Comparison of Isoproterenol – Induced Changes in Lysosomal Enzyme Activity *in vivo* and *in vitro*

T Mačičková[1], J Navarová[1], M Urbančíková[2] and K Horáková[3]

1 *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia*
2 *Institute of Preventive and Clinical Medicine, Bratislava, Slovakia*
3 *Faculty of Chemical Technology, Slovak University of Technology, Bratislava, Slovakia*

Abstract. Isoproterenol was used as a drug which, when administered in high doses, is able to induce lysosomal enzyme activity changes in *in vivo* conditions We correlated lysosomal enzyme activity in the absence and presence of isoproterenol, obtained in whole animals and in HeLa and HepG2 cells in tissue culture *In vivo* experiments male Wistar rats (270–300 g) were treated subcutaneously with isoproterenol in various doses Effect of isoproterenol on lysosomal enzyme activity was assayed in the heart after differential centrifugation *In vitro* experiments Isoproterenol in concentrations 0 1–100 μg/ml was added to HeLa and HepG2 cells and the activity of lysosomal enzyme was measured in the cell homogenate In the sedimentable and nonsedimentable fractions of the rat myocardium, the isoproterenol-induced changes in the activity of lysosomal enzyme were time-and dose-dependent In HeLa cells, isoproterenol administration caused a dose-dependent increase of lysosomal enzyme activity, while in HepG2 cells the activity remained unchanged Thus the isoproterenol-induced changes in lysosomal enzyme activity in the rat myocardium were comparable with the results found *in vitro* in HeLa cells

Key words: Isoproterenol — Lysosomal enzyme activity — Rat — *in vivo* — *in vitro*

Introduction

It is well known that the synthetic catecholamine isoproterenol (IPN) is capable of inducing massive myocardial necrosis in most mammals when administered in high doses (Csapo et al 1972, Noa et al 1994, Seth et al 1998) Animals injected

Correspondence to Tatiana Mačičková, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravska cesta 9, 842 16 Bratislava, Slovakia E-mail exfatama@savba sk

with IPN developed "infarct-like lesions" (Marjomaki et al. 1994) resembling myocardial infarction in man (Milei et al 1978). Irreversible necrosis is mediated through abnormal degradation of cellular constituents by lysosomal hydrolases, and this could indicate that some of the latent hydrolases in lysosomes, which are labilised by ischaemia, may be present besides lysosomal vacuoles, possibly from other membrane-bound sites. In experimentally induced myocardial infarction, a decrease in lysosomal stability increases the levels of lysosomal enzymes, leading to altered metabolism of different connective tissue constituents and collagen (Takahashi et al. 1990; Ravichandran et al. 1991).

The aim of the present study was to compare isoproterenol-induced changes in lysosomal enzyme activity *in vivo* and in HeLa and HepG2 cells in tissue culture The lysosomal enzymes – N-acetyl-beta-D-glucosaminidase (NAGA), acid phosphatase (APH), beta-D-glucuronidase (BGN) and cathepsin D (CAT D) were used as indicators of cellular damage.

Materials and Methods

Animals Male Wistar rats weighing 270-300 g (Breeding Facility, IEP SASc, Dobrá Voda, Slovakia) were fed *ad libitum* until the time of sacrifice To induce myocardial damage, the rats were given DL-isoproterenol hydrochloride (IPN, Sigma, USA) in a single subcutaneous injection of 50 mg/kg or in two doses of 40 mg/kg s c at an interval of 1 h Control animals received equivalent volumes of saline The animals were sacrificed by decapitation 1-18 h following administration of IPN The hearts were removed rapidly, washed thoroughly with ice-cold saline (0 25 mol/l KCl), dried on a filter paper, weighed and frozen individually in liquid nitrogen Each sample consisted of the hearts from two animals The cardiac tissue was homogenised (20 % w/w homogenate) in ice-cold buffer D, pH 7 4, containing 0 6 mol/l KCl, 40 mmol/l EDTA, 1 mmol/l $MgCl_2$, 10 mmol/l imidazole The homogenates were processed according to Asano et al (1979) After centrifugation, the pellets were suspended in ice-cold buffer D, pH 7 4, containing Triton X-100 (final concentration 0 1%) and homogenised Lysosomal enzyme activities and levels of proteins in individual fractions of the myocardium homogenate were assayed after differential centrifugation

In vitro experiments Human cervix carcinoma cell lines, HeLa and human hepatoma cell line HepG2 were cultured in Basale Eagle Medium with 10% foetal calf serum without antibiotics at 37°C with 5% CO_2 humidified atmosphere In experiments, the cells were inoculated in a density 3×10^5 cells/dish (5 ml) for 24 h before treatment IPN was added in the concentration of 0 1–100 μg/ml for 24 h After treatment, the cells were washed with saline buffer, homogenised in ice-cold phosphate buffer, pH 7 4, containing 0 1% Triton X-100 and centrifuged at 5000 × g for 15 min (at 4°C) Lysosomal enzyme activities and protein content were determined in the obtained supernatants

Biochemical analysis The activities of acid phosphatase (APH), cathepsin D (CAT D), beta-D-glucuronidase (BGN) and N-acetyl-beta-D-glucosaminidase (NAGA) were assayed according to standard methods (Barrett and Heath 1977) Proteins were determined by the method of Lowry et al (1951) All chemicals used (Lachema, Czech Republic, Serva, Germany, Sigma, USA) were of analytical grade

Statistical analysis All parameters measured are presented as means ± S E M , with significance determined by Student's t-test

Results and Discussion

IPN-induced myocardial infarction results in increased lysosomal hydrolase activities that may be responsible for tissue damage and the infarcted heart (Ravichandran et al 1991) Decreased activity of lysosomal enzymes in particulate fractions of ischaemic heart homogenates has been a common finding (Decker and Wildenthal 1978, Okuda and Lefer 1979, Nirmala and Puvanakrishnan 1996) The differences in the effect of IPN in our experiments varied in the time intervals studied After 3 and 5 h, a single subcutaneous dose of IPN (50 mg/kg) did not induce significant alterations in the shift of NAGA and APH activity from sedimentable fractions to supernatant (Tables 1, 2) After 12 h, single s c administration of IPN in the dose of 50 mg/kg resulted in a statistically significant decrease of the specific activity of APH in the lysosomal fraction of the myocardium homogenate, along with simultaneous increase of enzymatic activity in the supernatant This is indicative of damage of lysosomal membranes in myocytes due to ischaemia of the myocardium induced by IPN (Table 2) After 18 h however, administration of the same dose of IPN resulted in a statistically significant increase of enzymatic activity, and that both in the lysosomal fraction and in the supernatant This increase is ascribable to the influx and proliferation of nonmyocytic cells participating in the reparatory process in the necrotising myocardial tissue (Table 2) When myocardial damage was induced by two doses of IPN (2 × 40 mg/kg s c), the sedimentable fraction of the rat heart homogenate exibited a statistically significant decrease observed in the specific activity of CAT D after 3 h and of APH after 4 h After 5 or 6 h, a significant increase was recorded in the activity of both enzymes (Table 3) After 9 h, IPN administration in the dose of 50 mg/kg s c induced a statistically significant decrease of the specific activity of all four lysosomal enzymes studied in the sedimentable fraction (Table 4) This reduction was the highest of the IPN-induced damage observed during the 1-18 h period The obtained results indicate that it is the interval of 9-h IPN action that in terms of lysosomal activity distribution may correspond to 60-min ischaemia induced by ligation of the coronary artery (Decker

Table 1. Release of N-acetyl-beta-D-glucosaminidase (NAGA) in individual fractions of the rat myocardium homogenate after isoproterenol-induced damage

FRACTION	CONTROL	IPN (50 mg/kg)	
		3 h	5 h
P 1	1 32 ± 0 15	1 65 ± 0 18	0 94 ± 0 09
P 2	2 13 ± 0 22	3 40 ± 0 28**	1 63 ± 0 21*
P 3	0 31 ± 0 04	0 37 ± 0 10	0 16 ± 0 08*
SUPERNATANT	0 41 ± 0 05	0 39 ± 0 05	0 41 ± 0 04

P1 – 1700 × g pellet, P2 – 20,000 × g pellet, P3 – 105,000 × g pellet, Number of animals 9–12 in each group, Values are means ± S E M , *$p < 0\,05$ versus control **$p < 0\,01$ versus control Activity of NAGA is expressed in nmol p-nitrophenol/min/mg protein

Table 2. Release of acid phosphatase (APH) in individual fractions of the rat myocardium homogenate after isoproterenol-induced damage

FRACTION	CONTROL	IPN (50 mg/kg)		
		3 h	12 h	15 h
P1	10 36 ± 1 08	11 00 ± 1 52	10 82 ± 2 50	14 90 ± 2 02
P 2	23 45 ± 4 40	24 27 ± 3 42	21 35 ± 4 76	29 73 ± 1 46**
P 3	11 07 ± 1 54	10 80 ± 1 08	10 05 ± 2 40	4 80 ± 2 19*
SUPERNATANT	18 96 ± 1 86	18 14 ± 1 52	21 70 ± 2 20*	22 36 ± 1 01*

P1 – 1700 × *g* pellet, P2 – 20,000 × *g* pellet, P3 – 105,000 × *g* pellet Number of animals 9–12 in each group, Values are means ± S E M , *$p < 0\ 05$ versus control, **$p < 0\ 01$ versus control Activity of APH is expressed in nmol *p*-nitrophenol/min/mg protein

Table 3. Lysosomal enzyme release in sedimentable fraction of the rat myocardium homogenate after isoproterenol-induced damage

SAMPLE	TIME	CAT D	APH
CONTROL		1 34 ± 0 09	11 0 ± 0 08
IPN (2 x 40 mg/kg)	1 h	1 22 ± 0 10	9 30 ± 0 73
	2 h	1 41 ± 0 09	11 60 ± 0 44
	3 h	0 84 ± 0 05*	11 70 ± 0 37
	4 h	1 4 ± 0 03	9 80 ± 0 77
	5 h	1 74 ± 0 12*	13 10 ± 0 08*
	6 h	1 94 ± 0 07*	14 70 ± 0 07

CAT D – cathepsin D, APH – acid phosphatase, sedimentable fraction – 40,000 × *g* pellet Number of animals 9–12 in each group, Values are means ± S E M , *$p < 0\ 05$ versus control Activity of CAT D is expressed in μg tyrosine/min/mg protein and activity of APH is expressed in nmol *p*-nitrophenol/min/mg protein

and Wildenthal 1978, Kennett and Weglicki 1978). The given interval appears to be suitable for assessing the effect of lysosomal membrane stabilising substances. An early change in the integrity of the lysosomal membrane may crucially affect the moment when a potentially reversible ischaemic stress turns to be irreversible.

No information has come to our attention concerning IPN-induced damage accompanied by alteration of lysosomal enzyme activities *in vitro*. We studied this effect on two human carcinoma cell lines of different origin – HeLa and HepG2. Administration of IPN caused a dose-dependent increase of lysosomal activity in HeLa cells (Fig. 1). On the other hand, HepG2 cells were not found to be sensitive to IPN-treatment, as concluded from the unchanged lysosomal enzyme activity (data not shown) HepG2 cells were originally derived from liver carcinoma-hepatoma and, although they do not express all the characteristics of hepatocytes, they do possess

Table 4. Changes of specific activity of lysosomal enzymes in sedimentable fraction of the rat myocardium homogenate after isoproterenol-induced damage

ENZYME	CONTROL	IPN (50 mg/kg) s c 9 h
APH	9 89 ± 0 44	7 99 ± 0 20*
NAGA	0 65 ± 0 02	0 49 ± 0 02*
CAT D	1 39 ± 0 02	1 20 ± 0 01*
BGN	23 45 ± 4 80	14 75 ± 1 56*

APH – acid phosphatase, NAGA – N-acetyl-beta-D-glucosaminidase, CAT D – cathepsin D, BGN – beta-D-glucuronidase Lysosomal enzyme activities are presented as follows APH and NAGA – nmol *p*-nitrophenol/min/mg protein, CAT D – μg tyrosine/min/mg protein, BGN – nmol phenolphtalein/hod/mg protein, sedimentable fraction – 40,000 × *g* pellet Number of animals 9–12 in each group, Values are means ± S E M, $*p < 0\ 05$ versus control

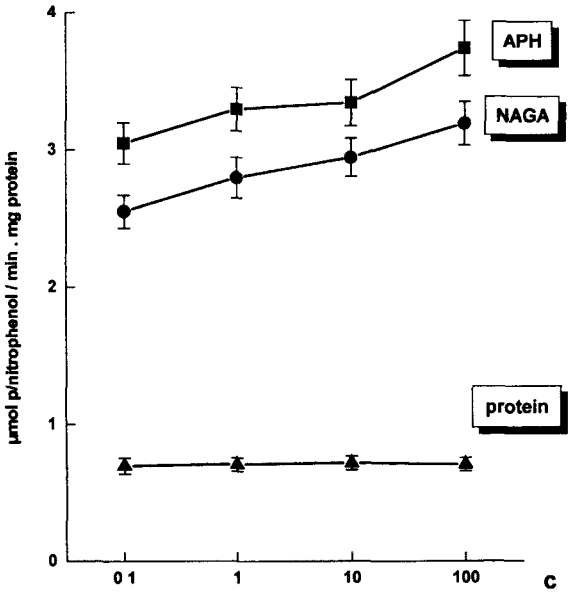

Figure 1. Changes of lysosomal enzyme activities and levels of proteins in HeLa cell homogenate after isoproterenol-induced damage APH – acid phosphatase, NAGA – N-acetyl-beta-D-glucosaminidase Activity of APH and NAGA is expressed in μmol *p*-nitrophenol/min/mg album Protein levels are presented in μg/ml

many metabolic enzymes specific for liver cells (Javitt 1990). One of the possible explanations of the controversial results obtained on the two *in vitro* systems used may be that HepG2 cells, contrary to HeLa cells, may metabolise IPN into an inactive compound before this drug could exert its effect Differences in lysosomal enzymes between HeLa and HepG2 cells may be also due to greater vulnerability

of HeLa cells to isoproterenol. In addition, a recent study showed that warm ischaemia of isolated rat liver did not increase the activity of acid phosphatase in effluent perfusate, while cytosolic enzymes were markedly elevated in this model (Smreková et al. 1998).

The results established *in vitro* in HeLa cells, however, were comparable with the IPN-induced changes in the activity of lysosomal enzymes in the rat heart *in vivo*.

Acknowledgements. This work was supported by the grants from VEGA 95/5305/152 and 2/6025/99, Bratislava, Slovakia

References

Asano S , Komoryia H , Hayashi E , Sawada H (1979) Changes in intracellular activities of lysosomal enzymes in tissue of rats during ageing Mech Age Dev **10**, 81–92

Barrett A J , Heath M F (1977) Lysosomal enzymes In Lysosomes A Laboratory Handbook, 2nd Ed (Ed J T Dingle) pp 19–147, Elsevier/North-Holland Biochemical Press, Amsterdam

Csapo Z , Dusek J , Rona G (1972) Early alterations of the cardiac muscle in isoproterenol-induced necrosis Arch Pathol **93**, 356–365

Decker R S , Wildenthal K (1978) Sequential lysosomal alterations during cardiac ischaemia II Ultrastructural and cytochemical changes Lab Invest **38**, 662–673

Javitt N B (1990) HepG2 cells as a resource for metabolic studies lipoprotein, cholesterol, and bile acids FASEB J **4**, 161–168

Kennett F F , Weglicki W B (1978) Effect of well-defined ischaemia on myocardial lysosomal and microsomal enzyme in a canine model Circ Res **43**, 750–758

Lowry O H , Rosenbrough N J , Farr A L , Randall R (1951) Protein measurement with Folin phenol reagent J Biol Chem **193**, 265–275

Marjomaki V , Ritamaki V , Gruenberg J (1994) Isoproterenol-induced redistribution of endosomes in cardiac myocytes Eur J Cell Biol **65**, 1–13

Milei J , Nunez R G , Rapaport M (1978) Pathogenesis of isoproterenol-induced myocardial lesions Cardiology **63**, 139–151

Nirmala Ch , Puvanakrishnan R (1996) Effect of Curcumin on cetrain lysosomal hydrolases in isoproterenol-induced myocardial infarction in rats Biochem Pharmacol **51**, 47–51

Noa M , Herrera M , Magraner J , Mas R (1994) Effect of policosanol on isoprenaline-induced myocardial necrosis in rats J Pharm Pharmacol **46**, 282–285

Okuda M , Lefer A H (1979) Lysosomal hypothesis in evolution of myocardial infarction Jpn Heart J **20**, 643–655

Ravichandran L V , Povanakrishnan R , Joseph K T (1991) Influence of isoproterenol-induced myocardial infarction on certain glycohydrolases and cathepsins in rats Biochem Med Metab Biol **45**, 6–15

Seth S D , Maulik M , Katiyar C K , Maulik S K (1998) Role of Lipistat in protection against isoproterenol-induced myocardial necrosis in rats Indian J Physiol Pharmacol **42**, 101–106

Smreková R , Vajdová K , Kukan M , Horecký J (1998) Release of lysosomal and cytosolic enzymes into the effluent perfusate after warm and cold ischaemia of rat liver Chem Papers **52**, 461–462

Takahashi S , Barry A C , Factor S M (1990) Collagen degradation in ischaemic rat heart Biochem J **25**, 233–241