Effect of Calcium Pantothenate on the Diurnal Rhythm and Rate of Epithelial Cell Proliferation in the Gastric Mucosa of Rats

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Recent years have seen an increasing number of applied studies on the use of pantothenic acid (the vitamin component of coenzyme A) and its analogs in various areas of medicine [4]. In a number of studies, evidence has been obtained that pantothenates afford protection to epithelial tissues, in particular by accelerating wound healing [7, 11, 14] and by preventing damage to the gastric and intestinal mucosas in radiation sickness [2]. Epithelial ulceration in the upper gastrointestinal tract due to a deficiency of this vitamin has also been reported [12, 15], as have been beneficial clinical effects of pantothenate-containing drugs in gastrointestinal ulcers [6, 10]. However, the cellular mechanisms underlying these effects have not been fully elucidated.

This study was undertaken to test calcium pantothenate for its impact on cell proliferation in the gastric mucosal epithelium of rats.

MATERIALS AND METHODS

Four groups of randomly bred rats weighing 220-240 g were used. Groups 1 and 3 served as controls, while groups 2 and 4 were injected with calcium pantothenate intraperitoneally in a pharmacotherapeutic dose of 30 mg/kg body weight once daily for 10 days before sacrifice [5]. Groups 1 and 2 were injected with ^{3}H-thymidine intraperitoneally in a dose of 3.7 MBq (0.1 ml) per 100 g body weight every 6 h for 24 h before sacrifice. In groups 3 and 4, five rats were killed at a time every 3 h over a period of 24 h after being given vinblastine at 2.5 mg/kg body weight and ^{3}H-thymidine at 3.7 MBq (0.1 ml) per 100 g body weight 3 h and 1 h before being killed, respectively. In all tests, ^{3}H-thymidine had a specific activity of 0.85 TBq. For histological examination, the rat stomachs were fixed in Carnoy's fluid, and autoradiographs were prepared. The mitotic activity (the number of colchamine-blocked mitoses over 3 h [MIch]) and the nuclear labeling index (NLI) were determined by examining, in each specimen, 50 longitudinally sectioned fundic or pyloric glands and were expressed in pro mille. The size of the proliferative pool (growth fraction) was expressed in percent of the total number of epithelial cells. The Fisher-Student test was used for statistical treatment of the results.

RESULTS

In control rats, the number of DNA-synthesizing cells per 24 h in the fundic epithelium was 2.7 times lower than in the pyloric epithelium. In test (calcium pantothenate-treated) rats, the size of the proliferative cell pool in the gastric mucosal epithelium was smaller than in control rats. Differences in the size

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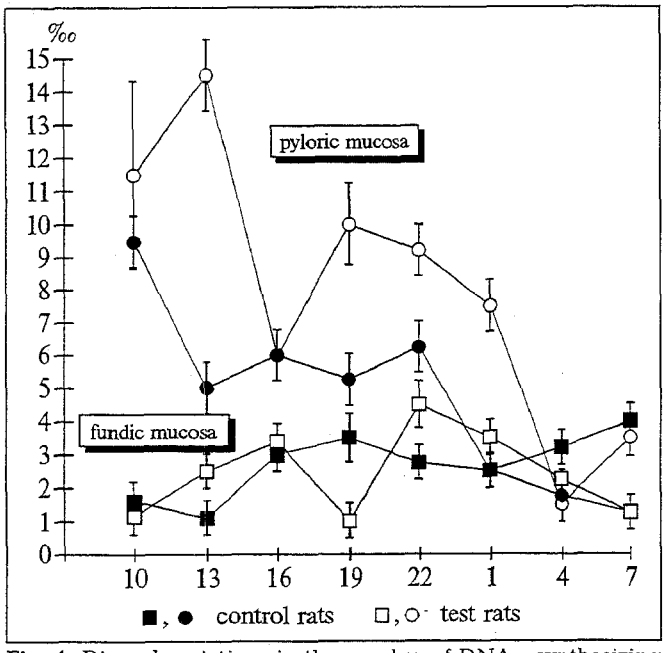

Fig. 1. Diurnal variations in the number of DNA-synthesizing epithelial cells in the gastric mucosa of control and test (calcium pantothenate-treated) rats.

of the proliferative pool between the control rats of group 1 and the test rats of Group 2 were statistically insignificant in the fundic epithelium and significant in the pyloric epithelium, where the growth fraction was decreased by 16.9% in the test group ($p<0.05$).

Diurnal variations in the rhythm of DNA synthesis and in the number of mitotic epithelial cells are shown in Figs. 1 and 2, respectively. In control rats, DNA-synthetic activity of fundic epithelial cells was characterized by a single-peak NLI curve with a maximum at 01 h (NLI=43.95‰) and a minimum at 10 h (NLI=10.01‰) (the mean diurnal NLI was 18.58‰), while that of pyloric cells had two peaks: the first peak occurred at 22 h (NLI = 83.48‰) and was followed by a decline (55.61‰) at 01 h and by a second peak at 04 h (65.82‰), which was succeeded in turn by a decrease of the NLI to a minimum (49.15‰) at 07 h followed again by a rise that started at 10 h (the mean diurnal NLI was 65.27‰). The higher mean diurnal value of the NLI in the fundic cells of control rats thus corresponded to the results obtained in experiments determining the proliferative pool for these rats.

The calcium pantothenate-treated rats differed from the controls in the diurnal rhythm of DNA synthesis, as shown in Fig. 1. Thus, the NLI of fundic cells was at its peak (24.62‰) at 04 h and had its lowest values at 16 h (9.42‰) and 07 h (9.75‰); the mean diurnal NLI was as low as 13.71‰, and the absolute values of this index between 13 and 19 h and between 22 and 01 h were significantly lower than in the controls. The amplitude of the DNA-synthetic rhythm was only 2.53‰ as compared to 4.39‰ in the controls, indicating that calcium pantothenate desynchronized the entry of cells into the synthetic stage of the mitotic cycle.

The DNA-synthetic activity of pyloric cells in test rats showed three peaks, occurring at 13, 22, and 04 h (NLI values were 68.19, 63.26, and 69.74‰, respectively) and had lowest values at 19, 01, and 10 h (35.72, 43.14, and 43.79‰, respectively). The mean diurnal NLI was also lower (by 15.5%) than in the control rats, and significant reductions in the absolute values of this index were recorded between 16 and 01 h and at 10 h, although the amplitude of variation in the number of labeled nuclei was not significantly altered as a result of calcium pantothenate treatment.

In control rats, the mitotic activity of both fundic and pyloric cells displayed a diurnal rhythm with two peaks. In the fundic epithelium, the highest MIch values were recorded at 19 h (3.65‰) and 01 h (4.57‰) and the lowest at 22 h and 10 h (1.0 and 1.02‰, respectively). The mean diurnal MIch value was 2.42‰. In the pyloric epithelium, mitotic activity was highest at 16 h (MIch was 14.20‰), declined substantially by 19 h (6.03‰), and began to rise again at 10 h, reaching a second peak at 11 h (9.89‰). The lowest MIch value was recorded at 07 h (1.64‰). The mean diurnal MIch in the pyloric epithelium was 7.93‰. This epithelium also showed a high amplitude of diurnal variation in MIch (8.66‰ vs 4.57‰ in the fundic epithelium). These findings agree with those reported by other authors [9] and indicate that

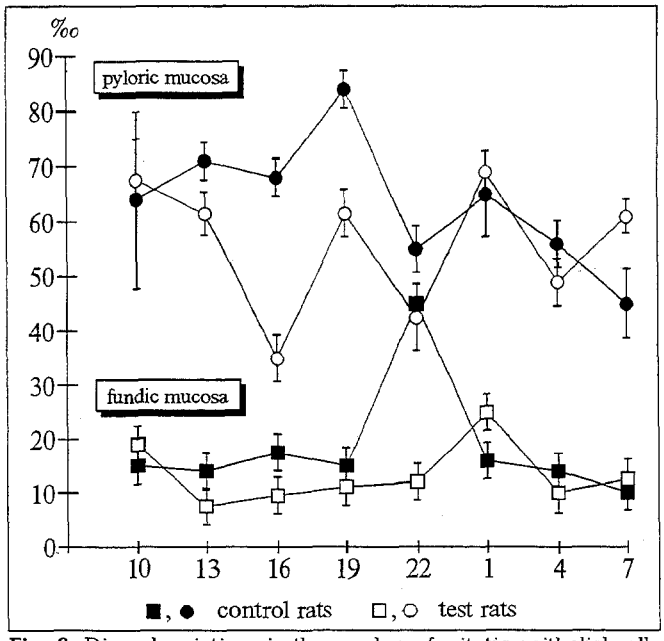

Fig. 2. Diurnal variations in the number of mitotic epithelial cells in the gastric mucosa of control and test (calcium pantothenate-treated) rats.

proliferative activity is higher in the pylóric than in the fundic epithelium. In our study, the mean time of epithelial cell renewal in the pylorus was only 15.7 days as compared with 51.6 days in the fundus.

Calcium pantothenate treatment altered the times at which mitotic activity had its highest and lowest values (Fig. 2). In test rats, the mean MIch decreased to 1.97‰ in the fundic epithelium and to 5.24‰ in the pyloric epithelium, and, as compared with the controls, the absolute values of mitotic activity were significantly lower at 16 h and 01 h in the fundic epithelium and at 16 h and 10 h in the pyloric epithelium. Calcium pantothenate also delayed cell renewal (by 11.9 days and 8.9 days in the fundic and pyloric epithelia, respectively) and decreased the amplitude of diurnal variations in mitotic activity, particularly (2.5-fold) in the pyloric epithelium.

The present study has thus shown that calcium pantothenate in a pharmacotherapeutic dose desynchronizes the entry of cells into the DNA-synthetic phase of mitosis and reduces the rate of cell proliferation in the gastric mucosal epithelium of rats. Our findings argue against the view that pantothenates enhance mitotic activity [11]. The antiinflammatory and cell-protecting actions of this vitamin appear to be mediated by other mechanisms.

Since alterations in proliferative activity similar to those observed in this study have been reported to be caused by hydrocortisone in mucocytes of the gastric fundus [1], and since pantothenates have been found to possess steroidogenic activity [8, 13]; it seems likely that calcium pantothenate exerts its effects on cell reproduction by altering the level of corticosteroid secretion by the adrenal glands. One cannot, however, rule out direct effects of this compound on histone acetylation processes, which are known to determine the transcription activity in cells during their transition from the resting state to the state of proliferation [3].

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