Glucose-insulin-potassium treatment in combination with dipyridamole inhibits ischaemia-reperfusion-induced damage

S. Bertuglia, A. Giusti, S. Fedele, E. Picano

CNR Institute of Clinical Physiology, Medical School, University of Pisa, Pisa, Italy

Abstract

Aims/hypothesis. Treatment with intravenous glucose-insulin-potassium has beneficial effects in reperfused patients, reducing mortality in patients with myocardial infarction by 28 %. We hypothesized that insulin response to glucose-insulin-potassium infusion might lead to vasodilation in ischemia/reperfusion (I/R). Hyperglycaemia and hyperinsulinaemia determine oxidative stress. We therefore investigated the microcirculatory changes following I/R after glucose-insulin-potassium or in association with glucose-insulin-potassium dipyridamole in hamster cheek pouch.

Methods. The control (I/R), glucose-insulin-potassium groups with and without dipyridamole were treated with saline, 300 g/l, 50 U/l insulin and 80 meq/l KCl infused at $0.2 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{h}^{-1}$, and GIK plus dipyridamole ($0.084 \text{ mg} \cdot 100 \text{ g}^{-1}$ intravenously) at beginning, 30 min before ischaemia, and continuing through reperfusion. We measured microvessel diameter changes, arteriolar red blood cell velocity, permeability increase, capillary perfused length, leukocyte and platelet adhesion.

Results. Hyperglycaemia and hyperinsulinaemia did not cause vasodilation whereas in the glucose-insulin-potassium group with dipyridamole there was a marked arterial vasodilation with increased red blood cell velocity and perfused capillary length at reperfusion. Glucose-insulin-potassium infusion reversed the arterial vasoconstriction caused by I/R at reperfusion. Adhering leukocytes to venules decreased by 56 and 86 % while platelets adhering to microvessels was reduced by 52 and 72 % at reperfusion in glucose-insulin-potassium groups with and without dipyridamole, respectively. The permeability was decreased by GIK and completely suppressed by GIKD after I/R.

Conclusion hypothesis. We demonstrated that GIK, when used in combination with dipyridamole, had beneficial effects on the capillary perfusion against I/R-induced injury. There was a marked reduction of leukocyte and platelet adhesion that can be explained by the antioxidant properties of dipyridamole. [Diabetologia (2001) 44: 2165–2170]

Keywords Glucose-insulin-potassium, dipyridamole, ischaemia-reperfusion, capillary perfusion, arterioles, leukocytes, platelets.

The beneficial effects of high dose glucose-insulin-potassium (GIK) infusion have been reported in re-

perfused patients after by pass graft surgery [1], in stable coronary artery disease [2], after acute myocardial infarction [3, 4] and during pacing stress testing [5]. GIK exert beneficial effects on the ischaemic heart by reducing infarct size, improving post ischaemic left ventricular function and reducing mortality in myocardial infarction by 28 % [6].

Ischaemia reperfusion (I/R) is associated with irreversible injury to the microcirculation. It is recognized that reperfusion of ischaemic tissues leads to an injury response that is accompanied by endothelial

Received: 23 July 2001 and in revised form: 30 August 2001

Corresponding author: S. Bertuglia, CNR Institute of Clinical Physiology, Medical School, University of Pisa, Pisa, Italy, e-mail: sibert@ifc.cnr.it

Abbreviations: GIK, Glucose insulin potassium; GIKD, glucose-insulin-potassium dipyridamole; I/R, ischaemia-reperfusion; PCL, perfused capillary length; RBC, red blood cell

injury, enhanced protein efflux and increased leukocyte and platelet adhesion in postcapillary venules [7–10].

Furthermore, the haemodynamic effects of hyperglycaemia and hyperinsulinaemia in microcirculation in normal and ischaemic conditions are not clear. Studies show that insulin causes vasodilation in cheek pouch microcirculation [11] while hyperglycaemia modifies the reaction of vessels to insulin in rat skeletal muscle [12]. There is also a direct vasodilatory effect of physiological hyperinsulinaemia in human skeletal muscle [13] and an increase in coronary blood flow with acute GIK infusion in human beings [14]. Insulin is crucial in the regulation of local microvascular perfusion because the insulin-induced increase in blood flow is mediated by nitric oxide (NO) as shown in isolated skeletal muscle arterioles [15] and in the human forearm [16].

Accumulating evidence indicates that oxygen free radicals have been implicated in tissue injury that follows I/R [9, 17, 18]. Dipyridamole, that blocks adenosine transport and determines endogenous adenosine accumulation protects reperfused myocardium of perfused rat heart and improves myocardial contractility of postischaemic myocardium [19–22]. Adenosine has been shown to have an antioxidant effect thus avoiding the production of oxygen free radicals by high insulin and hyperglycaemia [23, 24]. Furthermore, oxidative stress impairs insulin endocytosis in endothelial cells of both arterioles and venules [25]. This was not a consequence of the modified insulin binding capacity but could involve insufficient insulin receptor activation [26].

We hypothesized that insulin response to GIK infusion might lead to vasodilation thus improving capillary perfusion against I/R in cheek pouch microcirculation. Because oxidative stress by GIK causes microvascular dysfunction, we investigated the effects of GIK plus dipyridamole. We measured the arteriolar responses, the red blood cell (RBC) velocity, the functional capillary length (perfused capillary length, PCL), the leukocyte and platelet adhesion and the permeability changes induced by GIK or GIKD infusion during I/R.

Materials and methods

Male Syrian hamsters (80–100 g, Charles River, Calco, Lc Italy) were anaesthetised by intraperitoneal injections of sodium pentobarbital, 50 mg · kg⁻¹ body wt. The hamsters were tracheotomized and the right carotid artery and femoral vein were cannulated to measure blood pressure. Animal handling and care followed the procedures outlined in the Guide for the Care and Use of the Laboratories of the Italian Research Council.

Experimental groups. The control (I/R) ($n = 7$) group received an intravenous infusion of saline (0.2 ml/100 g/h). The GIK ($n = 7$) group was intravenously treated with continuous infu-

sion of GIK (glucose 300 g/l; insulin 50 U/l potassium 80 meq/l) infused at 0.2 ml · 100 g⁻¹ · h⁻¹). GIKD ($n = 7$) group was infused with GIK plus dipyridamole (Persantin, Boehringer Ingelheim s.p.a., Milan, Italy) (0.084 mg · 100 g⁻¹ injected intravenously at baseline and at the beginning of reperfusion). Immediately after baseline systemic measurements (15 min) saline or GIK or GIKD were infused by using a micropump for 15 min in control conditions, and throughout the 15 min of ischaemia and the 30 min of reperfusion. Insulinaemia and glycaemia were determined before saline, GIK, or GIKD infusion and after reperfusion using a glucose oxidase method (Beckman, Fullerton, Calif., USA) and a radioimmunoassay method (Linco Res, St. Charles, Miss., USA), respectively.

Experimental protocol. The cheek pouch was surgically prepared as previously reported [9, 10]. The cheek pouch was gently turned inside out and fixed to a special stage of the microscope and a thin black blade was inserted through a small incision between the upper and lower layers of the pouch. The membrane was suffused with a 36 ± 0.5 °C Ringer's solution (4 ml/min), with 5% CO₂ in 95% N₂ adjusted to pH 7.35.

Atraumatic microvascular clips were placed on the proximal part of the cheek pouch to achieve complete ischaemia for 30 min. The clamp was then removed and the microcirculation was observed after 30 min.

Observations were made with a fluorescent microscope (Orthoplan, Leica Microsystem GmbH, Wetzlar, Germany) and a filter block (Ploempak, Leica Microsystem GmbH, Wetzlar Germany) fitted with a long working distance objective (× 4, na 0.14, · 20, na 0.25) and a × 10 eyepiece. Epi-illumination was provided by a xenon 150-W lamp using the appropriate filters for fluorescein isothiocyanate bound to dextran MW 150,000 (Sigma Chemical, St. Louis, Miss., USA) (50 mg/100 g b.wt., intravenously injected as 5% W/V solution in 5 min) (Leitz I₂), for acridine red (Leitz N₂) and a heat filter (Leitz KG1). The area of interest was televised with a COHU 5253 SIT low light level camera, observed from a Sony PVM 122 CE monitor. Video images were videotaped and microvascular measurements were made off-line by a computer-assisted imaging software system (MIP Image, CNR, Institute for Clinical Physiology, Pisa, Italy).

Measurements of microvascular parameters. The animals of all the groups received an intravenous injection of acridine red (1 mg/100 g) to visualize the leukocytes and the platelets at baseline and after reperfusion. The number of adherent leukocytes was expressed as the number per 100 µm length of venule (diameters: 16 ± 8 µm, length > 250 µm). The number of adherent platelets was expressed as number per mm² of vessel surface (diameters: 16 ± 8 µm). In each animal five arterioles and five venules were recorded on videotape.

PCL, defined as the length of capillaries that have a RBC transit of at least one RBC in a 30 s period, was analysed from four to six different microscopic fields. Microvessel diameters were measured by an image shearing system (Digital Image Shearing Monitor Mod 907, I.P.M., San Diego, Calif., USA). Red blood cell (RBC) velocity was determined using dual slit cross correlation (velocity tracker Mod 102 B, I.P.M., San Diego, Calif., USA).

To quantify the permeability across the venules the values fluorescence intensity in the perivascular space were reported as normalized to baseline grey levels: $NGL = (I - I_r)/I_r$, where I is the average baseline grey level and I_r is the same parameter after reperfusion [9]. Grey levels, ranging from 0 to 255, were determined by the MIP Image program. The windows to measure average fluorescence intensity were set at 50 µm long and 50 µm wide.

Table 1. Mean arterial pressure (MAP), heart rate (HR), insulin and glucose concentrations in saline (I/R), glucose-insulin-potassium (GIK) and glucose-insulin-potassium-dipyridamole (GIKD)- treated groups at baseline and 30 min of reperfusion

Group	I/R	GIK	GIKD
MAP (mmHg)			
Baseline	90 ± 5	92 ± 4	88 ± 2
Reperfusion	87 ± 4	95 ± 5	87 ± 4
HR (beats/min)			
Baseline	260 ± 13	270 ± 25	250 ± 20
Reperfusion	230 ± 15	250 ± 15	200 ± 12
Insulin (μU/ml)	90 ± 20	900 ± 60 ^a	870 ± 40 ^a
Glucose (mg/dl)	50 ± 2	200 ± 15 ^a	190 ± 12 ^a

Values are means ± SEM

* $p < 0.05$ when compared with control group (I/R)

RBC velocity was determined using dual slit cross correlation (velocity tracker Mod 102 B, I.P.M., San Diego, Calif., USA). The measured centreline velocity was corrected according to vessel size to obtain the mean RBC velocity. The video image shearing technique was used to measure vessel diameter on-line.

Mean arterial blood pressure (MAP) (Viggo-Spectramed P10E2 transducer, Oxnard, Calif., USA) and heart rate (HR) were measured by a Gould Windograf recorder (Mod. 13-6615-10S Gould Instruments System GmbH, Dietzenbach, Germany).

All reported values are means ± SEM. Due to the limited number of animals non-parametric tests were used. We used the Kruskal-Wallis test for analysis between groups. A p value of less than 0.05 was considered to be statistically significant.

Results

MAP and HR did not change significantly after reperfusion in comparison with the baseline (Table 1). GIK and GIKD groups showed a significantly higher level of insulinaemia and glycaemia in comparison with the baseline and I/R group. Insulinaemia increased after 15 min compared with saline injection (Table 1).

In I/R group the arteriolar diameter decreased after reperfusion in comparison with the baseline ($p < 0.001$) whereas the venules dilated slightly. The changes in the diameter of A1 and A2 (range, 18–50 μm) and small collecting and large venules, Vc and VL (range 37–115 μm) measured at baseline and after reperfusion are shown in Figure 1. RBC velocity decreased in arterioles after reperfusion (1.98 ± 0.35 mm/s at baseline, $p < 0.01$) (Fig. 2). These changes were associated with a marked permeability increase in postcapillary and collecting venules after reperfusion (baseline: 0.09 ± 0.01 normalized grey levels, $p < 0.01$) (Fig. 1). PCL decreased by $47 \pm 3\%$ of baseline ($9,130 \pm 430$ μm) ($p < 0.01$) (Fig. 2). The number of leukocytes adhering to the venular wall increased after reperfusion ($p < 0.01$) (Fig. 3). The platelets adhering to microvessels and to arterioles

Fig. 1. Changes (%) of diameter of arterioles (□) and venules (▒), and arteriolar RBC velocity (▨), and permeability levels (■), at baseline in control (I/R), glucose-insulin potassium (GIK) and glucose-insulin-potassium-dipyridamole (GIKD) treated groups at baseline and 30 min of reperfusion. Data are means ± SEM, * $p < 0.05$ vs baseline, $p < 0.01$ vs I/R; ** $p < 0.01$ vs GIK

Fig. 2. Changes (%) of perfused capillary length (PCL) at baseline (Bsl), in the control (I/R) group treated with saline, glucose-insulin-potassium (GIK), glucose-insulin-potassium infusion plus dipyridamole (GIKD), respectively, at reperfusion. Means ± SD, * $p < 0.01$ vs baseline, $p < 0.01$ vs I/R; ** $p < 0.01$ vs GIK

increased significantly in comparison with baseline (Fig. 4).

In GIK group, arterioles did not constrict at reperfusion (Fig. 1). Under baseline conditions arteriolar diameter were mostly constant during the observation period whereas irregular arteriolar vasomotion appeared in control conditions during GIK infusion. During the period of ischaemia, some irregular contraction and dilation of arterioles caused a backward and forward flow in the network. A significant increase in diameter and RBC velocity in arterioles was present from the beginning of the reperfusion but did not persist to the end. The permeability de-

Fig. 3. Number of adherent leukocytes/100 μm venules in the groups treated with saline (I/R), with glucose-insulin-potassium (GIK) and GIK infusion plus-dipyridamole (GIKD), respectively, at baseline and 30 min of reperfusion. Means $\pm$ SEM, $n = 25$ for each entry * $p < 0.01$ vs baseline, ° $p < 0.01$. vs I/R; ** $p < 0.01$ vs GIK

Fig. 4. Number of adherent platelets/ mm^2 of vessel surface at baseline and after reperfusion in the control group (I/R) and those treated with glucose-insulin-potassium (GIK) and insulin-glucose infusion plus-dipyridamole (GIKD), respectively, at baseline and 30 min of reperfusion. Means $\pm$ SEM, $n = 25$ experimental observations for each entry, * $p < 0.01$ vs baseline, ° $p < 0.01$. vs I/R; ** $p < 0.01$ vs GIK

creased in comparison with I/R group but there were some leakages in the postcapillary venules (Fig. 1).

PCL increased significantly in comparison with the control group whereas it decreased in comparison with the baseline ($8950 \pm 780 \mu\text{m}$) (Fig. 2). GIK reduced the number of adherent leukocytes by 56% at reperfusion in comparison with I/R group (Fig. 3). The platelets adhering to microvessels and to arterioles decreased by 52% in comparison with I/R group (Fig. 4).

In the GIKD group arterioles dilated significantly during the period of reperfusion and the RBC velocity increased significantly in arterioles at reperfusion ($p < 0.01$) (Fig. 1). These changes were associated with no permeability increase in postcapillary and collecting venules. The PCL increased in comparison

with the control group whereas it was not reduced after I/R in comparison with baseline ($10250 \pm 550 \mu\text{m}$) (Fig. 2). The number of leukocytes adhering to the venular wall decreased by 86% at reperfusion in comparison with I/R group (Fig. 3). The platelets adhering to microvessels and to arterioles decreased by 72% in comparison with I/R group (Fig. 4).

Discussion

The main result of our findings is that GIK infusion attenuated the microvascular changes induced by I/R when used in association with dipyridamole which caused arteriolar vasodilation and consequently determined a significant increase in the capillary perfusion. The I/R injury caused arterial vasoconstriction that was suppressed by functional changes induced by hyperglycaemia and hyperinsulinaemia. However, the increase in the capillary perfusion was lower than in GIKD group. The increase in capillary perfusion could be essential in the mechanism of protection, thus determining glucose and oxygen delivery to the tissue and permitting the extraction of by products of cellular metabolism during reperfusion. The beneficial effects of dipyridamole seem to derive from a more marked reduction of leukocyte and platelet adhesion via the inhibition of oxygen free radical formation.

Our findings show that hyperglycaemia and hyperinsulinaemia inhibited the arterial vasoconstriction during ischaemia and improved the capillary perfusion at 30 min of reperfusion. Previously, we showed that insulin caused vasodilation in cheek pouch microcirculation in control conditions [11] whereas there is a different effect of hyperglycaemia and hyperinsulinaemia on the vascular endothelium during I/R; namely there is no vasodilation during reperfusion and blood flow is reduced below baseline after reperfusion. In healthy individuals, insulin administration caused an increase in blood flow which is dependent on the effect of insulin on the vascular endothelium that could cause increased production of NO [13]. Furthermore, hyperglycaemia modified the reaction of microvessels to insulin and increased arteriolar vasomotion in rat skeletal muscle [12].

During ischaemia the arteriolar tone improved after insulin injection and this should have additional and beneficial effects thus avoiding the endothelium impairment and re-establishing the shear stress mechanism on the vessel wall during the early period of reperfusion. Hyperinsulinaemia and hyperglycaemia might maintain a normal vascular reactivity thus permitting an increase in flow with increased vasodilation in association with dipyridamole injection.

Our data show that there was a significant reduction in leukocyte adhesion to venules with a significant increase in arteriolar and capillary RBC velocity

in hamster treated with GIK infusion and dipyridamole. The interaction of leukocytes with endothelial cells dramatically increase the oxygen-free radical formation especially in the latter part of reperfusion [18]. The beneficial microvascular protection might come from relief from oxidative stress, synergistically achieved by the addition of dipyridamole with its scavenger effect. There are several reports that provide evidence that adenosine acts as an anti-inflammatory agent. This evidence is largely based on observation that adenosine inhibits $O_2^{\cdot-}$ and H_2O_2 production by PMNs [27]. Previously, it has been shown that insulin decreases circulating vitamin E levels in humans during the euglycaemic hyperinsulinaemic clamp thus suggesting that high level of insulin increases ROS production [24]. In accordance with our data, insulin and dipyridamole have been observed to be protective on cultured central nervous system neurons, and more specifically, the antioxidant properties of dipyridamole were suggested to be protective [28]. Dipyridamole increases endogenous adenosine but it has a direct antioxidant effect and also acts via adenosine independent pathways, including inhibition of phosphodiesterases, blockade of chloride transport, interference with the multidrug resistance protein, and enhancement of prostacyclin synthesis [28]. Adenosine also has protective effects in patients as an adjunct to thrombolytic therapy for acute myocardial infarction in multicentre, randomized, placebo-controlled trial [29].

In agreement with our data, platelet-endothelial interactions have been observed in arterioles during I/R and not just in postcapillary venules [30]. Our data show that there is a decrease of platelet adhesion during GIK infusion in arterioles but the decrease is much more evident in GIKD group. The decreased perfusion in the capillary network during reperfusion might be related to platelet adhesion in arterioles. The adhesion of platelets to the arteriolar endothelium might impede restoration of blood flow by occlusion of the vascular lumen or by the release of vasoconstrictive platelet mediators [30]. Platelets are not activated by hyperglycaemia and hyperinsulinaemia [31]. However, the PKC is sensitive to oxidation and could be up-regulated during the oxidative stress caused by I/R. It was shown that many proaggregatory stimuli such as the oxidative stress might activate PKC that play an important role in platelet activation [32].

Therefore, the protective effects of dipyridamole might determine the inhibition of platelet adhesion in the arterioles as well as the inhibition of the following vasoconstriction and reduced capillary perfusion.

In conclusion, this study provides evidence that GIK, when used in combination with dipyridamole, had beneficial effects on capillary perfusion against I/R-induced injury. Hyperglycaemia and hyperinsulinaemia caused no vasodilation and less capillary perfusion. There was also a marked reduction of leu-

kocyte and platelet adhesion in response to the antioxidant properties of dipyridamole. We suggest that the hyperglycaemia and hyperinsulinaemia during GIK infusion is less beneficial than when used in combination with dipyridamole which has specific haemodynamic effects on the microcirculation. Insulin can improve capillary perfusion but not all the microvascular changes induced by I/R.

These results focus on the microcirculation changes as an important potential target of the therapeutic beneficial effects of GIK infusion used in combination with dipyridamole to avoid microvascular damage and afford protection against I/R.

References

1. Apstein CS (1998) Glucose-insulin-potassium for acute myocardial infarction. Remarkable results from a new prospective, randomized trial. *Circulation* 98: 2223–2226
2. Stanley AW, Moraski RE, Russel RO et al. (1975) Effects of glucose-insulin-potassium on myocardial substrate availability and utilization in stable coronary artery disease. Studies on myocardial carbohydrate, lipid and oxygen arterial-coronary sinus differences in patients with coronary artery disease. *Am J Cardiol* 36: 929–937
3. Taegtmeier H, Goodwin GW, Doenst T, Frazier OH (1997) Substrate metabolism as a determinant for postischemic functional recovery of the heart. *Am J Cardiol* 80: 3A–10A
4. Diaz R, Paolasso EA, Piegas LS et al. (1998) Metabolic modulation of acute myocardial infarction: the ECLA- glucose-insulin-potassium pilot trial. *Circulation* 98: 2227–2234
5. Vanoverschelde JJJ, Janier MF, Bakke JE, Mrshall DR, Bergmann SR (1994) Rate of glycolysis during ischemia determines the extent of the ischemic injury and functional recovery after reperfusion. *Am J Physiol* 267: H1785–H1794
6. Arnott WMD, Barrit AS, Douglas AS et al. (1968) Potassium, glucose and insulin treatment for acute myocardial infarction. *Lancet* ii: 1355–1368
7. Weyrich AS, Ma X, Lefer DJ, Albertine KH, Lefer AM (1993) In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest* 91: 2620–2632
8. Eppihimer MJ, Granger DM (1997) Ischemia reperfusion induced leukocyte endothelial interaction in postcapillary venules. *Shock* 8: 16–25
9. Bertuglia S, Marchiafava PL, Colantuoni A (1996) Melatonin prevents ischemia reperfusion injury in hamster cheek pouch microcirculation. *Cardiovasc Res* 274: 947–952
10. Bertuglia S, Colantuoni A (2000) Protective effects of leukopenia and t-PA in ischemia reperfusion injury in hamster cheek pouch microcirculation. *Am J Physiol* 278: H755–H761
11. Bertuglia S, Colantuoni A (1998) Insulin-induced arteriolar dilation after tyrosine kinase and NO synthase inhibition in hamster cheek pouch microcirculation *J Vasc Res* 35: 20–26
12. Renaudin C, Michoud E, Rapin JR, Lagarde M, Wiernsperger N (1998) Hyperglycaemia modifies the reaction of microvessels to insulin in rat skeletal muscle. *Diabetologia* 41: 26–33

13. Tack CJ, Schefman AE, Willems JL, Thien T, Lutterman JA, Smits P (1996). Direct vasodilatory effect of physiological hyperinsulinemia in human skeletal muscle. *Eur J Clin Invest* 26: 772–789
14. Rogers WJ, Russell RO Jr, McDaniel HG, Rackley CE (1977) Acute effects of glucose-insulin-potassium infusion on myocardial substrates, coronary blood flow and oxygen consumption in man. *Am J Cardiol* 40: 421–428
15. Ya-Li C, Messina EJ (1996) Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependent and nitric oxide mediated. *Am J Physiol* 270: H2120–H2124
16. Van Veen S, Chang PC (1997) Prostaglandin and nitric oxide mediate insulin-induced vasodilation in the human forearm. *Cardiovasc Res* 93: 223–229
17. Granger DN, Korthuis RJ (1995) Physiologic mechanism of post-ischemic tissue injury. *Annu Rev Physiol* 57: 311–332
18. Kurose I, Granger DN (1994) Evidence implicating xanthine oxidase and neutrophils in reperfusion-induced microvascular dysfunction. *Ann NY Acad Sci* 723: 158–179
19. Ely SW, Berne RM (1992) Protective effect of adenosine in myocardial ischemia. *Circulation* 85: 893–904
20. Wyatt DA, Edmunds MC, Rubio R et al. (1989) Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A1-adenosine receptors. *Am J Physiol* 257: H1952–H1957
21. Finegan BA, Lopaschuk GD, Coulson CS, Clanachan AS (1993) Adenosine alters glucose use during ischemia and reperfusion in isolated hearts. *Circulation* 87: 900–908
22. Lasley RD, Mentzer RM Jr (1997) Adenosine improves recovery of postischemic myocardial function via an adenosine A1 receptor mechanism. *Am J Physiol* 2263: H1460–H1465
23. Kusmic C, Petersen C, Picano E (2000) Antioxidant effect of oral dipyridamole during cerebral hypoperfusion with human carotid endarterectomy. *J Cardiovasc Pharmacol* 36: 41–45
24. Kusmic C, Picano E, Busceti C (2000) The antioxidant drug dipyridamole spares the vitamin E and thiols in red blood cells after oxidative stress. *Cardiovasc Res* 47: 510–514
25. Ceriello A (1997) Acute hyperglycaemia and oxidative stress formation. *Diabet Med* 14: S45–S49
26. Bertelsen M, Angard EE, Carrier MJ (2001) Oxidative stress impairs insulin internalization in endothelial cells in vivo. *Diabetologia* 44: 605–613
27. Cronstein BN, Kramer SB, Wissman G, Hirschorn R (1983) Adenosine a physiological modulator of superoxide anion generation by human neutrophils. *J Exp Med* 158: 1160–1173
28. Farinelli SE, Greene LA, Fridman WJ (1998) Neuroprotective action of dipyridamole on cultured CNS Neurons. *J Neurosci* 18: 5112–5123
29. Mahaffey KW, Puma JA, Barbagelata NA et al. (1999) Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: result of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial. *J Am Coll Cardio* 34: 1711–1720
30. Massberg S, Enders G, Eisenmenger S et al. (1999) Platelet-endothelial cell interactions in the postischemic microvasculature. *Prog Appl Microcirc* 23: 66–81
31. Xing M, Firestin BL, Shen GH, Insel PA (1997) Dual role of Protein Kinase C in the regulation of cPLA2-mediated arachidonic acid release by P2U receptors in MDCK-D1 cells: involvement of MAP kinase dependent and independent pathways. *J Clin Invest* 99: 805–814
32. Baldassarre JJ, Henderson PA, Burns D, Fisher JY (1992) Translocation of protein kinase C isozymes in thrombin stimulated human platelet. Correlation with 1,2 diacylglycerol levels. *J Biol Chem* 267: 15585–15590