ORIGINAL ARTICLE

Sequential Changes of the Autonomic Nervous System in the Development of Cysteamine-induced Duodenal Ulcer, Histochemical and Quantitative Studies

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Abstract

We studied how the autonomic nervous system participated in the development of experimental duodenal ulcers due to cysteamine hydrochloride. Sympathetic fibers showed a slight increase in activities in the duodenal mucosa 1 h after cysteamine administration, and a marked decrease thereafter. The release of noradrenaline (NA) into the blood was suggested. Of the activities of parasympathetic fibers, true cholinesterase activities, that are concerned with secretions, began to increase 1 h after cysteamine administration, which was observed most notably around the Brunner's glands in the duodenal submucosa, peaked at 8 h, and then decreased or disappeared at 24 h when ulcer formation was established. Pseudo cholinesterase activities that are concerned with motor function, decreased over time with a resulting decrease in gastroduodenal movements and emptying. Meanwhile, it was confirmed by estimation of NA and cholinesterase (ChE) by means of a fluorescent histochemical study. The above findings suggest that, though the sympathetic and parasympathetic systems play their roles with a complicated interrelationship in the development of cysteamine-induced duodenal ulcers, sympathetic fibers are initially depressed, and ulceration develops and advances under the parasympathetic dominance. In other words, the development of ulcers seems to depend more on the parasympathetic system than on the sympathetic system.

Key words: sympathetic fibers, parasympathetic fibers, fluorescence histochemistry, cysteamine, duodenal ulcer

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Etiology of Cysteamine-induced Duodenal Ulcer

Introduction

As we produced an experimental model of a duodenal ulcer closely resembling a clinical ulcer and tried fluorescent histochemical and biochemical studies with this model, it became clear to us that early changes in catecholamines in the duodenal mucosa have important bearings on the development and progress of ulcers. Recent views on the etiology of cysteamine-induced duodenal ulcers agree in that they result from the loss of equilibrium between aggressive and defensive factors such as hypersecretion of gastric acid and pepsin, elevated serum gastrin levels, a decrease in serum secretion and a reduction in somatostatin levels of gastric and duodenal tissue. However the pathophysiology of a duodenal ulcer is diverse and complicated with individual factors varying from case to case, so that the exact mechanism of its development is still unclear. In the present experiments, we studied the change in the autonomic nervous system for the occurrence and the development of an experimental duodenal ulcer.

Materials and Methods

1) Subjects

The experiment was carried out on male Wistar rats, (170–210 g weight) allowed to take water and solid food ad libitum.

2) Methods

a) Production of experimental duodenal ulcer

Cysteamine was prepared as 10% aqueous solution with physiological saline. Cysteamine was injected subcutaneously in a single dose of 40 mg/100 g body weight. Control rats were injected with the same quantity of physiological saline.

b) Morphological observation

The Falck-Hillarp method was used for observation of the distribution and tissue localization of sympathetic fibers. The distribution and localization of parasympathetic fibers were observed by the modified Karnovsky’s method with acetylcholinesterase (AChE) as the marker. In the other histochemical studies, Cresyl-violet staining was used for identification of the nerve plexuses and cells. Finally, all the tissue sections for histological study were stained with haematoxylin-eosin.

c) Determination of catecholamines and cholinesterase

Tissue noradrenaline (NA) level was measured by high performance liquid chromatography (HPLC) and cholinesterase (ChE) by the Routh method.

d) Cysteamine-treated groups

i) Dose response study

Cysteamine was administered subcutaneously in a single dose of 20 mg, 40 mg,
80 mg or 100 mg/100 g weight, and the rats were sacrificed by decapitation after 1 h.

ii) Time response study
Cysteamine was administered subcutaneously in a single dose of 40 mg/100 g body weight, and the rats were sacrificed by decapitation respectively at 1, 4, 8 and 24 h, and the stomach and duodenum were immediately taken out.

3) Statistical analysis
All results are expressed as the mean ± SD. Statistical significance was calculated by Student's t-test and differences was considered significant when p value of < 0.05 was obtained.

Results

1). Dose response study
Animals treated with 20 mg/100 g body weight of cysteamine showed no significant change as compared with the control animals. In the high dose groups such as 80 mg/100 g and 100 mg/100 g body weight groups, however, NA fluorescence and

![Fig. 1 Adrenergic fibers in the duodenum after the administration of cysteamine in dosages of 20 mg, 40 mg, 80 mg and 100 mg/100 g body weight. ×50](image_url)

In the high dose groups such as 80 mg and 100 mg/100 g body weight of cysteamine, noradrenergic fluorescence decreased or disappeared, and destruction of mucosa and the Brunner's glands was observed.
AChE activities decreased or disappeared, and destruction of the duodenal mucosa and of Brunner's glands was observed (Fig. 1). On the other hand, NA and AChE activities did not change at the stomach.

2) Time response study

A) Adrenergic innervation, Adrenergic fibers (Ad-fibers)
   a) Duodenum
      In the control group, Ad-fibers with a green fluorescence were found in abundance in Auerbach's plexus, Meissner's plexus, mucosal plexus and around arteries, with a part of the Ad-fibers from the mucosal plexus rising along the blood vessels within mucosa like fibers but not reaching the mucosal surface. Ad-fibers were distributed to the Brunner's glands too, but only very sparsely. In the cysteamine-treated group, the fluorescent zone in the duodenum slightly increased at 1 h after cysteamine administration, but markedly decreased thereafter. The fluorescent histochemical activities of Ad-fibers reduced at 4 h after the administration of cysteamine particularly in Auerbach's and Meissner's plexuses, markedly reduced at 8 h, and were no longer detected at 24 h (Fig. 2). Observation of the Brunner's glands at the prescribed time intervals disclosed that slight fluorescent activities of Ad-fibers remained around the Brunner's glands at 1 h, then began to disappear at 4 h when erosion was present, and ceased to be detected thereafter (Fig. 3).
   b) Corpus of the stomach
      In both the control and cysteamine group, the fluorescent activities of Ad-fibers were found in abundance in Auerbach's and Meissner's plexuses, and the deep muscular plexus was detected on the innermost side of the circular muscle layer. There was no significant difference between the control and cysteamine-treated group in this respect (Fig. 4).
   c) Pyloric region
      In both the control and cysteamine group, the fluorescent activities of Ad-fibers were detected only in Auerbach's plexus and muscle layer. As in the corpus of the stomach, there was no significant difference between the control and cysteamine-treated group.

B) Cholinergic innervation, cholinergic-fibers (Ch-fibers)
   a) True cholinesterase (true ChE) activities
      In the control group, true ChE activities that are concerned with secretions were found in abundance in all the layers of the duodenum, gastric corpus and pyloric region, especially in Auerbach's and Meissner's plexuses, the muscle layer and around the Brunner's glands. Within mucosa, true ChE activities were distributed like fibers
Fig. 2  Sequential changes of adrenergic fibers in the duodenum in the control group and 1 h, 4 h, 8 h and 24 h after the administration of cysteamine. ×50
The fluorescent activities of adrenergic fibers showed remarkable decrease after 8 h and complete disappearance after 24 h.

Fig. 3  Adrenergic fibers in the Brunner's glands in the control group (upper) and 4 h after the administration of cysteamine (lower). ×100
Adrenergic fibers decreased with time after the administration of cysteamine.
roughly homogeneously from the basal part of the mucosal layer to the mucosal surface.

In the cysteamine-treated group, true ChE activities increased with time 1 h after cysteamine administration, to peak at 8 h when erosion became severe, and markedly decreased or disappeared at 24 h when ulcer formation was established. In the tissues showing erosion or ulcer, true ChE activities remained to the end in spite of destruction of the Brunner's glands (Fig. 5). In the stomach, true ChE activities did not change with time at the corpus, but began to increase at the pyloric region from 4 h after the
administration of cysteamine (Fig. 6).

b) Pseudo cholinesterase (pseudo ChE) activities

In the control group, pseudo ChE activities that are concerned with motor function\textsuperscript{17} were markedly recognized only in the bundle of smooth muscle which corresponded to the muscularis mucosae in the duodenum, gastric corpus and pyloric region, and not detected within mucosa or the muscle layer though the activities were detected
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around Brunner's glands in the duodenum. In the cysteamine-treated group, pseudo ChE activities decreased with time as erosion developed and advanced until ulcer perforation occurred (Fig. 7).

C) Results of determination of NA and ChE

(1) NA content

In the control group, NA content of the duodenum, gastric corpus and pyloric region were respectively 0.84 ± 0.06 (M ± SD) µg/g (wet weight), 0.36 ± 0.07 µg/g and 0.27 ± 0.09 µg/g.

After the administration of 40 mg/100 g body weight of cysteamine, NA content

![Graph showing sequential changes of noradrenaline content](image)

**Fig. 8 Sequential changes of noradrenaline content after the administration of 40 mg/100 g body weight of cysteamine.**
of the duodenum significantly increased to $0.94 \pm 0.07 \mu g/g$ at 1 h as compared with the control group ($p<0.01$), then significantly decreased to $0.69 \pm 0.08 \mu g/g$ at 8 h and $0.47 \pm 0.07 \mu g/g$ at 24 h ($p<0.01$). NA content of the gastric corpus and pyloric region did not significantly differ from those in the control (Fig. 8).

(2) ChE content

(a) True ChE content

In the control group, true ChE content of the duodenum, gastric corpus and pyloric region were respectively $2.23 \pm 0.54$ (M ± SD) U/g, $2.1 \pm 0.56$ U/g and $2.29 \pm 0.74$ U/g.

In the cysteamine-treated group, the ChE content of the duodenum slightly in-
Fig. 10 Sequential changes of pseudo cholinesterase content after the administration of 40 mg/100 g body weight of cysteamine.

Increased to 2.98 ± 0.51 U/g at 1 h as compared with the control group (p<0.05), then significantly increased to 4.32 ± 0.69 U/g at 4 h and 6.67 ± 1.25 U/g at 8 h (p<0.01), and decreased to 1.68 ± 0.7 U/g at 24 h.

True ChE content of the gastric corpus and pyloric region did not significantly differ from those in the control group (Fig. 9).

(b) Pseudo ChE content

In the control group, pseudo ChE content of the duodenum, gastric corpus and pyloric region were respectively 9.50 ± 1.27 U/g, 2.87 ± 0.5 U/g and 4.14 ± 0.67 U/g.

In the cysteamine-treated group, pseudo ChE content of the duodenum significantly decreased to 6.47 ± 1.04 U/g at 1 h, 5.36 ± 1.77 U/g at 4 h, 4.45 ± 2.04 U/g at 8 h.
and 3.23 ± 1.38 U/g at 24 h as compared with the control group (p<0.01). Pseudo ChE content of the gastric corpus and pyloric region did not significantly differ from those in the control group (Fig. 10).

Discussion

It was Rokitansky\textsuperscript{18} who first pointed out that peptic ulcer was closely related to the autonomic nervous system. Similar statements were subsequently made by Schiff\textsuperscript{19}, Hoffman\textsuperscript{20} and Bergmann\textsuperscript{21}. Cushing\textsuperscript{22} reported that the hypothalamus—vagus system was involved in the development of ulcers. Selye\textsuperscript{23} pointed out autonomic ataxia as an etiologic factor for ulcers. French\textsuperscript{24} and Porter\textsuperscript{25} published their view that not only the vagus nerve but the sympathetic nervous system participated in the development of acute ulcers. Many authors have thus studied the pathogenesis of peptic ulcers from the viewpoint of autonomic innervation. In the autonomic nervous system, either adrenergic fibers of the sympathetic system or cholinergic fibers of the parasympathetic system (vagus nerve) can gain dominance over the other. It has been suggested that excitation of sympathetic fibers is associated with disturbance of mucosal blood flow resulting from vasoconstriction and thrombosis, and that of parasympathetic fibers with the development of peptic ulcer resulting from hypersecretion of hydrochloric acid, pepsin and gastrin and increased movements and tonus of the stomach. However, opinions differ on whether more importance should be attached to the sympathetic system\textsuperscript{26} which mediates vasoconstriction and ischemia or the parasympathetic system\textsuperscript{27} which mediates secretions. This controversy is still unresolved. We suspected that a cysteamine-induced duodenal ulcer, which closely resembles a clinical ulcer, was etiologically dependent on the abnormal state of the autonomic nervous system, i.e., sympathetic and parasympathetic fibers, and examined changes in the intrinsic nerves of the stomach and duodenum, chiefly those in NA and ACh activities, through fluorescent, histological and biochemical approaches.

The cysteamine dose used in the production of an experimental duodenal ulcer varies among different authors, from 30 mg/100 g body weight\textsuperscript{28,29} to 35 mg/100 g\textsuperscript{30} and 40 mg/100 g.\textsuperscript{31} Generally speaking, if the dose is increased, the frequency of the development of ulcers rises, but mortality also increases.\textsuperscript{32} Since our objective was to observe early changes in the gastric and duodenal tissues after cysteamine administration, we used a dose of 40 mg/100 g body weight, which allowed ulcer perforation to occur in 100\% of the cases while keeping the mortality rate low and preventing loss of NA activities.

In our observation of the sympathetic system, Ad-fibers were distributed in abundance throughout Auerbach’s plexus, Meissner’s plexus, mucosal plexus, and the muscular and mucosal layer of the stomach and duodenum in the control group. In the cysteamine-treated group, the fluorescent activities of Ad-fibers in duodenal mucosa
slightly increased 1 h after cysteamine administration. This transient increase in NA seemed to represent the host’s reaction initiating preparation for erosion. At 4 h after cysteamine administration, the fluorescence of NA began to weaken gradually at Auerbach’s plexus, then around the Brunner’s glands and subsequently at Meissner’s plexus. This fact suggested the release of NA into the blood. Because of the pharmacological property of NA, this would be followed by constriction of intrinsic arterioles in the duodenal submucosa with resulting ischemia of its mucosa. Meanwhile, it has been recently reported that administration of exogenous NA suppressed the development of experimental ulcers. More detailed studies are therefore necessary concerning this point.

We studied the parasympathetic system from the viewpoint of histochemical localization of AChE. True ChE activities, concerned with secretions, increased with time after cysteamine administration, with the result that parasympathetic fibers within gastric mucosa were excited and hydrochloric acid, pepsin and gastrin seemed to be secreted through the vagus nerve, that is, aggressive factors were reinforced. Within the duodenal mucosa, an increase in true ChE activities was detected at 1 h and 4 h, when erosion of the tissue was present. This suggested that the parasympathetic system acted to reinforce defensive factors in reaction to the destruction of mucosa and its spread. At 8 h when a shallow ulcer was present, the Brunner’s glands showed a marked increase in activity in spite of dilatation and flattening of their glandular structure. Clearly, the parasympathetic system was acting to further accelerate secretion and prevent ulcer formation. At 24 h, however, Brunner’s glands were destroyed and true ChE activities nearly disappeared. In this stage, depletion of mucus and deterioration of defensive mechanism occurred, inviting ulcer perforation. It was interesting to observe the two-facedness of the action of parasympathetic fibers in the stomach and duodenum, as described above. Pseudo ChE activities, concerned with motor function, decreased with time, which seemed to signify a decrease in the movements and emptying of the stomach and duodenum. Lichtenberger reported a delay of gastric emptying after cysteamine administration, proposing that this change depended on the vagus nerve, which supported our results. Those changes in the parasympathetic system suggest the importance of vagus nerve in the development of a cysteamine-induced duodenal ulcer.

To summarize the time-course changes in the sympathetic and parasympathetic systems, sympathetic fibers of the duodenum were initially stimulated after cysteamine administration but stimulation lasted only briefly, and they fell into a state of hypofunction subsequently. With the sedation of sympathetic activities, stimulation of the vagus nerve increased to induce secretion of gastric acid, pepsin and gastrin. Finally, secretion from Brunner’s glands was suppressed with the resulting depletion of mucus, which favored ulcer development. The present experiment suggested that, while the
sympathetic and parasympathetic systems each play a part under a complicated system of interrelationship in the development of a cysteamine-induced duodenal ulcer, hypofunction of the sympathetic system occurred first to allow the parasympathetic system to gain dominance, and an ulcer developed and advanced under this state of parasympathetic dominance. From the above findings, the parasympathetic system, rather than the sympathetic system, may play a very important role in ulcer formation.

Recent studies demonstrated the existence of non-cholinergic and non-adrenergic purinergic nerves with ATP as the transmitter of impulses,35 VIP-ergic nerves36 as observed by immunofluorescence and somatostatin-containing nerves.37 Since these nerves are all considered to participate in the control of secretions from Brunner's glands, it is necessary to investigate the etiology of cysteamine-induced duodenal ulcers further with attention paid not only to intrinsic neurons but to peptidic neurons.

References

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