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UPTAKE, SUBCELLULAR DISTRIBUTION AND BIOTRANSFORMATION OF ^{3}H-LABELLED ASTEMIZOLE IN CULTURED RAT HEPATOCYTES

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Abstract—When incubated with ^{3}H-astemizole, a potent antagonist of H_1 receptor, cultured rat hepatocytes, which do not express specific receptors for this ligand, avidly take up ^{3}H-label proportionally to the drug concentration. HPLC analysis indicates that at 10 ng ^{3}H-astemizole/ml, cells almost entirely deplete the culture medium of the drug within 4 hr of incubation. At 37°, astemizole is metabolized and released into the culture medium mainly under the form of glucuronoconjugated metabolites.

Differential centrifugation of homogenates from hepatocytes incubated with ^{3}H-astemizole indicates that astemizole and unconjugated metabolites are found in the particulate fraction, whereas astemizole and conjugated metabolites are present in the cytosol. Isopycnic centrifugation on sucrose gradient shows that the major part of the ^{3}H-label in the particulate fraction distributes like phospholipids and NADPH cytochrome *c* reductase, suggesting an association with membranes and, in particular, with the endoplasmic reticulum.

Chloroquine, a drug accumulating within lysosomes and acidic endosomes, decreases the uptake of ^{3}H-astemizole by hepatocytes and induces, during isopycnic centrifugation of a particulate fraction, a shift of the ^{3}H-label towards lower densities where it closely accompanies cathepsin B. This suggests that a minor part of astemizole accumulated in the hepatocytes could be trapped within lysosomes. These results could support the hypothesis that aspecific binding of astemizole to cellular membranes and, to a lesser extent, trapping in lysosomes could play a role in the pharmacokinetics of the drug.

Astemizole, whose chemical structure is illustrated in Fig. 1, is a safe, potent and long-acting histamine H_1 antagonist, marketed under the name "Hismanal"®, by Janssen Pharmaceutica [1].

The long duration of action of astemizole seems directly related to unusual receptor binding properties and more precisely to the extremely slow dissociation rate from the H_1 receptor [2]. After oral administration of the ^{14}C-labelled drug to man, plasma radioactivity peaks within 1 hr indicating a rapid absorption from gastrointestinal tract. Less than 5% of the radioactivity is associated with native astemizole, suggesting that there is a first-pass effect

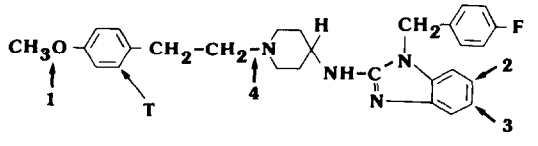

Fig. 1. Chemical structure of ^{3}H-astemizole and its main metabolites: 1, desmethylastemizole; 2, 6-hydroxyastemizole; 3, 5-hydroxyastemizole; 1 + 2 or 1 + 3, 6- or 5-hydroxydesmethylastemizole; 4, norastemizole; T, position of the ^{3}H-label.

followed by liver metabolization and plasmatic release of labelled metabolites. Astemizole and its metabolites distribute extensively within well-perfused organs, such as liver, lungs and kidneys; much lower concentrations are found in muscles and brain [3].

Study of astemizole metabolism carried out in rat, guinea pig, dog and man indicates large qualitative similarities [4]. As illustrated in Fig. 1, the main metabolic pathway of astemizole is, in man, *O*-demethylation resulting in a desmethylastemizole. Other metabolites appeared after aromatic hydroxylation giving hydroxyastemizole or hydroxydesmethylastemizole. These phenolic metabolites can thereafter be further glucuronoconjugated. Since the biotransformation of astemizole in the various species resulted in relatively minor modifications of the parent drug, the major metabolites of astemizole have been tested for pharmacological activity and it was concluded that, in addition to the parent drug, desmethylastemizole could contribute to the pharmacologic activity in animals and man [5].

The excretion of astemizole after oral administration in man is slow, amounting to only 40–54% of the dose at 4 days and to 61–79% at 14 days. Metabolites are mainly found in faeces pointing out an extensive biliary excretion, which probably further involves an enterohepatic circulation of the drug [4].

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Both pharmacokinetic and biotransformation studies point out that the liver plays a key role in the bioavailability of astemizole. This prompted us to study further the pharmacology of astemizole at the cellular level in a well-characterized model. Rat hepatocytes, in short term culture up to 2–3 days indeed keep most of their physiological functionality and have been widely used for cell biology and pharmacotoxicology studies (see e.g. refs. 6–12). The kinetics of uptake, the intracellular accumulation and the metabolism of ^{3}H-labelled astemizole were carefully investigated. Cell fractionation techniques were used to establish the subcellular localisation of ^{3}H-labelled astemizole and its metabolites.

Astemizole is a lipophilic weak base (log P between *n*-octanol and aqueous buffer at pH 9.1 is 5.10; $pK_{a1} = 5.64$; $pK_{a2} = 8.35$) and could, like many other similar substances, be concentrated within lysosomes [13] but also in other acidic intracellular organelles such as endosomes [14]. This could result in the alteration of cellular functions such as, for example, the endocytic pathway [15], but also induce the intracellular trapping of the drug and its lipophilic basic metabolites, affecting therefore the pharmacokinetics of the drug. We therefore also studied the effect of chloroquine, a drug known to accumulate within lysosomes [13, 16] and to inhibit the intralysosomal accumulation of drugs such as, for example, muscarinic receptor ligands [17].

MATERIALS AND METHODS

Isolation and culture of hepatocytes. Hepatocytes were isolated as previously described [6] from adult Wistar male rats (*ca.* 250 g) obtained from the local animal house. They were cultivated on 20 cm^2 gas-permeable Petri dishes (Petriperm, Heraeus Danau, F.R.G.), previously coated with collagen (Vitrogen, Flow laboratories, Brussels, Belgium) at 15 μg/ml in phosphate buffered saline (PBS), in Dulbecco's modified Eagle's medium (Gibco-BRL, Ghent, Belgium) supplemented with 15% (v/v) fetal calf serum (FCS: Gibco-BRL), 4 mM glutamine, 20 mM glucose, 100 U/ml of penicillin and 100 μg/ml of streptomycin, under 10% CO_2 (v/v) in air. After a few hours, the cells adhered to the dish and started to reassociate; after 4–6 hr hepatic-like traberculae and bile canaliculi progressively reappeared; after about 16 hr, a complete monolayer was reformed for at least one week [6]. For the experiments, only cells cultured from one day were used.

Binding to H_1 receptor. Cultured hepatocytes were harvested with a rubber policeman in 10 mM Tris–HCl pH 7.4 and homogenized in a dual tube by 3 up and down strokes. The homogenate was then diluted with the same buffer to 0.5 mg protein/ml. The binding was carried out using ^{3}H-pyrilamine (27.3 Ci/mmole, The Radiochemical Centre, Amersham, U.K.) as labelled ligand and different concentrations of astemizole, during a 30 min assay at 37°, as in ref. 2.

Kinetic experiments. Confluent cultures of hepatocytes were incubated with 2 ml of medium containing 15% of FCS* with or without different concentrations of ^{3}H-astemizole, in the presence or absence of 50 μM chloroquine, for different durations at 37°. At the end of the incubation, the culture medium was removed, centrifuged for 5 min at 2000 r.p.m. in order to eliminate dead cells and analysed for the presence of ^{3}H-labelled material as well as, in some experiments, for the presence of labelled metabolites (see below). Cells were then washed 3 times with 2 ml of PBS, once with 2 ml of culture medium containing FCS and twice with 2 ml of PBS. For some experiments, cells were reincubated in 2 ml of culture medium containing FCS with or without chloroquine for different durations and then handled as above. After washings, cells were dissolved in 1.5 ml of 1% (w/v) Na deoxycholate adjusted to pH 11.3 with NaOH and analysed for protein content according to [18] using bovine serum albumin as standard and for radioactivity after dispersion of 1 ml of sample in 10 ml Aqualuma cocktail (Lumac, Basel, Switzerland) in a Tri-Carb CD 460 (Packard Instruments, San Diego, CA).

Chloroquine was purchased from local pharmacy; ^{3}H-astemizole labelled on the ortho position (Fig. 1) of the methoxyphenylgroup (28.7 Ci/mmole, radiochemically pure) was obtained from Janssen Life Sciences Products (Beerse, Belgium) after synthesis by selective catalytic dehalogenation of the 2-Br analogue with tritium [19].

Cell fractionation. After washings (as for kinetic experiments) the contents of five dishes were harvested in 3 ml of 0.25 M sucrose containing 3 mM imidazole pH 7.0 and homogenized by 5 strokes of the tight pestle of the Dounce homogenizer (Kontes Glass Co., Vineland, NJ). The nuclear fraction (N) was separated, as in ref. 6, from the cytoplasmic extract by a 10 min centrifugation at 1700 r.p.m. (rotor 259, IEC centrifuge, Needham Heights, MA) followed by two washings in the same conditions. The cytoplasmic extract was further separated into a particulate fraction (MLP) and a final supernate (S) or into a heavy mitochondrial fraction (ML), a microsomal fraction (P) and a final supernate (S) by centrifugation for respectively 6 min at 25,000 r.p.m. (ML) or 30 min at 40,000 r.p.m. (P or MLP) in a Ti50 rotor and a Beckman L5-50 centrifuge (Beckman, Palo Alto, CA) as described in ref. 6.

The MLP, ML or P fraction resuspended in 0.25 M sucrose–3 mM imidazole at pH 7.0 was then applied at the top of a linear sucrose gradient ranging from 1.10 to 1.30 g/ml and centrifuged for 90 min at 49,000 r.p.m. in a VTi50 rotor (Beckman). Fractions were collected and analysed; results were presented as in ref. 20; 5′-nucleotidase activity was assayed as in ref. 21; cathepsin B as in ref. 22; cytochrome *c* oxidase as in ref. 23; NADPH cytochrome *c* reductase as in ref. 24, and phospholipids as in ref. 25.

* Abbreviations used: FCS, fetal calf serum; IC_{50}, drug concentration that inhibits 50% of the specific binding; MLP is the combination of the M, L and P fraction and ML, that of M and L; N, M, L, P and S correspond respectively to the nuclear, heavy mitochondrial, light mitochondrial and microsomal fractions and to the final supernate; PBS, phosphate buffered saline (0.14 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4–KH_2PO_4 pH 7.4); enzymes, cathepsin B (EC 3.4.22.1), cytochrome *c* oxidase (EC 1.9.3.1), NADPH cytochrome *c* reductase (EC 1.6.2.3); 5′ nucleotidase (EC 3.1.3.5).

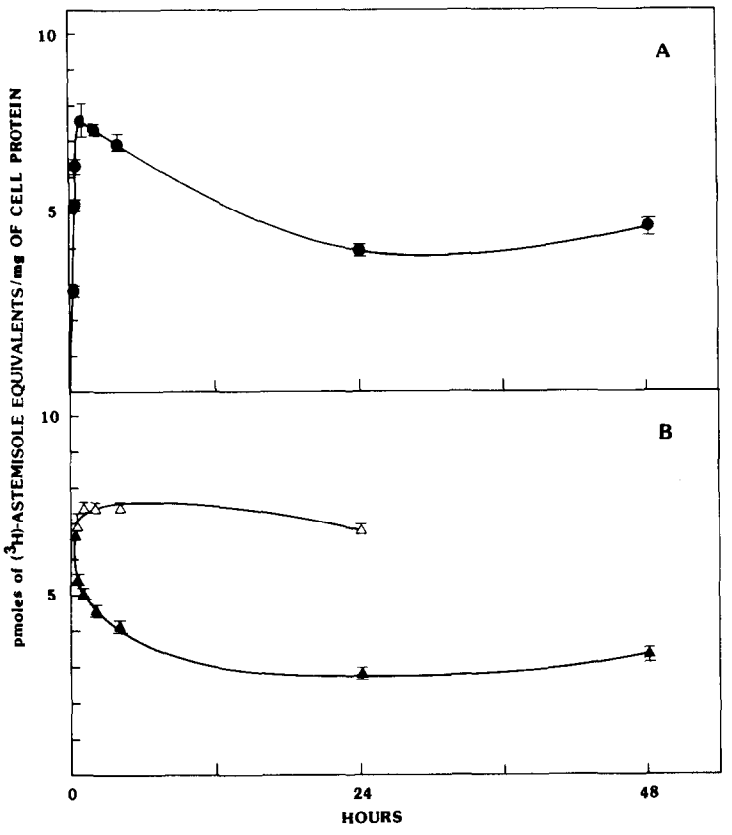

Fig. 2. Uptake and release of ^{3}H-astemizole by cultured rat hepatocytes. The cells were either incubated for different durations at 37° in the presence of 10 ng/ml of ^{3}H-astemizole (A, ●) or preincubated for 4 hr at 37° with 10 ng/ml of the drug and reincubated for different durations in a fresh culture medium without drug at 4° (B, △) or at 37° (B, ▲). Experimental protocol as described in Materials and Methods; mean results of three independent experiments ±SD are given.

Detection of metabolites. Culture media, cell lysates (obtained by dispersing cells with 1% (w/v) Triton X-100 and centrifugation for 30 min at 40,000 r.p.m. in a Ti50 rotor) or subcellular fractions were analysed by HPLC (Perkin Elmer, Norwalk, CT). The separation of metabolites was performed on a 30 cm × 4.6 mm column, packed with Lichrosorb RP-18 (10 μm) (Merck, Darmstadt, F.R.G.). The metabolites were eluted by a linear gradient running from 100% 0.1 M ammonium acetate at pH 8.5 (solvent A), to 10% (v/v) of solvent A and 90% of 1.0 M ammonium acetate–methanol–acetonitrile (10:45:45 v/v/v) over a 40 min period (flow rate 1 ml/min). UV monitoring (Perkin Elmer LC 85) was carried out at 280 nm and the radioactivity was monitored on-line using a Berthold Radioactivity Monitor LB 504 (Berthold, Wildbad, F.R.G.) using Picofluor TM30 (Packard) as scintillation medium [26]. Reference metabolites have been characterised previously [4].

For some experiments samples were previously incubated for 24 hr at 37° with beta-glucuronidase and arylsulfatase (Boehringer, Mannheim, F.R.G.) in 0.1 M acetate pH 5, in the presence or absence of 20 mM saccharo 1,4-lactone (Sigma, St Louis, MO) used as a specific inhibitor of beta-glucuronidase. ^{3}H-astemizole was also incubated for 6 hr at 37° in the presence of 100 μg/ml of purified lysosomal enzymes in 0.5 M acetate buffer at pH 4.8 containing 10 mM cysteine as in ref. 27.

RESULTS

Binding to H_1 receptor

When tested in a homogenate from hepatocytes, the IC_{50} of astemizole, using ^{3}H-pyrilamine as labelled ligand for the H_1 receptor, was higher than 10^{-6} M (not illustrated). When compared to the IC_{50} obtained with rat cerebellum (IC_{50}: 40 nM) [2], this strongly suggests the absence of specific receptor for astemizole on hepatocytes.

Kinetic experiments

In a first set of experiments, hepatocytes were incubated for 2 hr at 37° in the presence of different concentrations of ^{3}H-astemizole. At extracellular concentrations ranging from 0.1 nM to 10 μM, the amount of cell-associated ^{3}H-label is strictly proportional to the external concentration and no toxic effects can be visualized by phase contrast microscopy (not illustrated).

In a second set of experiments, hepatocytes were

incubated with 10 ng/ml of ^{3}H-astemizole for different durations. As shown in Fig. 2A, the amount of cell-associated ^{3}H-label increases rapidly to reach a maximum after 1 hr and then decreases until 48 hr. The maximal level of cell accumulation corresponds to a 51% depletion of the ^{3}H-astemizole present at the beginning of the experiment in the culture medium. Comparable profiles but respectively lower or higher accumulation levels were observed when cells were incubated in the presence of 1 or 100 ng/ml of ^{3}H-labelled drug (not shown).

Hepatocytes preincubated for 4 hr at 37° with 10 ng/ml of ^{3}H-astemizole were reincubated for different durations with a fresh medium in the absence of the drug. As shown in Fig. 2B, when the experiment is carried out at 4°, no release of labelled material was observed throughout the washout period; in contrast, at 37°, ^{3}H-label is quickly released from the cells and a plateau is reached after 24 hr, corresponding to about 50% of the initial accumulation level.

Since astemizole is a lipophilic base, which could concentrate within lysosomes and endosomes in a process which could be inhibited by high concentration of other lipophilic bases such as chloroquine [17], hepatocytes were incubated for different durations with 10 ng/ml of ^{3}H-astemizole in the presence or absence of 50 μM chloroquine. As shown in Fig. 3A, in the absence of chloroquine the amount of cell accumulated ^{3}H-label increases up to 4 hr, whereas in the presence of the drug, it increases up to 1 hr, reaching a level corresponding to 75% of the control, and then decreases. During a washout experiment (Fig. 3B) similar profiles were observed in the presence or absence of chloroquine, although a lower accumulation level was reached with the drug.

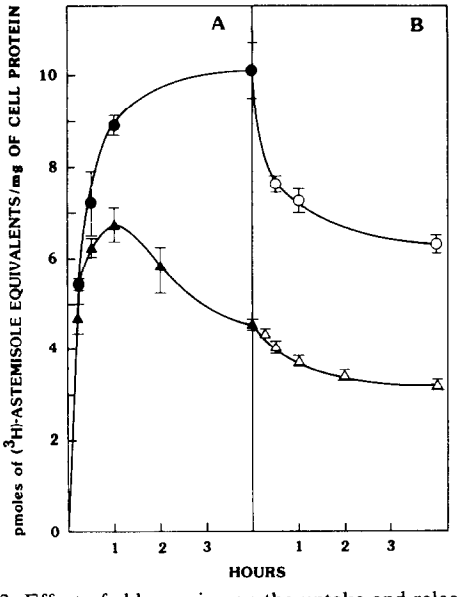

Fig. 3. Effect of chloroquine on the uptake and release of ^{3}H-astemizole. Cultured hepatocytes were either incubated for different durations at 37° with 10 ng/ml of ^{3}H-astemizole in the presence (A, ▲) or absence (A, ●) of 50 μM chloroquine or preincubated for 4 hr at 37° with 10 ng/ml of ^{3}H-astemizole and reincubated for different durations at 37° in a fresh culture medium in the presence (B, △) or absence (B, ○) of 50 μM chloroquine. Experimental protocol as described in Materials and Methods; mean results of three independent experiments ±SD are given.

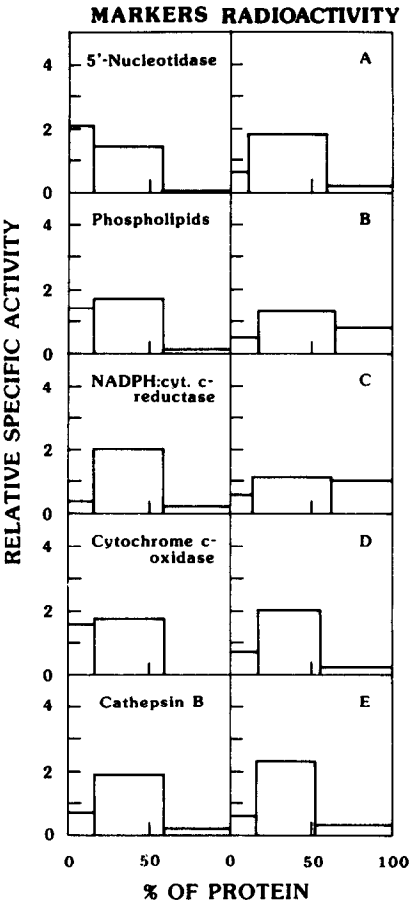

Fig. 4. Differential centrifugation in 3 fractions of homogenates from hepatocytes incubated with ^{3}H-astemizole. Cells were incubated for 1 hr at 37° in the presence of 1 (A), 10 (B) or 100 ng/ml (C) of ^{3}H-astemizole or for 4 hr with 10 ng/ml of ^{3}H-astemizole, washed and reincubated for 1 hr (D) or 16 hr (E) at 37° in a fresh culture medium in the absence of ^{3}H-astemizole. After incubation, the cells were homogenized and separated by differential centrifugation into N, MLP and S fractions as described in Materials and Methods. The fractions are respectively, from left to right, N, MLP and S. The distribution of the marker enzymes or constituents are the mean of the seven independent experiments.

Subcellular distribution

Cultured hepatocytes, to which ^{3}H-astemizole has been added after homogenization, or incubated for different durations with ^{3}H-astemizole with or without reincubation in the absence of the drug, have been fractionated. As indicated in Fig. 4, the bulk of the assayed enzymes, the phospholipids and the ^{3}H-label are found associated with the particulate fraction (MLP) (not illustrated for the experiment in which the drug was added to the homogenate); significant proportions of 5′-nucleotidase, phospho-

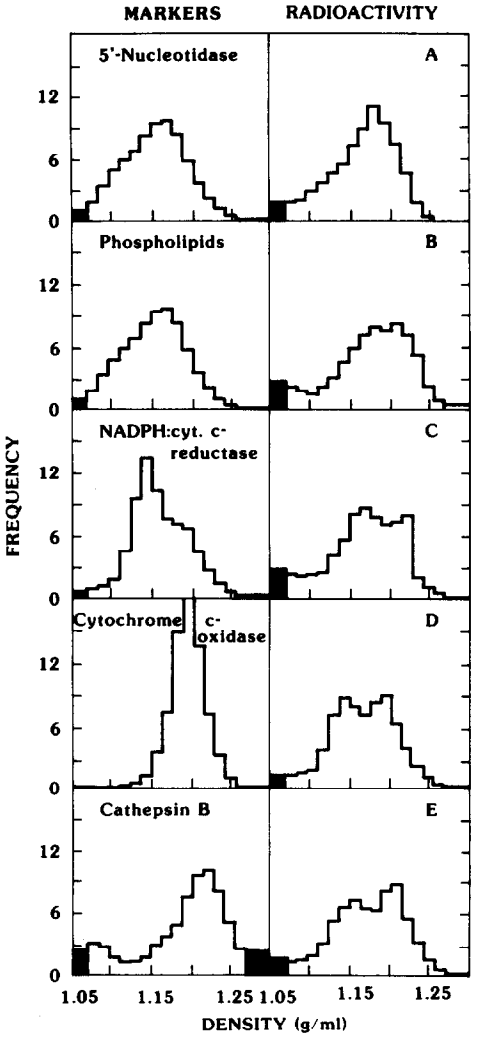

Fig. 5. Isopycnic centrifugation on sucrose gradients of MLP fractions from homogenates of cultured hepatocytes. (A) ^{3}H-astemizole added to the homogenate; (B) cells incubated for 1 hr with 10 ng/ml of ^{3}H-astemizole; (C) same as in B but with 100 ng/ml; (D) cells incubated for 4 hr with 10 ng/ml ^{3}H-astemizole followed by 1 hr washout; E: same as in D but with 16 hr reincubation. Results are expressed as in ref. 20. The distribution of marker enzyme and constituents are the mean of the seven independent experiments.

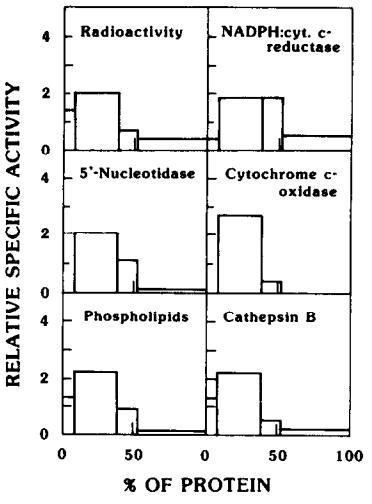

Fig. 6. Differential centrifugation in 4 fractions of homogenates from hepatocytes incubated with ^{3}H-astemizole. Cells incubated for 1 hr at 37° with 10 ng/ml of ^{3}H-astemizole, were homogenized and fractionated by differential centrifugation into N, ML, P and S fractions. Experimental protocol as described in Methods. The fractions are respectively from left to right N, ML, P and S.

lipids and cytochrome *c* oxidase are also present in the nuclear fraction (N). For the cells which have been incubated for 1 hr with 10 ng/ml (Fig. 4B) or 100 ng/ml (Fig. 4C) of ^{3}H-astemizole, important proportions of ^{3}H label are also found in the final supernate (S fraction).

The MLP fractions from these cells were further separated by isopycnic centrifugation on sucrose gradients. As indicated in Fig. 5, the marker enzymes are partially separated. When ^{3}H-astemizole is added to the homogenate, the label present in the MLP fraction distributes unimodally around densities where 5′-nucleotidase (marker enzyme of plasma membrane) and phospholipids (constituent of membranes) are detected. When cells have been incubated with the drug, variations can be observed between individual experiments which can, however, be correlated with variations in the equilibration profile of the marker constituents (not illustrated). However, in all the experiments, two peaks are detected. The first one equilibrates around densities where 5′-nucleotidase, NADPH cytochrome *c* reductase (marker enzyme of endoplasmic reticulum) and phospholipids are found; a second peak equilibrates at higher densities where cytochrome *c* oxidase (mitochondria), cathepsin B (lysosomes) and phospholipids are detected. No significant difference occurs in relation to the astemizole concentration in the culture medium nor to the duration of the washout.

The particulate fraction from hepatocytes cultured for 1 hr with ^{3}H-astemizole has been further fractionated into the heavy mitochondrial (ML) and the microsomal (P) fractions which were analysed by isopycnic centrifugation. Figure 6 illustrates that, except for NADPH cytochrome *c* reductase, the major part of marker enzymes, phospholipids and ^{3}H-label associated with the MLP fraction is recovered in the ML fraction. As shown in Fig. 7A, in the ML fraction, the equilibration profile of ^{3}H-label after isopycnic centrifugation is still bimodal overlapping largely those of phospholipids and NADPH cytochrome *c* reductase; in the P fraction (Fig. 7B) it becomes unimodal still accompanying closely those of these two markers, except for some label recovered at the top of the gradient.

In order to evaluate a possible accumulation of astemizole within the lysosomes, MLP fractions from hepatocytes treated or not with chloroquine for 1 hr in the presence of ^{3}H-astermizole were fractionated on sucrose gradients. The distributions of markers and ^{3}H-label between the fractions were not significantly different from those presented at Fig. 4.

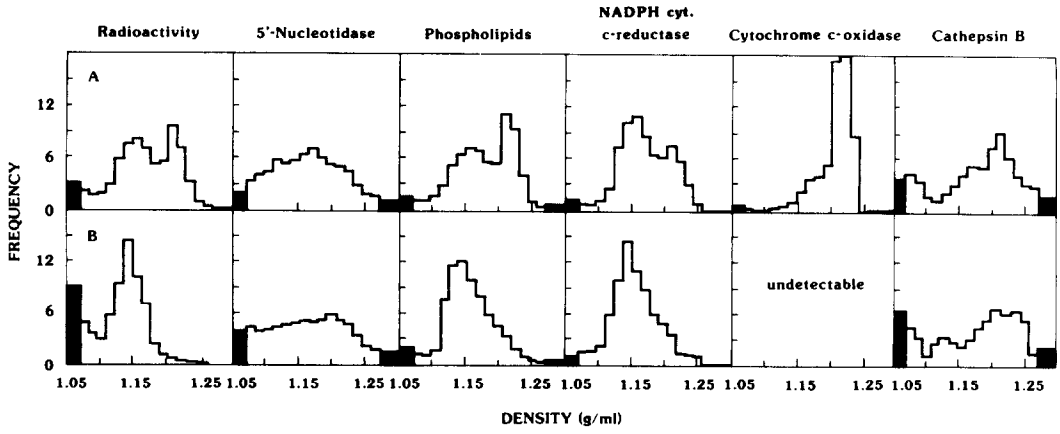

Fig. 7. Isopycnic centrifugation on sucrose gradients of ML (A) or P (B) fractions from the experiment described in Fig. 6.

As illustrated by Fig. 8A the equilibration profile of ^{3}H-label associated with the MLP fraction from control cells is bimodal, although the two peaks are less separated than in Fig. 5. In the MLP fraction from chloroquine-treated cells (Fig. 8B) two separated peaks appear: one equilibrates at high densities where the bulk of phospholipids is recovered, the other being found at lower densities where it accompanies closely the distribution of cathepsin B, which is considerably shifted in the presence of chloroquine. Both cathepsin B and ^{3}H-label are associated with organelles and do not correspond to solubilized material since a comparable equilibration profile is observed when the MLP fraction is introduced at the bottom of the sucrose gradient for a flotation centrifugation (not illustrated).

Biotransformation of astemizole

As shown in Table 1, after incubation at 37° for either 24 hr in complete culture medium or 6 hr in the presence of lysosomal enzymes at acid pH, more than 98% of the radioactivity behaves in HPLC as native astemizole suggesting that the label is still associated with intact drug and that the drug is not metabolized in these conditions.

When cultured hepatocytes are incubated with 10 ng/ml of ^{3}H-astemizole, the drug quickly disappears from the culture medium to reach undetectable concentrations after 24 hr continuous incubation or 4 hr incubation followed by 1 hr washout; concurrently, different labelled metabolites are recovered in the culture medium consisting mainly of desmethylastemizole, hydroxyastemizole and hydroxydesmethylastemizole. After 1 hr they are present under their unconjugated form whereas after 24 hr or after 4 hr incubation followed by washout they are almost entirely glucoronoconjugated; it should be noted that no sulfoconjugated metabolites have been detected. Cell associated ^{3}H-label consists mainly (after 1 hr or 4 hr of continuous incubation) or almost exclusively (after 24 hr) of labelled metabolites of astemizole. After 1 hr, the metabolites are present principally under their unconjugated form whereas after 24 hr the glucuronoconjugated metabolites become predominant.

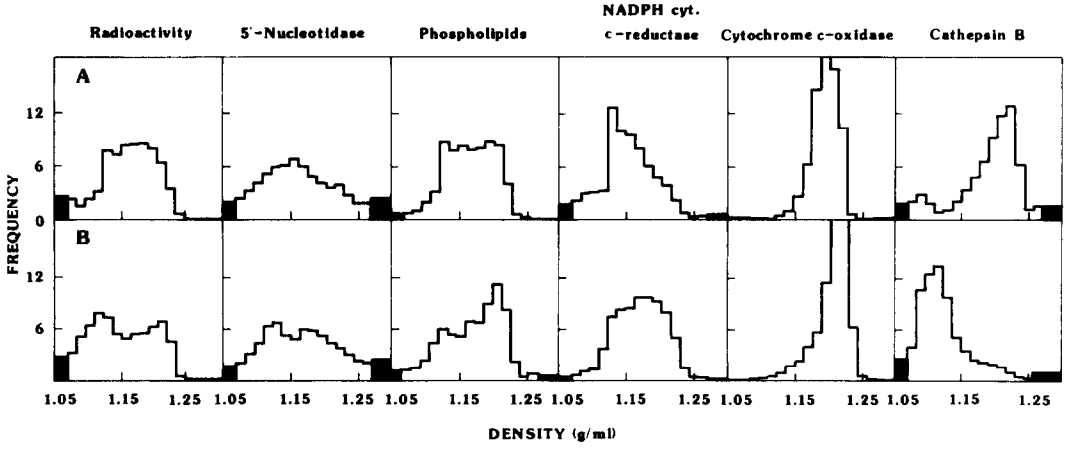

Fig. 8. Effect of chloroquine on subcellular distribution of astemizole. Isopycnic centrifugation on sucrose gradients of MLP fractions from cultured rat hepatocytes incubated for 1 hr at 37° with 10 ng/ml of ^{3}H-astemizole in the presence (B) or absence (A) of 50 μM chloroquine.

Table 1. ^{3}H-Astemizole and its labelled metabolites (in % of ^{3}H-label)

Incubation conditions	Astemizole	Metabolites				
		A	B	C	Others Identified	Others Unidentified
Culture medium for 24 hr at 37°	98.4	—/—	—/—	—/—	—	1.6
Lysosomal enzymes for 6 hr at 37°	99.0	—/—	—/—	—/—	—	1.0
Culture medium from hepatocytes incubated for:						
1 hr at 37°	71.7	3.9/—	13.6/—	—/—	6.7	4.1
4 hr at 37°	2.2	—/12.4	—/29.1	—/25.2	19.2	11.9
24 hr at 37°	—	—/12.8	—/11.9	—/40.1	22.8	12.4
4 hr at 37° + 1 hr washout	0.5	2.1/12.4	2.8/48.8	—/21.7	5.9	5.8
4 hr at 37° + 16 hr washout	—	0.3/18.8	—/13.0	—/42.6	7.9	17.4
Lysates of cells incubated for:						
1 hr at 37°	17.0	28.6/—	15.9/ 9.6	2.2 /—	5.1	21.6
4 hr at 37°	9.6	29.6/11.3	18.8/15.4	—/ 9.5	0.9	4.9
24 hr at 37°	—	18.7/ 9.8	7.0/14.2	8.3/11.8	1.4	28.8
Subcellular fractions from cells incubated for 1 hr at 37°						
N	90.0	9.4/—	—/—	—/—	—	0.6
ML	87.0	10.4/—	0.7—	—/—	—	1.9
P	88.0	10.0/—	—/—	—/—	—	2.0
S	23.1	1.2/16.1	1.7/41.9	—/—	—	16.0

On the basis of the chromatograms, the contribution of each metabolite was calculated in percent of the total area of the peaks. A: desmethylastemizole; B: hydroxyastemizole; C: desmethylhydroxyastemizole. The chemical structures are given in Fig. 1. The first figure corresponds to the aglycone metabolite, the second figure to the glucuronoconjugated one.

The presence of labelled metabolites was also investigated in the subcellular fractions obtained from hepatocytes incubated for 1 hr at 37° with ^{3}H-astemizole. It should, however, be noted that in this particular experiment astemizole appears to be metabolized much less rapidly than in the others. Whereas in the N, ML and P fractions, ^{3}H-label is almost exclusively associated with astemizole or unconjugated metabolites, in the final supernate glucuronoconjugated desmethylastemizole and hydroxyastemizole are predominant.

DISCUSSION

The kinetic experiments indicate that, at subtoxic doses, ^{3}H-astemizole is taken up by cultured rat hepatocytes in a concentration dependent process which on the basis of binding experiments does not appear to be mediated by an H_1 receptor. The uptake is rapid and after 1 hr incubation at 37°, the medium is depleted of about half of the labelled drug initially present (Fig. 2A). The washout experiments show that the hepatocytes release ^{3}H-label but only at 37°. The HPLC analysis reveals that after 24 hr continuous incubation or 4 hr incubation followed by 1 hr or 16 hr washout, the culture medium of hepatocytes does not contain any more detectable amounts of native astemizole. This indicates therefore that hepatocytes capture all ^{3}H-astemizole molecules present in the culture medium and thereafter release ^{3}H-label under the form of desmethyl-, hydroxy- or hydroxydesmethylastemizole, which are recovered mainly as glucuronoconjugates. It is important to note that these metabolites are those principally recovered *in vivo* in man, dog and rat [4].

All these results strongly suggest that ^{3}H-astemizole is quickly taken up by hepatocytes wherein it is metabolized before being excreted most probably into bile canaliculi-like structures which in the present culture conditions are not entirely separated from the culture medium [6]. The uptake of astemizole takes place until no drug is left in the extracellular fluid. The fact that label remains associated with the cells even after 48 hr washout could result from the recapture of labelled metabolites.

Chloroquine, a lipophilic weak base which concentrates within lysosomes [13, 16] and endosomes [28] and inhibits intralysosomal trapping of drug [17] decreases by about 25% the uptake of ^{3}H-astemizole by hepatocytes after 1 hr and by 50% after 4 hr (Fig. 3). This could indicate that a part of the cell-associated astemizole is accumulated within lysosomes or acidic endosomes.

Cell fractionation by differential centrifugation (Fig. 4) indicates that most of the cell-associated ^{3}H-label accumulated during 1 hr incubation of the cells with ^{3}H-astemizole is recovered in the particulate fraction (MLP) and represents, as indicated by the HPLC analysis (Table 1), native astemizole and unconjugated metabolites. Cytosol (S fraction) also contains ^{3}H-label in proportions increasing with the extracellular drug concentration in the culture medium (Fig. 4); a large proportion of it consists of glucuronoconjugated metabolites (Table 1). These data could suggest that the captured astemizole is associated with subcellular organelles where it is metabolized and that the glucuronoconjugated

metabolites are therefore released into the cytosol before being excreted out of the cell.

Isopycnic centrifugation (Fig. 5) of the particulate fraction (MLP) shows that ^{3}H-label distributes largely like phospholipids and NADPH cytochrome *c* reductase, suggesting its association with cellular membranes and in particular with those of the endoplasmic reticulum. This corresponds most probably to an aspecific binding since no H_1 receptor for astemizole was detected in hepatocyte homogenates (results not shown).

Subfractionation of hepatocytes cultured in the presence or absence of chloroquine, strongly suggests the association of a major part of the cell accumulated ^{3}H-label with endoplasmic reticulum (the labelled material present in the P fraction overlapping the distribution of the NADPH cytochrome *c* reductase (Fig. 7)) and of a minor part with lysosomes (the labelled material present in the MLP fraction shifted to lower densities after incubation of the cells with chloroquine (Fig. 8)).

On the basis of these results, we suggest that ^{3}H-astemizole is avidly taken up by cultured rat hepatocytes, most probably by diffusion through the lipid bilayer, due to its lipophilic character. The drug binds aspecifically to membranes, as indicated by its distribution similar to that of the phospholipids and therefore becomes largely associated with endoplasmic reticulum which represents, on a quantitative basis, the main hepatocyte membrane. The association of astemizole with endoplasmic reticulum could further facilitate its metabolization which precedes its excretion out of the cells. Due to its lipophilic weak base character, a part of the cell-accumulated astemizole and/or its metabolites seems also associated with lysosomes and/or acidic endosomes.

In addition to a direct interest for the cellular pharmacology of astemizole, these results further confirm that cultured rat hepatocytes could provide an alternative model for pharmacotoxicological studies. These results, indeed, clearly confirm that rat hepatocytes, in short term culture, are able to metabolize xenobiotics and to perform phase I and II biotransformations. These data extend previous studies showing that these cells keep the expression of biological receptors. Recent data from the literature indicate that all these biological activities can be maintained for a still longer period of time by cocultivating the hepatocytes with epithelial cells [29] or by culturing them in chemically defined media and/or on extracellular biomatrix [30].

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