Effect of isoproterenol and phenylephrine on the hearts of Mg-deficient rats*)

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Zusammenfassung

Nach Injektionen von Isoproterenol (3 mg/kg s.c.) oder Phenylephrin (10 mg/kg s.c.) war das relative Herzgewicht (Feucht- und Trockengewicht/Körpergewicht) in gleichem Maße erhöht. Bei Mg-Mangelratten waren diese Wirkungen doppelt so stark wie bei normalen Ratten.

Der geringere Mg^{2+} Gehalt bei Mg-Mangelratten wurde durch Isoproterenol stärker vermindert als durch Phenylephrin. Nach Injektionen von Isoproterenol nahm der Ca^{2+} Gehalt wesentlich stärker zu als nach Phenylephrin. Im Mg-Mangel war die Ca^{2+} Aufnahme in Gegenwart von Isoproterenol schneller.

Der Glykogengehalt war bei beiden Gruppen durch Isoproterenol um 15 % reduziert. Isoproterenol und Phenylephrin erzeugten bei normalen und Mg-armen Ratten gleich viel Nekrosen.

Summary

After injection of isoproterenol (3 mg/kg s.c.) or phenylephrine (10 mg/kg s.c.) the relative heart weights (wet and dry weight/body weight) were rapidly increased to about the same degree. In Mg deficient rats these effects were twice if compared with the controls.

The lower Mg^{2+} content in Mg-deficient rat hearts was more reduced by isoproterenol than by phenylephrine. Ca^{2+} content was much more increased by isoproterenol than by phenylephrine. In Mg deficiency Ca^{2+} was more rapidly taken up in presence of isoproterenol.

Glycogen content was reduced by 15 % in both groups only by isoproterenol. Isoproterenol and phenylephrine induced necroses without a significant difference between normal and Mg deficient rats.

Résumé

Après une injection d'isoprotérénol (3 mg/kg s.c.) ou de phényléphrine (10 mg/kg s.c.), les poids relatifs du cœur (poids frais et désséché/poids corporel) ont été rapidement accrus à peu près dans la même mesure. Chez les rats avec déficit magnésique ces effets ont été deux fois plus élevés que dans les contrôles.

La teneur plus faible en Mg^{++} dans les cœurs de rats avec déficit magnésique a été plus réduite par l'isoprotérénol que par la phényléphrine. La teneur en Ca a été beaucoup plus accrue par l'isoprotérénol et par la phényléphrine. Dans le déficit magnésique, le Ca^{++} a été capté plus rapidement en présence d'isoprotérénol.

La teneur en glycogène a été réduite de 15 % dans les deux groupes sous l'effet de l'isoprotérénol. L'isoprotérénol et la phényléphrine ont induit des nécroses sans différences significatives entre les rats normaux et déficitaires en Mg.

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Injection of isoproterenol in rats is a widely used model to study the mechanisms of myocardial necroses caused by Ca^{2+} overload and the protective action of so-called Ca^{2+}-antagonists (For reviews see 1, 9). In these experiments also Mg^{2+} exerted a protective effect as a Ca^{2+}-antagonist [2].

On the other hand, Mg-deficiency produced alterations in the electrolyte contents of the heart similar to those caused by injection of the β-agonist, isoproterenol, or the α-agonist phenylephrine, e.g. decrease in Mg^{2+} content and sometimes necroses [4, 5, 8]. As the Mg-deficiency-induced alterations in the heart are enhanced by stress and catecholamines [4, 14] and as adrenaline and noradrenaline had been found to exhibit both α- and β-adrenergic effects, we examined whether the effects of Mg deficiency were modified by phenylephrine or by isoproterenol.

Methods

Male Wistar rats weighing 80 g were fed a Mg-deficient diet (Sniff, Mg^{2+} content 2 mmol/kg, Ca^{2+} content 200 mmol/kg) and distilled water ad libitum for 3 weeks. Control rats received the same food enriched with Mg^{2+} (Mg^{2+} content: 25 mmol/kg) and distilled water for the same time.

Rats from both dietary groups were injected subcutaneously with

1. 0.9 % NaCl,
2. phenylephrine in 0.9 % NaCl, 10 mg/kg body weight,
3. isoproterenol in 0.9 % NaCl, 3 mg/kg body weight.

2, 6 and 12 h p.i., the hearts were removed, under nembutal anesthesia (30 mg/kg), cleaned from blood and immediately frozen in liquid N_2. After weighing, the hearts were freeze-dried, weighed and pulverized.

For determination of the Mg^{2+} and Ca^{2+} content, 20 mg of the dried hearts were ashed in a

low-temperature asher (Tracerlab), dissolved in 0.1 N HCl containing 1 % La^{3+} (as $LaCl_3$), and measured by atomabsorption spectrometry (Perkin-Elmer, model 300). For determination of glycogen, 20 mg of dried heart tissue were incubated together with amyloglucosidase [6]. The resulting glucose was estimated enzymatically (Enzymatischer Farbtest, Nr. 166 391, Boehringer, Mannheim). The rate of necrosis was estimated histologically according to [7].

Results

During the Mg-deficient period, the body weight of the control rats increased to 192 ± 8 g (mean ± SEM) and in the Mg-deficient group to 145 ± 8 g. The Mg^{2+} concentration in serum amounted to 0.84 ± 0.03 mmol/l and 0.26 ± 0.03 mmol/l for the controls and Mg-deficient rats.

Heart weight (Tab. 1)

The relative heart weight (wet and dry weight/body weight) was not altered by Mg-deficiency. After injection of isoproterenol and phenylephrine the relative heart weights were increased to about the same degree. In the Mg-deficient rats this increase was higher than in normal rats. The increase in wet weight was higher than the increase in dry weight, indicating an enhanced water content, particularly in the Mg-deficient groups.

N^+ and K^+ content (Tab. 2)

In the hearts of the Mg-deficient rats, the Na^+ content was increased by 18 % whereas the K^+ content was not significantly reduced. Normal and Mg-deficent rats were affected to the same degree by the catecholamines. However, isoproterenol had a greater effect on the Na^+ and K^+ content than phenylephrine. The effects on the K^+ content were rather small.

Mg^{2+} and Ca^{2+} content (Tab. 2)

In the Mg-deficient rats, the Mg^{2+} content of the heart was reduced by 8 %. In normal rats, isoproterenol and phenylephrine reduced the Mg^{2+} content by 13 %. The lower Mg^{2+} content in the Mg-deficient hearts was reduced to a small extent by phenylephrine and to a higher extent by isoproterenol.

In the hearts of the Mg-deficient rats, the Ca^{2+} content was increased by 25 %. The Ca^{2+} content was much more increased by isoproterenol than by phenylephrine. Whereas the Na^+ and Mg^{2+} content rapidly changed within the first two hours and remained constant thereafter, the Ca^{2+} content was permanently increased. The increase in the Ca^{2+} content due to isoproterenol was more rapid in the Mg-deficient rats.

The same dynamics of the Mg^{2+} and Ca^{2+} content was found in the homogenate of hearts from normal rats after injection of isoproterenol [10].

Glycogen content (Tab. 2)

There were no significant changes in the glycogen content of the hearts caused by Mg-deficiency. Phenylephrine had no significant effect, whereas isoproterenol reduced glycogen by about 15 % in both the normal and Mg-deficient rats.

Tab. 1: Relative heart weights (wet and dry weight × 10^3/body weight) of normal and Mg-deficient rats at various times after injection of phenylephrine (phe) (10 mg/kg s.c.) and isoproterenol (iso) (3 mg/kg s.c.). Mean ± S.E.M. of 3—4 rats in each group.

hours after injection	normal			Mg deficient rats		
	—	phe	iso	—	phe	iso
			wet weight × 10^3/body weight			
2	3.40 ± 0.14	4.30 ± 0.09	4.37 ± 0.06	3.80 ± 0.18	5.20 ± 0.50	5.42 ± 0.39
6	3.64 ± 0.12	4.23 ± 0.05	4.75 ± 0.14	3.77 ± 0.10	5.68 ± 0.13	5.82 ± 0.51
12	3.74 ± 0.03	4.33 ± 0.25	4.37 ± 0.17	3.61 ± 0.36	5.04 ± 0.20	4.87 ± 0.43
			dry weight × 10^3/body weight			
2	0.83 ± 0.05	1.01 ± 0.04	0.97 ± 0.12	0.86 ± 0.02	1.13 ± 0.08	1.09 ± 0.04
6	0.86 ± 0.05	0.99 ± 0.02	1.00 ± 0.02	0.89 ± 0.03	1.26 ± 0.05	1.23 ± 0.05
12	0.88 ± 0.03	1.05 ± 0.14	0.98 ± 0.04	0.86 ± 0.04	1.10 ± 0.04	1.09 ± 0.10

Tab. 2: Na^+, K^+, Mg^{2+}, Ca^{2+} and glycogen content in the hearts of normal and Mg-deficient rats at various times after injection of phenylephrine (10 mg/kg s.c.) and isoproterenol (3 mg/kg s.c.). Mean ± S.E.M. of 3—4 rats in each group.

hours after injection	normal rats —	normal rats phe	normal rats iso	Mg-deficient rats —	Mg-deficient rats phe	Mg-deficient rats iso
			Na^+ (mmol/kg dry weight)			
2	160 ± 6	179 ± 5	222 ± 11	187 ± 8	203 ± 10	254 ± 14
6	161 ± 3	176 ± 7	231 ± 4	191 ± 10	201 ± 11	251 ± 3
12	162 ± 3	183 ± 5	233 ± 6	192 ± 10	213 ± 7	250 ± 4
			K^+ (mmol/kg dry weight)			
2	274 ± 6	268 ± 4	253 ± 4	269 ± 2	261 ± 2	249 ± 4
6	268 ± 5	265 ± 4	256 ± 4	268 ± 3	262 ± 6	254 ± 3
12	268 ± 8	260 ± 4	254 ± 5	267 ± 2	257 ± 3	254 ± 3
			Mg^{2+} (mmol/kg dry weight)			
2	36.0 ± 0.8	31.4 ± 0.4	31.1 ± 0.8	33.0 ± 0.4	31.7 ± 1.0	29.3 ± 0.6
6	35.9 ± 1.0	31.4 ± 0.7	31.4 ± 0.4	33.2 ± 0.4	31.6 ± 0.6	30.0 ± 0.7
12	35.2 ± 0.9	31.1 ± 0.8	31.0 ± 0.8	33.3 ± 0.1	31.3 ± 0.8	30.1 ± 0.9
			Ca^{2+} (mmol/kg dry weight)			
2	2.79 ± 0.09	4.03 ± 0.19	6.11 ± 0.44	3.74 ± 0.30	4.78 ± 0.34	8.06 ± 1.20
6	2.86 ± 0.13	5.22 ± 0.84	8.95 ± 0.51	3.47 ± 0.10	5.16 ± 0.97	9.48 ± 0.62
12	2.87 ± 0.07	6.37 ± 0.52	9.30 ± 1.90	3.47 ± 0.05	5.49 ± 0.72	9.76 ± 1.37
			Glycogen [g glucose/100 g dry weight]			
2	7.54 ± 0.11	8.24 ± 0.35	6.21 ± 0.25	8.23 ± 0.08	7.27 ± 0.55	6.31 ± 0.28
6	7.20 ± 0.13	7.88 ± 0.30	6.76 ± 0.26	8.11 ± 0.65	6.95 ± 0.54	6.78 ± 0.14
12	7.86 ± 0.04	7.73 ± 0.21	6.59 ± 0.32	7.55 ± 0.41	6.79 ± 0.12	6.26 ± 0.03

Mg^{2+} and Ca^{2+} concentration in serum (Fig. 1)

After injection of phenylephrine, Mg^{2+} concentration in serum is strongly increased and almost normalized after 12 hours. After injection of isoproterenol, the increase in Mg^{2+} concentration is delayed and smaller. The effects on Mg^{2+} concentration were similar in normal and Mg-deficient rats. Whereas reduction of Mg^{2+} content in the hearts of normal rats was the same by isoproterenol and phenylephrine, the increase in serum Mg^{2+} concentration behaved differently, indicating a different excretion of Mg^{2+} or a different contribution of other organs. The Ca^{2+} concentration in serum was not significantly affected by isoproterenol and phenylephrine.

Rate of necroses (Tab. 3)

The rate of necroses is somewhat increased by phenylephrine and by isoproterenol. However, the amount of necroses induced by the catecholamines was not enhanced by Mg-deficiency.

Discussion

From the behaviour of the relative heart weights, it can be concluded that α- and β-agonists induce heart proliferation, as has been intensively studied in normal rats after injection of isoproterenol [16]. K^+ content was nearly unaffected. Mg^{2+} content in normal rats was reduced to the same amount by α- and β-agonists whereas the Na^+ and Ca^{2+} content were more increased by β-agonists, indicating a different action of α- and β-agonists on the electrolyte contents of the heart.

The most drastic effect was the increase in Ca^{2+} content caused by isoproterenol. This effect may in part have been due to an increase in extracellular fluid (blood content, swelling) of the heart. Regardless to the fact that Na^+ is taken up intracellularly by the action of catecholamines, the Na^+ content may be taken as a rough measure for extracellular fluid volume. The 30 to 40 % increase in the Na^+ content which has been constant between 2 and 12 hours, indicated that the strong increase in Ca^{2+} (200—300 %) is caused by intracellular Ca^{2+} uptake. The increase in extracellular fluid volumes agrees with an increase in extracellular fluid from 180 to 279 ml/kg found in frog hearts after injection of 200 mg/kg isoproterenol [13].

The presence of heart muscle necroses in the process of cellular Ca^{2+} uptake can be excluded because both in normal and Mg-deficient rats treated with isoproterenol and phenylephrine, we found no correlation between the increase in

Tab. 3: Rate of necroses in the hearts of normal and Mg deficient rats 24 hours after injection of phenylephrine (10 mg/kg s.c.) and isoproterenol (3 mg/kg s.c.). Values in %. Mean ± S.E.M. of 4 rats in each group.

normal rats			Mg deficient rats		
—	phe	iso	—	phe	iso
0.08 ± 0.05	0.66 ± 0.12	2.17 ± 0.33	0.07 ± 0.04	1.33 ± 0.22	1.07 ± 0.14

myocardial Ca^{2+} content and the rate of necroses.

The subcellular localization of the alterations in Mg^{2+} and Ca^{2+} content is not defined. After injection of isoproterenol to normal rats, there were no significant changes in the Mg^{2+} and Ca^{2+} content of the mitochondria when isolated in presence of ruthenium red to prevent mitochondrial Ca^{2+} uptake during preparation [12]. As cytosolic (Ca^{2+}) is very low (10^{-7} mol/l) and higher (Ca^{2+}) ($> 10^{-5}$ mol/l) are strongly inhibitory, such a strong Ca^{2+} uptake must be intracellularly bound. In Mg-deficient hearts with an increased Ca^{2+} content we observed electron-dense particles, probably Ca phosphate in the l-tubule system [5]. Therefore, one may conclude that in the catecholamine-injected rats, Ca^{2+} permeates into the heart muscle cells and is stored in the sarcoplasmic reticulum. With regard to the Na^+, Mg^{2+} and Ca^{2+} content, Mg-deficiency induces similar alterations in the heart as do α- and β-agonists. To analyse the quantitative interrelation between Mg-deficiency and the catecholamines, the differences in the Mg^{2+}, Ca^{2+} and Na^+ content of normal and Mg-deficient rats that had been treated with isoproterenol and phenylephrine were plotted (Fig. 2, 3, 4). It is seen that in Mg-deficient rats, the β-agonist, isoproterenol has a stronger effect on Mg^{2+} and Ca^{2+} contents than the α-agonist, phenylephrine. Isoproterenol had the same effect on the Na^+ and Ca^{2+} content in normal and Mg-deficient rats. Therefore, the

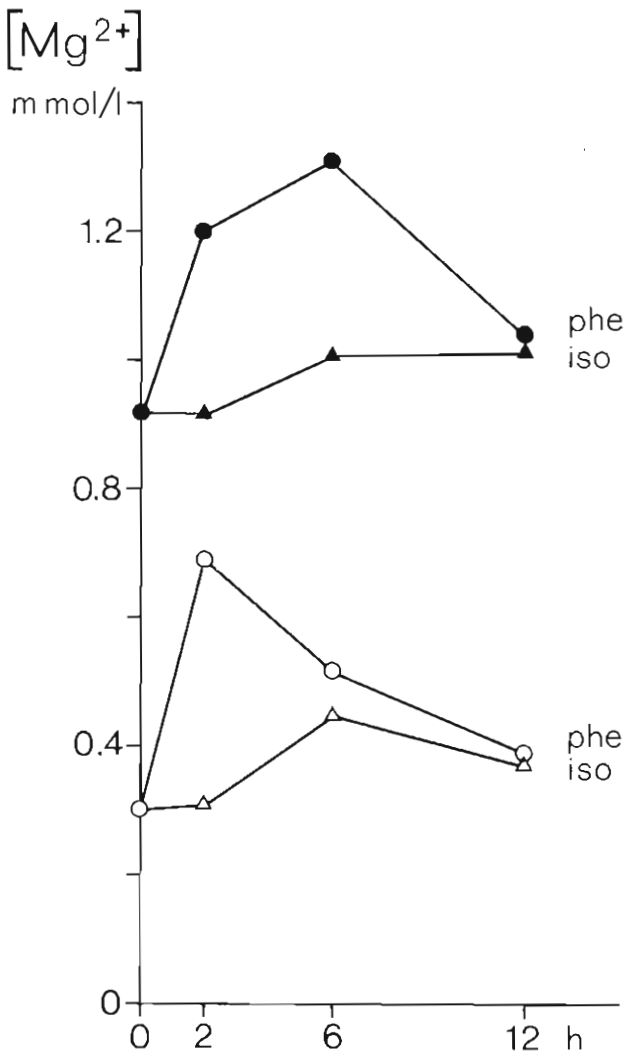

Fig. 1: Mg^{2+} concentration in the serum of normal (●, ▲) and Mg-deficient rats (○, △) after injection of isoproterenol (▲, △, iso) and phenylephrine (●, ○, phe).

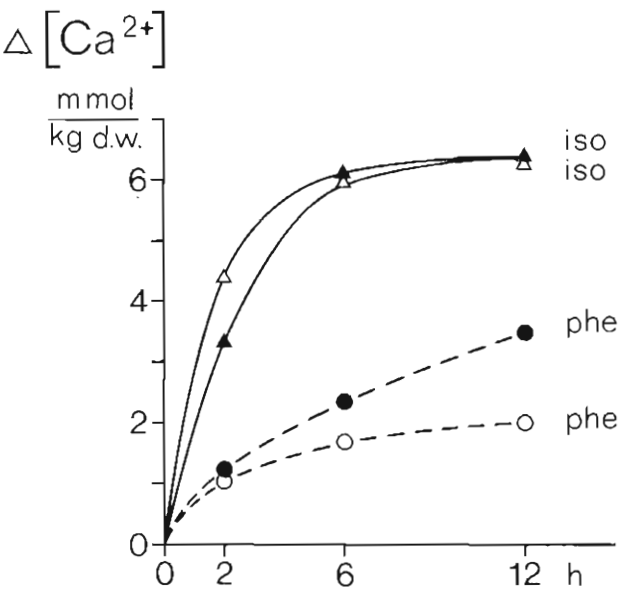

Fig. 2: Effect of isoproterenol (▲, △) (iso) and phenylephrine (●, ○) (phe) on Ca^{2+} content of rat hearts. The differences (Δ Mg^{2+}, Δ Ca^{2+}) between injected and non-injected rats (normal feed) (▲, ●) and Mg-deficient diet (△, ○) were plotted as a function of time after injection. Values were taken from Tab. 2.

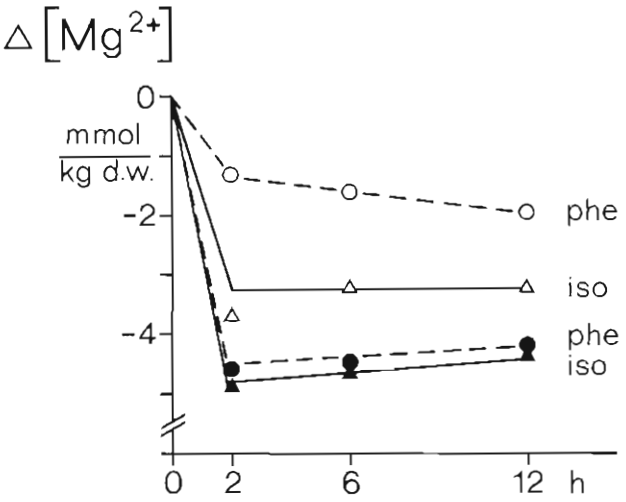

Fig. 3: Effect of isoproterenol (iso) and phenylephrine (phe) on Mg^{2+} content of rat hearts. According to Fig. 2.

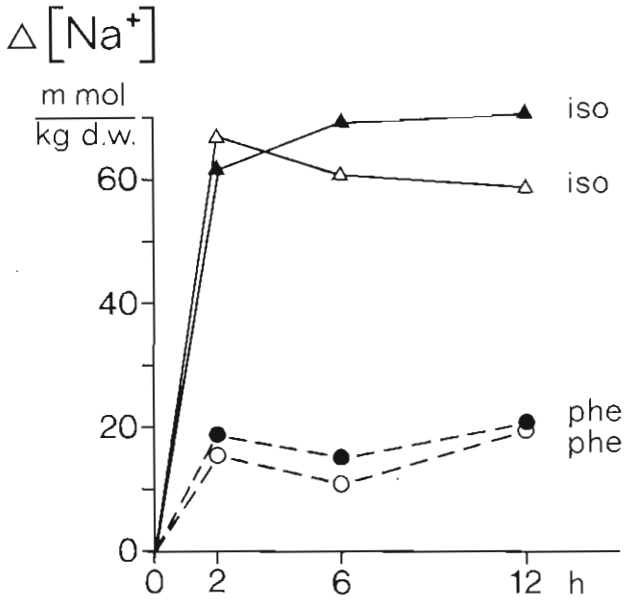

Fig. 4: Effect of isoproterenol (iso) and phenylephrine (phe) on Na^+ content of rat hearts. See Fig. 2.

effect of Mg-deficiency and isoproterenol on Ca^{2+} and Na^+ is an additive one.

Moreover, steroid hormones injected or released by stress may sensitize the myocardium to β-agonists [3]. In Mg-deficiency and stress, desensitization of β-receptors seems to be defective [15]. Both effects may cause an increased sensitivity to β-agonists. On the other hand, the simultaneously occurring α-adrenergic effect of endogenous adrenaline and noradrenaline may provide some protection against isoproterenol [3]. This effect may be enhanced because isoproterenol can increase the release of catecholamines from the adrenal glands [11].

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