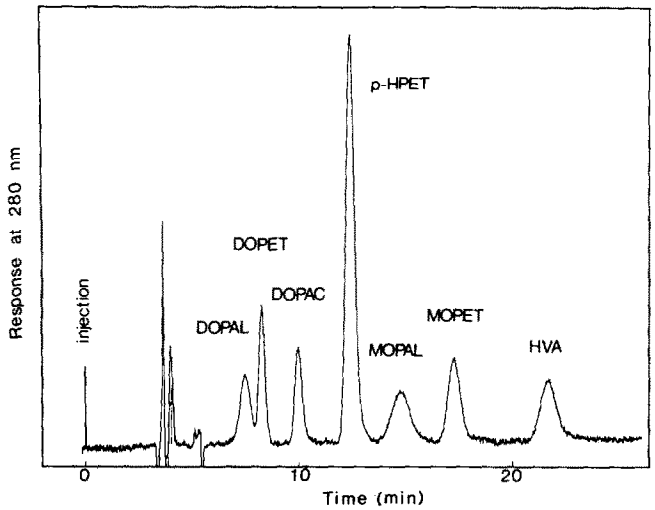

Fig. 3. High performance liquid chromatogram of standard DA metabolites and an internal standard (*p*-HPET). Injected amount: DOPAL (36.5 ng), DOPET (37.0 ng), DOPAC (40.3 ng), MOPAL (39.8 ng), MOPET (40.3 ng), HVA (43.7 ng), and *p*-HPET (265 ng). Conditions: see Materials and Methods.

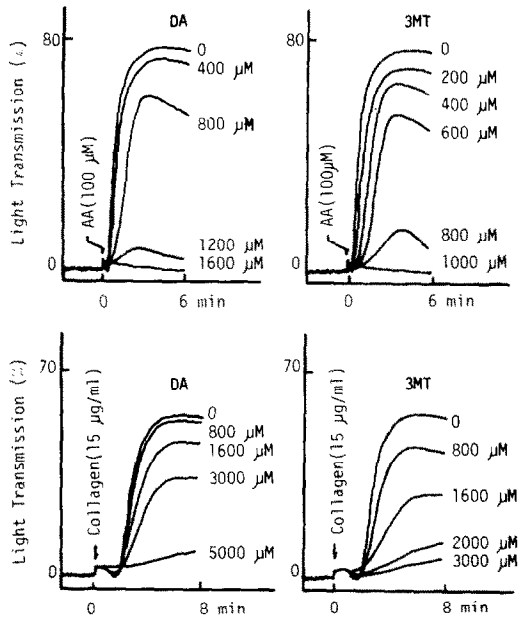

Fig. 4. Inhibitory effects of various concentrations of DA and 3MT compared to control responses on platelet aggregation. Each amine was added to PRP 3 min before induction of aggregation with arachidonic acid (AA) or collagen (at the arrows). The respective number in each panel represents a final concentration of the amine added. Superimposed tracings of platelet aggregation are representative of three to four observations.

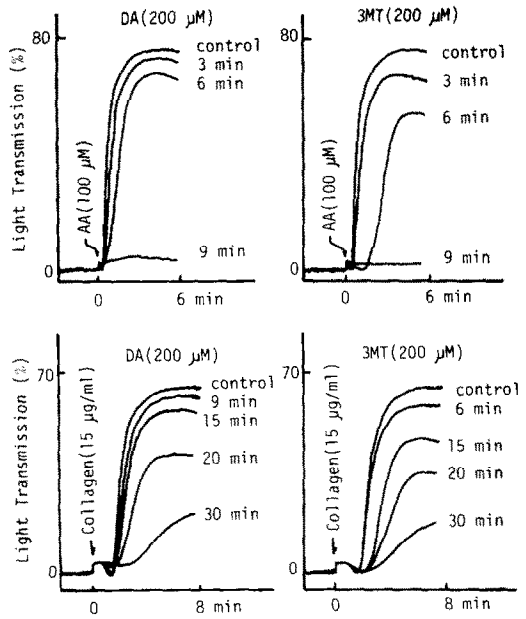

Fig. 5. Time-dependent inhibition of arachidonic acid (AA)- and collagen-induced platelet aggregation by DA and 3MT. The amount of each amine added was fixed at a final concentration of 200 μM. The respective number in each panel indicates the incubation time period before the addition of the aggregating agents (at the arrows). Control responses (saline only) were similar at each incubation time; a typical curve is shown. Superimposed tracings of platelet aggregation are representative of three to four observations.

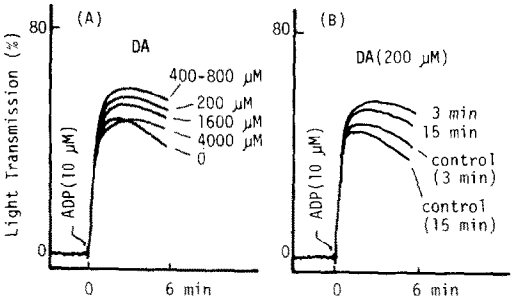

Fig. 6. Enhancement of ADP-induced platelet aggregation by DA. (A) After a 3-min incubation of PRP and various concentrations of DA (μM, shown in the panel). ADP (10 μM) was added to the mixture (at the arrow). (B) After a 3- or 15-min incubation of PRP and DA (200 μM), ADP (10 μM) was added to the mixture (at the arrow). Superimposed tracings of platelet aggregation are representative of three observations.

3MT was found to be less effective. DA began to potentiate the aggregation at 4 μM; this potentiating action reached a maximum at 400–800 μM and did not disappear at the concentration used (4.0 mM), though there was a decrease with increasing concentrations (Fig. 6A). This potentiating action of DA (200 μM) was not affected by prolonging the preincubation time up to 15 min (Fig. 6B).

Time-course of the decrease of DA and 3MT in PRP. The time-course of the decrease of DA and 3MT in PRP during the incubation period up to 60 min was investigated by HPLC (Fig. 2, see Materials and Methods). As shown in Fig. 7, the concentrations of DA and 3MT (200 μM each) in PRP after incubation decreased time-dependently. The incubation time of each amine that inhibited 50% of AA-induced aggregation was 6–9 min (Fig. 5), and the decrease in concentration of each amine at the time was 20–30 μM. In the case of collagen-induced aggregation, each amine inhibited 50% of the control response by preincubation for 20–30 min (Fig. 5), and the extent of decrease of each amine was 50–70 μM. With incubation for 60 min, 60–70% of each added amine had been lost.

Inhibitory effects of DA metabolites on platelet aggregation. We reported that DOPET and

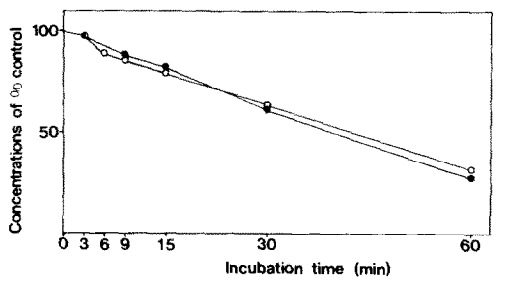

Fig. 7. Time-course of the decrease of DA and 3MT added to PRP. PRP (400 μl) was incubated with 200 μM DA or 3MT in an aggregometer at 37° for the time indicated, and the recovery of each amine was determined as described in Materials and Methods. Their recoveries at time 0 (no incubation) were corrected to 100%. Each point is the mean of the results from four experiments. Key: (—○—) DA, and (—●—) 3MT.

Table 1. Comparative potency of dopamine metabolites in blocking rabbit platelet aggregation*

Metabolites	IC_{50} (μM) of metabolite		
	Arachidonic acid† (100 μM)‡	Collagen (15 μg/ml)‡	ADP (10 μM)‡
DOPAL	12.5 (10.5–14.8)§	30.0 (21.4–42.2)	NE‖
DOPET¶	19.7 (13.6–28.6)	35.4 (23.9–52.6)	NE
DOPAC	1000	1600	NE
THP	800	153	700
MOPAL	16.7 (13.3–20.8)	34.0 (25.5–45.4)	NE
MOPET¶	13.9 (10.9–17.7)	30.5 (23.0–40.6)	NE
HVA	800	1200	NE
Aspirin¶	32.3 (24.8–42.0)	20.0 (14.5–27.6)	NE
Indomethacin¶	1.01 (0.80–1.26)	0.91 (0.77–1.09)	NE

* For each assay, rabbit PRP (440 μl) was preincubated with an inhibitor (2 μl in ethanol or dimethyl sulfoxide) for 3 min at 37° before the addition of the aggregating agents (40 μl). Figures represent IC_{50} values (concentration that inhibited 50% of the agonists' effects) evaluated from two to three different concentrations of the inhibitors on a log probit paper.

† Sodium arachidonate.

‡ Final concentration.

§ Mean (and its 95% confidence limits) for individual determinations in five to eight different platelet preparations.

‖ No effect.

¶ Data taken from our previous paper [7]. Aspirin and indomethacin are positive controls.

MOPET, DA metabolites, possess potent inhibitory effects on platelet aggregation, as induced by AA or collagen [7]. In the present work, other DA metabolites (not DOPET and MOPET) were investigated with regard to effects on platelet aggregation.

Table 1 shows the results obtained. The metabolic aldehydes (DOPAL and MOPAL) were found to have potent inhibitory activities nearly equal to those of DOPET and MOPET on AA- and collagen-induced platelet aggregation and were about twice as effective as aspirin in AA-induced aggregation, in the case of preincubation for 3 min before the addition of the aggregating agent. The carboxylic metabolites (DOPAC and HVA) were less effective, compared to other DA metabolites. THP proved to be a weak inhibitor on AA- and collagen-induced platelet aggregation, but did inhibit ADP-induced aggregation, at higher concentrations. Other DA metabolites had no effect on ADP.

Formation of DA and 3MT metabolites in PRP. Tables 2 and 3 show the results obtained by HPLC analysis of concentrations of DA and 3MT metabolites in PRP after various incubation periods (Fig. 3, see Materials and Methods). DA and 3MT decreased time-dependently during the incubation in PRP (Fig. 7). On the other hand, DA and 3MT metabolites did form and the concentrations increased time-dependently and in parallel to decreases in the concentrations of the amines. Main metabolites at each incubation time were found to be the corresponding aldehydes, DOPAL and MOPAL. Both aldehydes possessed anti-platelet-aggregating activities; the IC_{50} values for AA-induced aggregation were DOPAL, 12.5 μM and MOPAL,

Table 2. Concentrations of DA metabolites in PRP with various incubation periods*

Amine added	Incubation time (min)	Concentration (μM)						
		DOPAL	DOPET	DOPAC	MOPAL	MOPET	HVA	THP
DA (200 μM)	3	5.9 ± 0.8						
	6	12.5 ± 1.7		ND				
	9	18.6 ± 2.9		0.8 ± 0.3				
	12	22.0 ± 2.4		1.0 ± 0.1				
	15	28.0 ± 2.8		1.8 ± 0.5				
	20	35.5 ± 5.1		2.1 ± 0.5	ND			
	30	50.4 ± 7.3	ND†	4.0 ± 0.7	2.5 ± 0.8			
	45	72.5 ± 8.6	1.6 ± 0.3	6.4 ± 0.9	9.2 ± 1.9			
	60	88.9 ± 7.2	2.0 ± 0.5	8.0 ± 1.8	11.5 ± 2.1	ND	ND	ND

* PRP (440 μl) was incubated with 200 μM DA for the time indicated at 37°, in an aggregometer, and each concentration of its metabolites was determined as described in Materials and Methods. Each value is the mean ± S.E.M. of three experiments.

† Not detected.

Table 3. Concentrations of 3MT metabolites in PRP with various incubation periods*

Amine added	Incubation time (min)	Concentration (μM)		
		MOPAL	MOPET	HVA
3MT (200 μM)	3	6.5 ± 1.9		0.5 ± 0.3
	6	13.5 ± 1.6	ND†	1.6 ± 0.5
	9	19.6 ± 3.2	0.7 ± 0.2	2.4 ± 0.4
	12	23.0 ± 2.1	1.2 ± 0.3	2.8 ± 0.4
	15	29.7 ± 3.7	1.3 ± 0.4	4.3 ± 1.1
	20	39.6 ± 9.8	1.6 ± 0.3	6.0 ± 1.2
	30	53.2 ± 11.4	3.2 ± 1.8	9.5 ± 1.8
	60	95.5 ± 15.6	5.0 ± 1.0	18.6 ± 3.0

* PRP (440 μl) was incubated with 200 μ 3MT for the time indicated at 37°, in an aggregometer, and each concentration of its metabolites was determined as described in Materials and Methods. Each value is the mean ± S.E.M. of three experiments.
† Not detected.

16.7 μM and the IC_{50} values for collagen-induced aggregation were DOPAL, 30.0 μM and MOPAL, 34.0 μM. Preincubation of DA or 3MT in PRP for 6–9 min caused 50% inhibition of AA-induced aggregation (Fig. 5). The concentrations of aldehydes formed in PRP at the incubation time were: DOPAL, 12.5 to 18.6 μM and MOPAL, 13.5 to 19.6 μM. On the other hand, with preincubation for 20–30 min, DA and 3MT exhibited 50% inhibition of collagen-induced aggregation (Fig. 5). The concentrations of the aldehydes at this time were: DOPAL, 35.5 to 50.4 μM and MOPAL, 39.6 to 53.2 μM.

DA and 3MT inhibited AA- and collagen-induced platelet aggregation, respectively, in a concentration-dependent manner when preincubation was for 3 min (Fig. 4). Therefore, we determined the concentrations of DOPAL and MOPAL formed with increasing concentrations of DA and 3MT during the 3-min preincubation in PRP. As shown in Table 4, the formation of each aldehyde was much the same, that is, an increase to 1.2 mM or higher of DA or 3MT produced no further increases in the corresponding aldehydes. DA (0.8 to 1.2 mM) and 3MT (0.4 to 0.8 mM) inhibited 50% of AA-induced platelet aggregation. At these amine concentrations, the concentrations of the aldehydes formed were: DOPAL, 5.5 to 7.0 μM; and MOPAL, 3.5 to 4.5 μM.

Table 4. Concentrations of DOPAL and MOPAL in PRP after a 3-min incubation*

	DA	3MT
Amine added concentration (μM)	DOPAL concentration (μM)	MOPAL concentration (μM)
400	4.5	3.5
800	5.5	4.5
1200	7.0	9.0
1600	9.0	8.5
2000	11.0	10.0
3000	11.2	10.1
5000	10.2	9.0

* PRP (440 μl) was incubated with various concentrations of DA or 3MT for 3 min at 37°, in an aggregometer, and each concentration of DOPAL and MOPAL was determined as described in Materials and Methods. Each value is the mean of duplicate determinations.

DISCUSSION

On the basis of anti-platelet-aggregating activities of DOPAL and MOPAL and their concentrations detected in PRP, DOPAL and MOPAL would seem to contribute significantly to the time-dependent inhibition by DA and 3MT of AA- and collagen-induced platelet aggregation, respectively. DOPET and MOPET did not appear to contribute to the inhibition, as determined on the basis of their concentrations in PRP. As the concentration-dependent inhibition of DA and 3MT of both AA- and collagen-induced aggregation could not be explained by the amounts of their metabolites, DOPAL and MOPAL, respectively, the effects of these two compounds were probably due to an innate activity.

DA potentiated ADP-induced platelet aggregation, regardless of a preincubation period of up to 15 min before addition of the aggregating agent (Fig. 6). This may relate to the finding that DA accelerated ADP-induced aggregation at low concentrations and that there were no metabolites except THP [not detected in PRP with 60 min of incubation (Table 2)] to inhibit ADP-induced aggregation (Table 1). The potentiating action of DA on ADP-induced aggregation is not affected by pretreatment with aspirin [6], indicating that the action is not mediated by prostaglandins [20]. However, this action disappeared with addition of an alpha-adrenergic antagonist (phentolamine), thereby suggesting that DA may exert its effect via platelet alpha-adrenoceptors [6]. 3MT, an O-methylated metabolite of DA, was less effective than DA with regard to the potentiating action of ADP-induced platelet aggregation. In general, the essential physiological action of catecholamine disappears with O-methylation [8]. The potentiating action of DA on ADP-induced aggregation showed a similar tendency. However, the inhibitory effects of 3MT on AA- and collagen-induced platelet aggregation were enhanced to a greater extent than those of DA, both time- and concentration-depen-

dently (Figs. 4 and 5). DA and its metabolites, not including the amines, had different effects on various inducers, respectively, in that DA accelerated platelet aggregation induced by ADP, while, when DA was metabolized, the metabolites exhibited the inhibitory effects on AA- and collagen-induced platelet aggregation, at low concentrations (Table 1). Thus, a series of DA metabolites exhibited different effects on various inducers and these effects were accompanied by structural changes. These metabolites are the compounds actually formed *in vivo* during DA metabolism.

Dewhirst [21] examined the ability of sixty-three phenolic compounds to inhibit sheep vesicular gland prostaglandin cyclooxygenase and showed that a compound with a more potent cyclooxygenase inhibition also exhibits a potent inhibitory effect on AA-induced human platelet aggregation. For example, eugenol, a widely used anti-inflammatory and analgesic agent in dental practice, inhibits both cyclooxygenase and AA-induced platelet aggregation, at very low concentrations. DOPAL, MOPAL, DOPET, and MOPET, which are potent inhibitors of AA-induced platelet aggregation, are structurally analogous to eugenol; therefore, the possibility that these metabolites have an inhibitory activity on cyclooxygenase has to be considered.

When DA or 3MT was incubated in PRP, metabolites formed and increased, time-dependently (Tables 2 and 3). There was no difference in the amounts of DOPAL and MOPAL formed even when DA or 3MT was incubated in PRP (data not shown). This indicates that DOPAL and MOPAL in the former case were formed by the action of both platelet monoamine oxidase (MAO) [22, 23] and plasma MAO [23, 24], and in the latter case by the action of plasma MAO. In our experiment, the incubation was performed in a cuvette, while stirring with a bar and without addition of ascorbic acid or EDTA, compounds which reduce non-enzymic oxidation of catecholamines [25]. Non-enzymic oxidation, therefore, may also contribute to formation of these aldehydes. It is certain that DA and 3MT were deaminated to their corresponding aldehydes, in both PRP and PPP. MOPAL was detected in PRP (Table 2) but not in PPP with incubation for 60 min (our observation), indicating that DA or its aldehyde, DOPAL, underwent the action of catechol-*O*-methyltransferase (COMT) [8, 26] in platelets. There may be no or only weak COMT activity in PPP.

Whether DOPAL and MOPAL also occur in the free state or are continuously metabolized to alcholic or carboxylic metabolites, as end-products *in vivo*, remains to be determined. However, THP which is formed by a non-enzymic reaction [27] between DA and DOPAL has been detected in rat brain [28] and the urine of patients with Parkinson's disease [29] after the oral administration of L-DOPA, thereby suggesting the existence of these compounds, in the free state.

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