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Comparison of the Effects of Ouabain and Isoproterenol on Ischemic Myocardium of Conscious Dogs

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SUMMARY The effects of ouabain, 20 μg/kg, and isoproterenol, 0.03 μg/kg/min, were compared in conscious dogs with acute myocardial ischemia. The animals were instrumented with miniature pressure gauges for measurements of left ventricular (LV) pressure and dP/dt, miniature ultrasonic transducers for measurements of both regional myocardial fiber shortening and ECGs from the same sites and left atrial and aortic catheters for measurements of pressures and regional myocardial blood flow using the radioactive microsphere technique. Coronary occlusion increased heart rate and LV end-diastolic pressure but did not change LV systolic and mean aortic pressures or dP/dt significantly. In the ischemic zone, coronary occlusion reduced systolic segment shortening and blood flow markedly while increasing ST segment elevation. Isoproterenol in the presence of coronary artery occlusion increased heart rate 33 ± 4 beats/min and dP/dt 630 ± 90 mm Hg/sec and decreased mean arterial pressure by 6.3 ± 1.6 mm Hg. In the ischemic zone, isoproterenol reduced flow by 31 ± 9.1%, P < 0.01, and increased paradoxical bulging by 0.20 ± 0.07 mm, P < 0.02, and ST elevation by 3.0 ± 0.6 mV, P < 0.01. Ouabain increased dP/dt similarly by 600 ± 90 mm Hg/sec, but did not change heart rate or mean arterial pressure significantly. In contrast to isoproterenol, ouabain increased flow 46 ± 9.2%, P < 0.01, systolic segment shortening 0.35 ± 0.10 mm, P < 0.01, and reduced ST elevation 3.1 ± 0.4 mV, P < 0.01, in the ischemic zone. In conclusion, equi-inotropic doses of ouabain and isoproterenol induced opposite effects on the mechanical function, ECGs and blood flow of severely ischemic tissue. Ouabain appeared to alleviate the ischemic condition, whereas isoproterenol intensified ischemia.

INOTROPIC STIMULATION can be deleterious to the ischemic heart, since increasing myocardial metabolic demands in a situation of limited oxygen supply can exaggerate the imbalance between these two critical determinants of myocardial function.1-3 Recent studies in our laboratory indicated that ouabain in combination with propranolol4 or alone5 not only improved overall cardiac function but also enhanced function of the most ischemic area of the heart. This salutary action on mechanical function was associated with an increase in blood flow to ischemic myocardium and a reduction in ST segment elevation.4, 5 In order to determine if this action was peculiar to ouabain or was a result of its nonselective inotropic stimulation, i.e., that ouabain, by increasing contractility, caused a secondary rise in blood flow, the effects of ouabain on severely ischemic myocardium were compared with equi-inotropic doses of a β-adrenergic agonist, isoproterenol.

Methods

Eighteen dogs, weighing between 25-35 kg, were anesthetized with pentobarbital sodium, 30 mg/kg I.V. Through a thoracotomy in the fifth left intercostal space, miniature pressure gauges (P22 Konigsberg Instruments) were implanted within the left ventricle through a stab wound in the apex, and Doppler ultra-
sonic flow transducers were placed around the left anterior descending coronary artery, 2–3 cm from its origin. Hydraulic occluders were implanted just distal to the flow transducers and heparin-filled Tygon catheters (Norton Co) were implanted in the left atrium and aorta. Up to six pairs of miniature ultrasonic transducers were implanted intramyocardially, parallel to the muscle fibers, 1–2 cm apart and varying in depth from 4–15 mm, in the potentially non-ischemic and severely ischemic zones. The severely ischemic zone was predicted at operation and confirmed after completion of the experiment by finding reductions in regional myocardial function of greater than 90% and in regional blood flow of greater than 60%. The non-ischemic zone was in the central area of distribution of the left circumflex coronary artery on the posterior wall of the left ventricle.

The miniature pressure gauges were calibrated in vitro and in vivo against Statham P23 Db strain gauge manometers (Statham Instruments) connected to the left atrial and aortic catheters. At autopsy, the position of the gauge within the ventricular cavity was confirmed. Instantaneous coronary blood flow was measured with an ultrasonic Doppler flowmeter. An improved ultrasonic transit-time dimension gauge was used to measure regional myocardial segment length (SL). This instrument generates a voltage linearly proportional to the transit time of acoustic impulses traveling at the sonic velocity of approximately 1.5 × 10^8 mm/sec between the 3 MHz piezoelectric crystals, thereby giving a record of instantaneous myocardial fiber length. At a constant room temperature, the thermal drift of the instrument is minimal, i.e., less than 0.01 mm in 6 hours. The frequency response is flat to 60 Hz. Any drift in the measuring system, i.e., the instrument electronics, the data tape recorder, and the oscillograph that displayed data, was eliminated during the experiment by periodic calibrations. This involved substitution of pulses of precisely known duration from a crystal-controlled pulse generator having a basic stability of 0.001%. The instrument used in the present study was modified further to provide simultaneous measurements of multiple segment lengths and the regional ECGs from these crystal sites. These transducers were connected to Clevite-Brush preamplifiers for recording of electrographic potentials. The standard limb lead configuration was used for ground reference. The ECG preamplifier and strip chart were calibrated such that a 1 mV signal produced a 1 mm pen deflection. The position of the miniature ultrasonic transducers was confirmed at autopsy and minimal fibrosis, <1 mm, was observed at the site of implantation.

Regional myocardial blood flow and cardiac output were measured using the radioactive microsphere technique. The microspheres were shipped from the manufacturer (3M Co) in dry form, in multiple vials for each isotope. The solutions were prepared individually as needed, and the microspheres were in contact with the solution for less than seven days. The concentration of microspheres per ml of solution was adjusted appropriately to account for natural radioactive decay. The microspheres were suspended in 0.01% Tween solution (10% dextran) and placed in an ultrasonic bath for 60 minutes. They were subsequently agitated by direct application of an ultrasonic probe to insure dispersion of the spheres just before injection. Absence of microsphere aggregation was verified by microscopic examination. Before injection of microspheres, 0.7 ml of the Tween solution (without microspheres) was injected to determine if the diluent for the microsphere suspension was to have an adverse effect on cardiac dynamics. One to three million microspheres (15 ± 2 μ) labeled with 82Sr, 51Cr, 85Sr or 131I were injected through the catheter implanted in the left atrium for three determinations of blood flow during control, 10–15 minutes after the onset of coronary occlusion, and finally either 10–15 minutes after ouabain or 5–15 minutes after isoproterenol, in separate groups of dogs. A reference sample of arterial blood was withdrawn beginning 10 seconds before microsphere injection and continuing for 40 seconds after the injection was completed. After sacrifice of the animal, myocardial samples were obtained from the sites where function and ECGs were measured, dissected into epicardial and endocardial layers, weighed, placed in a multichannel gamma well counter (Searle Analytic, Inc), and counted in appropriately selected energy windows for 10 minutes. The gamma counting system uses three adjustable energy analyzers. The high voltage for the detector was adjusted with a 131I counting standard to optimize overall counting efficiency, and the individual base levels and energy windows were set around the main photon peak for each isotope, while at the same time minimizing the amount of crossover from the other isotopes. By counting measured aliquots of each isotope, the energy crossover of each isotope into adjacent channels was calculated before counting the samples from each experiment. Thus, the raw count data obtained for each tissue sample were corrected for background and converted to a true count value using matrix manipulation routines with a digital computer. The true counts were then compared with the reference blood sample to obtain flow expressed in ml/min/g of tissue. With this particular system there is essentially no problem with dead-time in terms of affecting the true count rate, as the time for processing individual events does not interfere with the overall counting schedule. Furthermore, total counts that may exceed the limits of the system, rarely encountered in practice, are easily recognized and the samples are simply recounted for a shorter period of time. In the case of measuring very small flows to the severely ischemic myocardium, the variability in this measurement is reduced by increasing the total count time and thus lowering the variance of the measured count. Furthermore, it should be noted that several animals were studied in this investigation, and the statistical significance of the results reported indicates that natural variability in the measurement of regional flows with the microsphere technique plays a minor role when compared with the direct effects of the interventions being studied.
Experiments were conducted one to four weeks after operation. While the conscious, unsedated dogs rested quietly, control records of left ventricular (LV) pressure (P), the rate of change of pressure (dP/dt), coronary blood flow, heart rate and SL shortening were recorded, along with intramyocardial ECGs. After control measurements were recorded and the first injection of microspheres was made, the coronary vessel was occluded, which was confirmed by absence of coronary flow measured with the Doppler probe until sacrifice of the animal. Measurements were recorded continuously and the second injection of microspheres was made 10–15 minutes after coronary occlusion, at a time when measurements of regional myocardial function and ECGs were stable. At 15–20 minutes after coronary occlusion, ouabain was injected in a dose of 20 μg/kg I.V. to a group of 12 conscious dogs, and isoproterenol was infused 0.03 μg/kg/min I.V. to another group of six conscious dogs. The third microsphere injection was made either 10–15 minutes after ouabain or 5–15 minutes after the beginning of the isoproterenol infusion. After 30 minutes of further recordings, the animals were anesthetized with 30 mg/kg of pentobarbital sodium and sacrificed to confirm placement of intramyocardial transducers and to obtain myocardial samples at the same sites for regional blood flow determination.

Data were recorded on a multichannel tape recorder and played back on two multichannel direct-writing oscillographs at a paper speed of 100 mm/sec. A cardiacthometer, triggered by the pressure pulse signal, provided instantaneous and continuous records of heart rate. Continuous records of dP/dt were derived from the LVP signal with a Philips (Teledyne Philbrick) operational amplifier connected as a differentiator having a frequency response of 700 Hz. A triangular wave signal with known slope (rate of change) was substituted for the pressure signal to calibrate the differentiator directly.

The effects of interventions on regional myocardial function were assessed by measurement of regional systolic myocardial fiber shortening per stroke. Mean and SEM values were calculated. The three states in each animal (control, occlusion and occlusion plus drug) were compared by the paired t test, while changes between two groups of treated animals were compared by the unpaired t test.

**Results**

**Effects of Coronary Occlusion (table 1)**

Coronary occlusion increased heart rate and LV end-diastolic pressure, *P < 0.01*, but did not affect LV systolic or mean aortic pressures or LV dP/dt significantly. In the non-ischemic zone, coronary occlusion increased end-diastolic length (0.34 ± 0.07 mm, *P < 0.01*), but did not affect systolic segment length shortening or the ST segment significantly. In the severely
ischemic zone, coronary occlusion increased ST segment elevation and end-diastolic segment length, decreased flow and reversed systolic segment shortening to lengthening. All these effects in the severely ischemic zone were significant, \( P < 0.01 \) (table 1).

**Effects of Isoproterenol and Ouabain in the Presence of Ischemia (table 1)**

Isoproterenol increased LV dP/dt by \( 630 \pm 90 \) mm Hg/sec (\( P < 0.01 \)), heart rate by \( 33 \pm 3.9 \) beats/min (\( P < 0.01 \)) and cardiac output by \( 27.3 \pm 8\% \) (\( P < 0.05 \)). Mean aortic pressure was decreased by \( 6.3 \pm 1.6 \) mm Hg (\( P < 0.01 \)), end-diastolic pressure by \( 1.3 \pm 0.4 \) mm Hg (\( P < 0.05 \)) and total peripheral resistance by \( 24.5 \pm 4.7\% \) (\( P < 0.02 \)). LV systolic pressure was not changed significantly (fig. 1). In the non-ischemic zone, isoproterenol increased ST segment elevation by \( 0.35 \pm 0.16 \) mV (\( P < 0.05 \)) and blood flow by \( 49.8 \pm 8.1 \% \) (\( P < 0.01 \)) (fig. 2). End-diastolic segment length was decreased by \( 0.17 \pm 0.06 \) mm (\( P < 0.02 \)) and systolic segment length shortening was increased by \( 0.39 \pm 0.11 \) mm (\( P < 0.01 \)) (fig. 3). In the ischemic zone, isoproterenol increased ST segment elevation by \( 3.0 \pm 0.6 \) mV (fig. 4) and reduced blood flow by \( 31 \pm 9.1\% \). The fall in blood flow was more pronounced in endocardial layers (-51.9 ± 7.8\%), as the endo/epi flow ratio fell from \( 0.53 \pm 0.14 \) to \( 0.28 \pm 0.07 \). These changes in the ischemic zone were significant, \( P < 0.01 \), and were associated with a fall in end-diastolic segment length and an increase in paradoxical bulging, \( P < 0.05 \) (fig. 5).

Ouabain increased LV dP/dt by \( 600 \pm 90 \) mm Hg/min, \( P < 0.01 \), and cardiac output by \( 27.6 \pm 7.9\% \), increments similar to those observed with isoproterenol; and ouabain decreased total peripheral resistance by \( 16.7 \pm 5.0\% \), \( P < 0.01 \). Ouabain also increased LV systolic and mean arterial pressures and decreased heart rate and LV end-diastolic pressure slightly; these changes were not significant. However, the responses of heart rate and mean arterial pressure were directionally opposite and significantly different, \( P < 0.01 \), from those observed with isoproterenol (fig. 1). In the non-ischemic zone, ouabain did not affect end-diastolic segment length, blood flow or ST segment elevation significantly, but it increased segment shortening by \( 0.23 \pm 0.07 \) mm (\( P < 0.01 \)) (fig. 3). Only the change in blood flow was significantly different, \( P < 0.01 \), from the response observed with isoproterenol (fig. 2). In the severely ischemic zone, ouabain did not affect end-diastolic segment length significantly, but it caused a substantial increase, \( P < 0.01 \), in segment shortening of \( 0.35 \pm 0.10 \) mm. The improvement in shortening per-
**REGIONAL MYOCARDIAL BLOOD FLOW**

**NORMAL ZONE**

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* p<.01

- *Isoproterenol*
- *Ouabain*

**ISCHEMIC ZONE**

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* p<.01

**REGIONAL MYOCARDIAL FUNCTION**

**NORMAL ZONE**

- Δ End Diastolic Length (mm)
  - 16.87
  - 15.94
  - * p<.05

- Δ Segment Length Shortening (mm)
  - 2.61
  - 2.68

**ISCHEMIC ZONE**

- Δ End Diastolic Length (mm)
  - 17.92
  - 16.31
  - * p<.05

- Δ Segment Length Shortening (mm)
  - -0.10
  - -0.07

*p<.01*

*p<.05*

**Figure 2.** A comparison of the effects of isoproterenol and ouabain on regional myocardial blood flow in the normal and ischemic zones is shown. Significant changes from control are denoted by the asterisks, while occlusion control values before intervention are shown at the base of the bars. The two drugs elicited opposite and significantly different (P < 0.01) effects on regional blood flow.
CHAPTER 4

A comparison of the effects of isoproterenol and ouabain on regional electrograms, i.e., ST elevation, is shown. Occlusion baseline values before intervention are shown at the base of the bars, while significant changes from these values are denoted by the asterisks. The two drugs had opposite and significantly different (P < 0.01) effects on ST elevation in the ischemic zone.

Discussion

The effects of cardiac glycosides on the ischemic heart in the absence of heart failure have been controversial. Several studies conducted either in man or experimental animals have failed to demonstrate a beneficial action of digitalis in the ischemic setting. In contrast, recent studies from this laboratory found that ouabain improved function in severely ischemic myocardium when administered alone or in combination with propranolol. The question arose whether ouabain's action of increasing the inotropic properties of ischemic myocardium could have caused an increase in blood flow to permit further augmentation of the inotropic effect of the drug. If this hypothesis were correct, isoproterenol, by increasing the inotropic state of ischemic myocardium, would have also improved blood flow and function.

In the present study, the effects of equi-inotropic doses of isoproterenol were compared with those of ouabain. The doses were matched to increase LV dP/dt by similar amounts in conscious dogs with acute myocardial ischemia. It is recognized that LV dP/dt yields only a rough indication of the inotropic state. However, it was felt that this would be a sufficiently accurate measurement for means of comparison. In comparison with ouabain, isoproterenol caused diametrically opposing effects on regional

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Figure 3. A comparison of the effects of isoproterenol and ouabain on normal and ischemic zone function, i.e., changes in end-diastolic segment length and systolic segment length shortening are shown. Significant changes from control are denoted by the symbols, while occlusion baseline values are denoted at the base of the bars. Whereas both drugs improved function in the non-ischemic zone, isoproterenol increased the extent of paradoxical bulging in the ischemic zone, while ouabain had the reverse effect and improved shortening.
function, blood flow and the ECGs in the ischemic zone. Isoproterenol caused a deterioration in function of the severely ischemic tissue by increasing the extent of paradoxical bulging in those segments. This finding of enhanced paradoxical bulging has also been observed by Kerber et al.20 and in a previous study from our laboratory9 where isoproterenol was administered for as long as 3 hours. However, in those studies the dose of isoproterenol was 10-100-fold greater than that employed in the present investigation.

Isoproterenol reduced blood flow to ischemic tissue significantly (fig. 2). This decrease in blood flow was more pronounced in endocardial layers, thereby reducing the endo/epi flow ratio. These results were in direct contrast to those for ouabain, where transmural and, in particular, endocardial flows, improved. Therefore, it is unlikely that the mechanism for the improvement in blood flow that was observed with ouabain could be related simply to inotropic stimulation, since when this occurs with a β-adrenergic agonist, such as isoproterenol, blood flow actually decreases to ischemic tissue. A decrease in retrograde flow and coronary artery pressure distal to the coronary occlusion with isoproterenol was observed by Cohen et al. in open-chest, anesthetized dogs.21 In that study the effects were attributed to isoproterenol’s action to dilate the coronary vasculature in non-ischemic zones and “steal” flow from the ischemic zones.21 The direct measurement of regional flow with micro-

**Figure 5.** A comparison of the effects of coronary occlusion (middle panel) and isoproterenol (right panel) is shown on the phasic waveforms for left ventricular (LV) pressure, dP/dt, segment length in an ischemic zone, the electrogram (ECG) blood flow and the pressure-length loop from that segment. The pressure-length loop was constructed by applying the pressure and length signals into the y and x inputs, respectively, of a storage oscilloscope. A polaroid photograph of the oscilloscope display is shown. With coronary occlusion, there was little change in LV pressure or dP/dt but a reversal of the normal systolic segment length shortening to paradoxical bulging. End-diastolic (ED) and end-systolic (ES) points are noted. There was also marked ST elevation and reduction in the LV pressure-segment length loop. With the addition of isoproterenol, there was further intensification of ischemia as reflected by more paradoxical bulging, a fall in the regional blood flow and the area of the pressure-length loop and a further elevation of the ST ECG. The post-systolic segment shortening is characteristic of ischemic segments.
spheres in the present study supports this concept. It appears that the key difference in the action of these two drugs is the response of regional blood flow. In the ischemic zone, blood flow increased, with a concomitant improvement in function with ouabain, whereas the fall in blood flow with isoproterenol was associated with a deterioration in function. In contrast, in the non-ischemic zone, blood flow rose slightly but not significantly with ouabain, while it rose significantly more with isoproterenol (fig. 2). The rise in blood flow observed with isoproterenol in the non-ischemic zone, along with the fall in the flow in the ischemic zone, tends to support the concept of coronary “steal.”

At the same time that isoproterenol caused a deterioration in function and a reduction in blood flow, it caused a rise in ST segment elevation (fig. 5). A rise in ST segment elevation with isoproterenol in anesthetized animals was described previously by Maroko et al. In contrast, ouabain caused ST segment elevation to decline significantly in the severely ischemic zone (fig. 4), a finding which was not observed in anesthetized animals. While considerable controversy exists regarding the use of ST segment mapping, it is important to note that the increases in ST segment elevation were observed along with a fall in blood flow and increase in paradoxical bulging with isoproterenol, and that the reduction in ST segment elevation with ouabain occurred with concomitant improvements in both flow and function.

The mechanism of the increased flow to the ischemic zone observed following ouabain was not determined. The slight, but not significant, elevation in mean arterial pressure and reduction in heart rate may have contributed to the increased flow. It is also
possible that in the presence of myocardial ischemia, ouabain can dilate coronary collateral vessels directly or indirectly through an action on Ca++, Na+ or K+, resulting in enhanced blood flow to the ischemic zone. It is unlikely that differences in peripheral vascular effects were important, since both drugs reduced total peripheral resistance. This finding is of interest in view of ouabain’s well-recognized action of increasing total peripheral resistance in the normal animal.26

In conclusion, two inotropic agents at equi-inotropic doses induced opposite effects on the mechanical function, ECGs and blood flow of severely ischemic tissue. On the one hand, ouabain acted to ameliorate the ischemic condition as reflected by improved flow and function and reduced ST segment elevation, while isoproterenol intensified ischemia, as reflected by increased paradoxical bulging and ST segment elevation and a fall in blood flow, particularly to endocardial layers. The difference between the effects of these two agents on severely ischemic myocardium is in part due to their disparate actions on regional hemodynamics, since responses of heart rate and mean arterial pressure were significantly different with the two drugs. Thus, these data imply that a positive inotropic agent may not necessarily affect ischemic myocardium adversely. As demonstrated with ouabain, concomitant actions on overall ventricular function, as well as on blood flow to the ischemic tissue, may result in a net salutary influence.

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References