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The Effect of Inosine on Blood Pressure in the Rat[1] (37753)

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(Introduced by George W. Thorn)

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Adenosine and the adenine nucleotides are known to be potent vasodilators (1, 2). Berne has suggested that the breakdown of adenosine triphosphate during myocardial hypoxia ultimately leads to the release of adenosine which acts to dilate the coronary vascular bed, increasing flow, thus improving oxygen supply (2). More recent studies by Dobson *et al.* (3), have suggested that adenosine may also play a role in regulation of skeletal muscle blood flow.

Adenosine is rapidly converted to inosine by plasma and erythrocyte adenosine deaminase. In ischemic exercising cardiac and skeletal muscle, inosine and inosine monophosphate (IMP) have been shown to rise as much as 58-fold (3, 4). Inosine is cleaved by purine nucleoside phosphorylase to form hypoxanthine and ribose-1-phosphate. Inosine, hypoxanthine, and ribose-1-phosphate have been reported to be without effect on the coronary circulation relative to the adenine nucleotides (2) and are generally assumed to be without vasoactivity (3, 5).

During studies of the hemodynamic effect of the adenine nucleotides and their metabolites, we noted that a small dose of inosine raised blood pressure (BP) in the intact rat. To our knowledge this effect has not been previously described. The present report describes the hypertensive response to inosine and the effect of various interventions on this BP rise.

Materials and Methods. Female, inbred Sprague–Dawley rats weighing 210–240 g were used for all experiments. The rats were anesthetized with sodium pentobarbital, 50 mg/kg ip with supplemental doses as necessary. The trachea was isolated and cannulated with a short piece of polyethylene tubing for removal of secretions. The external jugular vein was cannulated with PE 10 tubing; infusions were delivered with a microburette. A carotid artery was cannulated with a 2-cm length of PE 60 tubing and connected to a Statham P230b transducer attached to a Sanborn model 296 recorder. Electrocardiograms were simultaneously recorded with subcutaneous electrodes.

Inosine was purchased from Nutritional Biochemical Corporation, Cleveland and from Boehringer und Soehne, Mannheim, Germany. No difference in the results was noted between the two sources of inosine. Inosine-5′-monophosphoric acid (IMP) and hypoxanthine were also purchased from Nutritional Biochemical Corporation. The ribose-1-phosphate was from Boehringer und Soehne. Inosine, IMP, hypoxanthine, and ribose-1-phosphate were prepared to a concentration of 1×10^{-2} *M* with 0.14 *M* Tris–HCl. Final pH was adjusted to 7.34; all solutions were stored at −10°.

The experimental rats were divided into four groups.

Group I. Six rats were anesthetized with sodium pentobarbital. No attempt was made to alter the autonomic nervous system.

Group II. Ten rats were anesthetized as above; in addition, the autonomic nervous system was blocked with atropine 2 mg/kp ip and pentolinium, 4 mg/kg ip.

Group III. Four rats were prepared as in

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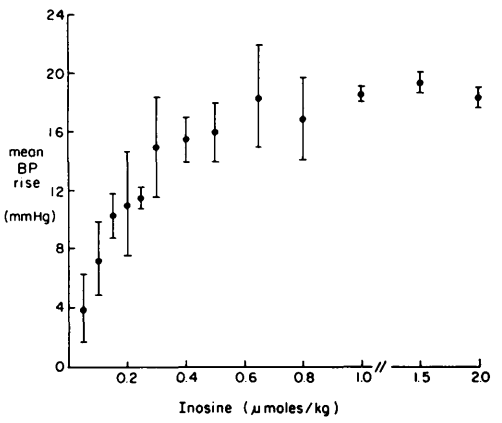

FIG. 1. Change in mean blood pressure (plus and minus one standard deviation) after intravenous inosine in doses from 0.05 to 2.0 μmoles inosine/kg body weight.

Group II; in addition, peripheral β-adrenergic receptor sites were blocked with propranolol, 15 mg/kg ip[2].

Group IV. Four rats were prepared as in Group II; in addition, peripheral α-adrenergic receptor sites were blocked with phentolamine, 20 mg/kg ip[3].

A dose-response curve was constructed from five rats prepared as in Group I. The mean BP change was measured after the administration of inosine in doses ranging from 0.01 to 2.0 μmoles/kg intravenously. The dose-response curve (Fig. 1) indicated that maximal BP elevation from inosine was obtained at about 0.8 μmoles/kg and that higher doses afforded no significant increase in hypertensive effect.

Experimental animals in all four groups were given rapid iv injections of 48.2 μg (0.18 μmoles) inosine in a total volume of 0.018 ml. This was equivalent to 0.749–0.857 μmoles/kg. Five constant infusions of inosine were given to animals in Group II at a rate of 0.6 μmoles/min (0.06 ml/min). Systolic and diastolic pressures were measured from the carotid artery pressure tracings; mean arterial pressure was determined by electronic integration of the arterial pressure curve. Heart rate was determined from the ECG. A total of 60 injections of inosine were given to the 24 rats in the four study groups. Each rat had at least two injections, and the results are expressed as the average of several injections for each experimental animal. Injections of 0.14 *M* Tris–HCl buffer were administered to five rats in various groups in a volume of fluid equal to the inosine solution. IMP was injected into two Group II rats at a molar concentration equal to inosine.

Inosine was boiled for 30 min in 0.2 *N* H_2SO_4 and adjusted to pH 7.34 with NaOH. This was done to insure no contamination of inosine with a vasoactive factor. The degraded inosine was then injected as in the other experiments. Xanthine and ribose-1-phosphate were tested for vasoactivity in molar doses comparable to those of inosine.

Results. All rats tested showed a prompt, significant rise in blood pressure. The *P* value was 0.001 for the change in blood pressure in all four groups when evaluated by the method of paired comparisons (paired Student *t* test) (6) (Fig. 2). The results were equally significant for changes of systolic, diastolic, or mean blood pressure. There was no significant change in heart rate or pulse pressure after inosine in any group. The onset of action was rapid (4–5 sec) and the duration of effect was transient (18–20 sec). No tachyphylaxis was noted after repeated injections. The blood pressure elevation was sustained during a 10-min infusion of inosine. Infusion of an equal volume of Tris–HCl had no effect.

Although the mean initial blood pressure was higher in Group I compared to the other three groups, this was significant only for Group I *versus* Group IV ($P < 0.05$). The rise in blood pressure, however, was not significantly different in any of the four groups (Table I).

The rise in BP after IMP was qualitatively and quantitatively similar to that produced by inosine. Tris–HCl injections caused no BP change. The inosine degraded by

[2] In separate experiments in our laboratory, it was determined that this dose effectively abolished the marked hypotensive response from 4 μg/kg of isoproterenol intravenously.

[3] This dose was found to block the marked hypertensive response resulting from an intravenous infusion of norepinephrine, 34 μg/kg/min.

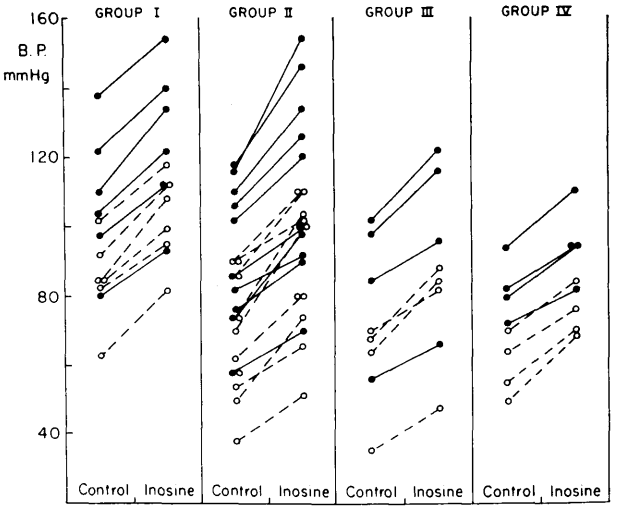

FIG. 2. Change in systolic and diastolic BP after inosine (0.75–0.85 μmoles/kg). Closed circles and bars (●—●) represent systolic BP; open circles and dashes (O- - -O) represent diastolic BP.

H_2SO_4 was inactive. This suggested that the rise in blood pressure was not due to contamination by trace elements. Hypoxanthine and ribose-1-phosphate were devoid of any effect on the rats' blood pressure in the doses studied.

Discussion. Berne and his co-workers found inosine concentration to increase 270-fold to 0.0028 μmoles/ml in the venous drainage of the exercised hindlimb of the dog (3). Our experiments have shown that iv inosine in minute amounts (about 0.004 μmoles/ml extracellular fluid) consistently caused an elevation of blood pressure in the rat. This hypertensive effect persisted after ganglionic blockade, blockade of parasympathetic muscarinic receptors, and selective blockade of alpha- and beta-sympathetic receptors. Thus, we conclude that the hypertensive effect of inosine is independent of the autonomic nervous system. The action of inosine is quantitatively similar over a wide range of initial blood pressures and apparently no tachyphylaxis occurs after repeated administration.

The data obtained in the present study do not allow conclusions regarding the mechanism of the blood pressure rise with inosine. Inosine has been reported to increase directly measured contractile force of isolated rabbit and dog hearts (7–9) and to decrease the

TABLE I. Change in Mean Arterial Blood Pressure.[a]

	Average mean blood pressure before inosine	Average mean blood pressure at peak of inosine action	Average change in mean blood pressure after inosine
Group I (n = 6)	92.5 ± 6.02	110 ± 6.4	16%
Group II (n = 10)	75.7 ± 8.4	96.3 ± 7.0	21%
Group III (n = 4)	68.0 ± 8.0	84.0 ± 10.2	19%
Group IV (n = 4)	67.5 ± 4.25	81.5 ± 4.15	17%

[a] Results are expressed as mm Hg ± SE. The rise in blood pressure (BP) after intravenous infusions of inosine (0.75—0.85 μmoles/kg) was significant in all four groups ($P < 0.01$). The magnitude of BP elevation did not differ significantly among the four groups.

contractile force of isolated rat atria (10). Buckley has demonstrated that propranolol prevents inotropic effects of inosine in the isolated, perfused rabbit heart (11). If applicable to the rat, this observation implies that blood pressure elevation after inosine injection in the propranolol-treated rat is probably not due to increased myocardial contractility. Since other investigations have not found inosine to act as a vasoconstrictor (2, 5), the sequence of events leading to BP elevation after inosine injection in the rat is not clear and requires further investigation. The observation that inosine concentration rises in the venous drainage of exercising skeletal muscle leads to the speculation that inosine may play a role in circulatory adjustments during exercise.

Summary. Adenosine and the adenine nucleotides decrease BP by direct vasodilatation. Adenosine is metabolized *in vivo* to inosine, the concentration of which is known to rise in exercising skeletal muscle and its venous effluent. Inosine is believed to be without effect on blood pressure although a positive myocardial inotropic effect has been suggested.

The present study concerns the influence of metabolic breakdown products of the adenine nucleotides on arterial pressure in the intact rat. BP was measured by direct arterial cannulation during the intravenous administration of inosine, inosine monophosphate (IMP), hypoxanthine, and ribose-1-phosphate. Inosine (0.75–0.85 μmoles/kg) caused a 16% elevation of mean BP. IMP caused a similar increase in BP; hypoxanthine and ribose-1-phosphate were inactive. BP rise after inosine persisted despite parasympathetic, sympathetic or selective α- and β-adrenergic blockade. There was no change in heart rate. Inosine, therefore, elevates BP in the rat by a mechanism which appears to be independent of the autonomic nervous system.

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