Inotropic Effects of Ouabain on Rabbit Left Atria in Presence of *Beta*-Adrenergic Blocking Drugs.* (30158)

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Conflicting reports exist on the question of whether or not the positive inotropic effects (PIE) of cardiac glycosides on the myocardium can be modified by prior or simultaneous administration of agents which are capable of blocking cardiac *beta*-adrenergic receptors. Thus, Moran and Perkins(1) showed that the PIE of digoxin or ouabain on the intact dog heart or isolated rabbit heart were not prevented by pretreatment with dichloroisoproterenol (DCI) in concentrations which blocked the stimulant effects of catecholamines on the heart. More recently, however, Tanz(2) has reported that the PIE of ouabain on the electrically driven cat papillary preparation could be blocked by pretreatment of the tissue with DCI in a concentration of 1×10^{-6} M.

It is recognized that although DCI does have *beta*-adrenergic receptor blocking actions, it also has stimulant and depressant actions on the heart(1) (depending on the doses used), and in this regard it is not the most suitable agent for producing *beta*-adrenergic receptor blockade. Recently, however, several drugs have been made available which are both highly specific and more potent than DCI in their *beta*-adrenergic receptor blocking action. Three such agents are pronethalol (2-isopropylamino-1-(2-naphthyl) ethanol HCl), propranolol (an oxy-propanol derivative of pronethalol), and MJ-1999 (4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide HCl). All 3 drugs are substituted N-isopropylphenylethylamines. Pronethalol has been shown to be superior to DCI in producing cardiac *beta*-adrenergic receptor blockade *in vitro* as well as *in vivo*(3-5). Propranolol and MJ-1999 are approximately 10-20 times more potent than pronethalol(6-9).

Because of the increased specificity and potency of these 3 drugs in producing *beta*-adrenergic receptor blockade compared to DCI, it was therefore felt that they offered an advantage and opportunity to reevaluate the basic question as to whether or not *beta*-adrenergic receptor blockade could modify the PIE or ouabain on cardiac muscle. The following report bears on this question.

Methods. Electrically driven left atria of rabbit hearts were used for studying contraction responses. The tissues were prepared according to procedures and technics described previously(10). The isolated atria were stimulated at a rate of 120 beats/min for 100 minutes after mounting the tissue in the muscle bath before any drug additions were made. Twenty minutes before the end of this equilibration period, the bath medium was exchanged once with fresh solution. The medium used in these experiments had the following composition (mmol/l): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.19; KH_2PO_4, 1.19; $NaHCO_3$, 12.5; glucose, 5.56. The solution was gassed with a 95% O_2, 5% CO_2 gas mixture *via* a sintered disc placed directly under the tissue. All experiments were done at 37.5°C. In those experiments where the effect of pronethalol, propranolol, or MJ-1999 on the PIE of ouabain was studied, the tissues were pretreated for 5 minutes (at 95 min post mount) with the *beta*-blocking drug before administering the ouabain. In the concentrations used, none of the drugs produced any significant change in contraction force during this 5-minute pretreatment period. Otherwise, all drugs were added at 100 minutes post equilibration (0 time in the Figures). The changes in isometric contraction force are expressed as per cent of the contraction force measured at the end of the 100-minute equilibration period.

Results. Fig. 1 shows the time course of the positive inotropic effects (PIE) of ouabain (6.9×10^{-7} M) on the driven left

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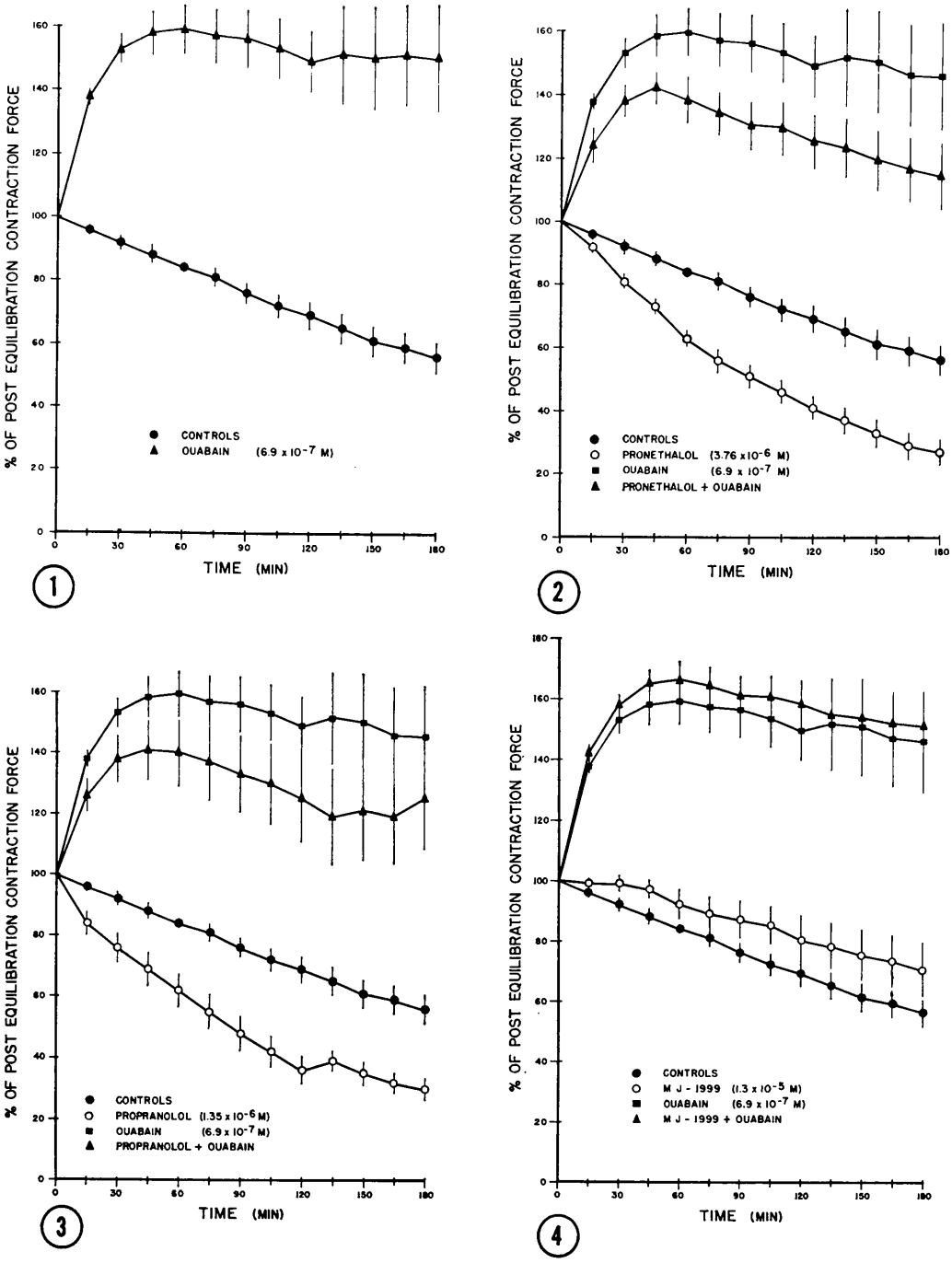

FIG. 1. Time course of positive inotropic effect (PIE) of ouabain (6.9×10^{-7}M) on driven (120 beats/min) rabbit left atrial preparations. Each point is mean value of 9 to 16 experiments. Vertical lines are standard errors of means. Ouabain added at 0 time (100 min post equilibration).

FIG. 2. Changes in contraction force produced by ouabain, pronethalol, and ouabain in presence of pronethalol. Each point is mean of 8 to 16 experiments. In the ouabain + pronethalol group, atria were pretreated (5 min) with pronethalol before adding ouabain (at 0 time).

FIG. 3. Changes in contraction force produced by ouabain, propranolol, and ouabain in presence of propranolol (5 min pretreatment). Each point is mean of 6 to 16 experiments.

FIG. 4. Changes in contraction force produced by ouabain, MJ-1999, and ouabain in presence of MJ-1999 (5 min pretreatment). Each point is mean of 5 to 16 experiments.

atria. The untreated control preparations showed a linear decline in contration force of approximately 15% per hour. The ouabain treated atria showed a highly significant ($P<0.001$, Student's "t" test) increase in the contraction force compared to the controls for all time periods measured.

Fig. 2 shows the inotropic effects of pronethalol alone, ouabain alone, and oubain in the presence of pronethalol. Pronethalol alone produced a significant ($P<0.05$) depression of contraction force in a concentration of 3.76×10^{-6} M compared to the untreated controls. This concentration is approximately 10 times the ED50 for antagonizing the inotropic effects of epinephrine on the driven cat papillary preparation(3). The results of experiments where the atria were pretreated for 5 minutes before adding ouabain indicate that although the mean responses for the ouabain + pronethalol group are lower than the responses seen with ouabain alone, none of these differences are significantly different ($P>0.05$).

Fig. 3 illustrates the effects of propranolol on the PIE of ouabain. Note that in a concentration of 1.35×10^{-6} M, propranolol alone caused a significant decrease in contraction force in relation to the controls ($P<0.05$). The mean contraction force values of ouabain + propranolol, although less than that seen with the glycoside alone, are still not statistically significant ($P>0.05$). In 6 experiments, the atria were pretreated for 30 minutes with propranolol before giving the ouabain. As with the 5-minute pretreatment, ouabain still produced its characteristic PIE in these experiments.

Fig. 4 compares the PIE of ouabain alone and in the presence of MJ-1999 (1.3×10^{-5} M). The *beta*-adrenergic receptor antagonist alone was found to have no significant inotropic effects compared to the controls. When ouabain was given after 5 minutes of pretreatment with this drug, the PIE response was nearly identical to that seen with ouabain alone. The concentration of MJ-1999 used is approximately the dose (1.24×10^{-5} M) needed to produce a 50% antagonism of the PIE of isoproterenol (9.4×10^{-8} M) on the electrically driven atria.

Discussion. Under the conditions of our experiments, the data fail to indicate that the PIE of ouabain on driven rabbit left atria can be significantly changed by pretreatment with and presence of 3 drugs known to have potent and specific blocking actions on *beta*-adrenergic receptors of the heart. In this regard, our results also fail to offer any support for the view that the PIE of ouabain on isolated cardiac tissue may result from the release of endogenous catecholamines from the heart(2). In the concentrations used, all 3 of the *beta*-adrenergic receptor blocking drugs are known to block the effects of sympathomimetic amines on the heart(3,5-9). Therefore, our results are consistent with the earlier report by Moran and Perkins(1) showing that the less potent blocking drug DCI was also ineffective in blocking the PIE of ouabain and digoxin on both isolated and intact hearts.

Several reasons for the discrepancy of our results and those of Tanz(2) may be given. For example, it might be argued that prevention of the PIE of ouabain on the cat papillary by DCI was unrelated to its *beta*-adrenergic receptor blocking effects. DCI is known to have other myocardial effects which may be separate from its action as an antagonist of *beta*-adrenergic receptors (*e.g.*, 11,12). However, pronethalol and propranolol are also known to have actions on the heart similar to DCI besides *beta*-adrenergic receptor blockade(13-15). Yet, both these drugs were unable to block the PIE of ouabain. It should also be emphasized that MJ-1999, which is similarly ineffective in modifying the PIE of ouabain, is devoid of any of the cardiac depressant ("quinidine-like") effects which have been described for DCI, pronethalol, and propranolol(13,15). In any case, whatever properties these *beta*-adrenergic receptor blocking drugs have besides their ability to antagonize the actions of sympathomimetic amines on the heart, they are unable to block the PIE of ouabain.

Of course, species differences or differences in the type of cardiac preparations used might be offered as explanations for the discrepancy of findings seen in the present study and those reported by Tanz(2). Arguing

against this view, however, are the findings by Black and Stephenson(3). These authors, in noting the specificity of pronethalol action, remarked that they failed to get a blockade of the PIE of ouabain with pronethalol using both cat and guinea pig papillary preparations.

While our findings appear to conflict with the evidence suggesting that the release of catecholamines may be involved in the PIE of ouabain on isolated tissue(2,10,16,17), it should be emphasized that this view is based largely on the fact that reserpine-treated hearts show an attenuated positive inotropic response to glycosides. However, our own findings showing an attenuation of the PIE of ouabain with a reserpine pretreatment known to deplete the heart of catecholamines did not exclude the possibility that this attenuation of the PIE could result from some other myocardial action of reserpine(10). Thus, failue to modify significantly the PIE of ouabain in the present experiments with agents known to block the PIE of catecholamines reemphasizes our previously stated possibility that attenuation of glycoside actions following reserpine may result from effects other than the lack of availability of endogenous catecholamines.

Nonetheless, whatever possible role the release of catecholamines may have in the development of the PIE of ouabain, this PIE is not apparently vulnerable to blockade by the presence of highly specific and potent *beta*-adrenergic receptor antagonists such as pronethalol, propranolol and MJ-1999.

Summary. The positive inotropic effects of ouabain on electrically driven rabbit left atrial preparations was studied in the presence of 3 drugs known to be potent and specific *beta*-adrenergic receptor antagonists (pronethalol, propranolol and MJ-1999). Ouabain alone (6.9×10^{-7} M) produced a highly significant increase in contraction force compared to untreated controls. Pronethalol (3.76×10^{-6} M) and propranolol (1.35×10^{-6} M) were found to produce significant decreases in contraction force. The methanesulfonanilide substituted isopropylphenylethylamine (MJ-1999) had no significant effects on contraction force in a concentration of 1.3×10^{-5} M. Administering ouabain after pretreatment with and in the presence of either pronethalol, propranolol, or MJ-1999 failed to modify significantly the normal positive inotropic effect of the glycoside. Therefore, no evidence was found to indicate that *beta*-adrenergic receptor blockade could prevent the positive inotropic effect of ouabain on isolated rabbit left atria.

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