EFFECTIVENESS OF INSULIN-GLUCOSE IN PREVENTING ADRENALINE-INDUCED MYOCARDIAL AND SYSTEMIC DISTURBANCES IN THE DOG*

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The aim of this study was to investigate whether insulin-glucose (IG) is able to prevent detrimental systemic and myocardial changes induced in healthy dog by adrenaline (AD) infused at a rate which mimics spontaneous secretion after coronary occlusion. Insulin (0.3 u/kg) and glucose (10%, 10 ml/kg) mixture was infused intravenously concurrently with AD (1.2 μ g/kg/min) for 4 h and blood values of FFA, triiodothyronine (T_3) immunoreactive insulin (IRI) and glucose measured initially, after 2 and 4 h of infusion were compared with the values found in dogs infused with AD alone and with saline. IG suppressed a rise in FFA, attenuated a fall in T_3 , reversed AD-induced histoenzymatic changes in SDH and ATPase activity and completely prevented the development of mitochondrial alterations shown by electron microscopic study. These data provide evidence for usefulness of IG in preventing the consequences of catecholamine excess in acute stage of MI.

THE phenomenon of enhanced response of sympathetic nervous system in acute myocardial infarction (MI) and the significance of this reaction for the course of the disease recently received much attention. Deleterious consequences of sympathetic overactivity have been suspected in clinical¹⁻³ and documented in experimental^{4,5} studies. We have previously shown⁶ that adrenaline infused to intact dogs at a rate equivalent to spontaneous release of this amine after coronary occlusion, is able to evoke profound myocardial and systemic metabolic effects similar to those seen in acute MI. All these findings indicate the necessity for introducing meta-

bolic or pharmacological interventions which might prevent myocardial and systemic alterations occurring as a consequence of catecholamine excess.

Treatment with potassium-insulin glucose (KIG) is an intervention applied in acute MI with an aim to reduce myocardial ischemic damage. Benefits of this therapy are attributed to reduced FFA,⁷ restoration of intracellular potassium, stabilization of membrane potential⁸ and reduced frequency of serious arrhythmias.⁹ Favourable clinical effects were reported by many authors,⁹⁻¹² although some trials failed to show beneficial results.^{13,14}

The aim of the present study was to investigate whether insulin-glucose (IG) is able to prevent detrimental myocardial and systemic alterations induced in intact dogs by adrenaline

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Fig.1. The effect of concurrent infusion of AD and IG upon the level of FFA, together with the effects of AD alone and of saline.

infused at a rate which mimics spontaneous secretion of this amine after coronary occlusion.¹⁵ Apart from known metabolic benefits resulting from provision of insulin and glucose, the concept of this study has also been based on the evidence for stimulatory effect of insulin upon phosphodiesterase activity,¹⁶ which is known to result in the decrease of tissue concentration of cAMP. Since this nucleotide mediates metabolic effects of catecholamines, the above action of insulin is likely to attenuate their metabolic effects. Such possibility might be another suggestive mechanism of therapeutic action of KIG.

MATERIAL AND METHODS

Animal preparation

Experiments were performed on 8 mongrel dogs of either sex ranging in weight from 5.5 to 18 kg, fasted overnight and anaesthetized with hexobarbitone sodium (30 mg/kg iv) followed by urethane (375 mg/kg) and chloralose (75 mg/kg) mixture iv. Artificial ventilation with atmospheric air was maintained via endotracheal tube. Jugular and femoral veins and femoral arteries were cannulated for infusions, blood sampling and blood pressure (BP) measurement. BP measured with Statham transducer (P23Db) was recorded together with ECG from standard limb leads on

Watanabe WA 294 recorder.

Experimental procedure

Total dose of anaesthetic agents was given throughout the preparation of the animal. Control registrations were made 30 min after the preparation was completed. Arterial and venous blood samples were then obtained for PO_2 , PCO_2 , pH measurements and venous samples for free fatty acids (FFA), triiodothyronine (T_3), immunoreactive insulin (IRI) and glucose determinations. Subsequently, intravenous infusions of adrenaline and IG were initiated and maintained for 4 hours. Adrenaline (AD) was infused at a rate of 1.2 μg/kg/min, insulin (I) was given in a dose of 0.3 μg/kg and 10% glucose (G) was administered in a dose of 10 ml/kg. Recordings of BP, ECG and measurements of PO_2 , PCO_2 , pH were repeated every hour. Blood samples for FFA, T_3 , IRI and glucose were taken after 2 and 4 hours of infusion. Each sample of the blood withdrawn was replaced with equivalent volume of dextran. After the last samples were obtained, thoracotomy was performed and the heart excised. Myocardial samples from both ventricles were taken for histochemical detection of succinic dehydrogenase (SDH) and ATPase and the section from the left ventricle for electron microscopic study.

Fig.2. Changes in serum T_3 concentration throughout infusion period in dogs infused with AD and IG in comparison with values obtained in dogs infused with AD alone and with saline.

The results obtained previously⁶ in dogs infused with AD ($n = 17$) and in dogs infused with 0.9 % sodium chloride ($n = 14$) provided the reference data for the present study.

Metabolic and hormonal measurements in blood

Arterial and venous blood for PO_2 , PCO_2 and pH was withdrawn anaerobically by micromethod of Astrup (Radiometer, Copenhagen). FFA were determined by the method of Dole¹⁷ in modification of Itaya and Ui.¹⁸ T_3 was determined by direct radioimmunoassay using merthiolate to block TBG binding and dextran-coated charcoal to separate free and bound hormones.¹⁹

Insulin was determined by radioimmunoassay kits provided by the Institute of Nuclear Research (Swierk, Poland). Free insulin was separated from antibody-bound hormone on charcoal-coated dextran²⁰. Glucose was determined by the hexokinase method.²¹ All biochemical determinations were performed in duplicate.

Histochemical study

Myocardial specimens for histochemical examination were immediately placed in dry ice-acetone mixture (-70°C) for cryostat sectioning. SDH was demonstrated by the technique of Nachlas et al.²² on 8μ cryostat sections at pH 7.4, temperature 37°C , incubation time 30 min,

with Nitro BT as hydrogen acceptor. Staining reaction for ATPase was performed by the method of Wachstein and Meisel²³ at pH 7.2, incubation time 120 min at 37°C . Sections stained for the detection of SDH and ATPase activity were classified as normal, or showing moderate or low histochemical activity. Criteria for classification included uniformity and intensity of staining reaction. As in the case of saline-infused and AD-infused dogs⁶ tissue sections from IG-treated hearts were examined blind. Samples were graded according to the following criteria:

- normal histochemical activity was uniformly distributed within myofibrils, ATPase staining intensified within cellular membranes and vascular smooth muscles, SDH activity prominent in mitochondria,
- moderate decrease in activity was reflected by irregular areas of reduced staining interspersed between myofibrils with normal deposition of reaction product,
- low histochemical activity was assessed in samples showing the areas of greatly reduced staining intensity and/or complete depletion of histochemical activity.

Electron microscopic examination

Sections of left ventricular wall were fixed immediately after removal in 4.5% buffered

TABLE I EFFECTIVENESS OF INSULIN-GLUCOSE

INFUSION	TIME of sampling	GLUCOSE (mg%)		IRI (μ U/ml)		IRI/GLUCOSE	
		n	mean $\pm$ SE	n	mean $\pm$ SE	n	mean $\pm$ SE
NaCl 0.9%	0	14	87.83 $\pm$ 6.07	13	14.92 $\pm$ 2.32	13	0.19 $\pm$ 0.04
	2h	14	93.47 $\pm$ 6.02	13	16.77 $\pm$ 2.30	13	0.20 $\pm$ 0.03
	4h	13	91.72 $\pm$ 5.37	13	13.62 $\pm$ 1.96	12	0.17 $\pm$ 0.03
Adrenaline (AD) 1.2 μ g/kg/min	0	12	89.90 $\pm$ 7.62	12	11.92 $\pm$ 1.91	12	0.14 $\pm$ 0.03
	2h	11	190.62 $\pm$ 36.50	11	15.91 $\pm$ 2.78	11	0.11 $\pm$ 0.03
	4h	11	190.64 $\pm$ 26.75	12	16.17 $\pm$ 3.63	11	0.10 $\pm$ 0.02
AD + Insulin 0.3U/kg Glucose 10%- 10mg/kg	0	5	88.41 $\pm$ 8.88	5	13.30 $\pm$ 0.93	5	0.15 $\pm$ 0.01
	2h	4	249.17 $\pm$ 67.84	4	60.25 $\pm$ 27.77	4	0.25 $\pm$ 0.10
	4h	5	189.86 $\pm$ 45.10	5	64.00 $\pm$ 20.52	5	0.37 $\pm$ 0.11

glutaraldehyde and 1% osmium tetroxide with addition of saccharose and embedded in epon. Sections of 300 Å were made with LKB ultramicrotome (Ultratom III), stained with lead nitrate and uranyl acetate and examined with electron microscope (JEM 100 S).

RESULTS

As shown in Fig. 1, a rise in FFA was greatly suppressed with IG.

Considerable decrease in serum T_3 , found in dogs infused with AD alone, was completely abolished in the animals given combined infusion of AD and IG (Fig. 2).

Blood pH was not influenced by IG and did not differ significantly from the values found in animals receiving AD alone.⁵

Table I shows IRI and G content in blood of animals treated with IG in comparison with the values detected in dogs infused with AD alone and with saline. In AD-infused dogs, increased level of glucose was not accompanied by relevant elevation of insulin activity, thus IRI/G index decreased showing inadequate release of the hormone. In dogs receiving concurrently AD and IG, in spite of glucose administration, blood glucose concentration was of similar range as that found in dogs given AD alone, suggesting enhanced influx of glucose to tissues. As expected, blood insulin was elevated and thus IRI/G index increased above the values obtained in saline-infused dogs.

Results of histoenzymatic examination are shown in Fig. 3. In all preparations from IG-treated hearts histochemical reaction for ATPase revealed the activity uniformly distributed within myofibrils. Staining intensity was even greater

than in the preparations from the control, saline-infused dogs. Histochemical reaction for SDH system showed normal activity in majority of preparations, only in two dogs moderate decrease of histoenzymatic activity was detected. This was in contrast with the results obtained in dogs infused with AD alone, in which considerable depletion of histoenzymatic activity was seen in

MYOCARDIAL SDH AND ATP-ase ACTIVITY

Fig.3. Prevention with insuline + glucose (IG). Histochemical determination of ATPase and SDH in dogs infused concurrently with AD and IG in comparison with the results obtained in dogs infused with AD alone and with saline.

Fig.4-a. Electron micrograph from the heart of dog infused concurrently with AD and IG. Normal shape and structure of mitochondria, endoplasmic reticulum and glycogen content unchanged ($\times 30,000$).

Fig.4-b. Electron micrograph from the heart of dog infused with AD alone. Disorganization of mitochondria, uneven and decreased matrix density, local cristolysis. Enlargement of endoplasmic reticulum, lipid droplets and aggregates of alpha glycogen are also visible ($\times 42,000$).

Fig. 4-c. Electron micrograph from the heart of dog infused with 0.9% saline ($\times 30,000$).

majority of preparations stained for SDH and also in those for ATPase activity.

Electron microscopic examination revealed evident differences in the ultrastructure of IG-treated dogs (Fig. 4a) as compared to those receiving AD alone (Fig. 4b). In IG-treated dogs, mitochondrial shape and structure was unchanged, as in saline-infused dogs (Fig. 4c) with matrix granules of normal density and abundant cristae. Glycogen content was also similar to that seen in control, saline-infused dogs. The aggregates of alpha glycogen granules previously detected in dogs infused with AD alone, were absent and enlargement of perivascular space was not evident in IG-treated animals. The Z band appeared normal, the capillary endothelial cells contained abundant pinocytic vesicles, the T system and endoplasmic reticulum were unchanged.

No rhythm disturbances were observed throughout the combined infusion of AD with IG and ST segment deviation exceeding 0.1 mV was recorded only in one experiment, whereas in dogs infused with AD alone, ST segment changes were noted in 13 out of 16 experiments and ventricular ectopic beats ($> 6/\text{min}$) were recorded in 3 dogs.

DISCUSSION

Results of this study indicate that concurrent administration of IG to dogs subject to AD infusion in a dose equivalent to spontaneous release of AD after coronary occlusion¹⁵ attenuates systemic metabolic and hormonal disturbances previously shown to be evoked by AD alone⁶ and lessens markedly myocardial histochemical and ultrastructural alterations.

IG prevented a rise in FFA, the effect presumably resulting from inhibition of AD-stimulated lipolysis²⁴ and stimulation of FFA uptake and esterification in adipose tissue^{25,26}. Theoretically, the benefits of this effect for the myocardium seem obvious in view of reported in the literature arguments for FFA toxicity to the heart. It has to be realized, however, that deleterious effects of FFA, such as an increase in myocardial oxygen requirements²⁷, decrease of energy production²⁸, promotion of arrhythmias^{29,30} and impairment of contractility^{30,31} have been documented in experimental models different from that used in the present study. It is an open question to what extent cellular and biochemical indices of myocardial damage, detected in intact, AD-stimulated hearts in our study result from the effects of high FFA. There

is indirect experimental evidence for the role of FFA in facilitating catecholamine-induced myocardial damage. Catecholamines increase myocardial uptake of circulating FFA³² and stimulate myocardial lipolysis.³³ Intracellular accumulation of FFA is thought to affect membrane integrity with subsequent enzyme leakage and potassium loss³⁴ and to uncouple mitochondrial respiration.^{27,35} Indeed, hearts perfused with FFA release more cytoplasmic enzymes than hearts perfused with glucose³⁶ and show more intense ultrastructural damage.³⁷ The role of FFA in catecholamine-induced myocardial injury is more directly confirmed by an observation that catecholamine-induced myocardial necrosis can be attenuated by treatment with antilipolytic agent.³⁸ Thus, protection of the myocardium by IG in our study may be related, at least in part, to reduction of lipolysis resulting in lowering of circulating FFA with subsequent decrease of their uptake by the myocardium. Importance of this mechanism of KIG action for the survival of ischemic dog myocardium³⁹ and for the improvement of myocardial metabolism in patients with coronary artery disease⁴⁰ has been documented.

As suggested by this study, another contributory mechanism to protective action of IG may be related to its effect upon the concentration of T_3 , the most metabolically active thyroid hormone in blood. IG effectively prevented a fall in T_3 , previously detected in AD-treated animals.⁶ Preliminary experimental evidence suggests that decrease in serum T_3 results from enhanced uptake of this hormone by tissues, also by the myocardium and this is promoted by catecholamines.⁴¹ Increased saturation of nuclear receptors for T_3 results in stimulation of cellular metabolism⁴² which is unfavourable effect. The mechanism by which IG restores blood T_3 level to normal remains unexplained at the moment. Speculatively, it might be related to insulin-induced decrease of cAMP. If cyclic nucleotide is involved in catecholamine stimulation of T_3 uptake by tissues, decrease in cAMP level might diminish tissue uptake leading to relative increase in blood level of the hormone. Another possible mechanism might be related to suppression of endogenous catecholamines by IG, suggested by our previous studies.⁴³ Since blood adrenaline level increases with time in anaesthetized saline-infused dogs,⁶ this might contribute to gradual decline of serum T_3 , due to increased tissue uptake of the hormone. IG might prevent

this phenomenon by suppressing blood level of endogenous adrenaline.

Amelioration of myocardial alterations detected in this study may also result in part from other independent effects of I or G. There is an evidence that insulin itself, exerts favourable effect upon myocardium. Insulin decreases enzyme release from ischemic myocardium,⁴⁴ presumably by protecting lysosomal membranes.⁴⁵ Moreover, insulin exerts protective effect upon vulnerability to fibrillation in both ischemic⁴⁶ and normal⁴⁷ canine heart and suppresses positive chronotropic⁴⁸ and inotropic⁴⁹ effects of catecholamines upon myocardium. Suggested mechanism through which insulin modifies catecholamine effects include alterations of intracellular ionic concentration and/or suppression of cAMP with elevation of cGMP.^{48,49} The latter possibility is favoured by the fact that insulin stimulates the activity of phosphodiesterase¹⁶ resulting in the decrease of cAMP levels. There is also convincing evidence that provision of glucose alone is beneficial for the myocardium. Glucose increases viability of ischemic canine heart,⁵⁰ decreases ventricular vulnerability,⁵¹ protects hypoxic isolated hearts from detrimental effects of FFA upon rhythm and contractility.⁵² This is due to well known metabolic alterations of the myocardial cell with enhancement of glycolysis, reduction of FFA utilisation, increase in tissue glycogen and high energy phosphate compounds. Although some experimental studies suggest that glucose is the most important component of KIG therapy,⁵⁰ close links between I and G in their metabolic action make it difficult to separate their effects in the present study. Thus, it was deliberately conceived to assess combined effects of I and G in order to provide the basis for the use of this therapy in counteracting the consequences of adrenergic overactivity in MI, amplifying the evidence for the mechanisms of KIG action.

In summary, this study demonstrated that IG effectively prevents systemic metabolic and myocardial alterations which develop in dogs subjected to prolonged infusion of AD, quantitatively imitating endogenous release after coronary occlusion. This suggests that metabolic intervention with IG may be of value in antagonizing the consequences of sympathetic overactivity in acute MI.

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