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Metabolic interactions between restraint stress and L-lysine: The effect on urea cycle components

Rapid Communication

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Summary. We studied the effects of L-lysine on wrap-restraint stress-induced changes in ureagenesis. An exposure to wrap-restraint stress did not affect the plasma concentration of L-lysine, but did decrease plasma urea and arginine. Oral L-lysine (1 g/kg) blocked the effect of stress on ureagenesis, and enhanced the effect of stress on L-arginine. No influence of L-lysine were found in controls. The results imply a stress-specific, ureagenesis-stimulating effect of L-lysine, and suggest an increased requirement for L-arginine during the above conditions.

Keywords: Wrap-restraint stress – Urea – Arginine – Lysine – Rat

Abbreviations: Arg, L-arginine; Orn, L-ornithine; Lys, L-lysine; p.o., oral; WRS, wrap-restraint stress

Introduction

Stress exacerbates both gastrointestinal and metabolic functional complications, and mood disorders (Roth et al., 1980; Barone et al., 1990; Ballenger et al., 2000; Mayer et al., 2001). The relationship between stress, gastrointestinal and metabolic disorders is not yet understood, but it reportedly involves interrelated humoral and neural pathways (Farthing, 1999; Kamm, 2002). Considering the heterogeneity of stress-related symptoms, it is not surprising that besides pharmacological treatments, other therapeutic approaches, namely nutritional ones, are sought (Matsueda, 2001). We have recently shown that deficiency in a dietary essential amino acid, L-lysine (Lys), increases wrap-restraint stress (WRS)-induced fecal excretion and stress-induced anxiety via modification of the serotonergic system (Smriga et al., 2002). In normally fed rats, Lys partly blocked the anxiogenic effects of stress. This effect of Lys was strengthened, when it was concomitantly applied with L-arginine (Arg) (Smriga and Torii, 2003). In parallel, Han and Baker (1993) reported specifically increased Lys requirements during acute stress in broilers.

The "anti-stress" effect of Lys in rats involved the hypothalamo-pituitary-adrenal axis (Smriga and Torii, 2003). Thus, we have hypothesized that, during acute stress, Lys might modify ureagenesis, via corticosterone-related changes in the protein catabolism, or Lys-induced changes in Arg availability (Anderson and Raiten, 1992; Zieve, 1986). The hypothesis was tested using a non-ulcerogenic form of restraint, WRS, a rodent model for irritable bowel syndrome (Williams et al., 1988).

Materials and methods

Male Wistar rats (body weight, 250–280 g) (Charles River Japan, Tokyo, Japan) were housed individually in conventional hanging cages (12 h light/dark cycle; dark period, 19:00 to 07:00). Rets consumed distilled water and chow diet *ad libitum*. Rats were handled for a week and, thereafter, treated with a single oral (p.o.) infusion of water (N = 6) or Lys solution (0.6 g/kg, N = 6; 1.0 g/kg, N = 6). The infusion volume was kept constant (5 mL).

Rats were exposed to WRS 40 min after p.o. treatments (WRS exposure time, 14:00–16:00), as previously described (Williams et al., 1988). Animals were briefly and lightly anesthetized with ether, and their forepaws, upper forelimbs, and thoracic trunks were wrapped using adhesive tape. Similarly to Williams et al. (1988), WRS did not cause diarrhea, feces were mostly dry and formed.

To avoid any influence of circadian variations (Smriga et al., 2000), blood samplings for amino acid analysis were done at the same time of day (16:00). Naïve (non-stressed) rats (N = 5 per group) were p.o. infused with water or Lys (0.6 g/kg, or 1.0 g/kg) at 13:20, but were not exposed to WRS.

Blood samples were collected from the *posterior vena cava* of all rats. Blood samples were centrifuged for 15 min (3000 rpm, 40°C). Thereafter, 300 μL plasma was removed and mixed with 600 μL of a 5% TCA solution. Samples were again centrifuged for 15 min (10,000 rpm, 4°C), and supernatant was filtered through low-binding regenerated cellulose (Millipore Co., Bedford, MA, USA). Plasma urea, Arg, Lys and L-ornithine (Orn) were evaluated using the automated L-8500 Amino Acid Analyzer (Hitachi, Tokyo, Japan).

Results are expressed as means + SEM. Comparisons within multiple groups were performed using a two-way analysis of variance (ANOVA) followed by Duncan's multiple range test. P values < 0.05 were considered statistically significant.

Results and discussion

An essential amino acid, Lys, orally treated at a dose of 0.6 and 1.0 g/kg body weight, significantly elevated plasma Lys concentrations, without regards to stress exposure (Fig. 1). Lys had no effect on plasma urea, Arg or Orn in non-stressed rats, suggesting absence of ureagenic effects of Lys during normal (non-stress) conditions. However, the effects of Lys were qualitatively different during WRS. The interactions of WRS and Lys on the urea cycle components can be characterized in threefold way.

Firstly, WRS significantly depressed both the circulating Arg, and the urea production, in controls (Fig. 1). This finding suggests that WRS inhibited ureagenesis, while shifting Arg production from the catabolic into the functional pathway, for example nitric oxide formation. Although the WRS-triggered down-regulation of ureagenesis might be interpreted as an initial trigger of stress-related hyper-ammonemia (Cooper, 2001), the finding contradicts reports of Beattie (1978) and Takeuchi et al. (2000), who documented a rise in plasma urea in rats exposed to water immersion and immobilization stress. This discrepancy might have been caused by different stress models, namely by an independent and additive effects of cold exposure and immobilization, as suggested already by Beattie (1978). Differently from milder forms of stress, cold water immersion decreases protein utilization and, consequently, increasing urea production. Our data suggest that stress may have dual effects on ureagenesis, a stimulatory effect via decreased protein utilization, and an inhibitory effect via increased functional use of free amino acids (Arg and Orn). The final metabolic consequence of these processes is probably

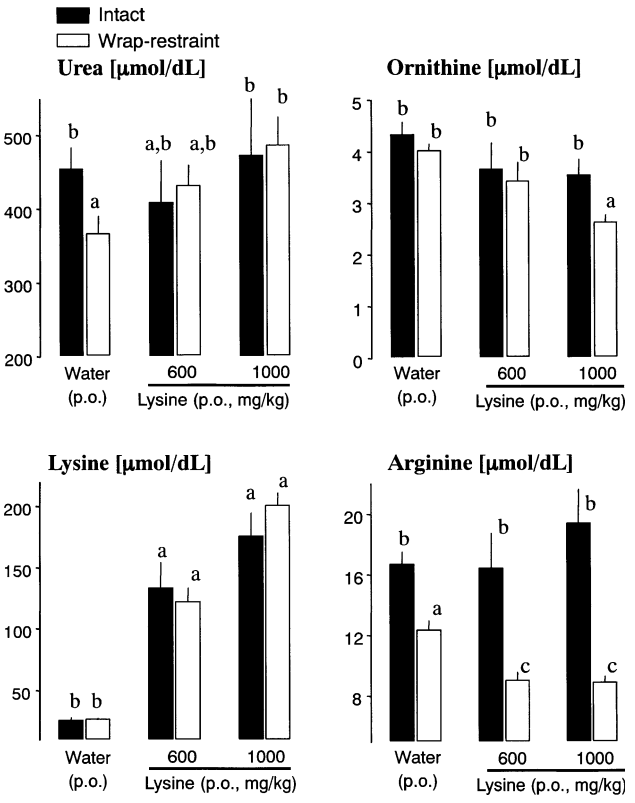

Fig. 1. Plasma concentration or urea, L-ornithine, L-lysine and L-arginine, measured 160 min after oral administration of water or L-lysine (600 mg/kg or 1 g/kg) in intact (black columns) and wrap-restraint stress-exposed (white columns) rats. Rats were exposed to 2-hour-long wrap-restraint stress 40 min after oral treatments. Blood was taken at the end of stress exposure. Means of 5, 6 rats + SEM. The bars with different superscript letters differ significantly at $P < 0.05$ by Duncan's multiple range test

related to stress model used. Secondly, Lys dose-dependently ameliorated urea formation. The Lys-normalized ureagenesis at the end of WRS could be interpreted as a compensatory mechanism for the detoxification of blood (Jorda et al., 1988).

Thirdly, a combined reductive effect of WRS and Lys on Arg, respectively Orn, suggests a stress-specific, Lys-triggered, "re-direction" of Arg and Orn use from functional pathways to ureagenesis. Thus, in Lys treated stressed rats Arg and Orn were increasingly utilized in both the catabolic and functional pathways, improving the animals' ability to accumulate energy and deal with a stressful event (Han and Baker, 1993; Smriga et al., 2002; Smriga and Torii, 2003). Correspondingly, Galili et al. (2001) suggested that the metabolic effects of Lys, in both mammals and plants, are conditionally related to the complex regulation of the general stress response.

In conclusion, present data show that Lys helps to provide energy for the body during acute stress, thus adding to the evidence (Smriga and Torii, 2003) that Lys ameliorates stress-induced pathologies. Matsueda (2001) proposed that those pathologies might be well served by a long-term dietary therapy, combined with stress management, because patients do prefer "non-pharmacological" treatments for life-style related diseases. In this respect, our data point to Lys, respectively Lys combined with Arg, as a promising target for such dietary therapies.

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References

Anderson SA, Raiten DJ (1992) Safety of amino acids used as dietary supplements. Life Science Research Office, FASEB Special Publications Office, Bethesda

Ballenger JC, Davidson JR, Lecrubier Y, Nutt DJ, Foa EB, Kessler RC, McFarlane AC, Shalev AY (2001) Consensus statement on posttraumatic stress disorder from the International Consensus Group on depression and anxiety. J Clin Psychiatry 61S: 60–66

Barone FC, Deegan JF, Price WJ, Fowler PJ, Fondacaro JD, Ormsbee III HS (1990) Cold-restraint stress increases rat fecal pellet output and colonic transit. Am J Physiol 258: G329–G337

Beattie D (1978) Physiological changes in rats exposed to cold/restraint stress. Life Sci 23: 2307–2314

Cooper AJ (2001) Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. Ment Retard Dev Disabil Res Rev 7: 280–286

Enck P, Merlin V, Erckenbrecht JF, Weinbeck M (1989) Stress effects on gastrointestinal transit in the rat. Gut 30: 455–459

Farthing MJG (1999) Irritable bowel syndrome: new pharmaceutical approaches to treatment. Bailliere's Clin Gastroenterol 13: 461–471

Galili G, Tang G, Zhu X, Gakiere B (2001) Lysine catabolism: a stress and development super-regulated metabolic pathway. Curr Op Plant Biol 4: 261–266

Han Y, Baker DH (1993) Effect of sex, heat stress, body weight and generic strain on the dietary lysine requirement of broiler chicks. Poultry Science 72: 701–708

Jorda A, Zaragoza R, Portoles M, Baguena-Cervellera R, Renau-Piqueras J (1988) Long-term high protein diet induces biochemical and ultrastructural changes in rat liver mitochondria. Arch Biochem Biophys 265: 241–248

Kamm KM (2002) Review article: the complexity of drug development for irritable bowel syndrome. Aliment Pharmacol Ther 16: 343–351

Roth E, Funovics J, Schulz F, Karner J (1980) Biochemical methods for the determination of a clinical protein catabolism. Infusionsther Klin Ernahr 7: 306–309

Matsueda K (2001) Nutritional support team. Nippon Rinsho 59: 30–34

Mayer EA, Craske M, Naliboff BD (2001) Depression, anxiety and the gastrointestinal system. J Clin Psychiatry 62S: 28–36

Smriga M, Mori M, Torii K (2000) Circadian release of hypothalamic norepinephrine in rats in vivo is depressed during early L-lysine deficiency. J Nutr 130: 1641–1643

Smriga M, Torii K (2003) Chronic pretreatment with L-lysine and L-arginine reduces stress-induced anxiety in an elevated plus maze. Nutr Neurosci 6 (in press)

Smriga M, Kameishi M, Uneyama H, Torii K (2002) Deficiency of dietary L-lysine increases stress-induced anxiety and fecal excretion in rats. J Nutr 132: 3744–3746

Takeuchi H, Kondo Y, Yanagi M, Yoshikawa M (2000) Accelerative effects of olive oil on adrenal corticosterone secretion in rats loaded with single or repetitive immersion-restraint stress. J Nutr Sci Vitaminol 46: 158–164

Williams CL, Villar RG, Peterson JM, Burks TF (1988) Stress-induced changes in intestinal transit in the rat: a model for irritable bowel syndrome. Gastroenterology 94: 611–621

Zieve L (1986) Conditional deficiencies of ornithine or arginine. J Am Coll Nutr 5: 167–176

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