J. Pharm. Pharmacol. 1996, 48: 323–326
Received February 27, 1995
Accepted December 5, 1995

Antihypertensive Drugs Reduce Noradrenaline-induced Hypertrophy of Cultured Myocardial Cells

T. A. LÄHTEENMÄKI, E. SIEVI AND H. VAPAATALO

Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Helsinki, PO Box 8, FIN-00014 University of Helsinki, Finland

Abstract

The cellular mechanisms of cardiac hypertrophy are still largely unknown. In-vivo studies have demonstrated that antihypertensive drugs can regress hypertrophy independently of reductions in blood pressure. The antihypertrophic effects of metoprolol, propranolol, felodipine, verapamil and captopril were studied in neonatal cardiac myocyte culture. Prazosin was used as a positive control. Hypertrophy was defined as an increase in protein content measured by [^{3}H]leucine incorporation.

Noradrenaline induced a 1·5-fold increase in protein synthesis over 48 h. Prazosin prevented the hypertrophic effect of noradrenaline. Adrenergic β-receptor blocking agents and calcium antagonists reduced myocyte hypertrophy in a dose-dependent manner. The angiotensin-converting enzyme inhibitor captopril was ineffective.

These results indicate that adrenergic β-receptor blockers and calcium antagonists may have direct nonhaemodynamic effects on the growth of cultured cardiac myocytes.

Myocardial hypertrophy is an important physiological and structural mechanism to compensate for increased work by the heart. The development of left ventricular hypertrophy is a complex process which involves mechanical, haemodynamic, neural and endocrine factors. Sodium chloride loading has been shown to produce hypertrophy by blood pressure-independent mechanisms (Kihara et al 1985; Mervaala et al 1992). Both in-vivo and in-vitro studies have demonstrated that adrenergic stimulation induces cardiac hypertrophy (Laks et al 1973; Simpson 1983). Noradrenaline has been considered under some experimental conditions to be a critical initiating factor, which may have direct or indirect effects on cardiac hypertrophy (Dahlöf 1988).

In addition to lowering blood pressure, some antihypertensive drugs also have the capacity to regress hypertrophic growth of the heart both in man and in experimental animals. Angiotensin-converting enzyme (ACE) inhibitors, adrenergic β-receptor blockers and calcium antagonists have been shown to decrease ventricular mass (Messerli et al 1988). We have found recently that the antihypertrophic effect of metoprolol does not necessarily correlate with its antihypertensive effect (Mervaala et al 1994b). The mechanisms by which antihypertensive agents cause this blood pressure-independent regression of cardiac hypertrophy are still unclear.

In the present study we have compared the antihypertrophic effect of different antihypertensive drugs using cultured neonatal ventricular myocytes, which allowed us to investigate the drugs in the absence of their haemodynamic effects.

Correspondence: T. Lähteenmäki, Institute of Biomedicine, Department of Pharmacology and Toxicology, University of Helsinki, PO Box 8, FIN-00014 University of Helsinki, Finland.

Materials and Methods

Cell culture and experiments

Cultures of neonatal rat cardiac myocytes were obtained from minced ventricular myocardium of 1–3 day-old Wistar rats. The fragments were dissociated by several incubations with Hanks' balanced salt solution containing 0·02% type II collagenase, 20 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulphonic acid] and 50 μM Ca^{2+} at 37°C. The cells released were collected by decantation, strained and washed twice with Medium 199 (M199) containing 10% foetal calf serum, 20 mM HEPES, 100 int. units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. The resulting cell suspension was plated for 30 min in plastic culture dishes (differential attachment technique). Unattached myocardial cells were replated at $6·5 \times 10^5$ cells in 35-mm diameter culture dishes with the same culture medium supplemented with 5-bromo-2'-deoxyuridine (BrdU, 100 μM). Cultures were incubated at 37°C in a humidified atmosphere of CO_2 (5%) and air (95%). After 18 h, cells were washed several times and cultured in M199 containing 10 μg mL^{-1} transferrin, 10 μg mL^{-1} insulin, 20 mM HEPES, 100 μM BrdU and antibiotics. This serum-free medium maintains cells in quiescent state. All experiments were started after 24 h in serum-free medium. The cells were rinsed and the medium was replaced with fresh serum-free medium (without BrdU) containing 0·5 μCi mL^{-1} [^{3}H]leucine. To inhibit noradrenaline oxidation and to prevent the formation of toxic metabolites, the culture medium was supplemented with ascorbic acid (100 μM) (Cooper et al 1986). Noradrenaline (20 μM) and the drugs to be studied were added and the cells were incubated for 48 h. An additional dose of noradrenaline was added after 24 h. The concentrations of the drugs and the incubation conditions were chosen after pre-testing. Phase-contrast microscopy was used to inspect the cells for evidence of cell detachment or changes in cell morphology

Table 1. Effects of metoprolol, propranolol, felodipine and verapamil on noradrenaline-induced [^{3}H]leucine incorporation over 48 h.

	[^{3}H]leucine incorporation relative to control
Noradrenaline (20 μM)	1·41 ± 0·22
+ metoprolol	
1 μM	1·35 ± 0·22
10 μM	1·34 ± 0·15
100 μM	1·06 ± 0·25*
Noradrenaline (20 μM)	1·48 ± 0·09
+ propranolol	
0·1 μM	1·23 ± 0·04
1 μM	1·26 ± 0·08
10 μM	0·92 ± 0·20***
Noradrenaline (20 μM)	1·54 ± 0·15
+ felodipine	
0·01 μM	1·45 ± 0·22
0·1 μM	1·37 ± 0·26
1 μM	1·15 ± 0·35
Noradrenaline (20 μM)	1·46 ± 0·20
+ verapamil	
0·1 μM	1·37 ± 0·22
1 μM	1·19 ± 0·22
10 μM	1·03 ± 0·17*

Results are the normalized means (treatment/control) ± s.e.m. (n = 4–7). *$P < 0·05$, ***$P < 0·001$ compared with treatment with noradrenaline alone.

in the presence of the drugs studied. Cell number and viability were determined by counting the trypan blue-negative cells. At the highest concentrations used in this study the drugs were not found to reduce cell viability.

Experiments were terminated by washing the cells three times with ice-cold Hanks' solution and precipitating with 10% trichloroacetic acid (TCA) at 4°C for 30 min. The precipitate was washed twice with 10% TCA and solubilized in 2M NaOH for 60 min. After neutralization, aliquots were counted in a liquid scintillation counter (Rackbeta, LKB, Wallac, Turku, Finland). The experimental design was approved by the Animal Experimentation Committee of the University of Helsinki.

Materials

Foetal calf serum and HEPES were from Gibco Europe (Paisley, UK). Transferrin was from Hyclone (Cramlington, UK), antibiotics from Biological Industries (Israel) and collagenase from Worthington Biochemical (Freehold, NJ, USA). [^{3}H]Leucine was purchased from Amersham International (Bucks, UK) and cell-culture dishes from Nunc (Copenhagen, Denmark). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA) or from E. Merck (Darmstadt, Germany). Felodipine was a gift from Suomen Astra Oy (Masala, Finland) and captopril, prazosin and verapamil from Orion Oy (Espoo, Finland).

Statistics

Results are expressed as a mean ± s.e.m. Statistical analysis of the data was performed by unpaired Student's t-test or by analysis of variance supported by Tukey's test. A value of $P < 0·05$ was considered significant.

Results

The hypertrophy of cardiac myocytes was induced by noradrenaline (20 μM) and was measured by [^{3}H]leucine incorporation into newly formed proteins. Noradrenaline induction increased protein synthesis 1·5-fold compared with control over 48 h. Prazosin (0·2 μM), an α_1-adrenergic antagonist, inhibited noradrenaline-induced hypertrophy by 85% ($P < 0·001$, n = 5, data not shown). Incubation of neonatal myocytes with increasing concentrations of the cardioselective adrenergic β-blocker metoprolol for 48 h caused a concentration-dependent decrease in the protein synthesis induced by noradrenaline (Table 1). Protein synthesis was reduced 17, 21 and 88% at 1, 10 and 100 μM metoprolol, respectively. The non-selective β-blocker propranolol was able to inhibit protein synthesis at lower concentrations. At concentrations of 0·1 and 1 μM, protein synthesis was reduced by 53 and 45%, respectively. At 10 μM propranolol, protein synthesis was below basal levels.

Table 1 also shows the effect of the calcium antagonist felodipine on protein synthesis. At concentrations of 0·01, 0·1 and 1 μM, [^{3}H]leucine incorporation was reduced 19, 38 and 74%, respectively. Another calcium-channel blocker, verapamil, required higher concentrations and reduced protein synthesis by 20, 59 and 94% at 0·1, 1 and 10 μM concentrations, respectively. The ACE inhibitor, captopril, had almost no effect on the myocyte growth induced by noradrenaline. At the highest concentration used (100 μM) it reduced [^{3}H]leucine incorporation by only 12% (n = 6, data not shown). Table 2 summarizes the effects of the antihypertensive drugs used in this study in the absence of noradrenaline induction. It shows that some of these drugs in certain concentrations were able to reduce [^{3}H]leucine incorporation.

Discussion

Left ventricular hypertrophy has long been regarded as an adaptation mechanism to increased blood pressure. Several

Table 2. The effect of antihypertensive agents on myocyte protein synthesis.

		[^{3}H]Leucine incorporation				
Treatment	Concentration (μM)	0·01	0·1	1	10	100
Metoprolol				0·9 ± 0·1	0·9 ± 0·1	0·9 ± 0·1
Propranolol			0·9 ± 0·1	0·8 ± 0·1	0·7 ± 0·1	
Felodipine		1·0 ± 0·2	1·0 ± 0·1	0·8 ± 0·2		
Verapamil			1·2 ± 0·1	1·1 ± 0·1	0·9 ± 0·2	
Captopril				0·9 ± 0·2	1·0 ± 0·2	0·9 ± 0·2

Values are relative means (treated/control) ± s.e.m.

studies indicate, however, that nonhaemodynamic factors (catecholamines, growth factors, renin-angiotensin system) can also participate in the development of hypertrophy in the absence of changes in blood pressure. Growth-promoting processes are multifactorial and are probably regulated in a number of ways. Catecholamines and angiotensin II have been proposed to play a major role as growth-promoting factors (Dahlöf 1988; Schelling et al 1991).

In the present study we investigated the direct effects of different antihypertensive drugs on noradrenaline-induced cell growth using cultured rat neonatal myocytes. This in-vitro model excludes haemodynamic effects which complicate the situation in-vivo. In our study, calcium antagonists and adrenergic β-blockers were able to reduce cell growth induced by noradrenaline. In contrast, the ACE inhibitor, captopril, had only a slight inhibitory effect on hypertrophy.

In cultured neonatal myocytes the hypertrophic effect of noradrenaline has been demonstrated to be mediated mainly through α_1-adrenergic receptors (Simpson 1983; Meidell et al 1986). The hypertrophic response is independent of contractile activity and results in an increase in cell volume, surface area, protein synthesis and protein content as well as activation of certain proto-oncogenes. The stimulation of protein accumulation by noradrenaline has been reported to be maximal between 24 and 48 h after treatment (Simpson 1985). In our study, noradrenaline induced a 1·5-fold increase in protein synthesis. This result is similar to that previously found using the same concentration of noradrenaline (Sadoshima & Izumo 1993). Recently it has been reported that the α_{1A}-adrenergic-receptor subtype mediates biochemical, molecular and morphologic changes induced by adrenergic stimulation of cultured myocardial cell hypertrophy (Knowlton et al 1993). In our experiment the α_1-adrenergic antagonist, prazosin, had a marked inhibitory effect on noradrenaline-induced hypertrophy. However, prazosin is a non-specific α_1-adrenoceptor antagonist (Ford et al 1994) and cannot therefore differentiate the type of α-receptor involved in hypertrophy in the present study.

Noradrenaline activates both α- and β-receptors. Decker et al (1993) observed that the adrenergic non-selective β-blocker, propranolol, blocked growth in rabbit cardiac myocyte culture by inhibiting noradrenaline-induced changes in protein turnover. Meidell et al (1986) did not observe any significant inhibition of protein synthesis by propranolol (10 μM) during a 24 h treatment in neonatal myocyte culture. Simpson (1985) has reported 15% reduction of noradrenaline-induced hypertrophy by 2 μM propranolol. In the present study propranolol was more effective; at a concentration of 10 μM, it inhibited protein synthesis below the basal level. The β_1-selective blocker, metoprolol, was clearly less effective. The explanation for this could be that most of the metabolic effects of the adrenergic stimulation are related to β_2-receptor activity (Walle et al 1988).

Calcium antagonists are structurally and pharmacologically a heterogeneous group of drugs. Therefore we studied two different chemical compounds, a dihydropyridine derivative, felodipine, and a papaverine derivative, verapamil. In-vivo, the reduction of ventricular hypertrophy by calcium antagonists seems to be mediated primarily by afterload reduction, but it has also been proposed that a lowered concentration of intracellular calcium and reduced sensitivity to angiotensin II also contribute to this effect (Messerli et al 1988). Calcium ions take part in the signal transduction of proto-oncogenes, which are involved in cell growth (Marban & Koretsune 1990). α_1-Adrenergic stimulation increases the concentration of intracellular calcium and the activity of protein kinase C (Henrich & Simpson 1987), which in turn induces proto-oncogenes leading to an increase in protein synthesis. In the present study felodipine (1 μM) and verapamil (10 μM), were effective in reducing protein synthesis induced by noradrenaline. This effect might be a consequence of decreased calcium influx to the cell. It is also possible that the antihypertrophic action is due to non-specific effects of calcium antagonists, since it has been shown that calcium antagonists can inhibit α_1-adrenergic receptors (Karliner et al 1982). In vascular smooth-muscle cell culture it has been demonstrated that dihydropyridine-type calcium antagonists prevent DNA synthesis without affecting the angiotensin II-induced increase in intracellular calcium concentration, indicating that calcium channel-independent mechanisms may also participate in the inhibition of cell growth (Ko et al 1993).

We have previously shown in-vivo, both in spontaneously hypertensive and in normotensive rats, that cardiac hypertrophy induced by increased salt loading can be prevented by felodipine and metoprolol (Mervaala et al 1994a, b), but not by enalapril or ramipril (Mervaala et al 1994c, d). In-vivo experiments show conflicting results as to the effectiveness of adrenergic β-blockers to induce regression of left ventricular hypertrophy. This has been attributed to differences in experimental conditions and the haemodynamic properties of the drugs (Monopoli & Ongini 1994).

In-vivo ACE inhibitors have been suggested to decrease ventricular mass by reducing afterload and inhibiting angiotensin II-dependent myocardial protein synthesis. They may also have an effect in decreasing angiotensin-mediated adrenergic outflow (Messerli et al 1988). ACE inhibitors are the most effective antihypertensive compounds in reducing ventricular hypertrophy in-vivo (Dahlöf et al 1992). It was therefore unexpected that in the present study, captopril was ineffective. However, in our model system, hypertrophy was induced by noradrenaline whereas in the in-vivo situation the development of hypertrophic growth is multifactorial and involves strong participation of the intracardiac renin-angiotensin system (Re 1987).

In the present study, we have shown that antihypertensive drugs such as adrenergic β-receptor antagonists and calcium channel blockers, but not an ACE inhibitor, are able to reduce noradrenaline-induced cardiac myocyte hypertrophy in-vitro. There are differences in the potencies of individual compounds inside a drug group. These results indicate that adrenergic β-blockers and calcium antagonists may have direct nonhaemodynamic effects on the growth of cultured cardiac myocytes.

Acknowledgements

This study was supported by the Sigrid Jusélius Foundation, the Academy of Finland and the University of Helsinki.

References

Cooper, G., Mercer, W. E., Hoober, J. K., Gordon, P. R., Kent, R. L., Lauva, I. K., Marino, T. A. (1986) Load regulation of the properties of adult feline cardiocytes. The role of substrate adhesion. Circ. Res. 58: 692–705

Dahlöf, B. (1988) Factors involved in the pathogenesis of hypertensive cardiovascular hypertrophy. Drugs 35 (Suppl.5): 6–26

Dahlöf, B., Pennert, K., Hansson, L. (1992) Regression of left ventricular hypertrophy. A meta-analysis. Clin. Exp. Hypertens. A14: 173–180

Decker, R. S., Cook, M. G., Behnke-Barclay, M. M., Decker, M. L., Lesch, M., Samarel, A. M. (1993) Catecholamines modulate protein turnover in cultured, quiescent rabbit cardiac myocytes. Am. J. Physiol. 265: H329–H339

Ford, A. P. D. W., Williams, T. J., Blue, D. R., Clarke, D. E. (1994) α_1-Adrenoceptor classification: sharpening Occam's razor. Trends Pharmacol. Sci. 15: 167–170

Henrich, C. J., Simpson, P. C. (1987) Activation of protein kinase C in hypertrophy of cultured rat heart myocytes. Circulation 76(Suppl. IV): 235

Karliner, J. S., Motulsky, H. J., Dunlap, J., Brown, J. H., Insel, P. A. (1982) Verapamil competitively inhibits α_1-adrenergic and muscarinic but not β-adrenergic receptors in rat myocardium. J. Cardiovasc. Pharmacol. 4: 515–520

Kihara, M., Utagawa, N., Mano, M., Nara, Y., Horie, R., Yamori, Y. (1985) Biochemical aspects of salt-induced, pressure-independent left ventricular hypertrophy in rats. Heart Vessels 1: 212–215

Knowlton, K. U., Michel, M. C., Itani, M., Schubeita, H. E., Ishihara, K., Brown, J. H. (1993) The α_{1A}-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. J. Biol. Chem. 268: 15374–15380

Ko, Y., Totzke, G., Graack, G. H., Heidgen, F. J., Meyer zu Brickwedde, M. K., Düsing, R., Vetter, H., Sachinidis, A. (1993) Action of dihydropyridine calcium antagonists on early growth response gene expression and cell growth in vascular smooth muscle cells. J. Hypertens. 11: 1171–1178

Laks, M. M., Morady, F., Swan, H. J. (1973) Myocardial hypertrophy produced by chronic infusion of subhypertensive doses of norepinephrine in the dog. Chest 64: 75–79

Marban, E., Koretsune, Y. (1990) Cell calcium, oncogenes and hypertrophy. Hypertension 15: 652–658

Meidell, R. S., Sen, A., Henderson, S. A., Slahetka, M. F., Chien, K. R. (1986) α_1-Adrenergic stimulation of rat myocardial cells increases protein synthesis. Am. J. Physiol. 251: H1076–H1084

Mervaala, E. M. A., Himberg, J.-J., Laakso, J., Tuomainen, P., Karppanen, H. (1992) Beneficial effects of a potassium and magnesium-enriched salt alternative. Hypertension 19: 535–540

Mervaala, E. M. A., Laakso, J., Karppanen, H. (1994a) Cardiovascular effects of felodipine are not antagonized by dietary salt. Eur. J. Pharmacol. 255: 73–79

Mervaala, E. M. A., Laakso, J., Vapaatalo, H., Karppanen, H. (1994b) Improvement of cardiovascular effects of metoprolol by replacement of common salt with a potassium- and magnesium-enriched salt alternative. Br. J. Pharmacol. 112: 640–648

Mervaala, E. M. A., Laakso, J., Vapaatalo, H., Karppanen, H. (1994c) Effects of enalapril and hydrochlorothiazide on the salt-induced cardiac and renal hypertrophy in normotensive rats. Naunyn Schmiedebergs Arch. Pharmacol. 350: 416–425

Mervaala, E. M. A., Paakkari, I., Laakso, J., Nevala, R., Teräväinen, T.-L., Fyhrquist, F., Vapaatalo, H., Karppanen, H. (1994d) Replacement of salt by a novel potassium- and magnesium-enriched salt alternative improves the cardiovascular effects of ramipril. Br. J. Pharmacol. 111: 1189–1197

Messerli, F. H., Oren, S., Grossman, E. (1988) Left ventricular hypertrophy and antihypertensive therapy. Drugs 35(Suppl.5): 27–33

Monopoli, A., Ongini, E. (1994) Effects of antihypertensive drugs on cardiac hypertrophy. Pharmacol. Res. 29: 197–215

Re, R. N. (1987) Cellular mechanisms of growth in cardiovascular tissue. Am. J. Cardiol. 60: 104–109

Sadoshima, J., Izumo, S. (1993) Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT_1 receptor subtype. Circ. Res. 73: 413–423

Schelling, P., Fischer, H., Ganten, D. (1991) Angiotensin and cell growth: a link to cardiovascular hypertrophy? J. Hypertens. 9: 3–15

Simpson, P. (1983) Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an α_1-adrenergic response. J. Clin. Invest. 72: 732–738

Simpson, P. (1985) Stimulation of hypertrophy of cultured neonatal rat heart cells through an α_1-adrenergic receptor and induction of beating through an α_1- and β_1-adrenergic receptor interaction. Evidence for independent regulation of growth and beating. Circ. Res. 56: 884–894

Walle, T., Webb, J. G., Bagwell, E. E., Walle, U. K., Daniell, G. B., Gaffney, T. E. (1988) Stereoselective delivery and actions of β-receptor antagonists. Biochem. Pharmacol. 37: 115–124