The Influence of (+)-Propranolol on the Inotropic Effect of Dihydro-Ouabain in Relation to Stimulation Frequency

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Summary. 1. The influence of (+)-propranolol, 10^-6 to 4 x 10^-5 mol/l, upon the positive inotropic effect of dihydro-ouabain was studied on guinea-pig papillary muscle. At 1 Hz, negative inotropically effective concentrations of (+)-propranolol decreased the inotropic effectiveness of the glycoside.

2. For equivalent diminution of the basal force of contraction, either by reduction of stimulation frequency or by increasing concentrations of (+)-propranolol, a similar decrease in the inotropic effectiveness of dihydro-ouabain was observed. In contrast, reduction of [Ca^2+]_o had almost no effect on the effectiveness of the glycoside.

3. The inhibitory effect of (+)-propranolol on the inotropic effectiveness of dihydro-ouabain declined with reduction of stimulation frequency. At any stimulation frequency tested, the inhibitory effect of (+)-propranolol was proportional to the effect of a reduction of that particular frequency to the rested-state condition in the absence of (+)-propranolol.

4. The results are consistent with the hypothesis that the inhibition of the excitation-coupled sodium influx by (+)-propranolol is the cause of its inhibiting effect on the effectiveness of dihydro-ouabain.

Key words: Inotropic effect of dihydro-ouabain – Inhibition of sodium influx – (+)-propranolol – Functional antagonism – Stimulation frequency.

Introduction

Recently we have reported a marked decrease in the inotropic effectiveness of dihydro-ouabain when the basal force of contraction was reduced by lowering the frequency of stimulation. This was in contrast to the effect of a reduction in extracellular calcium concentration (Ebner and Reiter, 1977). The finding was explained as the result of a different influence of the two experimental conditions on intracellular sodium load in the presence of the glycoside, i.e., sodium load was reduced by lowering stimulation frequency, but not by lowering [Ca^2+]_o if the frequency remained unchanged. In order to provide further evidence for the decisive role of the sodium load in the positive inotropic effect of dihydro-ouabain the following hypothesis was examined: a substance which depresses sodium influx during depolarization should have an influence on the inotropic effectiveness of dihydro-ouabain similar to that of a reduction of stimulation frequency.

The depression of the sodium influx during excitation is a well established non-stereospecific effect of propranolol (e.g. Tarr et al., 1973). At 1 Hz 3.4 x 10^-6 mol/l propranolol reduced by about 20% the upstroke velocity of the action potential in guinea-pig papillary muscle (Trithart et al., 1971; Ban, 1977). This concentration is far below the threshold concentration of 5 x 10^-5 mol/l for the impairment of Ca uptake into the sarcoplasmic reticulum (Scales and McIntosh, 1968). In a preceding investigation (Ebner and Reiter, 1979) we had observed a similar reduction of the inotropic effectiveness of dihydro-ouabain by (+)- or (−)-propranolol. This effect was accompanied by a decrease in electrical excitability (i.e., a negative bathmotropic action) which was to be expected in view of the inhibition of the sodium influx. Due to the combined negative bathmotropic effects, an increasing number of muscles became inexcitable with increasing concentrations of dihydro-ouabain in the presence of (+)-propranolol but not (−)-propranolol. The difference pointed to the facilitating influence on excitability of small amounts of endogenous noradrenaline which were probably released by high concentrations of dihydro-ouabain without adding to its inotropic effect. By using (+)-propranolol, therefore, it is possible to...
study the antagonism between propranolol and dihydro-ouabain over a sufficiently wide range of concentrations. In order to examine the assumed similarity between the diminution of the glycoside effectiveness caused by propranolol and that due to a reduction in stimulation frequency, we investigated the influence of (+)-propranolol on the effectiveness of dihydro-ouabain in relation to stimulation frequency. The influence of (+)-propranolol was compared with the effects of either a variation of stimulation frequency or a reduction in calcium concentration, both in the absence of propranolol. The results were presented in part at a joint meeting of the German and Polish Pharmacological Societies (Ebner and Reiter, 1976).

Methods

Isolated papillary muscles from the right ventricle of guinea pigs (250–350 g) of either sex were mounted in a two-chambered vessel (volume 50 ml) with internal circulation of the bath solution (Reiter, 1965). To provide sufficient oxygen supply, muscles were selected with a diameter of less than 1.0 mm (determined by length and wet weight). The muscles were stimulated electrically at their ventricular end via two electrodes with square wave pulses of 3 ms duration and at an intensity slightly above stimulation threshold. The muscles contracted isometrically from a resting force of 3.92 mN. For the measurement of the force developed inductive force transducers (Q 11/10 p, Hottinger Baldwin Messtechnik, Darmstadt) were used. The contraction curves were displayed on an oscilloscope and evaluated on line by means of an electronic computer (PDP 12, Digital Equipment Corporation). The composition of the bath medium was (in mmol/l): NaCl, 115; KCl, 4.7; CaCl2, 3.2; NaHCO3, 25; K2HPO4, 1.2; MgSO4, 1.2; glucose, 10. In the muscle chamber, the medium was vigorously gassed and circulated by a mixture of 95% O2 and 5% CO2; pH 7.55; the temperature was kept constant at 35°C.

Experimental Procedure. All papillary muscles had an initial equilibration period of 1 h during which they were stimulated at 1 Hz. The influence of five different concentrations of (+)-propranolol on the inotropic effect of dihydro-ouabain was studied at 1 Hz on five series of muscles. For each series (n = 8) two dihydro-ouabain concentration-effect curves were obtained by increasing the concentration cumulatively to 3 × 10^{-5} mol/l without changing the stimulation frequency. After the control concentration-effect curves had been established in the different stimulation frequencies, the glycoside was washed out by changing the bath solution three times. (+)-Propranolol, 2 × 10^{-5} mol/l, was then added and, 45 min later, the frequency dependence of the inotropic effect of dihydro-ouabain was again examined on the same muscles and as outlined above.

Without any other alteration in experimental procedure, the stepwise change in stimulation frequency was used in a second group of muscles (n = 8) from 1 Hz to 1/16, 1/32, 1/64 Hz and in a third one (n = 5) from 1 Hz to 1/100 Hz. The combined glycoside concentration-effect curves at 1 Hz in the absence of (+)-propranolol served as control for all three groups (n = 21). A comparison of these curves obtained in the absence or presence of (+)-propranolol was performed by Student's t test. Linear regressions were calculated according to the method of least squares. The values presented are arithmetic means ± standard error.

The Following Substances Were Used. Dihydro-ouabain (DHO), Hommel AG (Adliswil, Switzerland), (+)-propranolol-HCl, Rheinpharma (Heidelberg, Germany).

Results

A. The Influence of (+)-Propranolol on the Inotropic Effect of Dihydro-Ouabain

In Fig. 1, concentration-effect curves of dihydro-ouabain are depicted which were obtained in the presence of increasing concentrations of (+)-propranolol (part of the data had been shown in Fig. 1 of Ebner and Reiter, 1979). Instead of the usual semilogarithmic plot we chose a linear abscissa in order to stress the negative inotropic effect of (+)-propranolol as an integral part of its interference with the inotropic effect of dihydro-ouabain. Under the influence of (+)-propranolol the basal force of contraction was reduced. Only the amount of decrease in force, shown for 10^{-6} mol/l (+)-propranolol, was due to a decrease in basal force which occurred after the control concentration-effect curve; by its own this concentration of (+)-propranolol was without effect on the