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Effect of Inosine on Contractile Force and High-Energy Phosphates in Ischemic Hearts (40575)[1]

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During pathophysiologic stresses such as ischemia or hypoxia, myocardial stores of the adenine nucleotides are reduced, and there is a transient but significant increase in the myocardial content of the various nucleotide derivatives (1, 2). Much attention has been focused upon the effects of these derivatives on the myocardium and the coronary vasculature. In particular, the work of Berne (3), has led to the hypothesis that the purine nucleoside adenosine may be important in the regulation of coronary blood flow. Other investigations have shown that several of these purine and pyrimidine nucleosides and nucleobases enhance contractility in the isolated frog (4), dog (5, 6), and rabbit (6) heart preparation. Other studies in isolated preparations have suggested that the positive inotropic actions of one of these purine nucleosides, inosine, is mediated by adrenergic mechanisms (7, 8). Although the inotropic response to inosine in the normal *in situ* heart has been well established (9, 10), the precise mechanism remains obscure, since the inotropic actions of inosine were not abolished in either sympathectomized dog hearts (11) or those pretreated with propranolol (9, 10). In addition to its inotropic actions, several studies have indicated that inosine may have an important direct vasodilator effect in the coronary vascular bed (12–14), as well as in other vascular beds (15).

A preliminary study from our laboratory demonstrated that inosine enhanced contractility in the ischemic myocardium following acute coronary occlusion (16). However, this effect of inosine in ischemic myocardium has not been systematically explored. Therefore, one purpose of the present experiments was to obtain a better definition of inotropic actions of this nucleoside in the ischemic myocardium.

A second objective of the present study was to investigate a mechanism of action of inosine in the ischemic myocardium. The content of myocardial high-energy phosphates degrades rapidly in ischemic myocardium following coronary occlusion (2). Accumulation of the derivatives, including inosine, might be important either when flow is restored, or with sufficient collateral blood flow development as these derivatives may serve as substrates for resynthesis. It has been proposed that the inotropic effects of inosine may be due to the enhancement of high-energy phosphate levels (11, 17). It is attractive to speculate that such a mechanism of action may be especially effective in the ischemic heart when high-energy phosphate levels are declining. However, one report has shown that perfusion of isolated rabbit hearts with inosine is accompanied by an increased breakdown of myocardial high-energy compounds, presumably secondary to the enhanced energy requirements of the augmented cardiac contractility (17). In the present experiments, the effects of inosine on the myocardial contents of adenosine triphosphate (ATP) and creatine phosphate (CP) following coronary occlusion were determined in order to obtain a better understanding of the relationship of this nucleoside to energy conservation in the ischemic heart.

Methods. Seventeen mongrel dogs of either sex weighing 12–17 kg were divided into two groups. The protocols in both groups were identical. Dogs were anesthetized with sodium pentobarbital (30 mg/kg IV). Each an-

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imal was intubated and artificially respired using a Harvard positive-pressure respiration pump with room air. Polyvinyl catheters filled with heparinized saline were placed in the left jugular vein (8F), left femoral vein (8F), and left femoral artery (6F). The catheter in the jugular vein was advanced approximately to the level of the right atrium and attached to a Harvard Model 906 syringe pump for infusion of inosine or saline solutions. Catheters in the femoral artery and vein were attached to Statham P23AC pressure transducer for continuous arterial pressure measurement and to a fluid administration set, respectively. The chest was opened through the fifth intercostal space on the left side and the pericardium was carefully incised and retracted forming a cradle.

Contractile force. In the first group of seven dogs, the effects of inosine on myocardial contractile force following localized coronary occlusion were studied. Three Walton–Brodie strain gauge arches (13-mm total length) were sutured to the surface of the left ventricle with silk ligatures placed in the superficial epicardial muscle layers. The arches were oriented parallel to the epicardial muscle fibers as previously described (2).

Silk ligatures were placed around several small branches of the left anterior descending and left circumflex coronary arteries creating a circle of ligatures. One gauge was placed in the center of this area (IMCF). A second gauge was placed just outside the circle of ligatures and served to indicate the function of the peripherally ischemic muscle (PIMCF). A third gauge was placed in an area supplied by normal arteries (NIMCF). The outputs of the arterial pressure transducer and strain gauge arches were recorded on a Grass Model 7 polygraph.

After waiting 5–10 min to allow stabilization of the preparation, the isolated vessels were ligated producing an ischemic area of approximately 5–10 cm^2. With occlusion, contractile force rapidly declined and stabilized at a lower level usually within 1–2 min (2). At this time, 50 ml of normal (0.9%) saline was infused at a rate of 10 ml/min and the responses were recorded for a period of 10 min following termination of the infusion. The occlusion was released and approximately ¾ hr passed before the next occlusion, to allow all parameters to return to control levels. The occlusion was repeated and 50 ml of 50 m*M* (0.50 mmol/min) inosine (Sigma Chemicals) was infused at the same rate. This concentration of inosine has previously been shown to produce arterial inosine levels of approximately 120 μM/ml (9). All parameters were monitored for a period of 10 min after the infusion. The order of the infusions was reversed without consequence. Contractile force is expressed in terms of percentage change from the preinfusion level. The data was analyzed for significance using an analysis of variance and a Student's t test for paired data. Significance was established when $P < 0.05$.

ATP and CP. The second group of 10 dogs was subdivided into 5 inosine-treated and 5 saline-treated dogs. The experimental protocol was identical to that described above except no strain gauges were sutured to the heart. In each of these subgroups the procedure for obtaining myocardial tissue samples was identical and was performed as follows: Small samples of left ventricle (50–100 mg) were obtained from within the circle of ligatures by using stainless-steel tongs that had been precooled in liquid nitrogen. The muscle samples were frozen *in situ*, rapidly excised, and immersed in liquid nitrogen within 2–3 sec following excision. Immediately prior to the occlusion of the arterial branches, a muscle sample (1) was taken from the center of the area to be made ischemic. After sample 1 had been taken, the isolated vessels were rapidly ligated. Exactly 10 min after coronary occlusion, sample 2 was taken from the ischemic area, being careful not to sample twice from the same wound. After sample 2 was taken, the infusion of 50 ml of either normal saline or 50 m*M* inosine at a rate of 10 ml/min was begun. At the end of the infusion, sample 3 was taken. A final sample (4) was taken 10 min after completion of the infusion.

Myocardial contents of ATP and CP were determined as previously described (2). The values obtained from inosine-treated dogs were compared to those from saline-treated dogs at the specific sampling times and analyzed for significance using the Student's t test for unpaired data at the $\alpha = 0.05$ significance level.

Results. Contractile force. Figure 1 shows a representative tracing at the time of occlusion (Panel A, arrow). Mean arterial pressure (MAP), arterial pressure (AP), peripheral ischemic myocardial contractile force (PIMCF), nonischemic myocardial contractile force (NIMCF), and ischemic myocardial contractile force (IMCF) are shown. Note that following an initial period of arrhythmia, IMCF stabilized at a lower level. Panel B shows the same parameters during a subsequent inosine infusion (black line at bottom of tracing). Note the gradual and sustained increases in contractile force in all regions throughout the infusion.

Inosine increased contractile force by 31.57 ± 7.18% in IMCF ($P < 0.005$), 40.64 ± 5.02% in PIMCF ($P < 0.001$), and by 41.57 ± 4.46% in NIMCF ($P < 0.001$), above control levels. However, utilizing the analysis of variance test, the responses in each of three regions were not different from each other ($F = 1.09$, $P = 0.3680$).

ATP and CP. Table I shows the changes in ATP and CP content in the ischemic myocardium during saline and inosine infusions. Sample 1 was taken immediately prior to occlusion of the arterial branches. Since these samples represent control levels of ATP and CP regardless of the subsequent infusion, the values for sample 1 ATP and CP represent the pooled values of the two groups ($n = 10$). Samples 2–4 represent values from five dogs in each group. There were no significant differences in myocardial ATP or CP content in samples 2, 3, or 4 between the inosine- and saline-infused dogs ($P > 0.05$ in each). Inosine infusion did not enhance the degradation of ATP or CP. That is, the values in samples 3 and 4 in the saline-infused group are not significantly different from those in the inosine-infused group. Thus, inosine infusion did not result in lower values of either ATP or CP.

Discussion. The experiments reported herein demonstrate that inosine infused intravenously consistently causes an increase in ischemic, peripheral, and nonischemic myocardial contractile force following coronary occlusion. Our results also indicate that this augmentation in contractile force in the ischemic area is not associated with either increased or decreased levels of myocardial ATP or CP.

It might be argued that inosine does not have a direct effect in the ischemic area, but rather the increase in IMCF is merely a reflection of inotropic changes occurring in the surrounding muscle. This interpretation of our data is not tenable for two reasons. First, the model of ischemia used in this study only reduces contractile force by approximately 60%. Evidently, after occlusion there remains some flow which supports active contraction, though at a greatly reduced level. Second, if the changes in contractile force were primarily due to changes outside the ischemic area, the feet of the strain gauge arch would have been pulled apart, rather than forced to-

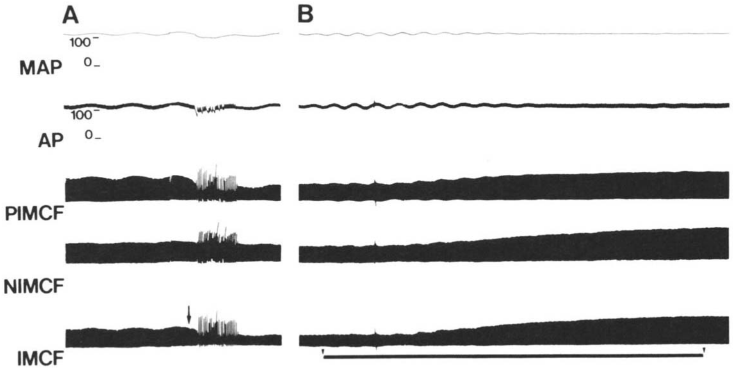

FIG. 1. Original tracing showing recordings of mean arterial pressure (MAP), arterial pressure (AP), nonischemic myocardial contractile force (NIMCF), peripheral ischemic myocardial contractile force (PIMCF), and ischemic myocardial contractile force (IMCF). Panel A shows the effects of occlusion of the isolated arteries (at arrow). Panel B is a continuous recording during inosine infusion following the occlusion (dark line at bottom). Both panels are from the same experiment.

TABLE I. ALTERATIONS IN MYOCARDIAL *ATP* AND *CP* CONTENT[a]

	Saline-infused group		Inosine-infused group	
	ATP	CP	ATP	CP
Sample 1[b] (control)	3.59 ± 0.16	6.50 ± 0.39	3.59 ± 0.16	6.50 ± 0.39
Sample 2 (occlusion)	2.43 ± 0.12	2.66 ± 0.79	2.46 ± 0.20	3.09 ± 0.26
Sample 3 (infusion)	1.66 ± 0.18	1.89 ± 0.09	1.77 ± 0.14	1.89 ± 0.38
Sample 4 (postinfusion)	1.60 ± 0.35	1.49 ± 0.10	1.11 ± 0.15	1.36 ± 0.35

[a] Expressed as μmol/g weight ± standard error of the mean.

[b] Control values represent pooled values from the two groups.

gether, yielding a downward deflection during ventricular systole rather than an upward displacement. For these reasons, we believe the increase in contractile force in all areas including IMCF to be due to the direct action of inosine.

Certain differences in the inotropic actions of inosine and other well-known inotropic agents such as isoproterenol and norepinephrine in the ischemic heart are striking and warrant further discussion. As clearly shown in the present experiments, inosine infusion resulted in a gradual and progressive increase in contractile force in the ischemic myocardium which was sustained for the duration of the infusion. In contrast, both isoproterenol and norepinephrine when infused into the ischemic heart for a 5-min period, resulted in prompt and profound though transient increases in contractile force; within 1 min contractile force in the ischemic area is reduced to well below control levels (18, 19). This response to isoproterenol or norepinephrine in ischemic myocardium is presumably due to the increased oxygen requirements caused by the inotropic agents without an accompanying increase in blood flow to that region. It is also noteworthy that both isoproterenol and norepinephrine significantly increase infarct size following coronary occlusion (20), while inosine significantly reduces infarct size by approximately 25% (14). The sustained increase in contractile force of ischemic myocardium during inosine infusion implies that inosine, in addition to its inotropic actions, may also augment blood flow to the ischemic region.

Inosine has generally been considered to have no vasoactive properties in the coronary vasculature. This consensus was based on the work of Wolf and Berne (21) who showed that levels of inosine (0.1–0.3 μM/min) were not vasoactive. However, more recent data indicates that inosine may indeed be vasoactive, especially at the higher arterial concentrations used in this study. Thus, Faucon *et al.* (12) and Aurrouseau *et al.* (13) found evidence of a direct vasodilator action in the coronary circulation. Furthermore, significant increases in circumflex blood flow (9) and anterior descending blood flow (10) have been reported with inosine infusion. Granger *et al.* (15) reported that inosine was 50% as potent as adenosine in causing vasodilatation of the mesenteric bed. In relation to the present experiments, Devous *et al.* (11), using tracer microspheres, observed that inosine increases collateral perfusion of ischemic myocardium following coronary occlusion.

The observations that inosine increases flow to the ischemic myocardium while simultaneously increasing contractile force is perhaps strengthened by the present data regarding ATP and CP degradation in the ischemic myocardium. Kypson and Hait have reported that inosine does not augment ATP levels, but rather promotes the breakdown of ATP in both the normal (17) and hypoxic (24) isolated rabbit heart. Our data indicates that inosine does not result in lower levels of either ATP or CP following coronary occlusion. The reasons for the differences in the results from Kypson and Hait's studies and the present investigation are not readily apparent, although perhaps dose- or species-related factors could help explain them. However, since inosine administration does not result in lower levels of ATP or CP in the *in situ* ischemic canine heart, one explanation is that flow was augmented to such an extent, that it remained essentially constant with regard to demand from the myocardium. As mentioned above, tracer microsphere studies have demonstrated increases in collateral flow to an ischemic area following inosine administration (14).

The mechanism of the inotropic action of inosine in the *in situ* canine heart remains unclear, although the present data indicate that it does not appear to act through augmentation of myocardial high-energy compounds. However, the fact that this inotropic agent does not result in further decay of ATP or CP, perhaps through increases in blood flow, points to its potential usefulness. An inotropic agent which enhanced function in normal and ischemic myocardium without further compromising the critical balance between energy supply and demand in ischemic myocardium has quite exciting implications.

Summary. The effects of intravenously infused inosine on myocardial contractile force (MCF) and high-energy phosphate content were studied in 17 open-chest, pentobarbital-anesthetized mongrel dogs following occlusion of small branches of the left anterior descending and circumflex coronary arteries. In 7 dogs, MCF was measured using strain gauge arches sutured in the center of the ischemic area (IMCF), in the periphery of the ischemic area (PIMCF), and in a nonischemic area (NIMCF). After the arteries were ligated and IMCF had stabilized, an infusion of either 50 ml of 50 m*M* inosine or saline was begun at a rate of 10 ml/min. Inosine infusion produced increases in IMCF, PIMCF, and NIMCF of 32, 41, and 42% of preinfusion levels, respectively. In an additional 10 dogs, adenosine triphosphate (ATP) and creatine phosphate (CP) content of the ischemic myocardium was determined at intervals prior to and following coronary arterial occlusion, during which time either inosine ($n = 5$) or normal saline ($n = 5$) was infused. The degradation of ATP or CP was not altered with inosine infusion as compared to the saline-infused group. It has been concluded from the above data that inosine significantly increases MCF in ischemic hearts and that this augmentation in MCF is not associated with alterations in levels of ATP or CP.

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