Pathobiology of magnesium deficiency: a cytokine/neurogenic inflammation hypothesis

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Weglicki, William B., and Terry M. Phillips. Pathobiology of magnesium deficiency: a cytokine/neurogenic inflammation hypothesis. Am. J. Physiol. 263 (Regulatory Integrative Comp. Physiol. 32): R734–R737, 1992.—During the progression of Mg deficiency in a rodent model, we have observed dramatic increases in serum levels of inflammatory cytokines [interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)] after 3 wk on a Mg-deficient diet. Sequential analyses of these cytokine changes in the serum of rats revealed an initial rise at day 12, followed by a major elevation in all three cytokine levels by day 21. Of greater interest was an early peak in the serum level of the neuropeptide substance P after only 5 days on the diet. This “neuronal” tachykinein is thought to be released from neural tissues, and it is known to stimulate production of certain cytokines, including IL-1, IL-6, and TNF-α. In addition, there was a concomitant increase in histamine levels, which may have resulted from stimulation and degranulation of mast cells by substance P. Thus we hypothesize that the release of substance P may be the earliest pathophysiological event leading to stimulation of the inflammatory cytokines, which may then stimulate the free radical mechanisms of injury previously confirmed by our work.

substance P; cardiovascular injury

THE PATHOPHYSIOLOGICAL SEQUENCE of events leading to cardiovascular injury and the formation of lesions in animal models of Mg deficiency remains unclear (1). The observation that antioxidant agents such as α-tocopherol are able to block the cardiovascular lesions to a significant degree suggests a role for free radicals in Mg deficiency (6-8, 24). A number of cell types are capable of generating free radicals, but the cellular origin of these free radicals that may be producing the Mg-deficiency lesions in animals is unknown. The recent finding that the inflammatory cytokines are elevated after 2 wk on the Mg-deficient diet suggests that they contribute to this sequence of pathophysiological events (25). However, the question still remains: what occurs first, cytokine stimulation of free radical production or free radical-induced tissue injury followed by an inflammatory cytokine response to injury?

Our data suggest that the early elevation of substance P, after only 5 days on the Mg-deficient diet, may be the initial trigger stimulating a number of host defense systems: macrophages to produce inflammatory cytokines, mast cells to release histamine, and the production of free radicals from a variety of different cells. Whether the amounts of substance P, released into the serum and generated locally in the tissue, are adequate to initiate these processes is unclear. Recent in vitro studies (17) reported that the concentration of substance P found in our animals at day 5 is sufficient to stimulate monocytes to produce interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Therefore, we submit that substance P may be the earliest initiating stimulus for the subsequent pathobiology of Mg deficiency.

METHODS

The Mg-deficiency models. We have developed two rodent models of Mg deficiency to study the problem of micronutrient deficiency and cardiovascular disease. The first model is the Sprague-Dawley rat, which is kept under a 12:12-h light-dark cycle and provided deionized water plus a diet that is either deficient in Mg or is Mg supplemented (Teklad, Madison, WI). Rats placed on the Mg-deficient diet become Mg deficient by 2 wk, after initiation of the diet. The second model uses BALB/c mice, which are kept under identical conditions with access to the same diet and water; this model develops hypomagnesemia before 2 wk.

Methodology section. Circulating levels of substance P were measured by a modified (T. M. Phillips, personal communication) antigen-capture enzyme-linked immunosorbent assay (ELISA) (11) using a polyclonal antibody to substance P (Chemicon International, Temecula, CA). Biotinylated anti-substance P antibodies were immobilized on avidin-coated eight-well ELISA strips (Flow Laboratories, McLean, VA), and the strips were incubated with 25 μl of rat plasma overnight at 4°C. After this incubation, the strips were washed 3 times in 0.01 M phosphate buffer-0.01% Nonidet, and the supernatant was recovered by centrifugation at 15,000 g for 10 min at 4°C. One μl of the clarified supernatant was injected into a BioRad model 100 HPCF (BioRad Laboratories, Richmond, CA) and developed at a potential of 5 kV in a 75 μm ID × 25 cm fused-silica capillary for 40 min. The chromatogram was monitored at 214 nm, 0.1 absorbance

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units full scale, the resolved peaks were analyzed by the HPCE supporting software, and fractions were collected for ELISA analysis.

RESULTS

The levels of inflammatory cytokines and substance P in plasma and cardiac tissues were determined in Mg-sufficient and Mg-deficient rats that were maintained on the diets for 3 wk. Plasma samples were taken at days 1, 3, 5, 10, 12, 14, 18, 19, and 21 and analyzed by chemiluminescence-enhanced ELISA (11). Figure 1 shows the time course of substance P plasma levels in Mg-sufficient and Mg-deficient rats. The Mg-deficient group had an early peak of substance P at day 5 and a second, more prolonged peak starting at day 14 and continuing to rise until day 21, when IL-1, IL-6, and TNF-α were also significantly elevated.

In the mouse model, dramatic elevations of substance P were seen in the plasma at 2 wk (Fig. 2). Cardiac tissue from these animals was taken, and the levels of substance P were measured in 4-μm frozen sections. Although the levels of substance P in the Mg-sufficient hearts were barely detectable, high levels were demonstrated in the Mg-deficient hearts. Further analyses of the Mg-deficient hearts revealed that nonlesion (normal appearing) areas contained only a modest amount of substance P, while dramatic increases of substance P could be demonstrated in the lesions (Fig. 3).

Analyses of other factors present in the plasma from both models demonstrated elevated histamine levels that could be detected at day 5 on the Mg-deficient diet, with a continuing rise until day 12 (Fig. 4); in addition, a rise in the plasma levels of prostaglandin E2 (PGE2) was also seen corresponding to the initial substance P peak and histamine release (Fig. 4).

DISCUSSION

Our observations of elevated histamine are consistent with previous studies by Bois (2), who observed that “the syndrome of Mg deficiency develops more or less rapidly according to age. In young animals, the first sign of peripheral vasodilation appears within 1 or 2 days and lasts for ~10–12 days. The erythema is generalized and is accompanied by slight edema of the torso, nose, and paws.” Substance P is a potent vasodilator, along with histamine, thrombin, bradykinin, ATP, ADP, and acetylcholine (10, 23). Other investigators have shown that substance P is one of the most potent vasodilators acting on the endothelium of blood vessels (21, 27). A recent study (17) stated that “our findings that substance P and SK augment the release of IL-1, TNF-α, and IL-6 provide evidence for the potentially pivotal role of neuropeptides in the pathogenesis of a wide range of inflammatory diseases.” We submit that the concomitant increases in histamine and PGE2 (Fig. 4) may result from substance P-triggered cellular responses due to concentrations in the 10^-10 M range (Fig. 1). Others (14) have proposed that “substance P would be expected to immediately induce vasodilation, increase vascular permeability, provoke the release of histamine and leukotrienes from mast cells, and promote the margination, emigration, and activation of polymorphonuclear leukocytes.” Thus both histamine and substance P may contribute to the erythema and edema seen in the Mg-deficient animals during the first week. Wozniak et al. (26) studied the role of substance P as a mediator of “neurogenic inflammation,” and they found that substance P increased neutrophil cytotoxic activity and enhanced superoxide anion production; they concluded that “the regulatory effects of substance P on the neutrophil functions studied appear to be similar to those of a number of cytokines that have
been previously implicated in inflammation." Brunelleschi et al. (3, 4) have reported that substance P and NKA, another neuropeptide, prime the neutrophils that have been treated with platelet-activating factor (PAF), and that pulmonary alveolar macrophages could be activated by substance P to produce superoxide. Kroegel et al. (15) postulated that substance P may act through nonspecific peptide-membrane phospholipid interactions in the eosinophils to induce degranulation and an oxidative burst. Substance P is found in central neurons and may be released after inflammatory stimuli. Other investigators (13) have described receptors for substance P on human astrocytoma cells, and they propose the potential relevance of the increased density of substance P receptors in neuronal injury; they state that “elevated levels of substance P or elevated levels of substance P receptors may be considered as proinflammatory and possibly neurotoxic events.” Other workers (9) suggested “a role for substance P in mediating the response of the superior cervical ganglion or its efferent and afferent innervation.” The irritability expressed by our fibers innervating it; these authors postulate that “substance P, contained in dermal nerve fibers, may be released after inflammatory stimuli. Other investigators (13) have described receptors for substance P on human astrocytoma cells, and they propose the potential relevance of the increased density of substance P receptors in neuronal injury; they state that “elevated levels of substance P or elevated levels of substance P receptors may be considered as proinflammatory and possibly neurotoxic events.” Other workers (9) suggested “a role for substance P in mediating the response of the superior cervical ganglion or its efferent and afferent innervation.” The irritability expressed by our Mg-deficient animals and the preliminary data showing accumulation of leukocytes during early vascular phases represent a critical initial mediator of the cascade of cellular events involving mast cell degranulation and release of pro-inflammatory cytokines (e.g., TNF-α), with subsequent induction of adhesion molecules on adjacent vascular endothelium. This would then, facilitate the local accumulation of leukocytes during early vascular phases of the inflammatory response.” We feel that a similar mechanism may be involved in the Mg-deficiency-enhanced release of substance P.

Fig. 4. Time course of plasma histamine and prostaglandin E2 (PGF-2α) levels in Mg-deficient and Mg-sufficient rats. A: histamine levels. B: PGE2 levels. Circles represent 2 Mg-deficient animals; squares represent 2 Mg-sufficient animals.

REFERENCES


