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Synergistic effect of nicorandil and amlodipine on mitochondrial function during isoproterenol-induced myocardial infarction in rats

Venkatachalem Sathish, Veereshwarayya Vimal, Kesavarao Kumar Ebenezar and Thiruvengadam Devaki

Abstract

The synergistic effects of nicorandil (K_{ATP}-channel opener) and amlodipine (calcium-channel blocker) on heart mitochondrial enzymes and the mitochondrial antioxidant defence system was examined on isoproterenol-induced myocardial infarction in rats. The rats given isoproterenol (150 mg kg^{-1} daily, i.p.) for two days showed significant changes in marker enzymes, mitochondrial enzymes and the mitochondrial defence system. Pre-co-treatment with nicorandil (2.5 mg kg^{-1} daily, p.o.) and amlodipine (5.0 mg kg^{-1} daily, p.o.) for 3 days significantly prevented these alterations and restored enzyme activity to near normal. These findings demonstrate the protective and synergistic effect of nicorandil and amlodipine in combination against isoproterenol-induced cardiac damage.

Introduction

Nicorandil (*N*-(2-hydroxyethyl) nicotinamide nitrate ester) is an orally efficacious drug for the treatment of ischaemic heart disease (Kitajima et al 1998). It is unique in having two vasodilator mechanisms, namely, a nitrate-like guanylate cyclase/cyclic guanosine monophosphate (cGMP)-dependent component and an adenosine triphosphate (ATP)-sensitive potassium-channel opener component (Humphrey 1998). Garlid et al (1996) suggested that the mitochondrial K_{ATP} (mitoK_{ATP}) channel is an important intracellular receptor that should be taken into account in considering the pharmacology of potassium-channel-opener activators in in-vitro experiments. It has been reported that nicorandil given orally to rats is preferentially distributed into heart mitochondria (Sakai et al 1999). Sato et al (2000) reported that nicorandil exerts a direct cardioprotective effect on heart muscle cells mediated by the selective activation of mitoK_{ATP} channels.

Amlodipine, a calcium-channel antagonist has been used as an effective antihypertensive agent (Cai et al 1996). Calcium-channel antagonists exert beneficial effects on the myocardium via their inhibiting action on the slow Ca^{2+} inward current through L-type Ca^{2+} channels into cardiac cells (Sandmann et al 2000). Isoproterenol-induced myocardial necrosis is a multifactorial process that occurs due to relative hypoxia, coronary microcirculatory defects and excessive formation of free radicals (Ostadal et al 1979). Isoproterenol administration causes intracellular calcium overload and leads to a deleterious high-energy-phosphate deficiency by the excessive activation of Ca^{2+}-dependent intracellular ATPases and by impairing the phosphorylating capacity of mitochondria (Janke et al 1975).

Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai 600025, India

Venkatachalem Sathish, Veereshwarayya Vimal, Kesavarao Kumar Ebenezar, Thiruvengadam Devaki

Correspondence: T. Devaki, Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai 600025, India. E-mail: devakit@yahoo.co.uk

These reports have attracted our great interest on studying these two drugs, as the mitochondrial K_{ATP} channels and calcium channels are involved in the control of mitochondrial functions. The aim of this study was to examine the synergistic effects of nicorandil and amlodipine on mitochondrial function during isoproterenol-induced myocardial infarction in rats.

Materials and Methods

Chemicals

Nicorandil and amlodipine were procured from Sun Pharmaceutical Ltd, India. Isoproterenol hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of analytical grade.

Animals

Adult male albino rats of the Wistar strain, weighing approximately 120–140 g, were obtained from King Institute of Preventive Medicine, Chennai, India. They were acclimatized to animal-house conditions, were fed commercial pelleted rat chow (Hindustan Lever Ltd, Bangalore, India), and had free access to water (ethically approved by Ministry of Social Justices and Empowerment, Government of India).

Experimental protocols

The rats were divided into eight groups (n = 6 in each group): group 1, control; group 2, isoproterenol-induced; group 3, nicorandil only; group 4, amlodipine only; group 5, nicorandil + amlodipine; group 6, nicorandil + isoproterenol; group 7, amlodipine + isoproterenol; group 8, nicorandil + amlodipine + isoproterenol. Drug administration was as follows: isoproterenol given intraperitoneally for 2 days (150 mg kg^{-1} daily) (Sreepriya et al 1999); nicorandil given orally (2.5 mg kg^{-1} daily) for 3 days; amlodipine given orally (5.0 mg kg^{-1} daily) for 3 days.

After the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected and the separated serum was used for estimation of protein (Lowry et al 1951) and assaying the activity of lactate dehydrogenase (LDH) (King 1965a), creatine phosphokinase (CPK) (Okinaka et al 1961), aspartate transaminase (AST) and alanine transaminase (ALT) (Bergmeyer & Bernt 1974).

The heart was excised, rinsed in ice-cold isotonic saline, blotted with filter paper, weighed, homogenized in 0.25 M sucrose at 4°C and then mitochondria were isolated by the method of Johnson & Lardy (1967). The activity of mitochondrial enzymes such as isocitrate dehydrogenase (ICDH) (King 1965b), malate dehydrogenase (MDH) (Mehler et al 1948), succinate dehydrogenase (SDH) (Slater & Bonner 1952), α-ketoglutarate dehydrogenase (α-KGDH) (Reed & Mukherjee 1969) and NADH dehydrogenase (Minaakami et al 1962) were assayed. Also the mitochondrial lipid peroxides (LPO) (Ohkawa et al 1979), glutathione (GSH) (Ellman 1959), glutathione peroxidase (GPx) (Paglia & Valentaine 1967), glutathione-S-transferase (GST) (Habig et al 1974), superoxide dismutase (SOD) (Misra & Fridovich 1972) and catalase (CAT) (Takahara et al 1960) were assayed.

Statistical analysis

Results were statistically evaluated using one-way analysis of variance for repeated measurements. They were further evaluated with the calculation of least significant difference to check whether the mean differences were significant.

Results and Discussion

Increased activity of serum ALT, AST, CPK and LDH is a well known diagnostic marker of myocardial function. In myocardial damage with myofibrillar degeneration and myocyte necrosis, these enzymes are released from the heart into the blood stream (Wexler & Kittinger 1963).

In this study there was marked elevation in the activity of these enzymes in the serum of isoproterenol-intoxicated rats (Table 1). Pre-treatment with oral nicorandil plus amlodipine resulted in a significant reduction in the levels of these enzymes towards near normal as compared with the rats induced with myocardial infarction, establishing the cardioprotective effect of the combination.

Mitochondrial enzyme studies

The activity of the enzymes involved in the aerobic oxidation of pyruvate in mitochondria (ICDH, α-KGDH, SDH, MDH) (Table 2) was significantly lower in myocardial infarcted rats as compared with those in controls. Pre-treatment with nicorandil plus amlodipine significantly prevented these alterations when compared with the group treated with nicorandil or amlodipine individually. Significant decrease in the activity of the mitochondrial oxidative enzymes (ICDH, α-KGDH, SDH, MDH) after intraperitoneal administration of

Table 1 Activity of lactate dehydrogenase, creatine phosphokinase and transaminases in serum of normal and experimental groups of rats.

Group (treatment)	Lactate dehydrogenase	Creatine phosphokinase	Oxaloacetate transaminase	Pyruvate transaminase
1 (Control)	1024.62±101.89	89.79±6.50	73.80±6.39	26.50±1.97
2 (Isoproterenol)	1576.39±140.32[a]	209.13±19.00[a]	147.21±13.96[a]	47.33±1.69[a]
3 (Nicorandil only)	1025.04±101.12	90.91±6.31	74.77±5.91	27.14±2.04
4 (Amlodipine only)	1025.82±109.33	91.05±6.54	74.39±6.03	26.93±1.92
5 (Nicorandil+amlodipine)	1026.91±102.77	90.07±6.93	74.23±6.88	27.00±1.29
6 (Nicorandil+isoproterenol)	1156.21±101.52[ab]	124.70±7.10[ab]	96.07±6.91[ab]	39.09±1.86[ab]
7 (Amlodipine+isoproterenol)	1163.73±103.47[ab]	127.16±7.27[ab]	97.12±7.01[ab]	39.88±1.94[ab]
8 (Nicorandil+amlodipine +isoproterenol)	1033.00±101.84[bcd]	92.6±8.90[bcd]	79.6±6.98[bcd]	32.30±3.14[bcd]

Results are mean±s.d. (n = 6). $P < 0.05$ compared with [a]group 1 (control), [b]group 2 (isoproterenol), [c]group 6 (nicorandil+isoproterenol), or [d]group 7 (amlodipine+isoproterenol). Activity is expressed as: μmol pyruvate liberated per mg of protein per hour for LDH; μmol phosphorus liberated per mg of protein per hour for CPK; and μmol of pyruvate liberated per mg of protein per hour for transminases.

Table 2 Activity of mitochondrial enzymes in the heart of normal and experimental groups of rats.

Group (treatment)	ICDH	SDH	MDH	α-KGDH	NADH dehydrogenase
1 (Control)	736.7±58.6	243.2±18.2	347.2±28.5	73.80±6.40	132.80±9.60
2 (Isoproterenol)	528.4±47.3[a]	152.8±12.6[a]	274.0±23.7[a]	48.50±4.10[a]	93.24±7.50[a]
3 (Nicorandil only)	739.1±51.2	247.1±16.3	351.12±29.3	75.19±5.92	136.29±10.60
4 (Amlodipine only)	741.6±55.7	248.6±17.6	349.62±28.7	75.89±5.96	135.62±10.10
5 (Nicorandil+amlodipine)	754.3±62.8	252.6±19.8	359.82±29.3	79.32±6.70	142.20±11.30
6 (Nicorandil+isoproterenol)	626.2±59.2[ab]	191.96±13.9[ab]	292.3±24.6[ab]	61.52±4.69[ab]	104.62±8.50[ab]
7 (Amlodipine+isoproterenol)	621.4±58.3[ab]	186.92±14.2[ab]	290.75±24.9[ab]	61.62±4.64[ab]	102.97±8.70[ab]
8 (Nicorandil+amlodipine +isoproterenol)	684.8±55.4[bcd]	224.8±17.7[bcd]	310.6±27.1[bcd]	67.82±5.60[b]	120.35±10.30[abcd]

Results are mean±s.d. (n = 6). $P < 0.05$ compared with [a]group 1 (control), [b]group 2 (isoproterenol), [c]group 6 (nicorandil+isoproterenol), or [d]group 7 (amlodipine+isoproterenol). Activity is expressed as nmol of α-ketoglutarate formed per hour per mg protein for ICDH; nmol of succinate oxidised per min per mg protein for SDH; nmol of NADH oxidised per min per mg protein for MDH; nmol of ferrocyanide formed per hour per mg protein for α-KGDH; and nmol of NADH oxidised per min per mg protein for NADH dehydrogenase.

isoproterenol has already been reported (Sreepriya et al 1999). Jikko et al (1984) stated that the level of NADH increased in mitochondria only when the metabolic overload on cells was prolonged. Regitz et al (1981) reported that the NADH/NAD ratio decreased in ischaemic myocardium. This might be due to a reduction in the activity of tricarboxylic acid (TCA) cycle enzymes by the mechanism of mass action.

The activity of the respiratory marker enzyme NADH dehydrogenase was lower in isoproterenol-treated (group 2) rats as compared with normal (group 1) rats. Decreased activity of the respiratory enzyme in the rats induced with myocardial infarction has been previously reported (Calva et al 1966). The rats treated with nicorandil and amlodipine in our study showed a significant change in the activity of NADH dehydrogenase when compared with rats given the drugs individually as well as the isoproterenol-intoxicated group.

Mitochondrial lipid peroxidation and antioxidant enzymes

Lipid peroxidation reaction, a type of oxidative degeneration of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation (Comporti 1985). Significant elevations in the levels of mitochondrial LPO after isoproterenol administration was observed (Table 3) in our study. The rats treated with nicorandil plus amlodipine in our study showed a significant decrease in lipid peroxidation when

Table 3 Activity of mitochondrial lipid peroxides and antioxidant enzymes in the heart of normal and experimental groups of rats.

Group (treatment)	LPO	GSH	GPx	GST	SOD	CAT
1 (Control)	3.60±0.26	7.173±0.65	1.289±0.106	62.81±5.74	11.14±1.12	1.338±0.123
2 (Isoproterenol)	5.26±0.45[a]	4.364±0.41[a]	0.903±0.083[a]	43.17±3.80[a]	6.24±0.51[a]	0.857±0.072[a]
3 (Nicorandil only)	3.53±0.28	7.212±0.64	1.296±0.104	62.48±5.62	11.29±1.05	1.344±0.119
4 (Amlodipine only)	3.55±0.27	7.194±0.68	1.300±0.101	62.42±5.65	11.31±1.10	1.374±0.124
5 (Nicorandil+amlodipine)	3.49±0.26	7.262±0.67	1.319±0.098	62.34±5.51	11.64±1.04	1.357±0.118
6 (Nicorandil+isoproterenol)	4.68±0.39[ab]	5.621±0.59[ab]	1.053±0.093	49.62±4.26	8.09±0.72[ab]	1.011±0.089[ab]
7 (Amlodipine+isoproterenol)	4.64±0.38[ab]	5.614±0.58[ab]	1.059±0.094	50.21±4.59	8.16±0.76[ab]	1.019±0.091[ab]
8 (Nicorandil+amlodipine + isoproterenol)	4.10±0.36[abcd]	6.084±0.56[ab]	1.163±0.091[b]	55.63±5.38[b]	9.42±0.83[abcd]	1.147±0.097[ab]

Results are mean±s.d. (n = 6). $P < 0.05$ compared with [a]group 1 (control), [b]group 2 (isoproterenol), [c]group 6 (nicorandil+isoproterenol), or [d]group 7 (amlodipine+isoproterenol). Activity is expressed as: nmol of TBA reactants per mg protein for LPO; nmol per 100 mg protein for GSH; nmol of GSH oxidised per min per 100 mg protein for GPx; nmol of 1-chloro 2,4-dinitrobenzene (CDNB) conjugated per min per 100 mg protein for GST; units per mg per 100 mg protein for SOD; nmol of H_2O_2 decomposed per min per mg protein for CAT.

compared with those treated with nicorandil or amlodipine individually.

The intracellular calcium concentration in mitochondria has been reported to rise during myocardial infarction (Node et al 1997). This intracellular Ca^{2+} is an inducer of phospholipase A_2, which degrades membrane phospholipids (Hearse 1977). The free radical produced as a result of this lipid peroxidation may attack RNA polymerase corresponding to these mitochondrial enzymes. The potassium-channel opener depolarizes the mitochondrial membrane thereby reducing Ca^{2+} influx through the potential-dependent mitochondrial uniporter (Holmuhamedov et al 1999).

GSH has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen, followed by the formation of oxidized GSH and other disulphides (Meister & Anderson 1983). GSH protects the mitochondrial membrane from the damaging action of LPO (Tappel 1973). Nicorandil and amlodipine treatment caused a significant increase in GSH levels when compared with isoproterenol treatment.

GPx, an antioxidant enzyme, offers protection to the mitochondrial membrane from peroxidative damage (Anandan et al 1999). A decrease in the activity of GPx makes mitochondria susceptible to isoproterenol-induced myocardial damage, which leads to a change in mitochondrial function. Our results also showed decreased GPx activity in isoproterenol-intoxicated (group 2) rats (Table 3).

GST, another scavenging enzyme, binds to many different lipophilic compounds (Ishigaki et al 1989). It would be expected to bind isoproterenol and act as an enzyme for GSH conjugation. Oral administration of nicorandil plus amlodipine in this study prevented the isoproterenol-induced alterations in the GST activity when compared with rats treated with individual drugs.

These findings suggests that GSH- and GSH-dependent enzyme systems may be directly related to the pathogenic mechanism of isoproterenol-induced myocardial infarction and that nicorandil plus amlodipine treatment prevented these alterations.

Mitochondrial antiperoxidative enzymes

The activity of the antiperoxidative enzymes, SOD and CAT, was significantly lower in isoproterenol-treated (group 2) rats as compared with controls (group 1). This is due to the diminished scavenging of the $^{\bullet}OH$ formed by isoproterenol-induced lipid peroxidation. Oral administration of nicorandil plus amlodipine in our study prevented the alteration, when compared with the drugs given individually.

These findings led to the conclusion that the combination therapy is more effective in reducing the extent of mitochondrial damage and ensuring the activity of mitochondrial enzymes and the mitochondrial tissue defence system in experimentally induced myocardial infarction in rats.

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