Modification of the Action of Propranolol on Pacemaker Automaticity and Overdrive Suppression by Reserpine[1] (37369)

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Recently we have described a technique to determine the action of drugs on cardiac pacemakers which measures overdrive suppression and spontaneous pacemaker activity in the isolated perfused cat heart with surgically induced heart block (1). Overdrive suppression was measured by the interval between the last ventricular beat evoked by artificial stimulation and the first idioventricular beat. Using this technique we have found that not all beta blocking agents have the same effects on pacemaker automaticity and overdrive suppression. In this regard, practolol (ICI 50, 172) a beta blocker reported to be devoid of a direct myocardial depressant action increased atrial and ventricular pacemaker rates and decreased the overdrive suppression interval (ODI), whereas propranolol slowed pacemaker rates and lengthened ODI. In addition, it was found that the d-isomer of propranolol interferes with the depressant action of the l-isomer.

It has been shown by Blinks (2) that in the acutely denervated heart adrenergic nerve terminals remain viable for up to 48 hr. Since adrenergic activity may increase the activity of atrial and ventricular pacemakers resulting in an increase in rate, it is uncertain in such acutely denervated preparations whether propranolol decreases rate and lengthens ODI by an antiadrenergic action or by direct depression of cardiac membranes. The purpose of this study was to evaluate the importance of cardiac catecholamines in the effects of d- and dl-propranolol on atrial and ventricular pacemaker rates and overdrive suppression in the acutely denervated cat heart.

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Materials and Methods. Cats, weighing between 2 and 3 kg were rendered unconscious with electric shock while being ventilated with 100% oxygen through a cannula inserted into the trachea under local anesthesia (procaine 1%). Heparin (1000 USP units) was injected into a femoral vein prior to the electric shock. After opening the chest, a cannula was inserted into the aorta and the heart was removed and perfused according to the method of Langendorff with Krebs–Ringer solution bubbled with 95% O_2–5% CO_2. The solution contains in m*M*/liter: sodium, 145; potassium, 5.8; calcium, 1.1; chloride, 127; magnesium, 1.2; bicarbonate, 25; sulfate and phosphate, 1.2; and glucose, 5.6.

The temperature of the perfusion fluid was maintained at 37° by appropriate thermoregulation of the water jacket. The pH, pO_2, and pCO_2 of the perfusion fluid was monitored throughout the experiment. The pH ranged between 7.38–7.44, pO_2 between 600–700 mm Hg and pCO_2 between 28–30 mm Hg. The coronary arteries were perfused at a constant rate of 25 ml/min by passing the perfusion fluid through a constant rate infusion pump. Perfusion pressure was kept constant at 60 cm H_2O.

Stainless steel recording electrodes were sewn onto the right and left atrium and the left ventricle. The electrogram was recorded on a direct writing polygraph. Complete heart block was produced by using a modification of the method of Grumback (3). The right atrium was opened to visualize the bundle of His and complete heart block was produced by ligating the bundle. Bipolar stainless steel stimulating electrodes were placed on the base of the left ventricle. The

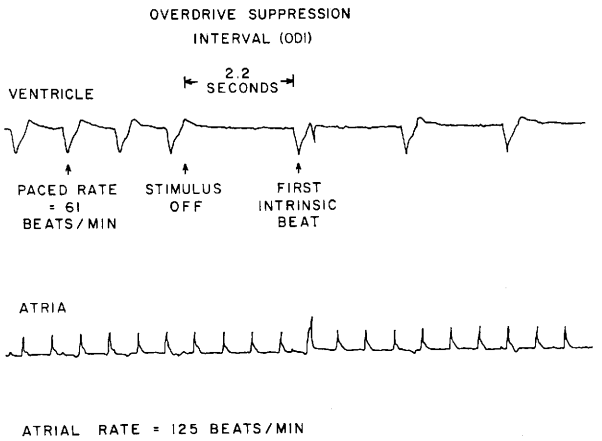

FIG. 1. Method for determining atrial and ventricular rates and overdrive suppression interval (ODI) in isolated cat hearts with heart block. ODI is the interval between the last response evoked by artificial stimulation and the first intrinsic beat. The ventricle was paced at 2/3 above its intrinsic rate for 2 min every 10 min. The intrinsic atrial rate is 125 beats/min and the intrinsic ventricular pacemaker rate is 37 beats/min. (not shown in figure). The ODI is 2.2 sec.

pacing rate of the ventricle was set at a frequency 2/3 greater than the idioventricular rate. The ventricle was paced for 2 min once every 10 min throughout the duration of the experiment. Other details of the method have been described in a previous publication (1).

Overdrive suppression was measured by the interval (ODI) between the last response evoked by artificial stimulation and the initiation of the first intrinsic beat (Fig. 1). We have reported previously that after the control ODI was established, the magnitude of ODI remained relatively constant for approximately 80 min (1). A 7% increase in ODI was the maximum variation which occurred during this time. After 80 min, however, the preparation in some cases began to rapidly change and within a 30-min period increases or decreases amounting to 25–30% of control spontaneously occurred. Thus, to avoid effects due to such spontaneous changes, drug actions were determined within a 60-min period. It should be emphasized that washout of the drug occurred in part during the unstable intervals and the results obtained during this time must be considered somewhat tenuous. In all cases, drug administration was not initiated until 3 successive ODI were within 3–5% of each other.

The effect of drugs on the intrinsic rate of the atrial or ventricular pacemaker was determined from the electrogram; a 5-sec interval was employed for this purpose. We have reported previously that in the absence of drugs, atrial and ventricular rates remained constant throughout the course of an experiment, *i.e.*, up to 90–120 min (1). dl-Propranolol[2] and the d-isomer were perfused in concentrations of 400 μg/liter. We have determined previously that this concentration of the drug produces significant effects on either pacemaker rate or ODI within the perfusion period (1). The amount of drug used is expressed in terms of the free base. The total dose administered up to any given time was determined by calculating the total volume of the perfusion fluid applied to the heart during the specified time period and then multiplying this volume by the concentration of the drug in the perfusion fluid. The deadspace (125 ml) was taken into account in these calculations. Reserpine (5 mg/kg) was injected intraperitoneally 24 hr prior to the experiment. This dose of reserpine has been reported to deplete myocardial catecholamine stores by more than 90% within 24 hr after administration (4).

[2] Propranolol and its dextro isomer were kindly supplied by Dr. Richard Davies.

Results. Effect of dl-propranolol on atrial and ventricular rates in reserpine-treated hearts. Nye and Roberts (6) have reported that reserpine pretreatment does not influence the rate of atrial or ventricular pacemakers in isolated hearts. Similar results were obtained in the present study. In 4 hearts removed from cats pretreated with reserpine, the average rate of the atrial pacemaker was 137.0 ± 7.2 beats per minute (BPM) which is not significantly different ($p > 0.05$) from the average atrial rate (118.0 ± 6.9 BPM) of isolated cat hearts not treated with reserpine, previously reported by us (1). The average rate of the ventricular pacemaker in the reserpine-treated hearts was 53.9 ± 4.7 BPM which is not different from the rate of 52.8 ± 1.9 BPM which we previously observed in hearts not treated with reserpine ($p > 0.05$) (1).

In the reserpine-treated hearts dl-propranolol caused a slight, but statistically significant, reduction in the rate of the atrial pacemaker after 50 min of perfusion at a cumulative dose of 500 μg; at this time the atrial rate was 91 ± 3% of control ($p < 0.05$; Fig. 2). The depressant effect on the atrial pacemaker was not reversible since the rate continued to decline throughout the washout period, *i.e.*, 70–100 min. In cats not treated with reserpine propranolol did not affect the rate of the atrial pacemaker (1).

The rate of the ventricular pacemaker in the reserpine-treated hearts was not affected by the perfusion of dl-propranolol at any of the cumulative doses achieved ($p > 0.05$; Fig. 2). This is in contrast to the effect of propranolol in hearts not treated with reserpine in which it caused a 22% depression of the ventricular rate at a cumulative dose of 600 μg ($p < 0.05$) (1). Thus, pretreatment of the animals with reserpine appears to diminish the depressant effects of propranolol on the ventricular

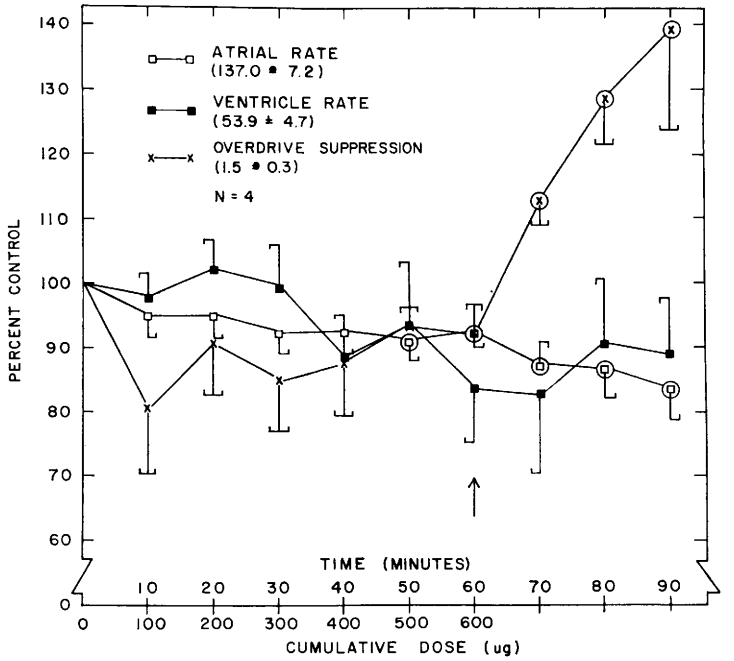

FIG. 2. The effect of dl-propranolol on atrial and ventricular rates and overdrive suppression after reserpine pretreatment in isolated cat hearts with heart block. Reserpine (5 mg/kg) was administered intraperitoneally 24 hr prior to the experiment. Perfusion with dl-propranolol (400 μg/liter was initiated at zero time. The arrow signals the termination of perfusion with the drug. The control atrial and ventricular rates and the control overdrive suppression interval are shown in the upper left-hand corner of the figure. The number of observations is indicated by *N*. The vertical lines represent the standard error of the mean. The circled values are significantly different from the control ($p < 0.05$).

pacemaker.

Effect of dl-propranolol on ODI in reserpine-treated hearts. We have reported that the average ODI in hearts removed from cats treated with reserpine is significantly longer than the ODI of hearts not treated with reserpine ($p < 0.05$) (1). In the present study, it was found that propranolol administered to hearts removed from cats treated with reserpine 24 hr prior to the experiment did not affect ODI through the 60-min perfusion period ($p > 0.05$; Fig. 2). We have previously found that in hearts not treated with reserpine, propranolol caused a significant prolongation of ODI within the first 10 min of perfusion producing a maximum prolongation of approximately 35% at a cumulative dose of 600 μg (1). Therefore, the data suggest that the action of dl-propranolol to prolong ODI is reduced by pretreatment with reserpine.

Effect of d-propranolol on atrial and ventricular rates in reserpine-treated hearts. It has been reported that the d-isomer of propranolol produces its antiarrhythmic effect by a direct depressant action (5). Therefore, we attempted to determine whether its effects on the rate of the pacemakers and on ODI were also due to this action. The perfusion of reserpine-treated hearts with d-propranolol produced markedly different effects on pacemaker rates than those seen in the untreated hearts. In hearts treated with reserpine, significant depression of the atrial rate did not occur until 30 min after perfusion with the drug was initiated (Fig. 3); this is three times longer than that required in untreated hearts (1). In addition, in reserpine-treated hearts, only a 7% change in atrial rate was achieved at a cumulative dose of 600 μg ($p < 0.05$) whereas in untreated hearts the maximum depressant effect was 20% and was achieved at a cumulative dose of 600 μg ($p < 0.05$) (1).

The effect of d-propranolol on the ventricular rate was also affected by reserpine

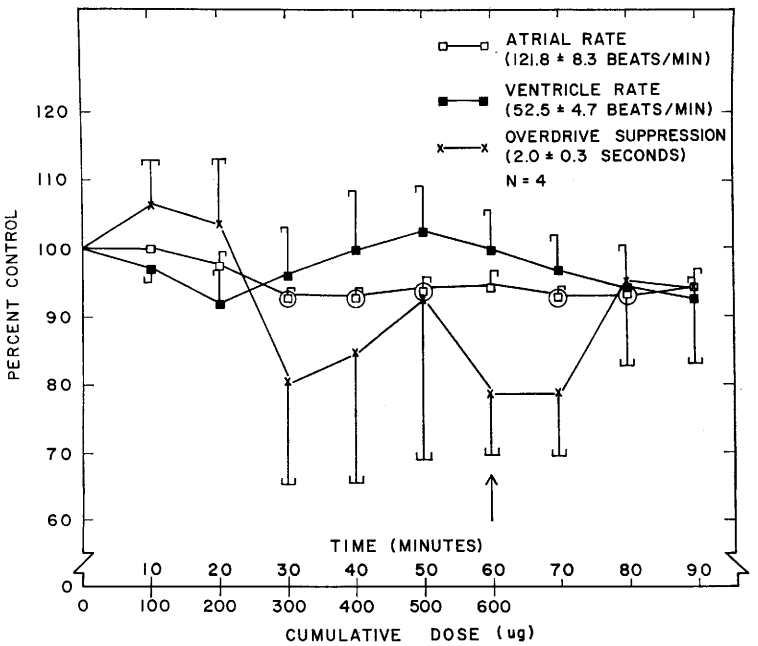

FIG. 3. The effect of d-propranolol on atrial and ventricular rate and overdrive suppression after reserpine pretreatment in isolated cat hearts with heart block. Reserpine (5 mg/kg) was administered intraperitoneally 24 hr prior to the experiment. Perfusion with d-propranolol (400 μg/liter) was initiated at zero time. The arrow signals the termination of perfusion with the drug. The control atrial and ventricular rates and the control overdrive suppression interval are shown in the upper right hand corner of the figure. The number of observations is indicated by *N*. The vertical lines represent the standard error of the mean. The circled values are significantly different from the control ($p < 0.05$).

pretreatment. In the present study, d-propranolol did not produce depression of the ventricular rate in the reserpine-treated hearts ($p > 0.05$; Fig. 3), whereas in our previous study using hearts not treated with reserpine d-propranolol produced a significant reduction in rate; the nadir was 35% and was achieved at a cumulative dose of 600 μg (1). The data suggest that, as in the case of the racemate, the depressant effect of d-propranolol on the rate of the ventricular pacemaker is diminished by pretreatment with reserpine.

Effect of d-propranolol on ODI in reserpine-treated hearts. In reserpine-treated hearts d-propranolol did not significantly influence ODI throughout the entire period of perfusion ($p > 0.05$; Fig. 3). This finding is in sharp contrast to the effect observed in hearts not treated with reserpine where d-propranolol produced a significant prolongation of ODI within the first 10 min of perfusion (1). The maximum prolongation of ODI produced by d-propranolol in that study was approximately 50% and occurred at a cumulative dose of 500 μg ($p < 0.01$). Thus, the effects of d-propranolol on ODI were diminished by pretreatment with reserpine.

Discussion. We have reported previously that overdrive suppression is a valuable method for analyzing drug action on cardiac pacemakers (1). The results of that study indicated that the overdrive suppression interval (ODI) is a more sensitive indicator of the reactivity of pacemakers to drugs than the intrinsic rate of pacemakers. We have now extended these observations to determine whether the effects of propranolol and its dextro isomer on pacemaker rate and ODI are influenced by pretreatment with reserpine.

We have reported previously that both d- and dl-propranolol cause a significant prolongation of ODI and that the d-isomer is more potent in this regard (1). The present study shows that reserpine pretreatment diminishes the action of these agents to prolong ODI, suggesting that both d- and dl-propranolol are acting through a similar mechanism. This mechanism probably involves catecholamines since reserpine in the dose used has been reported to produce nearly complete depletion of catecholamines in the heart (4). It has been shown that reserpine pretreatment prolongs ODI itself (1) indicating that catecholamines are involved in the overdrive suppression process. Some investigators have claimed that many of the cardiac actions of reserpine can be attributed to an action on the heart directly. However, Spilker and Cervoni (7) have reported that pretreatment with reserpine does not alter the characteristics of the transmembrane action potential recorded from isolated cardiac tissue while Choi and Roberts (8) have shown that reserpine pretreatment has no effect on the transmembrane exchange of sodium and potassium in cat papillary muscle. Thus, these data do not support the contention that reserpine has a direct action on the heart.

An important consideration in assessing the action of reserpine and propranolol on ODI is the study of Blinks (2) who has shown that adrenergic nerve twigs remain viable in acutely denervated isolated tissue for up to 48 hr, and that the adrenergic neurotransmitter contained in these twigs can be released by a suprathreshold stimulus. If d- and dl-propranolol prevent the stimulus-induced release of catecholamines from nerve terminals, then ODI would be prolonged. However, if pretreatment with reserpine had already produced an effect to prolong ODI by causing catecholamine depletion, then the action of the isomers on adrenergic nerve terminals would be of little consequence. It should be emphasized that the action by the isomers to reduce catecholamine activity and thereby prolong ODI probably occurs at the nerve twigs rather than at the receptor since the d-isomer of propranolol is also affected by reserpine pretreatment even though it is only 1/40th as potent as the racemate in producing receptor-blockade. In this regard it is important to note that Standaert *et al.* (9) have shown that d- and dl-propranolol are equally effective in producing depression of electrical activity in motor nerve terminals.

Although the interaction of propranolol, its d-isomer and reserpine on ODI appears to involve an effect on catecholamines, the mechanism involved in the action of reserpine to diminish the depressant action of the racemate and the d-isomer on pacemaker rate is uncertain. In this regard, reserpine pretreatment *per se* does not affect intrinsic pace-

maker rate suggesting that endogenous catecholamine stores are probably not involved in the maintenance of pacemaker activity in the isolated heart preparation. Thus, it would seem that an effect by d- and dl-propranolol on adrenergic activity would be of little importance in their action to depress pacemaker rates. Consequently the catecholamine depleting action of reserpine could not be responsible for its effect to diminish the depressant effect of these agents on pacemaker activity. It has been reported previously that although reserpine pretreatment does not affect the transmembrane exchange of sodium and potassium (8), it does diminish the effect of propranolol on ionic fluxes (10). Thus, the results of the present study together with our previous findings (8, 10) suggest that pretreatment with reserpine may interfere with the membrane effects of propranolol and in this way the capacity of the agent to decrease pacemaker activity by influencing ionic fluxes would be reduced.

Another possible explanation for the absence of a depressant effect by d- and d'-propranolol on both ODI and pacemaker rate in the reserpine-treated hearts relates to the report of Marcus *et al.* (11). These investigators found that pretreatment with reserpine reduces the uptake of digoxin into the myocardium. Thus, in the present study it is possible that the uptake of propranolol was less in the reserpine-treated hearts than in the untreated hearts; however, further experimentation will be required to explore this possibility.

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