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SHORT COMMUNICATIONS

The stereoselective metabolism of dimethylpropion and monomethylpropion

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As early as 1926, it was shown that the dextro- and levo-rotatory isomers of atropine, morphine and adrenaline differed strikingly in biological potency [1]. Because the two members of such pairs have otherwise identical chemical and physical properties and differ only in that their molecules are mirror images of one another, it became evident that the shape of a drug molecule can be crucial for its action and that a part of the molecule was obliged to fit a complementary three-dimensional structure.

Today it is widely accepted that the biological action of a drug is dependent upon three of its properties: lipophilicity, electron distribution (as demonstrated by ionisation and chelation) and a steric nature complementary to the receptor [2].

The stereoselective metabolic reduction of the ketonic group in the anorectic drug dimethylpropion (DMP I) and its metabolite monomethylpropion (DMP II), following their oral administration in man, is now reported and the probable conformation of dimethylpropion at the reductive active site considered in relation to the stereochemistry of the carbonyl reduction of another anorectic drug, diethylpropion [3].

A dose of 50 mg DMP I hydrochloride in aqueous solution was administered orally to three human volunteers. DMP II hydrochloride, in a dose of 30 mg in aqueous solution, was administered orally to a single volunteer. Acidic urine conditions were maintained, by means of the administration of ammonium chloride sustained release pellets, in order to minimize tubular reabsorption of the bases [4].

A Perkin–Elmer FII gas chromatograph with a flame ionisation detector was used for all GLC analyses. Samples to be analysed for DMP I and DMP II were extracted into chloroform (one-step extraction), acetylated *in situ*, using acetic anhydride, and then run on Column A, a 1 m, 4 mm i.d. glass column packed with Carbowax 20 M 2%, Apiezon L 10% on Chromosorb G (AW DMCS treated, 100–120 mesh); internal marker, ethylephedrine hydrochloride; N_2 flow 25 cm^3/sec, H_2 and air pressure 135 kPa; oven temperature 195°, injection port temperature 250°. Samples to be analysed for the diastereoisomers of methylephedrine (DMP IV) were run on Column A under the same conditions, but no *in situ* acetylation was carried out and pseudoephedrine hydrochloride was used as internal marker. The GLC column, Column B, and method of extraction of Wilkinson [5] was used for the quantitative analysis of ephedrine (DMP V) and norephedrine (DMP VI); nikethamide was used as internal marker. For the separation of the diastereoisomers of DMP V on Column B, the method of Testa and Beckett [6], based upon the differences in the rates of oxazolidine formation of the two isomers, was used. The method of Testa and Beckett [6] used for the quantitative determination of the enantiomers of the amino-alcohol metabolites of diethylpropion, was adapted for the determination of the enantiomers of methylpseudoephedrine (the threo diastereoisomer of DMP IV), i.e. the TLC solvent system used contained ethylacetate : methanol : concentrated ammonia (78:20:2) and optical rotation measurements were made at 257 nm.

The main routes of metabolism of DMP I and DMP II, in man, are shown in Fig. 1 [7]. An average of 91% of a dose of DMP I, and 89% of a dose of DMP II could be accounted for in the urine as compounds DMP I, DMP II, DMP IV, DMP V and DMP VI (see Table 1). The per-

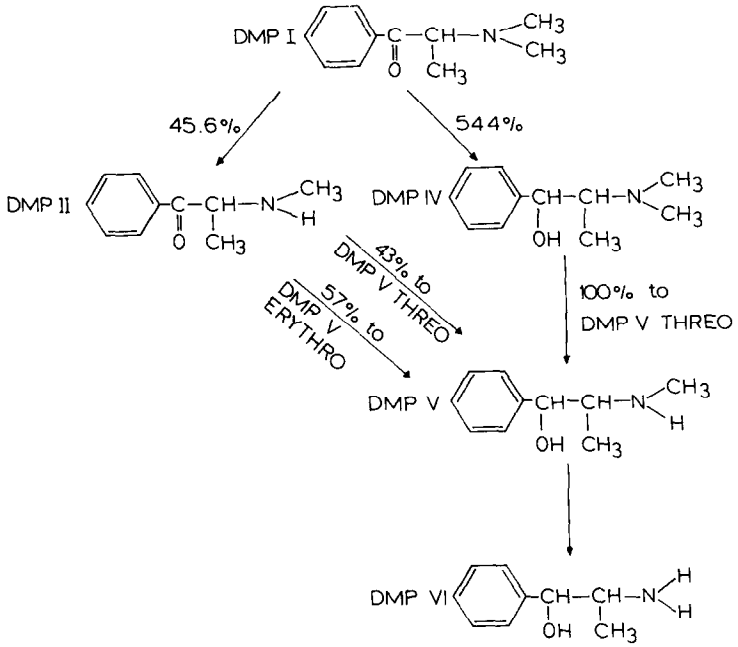

Fig. 1. Pathways for the production of the known metabolites of dimethylpropion (DMP I) and monomethylpropion (DMP II). The percentages shown are the percentage contribution of each route of metabolism calculated from the percentage recoveries of the diastereoisomers shown in Table 1.

centage stereoisomeric/enantiomeric composition of the amino alcohols DMP IV and DMP V recovered in the urine following the oral administration of racemic DMP I or DMP II hydrochlorides (in solution) are shown in Table 1. The amount of the amino alcohol DMP VI recovered in the urine was too small (a) to consider it an important metabolic product and (b) to allow the estimation of the percentage of each diastereoisomer [i.e. (±)-erythro and (±)-threo-norephedrine (DMP VI)] present.

The diastereoisomeric composition of the DMP IV formed by reduction of DMP I changed with time. Bulked urine samples analysed at certain intervals up to 36 hr after administration of the DMP I showed an increase with time in the percentage of (+)-DMP IV formed, i.e. 50 to 59%, 53 to 55% and 45 to 57% in the three subjects AHB, RP and SM, respectively. The percentages of (−)-DMP IV formed decreased accordingly.

The amino ketone DMP II from metabolic *N*-demethylation of DMP I was considered to be a racemic mixture because *N*-dealkylation of ephedrines and amphetamines is not a significantly stereoselective process [8] and because its enantiomorphs racemise rapidly since its asymmetric centre possessing one H atom is attached directly to a conjugated carbonyl group.

Following oral administration of DMP I, only (±)-threo DMP IV in approx. equal proportions of (+)- and (−)-isomers (i.e. 1S;2S and 1R;2R isomers) was formed by keto reduction of DMP I; (±)-erythro DMP IV was not recovered in the urine. However, both (±)-erythro and (±)-threo DMP V were found in the urine (see Table 1).

Following the oral administration of DMP II hydrochloride (in solution) to subject SM, (±)-erythro as well as (±)-threo DMP V were recovered in the urine (see Table 1).

With dimethylpropion [7], as with diethylpropion [3], carbonyl reduction plays the major role in the observed diastereoisomeric and enantiomeric proportions of the amino alcohols DMP IV and DMP V.

The carbonyl and phenyl groups of DMP I will be coplanar because of conjugation. The protonated molecule results in the coplanar portion binding to a flat portion of the active site of the reductase enzyme and this binding is reinforced by the ionic H-bond interaction from the ionized basic group to an anionic group in the reductive active site, so that directional influences are important. It is postulated that for the substrate to fit onto the active site of the enzyme, the phenyl and *C*-methyl groups have to be on the same side of the molecule with the *N*-methyl groups on the opposite side (see Figs. 2 and 3). Also the *C*-methyl, and especially the *N*-methyl, groups have to be orientated above the plane to allow a good fit between substrate and reductive active site. H transfer from the active site to the carbonyl group of DMP I occurs from the side of the enzyme (i.e. below the plane of the molecule in Figs. 2 and 3) and on the opposite side of the phenyl group (i.e. from the left- or right-hand side as shown in Figs. 2 and 3).

From the 2S and 2R configurations of DMP I only the 1S;2S and 1R;2R methylephedrine isomers can be formed respectively with the C_1—OH group on the side of the enzyme (i.e. below the plane of the molecule as shown in Fig. 2). The aforementioned change in the diastereoisomeric composition of DMP IV with time shows that the 2S configuration of DMP I is reduced at a slightly faster rate than the 2R configuration, suggesting that the 2S configuration fits slightly better on the active site of the reductase enzyme.

The erythro isomers of DMP IV, i.e. the 1S;2R and 1R;2S isomers cannot be formed by keto reduction of DMP I because the required orientation of the substrate (see Fig. 3) is prevented from fitting onto the reductive active site by the downward projecting *C*-methyl and *N*-methyl groups.

Of the *C*-methyl and *N*-methyl groups, the latter seems to play the major role in preventing the substrate from

Table 1. Percentage recovery of dimethylpropion (DMP I), monomethylpropion (DMP II) and their metabolites methylephedrine (DMP IV), ephedrine (DMP V) and norephedrine (DMP VI) in urine, under conditions of acidic urinary pH, after oral administration of 50 mg DMP I or 30 mg DMP II hydrochlorides in aqueous solution to various human subjects

Drug administered	Subject	% of dose recovered in urine as						% Stereoisomeric/enantiomeric composition of			
		DMP I	DMP II	DMP IV	DMP V	DMP VI	Overall	DMP IV (+)-threo	DMP IV (−)-threo	DMP V (±)-erythro	DMP V (±)-threo
DMP I	AHB	15.7	27.6	46.0	7.4	2.0	98.7	54.0	46.0	26.0	74.0
	RP	18.9	24.0	32.9	10.0	3.0	88.8	52.0	48.0	30.0	70.0
	SM	25.4	5.7	47.2	6.7	0.0	85.1	55.0	45.0	22.0	78.0
	Mean	20.0	19.1	42.0	8.0	1.7	90.8	53.7	46.3	26.0	74.0
DMP II	SM										
	Trial 1	—	35.0	—	48.0	4.0	87.0	—	—	59.0	41.0
	SM										
	Trial 2	—	37.0	—	48.0	5.0	90.0	—	—	55.0	45.0
	Mean	—	36.0	—	48.0	4.5	88.5	—	—	57.0	43.0

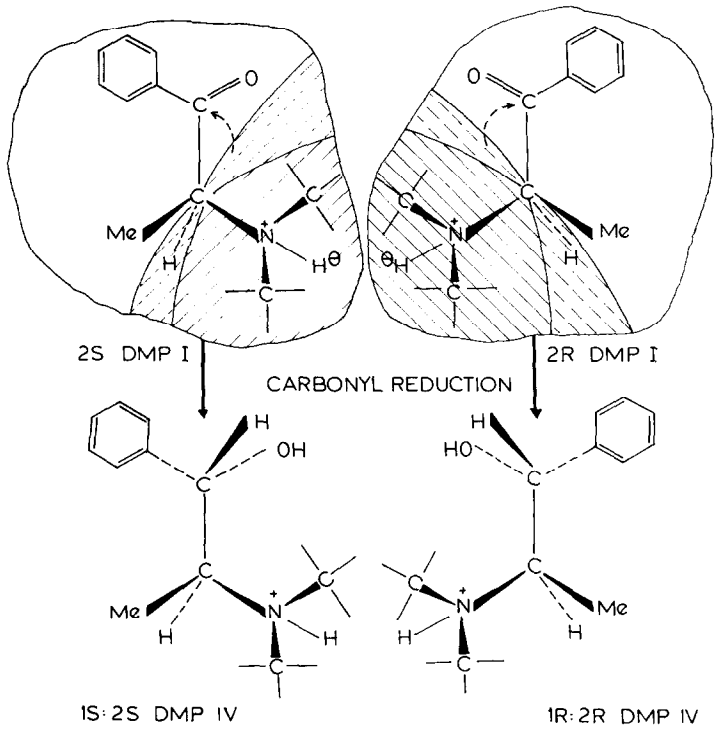

Fig. 2. Fit of the protonated (+)- and (−)-DMP I to the carbonyl reduction site which produces DMP IV Threo in the form of 1S;2S and 1R;2R enantiomers: ▨ sunken part of the reductive active site; ▨ raised part of the reductive active site (anionic site); ▭ planar part of the reductive active site.

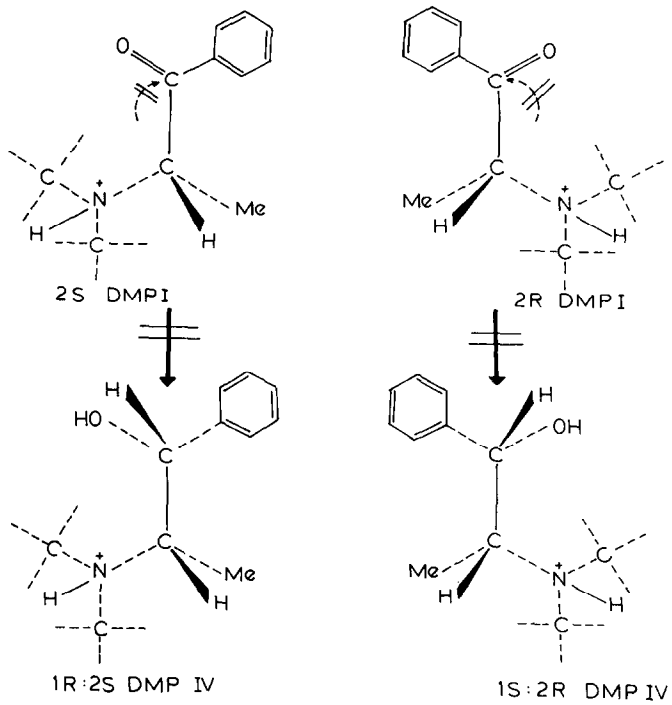

Fig. 3. The "forbidden" stereochemical fit of DMP I to the reductive active site, which would be required for the formation of the 1S;2R and 1R;2S DMP IV.

fitting onto the active site of the enzyme to allow the formation of the erythro isomers of methylephedrine (DMP IV). The results of Beckett and Mihailova [3] showed that by reduction of the size of the amino group of diethylephedrine (formed by keto reduction of diethylpropion) from *N*-diethyl to NH_2 the erythro isomer can be formed.

With DMP II, the bulk of the *N*-methyl groups in the molecule (of DMP I) is decreased, i.e. $N(CH_3)_2$ to $NHCH_3$, thus causing less steric hindrance, which seems to allow the molecule to be orientated either as shown in Fig. 2 or as shown in Fig. 3 (for DMP I). As a result of this both (±)-erythro and (±)-threo DMP V were produced by reduction of DMP II. Therefore, the erythro isomer of DMP V found in the urine after administration of DMP I (see Table 1) is not formed by reduction of DMP I to DMP IV followed by *N*-demethylation, but its demethylation first to DMP II and then reduction to form both isomers of DMP V.

Using the percentage stereoisomeric composition of the amino alcohols excreted in man after the oral doses of DMP I and DMP II (see Table 1), the contribution of each route of metabolism of DMP I to the formation of DMP V is calculated to be 45.6% from the route DMP I → DMP II → DMP V and 54.4% from the route DMP I → DMP IV → DMP V (see Fig. 1).

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Inhibition of type II procollagen to collagen conversion by lysine derivatives and related compounds
Mapping of the inhibitory structural features

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Collagens are a family of genetically distinct proteins which are initially synthesized and secreted as precursor forms, procollagens (for review on collagens, see Refs. 1–5). Type II procollagen, the hyaline cartilage-specific member of the family, contains non-collagenous extensions at both the amino- and the carboxy-terminal ends of the molecule. During the extracellular processing, these extensions are removed by specific proteinases, procollagen N-proteinase and C-proteinase respectively [6–8]. The removal of the extensions is necessary for the proper alignment of the collagen molecules to form fibril structures and for subsequent stabilization of the fibrils by intermolecular covalent cross-links [1–5, 7].

Previous studies have demonstrated that several naturally occurring amino acids, polyamines and structurally related compounds effectively inhibit the conversion of procollagen to collagen [9, 10]. Specifically, compounds such as lysine interfere with the removal of the carboxy-terminal extension, and consequently, pC-collagen accumulates in tissues incubated in the presence of the effective compounds. In this study, we have mapped the inhibitory structural features by testing several lysine derivatives and related compounds for their effectiveness in inhibiting the conversion of type II procollagen to collagen.

Materials and methods

The test compounds used in this study (see Table 1) were purchased from the Sigma Chemical Co. (St. Louis, MO), except for Tris and NH_3 which were from the Fisher Scientific Co. (Fair Lawn, NJ). The purity of the acetylated lysine derivatives was verified by amino acid analyses. To test the effects of these compounds on the conversion of type II procollagen to collagen, pulse-chase experiments were performed by incubating 17-day-old chick embryo sterna, as indicated previously [10, 11]. All test compounds were soluble in concentrations used. The sterna were labeled for 30 min with [^{14}C]proline (pulse) and, after inhibition of the protein synthesis by the addition of 500 μg/ml of [^{12}C]proline and 200 μg/ml of cyclohexamide, the test compounds were added; the incubations were continued for 120 min (chase). Radioactive proteins were extracted from the sterna with 3% sodium dodecyl sulfate (SDS) at 100° in the presence of protease inhibitors either at the end of the pulse or following the chase period. The collagenous polypeptides were separated by SDS-polyacrylamide slab gel electrophoresis, and the radioactive peptides were visualized by fluorography, as indicated previously [12, 13]. The bands were quantitated by scanning at 700 nm using an automatic computing densitometer (ACD-18, Gelman Instrument Co.). The conversion of procollagen to collagen was calculated, as indicated previously [10, 11] and shown in Table 1.

Results

When chick embryo sterna were pulse-labeled with radioactive proline for 30 min and then examined at the end of the pulse (t = 0), the major collagenous polypeptides could be identified migrating in the positions of proα and pCα chains of type II procollagen (Fig. 1, lanes A and B). When the further incorporation of radioactive proline was inhibited and the incubation continued for an additional 60 min (lane C) or 120 min (t = 120), most of the collagenous polypeptides were converted to α-chains (Fig. 1, lanes D–F). As demonstrated previously [10], addition of 50 mM L-lysine into the incubation medium at the beginning of the chase period inhibited the conversion of precursor polypeptides to α-chains (Fig. 1, lanes G–I).

Utilizing similar pulse-chase experiments, several lysine derivatives were tested for their inhibitory effects on the procollagen to collagen conversion (Table 1). The results indicated that *N*-α-acetyl-lysine inhibited the conversion by about 50% while *N*-ε-acetyl-lysine had little, if any, effect. In addition to L-lysine, 50 mM D-lysine was an effective inhibitor, and the conversion was abolished almost com-