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The Isoproterenol Stress Test in Unanesthetized Atherosclerotic Rabbits (40370)

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Cardiac stress testing is a necessary diagnostic prelude to selective coronary angiography. The most prevalent stress test utilized is exercise performed on a treadmill or bicycle ergometer. There are situations, however, in which exercise stress testing cannot be performed due to physical or psychological limitations (1). It is also difficult, at times, to obtain stable electrocardiographic (ECG) traces due to movement artifacts. Atrial pacing at rapid heart rates has also been utilized as a cardiac stress test (2, 3) but has the disadvantage of requiring cardiac catheterization.

Isoproterenol infusion has been reported to cause S-T segment and T wave changes indicative of ischemia in patients with coronary artery disease but not in normal individuals (4). In addition it has recently been reported to be more reliable than the Masters two-step (5) and as reliable as treadmill exercise (1) in predicting the presence of coronary artery disease in man. Because of its simplicity and great sensitivity it has been suggested that the "isoproterenol stress test" would appear to have a useful role in the clinical assessment of coronary artery disease (1). We have investigated the response to this isoproterenol stress test in an atherosclerotic rabbit model previously described (6, 7). This model is pathophysiologically similar to the patient with coronary artery disease, demonstrates a similar ECG response to the stress of atrial pacing, and responds similarly to pharmacological interventions.

Methods. Twenty-three male New Zealand white rabbits, weighing approximately 2 kg, were fed a standard laboratory chow pelleted with 2% cholesterol for 14–16 weeks. At that time surgical preparation of the animals was carried out as previously described (6). Briefly, a 14 G polyvinyl chloride catheter was implanted in the right external jugular vein under local anesthetic (procaine HCl) for subsequent infusion of isoproterenol. At least 24 hr later, the unanesthetized rabbits were lightly restrained on their backs. Surface leads were placed over the spine and sternum for recording the ECG on a Brush recorder (Mark 260) at a standard sensitivity of 1 mv/cm and on magnetic tape for computer analysis of S-T segment response. A solution of isoproterenol was infused continuously at a rate of 0.2 cc/min for 10 min using a commercial infusion pump (Sage Instruments, Model 255-1). The initial concentration of isoproterenol was such that a dose of 1 μg/kg/min was delivered. If S-T segment depression (i.e. at least 1 mm difference from control) was not seen after 3 min of infusion at this concentration, the infusion was stopped and the concentration of isoproterenol was increased to deliver 2–3 μg/kg/min. The rate of infusion and volume of fluid infused was always constant, however. Propranolol (Inderal) (0.01–1.0 mg/kg, $n = 6$), nitroglycerin (100 μg/kg, $n = 9$), dipyridamole (Persantin) (250 μg/kg, $n = 8$) or saline (0.2 cc/min, $n = 5$) was injected intravenously into the marginal vein of the left ear during the fifth or sixth min of the isoproterenol infusion to determine the effects on isoproterenol-induced heart rate and S-T segment depression. Dosages were selected based upon previous experience with these compounds in the paced rabbit model (6, 7).

Statistical analysis of the effects of propranolol, nitroglycerin (GTN) and dipyridamole were determined using Students t test (8).

Results. Effects of isoproterenol infusion. Heart rate began to increase almost immediately after the start of isoproterenol infusion and reached a steady-state value within 2 min as did ischemic S-T segment changes. Two

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distinct S-T segment changes occurred during isoproterenol infusion: S-T segment depression and S-T segment elevation. This paper will deal only with the S-T segment depression since this is the primary electrocardiographic response to stress in anginal patients. The occurrence of S-T segment elevation will be investigated in future experiments in an attempt to explain the hemodynamics and pharmacology involved.

Examples of S-T segment depression and T-wave inversion in response to isoproterenol infusion can be seen in Figs. 1 and 3. In each case isoproterenol infusion caused a significant tachycardia which was accompanied by the ischemic S-T segment changes. An indication of the reproducibility of the ECG changes in response to a given isoproterenol challenge in a given rabbit is evident in Fig. 3. The tracings are from the same rabbit on different experimental days. In both instances there is a classic, sagging S-T segment depression and T-wave inversion in response to the isoproterenol infusion.

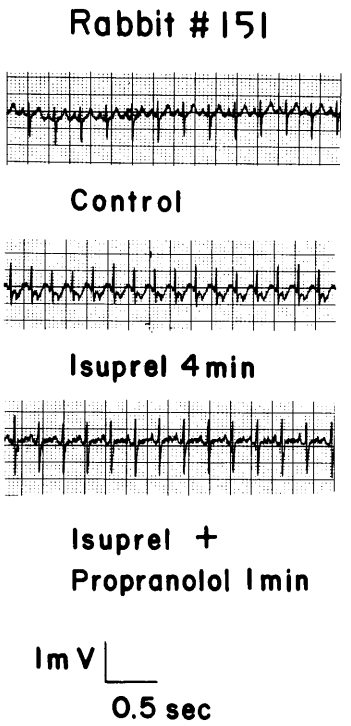

FIG. 1. ECG tracings demonstrating the reversal by propranolol (0.1 mg/kg) of the tachycardia, ischemic S-T segment depression and T-wave inversion caused by isoproterenol infusion.

Effects of propranolol on isoproterenol-induced ECG changes. The effects of a dosage of 0.1 mg/kg of propranolol on isoproterenol-induced tachycardia and ECG changes in one rabbit are shown in Fig. 1. Isoproterenol infusion caused an increase in heart rate from a control value of 240 to 310 beats/min, S-T segment depression and T-wave inversion. One min after intravenous injection of propranolol the heart rate decreased to 260 beats/min, and the S-T segment depression and T-wave inversion disappeared although the isoproterenol infusion continued. This is a typical response to a β-blocking dose of propranolol.

A dose-response relationship for propranolol can be seen in Fig. 2. in which the effects of three dosages of propranolol on heart rate and S-T segment depression are compared with those of saline (five animals per group). Propranolol did not significantly reduce heart-rate or S-T segment depression at the 0.01 mg/kg dose but did at the two higher doses.

Effects of nitroglycerin and dipyridamole on isoproterenol-induced ECG changes. Nitroglycerin was effective in reversing isoproterenol-induced S-T segment depression whereas dipyridamole had no beneficial effect. In fact, dipyridamole frequently caused

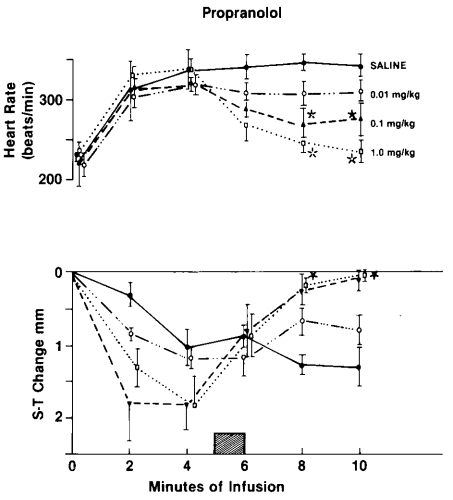

FIG. 2. Dose-response relationship of the effects of propranolol on isoproterenol-induced tachycardia and S-T segment depression (mean ± SEM). Asterisks in this and subsequent figures indicate significant differences ($P < 0.05$) when compared with the value at the fourth minute of isoproterenol infusion.

an exacerbation of the S-T segment depression. Figure 3 illustrates a typical response to both of these drugs in the same animal on different experimental days. The response to isoproterenol infusion was the same on both days resulting in a peak heart rate of 390 beats/min and a sagging S-T segment depression with T-wave inversion.

Nitroglycerin administration during isoproterenol infusion reversed the ischemic S-T segment changes without affecting the tachycardia (Fig. 3 left). Dipyridamole, on the other hand, caused further depression of the S-T segment (to 4 mm) without changing heart rate (Fig. 3 right).

A summary of the effects of nitroglycerin and dipyridamole on isoproterenol-induced heart rate and S-T segment changes is shown in Fig. 4. The heart rate and S-T segment responses to isoproterenol infusion were the same in both groups of animals. Neither drug had any effect on the isoproterenol-induced tachycardia in any of the animals. Following GTN administration the mean S-T segment depression was reduced from 1.3 to 0.4 mm, whereas dipyridamole tended to exacerbate the S-T segment depression (to 1.6 mm).

Discussion. Effects of Isoproterenol Infusion. Infusion of isoproterenol intravenously to unanesthetized atherosclerotic rabbits caused ischemic S-T segment depression and T-wave inversion similar to that seen when patients with coronary artery disease are similarly stressed (1, 4, 5, 9). Electrocardiographic S-T segment depression is thought to be a manifestation of subendocardial ischemia (10). The major portion of total coronary blood flow, and all of subendocardial blood flow, occurs during diastole (11). Therefore the supply of blood, and thus oxygen, to the subendocardium depends upon the diastolic perfusion pressure, the coronary resistance, and the duration of diastole. The diastolic blood pressure (afterload) is also a major determinant of oxygen consumption, and its net effect on myocardial oxygen balance is determined by the relative contribution to coronary flow (oxygen supply) and cardiac work (oxygen demand). Infusion of isoproterenol causes tachycardia and a positive inotropic effect thereby greatly increasing myocardial oxygen consumption. The presence of the tachycardia reduces the duration of diastole and thus the time during which subendocardial coronary flow can occur. Diversion of blood flow away from the subendocardium by isoproterenol has been reported (9). Thus, during infusion of isoproterenol to these animals with compromised coronary circulation, the highly susceptible subendocardium performs more work at a

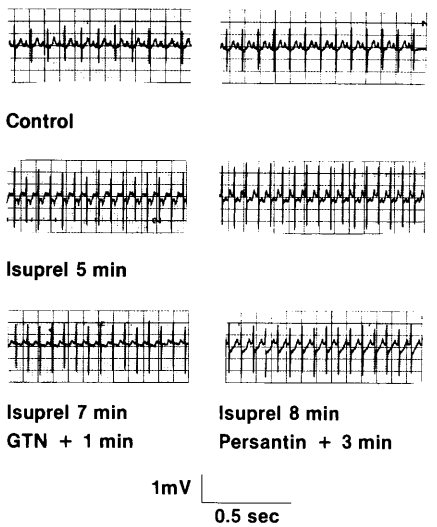

FIG. 3. ECG tracings showing reversal of isoproterenol-induced ischemic S-T segment depression by nitroglycerin and its exacerbation by dipyridamole (Persantin). Neither drug altered the isoproterenol-induced tachycardia. The tracings are from the same animal on different experimental days.

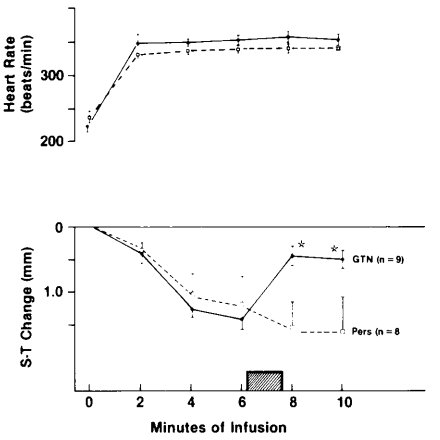

FIG. 4. Summary of the effects of nitroglycerin and dipyridamole on isoproterenol-induced heart rate and S-T segment changes (mean ± SEM). The only significant effect was the decrease in S-T segment depression following nitroglycerin (100 μg/kg) administration.

higher oxygen cost in the presence of decreased blood supply and becomes ischemic. This ischemia is manifested by electrocardiographic S-T segment depression.

Effects of propranolol on isoproterenol-induced ECG changes. Presumably the ischemic S-T segment depression elicited by isoproterenol infusion in these experiments was secondary to heart-rate and contractility increases brought about by beta-receptor stimulation. As might be expected from a competitive pharmacological antagonism, increasing doses of propranolol caused a dose-related inhibition of the effects of isoproterenol infusion (Fig. 2). The results of the present study have a clinical corollary in the work of Ushiyama *et al.* (5) who reported that propranolol (5 mg, iv) completely suppressed the isoproterenol-induced heart rate and ischemic S-T segment depression in each of eight anginal patients tested.

Propranolol is a clinically effective antianginal agent (12), the major beneficial effects of which are attributed to the reduction of heart rate and cardiac work due to blockade of beta-adrenergic stimulation by endogenous catecholamines. Its action in this animal model with coronary artery disease (13) mimics these clinical effects.

Effects of nitroglycerin and dipyridamole on isoproterenol-induced ECG changes. While differences of opinion regarding its mechanism of action exist, the efficacy of nitroglycerin in the therapy of angina pectoris is beyond question. Dipyridamole, however, has been shown to have only a benign coronary vasodilatory effect that has no significant therapeutic value when compared to placebo under double-blind conditions and may exacerbate the patient's angina (14). The vasodilation caused by dipyridamole is believed to be due to its inhibition of adenosine deaminase and cellular uptake of adenosine (15). It therefore might be expected to be active only in vascular beds that are not maximally dilated because of ischemic conditions. This is borne out by its tendency to cause "coronary steal" from ischemic regions. Nitroglycerin also causes vasodilation in a variety of vascular beds, however, its peripheral vasodilatory effects, particularly in the venous system, are felt to be chiefly (16, 17) if not solely (18) responsible for its antianginal effects. Its dramatic effect on isoproterenol-induced S-T segment depression in the present experiments suggests that this mechanism of venous pooling was operative since it is difficult to imagine coronary vasodilation being less than maximal under the conditions imposed by isoproterenol infusion in these animals with compromised coronary circulation. It is highly unlikely that the β-adrenergic blocking effects of nitroglycerin (19) were in any way involved since there was no effect on isoproterenol-induced tachycardia. In addition, the doses of nitroglycerin used to demonstrate β-adrenergic blockade were 30 times those used in this study.

Comparison of the effects of nitroglycerin and dipyridamole on ischemic S-T segment depression in this study shows a good correlation with clinical results, i.e. nitroglycerin has a beneficial effect whereas dipyridamole does not. The isoproterenol stress test appears to be a useful adjunct to atrial pacing in this experimental model of angina pectoris as well as in the clinical setting.

Summary. Isoproterenol infusion was employed as a cardiac stress test in unanesthetized, atherosclerotic rabbits. In addition to tachycardia, isoproterenol infusion caused ischemic S-T segment depression of the electrocardiogram. Propranolol (0.1 and 1 mg/kg, iv), given during isoproterenol infusion, reversed the tachycardia and S-T segment depression. Nitroglycerin (100 μg/kg, iv) reversed the ischemic S-T segment depression but did not affect the tachycardia. Dipyridamole (250 μg/kg) tended to exacerbate S-T segment depression, and had no effect on the tachycardia. The effects of nitroglycerin and dipyridamole in these animals correlated well with clinical results, i.e. nitroglycerin had a beneficial effect whereas dipyridamole did not. We conclude that the stress of isoproterenol infusion is as useful as atrial pacing in this experimental model of angina pectoris.

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