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A strain dependent sex difference in ouabain-induced cardiotoxicity in rats

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Male rats of the BDIX strain were about twice as sensitive to the lethal effects of intravenously infused ouabain than were their female counterparts. This sex difference in ouabain sensitivity was not exhibited by rats of the Wistar strain. In bile duct-cannulated animals, ouabain biliary excretion was much faster in female BDIX rats. This finding could partly explain the greater ouabain resistance, although certain aspects of the cardiac/plasma ouabain concentration data did suggest that there may have been a component of the sex-related resistance that was not explicable on a pharmacokinetic basis. The faster ouabain biliary excretion in female rats could also partly explain the fact that for similar rates of infusion, the plasma ouabain concentration during infusion was similar in both sexes, despite the body size of the female rats being about one-third smaller.

In ouabain infusion studies primarily designed to compare germ-free and conventional rats, it was found that with animals of the BDIX strain, female rats were markedly more resistant to ouabain-induced death than their male counterparts (Hewick & Wilson 1983). It was decided, therefore, to investigate the mechanism of this sex-related resistance in BDIX rats and to see if it occurred in the more commonly-used Wistar rat strain. When ouabain is given intravenously a major route of elimination is via the bile (Greef & Wirth 1981) therefore a feature of the study was to examine ouabain biliary excretion.

Methods

The BDIX and Wistar rats were bred from animals originally purchased from the Medical Research Council Experimental Embryology and Teratology Unit, Carshalton, Surrey and Charles River (UK) Ltd, Manston Road, Margate, Kent, respectively. Both strains of rat were housed similarly and had free access to standard rat diet and water.

In the first study, 10 and 7 male–female pairs (10–12 weeks old) of the Wistar and BDIX strains, respectively, were compared. The male–female pairs of each strain were anaesthetized (urethane 50%, 1·5 kg^{-1} i.m.) and electrocardiographic leads attached to each limb so that recordings could be made from lead II. Ouabain was infused (7·5 mg ml^{-1}, 1·9 ml h^{-1}) via the left femoral vein until death by cardiac arrest (defined as the absence of cardiac electrical activity for longer than 15 s) occurred.

In the second study, 5 male–female littermate pairs of BDIX rats (14–16 weeks old) were used. Ouabain was infused as before, but with [^{3}H]ouabain being added to the infusion fluid (5·0 μCi ml^{-1}). During the infusion period, blood (0·1 ml every 5 min) and bile samples were collected via cannulae in the right femoral artery and common bile duct, respectively. Bile was collected every 10 min, except at the end of infusion where the collection interval was governed by the survival time for each individual rat. In this study electrocardiographic monitoring was also used to give a measure of the onset of cardiotoxicity (the time taken for the first occurrence of a 'run' of at least 3 consecutive ventricular extrasystoles was noted).

After infusion the hearts were removed and weighed and the blood samples centrifuged to obtain plasma. The radioactivity in bile, plasma and cardiac tissue (atria and ventricles combined) was determined by standard liquid scintillation counting methods (Griffiths et al 1984). Since ouabain is a polar cardiac glycoside that is not metabolized (Klaassen & Strom 1978), ouabain concentration was calculated directly from radioactive content.

Comparisons were made using paired or unpaired Student's *t*-test as appropriate with $P < 0.05$ being taken as significant. Results are given as means ± s.e.

Results

In the first study, as found in our previous investigation, the lethal ouabain dose in male BDIX rats was about half that in females (22·5 ± 3·4 and 43·4 ± 3·0 mg kg^{-1}). The respective male/female body weights were 0·25 ± 0·002 and 0·21 ± 0·01 kg. However, in Wistar rats, the male and female animals showed a similar susceptibility to ouabain (lethal doses 27·0 ± 3·1 and 25·1 ± 3·1 mg kg^{-1}, respectively). As with the BDIX rats the female Wistar rats had lower body weights (0·232 ± 0·007 versus 0·317 ± 0·007 kg).

The sex-related resistance in female BDIX rats was also observed in the bile duct-catheterized animals both in terms of the higher lethal dose (38·5 ± 0·9 and 17·7 ± 1·1 mg kg^{-1}) and that causing ventricular dysrhythmias (34·9 ± 2·0 and 16·0 ± 1·0 mg kg^{-1}). The body weights of the male and female bile-duct catheterized rats were 0·353 ± 0·008 and 0·236 ± 0·006 kg, respectively.

In these animals the rate of bile flow, although it fell as infusion proceeded, was always significantly greater in female rats (Fig. 1a). This difference was associated with a more than doubled rate of ouabain excretion in the bile (Fig. 1b). During infusion, the concentration of ouabain in the plasma increased approximately linearly and was similar in both sexes (Fig. 1c).

At death the mean cardiac ouabain concentration was higher in female rats (28·3 ± 3·2 versus 20·2 ± 3·0 μg g^{-1}) but the difference was not statistically significant.

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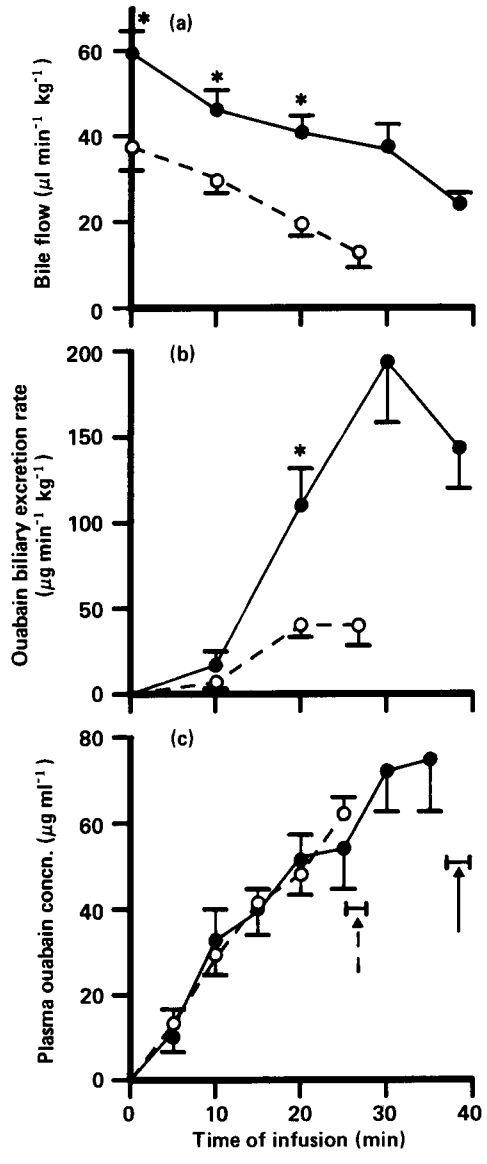

FIG. 1. The rates of bile flow and ouabain excretion, and plasma ouabain concentration in male and female BDIX rats during intravenous ouabain infusion (7·5 mg ml^{-1}, 1·9 ml h^{-1}). The dashed and solid lines correspond to male and female rats, respectively. The arrows indicate the mean times of death (±s.e.). The points are the means of five rats (±s.e.) apart from the final time points in panel (c) where only 4 and 3 male and female rats were surviving at these times. The asterisks indicate a significant difference ($P < 0·05$, Student's paired t-test) between male and female rats.

Discussion

The relative resistance of female rats of the BDIX strain to ouabain-induced cardiotoxicity seems in part due to a faster biliary elimination of the drug. Such sex-related differences in the biliary excretion of minimally-metabolized xenobiotics by rats have not been consistently reported (Klaassen & Watkins 1984). The present work suggests that the strain of rat used can be a factor contributing to such sex-related differences.

From the similar plasma ouabain concentrations in male and female rats infused at the same rate it seems that the one-third lower mean body weight of the female animals is compensated for by their faster elimination of the drug. Consideration of the present results indicates that such elimination probably does not involve solely biliary excretion. For instance at 20 min the male and female rats received 13·5 and 20·2 mg kg^{-1} ouabain, respectively, while the corresponding amounts excreted in the bile were 0·5 and 1·25 mg kg^{-1}. Therefore the 'residual' amounts of ouabain to be accounted for were 13 and 18·95 mg kg^{-1} in male and female rats, respectively. To explain similar ouabain plasma levels in male and female animals in this situation it is necessary to either postulate a different ouabain distribution in the two sexes, or, as is more reasonable, to consider additional routes of excretion. It is possible that in addition to some urinary excretion significant amounts of ouabain could be eliminated by direct excretion through the gut wall (Lauterbach 1981), and that in the female rats the amount of drug elimination via these routes could be greater than in male rats.

Finally, however, in addition to an obvious pharmacokinetic mechanism contributing to the ouabain 'resistance' of female BDIX rats, the higher 'female' mean values (although not statistically significant) for both the ouabain cardiac concentration at death and the final measured plasma ouabain concentration (Fig. 1c) could implicate some difference in cardiac sensitivity to the drug.

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