

Pancreatic juice secretion is modulated by histamine in the calf

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Abstract: The modulatory effect of histamine on pancreatic juice (PJ) secretion has been demonstrated in conscious preruminant calves. Earlier studies have shown that histamine stimulates pancreatic juice secretion in guinea-pig pancreatic segments and lobules, isolated dog pancreas, and in anaesthetised rabbit, but little data are available from conscious animals. Therefore, aim of this study was to determine the influence of histamine on pancreatic exocrine secretion in conscious preruminant calves. Cimetidine (H_2 receptor blocker) and promethazine hydrochloride (H_1 receptor blocker) were used to further investigate the enrolment of these receptors in the regulation of the exocrine pancreas in this animal species. Experiments were carried out on 6 calves (48.5±5.5 kg) fitted with a pancreatic duct catheter and a duodenal cannula. Experiments started in the morning after overnight fasting and, according to the Latin square principle. PJ was collected at 15 min. intervals for 45 min. before treatment and for 3 h after treatment. Control calves were treated with physiological saline (control group – 0.1 ml/kg b.w., i.m.) in experimental conditions with histamine (histamine group – 20.0 µg/kg b.w., i.m.), cimetidine 15 min. before histamine (cimetidine + histamine group – 3.6 mg/kg b. w., 20.0 µg/kg b.w., i.m., respectively), and promethazine hydrochloride 15 min. before histamine administration (promethazine hydrochloride + histamine group – 90.0 µg/kg b. w. and 20.0 µg/kg b.w., i.m., respectively). PJ samples were taken for analysis and the remainder was reintroduced into the duodenum. PJ was analysed for volume, total protein content and proteolytic activity. In conscious preruminant calves, histamine increased pancreatic juice volume (20.3±1.5 vs. 15.4 ±1.4 ml/15 min.), protein outflow (123.3±8.6 vs. control – 92.3±6.7 µg/kg b.w./min.) and proteolytic activity (89.7±6.6 vs. control – 40.5±2.6 U/mg of protein). Cimetidine administration before histamine treatment inhibited PJ fluid (8.6±2.0 vs. control – 17.0±1.4 µg/kg b w./min.) and protein outflow (47.3±6.4 vs. control – 70.8±5.7 µg/kg b w./min.), but not proteolytic activity (52.3±4.1 vs. control – 45.2±5.4 U/mg of protein). Promethazine hydrochloride administration eliminated the secretory effect of histamine on exocrine pancreas. We conclude that in the preruminant calf histamine has a stimulatory effect on pancreatic exocrine secretion, which is mediated by both H_1 and H_2 receptors.

Key words: histamine, cimetidine, promethazine hydrochloride, pancreatic juice, preruminant calf

INTRODUCTION

Pancreatic exocrine secretion in animals and humans is mediated by the vagal cholinergic pathway and gut regulatory peptides [1, 2, 3, 4, 5]. The pancreatic ganglia modulate and modify the efferent impulses from the dorsal vagal centre (DVC) in the brainstem and enteric nervous system supplying the glandular cells and vessels with postganglionic fibres. Thus, long vagovagal reflexes and short enteropancreatic reflexes regulate the exocrine pancreatic secretion [6]. These reflexes are fundamental for the action of gut hormones on pancreatic enzyme secretion. Among gut hormones secretin and cholecystokinin (CCK) are the major regulators of pancreatic juice secretion [7, 8, 9, 10]; however, other regulatory gut peptides acting as hormones or neuromediators can modulate this secretion [10, 11, 12]. Some species differences may exist in the regulation of pancreatic exocrine function. In humans and rats the action of CCK on pancreatic enzyme secretion is exclusively vagally mediated, while in the dog CCK acts partially as a classical humoral factor independently of the neural pathway [13]. In the pig and suckling calves the action

of CCK on pancreatic exocrine secretion is mainly mediated by short duodenopancreatic reflexes [7, 8, 10]. However, the role of histamine in the regulation of pancreatic secretion is less understood despite the fact that it is present in abundance in other parts of the digestive tract such as the stomach and intestines. In the pancreas of mouse, histamine immunoreactive cells are present in the glandular epithelium at late embryogenesis [14]. In guinea-pig pancreas, histamine immunoreactive cells are partially associated with the blood vessels and neural peptidergic nerve fibres, suggesting that histamine may play an important physiological role in the regulation of pancreatic blood flow [15].

It has been shown that the secretagogue effect of the paracrine hormone histamine on exocrine pancreas can be compared to that of secretin and vasoactive intestinal peptide [16, 17]; however, its action is moderate compared to acetylcholine and cholecystokinin [17]. In the exocrine pancreas histamine exerts its secretagogue effect through the activation of H_1 receptors evoking the mobilization of potassium ions (K^+) and cellular calcium, as well as through the H_2 receptors causing a shift in endogenous adenosine 3',5' cyclic monophosphate (cAMP) in pancreatic acinar cells [18, 19, 20, 21]. However, histamine can exert inhibitory effects on exocrine pancreatic secretion through the H_3 receptors present in the presynaptic parasympathetic nerve endings controlling the release of acetylcholine via restriction of Ca^{2+} influx [22].

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The role of histamine on pancreatic exocrine secretion has been studied mainly *in vitro* in isolated lobules or acinar cells of rats, mice, guinea pigs, rabbits and humans. Some studies have also been conducted on anaesthetised rabbits and dogs, and a few on conscious dogs and cats [17, 23, 24, 25]. In anaesthetized animals, the pancreatic secretion is markedly reduced due to the depressive effects of anaesthetics on the central nervous system (CNS), especially on the efferent pathway and presumably also on the gut vascular system [26]. Experiments on conscious animals have the advantage of preserving all neural and hormonal pathways influencing the complex regulation of the exocrine pancreatic secretion. The preruminant calf is a good model for studying pancreatic exocrine secretion and has been used in earlier studies [27, 28, 29]. During the first weeks of life calves are exclusively fed milk or milk substitutes containing proteins from vegetal origin causing often allergy-linked GIT disorders [30]. On the other hand, an elevated histamine level is associated with allergic reactions and pancreatic inflammation in animals and humans [31]. Thus, the aim of this study was to determine the influence of exogenous histamine and histamine H₁, H₂ antagonists on pancreatic exocrine secretion in conscious preruminant calves.

MATERIALS AND METHODS

Treatments and experiments were conducted according to the European Community regulations concerning the protection of experimental animals. In 6 male calves aged 15 days (48.5±5.5 kg) the accessory pancreatic duct was catheterised and a silastic cannula (Silastic®; Dow Corning Corp., Midland, USA) was placed in the duodenum, 5 cm distal to the pylorus, according to the method of Buttler [32], modified by Pierzynowski *et al.* [33]. Surgery was performed under combined neuroleptoanalgesia with xylazine (Rompun®, Bayer – 0.15 mg/kg i.m.) and ketamine (Narkamon 5%, Spofa, Czech Republic – 5 mg/kg i.v.) after premedication with atropine (0.01 mg/kg i.m.; Atropinum Sulfuricum, Polfa, Poland). After one week recovery, the experiments were carried out on 6 calves randomized to the all treatments according to Latin square principle protocol. This animal model allowed the maintaining of a constant re-entrant flow of juice to the duodenum between the collection periods, and the collection of pure pancreatic juice (PJ) during the experiments. In overnight fasted calves pancreatic juice (PJ) secretion was allowed to stabilize and collected at 15 min. intervals for 45 min. before treatment, and for 3 h afterwards. Four different sets of experiments were conducted: under control conditions physiological saline (0.1 ml/kg b. w. i.m.) was injected (control group). Treatments were conducted using histamine (20.0 µg/kg b.w., i.m. – histamine group), cimetidine (a H₂ receptor antagonist) 15 min. before histamine administration (3.6 mg/kg. b. w., i.m. and 20.0 µg/kg b. w. i.m., respectively – cimetidine+histamine group) and promethazine hydrochloride (a H₁ receptor antagonist) 15 min. before histamine (90.0 µg/kg. b. w. and 20.0 µg/kg b. w., i.m., respectively – promethazine hydrochloride+histamine group). PJ was collected continuously at 15 min intervals, measured, and 1.0 ml samples taken and stored at -20°C for further analysis. The remaining PJ was reintroduced into the duodenum during the next collection period in small portions using a syringe.

Pancreatic juice was analysed for total protein content according to the method of Bradford [34]. Proteolytic activity was determined spectrophotometrically using casein as substrate. One unit of proteolytic activity was defined as the amount of enzyme that hydrolyzed 1 mg of casein /20 minutes of incubation/mg of protein. Trypsin activity was measured according to the method described by Pierzynowski [35]. One-way ANOVA followed by the Tukey test was used for comparison of the 2 sets of data. The differences were recognised as significant when $p < 0.05$. Statistical analysis was carried out with InStat v2.03 software (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The effect of histamine and H₁ and H₂ receptors antagonists on PJ volume.

The PJ volume did not differ significantly before treatment in the control and experimental groups and oscillated from 14.0 – 18.0 ml/15 min. The PJ volume fluctuated within this range in the control group throughout the observation period (Table 1). The volume of pancreatic juice increased 90 min. after histamine administration (20.0 µg/kg b. w., i.m.) and lasted until the end of experiments. These changes were significantly different from the PJ basal level and the level of PJ secreted by control calves ($P < 0.05$, Table 1). Administration of promethazine hydrochloride (90.0 µg/kg b. w., i.m.) 15 min. before histamine administration (20.0 µg/kg b. w., i.m.) significantly decreased PJ volume at 60-90 min. compared to basal and control levels at the corresponding time of the experiment ($P < 0.05$, Table 1). Administration of cimetidine (3.6 mg/kg. b. w., i.m.) 15 min. before histamine treatment (20.0 µg/kg b. w., i.m.) significantly decreased PJ volume from the basal and control levels at 30 and 45 minutes after histamine administration. However, PJ volume increased to basal level 60-90 minutes after histamine administration, but was significantly lower than the level of PJ secreted in the control calves at the corresponding time of the experiment ($P < 0.05$, Table 1).

Table 1 Pancreatic juice volume (ml/15 min.) in control and experimental conscious preruminant calves (n=6±SE)

Time (minutes)	Control	Histamine	Cimetidine +Histamine	Promethazine hydrochloride +Histamine
-30	14.0±1.5 ^a	16.0±1.2 ^{aA}	15.3±1.7 ^{aA}	15.8±1.3 ^{aA}
-15	16.3±1.7 ^a	18.0±1.6 ^{aA}	17.5±1.3 ^{aA}	16.9±1.7 ^{aA}
0	14.0±1.4 ^a	17.0±1.9 ^{aA}	15.1±1.7 ^{aA}	15.4±1.9 ^{aA}
15	16.0±1.5 ^a	15.8±1.2 ^{aA}	12.6±1.4 ^{aA}	16.6±1.8 ^{aA}
30	17.0±1.4 ^a	16.6±1.3 ^{aA}	8.6±2.0 ^{bB}	13.6±1.4 ^{aA}
45	15.0±1.7 ^a	15.5±1.5 ^{aA}	10.1±1.8 ^{bB}	14.4±1.1 ^{aA}
60	17.8±1.7 ^a	16.5±1.3 ^{aA}	14.4±1.9 ^{bA}	13.2±2.0 ^{bA}
75	17.3±1.3 ^a	18.1±1.2 ^{bA}	14.9±1.5 ^{bA}	13.3±1.8 ^{bA}
90	15.4±1.4 ^a	20.3±1.5 ^{bB}	12.3±1.2 ^{bA}	11.4±1.3 ^{bB}
105	14.2±2.0 ^a	17.8±1.1 ^{bA}	14.5±1.1 ^{aA}	13.3±1.2 ^{aA}
120	14.3±1.3 ^a	16.9±0.5 ^{bA}	12.1±1.5 ^{aA}	12.6±1.1 ^{aA}
135	17.5±1.0 ^a	20.0±1.8 ^{aA}	14.4±1.4 ^{aA}	13.4±1.8 ^{aA}
150	15.1±1.4 ^a	19.8±1.5 ^{bA}	12.3±1.6 ^{aA}	17.0±1.1 ^{aA}
165	15.9±1.4 ^a	20.3±1.9 ^{bB}	13.4±1.9 ^{aA}	17.1±2.0 ^{aA}
180	16.9±0.8 ^a	21.6±1.1 ^{bB}	14.5±1.2 ^{aA}	18.9±1.5 ^{aA}

a,b – different lower case letters represent statistical significance between treatments ($P < 0.05$), A,B – different upper case letters represent statistical significance from the basal secretion in the treatment ($P < 0.05$) (one way Anova and Tukey test).

The effect of histamine and H_1 and H_2 receptors antagonists on PJ protein outflow. PJ protein outflow did not differ significantly during the pre-treatment period in both control and experimental calves and oscillated from 65-88 $\mu\text{g}/\text{kg b.w.}/\text{min}$. During the course of the control experiment, PJ protein outflow fluctuated from 63-97 $\mu\text{g}/\text{kg b.w.}/\text{min}$. (Table 2). Histamine (20.0 $\mu\text{g}/\text{kg b. w.}$, i.m.) evoked a significant protein outflow increase compared to basal and control levels 15 min. after its administration. This effect lasted for 2 h ($p < 0.05$, Table 2). Cimetidine (3.6 mg/kg. b. w., i.m.) administered 15 min. before histamine treatment (20.0 $\mu\text{g}/\text{kg b. w.}$, i.m.) significantly decreased protein outflow in PJ compared to basal and control levels 30 and 45 minutes after its administration ($p < 0.05$, Table 2). However, during the course of the experiment, PJ protein outflow reached basal and control levels, indicating that cimetidine effectively eliminated the histamine stimulatory effect. Promethazine hydrochloride administration (90.0 $\mu\text{g}/\text{kg b. w.}$, i.m.) before histamine treatment, significantly decreased protein outflow compared to basal and control levels. These changes were present from 30-150 minutes after promethazine hydrochloride injection ($P < 0.05$, Table 2).

Table 2 Protein outflow ($\mu\text{g}/\text{kg bw.}/\text{min}$) in the pancreatic juice of control and experimental conscious preruminant calves ($n=6 \pm \text{SE}$)

Time (minutes)	Control	Histamine	Cimetidine +Histamine	Promethazine hydrochloride +Histamine
-30	77.5 \pm 4.7 ^a	76.3 \pm 3.0 ^{aA}	65.3 \pm 5.1 ^{aA}	79.0 \pm 7.4 ^{aA}
-15	76.5 \pm 4.8 ^a	87.5 \pm 4.4 ^{aA}	79.8 \pm 6.1 ^{aA}	88.3 \pm 6.4 ^{aA}
0	84.0 \pm 4.7 ^a	73.5 \pm 4.8 ^{aA}	73.3 \pm 8.3 ^{aA}	83.5 \pm 9.6 ^{aA}
15	85.7 \pm 5.1 ^a	102.5 \pm 5.8 ^{bB}	42.5 \pm 5.1 ^{bB}	66.3 \pm 6.5 ^{bB}
30	70.8 \pm 5.7 ^a	91.7 \pm 3.5 ^{bB}	47.3 \pm 6.4 ^{bB}	58.0 \pm 3.9 ^{bB}
45	62.8 \pm 6.3 ^a	88.0 \pm 9.9 ^{bA}	59.0 \pm 5.0 ^{aA}	39.8 \pm 5.0 ^{bB}
60	76.8 \pm 4.9 ^a	113.7 \pm 9.8 ^{bB}	64.8 \pm 7.4 ^{aA}	54.0 \pm 4.0 ^{bB}
75	92.8 \pm 6.7 ^a	123.3 \pm 8.6 ^{bB}	80.0 \pm 6.1 ^{aA}	65.3 \pm 5.2 ^{bB}
90	74.0 \pm 5.2 ^a	114.0 \pm 8.9 ^{bB}	70.8 \pm 5.5 ^{aA}	61.0 \pm 4.0 ^{bB}
105	82.8 \pm 5.1 ^a	100.8 \pm 4.2 ^{bB}	71.0 \pm 6.7 ^{aA}	65.7 \pm 5.2 ^{bB}
120	78.5 \pm 5.7 ^a	108.3 \pm 4.2 ^{bB}	65.0 \pm 7.6 ^{aA}	56.0 \pm 5.8 ^{aB}
135	76.3 \pm 5.9 ^a	111.5 \pm 5.4 ^{bB}	69.0 \pm 5.1 ^{aA}	51.8 \pm 5.2 ^{bB}
150	97.5 \pm 6.2 ^a	103.2 \pm 5.8 ^{aA}	87.0 \pm 5.9 ^{aA}	79.8 \pm 5.3 ^{aA}
165	91.5 \pm 5.5 ^a	103.0 \pm 6.3 ^{aA}	78.5 \pm 5.3 ^{aA}	84.5 \pm 4.4 ^{aA}
180	94.8 \pm 6.5 ^a	100.3 \pm 4.9 ^{aA}	79.0 \pm 5.0 ^{aA}	89.3 \pm 5.6 ^{aA}

a,b – different lower case letters represent statistical difference between treatments ($P < 0.05$), A,B – different upper case letters represent statistical difference from the basal secretion in the treatment ($P < 0.05$) (one way Anova and Tukey test).

The effect of histamine and H_1 and H_2 receptors antagonists on PJ proteolytic activity. Proteolytic activity oscillated from 31.0-56.8 U/mg of protein in the pre-treatment period and did not differ between control and experimental values. During the next 3 h proteolytic activity in PJ did not change significantly in the control group (Table 3). Histamine (20.0 $\mu\text{g}/\text{kg b. w.}$, i.m.) significantly increased PJ proteolytic activity compared to control level 30 min. after administration ($P < 0.05$). Proteolytic activity significantly increased in comparison to both basal and control levels from the 45-120 min. after histamine administration ($p < 0.05$, Table 3). Afterwards, proteolytic activity reached basal levels. Cimetidine administration before histamine treatment evoked a biphasic increase of the proteolytic activity in the PJ. Significantly higher proteolytic activity compared to the control levels was showed at 15-30 min. (early phase) and from 105-165 min. (late phase) after histamine administration ($P < 0.05$, Table 3). Administration

Table 3 Proteolytic activity (U/mg of protein) in the pancreatic juice of control and experimental conscious preruminant calves ($n=6 \pm \text{SE}$)

Time (minutes)	Control	Histamine	Cimetidine +Histamine	Promethazine hydrochloride +Histamine
-30	38.4 \pm 5.7 ^a	51.5 \pm 5.8 ^{aA}	53.4 \pm 5.5 ^{aA}	41.5 \pm 7.4 ^{aA}
-15	39.3 \pm 5.4 ^a	47.9 \pm 6.9 ^{aA}	43.6 \pm 5.9 ^{aA}	31.1 \pm 6.4 ^{aA}
0	49.0 \pm 6.0 ^a	56.8 \pm 6.8 ^{aA}	46.6 \pm 4.4 ^{aA}	38.9 \pm 9.6 ^{aA}
15	35.7 \pm 6.1 ^a	44.1 \pm 7.8 ^{aA}	54.7 \pm 5.4 ^{bA}	30.6 \pm 6.5 ^{aA}
30	38.5 \pm 3.3 ^a	57.8 \pm 8.5 ^{aA}	63.7 \pm 4.3 ^{bB}	28.5 \pm 3.9 ^{aA}
45	45.2 \pm 5.4 ^a	73.6 \pm 8.4 ^{bB}	52.3 \pm 4.1 ^{aA}	41.5 \pm 5.0 ^{aA}
60	42.5 \pm 6.2 ^a	68.1 \pm 8.3 ^{bB}	48.2 \pm 3.7 ^{aA}	35.8 \pm 4.0 ^{aA}
75	50.3 \pm 5.5 ^a	70.8 \pm 6.3 ^{bB}	55.8 \pm 5.0 ^{aA}	40.3 \pm 5.2 ^{aA}
90	40.5 \pm 2.6 ^a	89.7 \pm 6.6 ^{bB}	46.2 \pm 4.9 ^{aA}	34.8 \pm 4.0 ^{aA}
105	54.0 \pm 4.5 ^a	68.6 \pm 5.2 ^{bB}	70.9 \pm 4.5 ^{bB}	41.8 \pm 5.2 ^{aA}
120	53.7 \pm 6.3 ^a	72.0 \pm 6.7 ^{bB}	72.7 \pm 5.5 ^{bB}	47.4 \pm 5.8 ^{aA}
135	49.4 \pm 7.6 ^a	56.7 \pm 8.4 ^{bB}	84.5 \pm 4.8 ^{bB}	42.3 \pm 5.2 ^{aA}
150	54.0 \pm 7.0 ^a	67.1 \pm 5.1 ^{aA}	67.4 \pm 7.6 ^{aA}	45.2 \pm 5.3 ^{aA}
165	51.3 \pm 6.4 ^a	63.8 \pm 5.2 ^{aA}	76.7 \pm 6.3 ^{bB}	55.3 \pm 4.4 ^{aA}
180	53.0 \pm 6.4 ^a	61.7 \pm 7.4 ^{aA}	59.5 \pm 3.8 ^{aA}	40.6 \pm 5.6 ^{aA}

a,b – different lower case letters represent statistical difference between treatments ($P < 0.05$), A,B – different upper case letters represent statistical difference from the basal secretion in the treatment ($P < 0.05$) (one way Anova and Tukey test).

of promethazine hydrochloride before histamine treatment did not affected PJ proteolytic activity, and the values were close to that of the basal and control levels (Table 3).

Trypsin activity in the control group did not differ from experimental groups in the pre-treatment period and was in the range from 4.0-5.2 U/mg of protein. Histamine significantly increased trypsin activity from 45-75 min. after administration ($p < 0.05$, Table 4). When cimetidine was administered before histamine an increase in PJ trypsin activity was observed from 60-90 min. after histamine administration ($p < 0.05$, Table 4). However, the administration of promethazine hydrochloride before histamine had no effect on trypsin activity in PJ (Table 4).

Table 4 Trypsin activity (U/mg of protein) in the pancreatic juice of control and experimental conscious preruminant calves ($n=6 \pm \text{SE}$)

Time (minutes)	Control	Histamine	Cimetidine +Histamine	Promethazine hydrochloride +Histamine
-30	4.4 \pm 0.3 ^a	4.0 \pm 0.9 ^{aA}	4.7 \pm 0.9 ^{aA}	4.2 \pm 0.9 ^{aA}
-15	5.1 \pm 0.6 ^a	4.4 \pm 0.6 ^{aA}	4.6 \pm 0.6 ^{aA}	4.3 \pm 0.5 ^{aA}
0	4.2 \pm 0.4 ^a	4.2 \pm 0.3 ^{aA}	3.9 \pm 0.4 ^{aA}	3.9 \pm 0.7 ^{aA}
15	3.7 \pm 0.2 ^a	4.7 \pm 0.9 ^{aA}	4.5 \pm 0.9 ^{aA}	3.6 \pm 0.5 ^{aA}
30	4.9 \pm 0.8 ^a	5.1 \pm 0.6 ^{aA}	4.6 \pm 0.6 ^{aA}	4.5 \pm 0.8 ^{aA}
45	4.0 \pm 0.4 ^a	6.0 \pm 0.8 ^{bB}	4.9 \pm 0.4 ^{aA}	3.9 \pm 0.6 ^{aA}
60	4.3 \pm 0.2 ^a	5.4 \pm 0.5 ^{bA}	5.4 \pm 0.5 ^{bB}	4.0 \pm 0.5 ^{aA}
75	4.9 \pm 0.3 ^a	6.5 \pm 0.6 ^{bB}	5.8 \pm 0.4 ^{bB}	4.3 \pm 0.6 ^{aA}
90	4.3 \pm 0.5 ^a	5.0 \pm 0.5 ^{aA}	5.9 \pm 0.5 ^{bB}	3.9 \pm 0.4 ^{aA}
105	5.0 \pm 0.9 ^a	4.6 \pm 0.9 ^{aA}	5.0 \pm 0.9 ^{aA}	5.1 \pm 0.5 ^{aA}
120	4.6 \pm 0.4 ^a	5.4 \pm 0.7 ^{aA}	4.1 \pm 0.8 ^{aA}	5.6 \pm 0.6 ^{aA}
135	5.4 \pm 0.8 ^a	5.5 \pm 0.6 ^{aA}	5.0 \pm 0.6 ^{aA}	5.9 \pm 0.8 ^{aA}
150	5.9 \pm 1.0 ^a	6.6 \pm 0.9 ^{aB}	5.6 \pm 0.4 ^{aA}	5.0 \pm 0.9 ^{aA}
165	5.5 \pm 1.1 ^a	5.2 \pm 0.8 ^{aA}	4.9 \pm 0.9 ^{aA}	5.4 \pm 0.6 ^{aA}
180	5.2 \pm 0.6 ^a	5.6 \pm 0.8 ^{aA}	4.2 \pm 0.6 ^{aA}	5.4 \pm 0.8 ^{aA}

a,b – different lower case letters represent statistical differences between treatments ($P < 0.05$), A,B – different upper case letters represent statistical differences from the basal secretion in the treatment ($P < 0.05$) (one way Anova and Tukey test).

DISCUSSION

Several studies have demonstrated that histamine – a powerful stimulant for gastric secretion – has a marked secretagogue effect on the exocrine pancreas [36, 37, 38, 39, 40] as well as on other exocrine secretory glands [41, 42]. Studies *in vitro* have demonstrated that dog and rabbit pancreas, as well as guinea-pig pancreatic segments and lobules, increase fluid output and enzyme secretion in response to histamine administration [16, 25, 37, 40]. In anaesthetised rabbits [43] and dogs [42, 44], the stimulatory effect of histamine on pancreatic secretion has been also shown, but this effect is not clear in the anaesthetised rat [24]. However, in conscious animals, the stimulatory effect of histamine on the exocrine pancreas has been shown only in dogs [36, 45, 46]. Moreover, only a few studies on the effect histamine H_1 and H_2 receptors antagonists on gastric and pancreatic secretion have been performed in conscious dogs [47] and in humans [48].

We report here the results of studies conducted on a chronic animal model – conscious preruminant calves – that allowed determination of the influence of histamine and H_1 and H_2 antagonists on exocrine pancreatic secretion.

In preruminant calves, exogenous histamine increased pancreatic juice volume, protein outflow and proteolytic activity. This is in accordance with data obtained on isolated dog pancreas [36] and in anaesthetised dogs [46, 49], rabbits and guinea pigs [15, 43]. The results from experiments on calves show that the stimulatory effects of histamine in this animal species concerns both fluid and enzymes. However, the effect on protein and enzymes activity was achieved earlier than the effect on fluid secretion. This suggests the direct action of histamine on pancreatic acinar cells H_1 and H_2 receptors, leading to increased levels of enzymes in the secreted PJ, and indirect action on ductal cells secreting water and electrolytes [50] through the increase of pancreatic blood flow, as suggested by Singh et al. [17].

Exogenous histamine was not able to stimulate PJ fluid and protein outflow when the H_2 receptor antagonist cimetidine was administered first. Moreover, cimetidine evoked a short decrease in PJ volume and protein outflow 30 min. after its administration, suggesting an effective binding to H_2 receptors that abolished both endogenous and exogenous histamine action on these receptors in pancreatic acinar cells. The latter pattern of PJ volume and protein suggests partial inactivation of cimetidine that allowed increase to control levels. On the other hand, when PJ volume and protein reached control levels an increase in proteolytic activity was achieved. This suggests that in the preruminant calf cimetidine may have a releasing effect on enzymatic protein from acinar cells, as has been shown in studies in dogs [44, 45] and in humans with duodenal ulcer [51]. It may be speculated that the synergistic effect does not occur when the cimetidine level in the pancreatic tissue is high, since it was observed after partial inactivation of cimetidine. However, proteolytic activity increased in a biphasic mode, but trypsin activity increased only for a short period during the first phase, 60 min. after histamine administration, suggesting that in the second phase enzymes other than trypsin were secreted. On the other hand, administration of promethazine hydrochloride – a H_1 receptor antagonist – before histamine treatment abolished its stimulatory effect on pancreatic exocrine secretion. Moreover, promethazine hydrochloride decreased PJ volume and protein outflow, but did not affected proteolytic activity. This observation suggests

that H_1 receptors may also substantially mediate the effect of histamine on exocrine pancreas in the preruminant calf, and confirms the results obtained *in vitro* [36, 52] and on resting pancreatic secretion in anaesthetised rabbits [43].

Our results strongly support the data obtained *in vitro* and *in vivo* and in anaesthetised animal models, and indicate that histamine plays a role in the modulation of the exocrine pancreatic secretion in the preruminant calf. We conclude that in the pre-ruminant calf, histamine has a stimulatory effect on pancreatic exocrine secretion and that these effects are mediated by both H_1 and H_2 receptors since administration of their antagonists promethazine hydrochloride and cimetidine decreased PJ volume and protein outflow, and abolished the stimulatory effect of histamine on PJ secretion.

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