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Ultrastructural Changes in the Intestinal Absorptive Cells of the Mouse Induced by Capsaicin in Relation to Thiamine Absorption

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The correlation between an inhibition of thiamine absorption and ultrastructural changes in the intestinal absorptive cells of the mouse jejunum induced by capsaicin was examined. In mice fed with capsaicin 1 and 2 mg/kg BW/day for 12 weeks, thiamine absorption was inhibited by 2.7% and 12.5% ($p < 0.001$) respectively. Ultrastructural alterations were observed in villus absorptive cells of jejunum in mice fed with capsaicin 1 and 2 mg/kg BW/day for 12 weeks. The most striking ultrastructural changes occurred in mice fed with capsaicin 2 mg/kg BW/day for 12 weeks. The organelle most affected by capsaicin was the mitochondria. The result shows that the ultrastructural changes of intestinal absorptive cells may be associated with the inhibition of thiamine absorption.

Keywords: capsaicin; thiamine absorption; jejunum; absorptive cells

INTRODUCTION

Thiamine deficiency is a nutritional problem in countries where polished rice is the staple food (Tanphaichitr and Wood, 1984). It is widespread among the population in South East Asia. The factors responsible for this are an inadequate intake of thiamine (Munger and Booton, 1990), a decrease of the thiamine activity by antithiamine factors (Vimokesant *et al.*, 1982) or an impairment of intestinal thiamine absorption by some unknown factors (Thomson *et al.*, 1970). In Asia, it has been known for a long time that hot, green, red and yellow peppers (capsicum fruit) are the most common spice used in cooking food. Capsaicin is the pungent material present in capsicum fruit (Nelson, 1919) and the average amount of capsaicin consumed by Thai people is about 1 mg/kg BW/day (Jonietz, 1983). It was found that capsaicin impaired thiamine absorption in mouse jejunum *in situ* (Toskulkao *et al.*, 1992). Therefore, it is of interest to investigate whether or not capsaicin can impair intestinal thiamine absorption *in vivo*. The ultrastructural alterations of villus absorptive cells induced by capsaicin were also studied.

MATERIALS AND METHODS

Materials. Capsaicin and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). ^{14}C-thiamine HCl (specific activity 24.2 mCi/mmol) was obtained from Amersham Radiochemical Centre Ltd. (Bucks, UK).

Animals. Male Swiss albino mice (20–25 g) obtained from the Animal Production Centre, Faculty of Science, Mahidol University were housed in stainless steel cages in a room at approximately 28 °C with a relative humidity of about 65%. The animals were fed with regular rat chow (Gold Coins Co., Singapore) and water *ad libitum*.

Effect of capsaicin on intestinal thiamine absorption. Groups of 12–15 mice were used in each experiment. Mice were given the solution every day in the morning by oral administration. For control, they were given water in group I and vehicle (absolute ethanol: Tween 80:09% NaCl, 10:10:80) in group II. Group III and IV mice were fed with capsaicin at doses of 1 and 2 mg/kg BW/day, respectively. At the end of 12 weeks, mice were fasted overnight and weighed prior to performing the thiamine absorption experiment. The animal was anaesthetized with ether and the abdomen was cut open. A carefully measured 6 cm of upper jejunum was isolated and the intestinal loop was prepared as previously described (Hoyumpa *et al.*, 1975). A 0.5 mL solution of Krebs–Henseleit–HCO_3^- buffer (pH 7.4) containing 1.5 μM ^{14}C-thiamine HCl filled the loop. After 30 min, the loop was excised and the content was flushed with 5 mL buffers and the volume was measured. The exact length in cm of the loop was measured. The loop was opened lengthwise and shaken vigorously in a flask containing 5 mL buffer. Aliquots of both washings and the initial test solution were each counted in scintillation fluid by a liquid scintillation counter (LKB, Rack beta 1219). The rate of thiamine disappearance from the intestinal lumen and the percent inhibition were calculated.

Electron microscopic study. At the end of 12 weeks, the animals (4–5 mice/group) were fasted overnight, weighed and anaesthetized with ether. The abdomen was cut open and the small intestine was removed rapidly. A portion around the mid-point of the small intestine was cut into short pieces. The specimens were

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Table 1. Effect of capsaicin on intestinal thiamine absorption in mice[a]

Treatment	Rate of thiamine disappearance[b] from intestinal lumen (ng/cm/5 min)	Inhibition (%)
Control (water)	6.25 ± 0.13	—
Control (vehicle)	6.28 ± 0.10	—
Capsaicin (1 mg/kg BW/day)	6.11 ± 0.21	2.70 ± 1.44
Control (water)	6.22 ± 0.11	—
Control (vehicle)	6.35 ± 0.08	—
Capsaicin (2 mg/kg BW/day)	5.86 ± 0.06[c]	12.46 ± 1.35

[a] All groups were fed with water or vehicle or capsaicin for 12 weeks.
[b] Rate of thiamine disappearance from the lumen is compared between the control (vehicle) and capsaicin-treated groups. Significant value (Student's *t*-test) is indicated by asterisk:
[c] $p < 0.001$.
Values are mean ± SEM of 8–10 mice/group.

rapidly immersed in cold 2.5% glutaraldehyde in cacodylate buffer. The wall of the jejunum was cut open and diced perpendicular to the luminal surface into small blocks about 1 mm^3. The blocks were transferred into cold fresh fixative for 1 h. Subsequently, they were washed in cacodylate buffer, post-fixed in 1% OsO_4 in cacodylate buffer, dehydrated in graded ethanol and embedded in Araldite 502 epoxy resin. Thick and thin sections were cut on a Sorvall MT-2 ultramicrotome. Thick sections (1 μm) were stained with methylene blue for routine examination by light microscopy, and the jejunal villi were selected for thin sectioning. Thin sections were stained with uranyl acetate and lead citrate. They were examined and photographed by using a Hitachi H-300 electron microscope.

RESULTS

Body weight

The differences in the growth rate between the control groups and capsaicin-treated groups (1 and 2 mg/kg BW/day) for 12 weeks were not statistically significant (data not shown).

Effect of capsaicin on intestinal thiamine absorption

Table 1 shows the intestinal thiamine absorption of mice fed with capsaicin 1 and 2 mg/kg BW/day for 12 weeks. In mice fed with capsaicin 1 mg/kg BW/day for 12 weeks, thiamine absorption of the jejunum was slightly inhibited (by 2.7%) when compared with that of the control (vehicle) mice. On the other hand, in mice fed with capsaicin 2 mg/kg BW/day for 12 weeks, the rate of thiamine absorption was significantly inhibited by 12.5% ($p < 0.001$) when compared with that of the control (vehicle).

Ultrastructural changes in the villus absorptive cells

Control animals. The villus columnar cells showed long slender microvilli, a few mitochondria with well-defined cristae and a few profiles of rough endoplasmic reticulum (Fig. 1). Lysosomes were occasionally seen in the apical cytoplasm.

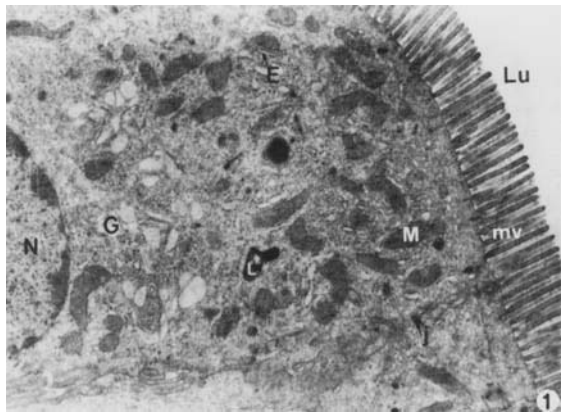

Figure 1. Electron micrograph of villus absorptive cells of the jejunum of a control mouse showing nucleus (N), golgi complex (G), rough endoplasmic reticulum (E), mitochondria (M), lysosome (L) and intercellular junction (j). Note abundant slender microvilli (mv) lining the jejunal lumen (Lu). (×6300).

Capsaicin-treated animals. In mice fed with capsaicin 1 mg/kg BW/day for 12 weeks, the villus absorptive cells underwent many alterations (Fig. 2). The cytoplasm was increased in density, it showed marked proliferation of rough endoplasmic reticulum. The number of mitochondria in most cell profiles was generally increased when compared with that of the control (data not shown). Some mitochondria in the columnar cells still have dilated cristae, the remainder showed no observable changes (Fig. 2). Residual bodies were occasionally seen in the apical cytoplasm, providing evidence of autophagocytosis. Figures 3 and 4 show the ultrastructural changes of the villus absorptive cells in mice fed with capsaicin 2 mg/kg BW/day for 12 weeks. Mitochondria with dilated cristae were more numerous when compared with those of the control. Degenerating mitochondria frequently showed myelin figures in the vicinity of the dilated cristae. Some of them showed intramatrical electron-dense particles and disruption of the mitochondrial membranes (Fig. 4). Many autophagic vacuoles and lysosomes appeared in the apical cytoplasm. The height of the microvilli of the

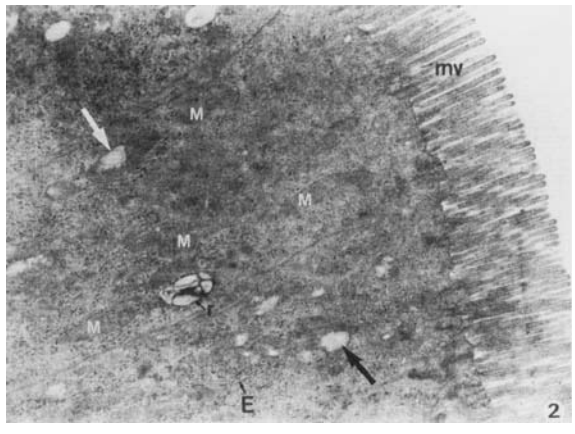

Figure 2. Electron micrograph of the apical part of villus absorptive cells from a mouse fed with capsaicin 1 mg/kg BW/day for 12 weeks. Note an increase in number of mitochondria (M) and amount of rough endoplasmic reticulum (E). Most mitochondria appear to be normal, some still show dilated cristae (arrows). A residual body (r) is seen in the cytoplasm. (×8850).

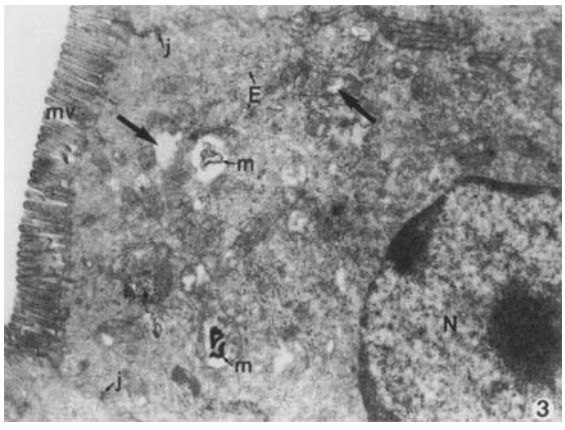

Figure 3. Electron micrograph of the upper part of the villus columnar cell of a mouse fed with capsaicin 2 mg/kg BW/day for 12 weeks. Most mitochondria show dilatation of some cristae (arrows). Myelin figures (m) are frequently found in the degenerating mitochondria. Note the lowering of the height of microvilli (mv). The nucleus (N), rough endoplasmic reticulum (*E*) and intercellular junction (j) appear to be unchanged. (×8750).

columnar cell (Fig. 3) was apparently lower than that of the control (Fig. 1). The nucleus and rough endoplasmic reticulum showed no observable alterations.

DISCUSSION

The growth rate of mice fed with capsaicin in the amount consumed by Thai people (1 mg/kg BW/day) or even at a higher level (2 mg/kg BW/day) for 12 weeks in this study was not different from that of the control (vehicle) mice. These results are in accordance with those of other investigators who reported that long term feeding of red pepper and capsaicin did not influence the growth rate in rats (Nopanitaya, 1973; Srinivasan *et al.*, 1980; Soontornchai *et al.*, 1989). This is in contrast to the finding by Monsereenusorn (1983) who reported that there was a significant reduction of the growth rate of rats fed with capsaicin. His results may be due to the high amount of capsaicin given to the rats exerting its effect on intestinal absorption of nutrients. As reported by Nopanitaya (1973), the slow growth rate seen in rats fed with a low protein diet supplemented with capsaicin may be accounted for by the reduction of absorptive ability of the epithelial cells of the small intestine.

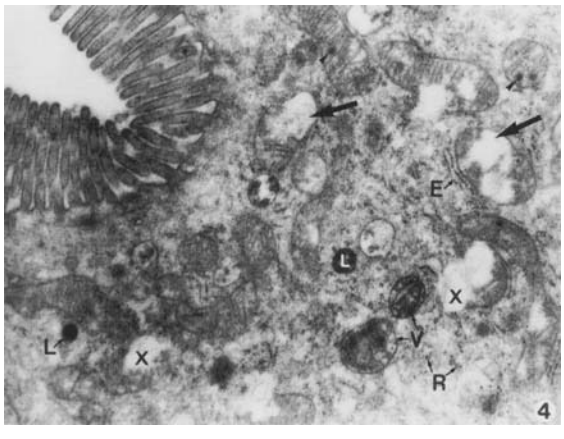

Figure 4. A higher magnification of the apical part of the villus columnar cell of a mouse fed with capsaicin 2 mg/kg BW/day for 12 weeks. Most mitochondria are swollen with loss of cristae and normal contour (arrows). Some mitochondria show disruption of outer and inner mitochondria membranes (X) and precipitation of electron dense material (arrow heads) in the mitochondrial matrix. Many lysosomes (L) and autophagic vacuoles (V) are evident. Rough endoplasmic reticulum (E) and free ribosomes (R) are still intact. (×13 350).

Considering the effect of capsaicin given to mice by oral feeding for 12 weeks, our results showed that capsaicin 1 mg/kg BW/day caused an inhibition of thiamine absorption in the jejunum by 2.7% (Table 1). It was shown earlier that the maximum inhibitory effect of capsaicin 1 mg/kg BW/day on the thiamine absorption occurred at 1 week after capsaicin administration (Hatthachote, 1992). The inhibition was reduced at the end of 8 weeks and tended to return to the normal thiamine absorption at the end of 12 weeks. This may be attributed to the intestinal adaptation of mice to the toxic effect of capsaicin. With regard to the effect of capsaicin on the morphology of the small intestine at light microscope level, our studies showed that no morphological alterations were observed in the absorptive area of the jejunum (data not shown). This may be explained by a decrease in the concentration of a given dose of capsaicin in the gastrointestinal lumen *in vivo* resulting from the absorption of capsaicin in the stomach (Kawada *et al.*, 1984) and dilution by gastrointestinal fluid (Nopanitaya and Nye, 1974; Soontornchai *et al.*, 1989). Perhaps capsaicin provoked early changes, but after a period of time the epithelial absorptive cells may adapt to it and gradually showed no signs of damage detectable at light microscope level. Our studies showed the adaptation of the intestinal epithelial absorptive cells of mice to the toxic effect of capsaicin by increasing the amount of mitochondria and rough endoplasmic reticulum (Fig. 2). This ultrastructural evidence could, at least partly, be related to the non toxic effect of capsaicin on the intestinal absorption of thiamine.

In mice fed with capsaicin at a dosage of 2 mg/kg BW/day for 12 weeks, thiamine absorption was significantly inhibited by 12.5% (Table 1). This value was higher than that of the lower capsaicin concentration (1 mg/kg BW/day) at the same period of time, suggesting that interference with the intestinal thiamine absorption was dependent on capsaicin concentration rather than on the duration of capsaicin feeding. The cytotoxic effect of capsaicin on mouse intestinal absorptive cells was apparently evident. Our results on the electron microscopy showed that quite a few mitochondria were altered (Figs. 3 and 4) and the microvilli of the villus absorptive cells were shorter (Fig. 4). Mitochondria showed dilatation of cristae and disruption of the mitochondrial membranes. Degenerating mitochondria were destroyed by auto-phagocytosis as evident from the presence of primary lysosomes and residual bodies which are rarely found in the normal intestinal absorptive cell. Consequently, it is likely that the toxic effect of capsaicin (2 mg/kg BW/day for 12 weeks) on the intestinal thiamine absorption in mice is brought about by a reduction of intestinal mucosal ATP content and surface area of the microvilli.

In conclusion, the inhibitory effect of capsaicin on intestinal thiamine absorption in mice is well correlated to the ultrastructural alterations of the intestinal absorptive cells.

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