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Comparative effects of ouabain on isolated papillary muscle from tree shrews, guinea-pigs and rats

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Abstract—Inotropic effects of ouabain were investigated in the isolated papillary muscle preparations of the tree shrew (*Tupaia glis*), guinea-pigs and rats. In the guinea-pig and shrew papillary muscles, ouabain at concentrations of 10^{-8} to 3×10^{-7} M caused a concentration-dependent positive inotropic response in a similar magnitude, while in the rat, ouabain at concentrations of 10^{-7} to 3×10^{-6} M elicited negative inotropic one. Either phentolamine or pindolol in a concentration sufficient to block the α- and β-adrenoceptors did not antagonize the positive inotropic effect of ouabain in the papillary muscle preparation of the shrews.

Even though it remains to be established whether the tree shrews (e.g. *Tupaia glis*) are closer phyletically to primates than to any other extant mammalian group, the animals have some anatomical (Le Gros Clark 1971) and serological (Goodman 1966; Moore & Goodman 1968) analogies to primitive primates. Some data have been gathered concerning the basic investigations on the shrews (Noyes 1968; McClure et al 1972; Sakai et al 1979), but little attention has been paid to explore their usefulness in applied medical research. Schwaier (1973; 1975) stated that the animals may be promising in many research fields, regardless of their low position in primate phylogeny. According to her suggestion, one of potential applications for the shrews appears to be infarct research: rats, used most popularly as an experimental animal, are known to be insensitive to the effects of cardiac glycosides (Fricke et al 1975; Ku et al 1976; Weinhouse et al 1983), and in this respect the shrews might be a 'better' model for that area.

The purpose of this study was to compare the inotropic effect of ouabain on the isolated papillary muscles of the shrews, guinea-pigs and rats, and to search the usefulness of the shrews as an animal model in this field.

Materials and methods

Male adult Hartley guinea-pigs, Sprague-Dawley rats and *Tupaia glis* (bred in the Chugai Farm) were killed by a blow to the back of the neck under light ether anaesthesia. Papillary muscles with a diameter of 1·5 mm or less were excised from the right ventricle of guinea-pigs, or from the left ventricle (the muscle from the right ventricle is too small) of rats or shrews. The muscles were mounted in a 20 mL bath maintained at 37°C containing a modified Krebs-Henseleit solution of the following composition (mM): NaCl, 119·0; KCl, 4·8; $MgSO_4$, 1·2; $CaCl_2$, 2·53; $NaHCO_3$, 24·8; KH_2PO_4, 1·2; glucose, 10·0 and ascorbic acid, 0·057; the bathing solution was continuously aerated with 95% O_2 and 5% CO_2. The muscles were stretched by a tension of 0·5 g, and stimulated by two platinum electrodes using field stimulation from a Nihon Kohden electric stimulator (model SEN-7103) (frequency, 0·5 Hz; intensity, 1·2–1·5 times above the threshold; duration, 5 ms). The developed tension of the muscle was measured isometrically with a Nihon Kohden FD pick up force transducer attached to a Yokogawa pen recorder (Type 3066). The muscle segments at 0·5 Hz stimulation were equilibrated for at least 30 min, with washes every 10-15 min before exposure to drugs. The agonists and antagonists were added to the 20 mL organ bath in a volume of 0·1 mL. In a preliminary experiment, it was confirmed that stable situation of the preparation was maintained for at least 2 h after equilibration, as evidenced by the constant response to submaximally effective concentration of either phenylephrine or isoprenaline (not shown). At the time point which the preparation became stable, ouabain was applied to the bath in a cumulative manner. When the response of the preparation to the preceding concentration of ouabain had reached a steady value, subsequent concentrations of ouabain were increased by a factor of about 3. The equilibration time for each concentration was usually 10-15 min.

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The influence of 10^{-6} M phentolamine or 10^{-7} M pindolol on the positive inotropic effect of ouabain was examined in the papillary muscle preparation of the shrews stimulated at 0·5 Hz. After equilibration, a single concentration of 10^{-5} M phenylephrine or 10^{-8} M isoprenaline was added to the bath. The equilibration time of each drug concentration was usually 3–5 min. Then, the preparation was washed out, and 10^{-6} M phentolamine or 10^{-7} M pindolol was allowed to act for 30 min before the application of successive concentrations of phenylephrine or isoprenaline. In the presence of each antagonist, 10^{-5} M phenylephrine or 10^{-8} M isoprenaline was added to the bath, and the cumulative concentration-response curve to ouabain was constructed.

Drugs used were ouabain octahydrate (Sigma), phentolamine methylate (ampoule, Ciba-Geigy), (−)-isoprenaline hydrochloride (ampoule, Nikken kagaku), (−)-phenylephrine hydrochloride (Sigma) and (±)-pindolol base (ampoule, Sandoz). All drugs were dissolved in distilled water, and diluted with 0·9% saline. Drug solutions were usually added to the bath in a volume of 0·1 mL using individual syringes (Terumo Co.) and the final bath molar (M) concentrations are given.

Values are mean ± s.e. The statistical significance of the differences between mean values was estimated by means of Student's *t*-test and expressed as *P* values.

Results and discussion

As depicted in Fig. 1, the inotropic effects of ouabain were examined in isolated papillary muscle preparations of tree shrews, guinea-pigs and rats. The basal-developed tension of the papillary muscle from the three species is as follows: shrews, 326·4 ± 38·8 mg; guinea-pigs, 519·5 ± 90·2 mg; and rats, 481·4 ± 88·6 mg; each n = 5, $P > 0.05$. Ouabain at a concentration-range of 10^{-8} to 3×10^{-7} M elicited a concentration-dependent positive inotropic response to similar extents in the preparations of shrews and guinea-pigs. Although in the guinea-pig preparation, ouabain at 10^{-6} M caused a further increase in developed tension, the same concentration produced a significant decrease in developed tension with a rise of basal tension in the shrew preparation. Ouabain, 3×10^{-6} M or more elicited ventricular arrhythmia in two of the five preparations of guinea-pigs. In the preparation of rats, unlike the shrews and guinea-pigs, ouabain (10^{-7}–3×10^{-6} M) caused a negative inotropic effect in a concentration-dependent fashion. The results are summarized in Fig. 1.

The effects of α- and β-adrenoceptor antagonists on the positive inotropic response to ouabain were examined. Either 10^{-6} M phentolamine or 10^{-7} M pindolol was applied to the papillary muscle preparation of tree shrews. The basal-deve-

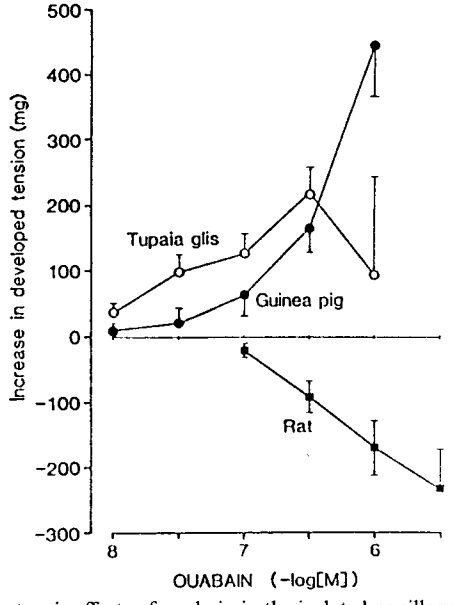

FIG. 1. Inotropic effects of ouabain in the isolated papillary muscle preparations of tree shrews, guinea-pigs and rats. Vertical bars represent mean ± s.e. of 5 observations on 5 preparations.

loped tension of the papillary muscle in the absence (control) or the presence of 10^{-6} M phentolamine or 10^{-7} M pindolol was as follows: control, 326·4 ± 38·8 mg; treated with phentolamine, 334·9 ± 70·8 mg; and treated with pindolol, 277·3 ± 36·6 mg; each n = 5, $P > 0.05$. As shown in Fig. 2, neither phentolamine nor pindolol significantly affected the positive inotropic response to ouabain, even though the ouabain (10^{-6} M)-induced decrease in developed tension was markedly attenuated after treatment with 10^{-6} M phentolamine. The concentration of the α- or β-adrenoceptor antagonist used was sufficient to inhibit the positive inotropic response seen with 10^{-5} M phenylephrine or 10^{-8} M isoprenaline.

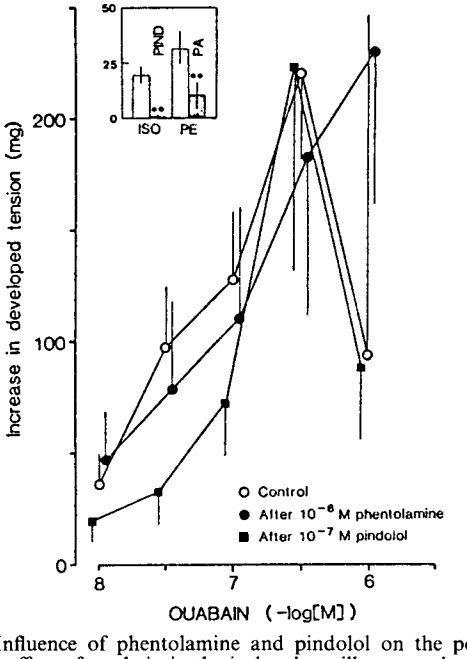

FIG. 2. Influence of phentolamine and pindolol on the positive inotropic effect of ouabain in the isolated papillary muscle of tree shrews. Vertical bars represent mean ± s.e. of 5 observations on 5 preparations. The positive inotropic responses to isoprenaline (ISO, 10^{-8} M) and phenylephrine (PE, 10^{-5} M) were inhibited significantly by pindolol (PIND, 10^{-7} M) and phentolamine (PA, 10^{-6} M) (closed columns), respectively. ** $P < 0.01$ vs control (open columns).

It is known that one of the remarkable characteristics of the positive inotropic effect of cardiac glycosides is the diverse species-dependent difference in sensitivity to the glycosides. Among most mammalian species, rats appear to be a digitalis-resistant species (Fricke et al 1975; Ku et al 1976; Weinhouse et al 1983), while man (Halkin et al 1978; Alken & Belz 1984), rabbits (Kelliher & Roberts 1976), guinea-pigs (Stephen et al 1976; Kafiluddi et al 1986) and other species (Cattell & Gold 1938; Horwitz et al 1977) are sensitive to the various cardiac glycosides.

In the present experiment, it was demonstrated that in the isolated papillary muscle of tree shrews, the therapeutic concentration range of ouabain is the same as that in digitalis-sensitive species such as guinea-pigs. Thus, the shrews appear to belong to the digitalis-sensitive species group. From our results, and because the tree shrew is a primitive primate characterized by rapid reproduction (Schwaier 1973; 1975), it would seem to be a suitable model for evaluation of cardiac glycosides for potential use in man.

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