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EFFECT OF DEXAMETHASONE ON THE PEPTIC ACTIVITY OF GASTRIC LUMEN AND MUCOSA

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Abstract—Dexamethasone (9α-fluoro-16α methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione-21-phosphate), a synthetic glucocorticoid, has a dual role on pepsinogen content of the gastric lumen and mucosa as measured by its peptic activity. Following stimulation the luminal peptic activity gradually decreases after 6 hr, then returns to basal levels at 18 hr and by 24 hr is inhibited by 50%. The luminal peptic activity induced by the secretory compound mercaptomethylimidazole (MMI) is also decreased. Dexamethasone effect on both basal and MMI-induced peptic activity can be reproduced by cycloheximide or puromycin, translational blockers of protein synthesis. This drug also has an independent time and dose-dependent inhibitory effect on gastric mucosal peptic activity which does not correlate with increased peptic activity of the lumen. Dexamethasone appears to be more effective than hydrocortisone and corticosterone in inhibiting the basal peptic activity of both lumen and mucosa. The inhibitory effect of this drug on tissue peptic activity is not mediated through induction of any inhibitory protein as evidenced by the insensitivity of the effect to actinomycin D. Studies on [^{14}C]phenylalanine incorporation into gastric protein indicate that the effect of dexamethasone on tissue pepsinogen content is not due to a generalized block of protein synthesis.

High doses of synthetic glucocorticoids such as dexamethasone, betamethasone and prednisolone are routinely used clinically for different pathological conditions. Among various tissues, gastric mucosa appears to be highly sensitive to glucocorticoids which cause gastric hyperacidity [1] and ulceration [2, 3]. The effect of glucocorticoids on gastric secretion and on different gastric enzymes are not firmly established. Corticotropin (ACTH†) and cortisone stimulate the volume of secretion and acidity without affecting pepsin activity [4]. Long-term administration of dexamethasone in pylorus-ligated rat shows an anti-secretory effect and a rise of pH, but single doses stimulate gastric secretion [5]. In adrenalectomized dogs, after cortisone administration, pepsin and the volume of secretion do not show significant change but acid secretion is significantly stimulated [6]. Prednisolone causes more than a three-fold increase in pepsin secretion [7]. The objective of the present study is to investigate critically the effect of dexamethasone on the peptic activity of the gastric lumen as well as its level in the gastric mucosa. Mercaptomethylimidazole (MMI), a newly established inducer of gastric secretion [8, 9], has been used to study the role of dexamethasone on the MMI-induced peptic activity of the gastric lumen. The present studies indicate that dexamethasone administration initially stimulates gastric luminal peptic activity but finally blocks the basal activity as well as the activity induced by MMI. Evidence is also presented to show that dexamethasone has an independent inhibitory effect on the mucosal peptic activity (tissue pepsinogen content) and the effect is not due to a generalized block of protein biosynthesis of the gastric mucosa.

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† Abbreviations: MMI, mercaptomethylimidazole; ACTH, adrenocorticotropic hormone; TCA, trichloroacetic acid.

MATERIALS AND METHODS

Materials

Porcine pepsin, cycloheximide, puromycin, actinomycin D, MMI, bovine serum albumin, hydrocortisone and corticosterone were procured from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Dexamethasone (decadron) was purchased from Merind Ltd (India) and all other reagents used were of analytical grade.

Methods

Male Wistar rats (120–150 g) were used throughout the experiments. As the measurement of the pepsin activity of the gastric lumen and mucosa is affected by food or water intake and associated gastric emptying, the animals were deprived of food and water for 24 hr before administration of the drug. Both the control and drug-treated (i.m.) animals were killed by cervical dislocation usually after 24 hr unless otherwise stated. After opening the abdomen, both the esophageal and pyloric ends of the stomach were ligated to block the loss of gastric fluid during dissection of the stomach. The gastric fluid was immediately (1 min) collected by flushing the stomach cavity with 2 mL of 0.9% saline through the pyloric end [8–10]. It was centrifuged at 5000 *g* for 10 min in a RC5B refrigerated Sorvall centrifuge. The clear supernatant was collected, the volume recorded and its peptic activity then measured.

Assay of pepsin activity. Pepsin activity of the luminal fluid was measured using porcine pepsin (1730 U/mg) as standard [8–10] according to the method of Schlamowitz and Peterson [11] and expressed as total unit of the gastric fluid collected. For the estimation of tissue peptic activity, a 5% homogenate was prepared from the fundic stomach in sucrose phosphate buffer (0.25 M sucrose, 0.05 M sodium phosphate buffer, pH 7.2) and the homogenate was spun at 12,000 *g* for 10 min in the Sorvall centrifuge. The postmitochondrial supernatant was assayed for pepsin activity of the tissue pepsinogen as described [8–10]. One unit of activity is defined as the amount which will give an absorbance of 0.014 at 280 nm under the standard assay condition. The peptic activity was expressed as U/g of stomach.

[^{14}C]Phenylalanine incorporation into gastric protein. [^{14}C]Phenylalanine (10 μCi) was administered to each rat 30 min after dexamethasone (1 mg/kg) administration (i.m.). The animals were killed after 24 hr and the postmitochondrial supernatant was prepared as described for the pepsin assay. Protein from an aliquot of this supernatant (1 mL) was precipitated with trichloroacetic acid (TCA) (final concentration 5%) and was washed three times with 3 mL of a mixture of ether–ethanol (1:3). The precipitate was finally dissolved in 3 M KOH and used for counting in a Beckman scintillation counter. The incorporation of [^{14}C]phenylalanine into gastric protein was expressed as cpm/g of protein. Protein was determined according to Lowry *et al.* [12].

Statistical evaluation. All data were expressed as mean ± SE. Significance was calculated from Student's *t*-test. In the case of multiple comparison, analysis of variance followed by an appropriate multiple comparison test was performed for the calculation of significance.

RESULTS

Effect of dexamethasone on peptic activity of gastric lumen and mucosa

Dexamethasone administration has a dual effect on the pepsin content of the gastric lumen as shown in Fig. 1. The luminal pepsin content was increased by 50% at 3 hr over the basal value and the content attained the peak value (300%) at 6 hr. This is followed by a gradual decline reaching the basal value at 18 hr, and falling to 50% after 24 hr. Figure 1 also shows the corresponding time course of pepsinogen content of the gastric mucosa under identical conditions. No significant inhibition of tissue pepsinogen content was observed up to 3 hr after drug administration and only about 20% inhibition was observed after 6 hr when the luminal content reaches the peak level. Almost 65% inhibition of tissue content was observed after 18 hr when the luminal content attains the basal level. The result thus shows no correlation between luminal pepsin content and inhibition of tissue pepsinogen level following dexamethasone administration. Dexamethasone itself has no direct effect on peptic activity when the control luminal fluid or the mucosal cytosolic preparation was preincubated with dexamethasone *in vitro* (data not shown).

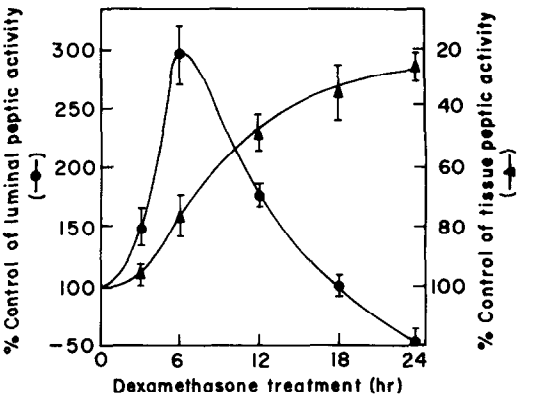

Fig. 1. Luminal and mucosal peptic activity after administration of dexamethasone. After intramuscular administration of dexamethasone (1 mg/kg body weight), the animals were killed at various time periods as indicated to collect the gastric fluid and to prepare postmitochondrial supernatant for the estimation of pepsin activity of tissue pepsinogen as described in Materials and Methods. The values used for normalization of total peptic activity of gastric fluid and tissue are 948 ± 130 and 210,007 ± 1382 U, respectively, and taken as 100%. N = 8–12.

We reported earlier that MMI *in vivo* induces high luminal content of pepsin activity [9, 10] which is insensitive to some established blockers of pepsinogen secretion [9]. MMI also induces a very high peptic activity in the gastric lumen of rat (more than 3-fold) but this induction is partially prevented by pretreatment at 24 hr with dexamethasone as shown in Table 1. The inhibitory effect of dexamethasone on MMI-induced peptic activity is similar to the effect of known protein synthesis blockers such as cycloheximide and puromycin. Both these translational blockers at the same dose used for dexamethasone (1 mg/kg body weight), inhibit MMI-induced luminal pepsin content almost to the same extent as did dexamethasone.

Figure 2 shows the dose-dependent inhibition of tissue pepsinogen content at 24 hr after administration of dexamethasone. The effect of cycloheximide was also studied for comparison. Dexamethasone at a dose of 0.25 mg/kg body weight could inhibit only 20% but 70% inhibition occurs at a dose of 1 mg/kg body weight. Cycloheximide appears to be slightly more potent than dexamethasone as it causes 40% inhibition at a dose of 0.25 mg/kg body weight and 82% inhibition at 1 mg/kg body weight.

In order to assess the effectiveness of dexamethasone, a long acting synthetic glucocorticoid, the effect of two naturally occurring glucocorticoids was also tested both on luminal pepsin content and tissue pepsinogen content as shown in Table 2. The result indicates that hydrocortisone and corticosterone are almost equally effective in inhibiting the peptic activity of both lumen and

Table 1. Effect of dexamethasone and protein translational blockers on basal and MMI-induced luminal peptic activity

	Peptic activity of gastric fluid (total units)
Basal	948 ± 130
+ MMI	3085 ± 516‡
+ MMI + dexamethasone	1413 ± 210§
+ MMI + cycloheximide	1592 ± 157§
+ MMI + puromycin	1809 ± 426§
+ Dexamethasone	520 ± 55*
+ Cycloheximide	400 ± 20*
+ Puromycin	617 ± 86†

Dexamethasone or translational blockers were administered intramuscularly with the same dose (1 mg/kg body weight) and after 24 hr, gastric fluid was collected as described in Materials and Methods for the estimation of basal pepsin activity. To study the effect of these drugs on MMI-induced luminal peptic activity, MMI (30 mg/kg body weight) was administered intraperitoneally 24 hr after administration of the above drugs; 2.5 hr after MMI administration, gastric fluid was collected for estimation of pepsin activity.

* $P < 0.001$, † $P < 0.05$ and ‡ $P < 0.001$ when compared with the basal value.

§ $P < 0.05$ (calculated from analysis of variance) when compared with the basal treated with MMI.

N = 8–12.

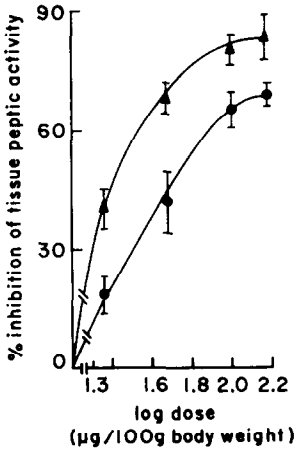

Fig. 2. Tissue peptic activity after administration of dexamethasone or cycloheximide. The animals were injected with varying doses of dexamethasone or cycloheximide as indicated. After 24 hr, the animals were killed and postmitochondrial supernatants measured for pepsin activity. Dexamethasone (●); cycloheximide (▲). N = 8–12.

mucosa but dexamethasone is more effective than these naturally occurring glucocorticoids.

Effects of inhibition of protein synthesis

The mode of inhibition of tissue pepsinogen content by dexamethasone is similar to that of translational blockers but unlike inhibition of transcription (Table 3). Dexamethasone inhibits about 70% of the tissue pepsinogen level after 24 hr. Cycloheximide inhibits almost to the same extent although puromycin is less effective under identical conditions. However, actinomycin D, the transcriptional blocker has no inhibitory effect under identical conditions. As the effect of dexamethasone on tissue pepsinogen level could not be reversed by pretreatment with actinomycin D, it is unlikely that dexamethasone-induced synthesis of protein is responsible for mediating the observed inhibition of tissue pepsinogen level.

In order to investigate whether the inhibitory effect of dexamethasone on mucosal pepsinogen content is due to a generalized block of protein synthesis, [^{14}C]phenylalanine incorporation into gastric protein was studied after administration of dexamethasone. Table 4 shows that dexamethasone has no significant inhibitory effect on protein synthesis in the gastric mucosa while it inhibits significantly the tissue pepsinogen content as indicated by the decrease in specific activity.

Table 2. Effect of other glucocorticoids on peptic activity of gastric lumen and mucosa

	Luminal peptic activity (total units)	Tissue peptic activity (U/g of stomach)
Control	948 ± 130	21,007 ± 2382
+ Hydrocortisone	620 ± 60*	12,200 ± 845*
+ Corticosterone	736 ± 42*	15,213 ± 1200†
+ Dexamethasone	520 ± 55	6269 ± 320*

Twenty-four hours after intramuscular administration of hydrocortisone or corticosterone or dexamethasone at a dose of 1 mg/kg body weight, the animals were killed and gastric fluid was collected immediately by flushing the gastric lumen with saline as described in Materials and Methods. The fundic stomach from control and drug-treated animals was homogenized to get the postmitochondrial supernatant for the estimation of pepsin activity as described in Materials and Methods.

* $P < 0.02$ vs control. † $P < 0.05$ vs control.

N = 10–12.

DISCUSSION

We have presented evidence showing that dexamethasone has a dual effect on the peptic activity of gastric lumen. A short term stimulatory effect is followed by a longer term inhibitory effect which also persists when the luminal peptic activity is not induced by MMI. MMI, an antithyroid drug of the thionamide group, is known to induce independently high acidity and peptic activity in the luminal gastric secretion [8, 9]. The present study indicates that 24 hr pretreatment with dexamethasone can block the MMI-induced peptic activity of the gastric lumen significantly. Since dexamethasone causes nearly 75% loss of tissue pepsin content after 24 hr (Fig. 1), MMI is unable to induce its secretion

Table 3. Effect of protein synthesis blockers on tissue peptic activity

	Tissue peptic activity (U/g stomach)
Control	21,007 ± 2382
+ Dexamethasone	6269 ± 320*
+ Cycloheximide	4663 ± 625*
+ Puromycin	14,732 ± 1833†
+ Actinomycin D	21,313 ± 1685‡
+ Actinomycin D + dexamethasone	7823 ± 1135*

Dexamethasone or protein synthesis blocker was administered intramuscularly with a dose of 1 mg/kg body weight. Dexamethasone was administered 30 min afgter administration of actinomycin D at the same dose. After 24 hr, tissue was processed for postmitochondrial supernatant to estimate pepsin activity.

* $P < 0.001$; † $P < 0.05$ when data were compared with the control.

‡ Not significant when data of actinomycin D treated rat were compared with the control.

N = 10–12.

Table 4. Effect of dexamethasone on protein biosynthesis and peptic activity of gastric mucosa

	[^{14}C]Phenylalanine incorporation (cpm/g)	Tissue peptic activity (U/g)
Control	28,330 ± 1220	105,030 ± 6600
+ Dexamethasone	23,987 ± 1164†	34,800 ± 3100*

[^{14}C]Phenylalanine (10 μCi) was administered (i.m.) to each rat 30 min after administration of dexamethasone (1 mg/kg). The rest of the procedure was described in Materials and Methods. The postmitochondrial supernatant was assayed for tissue peptic activity which was expressed as U/g of protein.

* $P < 0.001$ when data was compared with control.

† Not significant when compared with control.

N = 8–12.

due to the shortage of a ready-made pool of zymogen granules in the chief cells. The inhibition of basal peptic activity of the gastric lumen by dexamethasone or by other translational blockers after 24 hr may be due to inhibition of pepsinogen biosynthesis thereby depleting zymogen granules within the chief cells. However, dexamethasone-mediated increased peptic activity in the lumen during the initial 6 hr is not associated with any significant loss of tissue content. The mechanism of this short-term induction remains to be investigated.

Dexamethasone also inhibits tissue pepsin content but this is not due to augmented rate of secretion during the initial hours, as the stimulation of luminal pepsin activity and inhibition of tissue pepsin content are not correlated in time (Fig. 1). This raises the possibility that dexamethasone blocks pepsinogen biosynthesis leading to the long-term (24 hr) shortage of the tissue content of pepsinogen. There are numerous reports in the literature that glucocorticoid effects are mediated through the synthesis of new protein which inhibits different enzymes [13–16]. Such an effect associated with new mRNA formation is sensitive to inhibition by transcriptional blockers such as actinomycin D. However, simultaneous administration of actinomycin D with dexamethasone does not augment the tissue content of pepsinogen to the control value (Table 3). This eliminates the possibility of the involvement of induced protein synthesis in dexamethasone-mediated decrease in pepsinogen content of the gastric mucosa. Moreover, dexamethasone cannot cause a generalized block of protein synthesis in the gastric mucosa as there is no significant decrease in [^{14}C]phenylalanine incorporation into gastric proteins. Thus, the decrease in tissue pepsin content cannot be explained by a generalized block of protein biosynthesis by dexamethasone. As the fall in tissue content is not accounted for by the secretion into the lumen (Fig. 1), this suggests that either pepsinogen biosynthesis is specifically blocked or there is increased degradation of this protein or both. By western blot analysis using purified immunoglobulin G (IgG) specific for pepsin, we have observed (data not presented) that 35 kDa pepsin and 42 kDa pepsinogen bands are significantly reduced after dexamethasone

and cycloheximide treatment, indicating a loss of these proteins. It is interesting to note that dexamethasone shows similarity with cycloheximide in decreasing the basal peptic activity of both lumen and mucosa after 24 hr. It is plausible that the cycloheximide effect is mediated at the translational level of pepsinogen biosynthesis. Whether the effect of dexamethasone is mediated through decrease in pepsinogen biosynthesis or through increased degradation of this protein remains to be investigated.

Glucocorticoids are known to exert a variety of actions via genomic and nongenomic pathways [17–19]. Enzymes in the gastrointestinal tract are also sensitive to glucocorticoid action. Oral corticosteroids exert an enhancing effect on sucrase, enterokinase, alkaline phosphatase and aminopeptidase of intestinal brush border [20]. We have reported earlier that glucocorticoids modulate the gastric peroxidase activity [21] which is located mainly in the parietal cell and plays an important role in the control of acid secretion [10]. Our present study further shows that glucocorticoids also modulate the peptic activity of the gastric lumen and mucosa. It is thus clear that glucocorticoids play an important role in influencing the secretory activity of the gastric mucosa.

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