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The Thermosensitivity of Enterogastrone

KARL JOHAN ÖBRINK and HÅKAN WINBERG

Institute of Physiology, University of Uppsala, Uppsala, and Central Laboratories, Astra, Södertälje, Sweden

Very few facts are known about the enterogastrone (Eg) molecule. Öbrink [1] showed that the secretory depressant had no or a very slight electrophoretic mobility within wide pH-ranges. Katz, Dryer, Paul and Routh [2] obtained a similar result. This finding, together with the facts that the Eg activity is not destroyed by pepsin or trypsin [3] and easily dialyses through cellophane [4] makes it improbable that Eg is a protein or a protein derivative. Some of our active preparations lack any detectable amount of amino acids, whereas they all contain carbohydrate.

The present investigation of the thermosensitivity of Eg was undertaken in connection with work on the structure of the Eg molecule.

METHODS

Two enterogastrone preparations were used. They were both made from hog's intestine, but by two different methods which yielded preparations of different purity. The starting-material was in both cases the NaCl-precipitate or the "A-precipitate" [5]. One preparation (127 B) was in many respects similar to that of Gray, Bradley and Ivy [6]. The other one (111 B) was prepared according to a method which will be published in detail in this journal. Common to both the preparations was a dialysis in which the active material was recovered in the dialysate.

The activities of both the preparations were almost the same (one Eg unit in about 3 mg, cf. Öbrink [7]), but 127 B contained a large amount of amino-acids and peptides, whereas 111 B did not (ninhydrin and biuret reactions negative). The nitrogen content of 111 B was 0.24 % and of 127 B 14.8 %. NaCl formed more than 90 % of the 111 B.

The assay procedure was that worked out by Öbrink [7]. Heidenhain pouch dogs were used, and the secretion was induced by a continuous intravenous injection of histamine. When the secretion rate had become constant, the Eg was also injected intravenously at a slow constant rate during the rest of the experiment. After a latent period of about 60 minutes, the secretory rate decreased and a new steady level was reached. *The difference between the steady levels before and during the Eg administration was taken as a*

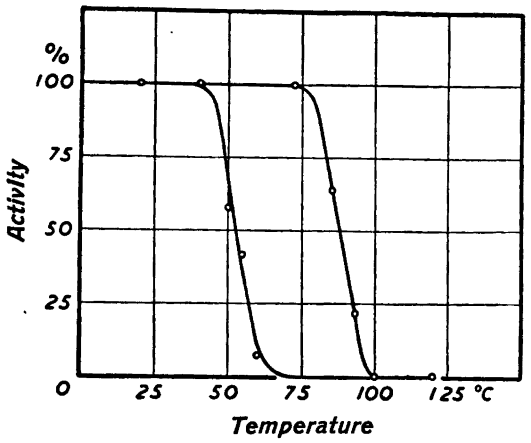

Fig. 1. The activity of two enterogastrone preparations after heating. Preparation 111 B to the left; 127 B to the right. The activity of unheated materials is defined as 100 %.

measure of the Eg activity. A curvilinear relationship was found to exist between the injection rate of Eg and its inhibitory effect. This inhibitory effect was expressed in per cent of the initial control secretion rate, which had to be kept within certain limits.

The Eg preparation was dissolved in 0.9 % NaCl to a concentration of 0.24 % (wt/v), and the injection rate was 6 mg Eg per hour in a 15 kg dog. When exposed to a high temperature, the solution was stored in a sealed vial of Pyrex glass. In most experiments the gas volume in the vial was air, but in some cases it was nitrogen.

RESULTS

It was found that enterogastrone cannot be kept for many days as an unbuffered and unheated water solution. Even after one day a decrease of activity was noticed, and after a month the preparation was completely inactive whether stored at room temperature or in an icebox (+ 4° C). Greengard, Atkinson, Grossman and Ivy [8] have kept a sterile solution potent for 10 days. In the present investigation no controls were made of sterility but all the solutions were freshly made. As a dry powder, however, the preparation has now been kept for some years, apparently without losing any activity.

The result of the investigations on the thermostability of the two preparations is seen in Fig. 1. The inhibitory effect of the Eg was determined with and without heating. The activity of the unheated preparations were defined as 100 %. The effect of the heated preparations was compared with the unheated ones and expressed in per cent of the original activity. *Cf.* Table 1.

It is obvious that the activity can be completely destroyed by heat and at some temperatures it was found that this happened whether oxygen was available or not (Table 1). It was also apparent that the two Eg preparations are characterized by different thermostabilities.

Table 1. Effect of heating of enterogastrone solutions.

Temp. °C	Time of heating, minutes	Inhibition of the secretion rate %	Activity of the heated solution %	Atmosphere in the vial
		Eg preparation 111 B.		
—	—	50.0 (average value)	100	air
41	60	50.0	100	»
50	60	29.0	58	»
55	60	21.0	42	»
60	60	4.0	8	»
100	10	0	0	»
100	10	0	0	N_2
		Eg preparation 127 B.		
—	—	60.8 (average value)	100	air
72	140	>60.8	100	»
84	120	39.0	64	»
93	120	13.2	22	»
100	120	0	0	»
100	60	0	0	»
120	120	0	0	»
120	120	0	0	N_2

DISCUSSION

In the present investigation it was found that two different enterogastrone preparations were unstable when stored as water solutions. When exposed to heat both the Eg preparations could be destroyed. This confirmed the earlier observations of Kosaka and Lim [9] that heating at 80—100° C for 10 minutes inactivated extracts derived from oil-treated intestinal mucosa.

The preparation 127 B showed a higher stability than 111 B, which was the purer with respect to organic constituents. The fact that the two preparations showed different stabilities to heat may be attributed to the higher content of organic impurities in the more stable 127 B. It may be thought that in this case the organic impurities have a protective effect on the thermolabile enterogastrone molecule.

It is clear that the Eg molecule is characterized by some very thermosensitive structure. The biological activity is completely lost if this labile structure of the molecule is destroyed by heat.

SUMMARY

Two enterogastrone preparations with different nitrogen contents (14.8 % and 0.24 %), but with equal biological activity, were shown to be very labile to heat. The preparation which had the lower content of organic material (N = 0.24 %) was the more thermosensitive one and did not withstand temperatures above 40° C.

The enterogastrone molecule is characterized by some not yet identified thermolabile structure.

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