

INHIBITION OF RESERPINE-INDUCED ULCERS BY CALCITONIN IN RATS

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Abstract: Reserpine has a net ulcerative effect, generating lesions of the glandular mucosa in rats at 8 hours after administration of a dose of 5 mg/kg ip. Gastric ulcerations can be present in a reduced proportion in the control group after a long fasting period. Calcitonin in repeated doses of 10 UI/kg and 100 UI/kg completely protects the animals against the ulcerative effects of reserpine and fasting. Interferences between calcitonin and reserpine, having as effect the gastric protection exert both peripheral and on the central nervous system.

Key words: calcitonin, reserpine, gastric mucosa, experimental ulcers, rats.

1. Introduction

At approximately 10 years after its discovery, the significant effects of the calcitonin on digestive system were observed [1]. Calcitonin inhibits basal and stimulated gastric secretion in humans [2, 3] and animals [4, 5, 6]. The hormone has favorable effect in peptic ulcers [7, 8, 9] and protective action on experimental models of ulcers [10, 11, 12, 13, 14, 15, 16]. Without being fully understood, the gastrointestinal mechanisms can be both peripheral [6, 13] and central [17, 18, 19], direct and indirect [20]. It is worthy to note that calcitonin is present, similar to other polypeptidic hormones, in endocrine and non-endocrine tissues [21], including central nervous system [22, 23, 24] and digestive tract [25]. Taking into account the remarkable experimental anti-ulcer effects, in this paper we studied the calcitonin action in acute gastric ulcerations induced by reserpine in rats.

2. Material and Methods

2.1. Biologic Material

We used albino male Wistar-Bratislava rats from UMF "Iuliu Haţieganu" Biobase, Cluj Napoca. Body weight was between 125 g and 150 g. Rats were adapted to laboratory conditions: ambient temperature 21°C, natural light-dark regimen, standard food and water ad libitum.

Four groups of 8 rats each were constituted by randomization. Before the onset of ulcerogenesis, animals were kept fasting for 12 hours in cages with large grills to avoid coprophagia. The first group, control, was injected with intraperitoneal (ip) saline at same time intervals as the other groups. The second group received ip reserpine 5 mg/kg. Groups III and IV were treated with ip

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reserpine and calcitonin ip, 10 UI/kg and 100 UI/kg. Three doses were administered within 15 minutes before and 3 and 6 hours after reserpine. Animals were sacrificed at 8 hours after the reserpine injection by general anesthesia with ethyl urethane.

Stomachs were harvested, opened on greater curvature and washed gently with saline. Gastric content and general aspect of the mucosa were taken into account. The number of ulcerations was determined with a magnifying glass 5x (Table 1).

Experimental protocol

Table 1

Groups (n=8)		Time [hours]					
		-12	-0.25	0	3	6	8
I	Control (C)	Fasting	Saline	Saline	Saline	Saline	Sacrification
II	Reserpine (R)		Saline	Reserpine	Saline	Saline	
III	R+ Calcitonin (CT) 10 UI		C10		C10	C10	
IV	I+CT 100 UI		C100		C100	C100	

2.2. Statistical analysis [26]

Incidence of ulcers and gastric hemorrhage was noted as percent and processed in 2 x 2 tables non-parametric test (Table 2).

Number of gastric ulcers was determined differentially, according to dimensions, in three types: total, large (> 1 mm) and confluent and expressed by the arithmetic mean and standard error ($\bar{x} \pm e. s.$). Comparison of parametric indexes was done by bilateral "t" Student test (Table 3).

Null hypothesis was rejected at $p < 0.05$. Severity of mucosal lesions was quantified by the Ulcer Index (U.I.). For comparison between groups, the bilateral "t" Student test was used (Table 3) and protection ratio (PR-Table 4). $PR (\%) = [(U.I. \text{ control} - U.I. \text{ treated}) / U.I. \text{ control}] \times 100$. The PR was considered relevant for absolute value greater than 33% [27].

2.3. Drugs

1. Synthetic calcitonin (eel) (Calcitonin[®], Sclavo Siena) lyophilised powder. After dissolving and diluting, doses of 10 UI/kg and 100 UI/kg were administered ip, in volume of 2 ml / 100 g.
2. Reserpine phosphate (Raunervil[®], Sicomed Bucureşti) f 2.5 mg/1 ml in dose of 5 mg/kg.
3. Ethyl urethane (Carlo Erba-Milano), water solution 20%, 1,25 g/kg
4. Saline.

Results

Sedative action of reserpine was progressive and evident after 3-4 hours after injection. Rats were immobile, with muscle tone up to rigidity, eyelid ptosis and diarrhea. Calcitonin, at used dosage, does not significantly modify the behavior of the animals.

Protective gastric effect of the calcitonin is total at both doses, on parametric and non-parametric indexes (Table 2, Table 3 and Table 4).

Ulcer's incidence (%)

Table 2

Groups (n=8)		%			Hemorrhage (%)	
		Total	> 1 mm	Confluent		
I	Control (C)	37.5	25	0	25	
II	Reserpine (R)	100	100	0	25	
III	R + Calcitonin (CT) 10 UI	0	0	0	0	
III	I + CT 100 UI	0	0	0	0	
p (tables 2 x 2)	I vs.	II	0.0256	0.0035	1	1
	II vs.	III	0.0001	0.0001	1	0.467
		V	0.0001	0.0001	1	0.467

Number of ulcerations ($\bar{x} \pm e. s.$)

Table 3

Groups (n=8)		Ulcerations	$\bar{x} \pm e. s.$	$\bar{x} \pm e. s.$	UI ($\bar{x} \pm e. s.$)	
		Total	> 1mm	Confluent		
I	Control (C)	5.88 ± 4.55	2.75 ± 1.89	0	0.69 ± 0.4	
II	Reserpine (R)	27.25 ± 5.6	11.75 ± 2.12	0.12 ± 0.12	2 ± 0.19	
III	R + Calcitonin (CT) 10 UI	0	0	0	0	
III	I + CT 100 UI	0	0	0	0	
p "t" Student	I vs.	II	0.01	0.0068	0.3343	0.0104
	II vs.	IV	0.0002	0.0001	0.3343	0.0001
		V	0.0002	0.0001	0.3343	0.0001

Protective ratio (%)

Table 4

Groups	Control	Reserpine
(R + C10) vs.	100	100
(R + C100) vs.	100	100

4. Discussions

At 8 hours after reserpine injections, all animals develop lesions of the gastric mucosa in the glandular region. Location of the ulcer lesions is predominant on mucosa rugae following the vascular tracts. Defects of the mucosa are superficial, variable in size, presenting at the base blood deposits. In some areas there is a deep congestion of the mucosa with dilated blood vessels that, most probably are evolving to ulcers. Reserpine does not cause ulcerations in rumen, the area of the stomach with keratinised epithelium. Also, in the glandular stomach, the antrum region is rarely affected after reserpine

administration and when the mucosa is involved, lesions are superficial and non-hemorrhagic. These macroscopic aspects of the gastric mucosa suggest a preponderant vascular action of reserpine in generating acute ulcerations [27].

Reserpine is one of the most used drugs for generating experimental gastric ulcers [28, 29, 30]. The ulcerative effect is manifested also in clinical condition [31, 32]. Central and peripheral mechanisms are involved in the gastric ulcers determined by reserpine [27]. Reserpine increases the acidity [33] and gastric motility and alters the blood vessels of the mucosa [34]. Both at peripheral level and central nervous system, reserpine depletes catecholamine,

serotonin and histamine stores. Also, it is releasing gastrin and corticosteroids [27]. From these released endogenous molecules, serotonin can act directly on the gastric mucosa. In fact, administered to laboratory animals, serotonin can generate by itself gastric ulcers. As for the catecholamines, the involvement in the ulcerative process is more complicated. In a first stage, released catecholamines act on gastric blood vessels and later, after the stores are depleted, they generate a functional adrenergic deficit. This phasic action on adrenergic mechanisms is prevalent at the central nervous system level. The result is an imbalance between the adrenergic and the cholinergic tonus with important consequences on gastric function [35]. Alteration of the adrenergic-cholinergic balance is transmitted to the gastric level via the vagus nerve. It is known the fact that vagotomy and M and N-cholinolytic drugs decrease the gastric ulcerative action of the reserpine [36, 37]. Restoration of the adrenergic activity by catecholamine precursors and drugs that stimulate the biosynthesis and release of the precursors [35, 38, 39] also have protective action at gastric level.

As for calcitonin, in the described experiments, protective effect on ulcerations is remarkable. And total. Gastric mucosa of the animals injected with reserpine and treated with the two doses of calcitonin has a normal color, without lacerations. It is notable the fact that animals treated with calcitonin have a lower severity index, in fact is null compared to the control group that did not received reserpine. This shows the fact that the hormone protects the stomach from the possible ulcerative action of prolonged fasting. Calcitonin has gastric anti-secretory effects via peripheral effects [40] and by acting on the central nervous system [17, 41, 42]. Calcitonin is present both in C thyroid cells [21] and in the

digestive tract [25] and the central nervous system [22, 23, 24]. In this way it is possible to intervene at central and peripheral levels with anti-ulcerative effects [13, 17, 18]. In an indirect way, calcitonin contributes at the maintenance of the tonus of gastric mucosa by synthesizing and releasing prostaglandins [20].

In similar reserpine models, calcitonin had evident anti-ulcerative effects [16]. Also, the protective gastric effect is evident on other experimental models as gastric ulcerations generated by nonsteroidal anti-inflammatory drugs [11, 43], pentagastrin [4], histamine [10], acetic acid [15], corticosteroids [12] and stress induced ulcers [44, 45].

5. Conclusions

1. Reserpine at dosage of 5 mg/kg induces acute gastric ulcerations in proportion of 100% in rats.
2. Long fasting periods can determine in a reduced proportion of animals, glandular gastric ulcerations.
3. Calcitonin in repeated doses of 10 UI / kg and 100 UI / kg has a total anti-ulcerative effect in the reserpine model.
4. Protective gastric effect can be explained by central and peripheral mechanisms.
5. Previous experiments and literature data shows a remarkable anti-ulcerative effect of calcitonin in experimental models of ulcers and in human peptic ulcer.

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