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Binding of amiodarone by serum proteins and the effects of drugs, hormones and other interacting ligands

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Amiodarone is chiefly bound to albumin (62·1%) and much of the remainder (33·5%) is carried on a high molecular weight protein, probably β-lipoprotein. Analysis of data for amiodarone binding to albumin revealed a high affinity primary binding site (K_a $5·6 \times 10^6$ litre mol^{-1}) with about four secondary sites (average K_a $1·9 \times 10^5$ litre mol^{-1}). Studies of the binding of amiodarone in serum revealed one type of binding site only with an affinity constant (K_a $4·2 \times 10^6$ litre mol^{-1}) similar to that of the primary site on albumin. The secondary albumin binding sites do not seem therefore to be utilized in whole serum and the affinity of the lipoprotein must be similar to that of the primary amiodarone binding site on albumin. The effects of a wide range of compounds on albumin binding of amiodarone were examined by equilibrium dialysis. Quinidine, amitriptyline, cephazolin and palmitate decreased albumin-bound [^{125}I]amiodarone. Neither warfarin nor digoxin affected the binding of amiodarone by albumin, thus of the three drugs known to be potentiated by concomitant amiodarone administration, only potentiation of quinidine could be explained by displacement from serum albumin. Rifampicin, frusemide, phenytoin, (−)-adrenaline, bromocresol green, (−)-noradrenaline and bromocresol purple were found to increase binding of [^{125}I]amiodarone by albumin. Amiodarone had no influence on the distribution of iodothyronines amongst their binding proteins nor were the concentration or binding properties of these proteins altered after prolonged treatment with the drug. Thus altered iodothyronine concentrations in amiodarone-treated patients cannot be attributed even in part to effects at the serum binding protein level.

Amiodarone which is coming into widespread use as an antiarrhythmic drug has a low solubility in water, is strongly tissue bound and in blood is largely (>90%) protein bound (Charlier et al 1968). It is not known on which serum proteins the drug is carried nor have the characteristics of the binding been determined.

Drug interactions with amiodarone, presumed to be due to displacement from binding sites on proteins, have been reported. Potentiation of anticoagulants by amiodarone has been described by Simpson (1979) and Rees et al (1981). Martinowitz et al (1981) found that warfarin potentiation persisted for up to 2 months after withdrawal of amiodarone, an observation consistent with the very long half-life of the drug (Broekhuysen et al 1969). Pharmacological interactions of amiodarone with digoxin (McComb et al 1980; Moysey et al 1981) and quinidine (Tartini et al 1982) have both been attributed to displacement.

Amiodarone is known to interfere with, or alter levels of, endogenously secreted compounds in the circulation. It acts as a non-competitive antagonist to the action of catecholamines on the heart and vascular system (Charlier et al 1962; Charlier 1970; Deltour et al 1962). The consequences of the administration of amiodarone on thyroid function have been widely reported (Pritchard et al 1975; Burger et al 1976; Rees et al 1979; Melmed et al 1981) and although these are chiefly due to effects on the thyroid and on the peripheral metabolism of thyroxine, no systematic study of the action of amiodarone on thyroid hormone transport by serum proteins has been published.

The experiments now reported were undertaken to establish the mode of transport of amiodarone by serum proteins, and to determine whether drug interactions can be explained by changes in serum binding.

MATERIALS AND METHODS

Human blood samples were allowed to clot at room temperature (20 °C). Serum was separated by centrifugation and stored at −25 °C. Total thyroxine (T4), triiodothyronine (T3) and reverse-T3 (rT3) concentrations were determined by radioimmunoassay. Serum concentrations, and all other subsequent

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measurements, of T4-binding globulin (TBG), prealbumin (TBPA), albumin, α_1-lipoprotein and β-lipoprotein were made by 'rocket' immunoelectrophoresis (Laurell 1966) using commercially available monospecific antisera.

Pure amiodarone and [^{125}I]amiodarone were gifts from Labaz, Brussels, Belgium. A stock solution (4×10^{-5} M) of amiodarone was prepared in ethanol and stored in the dark at −70 °C. Freeze-dried [^{125}I]amiodarone (6·03 Ci mmol^{-1}) was reconstituted in ethanol and stored at −25 °C.

The distribution of amiodarone between the serum proteins of a normal subject was determined by chromatographing serum trace-labelled with [^{125}I]amiodarone on a column of Bio-Gel P-200 (<400 mesh) in 50 mM phosphate buffer, pH 7·4. The radioactivity in each fraction was measured and the peaks analysed by 'rocket' immunoelectrophoresis.

The affinity of amiodarone for serum proteins was determined from equilibrium dialysis binding data. Pure human albumin (Behringwerke A G) or normal human serum were diluted in phosphate buffer, pH 7·4 (0·008 M Na_2HPO_4; 0·0015 M KH_2PO_4; 0·137 M NaCl) to give the same concentration of albumin ($1·5 \times 10^{-5}$ M). Visking bags, pre-hydrated for 30 min in the presence of EDTA, containing 0·5 ml of protein solution to which had been added [^{125}I]amiodarone ($74·4 \times 10^{-8}$ M) and stable amiodarone ($4·8 \times 10^{-8}$ M to $618·2 \times 10^{-8}$ M for pure albumin; $21·8 \times 10^{-8}$ M to $2501·8 \times 10^{-8}$ M for serum), were placed in 9·0 ml volumes of diluent buffer. The containers were rotated end-over-end for 22 h at 37 °C. At equilibrium the radioactivity in the dialysate and dialysand was measured. Association constants for the interaction between amiodarone and serum proteins were obtained by Scatchard (1949) analysis of the binding data. An estimate of the number of binding sites was possible by extrapolating the Scatchard plots (Feldman 1972), together with further analysis of the data by Hill (1910) plots.

The effects on [^{125}I]amiodarone ($74·4 \times 10^{-8}$ M) binding to serum albumin (3×10^{-4} M) in the presence of a variety of compounds was also studied by equilibrium dialysis as described above but with 1·0 ml of solution within the dialysis bag and 8·0 ml external buffer. Each test compound was added to the albumin solution at the same molar concentration as albumin. The compounds were dissolved in minimal amounts of water, aqueous sodium hydroxide, acetone, chloroform, ethanol, ether and methanol as necessary. Each compound was studied in duplicate.

The influence of amiodarone on iodothyronine distribution among the three principal thyroid hormone-binding proteins was examined by reverse-flow electrophoresis in three types of sample: (a) serum stripped of iodothyronines (by multiple passes through Amberlite CG 400 ion-exchange resin) to which 12·5 mg litre^{-1} amiodarone had been added; (b) serum from a normal healthy individual containing endogenous thyroid hormones with 12·5 mg litre^{-1} added amiodarone; (c) serum from two patients on treatment with amiodarone. Reverse-flow electrophoresis of serum labelled with high specific activity [^{125}I]T4, [^{125}I]T3 or [^{125}I]rT3 (Amersham International, UK) equilibrated for at least 30 min at room temperature, was carried out in 50 mM Tris-maleate buffer, pH 8·6, on Whatman 3MM paper strips at constant current (10 mA) for 16 h, employing a modified version of the technique described by Robbins (1956). The strips were dried and scanned for radioactivity (Panax scanner) and then cut into 2 mm sections and the radioactivity measured.

RESULTS

Gel filtration of serum trace-labelled with [^{125}I]amiodarone resulted in only two radioactive peaks (Fig. 1). The first peak coincided with β-lipoprotein and carried 33·5 per cent of the total amiodarone tracer recovered whilst the second was coincident with albumin and bound 62·1 per cent of tracer. The position of α_1-lipoprotein was between these two peaks.

Scatchard (1949) analysis of binding data from equilibrium dialysis experiments for the interaction between amiodarone and pure albumin showed two types of interaction (Fig. 2a). The association constants (K_a) for these interactions were K_a $5·6 \times 10^6$ litre mol^{-1} and K_a $1·9 \times 10^5$ litre mol^{-1}. An estimate of the number of binding sites revealed 1·0 high affinity site and 4·4 additional low affinity sites per molecule of albumin. Hill (1910) plot analysis (Fig. 2b) gave a straight line for the high affinity Scatchard data with a Hill coefficient of 0·98 confirming only one high affinity amiodarone binding site per albumin molecule.

In contrast, when binding studies were carried out using whole serum, Scatchard analysis (Fig. 3a) indicated only one type of binding site with a K_a of $4·2 \times 10^6$ litre mol^{-1} (mean of two estimates) and the Hill plot (Fig. 3b) was linear and gave a coefficient of 1·0. These results suggest that only one kind of amiodarone binding site is utilized in whole serum

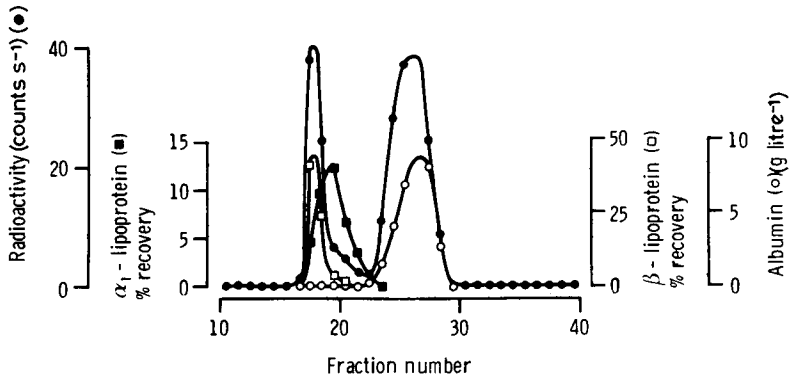

FIG. 1. Distribution of [^{125}I]amiodarone tracer in normal serum after chromatography on a column (70 × 1·5 cm) of Bio-Gel P200 (<400 mesh). The column was eluted (2 ml h^{-1}) with 0·05 M phosphate buffer, pH 7·4 and 2 ml fractions collected. Albumin, β-lipoprotein and α_1-lipoprotein concentrations were measured by 'rocket' immunoelectrophoresis.

and that it has an affinity similar to that of the primary amiodarone binding site on albumin.

The influence on [^{125}I]amiodarone binding to albumin by a variety of compounds is illustrated in Fig. 4. Low recoveries of [^{125}I]amiodarone (range 13·6–61·9%) observed are probably due to adsorption of the drug to the apparatus perhaps due to its low solubility in aqueous media. Binding was therefore expressed as a percentage of recovered activity. The percentage of [^{125}I]amiodarone bound to albumin at equilibrium was 87·4. In the presence of added test compounds the percentage bound ranged between 76·8 and 95·5. Lower percentage bound values, however, were not necessarily associated with low recoveries, nor higher percentage bound values with high recoveries. Changes in percentage bound when compounds were added therefore represent changes in binding. The overall mean responses to the additions were shown to be significantly different (F test $P < 0{\cdot}005$) using a linear regression model. The model was also used to calculate the mean for each drug. The change in binding of amiodarone caused by the presence of each compound is shown in Table 1.

The drugs warfarin, 99% bound to albumin (Wilding et al 1977), and digoxin, 23% bound to albumin (Lukas & De Martino 1969), are known to become more potent in patients also treated with amiodarone. Neither significantly affected albumin-bound [^{125}I]amiodarone when tested at concentrations equimolar to that of albumin and many times greater than occur during therapy (Fig. 4, Table 1). Thus, the interaction seen clinically between warfarin or digoxin and amiodarone must be other than at the amiodarone-binding sites on albumin. Amiodarone-treated patients have increased serum T4 concentrations (Pritchard et al 1975), however T4 had no effect on [^{125}I]amiodarone binding to albumin (Table 1). Thus, it is also unlikely that this hormone and amiodarone have common binding sites on albumin.

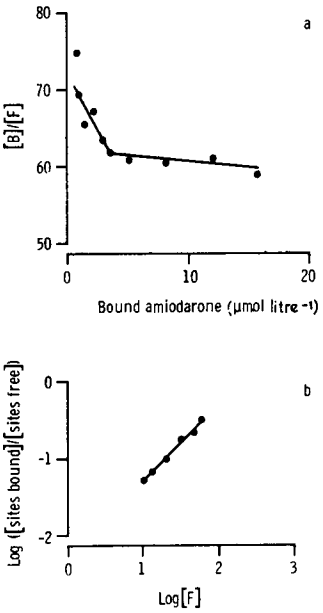

FIG. 2. Analysis of equilibrium binding data for pure albumin-amiodarone interactions. Fig. 2a. Scatchard plot showing two types of interaction with association constants of 5·6 × 10^6 and 1·9 × 10^5 litre mol^{-1}. Extrapolation of the plots to the abscissa after correction (Feldman 1972) revealed 1·0 high affinity site and 4·4 additional low affinity sites per molecule of albumin. Fig. 2b. Hill plot for the data representing the high affinity interaction in Fig. 2a was linear with a Hill coefficient of 0·98 thus confirming a single site.

Table 1. Linear regression analysis of the mean percentage albumin-bound [^{125}I]amiodarone ($74{\cdot}4 \times 10^{-8}$ M) response to the presence of compounds added at the same molar concentration as albumin (3×10^{-4} M). For [^{125}I]amiodarone alone the mean percentage bound was 87·38 with a standard error of 0·89. The compounds which significantly decreased or increased albumin-bound [^{125}I]amiodarone are shown by asterisks: * $P < 0{\cdot}05$; ** $P < 0{\cdot}001$.

Compound	Estimate	Standard error	t
Ca^{2+}	0·41	1·84	0·22
Mn^{2+}	−0·36	1·84	−0·20
L-Tryptophan	−0·06	1·84	−1·66
Cu^{2+}	−2·73	1·84	−1·48
Ni^{2+}	−2·37	1·84	−1·29
Palmitate	−9·64	1·84	−5·25**
(−)-Noradrenaline	8·33	1·84	4·53**
(−)-Adrenaline	5·45	1·84	2·96*
Bilirubin	1·24	2·44	0·51
L-Thyroxine	−0·53	2·44	−0·22
Bromocresol purple	8·74	1·84	4·76**
Bromocresol green	6·59	1·84	3·59**
Bromosulphophthalein	−0·08	2·44	−0·03
Frusemide	4·20	1·84	2·28*
Phenytoin	4·05	1·84	2·20*
Digoxin	1·92	1·84	1·04
Erythromicin	3·64	1·84	1·98
Tolbutamide	2·58	1·84	1·40
Rifampicin	5·42	1·84	2·95*
Diazoxide	1·09	2·44	0·45
Fenoprophen	0·85	2·44	0·35
Gentamicin	0·67	2·44	0·27
Warfarin	0·33	1·84	0·18
Glibenclamide	−1·23	2·44	−0·50
Propranalol	−2·22	2·44	−0·91
Trifluoperazine	−3·24	2·44	−1·33
Salicylate	−3·54	2·44	−1·45
Methadone	−2·13	1·84	−1·16
Cephazolin	−9·69	1·84	−5·27**
Amitriptyline	−15·31	1·84	−8·33**
Quinidine	−12·14	1·84	−6·61**

[^{125}I]Amiodarone binding to albumin was reduced in the presence of quinidine, amitriptyline, cephazolin and palmitate. Several compounds including frusemide, phenytoin, rifampicin, (−)-noradrenaline, (−)-adrenaline, bromocresol purple and bromocresol green apparently increase the binding of [^{125}I]amiodarone to albumin (Table 1).

The iodothyronine distribution among their binding proteins, TBG, TBPA and albumin in the presence of amiodarone was investigated by reverse-flow electrophoresis (Table 2). Amiodarone did not enhance or inhibit iodothyronine binding to any of their transport proteins nor their distribution among their binding proteins whether they were tested individually or in their natural combination. The distribution of iodothyronines amongst TBG, TBPA and albumin in serum was normal in the patients

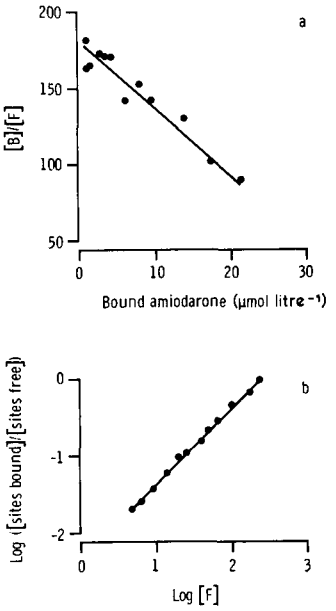

FIG. 3. Analysis of equilibrium binding data for whole serum-amiodarone interactions. Fig. 3a. Linear Scatchard plot giving a single affinity constant of $4{\cdot}2 \times 10^6$ litre mol^{-1}. Fig. 3b. Hill plot from the same data, with a Hill coefficient of 1·0. Both plots indicate a single order of binding sites in whole serum for amiodarone.

treated with amiodarone and so it is unlikely that chronic exposure to amiodarone or any metabolic product of amiodarone either enhances or inhibits iodothyronine binding to their transport proteins. Nor did prolonged amiodarone therapy significantly alter iodothyronine-binding protein levels in the serial serum samples from one patient. Before treatment the concentrations were TBG: 9·6 mg litre^{-1} (N.R. 6–16 mg litre^{-1}); TBPA: 172 mg litre^{-1} (N.R. 150–350 mg litre^{-1}); albumin: 31·5 g litre^{-1} (N.R. 35–50 g litre^{-1}), after 49 days treatment they were TBG: 9·8 mg litre^{-1}; TBPA: 190 mg litre^{-1}; albumin: 37·5 g litre^{-1} and six months later they were TBG: 9·3 mg litre^{-1}; TBPA: 184 mg litre^{-1}; albumin: 37·8 g litre^{-1}.

DISCUSSION

Amiodarone is largely (96%) bound by two serum components. Albumin transports two thirds of the available drug and the remainder is carried by β-lipoprotein (or a molecule of similar molecular weight). A number of compounds dramatically influence the binding of amiodarone to albumin. Some displace the drug whilst others enhance its binding. These effects explain some, but not all,

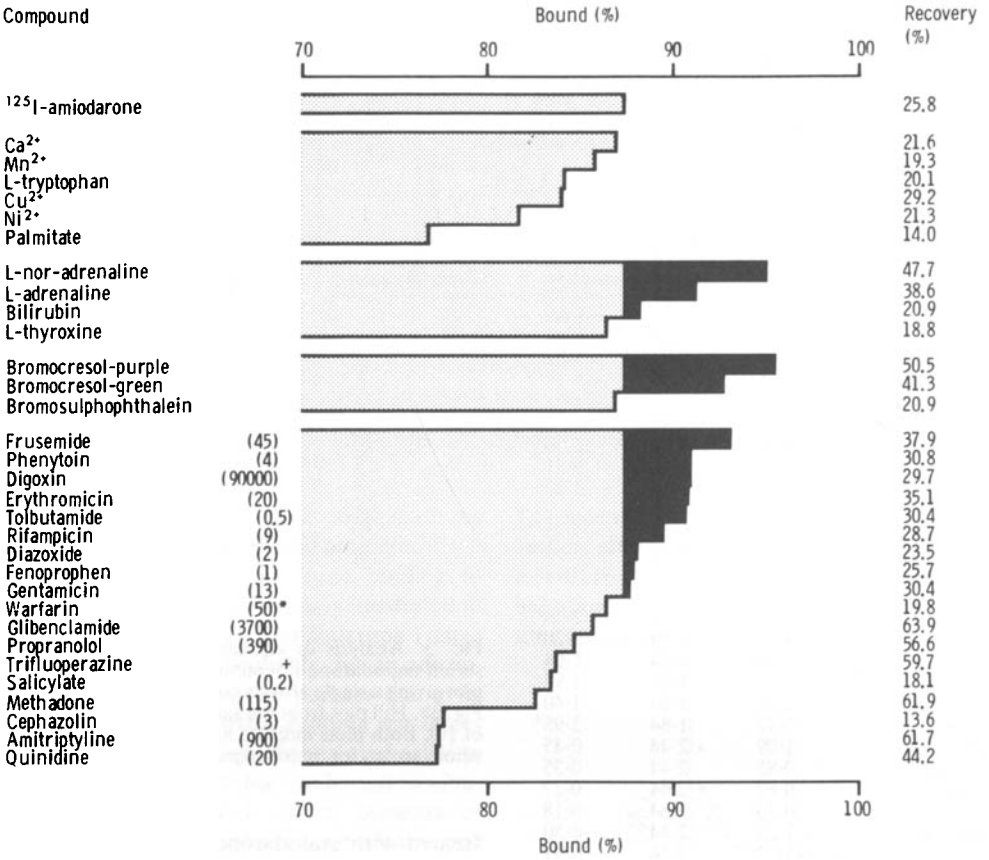

FIG. 4. The influence of various compounds on albumin-bound [^{125}I]amiodarone. Pure albumin (3×10^{-4} M) was pre-equilibrated with [^{125}I]amiodarone tracer ($74{\cdot}4 \times 10^{-8}$ M); each test compound was added to the trace-labelled albumin solution at the same molar concentration as albumin. The percentages of [^{125}I]amiodarone bound to albumin in the presence of added compounds are shown as histograms. The boundary between stippled and black areas is the percentage bound (87·4%) obtained with [^{125}I]amiodarone in the absence of added test compound. Recoveries of radioactivity in the presence of each compound are shown in the right hand column. The approximate ratio of the experimentally used concentration to the therapeutic circulating level of each drug is shown in brackets.

* Doses vary over wide range.

† Therapeutic circulating level not available.

Table 2. Percentage distribution of high specific activity [^{125}I]T4, [^{125}I]T3 and [^{125}I]rT3 among the serum binding proteins TBG, TBPA and albumin (Alb) separated by reverse-flow electrophoresis in the absence or the presence of amiodarone. Three types of sample were used. (1) Thyroid hormone-free serum. (2) Normal serum (the percentage distribution of [^{125}I]T4 with and without added stable T4 is shown). (3) Sera from two patients treated with amiodarone.

	% [^{125}I]T4 Distrib.			% [^{125}I]T3 Distrib.			% [^{125}I]rT3 Distrib.		
	TBG	TBPA	Alb	TBG	TBPA	Alb	TBG	TBPA	Alb
Thyroid hormone free serum	84·3	6·8	8·9	78·1	0	21·9	37·4	0	62·7
Thyroid hormone free serum + 12·5 mg litre^{-1} amiodarone	83·3	6·9	9·8	80·3	0	19·7	39·4	0	60·6
Normal serum	76·4	13·2	10·4	69·7	0	30·3	28·9	0	71·1
Normal serum + 12·5 mg litre^{-1} amiodarone	77·4	14·4	8·2	72·9	0	27·1	30·7	0	69·3
Normal serum + 1·0 μmol litre^{-1} T4	25·5	39·7	34·8						
Normal serum + 1·0 μmol litre^{-1} T4 + 12·5 mg litre^{-1} amiodarone	26·9	39·3	33·9						
Patient 1 serum	80·2	8·8	11·0	76·2	0	23·8	37·7	0	62·3
Patient 2 serum	76·4	14·3	9·3	73·4	0	26·6	33·3	0	66·7

amiodarone–drug/hormone interactions seen in practice and indicate some situations where interactions may be found in future.

Albumin has an uncommonly high affinity primary binding site for amiodarone (K_a $5{\cdot}6 \times 10^6$ litre mol^{-1}) and about four secondary sites of lower affinity (K_a $1{\cdot}9 \times 10^5$ litre mol^{-1}). These secondary sites do not appear to be utilized in serum since studies using whole serum indicated only one type of binding site with an affinity constant similar to that of the primary site found on pure albumin. It may thus be inferred that the affinity of amiodarone for β-lipoprotein is of the same order as for the primary binding site on albumin. The avidity of albumin for most synthetic compounds is generally lower ($K_a < 5 \times 10^5$ litre mol^{-1}) than we have determined for amiodarone although the antitumour agent camptothecin, which is 98·3% bound to protein in human plasma, has a K_a of $7{\cdot}9 \times 10^6$ litre mol^{-1} for a single site (Guarino 1973). Albumin has also been reported to have multiple binding sites for some other drugs (e.g. four sites for salicylate K_a $2{\cdot}2 \times 10^5$ litre mol^{-1}; and four to five sites for indomethacin K_a $8{\cdot}4 \times 10^5$ litre mol^{-1}; Hultmark et al 1975).

The possibility that albumin binding of amiodarone may be affected by other substances was examined by employing a wide range of natural and synthetic compounds. The potentiation of warfarin, digoxin and quinidine action in patients treated with amiodarone has already been noted. However, in our studies only quinidine significantly reduced [^{125}I]amiodarone binding to albumin (Fig. 4, Table 1). Tartini et al (1982) found raised plasma levels of quinidine in a healthy volunteer on amiodarone, and atypical ventricular tachycardia developed in two patients treated with both amiodarone and quinidine. Our findings indicate that the strong mutual potentiating effect of amiodarone and quinidine is due to a displacement interaction of both drugs on the albumin molecule. However, quinidine as well as other drugs may also affect amiodarone binding to lipoprotein; this was not investigated. Thus our results may only partially describe the multi-ligand-binding interactions which occur in-vivo and which may be complicated further by interactions on β-lipoproteins.

Both warfarin and amiodarone are strongly bound by albumin. In this study warfarin failed to displace albumin-bound [^{125}I]amiodarone (Table 1), indicating that the potentiation is not related to albumin binding. This supports the findings of Serlin et al (1981) who used equilibrium dialysis to examine warfarin binding to plasma from a patient also treated with amiodarone. They showed that the percentage unbound plasma warfarin did not alter during amiodarone treatment, and proposed that the mechanism of amiodarone-warfarin interaction is due to reduced warfarin catabolism. Digoxin also failed to reduce albumin bound [^{125}I]amiodarone in our study. The amiodarone-digoxin interaction also is therefore unlikely to be at the serum albumin level. This supports observations that digoxin is not very strongly bound to albumin (Lukas & De Martino 1969; Doherty & Hall 1971; Ohnaus et al 1972) but significantly tissue bound (Coltart et al 1972). The increased potency of digoxin in patients treated with amiodarone may be related more to a tissue effect rather than a circulating protein interaction or perhaps to interference with the biological clearance of digoxin.

Two other drugs were found to significantly reduce [^{125}I]amiodarone binding to albumin. These were amitriptyline and cephazolin. There is very little, if any, structural similarity between these two drugs and amiodarone, and hitherto there have been no reports of clinical interactions. The nutrient palmitate reduced [^{125}I]amiodarone binding to albumin which may indicate a potential dietary influence on amiodarone's efficacy.

Only trace amounts of [^{125}I]amiodarone were used in this study and so the drug would have been entirely bound to the high affinity site on albumin. Thus, any amiodarone-compound interaction observed is probably chiefly due to the influence of the compound on the primary amiodarone binding site on albumin. However, whether the amiodarone-compound interaction is allosteric or competitive in nature for the binding site on albumin remains to be elucidated. Fatty acid inhibition of drug binding has been reported by Rudman et al (1971) to increase with increasing concentration of fatty acid, and to result either in an apparent reduction in the number of drug binding sites or in a reduced K_a value for that binding site. Some of the drug interactions however may possibly be explained by anti-co-operative allosteric displacement effects (Kragh-Hansen 1983).

Several compounds both natural and synthetic apparently enhanced the binding of [^{125}I]amiodarone to albumin (Fig. 4, Table 1). These include the drugs frusemide, phenytoin and rifampicin, the catecholamines and the dyes bromocresol purple and bromocresol green. The mechanism for this enhanced binding of [^{125}I]amiodarone to albumin may be a conformational change in albumin induced by the binding of ligand resulting in an increased affinity

for amiodarone in the primary or possibly one of the secondary binding sites. If the converse to this allosteric effect holds true (i.e. co-operative allosterism) the presence of amiodarone on albumin may result in increased binding of drug, catecholamine or dye. Both bromocresol purple and bromocresol green are used in serum albumin estimations. Excessive binding of these dyes in the sera from patients on amiodarone therapy could lead to erroneous serum albumin measurements.

Amiodarone treatment tends to lead to an increase in total serum T4 concentration, a drop in T3 concentration and elevated free-T4 and rT3 serum levels. Our results show that these changes in iodothyronine levels are not related to transport effects. The hormone T4 failed to influence albumin-bound [^{125}I]amiodarone. Amiodarone did not change the distribution amongst the proteins of iodothyronines in sera from two patients on prolonged amiodarone treatment. Neither did the amiodarone or a metabolite of the drug in the sera of these patients affect the binding properties of the proteins nor did levels of the proteins alter significantly in the serial serum samples from one patient. It is possible to conclude that the widely observed effects of amiodarone on thyroid hormone levels are in no way due to influences on the three iodothyronine-binding proteins. The effects of amiodarone on thyroid hormone concentrations are most probably due to an inhibition of the monodeiodination of T4 to T3 (Lambert et al 1982) with reduced rT3 catabolism.

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