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The influence of conformational factors on the metabolic conjugation of aryloxyacetates

HAN VAN DE WATERBEEMD, BERNARD TESTA* AND JOHN CALDWELL†

School of Pharmacy, University of Lausanne, Place du Château 3, CH-1005 Lausanne, Switzerland and †Department of Pharmacology, St Mary's Hospital Medical School, University of London, Norfolk Place, London W2 1PG, UK

Among *p*-chlorophenoxyalkanoic acids, the acetate and 2-propionate are essentially inert towards metabolic conjugation, whereas the isobutyrate (clofibric acid) undergoes extensive glucuronidation, as well as amino acid conjugation in carnivores. To try to explain these differences, the conformational behaviour of three model compounds was studied by quantum mechanical calculations (PCILO method). All three compounds prefer *syn* (folded) conformers, but the isobutyrate, in contrast to its two lower homologues, also has *anti* (extended) conformers of relatively low energy. Based on these results, a hypothetical topographical model is proposed for the binding site of glucuronyltransferase.

The recognition and exploitation of the relationships between chemical structure and biological activity have been responsible for many advances in pharmacology. In contrast, the discernment of the relationships between structure and various aspects of absorption, distribution, metabolism and excretion has proved more elusive (for reviews see Hansch 1972; Seydel & Schaper 1982; Mayer & van de Waterbeemd 1985; and a study by Ong et al 1982). Absorption, distribution and excretion generally involve passive diffusion, hence it is no wonder that these processes are related to lipophilicity. It is to be expected that structure–metabolism relationships exist for the various drug-metabolizing enzymes, but their delineation is hampered by the fact that most of these enzymes exist as families of isoenzymes with (partially) overlapping substrate selectivities. These matters have been reviewed recently elsewhere (Testa 1984; Caldwell & Hutt 1984).

One of the few examples of a series of compounds exhibiting clear-cut structure-metabolism relationships in-vivo is seen with xenobiotic carboxylic acids (e.g. Fig. 1). Stated briefly, the carboxyl group of such acids may undergo conjugation with amino acids or glucuronic acid, and the relative extents of these two options depends in the main upon the steric features of substituents close to the -COOH group. Thus bulky substituents *ortho*- to the carboxyl group of benzoic acids or on the α-methylene carbon of arylacetic acids are associated with reductions in the extent of amino acid conjugations and increases in glucuronidation (Table 1; Caldwell 1982). These changes apparently arise from the steric features of the substituent and not other, possibly associated,

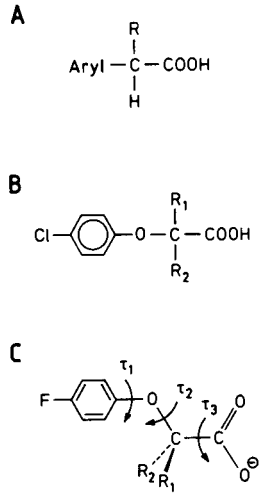

FIG. 1. Structures and compounds discussed in the text. A: R = H, arylacetic acids; R = Me, 2-arylpropionic acids. B: $R_1 = R_2 = H$, *p*-chlorophenoxyacetic acid; R_1 = Me, R_2 = H, 2-(*p*-chlorophenoxy)propionic acid; $R_1 = R_2$ = Me, *p*-chlorophenoxyisobutyric acid (clofibric acid). C: model compounds used in the calculations, shown here with $\tau_1 = \tau_2 = 180°$, $\tau_3 = 0°$; I: $R_1 = R_2 = H$; II(S): $R_1 = H$, R_2 = Me; III: $R_1 = R_2$ = Me.

changes in properties such as pK_a or lipophilicity (Table 1) (Caldwell 1978, 1982). Indeed, the abolition of amino acid conjugation seen in rodents and primates when phenylacetic acid is substituted with a methyl group to yield 2-phenylpropionic acid (Dixon et al 1977) is one of the most remarkable influences of structural change upon metabolism observed to date.

More recently, metabolic studies at St Mary's Hospital Medical School have been extended to aryloxyacetic acids (Emudianughe et al 1983), which

* Correspondence.

Table 1. Physicochemical properties and metabolic patterns of arylacetic and aryloxyacetic acids.

Acid	R′	R″	log P[1]	pK_A[2]	In-vivo conjugation[2]
Arylacetic acids (Ar–CR′R″–COOH)					
Phenylacetic	H	H	1·43	4·32	AA
2-Phenylpropionic	H	CH_3	1·93	4·60	GA > AA (rodents, primates) AA > GA (carnivores)
Diphenylacetic	H	C_6H_5	3·09	3·94	GA
p-Chlorophenoxy acids (Ar–O–CR′–R″–COOH)					
Acetic	H	H	1·99	3·20	Inert
Propionic	H	CH_3	2·31	4·35	Inert (little AA in carnivores)
Isobutyric	CH_3	CH_3	2·37	4·46	GA (+ AA in carnivores)

[1] Data from Hansch & Leo (1979).
[2] Data from Caldwell 1982; Sinclair 1986.
AA = amino acid conjugation: GA = glucuronide formation.

have importance as hypolipidaemic agents and herbicides. It has long been known that *p*-chlorophenoxyacetic acid (Fig. 1B) is metabolically inert (Williams 1959). Substitution of the α-methylene protons with two methyl groups yields clofibric acid, the active metabolite of the hypolipidaemic drug clofibrate (the ethyl ester). Clofibric acid is extensively metabolized, undergoing conjugation with glucuronic acid in all species tested except the cat, and with glycine and taurine in cat, dog and ferret (Emudianughe et al 1983). It was thus remarkable to find that the racemic mono-α-methyl congener, 2-(*p*-chlorophenoxy)-propionic acid (CPPA), is essentially inert to metabolism in the rat and rabbit, being extensively excreted unchanged, and only undergoes a small (<10% of dose) amount of amino acid conjugation in the cat. This suggests that structure-metabolism relationships of the aryloxyacetic acids are analogous to, but different from, those seen with arylacetic acids. As with the arylacetic acids, factors such as log P and pK_a do not appear to have a major influence upon the observed structure-metabolism relationships, as is illustrated in Table 1. In order to explore further the relationships between the steric features of the aryloxyacetic acids and their metabolism, we have studied the conformational behaviour of these compounds using semi-empirical molecular orbital calculations.

METHODS

A convenient method of studying the conformational behaviour of drugs is an all-valence-electron semi-empirical procedure, the PCILO method (Diner et al 1969; van de Waterbeemd & Testa 1983). This method provides a rapid calculation procedure of conformation energies in the vacuum. The version used (QCPE 272) is parametrized only for first row elements. Therefore, the chlorine atom in the compounds of Fig. 1B was replaced by fluorine, a change which is not expected to affect notably the conformational behaviour of the sidechain. The compounds were taken in their anionic form which is believed to be the chemical species involved in the relevant conjugation reactions (Caldwell 1982). The structures of the investigated model compounds I–III are shown in Fig. 1C. The PCILO method works with localized bonds and charges. For each conformation, two Kekulé structures were considered for the aromatic ring, and the energies calculated as the arithmetic mean as explained elsewhere (Anker et al 1984).

All calculations were performed on the CDC CYBER 170/855 computer of the Federal Institute of Technology in Lausanne (EPFL). Isoenergy plots τ_1 vs τ_2 (in two- and three-dimensions) were obtained using the DESNIV program written by A. Bouberguig of the EPFL.

RESULTS

Preliminary calculations were performed to investigate the orientation of the three oxygen atoms with respect to each other. The dihedral angles τ_1 and τ_2 (defined according to Klyne & Prelog 1960) were fixed at 180° (see Fig. 1C), and the torsion angle τ_3 was rotated in 30° steps. It was found that the three oxygen atoms are preferably coplanar, i.e. $\tau_3 = 0°$ or 180°. For the subsequent calculations, τ_3 was therefore fixed at 0°, as shown in Fig. 1C. Compounds I and III are achiral, whereas II is chiral; we have arbitrarily considered only the enantiomer (*S*)-II, the conformational behaviour of which can only be identical with that of the (*R*)-II form in the absence of diastereoisomeric interactions with the environment. The torsion angles τ_1 and τ_2 were rotated in 30° steps. From the energy thus obtained for each conformer, two-dimensional (not shown) and three-dimensional conformational maps (Fig. 2A–C) were plotted. The main results to emerge from these calculations are as follows:

(a) The three compounds have similar regions of very low conformational energy (0–2 kcal (0–8 kJ) mol^{-1}) for approximately $\tau_2 = 0 \pm 30°$, $\tau_1 = 30–90°$ and 210–300°. These global energy minima correspond to *syn* (folded) conformers, with the carboxylate group pointing towards the phenyl ring and R_1 and R_2 (H or CH_3) pointing away from it. These conformations produce an electron-rich zone on one side of the aromatic ring.

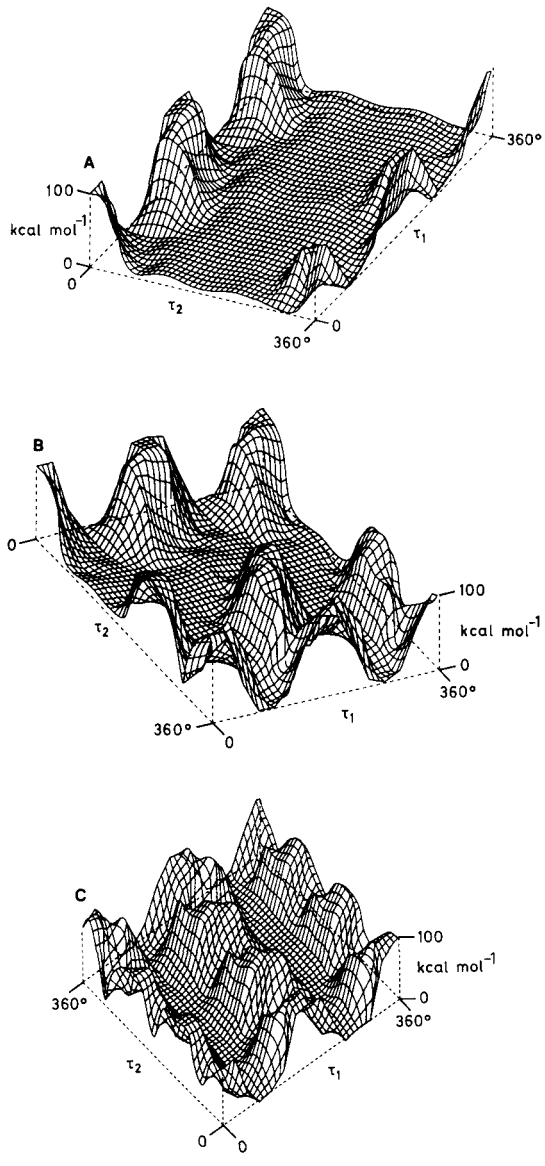

FIG. 2. Three-dimensional conformational maps for compounds I (A), II (B) and III (C). Energies above 100 Mkcal (420 kJ) mol^{-1} are not taken into consideration.

(b) In contrast to the above, the compounds markedly differ when regions of low conformational energy (2–5 kcal (8–21 kJ) mol^{-1}) are considered. For compounds I and II, these regions correspond essentially to *syn* (folded) conformers ($\tau_2 = 0 \pm 90°$). For compound IIIs on the other hand, regions of low conformational energy also exist for *anti* (extended) conformers ($\tau_2 = 180 \pm 90°$).

(c) When regions of higher conformation energies (5–15 kcal (20–60 kJ) mol^{-1}) are considered, compound III again differs from compounds I and II. Indeed, these regions occupy most of the conformational energy surface of compounds I and II, whereas for compound III much of this surface corresponds to virtually inaccessible regions (>25 kcal (100 kJ) mol^{-1}). This difference is particularly well illustrated by Fig. 2A–C.

DISCUSSION

From a metabolic point of view (Table 1), *p*-chlorophenoxyacetic acid and 2-(*p*-chlorophenoxy)-propionic acid behave comparably and are essentially inert, while *p*-chlorophenoxyisobutyric acid is well conjugated. How can these patterns be compared to, and also explained in terms of, the conformational behaviour of the model compounds I, II and III?

It was noted above that the three model compounds have comparable preferred conformations corresponding to folded forms, which maximally expose the methyl group(s) of the propionic acid and isobutyric acid derivatives. Thus, a possible explanation for the metabolic differences of the three acids is that they have preferred conformations which maximize the steric influence of the methyl group(s) in the higher homologues. However, such an explanation would dissociate the acetic acid derivative from its two homologues, and thus must be discarded.

Another explanation would involve the differences noted for the regions of higher conformational energy (5–15 kcal (21–63 kJ) mol^{-1}). But because higher conformational energies mean a very low probability of existence under normal conditions, the differences among high-energy conformers do not afford convincing evidence for metabolic differences.

In our opinion, the metabolic differences are best explained in terms of the conformational difference noted above under point (b), namely that compound III, as opposed to compounds I and II, has a fair probability (energy between 2–5 kcal (8–21 kJ) mol^{-1}) of existing as extended conformers. This leads to the suggestion that the active conformation of phenoxyacetates for glucuronide formation is an extended one, and that two α-methyl groups tend to render this conformation relatively favourable.

Based on this proposal, a topographical binding site for glucuronyltransferase is proposed in Fig. 3. This binding site involves electrostatic and hydrophobic interactions, and it binds *p*-chlorophenoxyisobutyrate in an extended conformation (Fig. 3A),

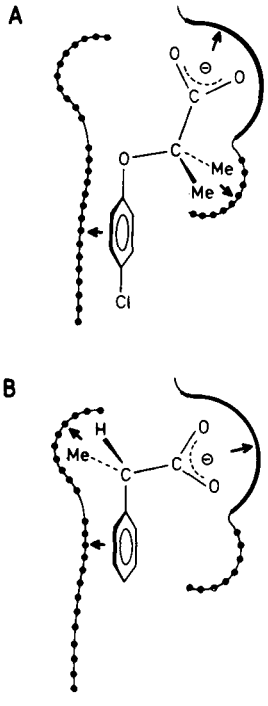

FIG. 3. Hypothetical 2-dimensional topographical model for the binding site of glucuronyltransferase. A: binding of phenoxyisobutyrates; B: binding of 2-arylpropionates. (——): electrostatic interactions; (—●—): hydrophobic interactions.

larger group of substrates, in particular 2-arylpropionates, the hydrophobic region in the enzyme from these animals has been viewed as being sterically less restrictive (see Fig. 4A).

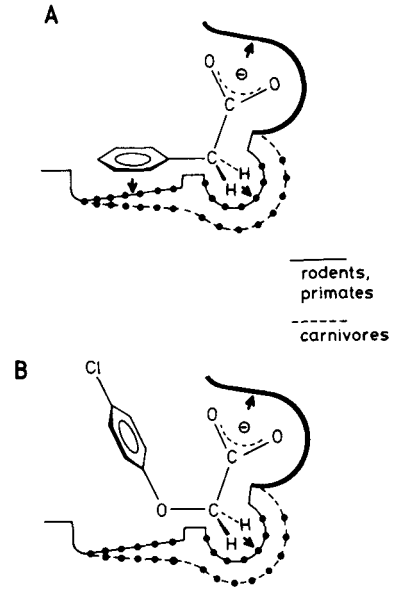

FIG. 4. Hypothetical 2-dimensional topographical model (according to Caldwell 1978, 1982) for the binding site of amino acid conjugation. A: binding of arylacetates; B: partial fit of *p*-chlorophenoxyacetate (here in a folded form). (——): Electrostatic interactions; (—●—): hydrophobic interactions.

but not phenoxyacetates in folded form. The flat hydrophobic surface interacting with the aromatic region must, in fact, extend beyond that shown in Fig. 3, since phenoxyisobutyric acids with a *para*-substituent much larger than a chlorine atom (e.g. a *p*-chlorophenyl substituent), also undergo glucuronidation (Sinclair 1986). As shown in Fig. 3B, this binding site also accommodates arylacetates and especially 2-arylpropionates which are known substrates of glucuronyltransferase (Caldwell 1978, 1982). In contrast, it appears difficult at present to equate this model with structure-glucuronidation relationships of alcohols and phenols (Okulicz-Kozaryn et al 1981; Schaefer et al 1981).

Structure-metabolism relationships of phenylacetates for amino acid conjugation have been rationalized by Caldwell (1978, 1982) in terms of the topographical binding site shown in Fig. 4A. Attractive interactions of electrostatic and hydrophobic nature were hypothesized. Because it is known that carnivores can form amino acid conjugates from a

Placing phenoxy acids into this binding site does not allow a good fit, neither for the folded (as shown in Fig. 4B) nor for the extended conformations of these compounds. However, both *p*-chlorophenoxypropionate and -isobutyrate undergo amino acid conjugation to a limited extent in carnivores, these reactions being more extensive with the isobutyrate. This is perhaps due to hydrophobic interactions involving the methyl group(s).

One structural element neglected by the topographical models shown in Figs 3 and 4 is the chirality of the α-carbon in 2-arylpropionates. If future studies reveal substrate enantioselectivity in the glucuronidation and/or amino acid conjugation of such acids, it will become possible to better model hydrophobic pockets binding the $CHCH_3$ group.

Modelling enzymatic binding sites from structural features of substrates is always hypothetical. However, such exercises allow a qualitative rationalization of biological data which provides an incentive

for further research. Additionally, since topographical models may ultimately contribute to the discovery of drugs with improved pharmacokinetics, their interest is worth assessing.

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