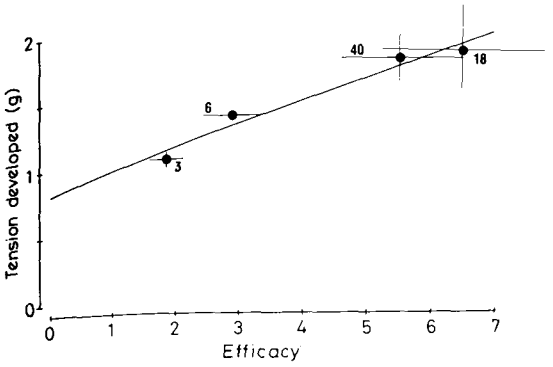

FIG. 3. A relationship between the efficacy of noradrenaline and the maximum tension developed. Ordinate: efficacy (as defined by Stephenson 1956: see text for detail). Abscissa: maximum tension (g) developed by noradrenaline. Each value is presented as a mean ± s.e. of 4 to 5 experiments. 3, 6, 18 and 40 are the ages of rats in weeks, respectively. A positive correlation was found ($r = 0.980$, $P < 0.02$). The best fitting line ($y = 0.163x + 0.811$) is shown.

the response of rabbit aorta to noradrenaline as developed tension (g) and concluded that tension (g) developed by noradrenaline increased with age.

The observations that the efficacy correlates with the maximum tension in g, but not to other expressions of tension, were not explained in that paper. It might be related to the present observation that the number of smooth muscles involved in a cross-sectional area (mm^2) or in a wet tissue weight (g) may decrease with increasing age.

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Effects of chronic treatment with amiodarone on hepatic demethylation and cytochrome P450

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The effect of chronic treatment with amiodarone on hepatic oxidative metabolism using an in-vivo [^{14}C]aminopyrine breath test and on hepatic cytochrome P450 was examined in Wistar rats. Aminopyrine demethylation was significantly impaired but returned to pretreatment values following amiodarone for 4 weeks. In contrast the levels of cytochrome P450 were significantly depressed during treatment and at 4 weeks following treatment. While an inhibitory effect on oxidative metabolism may explain the reported drug interactions with amiodarone, the discrepancy between its in-vivo effects and cytochrome P450 levels may suggest the development of 'compensatory' extra-hepatic site of drug metabolism.

Amiodarone, a benzofuran derivative, is now established in many countries as a highly effective antiarrhythmic agent for supraventricular and ventricular arrhythmias. The more widespread use of this drug has resulted in the identification of several side-effects, which range from corneal deposits and pulmonary toxicity to hepatic and thyroid dysfunction (Heger et al 1984). Case reports suggest possible pharmacokinetic interactions with warfarin, acenocoumarol, quinidine, digoxin, procainamide, phenytoin and aprindine. Only recently, however, has it been suggested that the underlying mechanism of many of these interactions may be inhibition of the hepatic metabolism (McGovern et al 1984; Saal et al 1984; Richard et al 1985).

Demethylation is an important route of metabolism for many drugs and is primarily catalysed by one or more of the forms of hepatic microsomal cytochrome P450. Several studies suggest that aminopyrine *N*-demethylation is a good probe for cytochrome P450 isozymes (Houston et al 1981). Therefore the aim of this study was to examine the effects of chronic doses of amiodarone on hepatic oxidative drug metabolism in

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the rat, using an in-vivo aminopyrine breath test (ABT) model, and to correlate our findings with the level of cytochrome P450.

Materials and methods

Male Wistar rats of similar weight (250 ± 15 g, mean ± s.e.m. 8 weeks-old) and age were maintained on a 12 h light-dark cycle. The environmental temperature (22 ± 2 °C) was controlled throughout and the animals had free access to a standard rodent chow and tap water before and after ABT. Rats were injected intraperitoneally with amiodarone (100 mg kg^{-1} day^{-1}) at the same hour every day for 4 consecutive weeks in the right lower quadrant. The intravenous formulation of amiodarone (Cordarone, Labaz: Sanofi, UK Ltd) which contains 50 mg amiodarone HCl, 100 mg of polysorbate 80 and 20 mg of benzyl alcohol mL^{-1} of water was used. The dosage was calculated from the data of Plomp et al (1985) showing that the serum concentration at the 'steady-state' in the rat after repeated i.p. injection of 100 mg kg^{-1} day^{-1} is between 2 and 3 μg mL^{-1} which is consistent with the concentrations reached at the 'steady-state' in man. A control group received an equivalent amount of normal saline. ABT were carried out by the method of Desmond et al (1980).

[dimethylamine-^{14}C] Aminopyrine (118 μCi $mmol^{-1}$, Amersham International, UK) was injected into the peritoneal cavity of unanaesthetized rats (1 μCi kg^{-1}). Rats were housed in individual metabolic cages. The expired gas was passed through concentrated sulphuric acid and then through a scintillation vial containing 10 mL of a 1 : 4 (v/v) ethanolamine–methanol mixture to trap all expired CO_2. Samples were collected for 25 min periods over 200 min. Trapped radioactivity ($^{14}CO_2$), measured after addition of 10 mL of an aqueous scintillation fluid by liquid scintillation spectroscopy, decreased exponentially during the collection period. The elimination rate constant (Kel) was calculated by a least square regression analysis of the logarithm of the amount of $^{14}CO_2$ produced with respect to time. The elimination half-life was calculated by dividing 0·693 by the Kel. All the ABT were performed at the same time every day to avoid the effect of diurnal rhythm. Control ABT were recorded 24 h before dosing with amiodarone or saline solution and at weekly intervals during and after the treatment for a total of 8 weeks.

Treated rats were killed at 1, 4 and 8 weeks after commencing the treatment and their livers removed for the preparation of hepatic microsomes by differential centrifugation. The concentration of cytochrome P450 in microsomal preparations was determined from the carbon monoxide difference spectrum of dithionite-treated samples (Omura & Sato 1964). The levels of P450 were expressed as nmol (mg protein)$^{-1}$. Protein concentrations were determined by the method of Lowry et al (1951). Rats receiving saline were killed after the completion of ABT, to determine a possible influence of repeated [^{14}C]aminopyrine on the cytochrome P450 levels.

Statistical analysis was performed by the Student's *t*-test for paired and unpaired sample means. Data are expressed as the mean ± s.e.m.; differences were assumed to be significant when $P < 0.05$.

Results

The saline group (n = 6), which was used to detect any day to day variation or a possible influence of repeated aminopyrine on hepatic drug metabolism, did not show any alteration over the study period (data not shown). The results are summarized in Fig. 1. In the rats treated with amiodarone 100 mg kg^{-1} day^{-1} i.p. for 4 weeks,

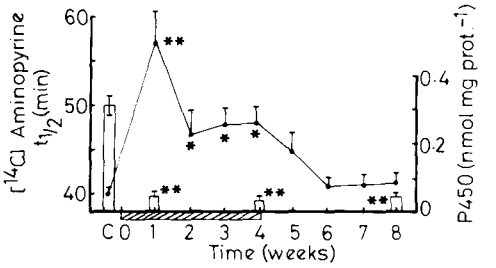

FIG. 1. The effect of four weeks treatment (hatched bar) with amiodarone (100 mg kg^{-1} day^{-1}) on the mean (±s.e.m.) elimination half-life of [^{14}C]aminopyrine (●——●) and hepatic cytochrome P450 (histogram). Differences from pretreatment control (C) values are indicated $^{*}P < 0.05$, $^{**}P < 0.01$.

the $t_{\frac{1}{2}}$ was significantly prolonged (compared with their own control, 40·7 ± 1·0 min) at 1, 2, 3 and 4 weeks by 40% ($P < 0.01$), 17% ($P < 0.05$), 19% ($P < 0.05$) and 19% ($P < 0.05$), respectively. After stopping treatment (weeks 5, 6, 7 and 8) there was no significant difference in the aminopyrine elimination half-life: 44·5 ± 2·5 min (week 5), 40·7 ± 2 min (week 6), 40·8 min (week 7) and 40·9 min (week 8).

The effects of amiodarone on cytochrome P450 are also presented in Fig. 1. There were significant differences ($P < 0.01$) between the saline group (0·31 ± 0·06 nmol (mg protein)$^{-1}$), and groups (n = 6 in each one) killed 1 week (0·05 ± 0·01 nmol (mg protein)$^{-1}$), 4 weeks (0·03 ± 0·01 nmol (mg protein)$^{-1}$) and 8 weeks (0·05 ± 0·01 nmol (mg protein)$^{-1}$) after commencement of treatment.

Discussion

These studies show that amiodarone is a potent inhibitor of hepatic microsomal mixed function oxidative drug metabolism in the rat and markedly reduces cytochrome P450, and suggest that our earlier observations (Barry et al 1986) on the acute effects of amiodarone are also seen during chronic treatment. A prolongation in the $t_{\frac{1}{2}}$ of antipyrine has recently been demonstrated in three humans treated with amiodarone (Staiger et al 1984) and impairment of hepatic drug metabolism may be an

explanation for the reported drug interactions. Regarding the cytochrome P450 data, our results also suggest that amiodarone has a long-term inhibitory effect on hepatic drug metabolism for at least 4 weeks following cessation of treatment. Possible explanations for the delay in reversal of reduced cytochrome P450 levels are unclear and other studies are required to elucidate the mechanism(s). Future work should also consider the study of specific enzyme activity markers, rather than the total P450 content.

Our study shows that the ABT returns to normal pretreatment findings (week 6) but the hepatic cytochrome P450 levels remained significantly reduced in week 8, Fig. 1 (i.e. there is no correlation between aminopyrine $t_{\frac{1}{2}}$ and concentration of cytochrome P450 in the liver at the end of the experimental period). A similar lack of correlation has also been reported (McManus & Ilett 1979; Vuitton et al 1981) using antipyrine. One possible explanation is a compensatory increase in extra-hepatic sites of drug metabolism (Babany et al 1985). Approximately 25% of aminopyrine demethylation takes place in tissues other than the liver, e.g. kidney (Lauterburg & Bircher 1976). Therefore, in the presence of a decreased hepatic cytochrome P450 content, some endogenous substrate(s) may be poorly metabolized, and thereby induce some renal cytochrome P450 isoenzyme (Babany et al 1985) which will result in a return to normal aminopyrine metabolism. Moreover, very recently we have shown (Barry et al 1987) that amiodarone (acutely) has an inducing effect on renal cytochrome P450 and NADPH cytochrome c reductase activity. If this hypothesis is confirmed, in some cases our pharmacological tools for assessing hepatic microsomal drug-oxidizing enzyme will need to be re-evaluated.

The liver and adipose tissue act as a reservoir for amiodarone both during and after withdrawal of therapy (Simon et al 1984; Plomp et al 1985). Studies in humans report a very long (many weeks) terminal elimination half-life (Heger et al 1984). Whether amiodarone has the same persistent inhibitory effect on the hepatic microsomal drug metabolism in humans as demonstrated in this study remains to be established and the possible contribution of 'compensatory' extra-hepatic metabolism studied.

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