Magnesium-deficient rats prefer magnesium-containing to pure water, but not to sweet tasting solutions

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Zusammenfassung

Ratten mit Magnesiummangel bevorzugen magnesiumhaltiges Trinkwasser gegenüber reinem Wasser, nicht aber gegenüber süß schmeckenden Lösungen.

Gruppen von 3 x 20 weiblichen Sprague Dawley Ratten mit einem Körpergewicht von 80–140 g erhielten eine Mg-Mangel-Diät (135 ppm MG) während 14 Tagen. Ab dem 9. Versuchstag erhielten 4 Tiere pro Käfig drei Tränkeflaschen in randomisierter Reihenfolge, die entweder Wasser oder 16 mmol Mg/l Wasser oder 5 mmol Saccharin/l Wasser enthielten (Dreiecks-Test). Eine Untergruppe erhielt 2 x täglich 10 mmol Mg pro kg Körpergewicht mittels Magensonde. Zusätzlich wurden Kontrollen mit Standardfutter mitgeführt.

Der Zusatz von Saccharin erhöhte die absoluten Trinkmengen; süß wurde eindeutig gegenüber Mg-haltigem oder purem Wasser bevorzugt. Andererseits wurde Mg-haltiges Wasser gegenüber reinem Wasser bevorzugt; die Hypomagnesiämie wurde hierdurch signifikant abgeschwächt. Die Gewichtsretardierung von 14% und der durch Mg-Mangel bedingte Verlust der Freßlust wurden nicht vollständig während der 5tägigen Behandlungszeit kompensiert.

Da Ratten ohne Mg-Mangel angereichertes Trinkwasser – im Gegensatz zu gesüßtem Wasser – nicht bevorzugen, lassen die vorliegenden Daten den Schluß zu, daß sich die Vorliebe für Mg erst während des Mangels entwickeln muß.

Summary

Groups of 3 x 20 female Sprague Dawley rats weighing 80–140 g were kept on a Mg-deficient diet (135 ppm Mg) during 14 days. Starting on the 9th day, 4 animals per cage were offered 3 drinking-bottles in a randomized order, containing either pure water, or water with 16 mmol Mg/l, respectively with 5 mmol saccharin/l (triangle test). One subgroup received 10 mmol Mg/kg body weight twice daily by gavage. Additional animals were fed a standard laboratory chow.

Saccharin increased total fluid consumption; sweet fluid was clearly preferred to Mg-containing or to pure water. On the other hand, Mg-containing water was preferred to pure water; hypomagnesemia was significantly attenuated by water-borne Mg. Weight loss of 14% and loss of appetite caused by Mg deficiency were not fully compensated during the 5-day treatment period.

Since Mg-sufficient rats did not prefer Mg-rich water in contrast to sweet solutions, these data allow to conclude that the preference for Mg has to develop during Mg deficiency.

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Introduction

Rats offered palatable foods, e.g., chocolate chips, cookies, cheese, peanut butter or "junk food" diet increase caloric intake and rapidly gain body weight relative to animals fed a standard laboratory diet. When rats were offered food and either a sucrose or saccharin solution for only one hour each day they avidly drank the sweet tasting solutions and failed to consume sufficient amounts of food to maintain their body weight. Despite continued weight loss they did not alter their pattern of behaviour and finally died of starvation [3]. So there is no doubt that both animal and human prefer sweet whereas, on the other hand, bitter is mostly aversive. Saltiness and sourness may be pleasant at low levels. In man the threshold for bitterness (quinine sulfate) is 0.008 μmol/l, for sourness (HCl) 0.9 μmol/l, for sweetness (saccharin) 0.023 μmol/l and for saltiness (NaCl; $CaCl_2$) 0.01 mmol/l [1].

In normal weight individuals stress usually reduces appetite. Most anoretic drugs produce their effects by interacting with the catecholaminergic or the serotoninergic system; cholecystokinin and opioid antagonists also suppress appetite and drinking behaviour [8]. The pathogenesis of anorexia in cancer comprises thermostatic, glucostatic and lipostatic mechanisms, including "anorexins" and "cachectin" (the latter being identical with TNF, see later). – Gastrointestinal and neurological disturbances, including anorexia and nausea, have been described by *Shils* [7] in severely Mg-deficient man. Under comparable drastic conditions, serum levels of substance P, interleukin-1, interleukin-6 and tumor necrosis factor-alpha (TNF-alpha) were increased in rats together with reduced food consumption and food utilization, and reduced weight gain [9, 11]. So far depressed food uptake could only be reversed when Mg-deficient rats were rendered diabetic with streptozocin [6].

The intensively bitter taste of magnesium sulfate solutions is well known [2] and *Wacker* [10] has speculated that "during the drought that year [1618] the cattle of Henry Wicker found the spring [Espsom Spa] but would not drink the water apparently because of its bitter taste owing to magnesium sulfate". On the other hand epidemiological data strongly suggest cardioprotective effects of Mg-rich drinking-water [4]. In view of these facts it seemed interesting to study the drinking behaviour of rats kept on a Mg-deficient diet. Using the so-called triangle test, the animals were offered 3 drinking-bottles containing water with or without added Mg or saccharin. If the animals prefered the Mg-containing solution to pure water this would indicate that they had developped a preference for the health-supporting, bitter-tasting mineral. Saccharin was also tested to demonstrate the preference for sweet.

Material and Methods

Identical experiments were performed on groups of 3 x 24 female Sprague

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Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen, respectively Harlan-Winkelmann, Borchen) in the years 1995–1997 within students' courses, permitted by the Local Authorities. The animals were randomly allocated to the following groups:

1: control group
2: Diet plus pure water (3 bottles)
3: Diet plus water (2 bottles) versus *saccharin* (1 bottle)
4: Diet plus water (2 bottles) versus *magnesium* (1 bottle)
5: Diet plus *saccharin* (1 bottle) versus *magnesium* (1 bottle) and water (1 bottle)
6: Diet plus Mg given by gavage.

The diet was provided by Altromin Comp., Lage/Lippe, (No C 1035) and contained 135 ppm Mg. Controls received standard chow (Altromin Haltungsfutter). Starting on the 9th day, 4 rats/group/cage Type III were offered 3 drinking-bottles à 250 ml containing distilled water or water with 16 mmol Mg/l ($MgCl_2$) or water with 5 mmol saccharin/l (Na-saccharin, süssin®, Süßstoffvertriebsgesellschaft, München). The position of the bottles (left or right side, respectively the center) and their content were randomly chosen and changed every day. Total fluid consumption per day and percental consumption per drinking-bottle were recorded daily at 9 a.m. together with food consumption and body weight. At the end of the experiments arterial blood was taken in deep pentobarbital anesthesia. Serum was produced and used for Mg determinations (AAS technique).

Results

Starting on days 7 to 9, rats kept on the Mg-deficient diet developed typical erythema and became somewhat "nervous"; no mortality occurred. Basic data are summarized in **figure 1:** In comparison to the controls (group 1), Mg-deficient rats of **group 2** consumed less food (–35%) and fluid (–14%) and their body weight had decreased by 14% at the end of the observation period. There was no preference of any of the three drinking-bottles containing water (data not shown). When one of the three drinking-bottles contained saccharin: **group 3** and **group 5,** total fluid consumption increased. The bottles containing saccharin were clearly preferred to pure water (79%, group 3) or to pure water and to Mg (65%, group 5) (see **figure 2**). When Mg was offered via drinking-water (**group 4**) the effects were less pronounced than those produced by stomach tube feeding (**group 6**): In group 6, food consumption increased as well as body weight.

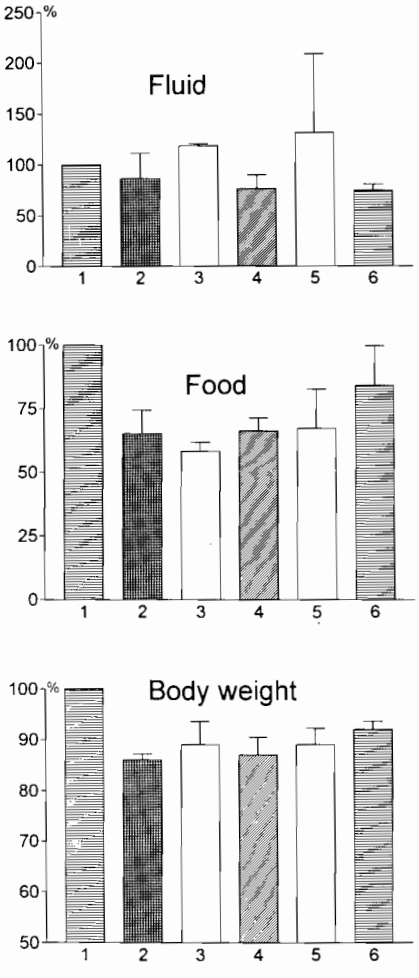

Fig. 1: Percental consumption of total fluid and food and the development of body weight on day 14, in relation to the controls (group 1 = 100%). Presented are the arithmetic means of 3 independent experimental series with their standard deviations. Group 1 (Control) received standard rat chow and distilled water (3 bottles) ad libitum. Groups 2 to 6 received a Mg-deficient diet and additionally:
Group 2: Distilled water, 3 bottles
Group 3: Saccharin: 1 bottle; distilled water: 2 bottles
Group 4: Magnesium: 1 bottle; distilled water: 2 bottles
Group 5: Magnesium: 1 bottle; saccharin: 1 bottle, distilled water: 1 bottle
Group 6: Distilled water: 3 bottles, 10 mmol Mg/kg b.w. by gavage

The percental consumption of the fluids offered to **group 3,** to **group 4** and to **group 5** on day 14 of the experiment are depicted in **figure 2:** Saccharin is clearly preferred to pure water and to Mg-containing water. However, the 16 mmol Mg/l solution is also clearly preferred to pure water (group 4), and slightly preferred to water when saccharin is offered in addition (group 5). Serum Mg levels determined in the different groups are summarized in **figure 3:** Hypomagnesemia persisted when the drinking-water was free of Mg (group 2 and 3). Hypomagnesemia was

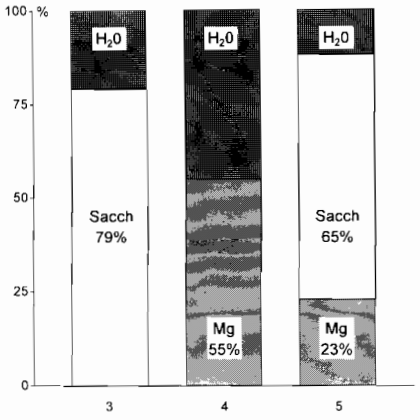

Fig. 2: Drinking behaviour of groups 3, 4 and 5 on day 14: Presented is the percental consumption of fluid, offered in 3 drinking-bottles and containing
Group 3: Saccharin: 1 bottle; distilled water: 2 bottles
Group 4: Magnesium: 1 bottle; distilled water: 2 bottles
Group 5: Magnesium: 1 bottle; saccharin: 1 bottle; distilled water: 1 bottle

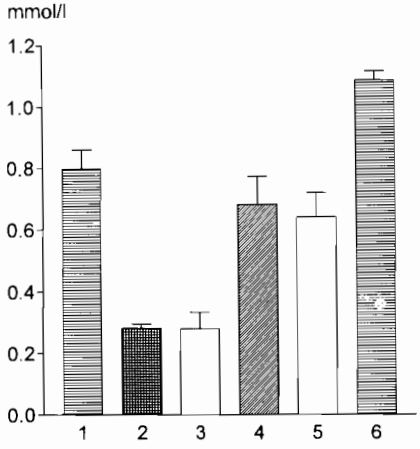

Fig. 3: Serum-Mg levels of groups 1–6. For details see Legend to Fig. 1.

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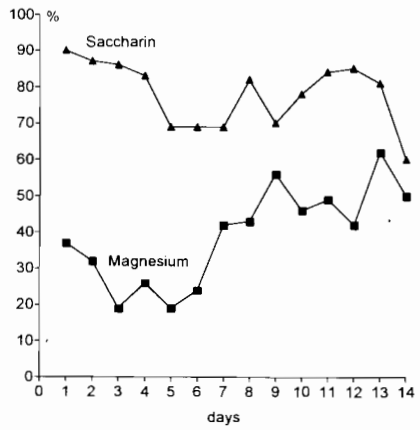

Fig. 4: Drinking behaviour of 2 x 2 rats offered during 14 days 3 drinking-bottles, one containing saccharin or magnesium, modified according to [5].

partly corrected when one of 3 bottles contained Mg (groups 4 and 5). Rats were moderately hypermagnesemic when Mg had been administered by gavage (group 6), although basic data (food consumption; body weight) had not yet completely normalized (figure 1).

In previous experiments of *Meyer* and *Classen* [5] following the same design Mg-rich solutions were offered already since the first day of feeding the Mg-deficient diet. As depicted in **figure 4** the Mg-rich solution was only preferred to pure water during the second half of the observation period, i.e. when Mg deficiency had developed. In contrast, saccharin was immediately preferred to water and was consumed to a much greater extent (70 to 90%) than Mg (50 to 55%).

Discussion

The recommended dietary content of Mg for rats is 400 mg/kg diet (90% dry matter); laboratory chow usually contains 4fold this amount. In the present experiments initial daily food consumption was approximately 18 g per rat (of 100 g weight). 18 g of a diet containing 400, respectively 1600 mg Mg/kg, corresponds to 7.2, respectively 28.8 mg Mg per rat, or to 72 respectively 288 mg Mg/kg rat's body weight. These figures demonstrate that the Mg requirement of small rodents is by far higher than human requirement. — The Mg-deficient diet used in the present experiments contained only 135 ppm Mg. Assuming again a daily food consumption of 18 g, the Mg uptake was thus reduced to 2.4 mg Mg per animal. Hence, a daily deficit resulted of at least 4.8 mg Mg per rat. In groups 4 and 5, drinking-water was offered in one out of three bottles that contained 16 mmol/l $MgCl_2$ (= 390 mg Mg/l). If the animals drank 12 ml of this solution per day their deficit would have been corrected.

In fact, hypomagnesemia of 0.25 mmol Mg/l was nearly corrected when 1 drinking-bottle was offered during 5 days containing 16 mmol Mg/l (group 4): serum-Mg levels increased to 0.681 - mmol/l. When saccharin was also offered (group 5), serum-Mg increased to only 0.640 mmol/l, since the Mg-containing was less preferred than sweet water (see figure 2) and also since urine volume increased due to higher fluid consumption (see figure 1). These effects could be reproduced in 3 independent experimental series within students' courses.

Previous experiments of *Meyer* and *Classen* [5] had shown that Mg-sufficient rats do not prefer Mg-containing water, probably due to the bitter taste. However, preference for Mg developed when the animals became Mg-deficient (figure 4). If these data were transferred to man, one could understand why people with high Mg requirement, e.g. stress-prone persons, stick to Mg supplements.

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