Time-course of the change in blood pressure level in magnesium-deficient Wistar rats

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The aim of the present study was to determine whether a severely Mg-deficient diet can modify blood pressure in rats and whether these alterations in blood pressure are associated with a change in *in vivo* cardiovascular reactivity, alteration in plasma lipids and modification of the production of hormones involved in blood pressure regulation. Weanling male Wistar rats were pair-fed for 40 weeks with control (960 mg Mg/kg) and Mg-deficient (80 mg Mg/kg) diets. At 2 weeks, blood pressure was lower in Mg-deficient rats, while heart rate was greater than in controls. Mg-deficiency-induced hypotension was transitory and the administration of antihistamine agents inhibited the appearance of this hypotensive phase, suggesting that histamine may play a role in lowering blood pressure. Until 15 weeks, blood pressures were similar for control and Mg-deficient rats. Thereafter, blood pressure rose gradually until the end of the experiment in Mg-deficient rats. Heart rate remained higher in hypertensive Mg-deficient rats. After 21 weeks, *in vivo* cardiovascular reactivity to noradrenaline was lower and reactivity to angiotensin II was unchanged in hypertensive Mg-deficient rats. At 2 and 21 weeks, hypomagnesaemia was accompanied by higher plasma levels of Ca, triacylglycerols and cholesterol. Plasma renin activity was higher at week 2, whereas levels of plasma angiotensin converting enzyme were lower at 2 and 21 weeks in Mg-deficient rats. The plasma aldosterone level was higher at 2 and 21 weeks while the vasopressin level did not change. Plasma corticosterone levels were lower at 2 weeks and higher at 21 weeks. It is concluded that Mg deficiency induced a transitory hypotension followed by a sustained hypertension in rats. The release of vasodilator inflammatory agents may contribute to the early hypotension. The hypertensive phase may be explained by the increased sympathetic nervous activity induced by Mg deficiency even though the contribution of several hormonal systems implicated in blood pressure regulation remains to be elucidated.

Magnesium: Blood pressure: Cardiovascular function

Mg, the fourth most abundant extracellular cation in vertebrates, is involved in numerous biological processes and is essential for cardiovascular function. Epidemiological and experimental studies indicate that Mg deficiency may be considered as a risk factor for cardiovascular diseases (Altura & Altura, 1995; Rayssiguier *et al.* 1996; Weglicki *et al.* 1996).

The effects of dietary Mg deficiency on blood pressure are still the subject of much debate and remain contradictory (Durlach *et al.* 1992). Generally, short-term Mg deficiency in rats induces hypotension, or does not change blood pressure (Cantin, 1970; Itokawa *et al.* 1974; Luthringer *et al.* 1988; Rayssiguier *et al.* 1992). Some other studies have shown that long-term Mg deficiency elevates blood pressure in normotensive rats (Altura *et al.* 1984, 1992). In spontaneously hypertensive rats, Mg deficiency accelerates the development of hypertension (Berthelot & Esposito, 1983). However, in spontaneously hypertensive rats with established hypertension, a Mg-deficient diet exerts no significant effect on blood pressure (Günther *et al.* 1984; Overlack *et al.* 1987; Evans *et al.* 1989; Laurant *et al.* 1997). Thus, the relationship between Mg deficiency and hypertension is complex and remains inconclusive because of differences in experimental design, diet composition, feeding protocols, levels of dietary Mg, duration of Mg deficiency, strain and age of the rats and/or blood pressure methodology.

The mechanisms by which Mg deficiency may increase or decrease blood pressure level have not been clearly investigated. Although the cellular concentration of Mg falls only slightly in most tissues, secondary effects on other cell constituents are well recognized. These include loss of K, and accumulation of Na and Ca (Tongyai *et al.* 1989). Numerous experimental and clinical data indicate that Mg deficiency can induce pathophysiological disorders in the cardiovascular system, such as vasospasm, increased vasoconstrictor activity, elevation in smooth muscle and cardiac intracellular Ca concentrations (Altura *et al.* 1984, 1992, 1995), formation of oxygen radicals, proinflammatory agents and growth factors (Bussière *et al.* 1994; Weglicki *et al.* 1996; Maier *et al.* 1997; Rayssiguier *et al.* 1997) and changes in membrane permeability and transport (Astier *et al.* 1996). All these phenomena may certainly contribute to the modification of blood pressure level during Mg deficiency.

The present study was undertaken to determine whether short- and long-term severe dietary Mg deficiency can modify arterial blood pressure in Wistar rats, and whether these alterations in blood pressure are associated with changes in *in vivo* cardiovascular reactivity, alterations in lipid metabolism and modifications in production of hormones involved in blood pressure regulation.

Materials and methods

Animals and diets

Weanling male Wistar rats (3 weeks old) weighing 60 g (IFFA CREDO, L'Arbresle, France) were used. Animal care was in accordance with the applicable guidelines from the French Ministry of Agriculture. On arrival, the rats were housed in stainless-steel cages at a constant temperature of 23°, with controlled humidity (50–60 %) and a daily 12 h light–dark cycle. The rats were randomly divided into Mg-deficient and control groups, and pair-fed with the appropriate diets for 40 weeks. The synthetic diets (ICN, Biomedicals, Orsay, France) contained (g/kg): casein 200, sucrose 705, maize oil 50, modified AIN-76 mineral mixture 35, AIN-76A vitamin mixture 10, DL-methionine 3, choline 2. Mg was given in the form of MgO. The Mg contents of the diets determined by analysis were 80 mg/kg (Mg-deficient diet) and 960 mg/kg (control diet). The rats were given free access to distilled water.

After 2 weeks of dietary treatment, seven Mg-deficient and seven control rats were treated with the histamine antagonists dexchlorpheniramine (H1 antagonist, 0·3 mg/kg body weight, subcutaneously) and cimetidine (H2 antagonist, 10 mg/kg body weight, intraperitoneally). Another group of seven Mg-deficient rats and ten control rats received the saline vehicle in the same way. Blood pressure was measured 2 h after histamine antagonist or vehicle administration.

In one set of experiments, six Mg-deficient rats, which had been fed on the Mg-deficient diet for 21 weeks, were given *ad libitum* a supplement of Mg in the form of control diet. Blood pressure and heart rate were measured during 3 weeks of Mg supplementation.

Blood pressure determination

Systolic blood pressure was measured in unanaesthetized restrained prewarmed rats by the indirect tail-cuff method using a sphygmomanometer (PE-3000; Narco Biosystem, Houston, TX, USA). The cuff was inflated rapidly at an inflation–deflation rate of 15 mmHg/s. The lowest and the highest values were discarded before calculating the mean systolic blood pressure of at least six clear readings for each rat. Heart rate was determined at the same time.

Biochemical analysis

At 2 and 21 weeks after the onset of dietary treatment, blood samples were drawn from the aorta of anesthetized rats and collected into heparinized tubes. Blood was immediately centrifuged at 2000 g for 15 min at 4°. After appropriate dilution of the plasma, Ca and Mg were analysed by atomic absorption spectrophotometry (Model 420; Perkin Elmer, St-Quentin Yvelines, France). Triacylglycerols and total cholesterol were determined by enzymic methods. Plasma renin activity was determined by radioimmunoassay of angiotensin I, as previously described (Safwate *et al.* 1991), with adjustments to the rat plasma: 250 μl thawed plasma was incubated for 30 min at 37° with 750 μl phosphate buffer containing 10 μl phenyl methyl sulfonyl fluoride. The pH of the buffer was adjusted to 6·00 after assays to determine the optimum pH for renin activity in rat plasma. Values for intra-run and inter-run precision were respectively 8 % and 10 %, and the sensitivity of the method was 0·02 ng. The serum angiotensin I converting enzyme (ACE) was assayed using [^{14}C]hippurryl-histidyl-leucine as substrate (Ryan *et al.* 1977). Plasma aldosterone and corticosterone were determined after dichloromethane extraction by radioimmunoassay using highly specific antibodies (gifts from Dr Bayard, CHU Rangueil, Toulouse and from Roussel-Uclaf, Paris, France respectively). Values for intra- and inter-assay CV with rat plasma were respectively 7 % and 9 % and the sensitivity was 10 pg/tube for both hormones. Arginine vasopressin was determined by radioimmunoassay using specific antibodies (Peninsula, Belmont, CA, USA) after acetone extraction. Values for intra- and inter-run precision were respectively 10 % and 12 % and the sensitivity was 2 pg/tube.

In vivo *cardiovascular reactivity*

After 21 weeks of dietary treatment, six control and six Mg-deficient rats were anaesthetized with sodium pentobarbital (40 mg/kg intraperitoneally). Polyethylene cannulas (Biotrol, Paris, France) were placed in the trachea (PE-205) to assist ventilation, in the carotid artery (PE-50) to measure blood pressure directly, and in the jugular vein (PE-50) to administer drugs. To minimize spontaneous blood pressure regulation, pentolinium (25 mg/kg) and atropine sulfate (0·25 mg/kg) were administered subcutaneously, and vagotomy was performed on both sides according to the method described by Dupont & Sassard (1974). The carotid arterial cannula (filled with a solution of sodium heparin, 1×10^5 IU/l in 9 g NaCl/l saline) was connected to a pressure transducer (model P 1000-B; Narco Biosystem) and coupled to a

physiograph (MK-III; Narco Biosystem) to record blood pressure and heart rate. Cardiovascular reactivity to noradrenaline and angiotensin II was expressed as the change in mean arterial pressure and heart rate evoked by 100 μl intravenous bolus injections of 125, 250, 500 and 1000 ng noradrenaline/kg, and 25, 50, 75, 100 ng/kg angiotensin II dissolved in saline, and calculated as the area under the curve (mm^2) of the mean arterial pressure peak (mean arterial pressure × duration of the effect for the mean arterial pressure peak). The experimental protocol was implemented 30–45 min after blood pressure had stabilized.

Drugs

The following drugs were used: L-noradrenaline hydrochloride (Flucka A.G., St-Quentin Fallavier, France), atropine sulfate, pentolinium bitartrate, indomethacin (Sigma, St-Quentin Fallavier, France), sodium pentobarbital (Sanofi, Libourne, France), cimetidine (Tagamet; Smith Kline and French, Paris, France), dexchlorpheniramine (Polaramine; Unicet, Levallois-Perret, France).

Statistical analysis

Values are presented as means with their standard errors. Differences between groups were assessed using one-way ANOVA and unpaired Student's t test. A P value less than 0·05 was considered statistically significant. In all experiments, n is the number of rats.

Results

Effect of magnesium-deficient diet on blood pressure, heart rate and body weight

The classic signs of Mg deficiency (including hyperaemia, growth retardation, hair loss, skin lesions, oedema of paws, brown pigmented mucus in eyes, nose and mouth, nervousness) were observed in Mg-deficient animals. At the beginning of the experiment (2 weeks), the blood pressure was significantly lower in Mg-deficient rats than in control rats ($P < 0·001$) (Fig. 1). Between 5 and 10 weeks, blood pressures were similar in the two groups. After 15 weeks, and until the end of the experimental period, the blood pressure of Mg-deficient rats gradually rose and was significantly higher than in the control rats ($P < 0·001$). Heart rate was significantly higher in Mg-deficient rats than in control rats throughout the experiment ($P < 0·001$). Body weights were similar in Mg-deficient and control rats during the first 8 weeks. After 8 weeks, Mg-deficient rats showed significant growth retardation ($P < 0·05$).

Effect of antihistamine agents on blood pressure and heart rate of hypotensive magnesium-deficient rats

After 2 weeks of dietary treatment, as the Mg-deficient rats were hypotensive, the administration of the combined antihistamine agents significantly increased their blood pressure to the control normotensive level, whereas in the control
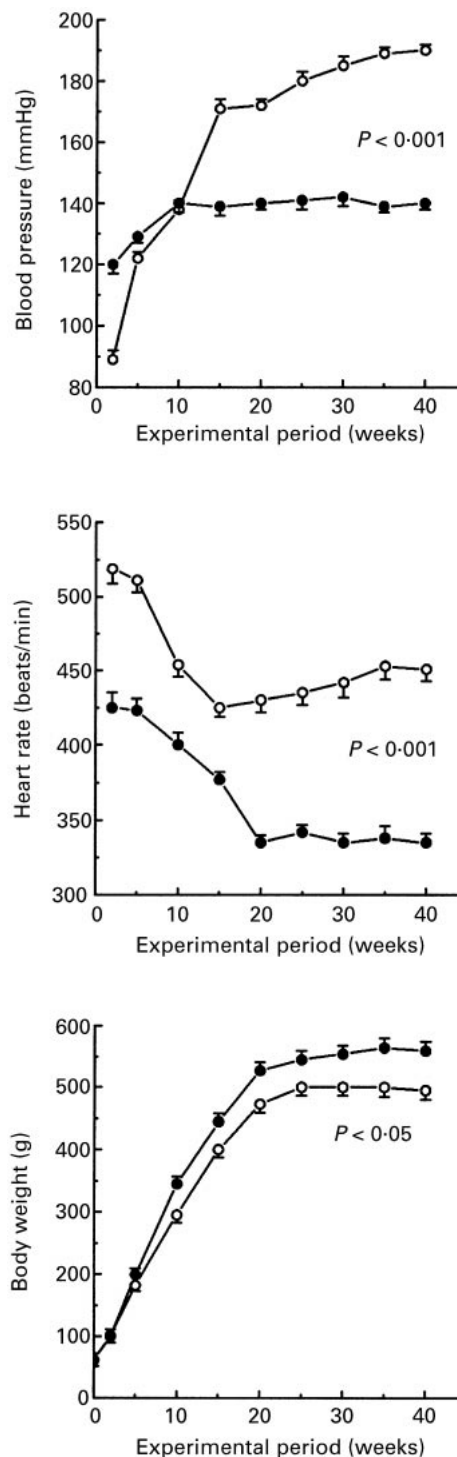

Fig. 1. Blood pressure, heart rate and body weight in rats fed on control (●) and magnesium-deficient (○) diets for 40 weeks. Values are means for seven to ten animals, with their standard errors represented by vertical bars.

rats, the antihistamine agents did not modify blood pressure (Fig. 2). Independently of the administration of combined antihistamine agents, heart rate remained significantly higher in the Mg-deficient rats than in the control rats (Fig. 2).
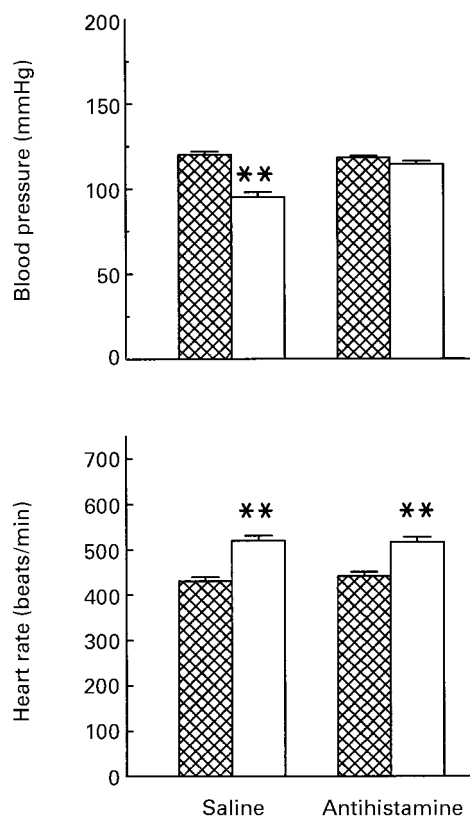
Fig. 2. Blood pressure and heart rate measured in rats fed on control (▨) and magnesium-deficient (□) diets for 2 weeks, 2 h after sub-cutaneous administration of antihistamine agents or saline solution. Values are means for seven to ten rats, with their standard errors represented by vertical bars. Mean values were significantly different from those for the corresponding controls: *$P < 0.05$, **$P < 0.01$.

Effect of magnesium-deficient diet on biochemical variables

In the rats fed on the Mg-deficient diet for 2 and 21 weeks, the plasma Mg concentration was significantly lower, whereas plasma Ca, total triacylglycerol and cholesterol concentrations were significantly higher than in control rats (Tables 1 and 2). In Mg-deficient rats, the plasma renin activity was significantly higher at 2 weeks whereas the plasma ACE levels were significantly lower at 2 and 21 weeks of dietary treatment as compared with controls. The plasma vasopressin level was not modified whereas the plasma aldosterone concentration was significantly higher at weeks 2 and 21 in Mg-deficient rats than in control rats. The plasma corticosterone level was slightly decreased after 2 weeks of Mg deficiency and was significantly increased after 21 weeks (Tables 1 and 2).

Effect of magnesium-deficient diet on *in vivo* cardiovascular reactivity

At 21 weeks, after anaesthesia and complete abolition of the ability to regulate cardiac and vascular activities by the nervous system, the blood pressure fell significantly in both groups of rats (61 (SE 3) *v.* 144 (SE 2) mmHg in control rats, 58 (SE 2) *v.* 186 (SE 2) mmHg in Mg-deficient rats; $P < 0.001$) and values were similar for the control and the Mg-deficient rats. The Mg-deficient rats displayed a blunted

Table 1. Biochemical variables in the plasma of rats fed for 2 weeks on a control diet or a magnesium-deficient diet†

(Mean values with their standard errors for ten rats per group)

	Control		Magnesium-deficient	
	Mean	SEM	Mean	SEM
Magnesium (mmol/l)	0·80	0·02	0·18***	0·04
Calcium (mmol/l)	2·40	0·02	2·65**	0·03
Triacylglycerols (mmol/l)	0·95	0·08	3·92***	0·33
Cholesterol (mmol/l)	1·29	0·10	1·75***	0·09
PRA (ng/ml per h)	7·9	0·9	16·5*	3·5
ACE (mU/ml per min)	68·9	4·4	33·1***	3·6
Aldosterone (pg/ml)	218·2	12·9	351·4**	40·2
Corticosterone (ng/ml)	250	12	202*	15
AVP (pg/ml)	8·86	0·89	10·20	0·64

PRA, plasma renin activity; ACE, angiotensin I converting enzyme; AVP, arginine-vasopressin.
Mean values were significantly different from those for the control group: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
† For details of diets and procedures, see pp. 244–245.

vasopressor response to noradrenaline. The increase in systolic blood pressure following the injection of 125 ng/kg was significantly smaller in the Mg-deficient rats than in the control rats ($P < 0.05$). The duration of the vasopressive response induced by the injection of 125, 250 and 500 ng noradrenaline/kg was significantly smaller in the Mg-deficient rats than in the control rats. The Mg-deficient diet did not affect either the increase in blood pressure or the duration of the vasopressive response induced by the injection of 25, 50, 75 and 100 ng angiotensin II/kg (Fig. 3). The low-Mg diet did not significantly affect the response in heart rate following the injection of noradrenaline or angiotensin II (results not shown).

Effect of magnesium supplementation on blood pressure and heart rate of hypertensive magnesium-deficient rats

At 21 weeks of dietary treatment, as the Mg-deficient rats were hypertensive, Mg supplementation for 3 weeks reduced

Table 2. Biochemical variables in the plasma of rats fed for 21 weeks on a control diet or a magnesium-deficient diet†

(Mean values with their standard errors for ten rats per group)

	Control		Magnesium-deficient	
	Mean	SEM	Mean	SEM
Magnesium (mmol/l)	0·75	0·03	0·25***	0·02
Calcium (mmol/l)	2·20	0·02	2·42**	0·05
Triacylglycerols (mmol/l)	1·00	0·02	2·67***	0·41
Cholesterol (mmol/l)	1·75	0·08	3·07***	0·16
PRA (ng/ml per h)	8·6	1·3	6·6	1·1
ACE (mU/ml per min)	58·2	2·3	50·0*	1·6
Aldosterone (pg/ml)	39·0	4·3	87·4***	6·9
Corticosterone (ng/ml)	56·5	11·2	100·3*	13·9
AVP (pg/ml)	8·70	0·57	10·21	1·40

PRA, plasma renin activity; ACE, angiotensin I converting enzyme; AVP, arginine-vasopressin.
Mean values were significantly different from those for the control group: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
† For details of diets and procedures, see pp. 244–245.

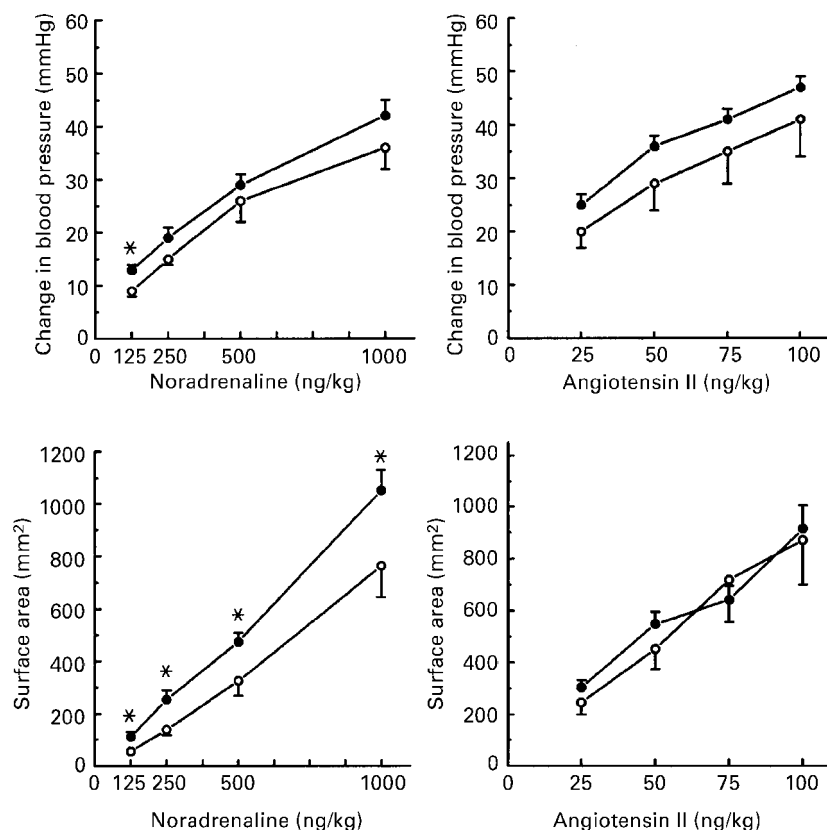**Fig. 3.** Cardiovascular reactivity to noradrenaline and angiotensin II in terms of systolic blood pressure in rats fed on control (●) and magnesium-deficient (○) diets for 21 weeks. Values are presented as mean change in systolic blood pressure (upper panels) and as area under the curve (lower panels). Values are means of six rats, with their standard errors represented by vertical bars. Mean values were significantly different for the two groups: *$P < 0.05$.

their blood pressure ($P < 0.05$). However, the blood pressure of these rats remained higher than in the control rats ($P < 0.05$). The heart rate of the Mg-deficient rats fed on the Mg-supplemented diet was significantly decreased compared with that of the Mg-deficient rats, but remained higher than the heart rate of the control rats ($P < 0.05$) (Fig. 4). The growth of the Mg-deficient rats was significantly increased by Mg supplementation ($P < 0.001$). After 3 weeks, the body weight of the Mg-deficient rats supplemented with Mg did not differ from the body weight of the control rats. Plasma Mg concentration returned to the normal level of the control rats after Mg supplementation of the Mg-deficient rats (0·72 (SE 0·04) mmol/l, $P < 0.001$).

Discussion

Magnesium deficiency and blood pressure

Although the relationship between Mg deficiency and the level of blood pressure remains to be elucidated, the present study provides evidence that the change in blood pressure induced by Mg deficiency is related to the time course of the dietary experiment. In rats maintained on a severely Mg-deficient diet for a period of less than 10 weeks, most studies have reported a decrease or no change in blood

pressure (Cantin, 1970; Itokawa *et al.* 1974; Luthringer *et al.* 1988; Rayssiguier *et al.* 1992), whereas in rats maintained on a Mg-deficient diet for more than 12 weeks, increases in blood pressure have been reported (Altura *et al.* 1984, 1992). Accordingly, it was shown in the present study that a chronic severely Mg-deficient diet induced in rats a decrease in blood pressure at the onset of the experimental dietary period and prolonged increase in blood pressure after the 15th week. Thus, dietary Mg deficiency results in an initial transitory hypotensive phase followed by sustained hypertension.

Magnesium deficiency and cardiovascular function

The precise mechanism by which Mg deficiency induces these different effects on blood pressure in rats has yet to be elucidated. It seems well established that changes in extracellular Mg concentration affect vascular reactivity and blood flow. Lowering Mg concentration enhances peripheral resistance and potentiates agonist-induced vasoconstriction (Altura & Altura, 1995). Previous experimental studies have reported that Mg-deficient-induced hypertension in rats is associated with reduced lumen diameter of mesenteric microvessels, increased vascular total Ca content, and increased vasoconstrictor activity to endogenous agonists
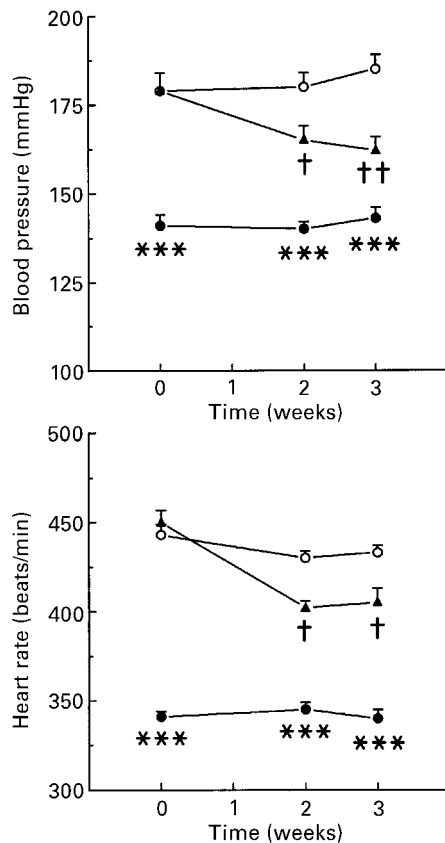
Fig. 4. Blood pressure and heart rate measured after 21 weeks of dietary treatment in rats fed on control (●) and magnesium-deficient (○) diets, and in magnesium-deficient rats fed on a magnesium-supplemented diet (▲). Values are means for six rats, with their standard errors represented by vertical bars. Mean values were significantly different from those for the magnesium-deficient and magnesium-supplemented deficient rats: ***$P < 0.001$. Mean values were significantly different from those for the magnesium-deficient rats: †$P < 0.05$, ††$P < 0.01$.

by antihistamine agents was not accompanied by a reduction in heart rate. These findings indicate that heart rate elevation is not a compensatory control in response to Mg deficiency-induced hypotension but may depend on the direct action of Mg deficiency on sympathetic nervous activity, as has been previously suggested (Durlach *et al.* 1987; Ising & Günther, 1997). Since the inhibition of histamine activity abolished hypotension but did not induce hypertension despite an increased heart rate and an increased vascular reactivity to several agonists, as has been previously reported (Luthringer *et al.* 1988), it could be postulated that some other vasodilating agents released during the inflammatory period of Mg deficiency may explain the absence of blood pressure elevation. Mg deficiency is also accompanied by an increase in plasma NO levels (Rock *et al.* 1995; Mak *et al.* 1996) which could result in vasodilation. However, because a low Mg concentration results in a high rate of free-radical formation (Weglicki *et al.* 1996) and because free radicals inactivate endothelium-derived relaxing factors, increased degradation of NO by superoxide anions could play a role in the modification of arterial contractile response (Yang *et al.* 1998).

The activation of the sympathetic nervous system would be the determining factor contributing to the development and maintenance of sustained hypertension during chronic Mg deficiency. The observation that arterial pressure decreased more in hypertensive Mg-deficient rats than in control rats after anaesthesia and the abolition of extrinsic regulation, clearly indicates that the sympathetic nervous system contributes to Mg-deficiency-induced hypertension. Although sympathetic nervous activity may be enhanced, it was shown in the present study that cardiovascular reactivity to noradrenaline was lower in hypertensive Mg-deficient rats than in control rats. The decrease of cardiovascular reactivity to noradrenaline was not due to a difference in cardiac output but rather to a lowered vascular reactivity. The origin of vascular hyporeactivity seen in hypertensive Mg-deficient rats is not clear but could be related to degenerative changes in blood vessel walls occurring in the later stages of Mg deficiency. The arterial change resulting from Mg deficiency has been extensively reviewed (Rayssiguier & Gueux, 1985). This includes intimal thickening, thinning and fragmentation of elastic membranes, collagen accumulation and calcification. Moreover, Mg deficiency can intensify cardiovascular lipid deposition and lesions in animals fed on atherogenic diets (Altura *et al.* 1990). As shown previously, in the present study, plasma cholesterol and total triacylglycerol concentrations were greater in Mg-deficient rats, indicating abnormalities in lipoprotein metabolism (Nassir *et al.* 1995). Moreover, other studies have indicated that Mg deficiency increases the susceptibility of lipoproteins to peroxidation (Rayssiguier *et al.* 1993). Thus, inflammatory events, oxidative modification of lipoproteins, free-radical damage, could play a significant role in the pathogenesis of vascular lesions following Mg deficiency (Rayssiguier *et al.* 1996; Shivakumar & Prakash Kumar, 1997). Interestingly, the vascular changes observed in Mg-deficient rats resemble those seen during the ageing process, which is accompanied by atherosclerosis, hypertension and also by hyporesponsiveness to some vasoconstrictor agonists (Cohen & Berkowitz, 1976;

such as noradrenaline and angiotensin II, suggesting that long-term Mg deficiency may elevate blood pressure level by increasing peripheral resistance (Altura *et al.* 1984, 1992). Enhanced cardiovascular reactivity to noradrenaline has, however, been reported in Mg-deficient rats during the early hypotensive phase, as well as an increased heart rate (Luthringer *et al.* 1988). Hyperaemia observed during the early phase of Mg deficiency corresponds to an increased production and release of circulating proinflammatory agents such as cytokines (tumour necrosis factor, interleukin 1, interleukin 6), prostacyclin and histamine (Weglicki *et al.* 1992, 1996; Rayssiguier *et al.* 1997). All these endogenous substances can exert vasodilatory activity, reduced myogenic tone and diminish blood pressure level (Van de Voorde & Leusen, 1983; McKenna, 1990; Vane *et al.* 1990). In the present study, the combined administration of the antihistamine agents, dexchlorpheniramine and cimetidine, totally abolished the appearance of the hypotensive phase in Mg-deficient rats, suggesting that release of the vasodilating agent histamine during the inflammatory period of Mg deficiency contributes to reduced blood pressure. Interestingly, the abolition of hypotension induced

Haudenschild *et al.* 1981; Haudenschild & Chobanian, 1984; Feletou *et al.* 1993; Folkow & Svanborg, 1993; Bilato & Crow, 1996). In the present study, however, it was shown that the vasoconstrictor activity to angiotensin II was not affected by Mg deficiency suggesting that alteration of the receptor-mediated intracellular signalling pathway could also account for the hyporesponsiveness to catecholamines observed in hypertensive Mg-deficient rats.

The present data also showed that hypertension induced by a low-Mg diet was only partially corrected if the hypertensive Mg-deficient rats were fed on the control diet for 3 weeks while heart rate was still elevated, suggesting that an activated sympathetic nervous system is still present. It could be stipulated that the severity and/or prolongation of the Mg deficiency induces some irreversible biological lesions related to cardiovascular function and blood pressure control.

Magnesium deficiency and the hormonal system

Few studies have investigated the effects of Mg deficiency on the hormonal systems which control blood pressure. The renin–angiotensin–aldosterone system plays an essential role in humoral and haemodynamic regulation (Hackenthal *et al.* 1990). In the present study, plasma renin activity was increased in Mg-deficient rats during the early hypotensive phase. No modification was found during the hypertensive phase. These observations suggest that lowered systemic blood pressure may increase the release of renin by juxtaglomerular cells. These findings are consistent with early studies showing that Mg deficiency in rats lowers blood pressure and induces hyperplasia of epithelial juxtaglomerular cells suggesting that the lower blood pressure observed in Mg-deficient rats would be the main stimulus of juxtaglomerular cell hyperactivity and that the increased plasma renin activity observed in Mg-deficient rats can be considered as a normal response to hypotension (Cantin, 1970). Despite a greater plasma renin activity in Mg-deficient rats, ACE levels were significantly lower at 2 and 21 weeks of Mg deficiency. ACE converts plasma angiotensin I to the potent vasopressor angiotensin II, and inactivates the vasodilator peptide bradykinin. Therefore, decreased plasma ACE activity would lead to decreased angiotensin II production and would stimulate bradykinin activity which would contribute to reduced vascular tone and reactivity and thus to decreased blood pressure. Whether a decrease in ACE activity contributes to the lower blood pressure of rats during the early stages of Mg deficiency remains to be determined. Further studies are also needed to show if the reduced plasma ACE activity induced by Mg deficiency is associated with a reduction in angiotensin II production and to ascertain how Mg deficiency reduces plasma ACE activity. In contrast, Mg deficiency elevated plasma aldosterone concentrations at 2 and 21 weeks of dietary treatment. These findings are consistent with previous studies in which Mg supplementation was shown to exert the opposite effect (Ginn *et al.* 1967; Solounias & Schwartz, 1975; Atarashi *et al.* 1989; Ichihara *et al.* 1993; Nadler *et al.* 1993; Corica *et al.* 1996). In the present study, plasma aldosterone increased regardless of the decreased ACE. This dissociation suggests that Mg has some direct effects on

aldosterone synthesis rather than indirect effects via the renin–angiotensin system. The precise mechanism by which Mg deficiency stimulates aldosterone production remains undetermined. It is known that aldosterone secretion and release by the zona glomerulosa of the adrenal gland are Ca-dependent processes (Kafunding *et al.* 1979; Guthrie *et al.* 1983). In human subjects it has been demonstrated that Mg infusion decreases aldosterone production by inhibiting cellular Ca influx (Ichihara *et al.* 1993). Thus, one could suggest that Mg deficiency, by facilitating cellular Ca entry, may promote aldosterone production and release. Another factor which regulates aldosterone production is adrenocorticotrophic hormone (Quinn & Williams, 1988). Plasma adrenocorticotrophic hormone concentration was not assessed in the present study. However, plasma corticosterone levels decreased and increased when rats were fed on the Mg-deficient diet for 2 and 21 weeks respectively, while aldosterone production was increased throughout the dietary experiment. These findings suggest that the adrenocorticotrophic hormone–corticosteroids axis does not contribute to enhanced aldosterone production in Mg-deficient rats. The reason for the differential effects of the Mg-deficient diet on corticosterone level during the time course of the experiment remains to be elucidated, as does the contribution of aldosterone to the change in blood pressure observed in Mg-deficient rats. Among the various endogenous vasoconstrictors, vasopressin has been shown to elicit increased vascular responses in hypertension (Larivière *et al.* 1989) suggesting that these hormones may play a role in the modulation of peripheral resistance and thus in the blood pressure level. However, the present study showed that the plasma vasopressin level was not modified at any time during the Mg deficiency treatment.

In conclusion, chronic consumption of a Mg-deficient diet induces severe and sustained hypertension in rats. However, the onset of dietary Mg deficiency is characterized by a transitory phase of hypotension. Although the sympathetic nervous system contributes, at least in part, to the elevation of blood pressure, the contribution of several hormonal systems implicated in blood pressure regulation and the origin of early hypotension remains to be elucidated. Our observations, however, suggest that vasodilator inflammatory agents (such as histamine) may contribute to the lower blood pressure during the hypotensive phase.

References

Altura BM & Altura BT (1995) Role of magnesium in the pathogenesis of hypertension updated: relationship to its actions on cardiac, vascular smooth muscle, and endothelial cells. In *Hypertension: Pathophysiology, Diagnosis, and Management*, 2nd ed., pp. 1213–1242 [JH Laragh and BM Brenner, editors]. New York, NY: Raven Press Ltd.

Altura BM, Altura BT, Gebrewold A, Ising H & Günther T (1984) Magnesium-deficiency and hypertension: correlation between magnesium deficient diets and microcirculatory changes in situ. *Science* **223**, 1315–1317.

Altura BM, Altura BT, Gebrewold A, Ising H & Günther T (1992) Noise-induced hypertension and magnesium in rats: relationship to microcirculation and calcium. *Journal of Applied Physiology* **72**, 194–202.

Altura BT, Brust M, Bloom S, Barbour RL, Stempak JG & Altura

BM (1990) Magnesium dietary intake modulates blood lipid levels and atherogenesis. *Proceedings of the National Academy of Sciences USA* **87**, 1840–1844.

Astier C, Rock E, Lab C, Gueux E, Mazur A & Rayssiguier Y (1996) Functional alterations in sarcoplasmic reticulum membranes of magnesium-deficient rat skeletal muscle as consequences of free radical-mediated process. *Free Radical Biology and Medicine* **20**, 667–674.

Atarashi K, Matsuoka H, Takagi M & Sugimoto T (1989) Magnesium ion: a possible physiological regulator of aldosterone production. *Life Science* **44**, 1483–1489.

Berthelot A & Esposito J (1983) Effects of dietary magnesium on the development of hypertension in the spontaneously hypertensive rat. *Journal of the American College of Nutrition* **4**, 345–353.

Bilato C & Crow MT (1996) Atherosclerosis and the vascular biology of aging. *Aging Clinical Experimental Research* **8**, 221–234.

Bussière L, Mazur A, Gueux E & Rayssiguier Y (1994) Hypertriglyceridemic serum from magnesium-deficient rats induces proliferation and lipid accumulation in cultured vascular smooth muscle cells. *Journal of Nutritional Biochemistry* **5**, 585–590.

Cantin M (1970) Relationship of juxtaglomerular apparatus and changes in magnesium deficiency. *Laboratory Investigation* **22**, 558–568.

Cohen ML & Berkowitz BA (1976) Vascular contraction: effect of age and extracellular calcium. *Blood Vessels* **13**, 139–154.

Corica F, Allegra A, Ientile R, Buemi M, Cucinotta G, Bonanzinga S & Ceruso D (1996) Effects of the intravenous magnesium administration on aldosterone and atrial natriuretic factor plasma concentration. *Nephron* **73**, 739–741.

Dupont J & Sassard J (1974) Vascular reactivity in spontaneously hypertensive, normotensive and hypotensive rats. *British Journal of Pharmacology* **50**, 185–188.

Durlach J, Durlach V, Rayssiguier Y, Bara M & Guiet-Bara A (1992) Magnesium and blood pressure. II. Clinical studies. *Magnesium Research* **5**, 147–153.

Durlach J, Poenaru J, Rouhani S, Bara M & Guiet-Bara A (1987) The control of central neural hyperexcitability in magnesium deficiency. In *Nutrients and Brain Function*, pp. 78–81 [WB Essman, editor]. Basel: Karger.

Evans GH, Weaver CM & Harrington DD (1989) Dietary magnesium does not affect blood pressure in spontaneously hypertensive rats. *Clinical and Experimental Hypertension Theory and Practice* A**11**, 619–632.

Feletou M, Moreau N, Boulanger M & Duhault J (1993) Effect of aging and drug-induced weight reduction of rat vascular reactivity. *Journal of Cardiovascular Pharmacology* **21**, 120–127.

Folkow B & Svanborg A (1993) Physiology of cardiovascular aging. *Physiological Reviews* **73**, 725–764.

Ginn HE, Cade R, McCallum T & Fregley M (1967) Aldosterone secretion in magnesium-deficient rats. *Endocrinology* **80**, 969–971.

Günther T, Ising H, Babish W & Vormann J (1984) Magnesium intake and blood pressure of spontaneously hypertensive rats. *Magnesium-Bulletin* **6**, 120–124.

Guthrie GP, McAllister RG & Kotchen TA (1983) Effect of intravenous and oral verapamil upon pressor and adrenal steroidgenic responses in normal man. *Journal of Clinical Endocrinology and Metabolism* **57**, 339–343.

Hackenthal E, Paul M, Ganten D & Taugner R (1990) Morphology, physiology, and molecular biology of renin secretion. *Physiological Reviews* **70**, 1067–1116.

Haudenschild CC & Chobanian AV (1984) Blood pressure lowering diminishes age-related changes in the rat aortic intima. *Hypertension* **6**, Suppl. 1, I-62–I-68.

Haudenschild CC, Prescott MF & Chobanian AV (1981) Aortic endothelial and subendothelial cells in experimental hypertension and aging. *Hypertension* **3**, Suppl. 1, I-48–I-53.

Ichihara A, Suzuki H & Saruta T (1993) Effect of magnesium on the renin–angiotensin–aldosterone system in human subjects. *Journal of Laboratory and Clinical Medicine* **122**, 432–440.

Ising H & Günther T (1997) Suboptimal magnesium, noise-induced stress, aging and cardiovascular risk. *Magnesium-Bulletin* **19**, 42–45.

Itokawa Y, Tanaka C & Fujiwara M (1974) Changes in body temperature and blood pressure in rats with calcium and magnesium deficiencies. *Journal of Applied Physiology* **37**, 835–839.

Kafunding JL, Chow R & Catt KJ (1979) The role of calcium in the stimulation of aldosterone production by adrenocorticotropin, angiotensin II, and potassium in isolated glomerulosa cells. *Endocrinology* **105**, 327–333.

Larivière R, St-Louis J & Schiffrin EL (1989) Vasopressin receptors and inositol triphosphate production in blood vessels of spontaneously hypertensive rats. *Canadian Journal of Physiology and Pharmacology* **67**, 232–239.

Laurant P, Robin S & Berthelot A (1997) Magnesium deficiency increases vasoconstrictor activity without affecting blood pressure of aged spontaneously hypertensive rats. *Magnesium Research* **10**, 107–117.

Luthringer C, Rayssiguier Y, Gueux E & Berthelot A (1988) Effect of moderate magnesium deficiency on serum lipids, blood pressure and cardiovascular reactivity in normotensive rats. *British Journal of Nutrition* **59**, 243–250.

McKenna TM (1990) Prolonged exposure of rat aorta to low levels of endotoxin in vitro results in impaired contractility. Association with vascular cytokine release. *Journal of Clinical Investigation* **86**, 160–168.

Maier JAM, Malpuech-Brugère C, Rock E, Rayssiguier Y & Mazur A (1997) Serum from magnesium-deficient rats affects endothelial cells in culture: role of hyperlipemia and inflammation. *Journal of Nutritional Biochemistry* **9**, 17–22.

Mak IT, Komarov AM, Wagner TL, Stafford RE, Dickens BF & Weglicki WB (1996) Enhanced NO production during magnesium deficiency and its role in mediating red blood cell glutathione loss. *American Journal of Physiology* **271**, C385–C390.

Nadler JL, Buchanan T, Natarajan R, Antonipillai I, Bergman R & Rude R (1993) Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. *Hypertension* **21** (2), 1024–1029.

Nassir F, Mazur A, Giannoni F, Gueux E, Davidson O & Rayssiguier Y (1995) Magnesium deficiency modulates hepatic lipogenesis and apolipoprotein gene expression in the rat. *Biochimica et Biophysica Acta* **1257**, 125–132.

Overlack A, Zenzen JG, Ressel C, Müller HM & Stumpe KO (1987) Influence of magnesium on blood pressure and the effect of nifedipine in rats. *Hypertension* **9**, 139–143.

Quinn ST & Williams GH (1988) Regulation of aldosterone secretion. *Annual Review of Physiology* **50**, 409–426.

Rayssiguier Y & Gueux E (1985) Magnesium and lipids in cardiovascular disease. *Journal of the American College of Nutrition* **5**, 507–519.

Rayssiguier Y, Gueux E, Bussière L, Durlach J & Mazur A (1993) Dietary magnesium affects susceptibility of lipoproteins and tissues to peroxidation in rats. *Journal of the American College of Nutrition* **12**, 133–137.

Rayssiguier Y, Malpuech C, Nowacki W, Rock E, Gueux E & Mazur A (1997) Inflammatory response in magnesium deficiency. In *Advances in Magnesium Research*: 1. *Magnesium in Cardiology*, Suppl. 1 *Magnesium Research*, pp. 415–421 [R Smetana, editor]. London: John Libbey & Co Ltd.

Rayssiguier Y, Mazur A, Gueux E & Rock E (1996) Magnesium deficiency affects lipid metabolism and atherosclerosis process by a mechanism involving inflammation and oxidative stress. In *Current Research in Magnesium*, pp. 251–255 [MJ Halpern and J Durlach, editors]. London: John Libbey & Co Ltd.

Rayssiguier Y, Mbega JD, Durlach V, Gueux E, Durlach J, Giry J, Dalle M, Mazur A, Laurant P & Berthelot A (1992) Magnesium and blood pressure. I. Animal studies. *Magnesium Research* **5**, 139–146.

Rock E, Astier C, Lab C, Malpuech C, Nowacki W, Gueux E, Mazur A & Rayssiguier Y (1995) Magnesium deficiency in rats induces a rise in plasma nitric oxide. *Magnesium Research* **8**, 237–242.

Ryan JW, Chung A, Ammons C & Carlton ML (1977) A simple radioassay for angiotensin-converting enzyme. *Biochemical Journal* **167**, 501–504.

Safwate A, Kati-Coulibalis S, Davicco MJ, Giry J & Barlet JP (1991) Renin–aldosterone system and arginine vasopressin in diarrhoeic calves. *British Veterinary Journal* **147**, 533–537.

Shivakumar K & Prakash Kumar B (1997) Magnesium deficiency enhances oxidative stress and collagen synthesis in vivo in the aorta of rats. *International Journal of Biochemistry and Cell Biology* **29**, 1273–1278.

Solounias MB & Schwartz R (1975) The effect of magnesium deficiency on serum aldosterone in rats fed two levels of sodium. *Life Science* **17**, 1211–1218.

Tongyai S, Rayssiguier Y, Motta C, Gueux E, Maurois P & Heaton FW (1989) Mechanism of the increased erythrocyte membrane fluidity during magnesium deficiency in weanling rats. *American Journal of Physiology* **257**, C270–C276.

Van de Voorde J & Leusen I (1983) Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. *European Journal of Pharmacology* **87**, 113–120.

Vane JR, Änggard EE & Botting RM (1990) Regulatory function of the vascular endothelium. *New England Journal of Medicine* **323**, 27–36.

Weglicki WB, Mak IT, Kramer JH, Dickens BF, Cassidy MM, Stafford RE & Phillips TM (1996) Role of free radicals and substance P in magnesium deficiency. *Cardiovascular Research* **31**, 677–682.

Weglicki WB, Phillips TM, Freedman AM, Cassidy MM & Dickens BF (1992) Magnesium deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Molecular Cell Biochemistry* **118**, 105–111.

Yang BC, Li DY, Weng YF, Lynch J, Wingo CS & Mehta JL (1998) Increased superoxide anion generation and altered vasoreactivity in rabbits on low-potassium diet. *American Journal of Physiology* **274**, H1955–H1961.