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BIOCHEMICAL PROPERTIES OF ANTI-INFLAMMATORY DRUGS—XII

INHIBITION OF URATE BINDING TO HUMAN ALBUMIN BY SALICYLATE AND PHENYLBUTAZONE ANALOGUES AND SOME NOVEL ANTI-INFLAMMATORY DRUGS

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Abstract—We have previously reported that 0·2 mM phenylbutazone and salicylate inhibit the binding of urate *in vitro* to human albumin by over 50 per cent. This communication describes similar studies with over 60 other compounds. Only gentisate, perfluorosalicylate, diiodosalicylate, salicylimide, Trimethazone, Diflumidone, the fenamic acids, 3-hydroxycinchophen and several acidic sulfonamides were as potent as phenylbutazone or salicylate. A structure–action relationship was delineated for salicylate analogues. There was a consistent relationship between the ability of an acidic compound to displace albumin-bound urate and its relative avidity for the primary dansylamide (DNSA)-binding site(s) of the albumin. Some advantages of using DNSA, *vis à vis* other dyestuffs, for measuring drug binding to albumin are discussed. Since some of the uricosuric action of certain drugs may depend on the displacement of urate from albumin or other proteins by the drugs, the assays *in vitro* for urate displacement which are described may facilitate pharmacological screening for potential uricosuric activity.

CERTAIN nonsteroid acidic anti-inflammatory drugs currently in use demonstrate uricosuric activity, i.e. they increase the excretion of uric acid by the kidney. We previously noted[1–4] that some of these anti-inflammatory drugs, including salicylates and phenylbutazone, decrease the binding of urate to plasma protein when administered *in vivo* or added *in vitro*. We have now examined the effects of a number of other compounds on urate binding to human albumin *in vitro*. This report describes our studies with three particular classes of drugs: (1) derivatives or chemical analogues of salicylic acid; (2) dioxopyrazolidines related to phenylbutazone, including two of its metabolites formed in man; and (3) chemically diverse acidic drugs for which anti-inflammatory or uricosuric activity has been claimed in the recent scientific, but non-patent, literature.

We also obtained a measure of the relative binding of each of these compounds to human albumin by determining the concentrations at which they displaced 50 per cent of a given quantity of 5-dimethylaminonaphthalene-1-sulfonamide (dansylamide, DNSA) from its binding site(s) on the albumin molecule.

EXPERIMENTAL

Piperazine-*N*,*N*′-bis(2-ethane sulfonic acid) (PIPES) buffer and DNSA were obtained from Calbiochem, Los Angeles. Hog liver uricase was obtained from Worthington Biochemical Corp., Freehold, N.J. Human crystallized albumin preparations

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were obtained from Dade Laboratories, Miami, Fla., and Calbiochem (Pentex, grade B, chromatographically pure). Uric acid was obtained from Pfanstiehl Laboratories, Waukegan, Ill., and Matheson, Coleman & Bell Company, Norwalk, Ohio. Other chemicals were obtained from the appropriate pharmaceutical companies and supply houses in the best grade available and used without further purification.

DL-γ-Hydroxyphenylbutazone was prepared from the tautomeric α-carboxy-*N*,*N*'-diphenylhydrazide δ-caprolactone,[5] kindly donated by Dr. R. Pfister, J. R. Geigy S. A., Basel, Switzerland. This lactone was dissolved in *N*,*N*-dimethylformamide (DMF) and immediately diluted with 99 volumes of buffer solutions ranging from pH 5 to 9. Rearrangement of the lactone to γ-hydroxyphenylbutazone (and its subsequent ionization) in these buffer media was monitored by the large increase in light absorption at 263 mμ. Corresponding solutions of the lactone in DMF were also diluted with 50 mM sodium hydroxide to ascertain the theoretical maximum absorption of γ-hydroxyphenylbutazone at 263 mμ. The rearrangement of the lactone to γ-hydroxyphenylbutazone was pH dependent but virtually complete within 10 min at pH 7·4 in phosphate or PIPES buffer at room temperature.

Disalicylimide (m.p. 208°) and *N*-acetyl-salicylamide (m.p. 143–144°) were prepared from salicylamide.[6]

The effect of a drug on the binding of urate by human serum albumin was determined by equilibrium dialysis carried out for 16 hr at 4°, exactly as described.[3] The concentrations of each component were: albumin (Pentex), 0·75 mM; PIPES buffer, pH 7·4, 50 mM; the drug under investigation, 0·1, 0·2 or 0·5 mM; and urate, 0·9 mM (15 mg/100 ml). The concentration of urate inside and outside the dialysis bag was determined spectrophotometrically after oxidation with uricase.[7] None of the drugs investigated interfered with the enzymatic assay at the concentrations used in our experiments. Data reported in the tables are the mean of duplicate studies, agreeing within 5 per cent, at each drug concentration.

The effect of a drug on the binding of DNSA by human serum albumin was determined by measuring the fluorescence due to albumin-bound DNSA at various concentrations of added drug at 25° (±1°) exactly as described.[3] The concentrations of each component were: albumin (Dade), 14·5 μM; phosphate buffer, pH 7·4, 0·1 M; DNSA 1·0 μM; and variable drug concentrations (5–500 μM). None of the drugs investigated, except for dinitrophenol, quenched the fluorescence of DNSA in a hydrophobic environment, as determined by adding the drug (at five or more times the I_{50} concentration) to a solution of 1 μM DNSA in methanol. The I_{50} values were determined as that drug concentration required to inhibit by 50 (±3) per cent the fluorescence of DNSA in the presence of albumin. The reproducibility of the I_{50} values was generally excellent: for example, six independent determinations with salicylate gave a value of 0·45 mM with S.E.M. of ±0·015, which did not vary significantly with different batches of the crystallized albumin used (Human Protein Standard from Dade Laboratories). Certain other commercial preparations of crystalline human albumin were less satisfactory, giving lower fluorescence intensities with given amounts of DNSA.

Addition of equivalent quantities of either salicylic acid or sodium salicylate to the albumin solutions displaced identical amounts of urate or DNSA from their respective albumin-binding sites. As a general procedure, we therefore added the acidic form of the drug from solutions in dimethylsulfoxide (DMSO) or DMF to the appropriate

buffer solutions, giving a final concentration of DMSO or DMF not exceeding 1 per cent by volume.

RESULTS

Studies with salicylate analogues

Table 1 indicates that none of the analogues studied was more potent than the salicylate anion in displacing albumin-bound urate. Gentisate (2,5-dihydroxybenzoate and a metabolite of salicylic acid in man), certain halogen-substituted salicylates, and salicylimide were comparable to the salicylate anion at low concentrations (0·1–0·2 mM) in their ability to displace urate ions and evidently had a higher affinity for the DNSA-binding portion of the albumin molecule than salicylate itself. Salicyluric acid (*N*-salicyl-glycine), one of the principal metabolites of salicylic acid in man, was also quite active in displacing both DNSA and urate.

The feeble activities of salicylamide, 2-hydroxybenzyl alcohol, 4-hydroxybenzoate and hexahydrosalicylate in displacing bound urate emphasize the fairly sharp structural requirements for salicylate-like activity in this respect (e.g. an *ortho*-phenolic group, the benzene nucleus and an unsubstituted carboxyl group or its equivalent as an

TABLE 1. EFFECTS OF SOME SALICYLATE ANALOGUES ON THE BINDING OF URATE AND DNSA TO HUMAN SERUM ALBUMIN *in vitro*

Compound added	I_{50} For displacement of DNSA (mM)	Urate binding (% drug-free control) in the presence of compound at		
		0·1 mM	0·2 mM	0·5 mM
Sodium salicylate	0·45	42	30	15
Salicylic acid				
Hexahydrosalicylic acid	12·0		81	68
Sodium 4-hydroxybenzoate	8·0		60	47
O-Acetylsalicylic acid	2·0		54	29
2-Phenoxybenzoic acid	0·2		51	25
2,3-Dihydroxybenzoic acid	0·5		36	22
2,4-Dihydroxybenzoic acid (β-resorcylic)	1·8		41	27
2-5-Dihydroxybenzoic acid (gentisic)	0·05†	47	21	4
2-6-Dihydroxybenzoic acid (γ-resorcylic)	0·16	65	40	4
4-Aminosalicylic acid	5·5		77	54
3,5-Di-*t*-butylsalicylic acid	0·01		37	24
3,5-Di-*t*-butyl-2,6-dihydroxybenzoic acid	0·1		43	33
3,4,5,6-Tetrafluorosalicylic acid	0·05	45	27	6
5-*p*-Fluorophenylsalicylic acid	†	58		12
3,5-Di-iodosalicylic acid	0·01		21	0
2-Hydroxybenzyl alcohol	> 5·0		98	98
Salicylamide	12·0		85	74
N-Acetylsalicylamide	0·01†		38	19
(Di)salicylimide	0·01	35	29	17
Salicylanilide	0·05		58	39
Salicyluric acid	0·5†		48	10
2,4-Dinitrophenol	0·02‡		40	20

* The I_{50} is the drug concentration inhibiting DNSA binding by 45–55 per cent.
† Determination uncertain because of the natural fluorescence of this compound.
‡ Corrected for quenching of the DNSA fluorescence.

acid). Since these four latter compounds were also very inefficient displacers of bound DNSA, it would appear that their chemical constitution precludes avid binding to the albumin molecule in general.

2,4-Dinitrophenol, which in many biochemical test systems behaves as a more potent phenolic drug than salicylic acid, proved to have some activity here as well. This fact, together with the other findings discussed in this section, suggests that only moderately lipophilic acidic phenols will mimic salicylate in seeking out, and binding to, the prime urate-binding site on human albumin.

Studies with phenylbutazone analogues

This survey was confined to cyclic β-diones, which are either being used clinically as uricosuric or antirheumatic drugs (i.e. alternatives to phenylbutazone) or which have been reported to manifest anti-inflammatory (or uricosuric) activity in small animals. We made no attempt to delineate the relationship between chemical structure and phenylbutazone-like activity. Instead, we tried to find out if some of the various

TABLE 2. EFFECT OF PHENYLBUTAZONE ANALOGUES AND OTHER CYCLIC β-DIONES ON THE BINDING OF URATE AND DNSA TO HUMAN SERUM ALBUMIN *in vitro*

Compound (Trivial name)	Ref. no.	I_{50} For displacement of DNSA (mM)	Urate binding (% drug-free control) in the presence of compound at		
			0·1 mM	0·2 mM	0·5 mM
Phenylbutazone		0·015	58	44	31
Oxyphenbutazone (Metabolite I)	8	0·045	67	53	45
DL-γ-Hydroxyphenylbutazone (Metabolite II)	8	0·020	57	49	36
γ-Ketophenylbutazone (Ketazone)	9	0·055		52	39
4′,4′,4′-Trimethyl-Ketazone (Trimethazone)	10	0·015		38	24
Sulphinpyrazone		0·035		50	30
5-*n*-Butyl-1-cyclohexyl-barbituric acid (Buculome, Paramidine)	11, 12	0·012		24	31
1,2-Propylmalonyl-3-dimethylamino-7-methyl-1,2-dihydro-1,2,4-benztriazine (Azapropazone, Mi-85)	13	0·015		40	27
1,2-(*n*-Pentylmalonyl)-4-phenyl-1,2-dihydrocinnoline (Cinnopentazone, Scha-306)	13	*		56	36
2-Phenyl-indan-1,3-dione (Phenindione)		0·025		39	24
2-Phenyl-5-trifluoromethyl-benzothiophene-3(2H)-one-1,1-dioxide (CP-12,070)	14	0·012		33	6
Warfarin		0·025		40	18
N-*p*-chlorophenyl-1,3(2H, 4H)-dioxo-isoquinoline-4-carboxamide (CP-13,608)	15	*		82	52

* Could not be determined due to fluorescence of the drug.

acidic diones which have been proposed as alternatives to phenylbutazone for clinical use did, in fact, match or exceed phenylbutazone in their ability to displace urate from human albumin (see Table 2).

The two principal metabolites of phenylbutazone in man could be distinguished by their albumin-binding characteristics. The so-called "metabolite II", L-γ-hydroxyphenylbutazone, is inactive as an antirheumatic agent but has uricosuric activity.[8] Synthetic DL-γ-hydroxyphenylbutazone was certainly more potent than the other phenylbutazone metabolite, oxyphenbutazone, in displacing both urate and DNSA, though neither metabolite equalled phenylbutazone in this regard. A hypothetical oxidation product of γ-hydroxyphenylbutazone being used in the clinic,[9] namely γ-ketophenylbutazone (Ketazone, Kebuzone), had a lower affinity for the DNSA-binding site on the albumin, but did not differ markedly from either of the hydroxyphenylbutazones (metabolites I or II) in urate-displacing activity. A trimethyl derivative of Ketazone, 1,2-diphenyl-3,5-dioxo-4-(4′,4′-dimethyl-3-oxopentyl) pyrazolidine (Trimethazone), which is claimed to have fewer side effects than phenylbutazone,[10] seemed to be as active as phenylbutazone in displacing both urate and DNSA from their respective binding sites on the albumin molecule.

Both bucolome and CP-13,608 have been reported to lower the serum urate levels in man,[11,15] but only bucolome matched phenylbutazone at low concentrations (0·1, 0·2 mM) in displacing urate. The two anticoagulants, phenindione and warfarin, and an anti-inflammatory sulfono analogue of phenindione without anticoagulant activity (CP-12,070) each displaced both DNSA and urate as efficiently as phenylbutazone.

Studies with other acidic anti-inflammatory drugs

The three fenamic (*N*-arylanthranilic) acids again mimicked the salicylate anion in these as in other studies.[16] An acidic sulfonanilide (Diflumidone)[17] and 3-hydroxycinchophen, which might be regarded as a lipophilic salicylate analogue, were the most potent of the compounds examined in this mixed group of acidic drugs (Table 3). Cinchophen and its hydroxy derivatives have long been known to have uricosuric properties[18,19] and were used extensively in Europe for treating gout until they fell into disfavor because of alleged hepatotoxicity.

The other acids listed in Table 3 were notably less active at 0·2 mM than either the salicylate anion or phenylbutazone in displacing urate from human albumin, though all these compounds have shown phenylbutazone-like anti-inflammatory activity either in the clinic or in animal models of arthritis.[22–28]

That it is primarily the drug anion which displaces the urate and DNSA was indicated by the fact that the primary alcohols (obtained by reducing the carboxylate group of salicylic acid, flufenamic acid, indomethacin and Ibuprofen) were each less potent *in vitro* than the corresponding carboxylic acids.

Studies with sulfonamides and other acidic drugs

The free acid corresponding to MK-185 (a novel hypolipidemic ester with uricosuric activity in man[29]), which is readily formed from MK-185 *in vivo*, was quite effective in displacing bound urate from albumin. Tolbutamide, which binds strongly to sites on the albumin molecule for which both warfarin and phenylbutazone have a high affinity,[30] also simulated these drugs in effectively displacing bound urate at relatively

TABLE 3. EFFECT OF SOME MISCELLANEOUS ANTI-INFLAMMATORY ACIDIC DRUGS AND RELATED ALCOHOLS ON THE BINDING OF URATE AND DNSA TO HUMAN SERUM ALBUMIN *in vitro*

Compound (Trivial name)	Ref. no.	I_{50} For displacement of DNSA (mM)	Urate binding (% drug-free control) in the presence of compound at 0·1 mM	0·2 mM	0·5 mM
N-(2,3-xylyl)anthranilic (Mefenamic) acid	20	0·04	50		
N-(α′,α′,α′-trifluoro-*m*-tolyl) anthranilic (Flufenamic) acid	20	0·035	80	53	
Flufenamyl alcohol*		0·08			
N-(2,6-Dichloro-*m*-tolyl) anthranilic (Meclofenamic) acid	21	0·035	63	53	
4-Isobutylphenylacetic acid (Ibufenac, U-21,880)	22	0·040		74	46
4-Isobutylphenylethanol		0·75			
2(4-Isobutylphenyl)-propionic acid (Ibuprofen, U-18,573)	23	0·20		78	44
2-(4-Isobutylphenyl)propanol		0·50		90	86
2-Phenylcinchoninic acid (Cinchophen)	18	0·04		55	21
3-Hydroxy-cinchophen	18	0·04		39	14
D-2-(6-Methoxy-2-naphthyl)-propionic acid (Naproxen)	24	0·24		68	34
2-(4-Chlorophenyl)-thiazol-4-yl-acetic (Fenclozic) acid (ICI-54,450)	25	0·30		67	39
β-(4,5-Diphenyl-oxazol-2-yl)-propionic acid (Wy-21743)	26	0·12		65	
2-(3,4-Dichlorobenzamido)-phenoxyacetic (Clamidoxic) acid (SNR-1804)	27	0·035		70	32
4-Allyloxy-3-chlorophenylacetic acid (Mervan)	28	0·32		86	63
3′-Benzoyl-1,1-difluoromethane sulfonanilide (Diflumidone)	17	0·03		39	17
Indomethacin (acid)		0·05	72	50	30
Indomethacin alcohol†		0·08	97		

* 2-(α′,α′,α′-Trifluoro-*m*-toluidino) benzyl alcohol.
† 2-(*N*-*p*-chlorobenzoyl-5-methoxy-2-methyl-indol-3-yl) ethanol.

low drug concentrations (0·1, 0·2 mM). Another oral hypoglycemic agent, acetohexamide, and its reduction product *in vivo* (1-hydroxyhexamide), both of which demonstrate uricosuric activity in man,[31] were also about as potent as phenylbutazone at 0·2 mM in displacing urate but less potent than phenylbutazone in displacing DNSA. Other relatively acidic sulfonamides such as sulfaethylthiadiazole (pK_a 5·4), sulfaphenylpyrazole (pK_a 6·1), sulfapyrimidine (pK_a 6·4) and sulfamethoxypyridazine (pK_a 7·0) exhibited pronounced urate-displacing activity (Table 4). Some of these sulfonamides are known to have a high affinity for human and bovine albumin[32,33] and are certainly effective displacers of DNSA (Table 4 and refs. 34, 35). By contrast, sulfacetamide, which is also relatively acidic (pK_a 5·4), failed to displace urate from its

TABLE 4. EFFECT OF SOME SULFONAMIDES AND OTHER ACIDIC DRUGS ON THE BINDING OF URATE AND DNSA TO HUMAN SERUM ALBUMIN *in vitro*

Compound (Trivial name)	I_{50} For displacement of DNSA (mM)	Urate binding (% drug-free control) in the presence of compound at	
		0·2 mM	0·5 mM
1-Butyl-3-*p*-tosylurea (Tolbutamide)	0·2	44	20
1-(*p*-Acetylphenylsulfonyl)-3-cyclohexyl-urea (Acetohexamide)	0·2	49	11
1-(1′-*p*-Hydroxyethylphenylsulfonyl)-3-cyclohexylurea (1-Hydroxyhexamide)	0·3	49	11
dl-Hydroxyhexamide	0·25	54	23
Sulfadimethoxypyrimidine (Sulfadimethoxine)	0·06	43	15
Sulfadimethylisoxazole (Sulfisoxazole)	0·1*	35	19
Sulfaphenylpyrazole (Sulfaphenazole)	0·075	33	13
Sulfamethoxypyridazine	0·15	48	27
Sulfamethylthiadiazole (Sulfamethizole)	0·4	30	18
Sulfaethylthiadiazole (Sulfaethidole)	0·05	29	11
Sulfapyrimidine (Sulfadiazine)	0·3	67	
N-acetylsulfanilamide (Sulfacetamide)	0·75	93	79
4-*p*-Chlorophenyl-3-trifluoromethyl-phenoxyacetic acid (MK-185 FA)†	0·035	40	14
α-(*p*-Chlorophenoxy)isobutyric acid	0·8	65	28
2,3-Dichloro-4(2-methylenebutyryl) phenoxyacetic acid (Ethacrynic acid)	0·1	71	30
Taurocholic acid	6·0	91	84
Taurochenodeoxycholic acid	12·0	100	83
p-(Dipropylsulfamyl)benzoic acid (Probenecid)	2·5	73	47
Mersalyl (Salyrgan)	0·1*		67‡

* Gave a very flat dose–response curve over the range 0·05 to 0·25 mM.
† The 2-acetoaminoethyl ester of this acid is MK-185 (Halofenate).
‡ At 1 mM.

albumin-binding site(s). This is explicable because the *N*-acetyl substituent on the amido nitrogen (of sulfanilamide) confers little or no lipophilic character as compared to the mixed aromatic-aliphatic substituents carried by this same N^1 atom in the other acidic sulfonamides examined.*

It would be interesting to know the incidence of further attacks of gout in former gouty patients who subsequently receive a sustained course of anticoagulant, antibiotic or hypoglycemic therapy with an acidic coumarin drug, sulfonamide or sulfonylurea. Our observations suggest that such drugs might serve as prophylactics for gouty arthritis, if the requisite plasma levels of the drugs could be sustained.

DISCUSSION

We have shown in previous studies that, when certain drugs such as phenylbutazone or aspirin are administered to normal subjects, the urate-binding capacity of the plasma

* Inspection of the aqueous solubilities of either the sulfonamides themselves or their sodium salts, as given in the *Merck Index*, clearly shows that sulfacetamide is by far the most water-soluble (solubility = 0·67%) and therefore least lipophilic of this group of acidic sulfonamides (cf. sulfaethylthiadiazole with the same pK_a, solubility = 0·00025%).

is significantly reduced, but rapidly returns to normal after discontinuing the drug.[2] Furthermore, most of the drugs currently being used for their uricosuric activity have the property of displacing urate from albumin *in vitro*.[3] We have postulated that this drug-induced displacement of urate from carrier proteins may be partially responsible for the uricosuric activity of these and similar anionic drugs.

One method of screening drugs for such potential uricosuric activity would be to test their ability to displace urate from albumin *in vitro*. A drug exhibiting urate-displacing activity comparable to or greater than that of salicylate would be a good candidate for further testing *in vivo*. On this basis, we have screened over 60 compounds for potential uricosuric activity using two indicators of activity: (1) the drug-induced displacement of urate from albumin as measured by equilibrium dialysis;[1-3] and (2) the displacement of dansylamide (DNSA) from albumin (presumably from the same site(s) involved in urate binding) measured fluorimetrically.[34]

Binding of DNSA to albumin

The DNSA-displacement studies were undertaken to find a more rapid technique than equilibrium dialysis for measuring the affinity of a drug for albumin. Solomon[34] assessed the competition between different drugs and the DNSA binding site(s) on human albumin. Certain acidic drugs could be ranked in the following order of diminishing ability to displace DNSA from albumin: phenylbutazone, warfarin, tolbutamide, chlorophenoxyisobutyric acid and probenecid.[35] The same order was found in our own studies comparing these drugs not only by their I_{50} values for DNSA displacement but also by their effects on urate binding to human albumin (Tables 2 and 3). In most cases, there was very good correlation between the ability of a drug to displace DNSA and urate, although there were some clear exceptions. For example, sulfaethylthiadiazole and sulfamethylthiadiazole showed about the same degree of urate-displacing activity, yet differed significantly in their ability to displace DNSA (I_{50} values are 0·05 and 0·4 mM respectively). Conversely, clamidoxic acid, an efficient displacer of DNSA, proved rather ineffective in displacing urate.

In our experiments, we used an albumin/DNSA ratio of 14·5 with the DNSA at 1 μM to ensure considerable (but still suboptimal) binding and fluorescence of the DNSA in the absence of added drug. Of the compounds tested, those which were the most effective in quenching the albumin-induced fluorescence of the DNSA gave I_{50} values of 12–20 μM implying that an albumin/drug ratio of approximately 1:1 was sufficient to dissociate 50 per cent of the bound DNSA. Thus the DNSA displacement is a sensitive index of drug–albumin interactions and can be used, literally, to titrate albumin with an efficient DNSA-competing drug such as phenylbutazone.

This DNSA-displacement procedure is of most value in studying drug interactions with human albumin, since animal albumins[34] and certain other proteins (e.g. pepsin, trypsin) give only 20 per cent or less of the fluorescence induced by human albumin. A further possible restriction arises from any natural or albumin-induced fluorescence of the drug under study.

Apart from these difficulties, the DNSA method offers a few distinct advantages over some of the other methods used to study drug–albumin interactions. It is rapid and not time-dependent, unlike the trinitrobenzaldehyde (TNBal) procedure.[36] It needs little albumin and indicator, unlike methods involving the colorimetric measurement of the displacement of albumin-sensitive dyestuffs such as HBABA.[37] It can be

used over a fairly wide pH range,[34] unlike the TNBal method or the commonly used nephelometric assessment of the drug-sensitive thermal denaturation of albumin described by Mizushima.[38]

Where data are available, there is good agreement between the findings with this fluorimetric assay (with DNSA), a colorimetric method (TNBal), and the nephelometric procedure, respectively, regarding the relative affinities for albumin of individual acidic compounds within a given chemical series, e.g. hydroxybenzoates (Table 1 and refs. 36, 39, 40).

Uricosuric activity

Drug-induced displacement of urate from carrier molecules, especially albumin, in the blood could promote uricosuria by affecting both glomerular filtration and tubular reabsorption of urate. First, increasing the amount of free urate in the blood would increase the amount of urate available for glomerular filtration. That free urate is actually increased upon administering these drugs is suggested by the fact that gouty patients given a salicylate preparation or phenylbutazone often suffer attacks of acute gout.[41] This "drug-induced agony" may occur because the unbinding of urate would be practically instantaneous, while the uricosuric process is much slower, resulting in a period during which the amount of free urate in the blood actually increases, so perhaps exacerbating the gouty symptoms.

Second, drug-induced displacement of urate from albumin could also inhibit tubular reabsorption of urate. It has been shown that adding albumin to the dialysis fluid during human peritoneal dialysis substantially increases the net amount of urate extracted across the peritoneal membrane.[42] A similar situation exists in the kidney where the albumin-containing fluid is on one side of the tubular membrane and the protein-free filtrate is on the other side. Thus albumin could facilitate movement of urate across the tubular membrane in a manner similar to that demonstrated in the peritoneal dialysis experiments. This mechanism would affect urate moving by diffusion but would not affect active transport of urate. A uricosuric drug might block the urate-binding sites on the albumin molecule, thus diminishing the effect of albumin

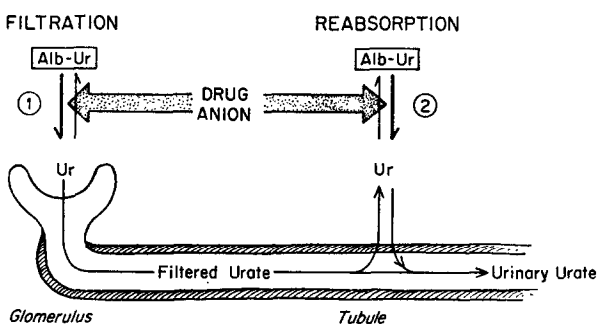

FIG. 1. Scheme illustrating how drug-induced displacement of urate from albumin within the kidney may promote uricosuria through two albumin-dependent, extracellular actions: (1) promoting glomerular filtration by increasing the unbound urate; and (2) decreasing urate extraction across the tubular membrane, i.e. decreasing tubular reabsorption (see text). This scheme does not include the effect of drugs on receptors within the tubular membrane. Key: Alb-Ur, albumin-bound urate; Ur, unbound urate in the renal blood flow.

in promoting urate reabsorption in this manner (Fig. 1). To avoid confusion with other modes of drug action causing uricosuria, the type of uricosuric activity involving albumin can be designated as "albumin dependent".

Another site of action, perhaps the primary one, of the uricosuric drugs would be at receptors intimately associated with the tubular membrane and regulating active transport and carrier-mediated passive diffusion of urate.[43] It is possible that the same drugs competing with urate for binding sites on the albumin molecule would also compete for the sites in the tubular membrane. Thus the assays used in this paper may measure the potential of a drug to displace urate from albumin *and* from sites within the kidney tubules.

The assays we have used would not measure the capacity of a drug to cause uricosuria by other means, such as by poisoning active transport mechanisms involved in tubular reabsorption. Drugs such as salyrgan (mersalyl),[18] probenecid and CP-13,608,[15] known to be uricosuric in man but showing low urate-displacing activity *in vitro* (Tables 2 and 4), probably have activities of this type.

We have previously shown the effects of pH, temperature and ionic strength on the binding of urate to albumin *in vitro*.[1] The experimental conditions for the present studies were chosen to maximize the urate-albumin binding rather than to duplicate conditions *in vivo*. The effects *in vivo* of the drugs may be much smaller than the activities *in vitro*. What we measure *in vitro*, however, is an indication of what might occur *in vivo*, not only with respect to displacement of urate from albumin but also with respect to displacement of urate from the other receptors as well. Furthermore, because of the large volume of blood which flows through the kidneys (over 20 per cent of the cardiac output), a given molecule of urate or albumin passes through the kidney many times in any given time period. The cumulative uricosuric effect over this time period is the result of many very small discrete interactions which might be unmeasurable except with the techniques described.

Analysis of our data suggests that those drugs which inhibit urate binding *in vitro* by 50 per cent or more at a drug concentration of 0·2 mM and a urate concentration of 0·9 mM (two to three time the normal plasma concentration) might inhibit binding *in vivo* with drug levels of only 0·05–0·1 mM. Therefore drugs failing to attain plasma levels of 10 μg/ml would probably not show uricosuric activity (unless they stimulated tubular secretion of urate). This would explain why flufenamic and meclofenamic acids are not uricosuric, although they are effective displacers of urate *in vitro*. The blood levels of these drugs with effective anti-inflammatory doses are less than 10 μg/ml (private communications from various scientists and clinicians associated with Parke, Davis & Company). Uricosuric drugs previously found to inhibit the binding of urate to plasma have been either those given with daily doses greater than 1 g (e.g. mefenamic acid and salicylate preparations) or those which have an exceptionally long half-life in man (e.g. phenylbutazone), allowing blood levels of 10 μg/ml or more to be sustained.

Where clinical data are available, the relative activities shown by the drugs *in vitro* generally agree with the relative activities shown by the drugs *in vivo*. Thus the methods described here may well provide a relatively rapid screening procedure for finding potential uricosuric drugs. Studies are currently under way to determine whether or not those drugs most potent in displacing urate *in vitro*, do in fact, have uricosuric properties *in vivo*.

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