dependent on the dose higher than 2·5 mg kg^{-1}. The relatively small systemic availability of propranolol after oral or portal administration thus increased with the dose, ranging from approximately 8–25% on average.

Provided that there is no pulmonary clearance, the total body clearance could be essentially assigned to the hepatic clearance, since renal clearance of this drug was insignificant in the rats older than 7 weeks (Iwamoto et al 1985). Estimated hepatic clearance of propranolol was constant at any present dose ranging from 1·0 to 10·0 mg kg^{-1}. In contrast, the hepatic intrinsic clearance was found to be largely dependent on the portal dose, suggesting evidence for the saturation kinetics in hepatic first-pass metabolism of propranolol.

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Modification of the inotropic effect of digoxin by diazepam in rat left atria

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There is a pharmacokinetic interaction between digoxin and diazepam that increases the elimination half-life of digoxin. It may be due to a reduction of digoxin tissue concentrations and to an enhanced effect of diazepam on digoxin binding to plasma albumin. Diazepam (10^{-5} M) also induces a positive inotropic effect in guinea-pig isolated atria. In a study of a possible pharmacodynamic interaction between both drugs, the inotropic response to digoxin has been examined in rat isolated atria in the presence of diazepam. The atria were kept in Tyrode at 37 °C, bubbled with 95% O_2 and 5% CO_2 and electrically stimulated at twice the threshold voltage. The results indicate that diazepam induces an inotropic effect at 10^{-5} M ($P < 0.05$) and reduces ($P < 0.05$) at 10^{-9}, 10^{-7} and 10^{-5} M the inotropic response to digoxin (10^{-5} M).

It has been shown in rats, dogs and healthy humans that, in the presence of diazepam, digoxin reaches higher plasma concentrations, its elimination half-life increases, its urinary excretion decreases and its tissue levels at several sites also decrease (Castillo-Ferrando et al 1980; Castillo-Ferrando & Carmona 1981). An enhanced effect exerted by diazepam and other benzodiazepines on digoxin binding to protein might account for the pharmacokinetic changes described above (Castillo-Ferrando 1983).

We have examined a possible pharmacodynamic interaction between diazepam and digoxin in the isolated rat atria.

Material and methods

Albino, Wistar rats of either sex, 200–300 g, were used (n = 26). Rats were killed and the left atria excised and tied by one end to a force-displacement transducer (Ugo-Basile) connected to a polygraph; the other end was attached to an electrode connected to a stimulator (SRI). The atria were stimulated at twice the threshold voltage at 1 Hz rate. The Tyrode bathing solution was at

* Correspondence.

37 °C and aerated with 95% O_2 and 5% CO_2. Its composition was as follows (mM): NaCl 1·8; $NaHCO_3$ 11·9; dextrose 5; $MgCl_2$ 1·05; NaH_2PO_4 0·4; $CaCl_2$ 1·8; distilled water to 1 litre. The atria were submitted to a resting tension of 0·5 g and after a 30 min stabilization period, digoxin (5×10^{-5} M) was added in one group. Other groups receiving also digoxin (5×10^{-5} M) had been pretreated with different concentrations of diazepam (10^{-9}, 10^{-7}, 10^{-5} M) until a stable response was obtained, which usually occurred within 10 min.

At 5×10^{-5} M digoxin, the inotropic response reached its maximum in about 10 min. This high concentration of digoxin was needed as the rat is relatively resistant to digitalis (Weinhouse et al 1983; Finet et al 1983).

Data were analysed by Student's *t*-test for group comparison.

Results and discussion

Digoxin 5×10^{-5} M, produced a positive inotropic response in the rat left atria amounting to a 89 ± 14% increase above the control ($P < 0.001$). The positive inotropic effect induced by digoxin (5×10^{-5} M) is significantly reduced ($P < 0.05$) in the presence of three different concentrations of diazepam (10^{-9}, 10^{-7}, 10^{-5} M). At 10^{-9} M diazepam the reduction amounted to 44·8 ± 9%, at 10^{-7} M it was 48·9% and at 10^{-5} M it was 47·2 ± 8·9%. Diazepam alone did not modify control inotropism at 10^{-9} and 10^{-7} M but at 10^{-5} M it induced a positive inotropic effect (32·3 ± 11% $P < 0.005$). All increases due to digoxin or diazepam were measured from the pre-diazepam or control level.

The results demonstrate the existence of a pharmacodynamic interaction between digoxin and diazepam in the experimental model presented. The interaction is not dose-related and does not respond to a simple model of pharmacological antagonism, indicating that other factors may play a role in addition to drug-receptor interplay. The existence of the interaction may partly explain why the association does not lead to clinical toxicity in spite of the increase in plasma levels and elimination half-life of digoxin when diazepam is used concurrently (Castillo-Ferrando et al 1980).

Binding studies have established the presence of benzodiazepine receptors in mammalian heart tissue, although the possible function they might serve is not yet defined (Davies & Huston 1981). The new compounds Ro 5-4864 (7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one) and PK 11195 (1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide) that act as agonist and antagonist, respectively, on peripheral receptors may help to elucidate their physiological significance (Le Fur et al 1983).

In the longitudinal smooth muscle from the guinea-pig ileum the depressor effect of diverse benzodiazepines on the electrically-induced contraction has been related to intracellular Ca^{2+} availability (Hullihan et al 1983). A specific inhibitory action of certain benzodiazepines on the sodium–calcium exchange in heart and brain mitochondria has been previously described (Matlib et al 1983). Cardiac glycosides have been shown to increase intracellular Ca^{2+} (Langer 1981). The association of diazepam and digoxin may, by the above mechanism, modify the intracellular Ca^{2+} equilibrium thus producing the interaction described.

An additive inhibition of Na-K-activated-ATPase cannot be excluded since diazepam has been shown to inhibit the enzyme in the microsomal fraction from rat ventricular muscle (Takemoto et al 1984). Cardiac glycosides are known to be potent inhibitors of membrane bound ATPase (Smith 1984; Hansen 1984).

Inotropism can be affected by diazepam in a dose-dependent manner; at 10^{-5} M we have found a positive inotropic effect, below that concentration no changes are produced. These data are in agreement with the results of other authors using the same experimental model, where a positive inotropic effect has been shown above 10^{-5} M (Kenakin 1982; Sugimoto et al 1978).

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