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Antiarrhythmic effect of amperozide, a novel psychotropic compound with class III antiarrhythmic properties, on digoxin-induced arrhythmias in the guinea-pig

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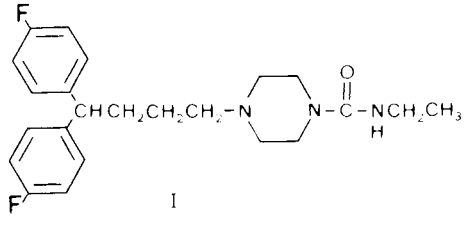
Amperozide is a novel psychotropic compound with specific effect in limbic brain areas. Preliminary findings have also indicated an antiarrhythmic effect in-vitro. Injections of saline, amperozide, melperone, thioridazine, bretylium or lignocaine, were given i.p. to anaesthetized guinea-pigs, which 10 min later were given digoxin s.c. to induce arrhythmia. In a series of control experiments none of these compounds caused arrhythmia in combination with the vehicle of digoxin. The time to arrhythmia was significantly prolonged after treatment with amperozide, melperone and bretylium compared with saline, but there were no differences between the treatments. The digoxin concentrations in plasma at death varied considerably within the groups and no statistical significance was found.

Amperozide (Hogpax) is a new psychotropic compound which is already established in veterinary medicine. It is used in the treatment of wasting pigs, a condition developed after weaning or regrouping.

Chemically, amperozide is a diphenylbutylpiperazine carboxamide (I) and thus belongs to a new group of compounds which, although containing a diphenylbutylamine moiety, are pharmacologically different from the diphenylbutylpiperidines (Gould et al 1983). It also has 5-HT_2 receptor-blocking properties in frontal cortex of the rat brain (Svartengren & Christensson 1985).



I

In pigs, amperozide has demonstrated potent antiaggression properties, without decreasing arousal (Björk et al 1984).

It has previously been suggested that amperozide has class III antiarrhythmic effects (according to Vaughan Williams' classification 1970) on isolated papillary muscles from ferrets and guinea-pigs (Arlock, unpublished observations). To examine this effect in-vivo, a model of digoxin-induced arrhythmias in anaesthetized guinea-pigs was used, and the antiarrhythmic effect of amperozide was compared with that of melperone, thioridazine, bretylium and lignocaine.

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Materials and methods

Method. Young male pigmented guinea-pigs (weight 288 ± 65 g, mean ± s.d.) purchased from Sahlins, Malmö, Sweden, were anaesthetized with sodium pentobarbitone 60 mg ml^{-1}, 35 mg kg^{-1} s.c. Most animals (91%) received a second subcutaneous injection of pentobarbitone 45–60 min after the first. The second dose was half the first, and was only given if the animals reacted vigorously when subdermal needle electrodes were inserted in the extremities. The electrodes were connected to a Grass 7 Polygraph. Continuous ECG recordings of a distinct lead were obtained throughout the experiments, at a paper speed of 2·5 mm s^{-1}, and intermittently for a detailed study of the ECG at 50 mm s^{-1}. Similar recordings were obtained for at least 90 min after the administration of an active substance in the control experiments, after which the animals were killed by an overdose of pentobarbitone. The following substances were administered intraperitoneally in a volume of 0·1 ml/100 g: amperozide hydrochloride (2 mg kg^{-1}), melperone chloride (2 mg kg^{-1}), thioridazine hydrochloride (10 mg kg^{-1}), bretylium tosylate (10 mg kg^{-1}) and lignocaine hydrochloride (2 mg kg^{-1}). Thioridazine hydrochloride (50 mg) was dissolved in 80% ethanol (0·5 ml) and 0·9% NaCl (saline) was added to a final concentration of 10 mg ml^{-1}; this solution was protected from light. Lignocaine hydrochloride 200 mg ml^{-1} was diluted with saline to a final concentration of 2·0 mg ml^{-1}. Solutions of amperozide hydrochloride (2·0 mg ml^{-1}), melperone chloride (2·0 mg ml^{-1}) and bretylium tosylate (10 mg ml^{-1}) were made in saline.

Ten minutes after the i.p. injection of drug, digoxin (10 mg kg^{-1}) was injected s.c. in a volume of 1 ml/250 g. Digoxin (25 mg) was dissolved in heated 80% ethanol (2·5 ml) and propylene glycol was added to a final concentration of 2·5 mg ml^{-1}.

In a series of control experiments the vehicles were treated as respective substances. The animals were divided into groups of six. Time to arrhythmia was measured from the administration of digoxin to an established grave arrhythmia (i.e ventricular fibrillation, atrioventricular block grade III) which proved fatal. Shortly after death, in all the digoxin-treated animals and in some controls, the chest was opened and the heart punctured and blood was collected in a heparinized blood collecting tube (Venoject). Samples

were centrifuged in a Hettich Universal 2 S centrifuge for 10 min at 2500 rev min^{-1}. Plasma was then separated and stored at −20 °C until analysis.

Assay. Analyses for digoxin were carried out with a commercial radio immunoassay (Farmos [^{125}I]digoxin RIA, lot KB01) routinely used in our laboratory for assessing plasma digoxin concentrations in patients.

As control we checked (i) that the native plasma from guinea-pigs did not interfere with the method, (ii) that addition of a known amount of digoxin to native plasma from guinea-pigs yielded the expected concentration, and (iii) that extraction of digoxin from the plasma with dichloromethane–2-propanol (98 : 2) yielded the same value as an unextracted sample.

As the method is for analysis of human plasma, we decided to dilute the samples with human plasma, obtained from the hospital blood bank, by a factor of 500 to obtain values within the range of the assay. The analyses were then performed in accordance with the manufacturers instructions.

Statistical analysis. The statistical calculations were made according to one way analysis of variance.

Drugs. Sodium pentobarbitone (Mebumal vet.) was purchased from ACO, Sweden; digoxin and propylene glycol from Sigma; lignocaine hydrochloride (lidocaine; Xylocard) from Hässle/Astra, Sweden. Thioridazine hydrochloride from Sandoz, and bretylium tosylate from Wellcome. Melperone chloride and amperozide hydrochloride were gifts from Ferrosan, Malmö, Sweden.

Results

In the control groups no animals died during the recording period, nor did any arrhythmias occur.

All the animals in the experimental groups died of arrhythmias. The times to lethal arrhythmia are given in Table 1. The time to arrhythmia was significantly ($P < 0{\cdot}01$) increased in the groups treated with amperozide, melperone or bretylium compared with the group treated with saline. Between the different antiarrhythmic treatments there were no significant differences.

The digoxin concentrations varied considerably within the groups and no statistical significance was found. One animal treated with melperone, had a considerably higher plasma digoxin concentration (18·5 μmol $litre^{-1}$) than any of the others; this was possibly due to contamination and the value was not included in the calculations.

No statistically significant differences were found regarding body weight between the treatment groups. Furthermore, a correction for body weight did not influence the statistics.

Table 1. N is the number of animals in each treatment group, all parameters are given as means ± s.d.; tA is the time to lethal arrhythmia and D is the level of digoxin in plasma at death in guinea-pigs treated with various antiarrhythmic drugs in addition to digoxin. The levels of significance (analysis of variance) in tA between control treatments and other treatments are also given.

Treatment	N	Body weight (g)	tA (min)	D (μmol $litre^{-1}$)	P
Control (saline)	6	278 ± 44	39 ± 4	1·08 ± 0·33	—
Amperozide	6	253 ± 31	60 ± 12	1·29 ± 0·38	<0·01
Melperone	6	293 ± 39	61 ± 15	1·05 ± 0·29	<0·01
Thioridazine	6	257 ± 15	52 ± 18	0·88 ± 0·27	n.s.
Bretylium	6	285 ± 81	60 ± 13	1·12 ± 0·23	<0·01
Lignocaine	6	240 ± 46	50 ± 11	1·14 ± 0·20	n.s.

Discussion

Traditionally the guinea-pig, like man, is considered a digoxin-sensitive species and the sensitivity to the glycoside increases with age, as does the proneness to ventricular arrhythmias from digitalis (Weinhouse et al 1983). Thus we have chosen animals of similar age as expressed by body weight. There was no significant difference in weight between the digoxin-treated groups. Various models for inducing arrhythmias with cardiac glycosides have been used. In the classical work of Vaughan Williams & Sekiya (1963) an infusion of ouabain was used, whereas in a recent study, Weinhouse et al (1983) administered digoxin subcutaneously and measured the time to onset of arrhythmias; our model has many similarities with the latter.

A previous investigation, on isolated papillary muscles from ferrets and guinea-pigs (Arlock, unpublished observations), has indicated an antiarrhythmic effect of amperozide, seemingly of class III according to Vaughan Williams' classification (1970). In addition it was found that amperozide has an antiarrhythmic effect on delayed afterpotentials caused by digitalis. A class III antiarrhythmic effect has also been described for melperone, a butyrophenone neuroleptic (Arlock et al 1978; Platou et al 1982). Further, melperone has been tested in the model of Vaughan Williams & Sekiya (1963) and, in a dose of 5–15 mg kg^{-1} i.v. it was found to increase significantly the dose needed for cardiac arrest (Petersen 1978). As for thioridazine, a phenothiazine neuroleptic, there is a report on both an antiarrhythmic effect and arrhythmogenic properties (Yoon et al 1979). Those authors found that, in dogs, a dose of 50 mg kg^{-1} i.v. was arrhythmia-promoting, whereas 10 mg kg^{-1} i.v. was antiarrhythmic. The antiarrhythmic effect on thioridazine is probably a local anaesthetic, membrane stabilizing effect (Jarvik 1970; Arlock et al 1978).

Bretylium is another class III antiarrhythmic drug which causes marked prolongation of effective refractory periods of ventricular muscle in dogs at a dose of 10 mg kg^{-1} i.v. (Waxman & Wallace 1972). Lignocaine is a class I antiarrhythmic drug which may have effects on delayed after potentials caused by digitalis (Bigger & Hoffman 1980).

In this study, we found significant increases in time to arrhythmia in the treatments with amperozide, melperone and bretylium compared with saline, indicating an antiarrhythmic effect of approximately the same magnitude by the doses used in guinea-pigs against digoxin-induced arrhythmias. These drugs share class III antiarrhythmic properties. As for the treatments with thioridazine and lignocaine the time to arrhythmia was increased but did not reach statistical significance.

The results of the present in-vivo study are in agreement with the recent findings (Arlock, unpublished observations) that amperozide has antiarrhythmic properties, and thus warrants further investigation in other models.

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Letter to the Editor

Bioavailability of sustained release acetazolamide

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A paper presented at the 1985 British Pharmaceutical Conference compared the bioavailability of two formulations of acetazolamide (Diamox 250 mg tablets and Diamox Sustets 500 mg capsules) (Ledger-Scott & Hurst 1985). Based on 0–24 h concentrations of acetazolamide in plasma after single doses, the authors concluded that the sustained release product was absorbed only 50% compared with the tablet. I would like to present a different interpretation of the reported information based on unique properties of acetazolamide.

Pharmacokinetic studies of drugs usually employ plasma concentration determinations to characterize the rate and extent of absorption of an administered dose. It is implied by this usage that a linear relationship exists between plasma values and concentrations of the drug in whole blood, the latter being a more definitive measurement of amounts absorbed. For acetazolamide, or any of the unsubstituted aromatic sulphonamides, this relationship does not hold because of a preferential uptake of these drugs by red blood cells (Maren 1967; Lehmann et al 1969; Wallace & Riegelman 1977). This phenomenon is well documented as due to an association of the drug with carbonic anhydrase to form an enzyme-inhibitor complex. What has apparently not been addressed, however, is the effect of this phenomenon on a comparison of two formulations having differing rates of absorption.

Lehmann et al (1969) have reported the mean concentrations of the two carbonic anhydrase isoenzymes in human red cells and their acetazolamide dissociation constants and used these values to describe the overall distribution of acetazolamide in the body. Using the Lehmann values, the concentration of acetazolamide in red cells in equilibrium with plasma can be determined as:

$$C_{rbc} = C_f + \frac{C_f \times C_{m(b)}}{K_b + C_f} + \frac{C_f \times C_{m(c)}}{K_c + C_f} \qquad (1)$$

where: C_{rbc} is the concentration in red blood cells, C_f is the concentration of free (unbound) acetazolamide in plasma, $C_{m(b)}$ and $C_{m(c)}$ are the maximum concentrations of acetazolamide which can be bound to carbonic anhydrases B and C (136 μm ml^{-1} and 20 μm ml^{-1},