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Myocardial inotropic responses and adrenoceptors in protein-deficient rats

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1 Inotropic effects of isoprenaline, phenylephrine and calcium were studied in left atria of 5 weeks old rats fed a low (5%) or a normal (21%) protein diet for 3 weeks. Rats maintained on a low (5%) protein diet consume about half the amount of food eaten by the same rats maintained on a normal (21%) protein diet and thus suffer from protein-calorie malnutrition (PCM).

2 Body weight did not increase in PCM, but heart weight adjusted to body weight was slightly increased compared to normal rats.

3 Atrial resting tension and peak developed tension in response to isoprenaline, phenylephrine or calcium were not diminished by PCM.

4 The number of α and β-adrenoceptors and the receptor affinity in ventricular membranes were not reduced by PCM.

Introduction

Protein-calorie malnutrition (PCM) has been shown to cause cardiac atrophy in children (Smythe, Swanepoel & Campbell, 1962), monkeys (Chauhan, Nayak & Ramalingaswami, 1965), rats (Cohen, Abelmann, Messer & Bing, 1976; Nutter, Murray, Heymsfield & Fuller, 1979) and guinea-pigs (Varma, 1980), structural damage to the heart of children (Smythe *et al.*, 1962; Piza, Troper, Cespedes, Miller & Berenson, 1971), monkeys (Chauhan *et al.*, 1965) and rats (Svoboda, Grady & Higginson, 1966), low cardiac output in children (Viart, 1977), and electrocardiographic abnormalities in children (Smythe *et al.*, 1962; Wharton, Balmer, Somers & Templeton, 1969) and monkeys (Chauhan *et al.*, 1965). It has been suggested that during PCM the cardiovascular system is in an adaptive hypocirculatory state comparable to hypothyroidism (Viart, 1977). PCM is associated with a decrease in circulating triiodothyronine in man (Chopra & Smith, 1975).

The response of the myocardium to sympathomimetic agents is an essential component of cardiovascular adaptation. To our knowledge there is no account of the inotropic effects of sympathomimetic agents during PCM.

Methods

Animals and diet

Five-weeks old male Sprague Dawley rats weighing 100–125 g were housed individually in suspended wire-bottom cages and fed water *ad libitum* and a 21% (control) or a 5% (deficient) protein diet for 3 weeks as described (Varma, 1979). The composition of the control diet was ($g\,kg^{-1}$): vitamin-free casein 231, sucrose 519, corn starch 150, corn oil 50, mineral mixture (Williams-Briggs) 40 and vitamin mixture (Teklad) 10. The 5% protein diet contained 55 $g\,kg^{-1}$ of vitamin-free casein and 695 $g\,kg^{-1}$ of sucrose; all other constituents were the same as those in the control diet. Both diets were isocaloric in composition and were purchased in pellet form from Teklad Test Diets, Madison, Wisconsin, USA.

Inotropic drug effects

The hearts were removed under ether anaesthesia. Left atria were suspended in a solution containing (mM): NaCl 114.9, $NaHCO_3$ 24.9, KCl 4.7 $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 and disodium edetate, 0.030, which was equilibrated with

5% CO_2 in O_2. The bath temperature was 37°C. Contractions were elicited with square-wave pulses (0.3–1 ms, voltage just above threshold) at a rate of 1 Hz and recorded isometrically. Resting diastolic tension was adjusted to give maximal control contractions. Dose-response curves were obtained cumulatively; only one dose-response curve was established in an atrial preparation (Benfey, Yong, Belleau & Melchiorre, 1979).

α and β-adrenoceptor assay

Membranes of ventricular tissue were prepared by the method of Baker, Boyd & Potter (1980). Ventricles were homogenized by Polytron blender in 15 ml ice-cold 10 mM Tris-HCl buffer (pH 8) and the homogenate was diluted with 15 ml 1 M KCl and left on ice for 10 min and then centrifuged at 48,000 *g* for 10 min. The pellet was suspended in 30 ml 50 mM Tris buffer (pH 8), centrifuged at 48,000 *g* and the pellet resuspended in 10 ml 50 mM Tris buffer. Membrane preparations corresponding to 4–8 mg tissue (0.3–0.5 mg membrane protein) were incubated for 20 min at room temperature with (−)-[^{3}H]-dihydroalprenolol (1–10 nM) with or without (−)-alprenolol (10 μM) for β-adrenoceptor assay (Baker *et al.*, 1980) or incubated with [^{3}H]-prazosin (0.1–2 nM) with or without phentolamine (10 μM) for α_1-adrenoceptor assay (Karliner, Barnes, Hamilton & Dollery, 1979). At the end of the incubation, ice-cold 50 mM Tris buffer was added and the suspension filtered through Whatman GF/C glass fibre filters. Filters were rinsed twice with 4 ml Tris buffer (50 mM) and placed in vials containing 10 ml Scintiverse (Fisher) for counting.

Assays were done in triplicate. Specific binding was defined as the difference between binding of [^{3}H]-dihydroalprenolol in the presence and absence of alprenolol and binding of [^{3}H]-prazosin in the presence and absence of phentolamine. Data were analyzed for number of binding sites and K_d values by the technique of Scatchard (1949).

Uptake of isoprenaline

Ventricular tissue was cut into pieces of approximately 100 mg and placed in 25 ml flasks containing 5 ml of the solution used for the determination of inotropic drug effects and 0.15 mM [^{3}H]-isoprenaline at 30°C. Tissues were removed after 5, 15, 30 and 60 min, washed with the incubation solution, blotted on filter paper and placed in vials containing 1 ml of hyamino hydro tissue solubilizer (NCS, NEN). Radioactivity was counted after adding 10 ml Scintiverse approximately 24 h later.

Protein, DNA and ATP contents

Protein was measured with bovine serum albumin as the standard according to Lowry, Rosebrough, Farr & Randall (1951). DNA was assayed by the diphenylamine method (Burton, 1956). ATP in freshly dissected atrial and ventricular tissue was determined by the luciferase technique (Karl & Holm-Hansen, 1976).

Drugs and radiochemicals

(−)-[^{3}H]-dihydroalprenolol HCl, 31.5 Ci mmol^{-1}; (±)-[^{3}H]-isoprenaline, 6.5 Ci mmol^{-1}; [^{3}H]-prazosin, 18 Ci mmol^{-1} (New England Nuclear); (−)-isoprenaline bitartrate dihydrate (Sterling-Winthrop Res. Inst.), (−)-phenylephrine HCl (K+K Labs.); (±)-propranolol HCl (Ayerst, McKenna & Harrison); phentolamine mesylate (Ciba-Geigy); ATP disodium and firefly luciferase (Sigma) were used.

Table 1 Characteristics of control and protein-deficient rats

Parameters	n	*Control*	*Protein-deficient*
Initial body weight (g)	28	121 ± 2	115 ± 2
Body weight after 3 weeks (g)	28	317 ± 11	118 ± 3*
Ventricular weight (g)	28	0.93 ± 0.03	0.42 ± 0.01*
Ventricular weight (g kg^{-1} body weight)	28	2.89 ± 0.08	3.56 ± 0.08*
Atrial weight (mg)	12	48 ± 4.5	29 ± 1.9
Atrial weight (mg kg^{-1} body weight)	12	145 ± 11.4	252 ± 16.9*
Ventricular protein (mg g^{-1} tissue)	17	170 ± 7.8	167 ± 13.6
Atrial protein (mg g^{-1} tissue)	5	122 ± 11.4	114 ± 18
Ventricular DNA (mg g^{-1} tissue)	17	5.0 ± 0.23	5.5 ± 0.25
Ventricular ATP (nmol mg^{-1} protein)	5	17.8 ± 1.6	29 ± 2.8
Atrial ATP (nmol mg^{-1} protein)	5	15.9 ± 0.5	21.5 ± 3.6

Control and protein-deficient rats were fed *ad libitum* a 21% or a 5% protein diet, respectively, for 3 weeks. Values are means ± s.e. *$P < 0.05$, compared to control.

Table 2 Effects of inotropic agents on left atrial contractile force in control and protein-deficient rats

Parameters	*Control*			*Protein-deficient*		
Resting tension (mg $^{-1}$ tissue)	2375	±187	(43)	2621	±224	(46)
Peak tension, isoprenaline (mg g^{-1} tissue)	5375	±833	(12)	5759	±552	(14)
Peak tension, phenylephrine (mg g^{-1} tissue)	5021	±458	(15)	5655	±483	(16)
Peak tension, calcium (mg g^{-1} tissue)	6063	±604	(8)	7207	±759	(8)
EC_{50} isoprenaline (nM)	1.21	± 0.027	(12)	3.57	± 0.85*	(14)
EC_{50} phenylephrine (μM)	2.45	± 0.37	(15)	3.45	± 0.67	(16)

Values are means ± s.e. *$P < 0.05$, compared to control. The number of experiments is in parentheses.

Results

Animal characteristics

Unlike the animals fed the normal diet, the rats fed the protein-deficient diet did not gain weight. Their heart and body weights were lower than those of the normal animals, but adjusted to body weight, heart weight was greater in the protein-deficient than in the normal rats (Table 1).

Ventricular and atrial protein and ATP concentrations and ventricular DNA content were similar in the two groups of rats.

Inotropic responses

Resting tension and peak tension developed in the presence of isoprenaline, phenylephrine or calcium did not differ in atria of normal and protein-deficient rats (Table 2). There was a significant loss of potency of isoprenaline, but not of phenylephrine, in the protein-deficient atria.

Adrenoceptor binding

We did not succeed in finding the cause of the reduced potency of isoprenaline in the protein-deficient animals. The number of α- and β-adrenoceptor binding sites and the receptor affinity were similar in ventricular membranes of protein-deficient and normal rats (Table 3).

Tissue uptake of isoprenaline

There was no difference in the uptake of [^{3}H]-isoprenaline by ventricular tissue from normal and protein-deficient animals (Table 4).

Discussion

Varma (1979) and Yue & Varma (1982) have previously reported that 5-weeks old rats fed a low (5%) protein diet do not gain weight. They consume about half the amount of food eaten by similar rats maintained on a normal (21%) protein diet and thus suffer from a deficiency of both protein and calories (PCM).

Heart weight in the PCM rats was lower than in the normal rats, but heart weight adjusted for body weight was significantly higher. This has also been found by Cohen *et al.* (1976) in rats kept on a low-food diet for 24 days and by Nutter *et al.* (1979) in rats subjected to 6 weeks of PCM.

Resting left atrial tension and peak tension developed in the presence of isoprenaline, phenylephrine or calcium were similar in normal and PCM rats. Likewise Varma (1980), who kept immature guinea-pigs for 4 weeks on a low-protein diet, found that ouabain had the same inotropic effect on papillary muscle and the left atrium in normal and PCM animals.

The results agree with previous observations in rats. Thus Cohen *et al.* (1976) measured intrinsic

Table 3 (−)-[^{3}H]-dihydroalprenolol and [^{3}H]-prazosin binding to ventricular membranes from control and protein-deficient rats

		Control		*Protein-deficient*	
Ligand	n	*Number of binding sites (fmol mg^{-1} protein)*	K_d *(nM)*	*Number of binding sites (fmol mg^{-1} protein)*	K_d *(nM)*
Dihydroalprenolol	7	156 ± 12.4	2.9 ± 0.62	146 ± 14.2	3.9 ± 0.7
Prazosin	6	173 ± 19.5	0.6 ± 0.08	227 ± 24.0	0.7 ± 0.11

Values are means ± s.e.

Table 4 Uptake of [^{3}H]-isoprenaline by ventricular tissue from control and protein-deficient rats

Incubation time (min)	*Uptake (pmol g^{-1} tissue)*	
	Control	*Protein-deficient*
5	14±2.3	15±3.6
15	23±4.4	25±5.8
30	36±2.1	31±8.5
60	51±7.1	37±3.5

The tissue was incubated with 0.1 μM [^{3}H]-isoprenaline. Values are means ± s.e. of 4 experiments.

contractile properties of isolated left ventricular trabecular muscles from male Sprague-Dawley rats weighing approximately 340 g and kept on a low-food diet for 24 days. Starvation did not reduce contraction mechanics measured when the muscles were stretched to the peak of their length-tension curves. Nutter *et al.* (1979) measured myocardial function in 2.5 months old Long-Evans rats weighing about 400 g and subjected to 6 weeks of PCM. Active length-tension curves for left ventricular papillary muscles showed normal or enhanced myocardial contractility. Haemodynamic parameters, such as heart rate, mean aortic pressure, left ventricular systolic pressure, stroke volume and cardiac output, were decreased in the PCM rats, but cardiac index was not. Thus although uncomplicated chronic PCM is associated with cardiac atrophy, left ventricular function had adjusted to the decrease in body mass and metabolic requirements.

These results may be applicable to the marasmic form of human cachexia arising from anorexia nervosa, cancer or chronic digestive disorders but probably cannot be extrapolated to all forms of undernutrition. The presence of complicating factors, such as depletion of certain ions or vitamins and severe protein deprivation (kwashiorkor), may result in pathological changes in the myocardium leading to depressed contractility and cardiac dysfunction (Nutter *et al.*, 1979).

We do not know why protein deficiency reduced the inotropic potency of isoprenaline (Table 2) and did not change β-adrenoceptor concentration or affinity (Table 3). We may refer to a related observation by Guarnieri, Filburn, Zitnik, Roth & Lakatta (1980): isoprenaline increased the maximum rate of force development of isolated perfused septa significantly less in senescent rats than in adult animals; β-adrenoceptor number and affinity were not different in the two age groups, and adult and senescent septa responded equally to an increase in perfusate [Ca^{2+}] to 1.0 mM, which enhanced contractility to the same extent as that obtained with isoprenaline. It appears that the number of β-blocker binding sites cannot be used as an index of sensitivity to an agonist, as has often been assumed.

In summary, keeping 5-weeks old rats for 3 weeks on a low-protein diet prevented gains in body and heart weight but did not impair the ability of the atria to respond to the inotropic action of isoprenaline, phenylephrine or calcium and did not change the concentration or affinity of adrenoceptors in the ventricles.

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