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Quantitative Histochemical Study of a Vitamin E Analog on Rat Gastric Cyclic AMP[1] (38894)

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It was reported by Schroeder (1) that 3 alpha-tocopherol analogs which lacked the polyisoprenoid side chain (2,2,5,7,8-pentamethyl-6-hydroxychroman; 2,2,5,7,8-tetramethyl-5,6-ortho-quinonechroman; 2,2,7,8 - tetramethyl - 5 - (α - ethoxy) - methyl-6-hydroxychroman) are noncompetitive inhibitors *in vitro* of beef liver cyclic AMP and cyclic GMP phosphodiesterases (PDE), although alpha-tocopherol itself is not. It was also found that vitamin E-deficient rats had an elevated liver cyclic AMP-PDE activity compared with normal controls.

It might be expected that administration of vitamin E analogs that inhibit PDE *in vitro* would suppress PDE *in vivo* and thereby elevate the cyclic nucleotide concentrations in the tissues. In the present study, this possibility was tested with the most inhibitory of the analogs (2,2,7,8-tetramethyl-5,6-ortho-quinonechroman), which Schroeder found to inhibit the cyclic AMP enzyme by 71%, compared with 20% for theophylline at the same concentration (0.1 m*M*) and under the same conditions.

We had shown earlier that the highest stomach concentration of cyclic AMP in the fasted rat occurs in the parietal-mucous neck cell region (2), and that injection of drugs that stimulate gastric secretion (pentagastrin, urecholine, theophylline, and histamine) elevates the cyclic AMP level in this region, while the secretory inhibitors (atropine, prostaglandin E_2) have little influence on it (3). That this elevation of cyclic AMP is related to stimulation of the parietal rather than of the mucous neck cells, is suggested by the finding that the acid-stimulating drugs causing this elevation do not appreciably increase cyclic AMP in the mucus-secreting epithelial zone.

While our evidence is in accord with involvement of cyclic AMP in the acid secretion in the rat stomach, unresolved questions and conflicting viewpoints remain. These matters have been discussed in a recent review by Kimberg (4).

Materials and Methods. Male albino Sprague–Dawley rats (350–450 g) were caged separately in a controlled-temperature room (22 ± 1°) with regulated lighting (on 6 AM, off 6 PM) and were fed Purina Laboratory Chow and water ad lib. They were deprived of chow but not water for 24 hr before intraperitoneal injection of the vitamin E analog, as indicated in Fig. 1, and killed by guillotine decapitation between 9:30 and 10:00 AM. The glandular stomach was quickly removed, cut open, emptied, pinned flat, and frozen with dry ice as in earlier work (2). It required about 90 sec to obtain the frozen tissue after death (15 sec to remove and freeze the stomach) and, as performed, no appreciable postmortem change in cyclic AMP content was found (2). In a cryostat at −15°, cylinders of the frozen tissue were bored through the wall of the body of the stomach with a drill press, transferred to a microtome, and serially sectioned (4 mm diam, 16 μm thick, 0.20 μl vol) from the epithelial surface down into the muscle. Four sections were taken for each cyclic AMP analysis, and the next adjacent section was taken for histological identification by toluidine blue staining following the procedure described in the earlier paper (2) and with our usual sectioning technique (5).

As in the procedure of Schroeder (1), the vitamin E analog was dissolved in ethanol

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and diluted with water. The final ethanol concentration was brought to 4% to obtain an approximately saturated solution of analog (1 mg/ml) that would provide sufficient compound for an intraperitoneal dose of 20 mg/kg in a practical volume (8 ml). Single injections of 8 ml of this solution and of a suspension of the analog (6.5 mg/ml) in 4% ethanol were given to separate rats.

The luminescence method for measurement of cyclic AMP has been described (2), and a subsequent simplification of the preparation of the myokinase reagent used was given (3). As before, concentrations of cyclic AMP were based on four tissue sections and also on micrograms protein–nitrogen, and the protein precipitated by $HClO_4$ extraction of cyclic AMP in the samples was determined by the bromsulfalein-binding method (6).

Results. The quantitative distribution of cyclic AMP in the histological regions of the stomach wall is shown in Fig. 1. The saline control curve is included for reference, and it was found earlier that there is no significant difference between the curves derived from stomachs of untreated rats and of those injected either subcutaneously or intraperitoneally with 0.9% NaCl (3).

Statistical analysis of the data shows that the differences between the corresponding values in the saline and 4% ethanol control curves is significant in the peak parietal region (P = 0.015, 0.009, in upper and lower graphs of Fig. 1, respectively), less so in the epithelial zone (P = 0.25, 0.15), and apparently not significant in any of the other regions (P = 0.6–0.8).

Compared with the ethanol control curve, administration of the vitamin E analog in a practically saturated solution (20 mg/kg) produced little significant effect on the cyclic AMP concentration in any histological region (P = 0.2–0.6). When a suspension of the analog (130 mg/kg) was injected, the effect was essentially the same, and even the increase in the mean concentrations in the chief cell zone and muscle had a significance only of P = 0.8, 0.4, and 0.6, 0.4, respectively, for the values in the upper and lower graphs Fig. 1.

These effects of the vitamin E analog contrast with those of theophylline in a similar

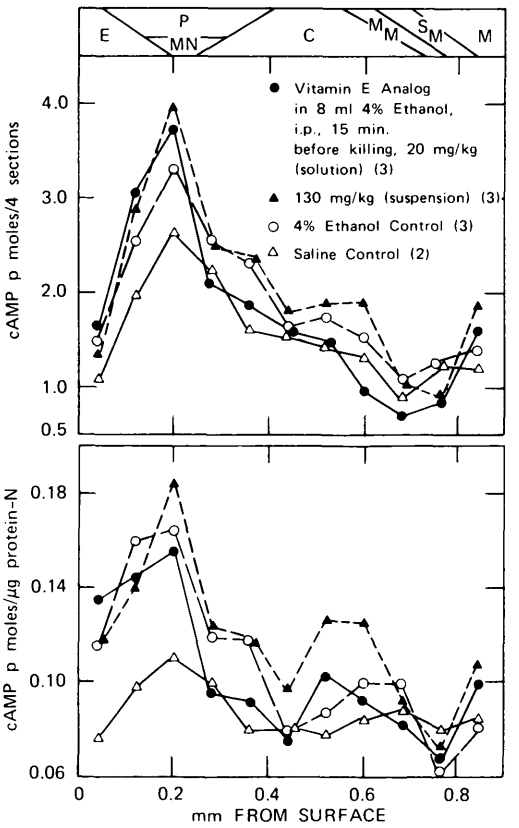

FIG. 1. Effects of injections of vitamin E analog on the quantitative histological distribution of cyclic AMP in the body of the rat glandular stomach. The ethanol solvent control curve is given with that for physiological saline solution to demonstrate the influence of the 4% ethanol alone. The points on curves represent mean values from experiments on separate stomachs, the number of which is indicated in parentheses. Histological regions are designated: E, epithelial; P, parietal; MN, mucous neck cell; C, chief cell; MM, muscularis mucosa; SM, submucosa; and M, muscle. Circular fresh-frozen microtome sections (4 mm diam, 16 µm thick, 0.20 µl vol) used for analysis.

experiment in which it, too, was injected intraperitoneally 15 min before killing, but gave an increase of about 100% in the cyclic AMP level in the parietal peak region (3). The effects reported here also contrast with what might have been expected from 71% inhibition of PDE by the analog *in vitro* (1).

The results obtained from injection of 0.4% ethanol alone which showed significant increases in cyclic AMP in the peak parietal region are of interest in relation to other

work on whole mucosa homogenates of rat stomach in which it was found that ethanol (5–20%) placed in the stomach for up to 15 min did not noticeably change cyclic AMP concentrations, that ethanol (2–20%) significantly inhibited PDE *in vitro*, and that *in vitro* and *in vivo* 20% ethanol inhibited the mucosal adenyl cyclase (7). The increase in the cyclic AMP level observed in the present study may have been due to a proportionally greater inhibition of PDE than of the adenyl cyclase. It should be noted that in our work the cyclic AMP increase occurred predominantly in the parietal cell zone and that the effect measured would have been diluted if a homogenate of the total mucosa had been analyzed as in the study cited (7).

Summary. The vitamin E analog, 2,2,7,8-tetramethyl-5,6-orthoquinonechroman, unlike vitamin E itself, had been reported to be a potent inhibitor of beef liver cyclic AMP-PDE *in vitro* (about 3.5 times more inhibitory than theophylline under the same conditions). In this *in vivo* study, single intraperitoneal injections were given to fasted rats, 15 min before killing, of a solution of 20, and of a suspension of 130, mg analog/kg in 4% ethanol. No significant increases over the 4% ethanol control values in cyclic AMP levels were produced in any of the histological regions of the glandular stomach. However, injection of the 4% ethanol itself produced a rise of about 50% (peak value 4.1 pmoles/mg wet wt, 0.16 pmoles/μg protein-nitrogen) in the cyclic AMP concentrations in the parietal cell region and had no significant effect in any of the other histological zones where the concentrations were much lower.

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