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Gastric Antiulcer Activity of Pentacaine: Possible Mechanism of Action

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Summary

The effect of pentacaine on different models of gastric and duodenal damage and on gastric acid secretion was studied after oral and parenteral administration. A proportional involvement of local and systemic effects of pentacaine was found in phenylbutazone-induced and cold-restraint stress-induced lesions, whereas in ethanol-induced lesions oral administration was the only effective way. On the other hand, duodenal lesions and gastric acid secretion were substantially affected by parenteral administration. The possible mechanisms involved in these differences are discussed.

Key words

Pentacaine – Gastric and duodenal lesions – Gastric acid secretion – Oral and parenteral administration – Rat

Introduction

Numerous studies have demonstrated the pronounced antiulcer, gastroprotective, and local anaesthetic effect of pentacaine (trapencaine, INN) (±)-trans-2-(1-pyrrolidinyl)cyclohexyl ester of 3(n)-pentyloxy carbanilic acid (Beneš *et al.* 1969, Nosáľová *et al.* 1987, Beneš 1991). The drug was selected from a series of carbanilates due to its potency, low toxicity, and special ability to remain active in an acid environment. Unlike the effect of most of the common local anaesthetics, the effect of pentacaine increases in low pH conditions as shown in *in vitro* experiments on action potential conduction (Štolc and Stankovičová 1986). This property would be of advantage with regard to the action of pentacaine in regions where pH is shifted toward acidity. It was therefore suggested that to exert its antiulcer activity pentacaine had to be locally present in the stomach and to act topically. This seemed to be supported by favourable results obtained after oral administration of pentacaine. The aim of the present study was to test this assumption and to compare the effect of pentacaine after oral and parenteral administration on various models of gastric and duodenal lesions and on gastric acid secretion. Preliminary results of these studies were reported in abstract form (Nosáľová and Babuľová 1991).

Methods

Female Wistar rats weighing 160–200 g were randomly assigned to experimental groups. Prior to the experiments the rats were fasted for 24 h and allowed free access to water. The antiulcer effect of pentacaine was studied in 3 experimental gastric models, i.e. phenylbutazone-, stress-, and ethanol-induced lesions and in a model of duodenal lesions induced by cysteamine. The gastric antisecretory effect of pentacaine was assessed in pylorus-ligated rats. The doses of pentacaine chosen were based on previous studies (Nosáľová *et al.* 1985, Nosáľová *et al.* 1986, Babuľová *et al.* 1988, Nosáľová *et al.* 1991).

In all antiulcer experiments, described in detail below, the animals were sacrificed, the stomach and/or duodenum were removed, opened along the greater curvature or in the case of the duodenum on the antimesenteric side, rinsed with tap water and examined for lesions under a dissecting microscope by an independent observer who was unaware of the treatment.

Phenylbutazone-induced gastric lesions

Female rats fasted 24 h before the experiments received an ulcerogenous dose of

phenylbutazone (200 mg/kg p.o.). Pentacaine was given orally or subcutaneously in doses of 5, 10, and 20 mg/kg 30 min before phenylbutazone administration. Control rats received a vehicle. The rats were killed 5 h after phenylbutazone administration by cervical dislocation. The length of lesions (mm) was measured and the number of lesions was recorded.

Cold-restraint stress-induced gastric lesions

Fasted female Wistar rats were immobilised and exposed to a temperature of 4 °C for 2 h. Control nonstressed rats were kept unrestrained at room temperature. Pentacaine was given in doses of 3, 10, and 30 mg/kg orally or subcutaneously shortly before the exposure to stress. The length and number of lesions were counted.

Ethanol-induced gastric lesions

Fasted female Wistar rats were given 96 % ethanol orally in a volume of 5 ml/kg b.w. Pentacaine was administered in doses of 3, 10, and 30 mg/kg orally or subcutaneously 30 min before ethanol. The control rats received an equal volume of distilled water or saline. The animals were sacrificed 60 min after ethanol intake. The length and number of lesions were recorded.

Duodenal lesions induced by cysteamine

Fasted rats received injections of cysteamine in a dose of 400 mg/kg s.c., given in two doses of 300 and 100 mg/kg 6 h apart, each preceded by oral or subcutaneous administration of pentacaine in doses of 5, 10, and 20 mg/kg. The rats were sacrificed 18 h after the second dose of cysteamine. The severity of duodenal lesions was graded as follows: 0 - normal, 1 - hyperaemia, 2 - superficial ulcer, 3 - deep ulcer, 4 - perforating ulcer (Szabo 1978).

Gastric acid secretion stimulated by histamine in pylorus-ligated rats

The pylorus was ligated under light ether anaesthesia after 24 h fasting (Shay *et al.* 1945). Pentacaine in doses of 10, 20, and 40 mg/kg was administered either orally immediately before ligation of the pylorus, or systematically by injection into the duodenum or by subcutaneous injection immediately after the pylorus ligation. Control animals received the vehicle in the same volume as the treated rats (5 ml/kg). Gastric acid secretion was stimulated by histamine in a dose of 25 mg/kg s.c. The rats were sacrificed 4 h after surgery by cervical dislocation. The pylorus and cardia were clamped, the stomachs were removed and the gastric content was collected and centrifuged at 1 500 rpm for 10 min. The volume (ml/100g b.w.) of gastric juice was measured, acidity (mmol/l) was evaluated by titration of an aliquot sample to pH 7 with 0.1 N NaOH and total acid output was calculated and expressed as μmol/100 g b.w.

The results expressed are means ± S.E.M., Student's t-test and Mann-Whitney U-test were used for statistical analysis. ID_{50} values were calculated by regression analysis.

The following drugs were used: pentacaine hydrochloride (trapencaine hydrochloride) (Galena), phenylbutazone (Spofa), histamine diphosphate (Merck). All chemicals were of analytical grade.

Results

The formation of acute gastric lesions induced by phenylbutazone was significantly inhibited by oral as well as subcutaneous administration of pentacaine (Fig. 1). The reduction of the gastric lesion length was dose-dependent and significant inhibition (70 %) was already achieved with the dose of 5 mg/kg pentacaine. There was no difference in the antiulcer activity of pentacaine between the routes of administration used. ID_{50} values were 2.96 mg/kg p.o. and 3.94 mg/kg s.c.

Similarly, gastric injury induced by cold-restraint stress was comparably affected by pentacaine administered orally or subcutaneously, with ID_{50} 7.2 mg/kg and 3.78 mg/kg, respectively (Fig. 2). The only difference was observed after administration of the lowest dose of pentacaine tested (3 mg/kg), when subcutaneous administration was found to be more effective (26 % versus 47 % inhibition).

Unlike the above results, the oral route was the only effective way of pentacaine administration in the case of ethanol-induced gastric damage. No significant inhibition of the gastric lesion length was found after the subcutaneous injections (Fig. 3). On the other hand, pentacaine given orally was very effective and prevented the development of necrotic haemorrhagic lesions caused by concentrated ethanol in a dose-dependent way (ID_{50} 6.1 mg/kg).

Cysteamine-induced ulcers were formed in the part of the duodenum just below the pylorus. In the majority of control untreated rats deep ulcers were found with a tendency to perforate. Oral administration of pentacaine was ineffective against duodenal injury induced by cysteamine. However, pentacaine given subcutaneously reduced the ulcer score significantly (Fig. 4).

Gastric acid secretion stimulated by histamine was effectively inhibited by pentacaine administered subcutaneously. A significant antisecretory effect was observed after the administration of all doses of pentacaine tested. There was a decrease in the volume, acidity as well as total acid output (ID_{50} 25.04 mg/kg s.c. for total acid output). An even more pronounced antisecretory activity was achieved after intraduodenal administration of pentacaine (ID_{50} 18.09 mg/kg i.d.). On the contrary, the oral administration of pentacaine was not significantly effective with the exception of the highest dose used (Table 1).

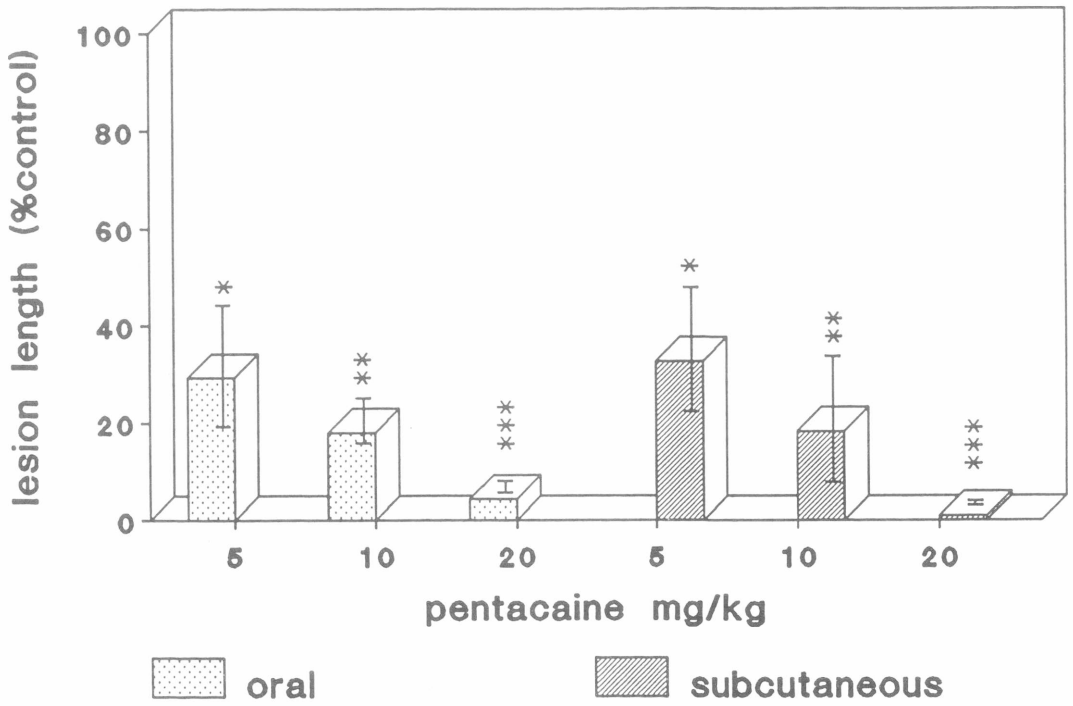

Fig. 1
Effect of pentacaine (administered orally and subcutaneously in doses of 5, 10, and 20 mg/kg) on the length of gastric lesions induced by phenylbutazone in rats. Data are expressed as percentage of control values. Controls received vehicle in a volume of 5 ml/kg, n = 8–10 animals per each dose, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus controls.

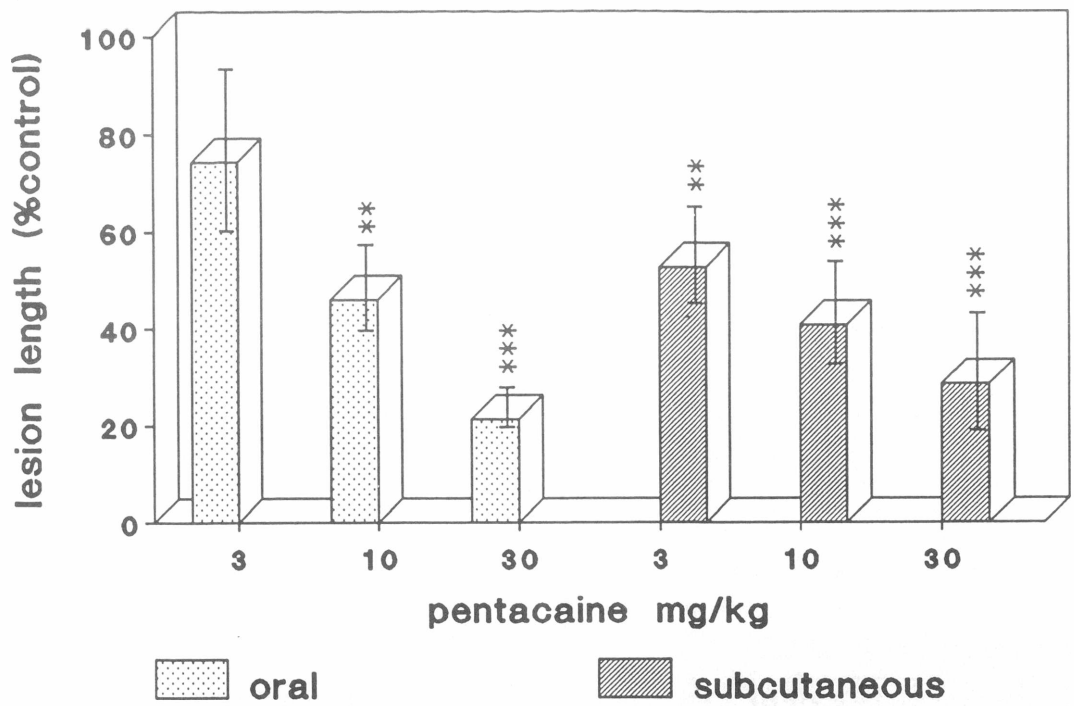

Fig. 2
Effect of pentacaine (administered orally and subcutaneously in doses of 3, 10, and 30 mg/kg) on the length of gastric lesions induced by cold-restraint stress in rats. Data are expressed as percentage of control values. Controls received vehicle in a volume of 5 ml/kg, n = 8–10 animals per each dose, ** $p < 0.01$, *** $p < 0.001$ versus controls.

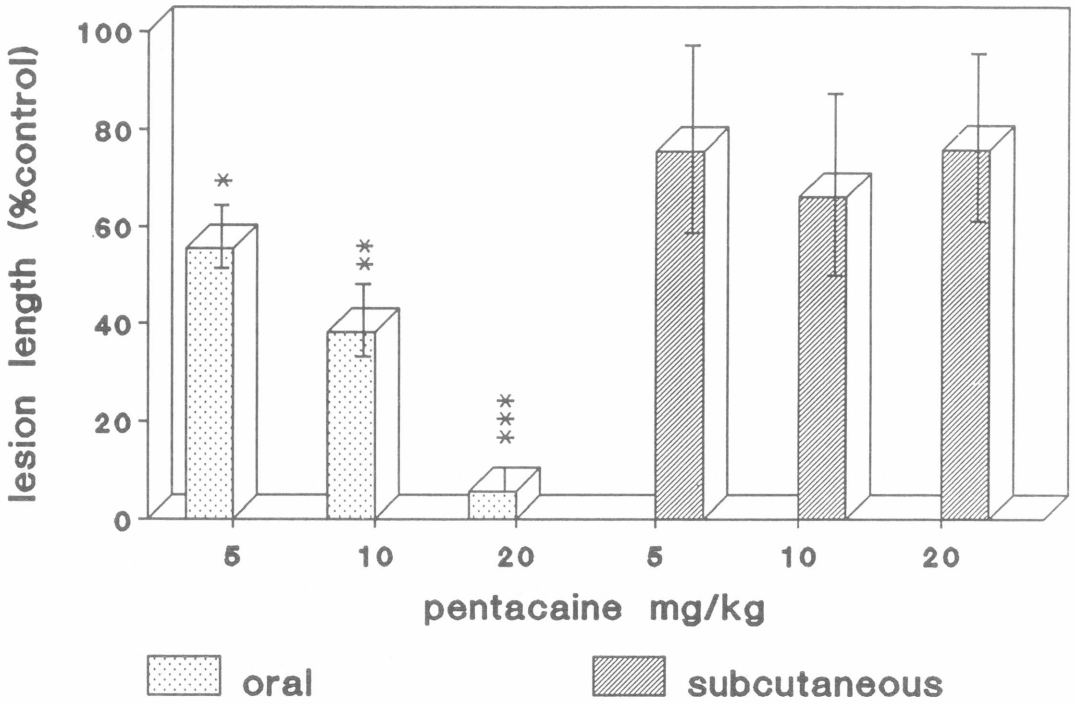

Fig. 3
Effect of pentacaine (administered orally and subcutaneously in doses of 5, 10, and 20 mg/kg) on the length of gastric lesions induced by 96 % ethanol in rats. Data are expressed as percentage of control values. Controls received vehicle in a volume of 5 ml/kg, n = 8–10 animals per each dose, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus controls.

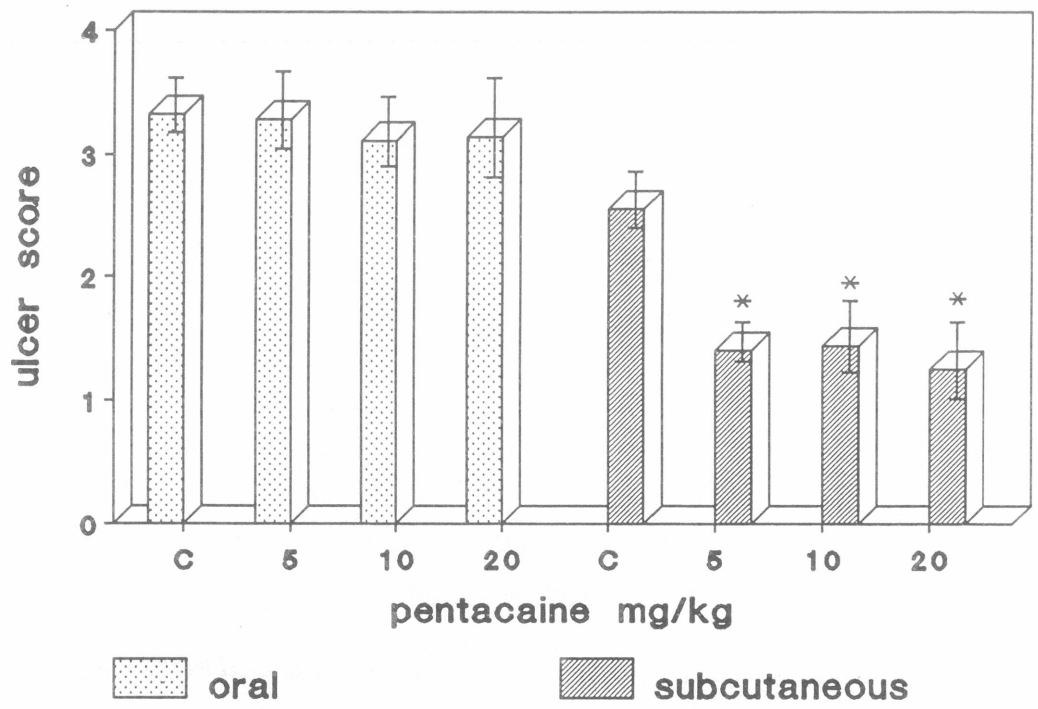

Fig. 4
Effect of pentacaine (administered orally and subcutaneously in doses of 5, 10, and 20 mg/kg) on cysteamine-induced duodenal ulcers in rats. Controls received vehicle in a volume of 5 ml/kg, n = 10–12 animals per each dose, * $p < 0.05$ versus controls.

Table 1
Effect of pentacaine on gastric acid secretion stimulated by histamine in pylorus-ligated rats

Treatment	Dose mg/kg	Volume ml/100 g	Acidity mmol/l	Total acid output μmol/100 g
Oral				
Controls		4.9±0.1	107.1±2.5	522.5±21.4
Pentacaine	10	4.7±0.2	109.2±1.9	513.3±25.9
	20	4.4±0.2	93.0±2.7	411.5±26.5
	40	2.8±0.2**	73.6±7.6**	207.8±29.9**
Intraduodenal				
Controls		4.7±0.2	107.5±3.0	510.6±25.5
Pentacaine	10	4.1±0.2*	94.6±4.6*	389.7±34.9*
	20	2.3±0.1***	81.4±6.2***	190.1±19.0***
	40	1.6±0.1***	79.3±3.5***	129.9±5.3***
Subcutaneous				
Controls		4.8±0.2	103.7±5.6	505.3±43.1
Pentacaine	10	3.5±0.3**	94.2±5.3	339.7±43.5*
	20	2.9±0.2***	85.2±6.0*	253.4±33.6**
	40	2.7± 0.2***	82.8±5.1*	232.8±27.8**

Mean ± S.E.M., n = 10–20 animals per each dose, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus controls, controls received vehicle in a volume of 5 ml/kg

Discussion

The present results confirmed the different antiulcer efficacy of pentacaine in various models of gastric and duodenal injury depending on its route of administration. A proportional involvement of both local and systemic effects was found only in phenylbutazone- and stress-induced gastric damage, which were equally influenced by oral and parenteral administration of pentacaine. Ethanol-induced extensive damage was prevented by orally given pentacaine with a virtual lack of antiulcer activity after parenteral administration. Thus the inhibition of the necrotic effect of ethanol requires the presence of pentacaine in the stomach. Since acid does not play a decisive role in the pathogenesis of ethanol-induced damage, the pronounced gastroprotective effect of oral pentacaine cannot be diminished by its weak antisecretory activity after oral administration. It is typical for most of the gastroprotective agents that there is a difference of one to two orders (Robert 1981) between the doses necessary for their gastroprotective and antisecretory actions. Their ability to protect cells of the gastrointestinal epithelium against a variety of noxious stimuli is independent of their antisecretory properties (Miller and Jacobson 1979, Robert 1981).

The route of administration also appears to play a role in the efficacy of prostaglandins in mediating their antiulcer properties. Oral or topical administration was demonstrated to be three to five times more effective compared with parenteral administration of prostaglandins (Robert *et al.* 1979). This may be related to the larger concentration of the agent exerting a topical action directly on the gastric epithelium when administered orally or topically, which fails to be the case when administered parenterally due to its distribution throughout the entire body and partial degradation (Miller 1983).

Duodenal ulceration induced by cysteamine was not affected by oral administration of pentacaine. Similar results were obtained with prostacyclin and its analogues (Náfrádi *et al.* 1989), which were ineffective in the same model of duodenal damage. Enhanced gastric acid secretion plays an important role in the pathogenesis of cysteamine-induced duodenal ulcers (Ishii *et al.* 1976, Kirkegaard *et al.* 1980). This can explain the antiulcer effect of subcutaneously given pentacaine because its antisecretory effect was also evident after this route of administration. Poor absorption of pentacaine from the stomach, even from the ulcerous one (Tomčíková *et al.* 1985), may be responsible for its inefficient antisecretory effect in pylorus-ligated rats after oral administration and suggests an indirect action of pentacaine by its systemic effect.

If clinical studies prove satisfactory, the observed differences in the antiulcer, gastroprotective and antisecretory activity of pentacaine caused by the

different route of administration, might indicate its future use particularly in the treatment of gastric ulcers (including stress ulcers) and under the conditions requiring gastroprotection.

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References

BABUĽOVÁ A., NOSÁĽOVÁ V., BAUER V., BURAN L.: The action of pentacaine on gastric acid secretion in rats. *Agents Actions* **23**: 286–288, 1988.

BENEŠ L.: Trapencaine hydrochloride. *Drugs Future* **16**: 627–630, 1991.

BENEŠ L., BOROVANSKÝ A., KOPÁČOVÁ L.: Alkoxycarbanilic acid esters with high local anaesthetic activity. *Arzneim.-Forsch. (Drug Res.)* **19**: 1902, 1969.

ISHII U., FUJII Y., HOMMA M.: Gastric acid stimulation action of cysteamine in the rat. *Eur. J. Pharmacol.* **36**: 331–336, 1976.

KIRKEGAARD P., POULSEN S.S., LOUD F.B., HALSE C., CHRISTIANSEN J.: Cysteamine-induced duodenal ulcer and acid secretion in the rat. *Scand. J. Gastroenterol.* **15**: 621–624, 1980.

MILLER T.A.: Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. *Am. J. Physiol.* **245**: G601–G623, 1983.

MILLER T.A., JACOBSON E.D.: Gastrointestinal cytoprotection by prostaglandins. *Gut* **20**: 75–87, 1979.

NÁFRÁDI J., BALINT G.A., VARRÓ V.: The effect of prostacyclin and its analogues on cysteamine-induced duodenal ulceration in rats. *Scand. J. Gastroenterol.* **24 (Suppl. 167)**: 90–92, 1989.

NOSÁĽOVÁ V., BABUĽOVÁ A.: Dependence of antiulcer activity of pentacaine on the routes of its administration (in Slovak) *Čs. fyziol.* **40**: 497, 1991.

NOSÁĽOVÁ V., BABUĽOVÁ A., BURAN L., BENEŠ L.: On the pharmacological treatment of phenylbutazone-induced lesions in rats. In: *New Trends in Pathological Physiology. Opuscula Pathophysiologica* J. VAŠKU, M. DOSTÁL (eds), **92**: 399–404, 1985. *Acta Facultatis Medicae Universitatis Brunensis,.*

NOSÁĽOVÁ V., BABUĽOVÁ A., BENEŠ L.: On gastric cytoprotective effect of pentacaine. *Acta Pharmacol. Toxicol.* **59 (suppl. V.)**: 201, 1986.

NOSÁĽOVÁ V., BABUĽOVÁ A., JAKUBOVSKÝ J., BENEŠ L.: Experimental peptic ulcer: effect of pentacaine. In: *New Pharmacology of Ulcer Disease.* S. SZABO, G. MÓZSIK (eds), Elsevier, New York, 1987, pp. 505–516.

NOSÁĽOVÁ V., JURÁNEK I., BABUĽOVÁ A.: Effect of pentacaine and ranitidine on gastric mucus changes induced by cold-restraint stress in rats. *Agents Actions* **33**: 164–166, 1991.

ROBERT A., NEZAMIS J.E., LANCASTER C., HANCHAR A.J.: Cytoprotection by prostaglandins in rats: prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* **77**: 433–443, 1979.

ROBERT A.: Prostaglandins and the gastrointestinal tract. In: *Physiology of the Gastrointestinal Tract.* L.R.JOHNSON (ed.). Raven Press, New York, 1981, pp. 1407–1434.

SHAY H., KOMAROV S.A., FEELS S.S., MERANZE D., GRUENSTEIN M., SIPLET H.A.: A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology* **5**: 43–61, 1945.

SZABO S.: Duodenal ulcer disease. Animal model. Cysteamine-induced acute and chronic duodenal ulcers in the rat. *Am. J. Pathol.* **93**: 273–276, 1978.

ŠTOLC S., STANKOVIČOVÁ T.: Effect of local anaesthetics and pH: new aspects. *Drugs Exp. Clin. Res.* **12**: 753–760, 1986.

TOMČÍKOVÁ O., BABUĽOVÁ A., ĎURIŠOVÁ M., TRNOVEC T., BENEŠ L.: Absorption of pentacaine from ulcerous rat stomach. *Die Pharmazie* **40**: 48–49, 1985.

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