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Exocrine Pancreatic Response to Secretin and Bethanechol in Rabbits under Hypothermia

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The effect of the administration of secretin and bethanechol on exocrine pancreatic secretion was studied in rabbits subjected to temperature changes; these involved a drop from 38°C ± 1 to 28°C ± 1 (hypothermia) and a subsequent return to 38°C ± 1 (normothermia). It was observed that hypothermia does not depress the action of secretin on the secretion of fluid, HCO^-_3 and Cl^-. Neither was the action of bethanechol on the enzyme secretion affected by changes in body temperature.

Key words: Exocrine pancreatic secretion, Secretin, Bethanechol, Hypothermia.

Previous studies have shown that changes in body temperature affect exocrine pancreatic secretion; the effect varies in the different animal species assayed: rat (17, 19, 20), dog (6, 12), man (2) and rabbit (4, 11). However, information concerning the action of physiological stimulants on pancreatic secretion under experimental conditions of hypothermia is scanty. Only LUPIANI *et al.* (11) working with the rabbit have studied the effect of secretin and CCK-PZ on the secretion of fluid and amylase, respectively; these authors observed a decrease in the sensitivity of the pancreas to these hormones under hypothermia.

The aim of the present work was to analyze the influence of temperature on the response to secretin of the secretion of fluid, HCO^-_3 and Cl^-, and to bethanechol, a cholinergic agent, on enzyme secretion (total protein, amylase, chymotrypsin and trypsin) in anaesthetized rabbits maintained under conditions of normothermia and hypothermia followed by a return to normothermia.

Materials and Methods

Preparation of animals. — Following a 20 h fast New Zealand rabbits (2-3 kg body wt) were anaesthetized with sodium pentobarbitone (Nembutal, Abott Laboratories, 30 mg/kg, i.v.). After performing a median laparotomy the main pancreatic duct was exposed and cannulated

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at its entrance to the duodenum following ligation of the pylorus and cannulation of the bile duct for derivation of bile to the exterior. The experiments were conducted on four groups of animals: 1) Rabbits which after a period of normothermia (A) were progressively cooled by contact with ice until a rectal temperature of 28°C ± 1 was reached; this state was maintained for 90 min (B), after which the animals were rewarmed by an electric heating system until normothermia was reached again. This state was also maintained for 90 min (C). These animals were injected secretin (0.5 U/kg b. w., i.v.) (Karolinska) at the start of periods A, B and C. 2) Rabbits subjected to the same temperature conditions as group 1 and injected with bethanechol (80 nmol/kg body wt, i.v.) (Sigma) during periods A, B and C. 3) and 4) Rabbits maintained in normothermia throughout the experimental period and injected with secretin and bethanechol respectively, at the start of the experiment, at 130 min and 310 min, times corresponding to the start of periods A, B and C of groups 1 and 2.

Throughout the experiments individual fractions of pancreatic juice were collected on ice.

Assays. — The volume of pancreatic juice of each fraction was determined by weighing on a fully automatic electronic balance, assuming the density of the juice to be 1. Bicarbonate concentrations were determined by measuring the CO_2 content in a Natelson microgasometer (mod. 600). Chloride concentrations were evaluated by a chloridemeter (mod. Analytical Control 1206). Total protein concentrations were estimated by the method of BRADFORD (3). Amylolytic activity was determined according to the method of NOELTING and BERNFIELD (13), adopting arbitrary units defined by HICKSON (9). Chymotrypsin activity was determined after chymotrypsinogen activation according to the method of GILLIAND and GLAZER (7) with modifications (4). Enzymatic activity was calculated spectrophometrically at 25°C using 1 mM of N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate (21). For the determination of trypsin, the trypsinogen of the pancreatic juice was previously activated with porcine enterokinase (18) and enzymatic activity was evaluated by spectrophotometry at 25°C by the method of SCHWERT and TAKENAKA (15), using 0.1 mM of N-benzoyl-L-arginine ethyl ester (BAEE) as substrate.

Statistical analysis. — All values are given as means ± standard deviation. Student's test was employed (5) for comparative analysis of the results obtained between groups 1 and 3, and between 2 and 4 after injection of secretin and bethanechol, respectively. Likewise, a comparison was made between the parameters studied after each injection in each of the groups of animals.

Results

Plotted graphically, the results show the variation in the parameters studied throughout the experimental period. Figure 1a shows that the increase in flow induced by secretin in the rabbits subjected to changes in temperature is very similar to that observed in the control animals; however, it may be seen that the effect of the hormone declines significantly ($p < 0.01$) after the second and third injection in both groups of animals. Similar findings were observed regarding bicarbonate secretion (fig. 1b) since secretin administration led to a significantly greater response ($p < 0.02$) at the start of the experimental period in both groups of animals. Chloride concentrations decreased proportionally to the increases noted in HCO^-_3 concentrations after the injections of secretin (fig. 1b).

Bethanechol affected enzyme secretion

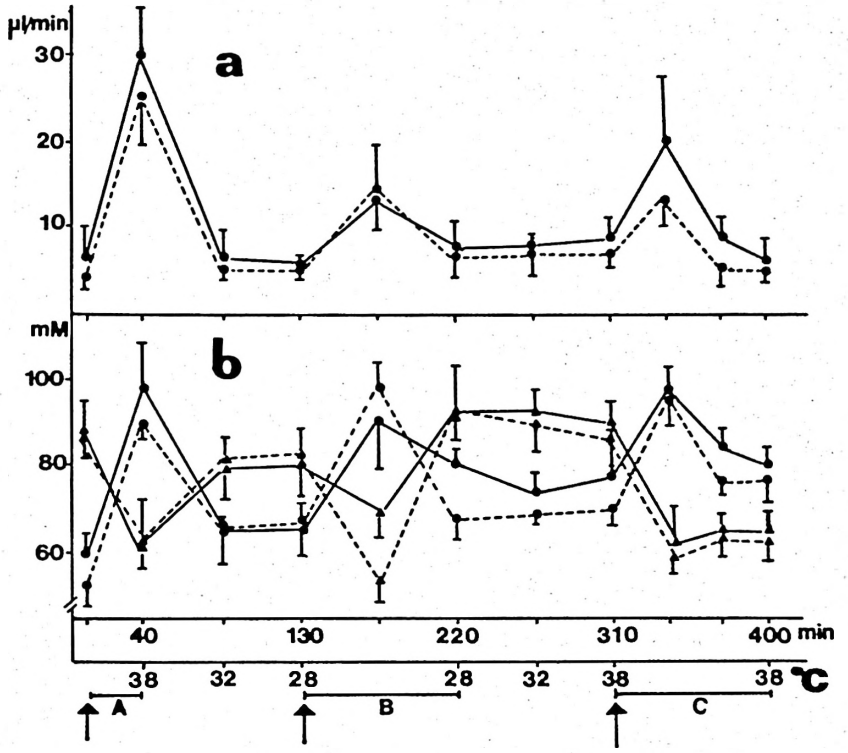
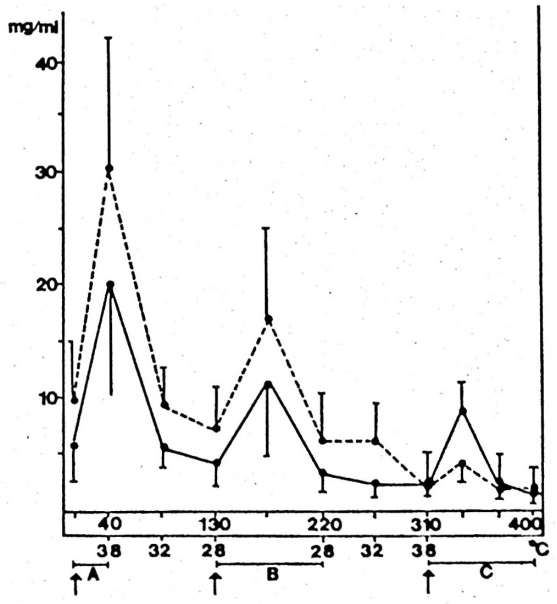


Fig. 1. *Effect of secretin on variation in flow, HCO^-_3 and Cl^-.*
a) Flow; b) HCO^-_3 (●) and Cl^- (▲). Controls (- - -), cooled and rewarmed animals (———). (↑) secretin injection. N = 5.



in the control animals in a similar way to what was observed in the animals subjected to hypothermia. There were no significant differences between either group of animals after administration of the drug. The increase in enzymatic activity recorded after the first injection was significantly greater ($p < 0.01$) both in the control rabbits and in those subjected to hypothermia. The correlation coefficients between the enzyme secretion were significant ($p < 0.001$) in both groups of animals after bethanechol treatment (figures 2, 3, 4 and 5).

Fig. 2. *Effect of bethanechol on total protein concentration.*
Controls (- - - -), cooled and rewarmed animals (———). (↑) bethanechol injection. N = 5.

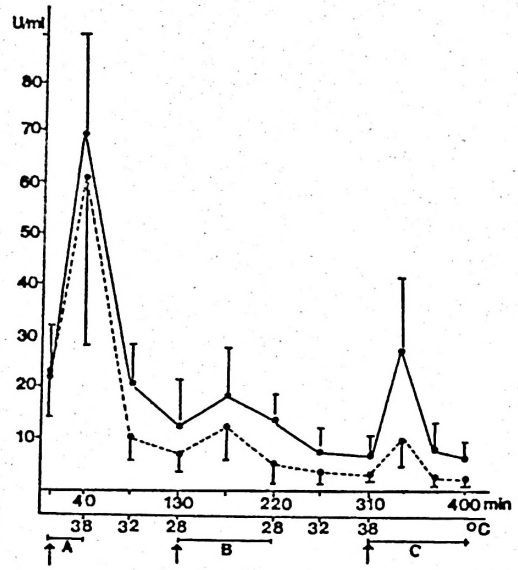


Fig. 3. *Effect of bethanechol on amylase secretion.* Legend as in fig. 2.

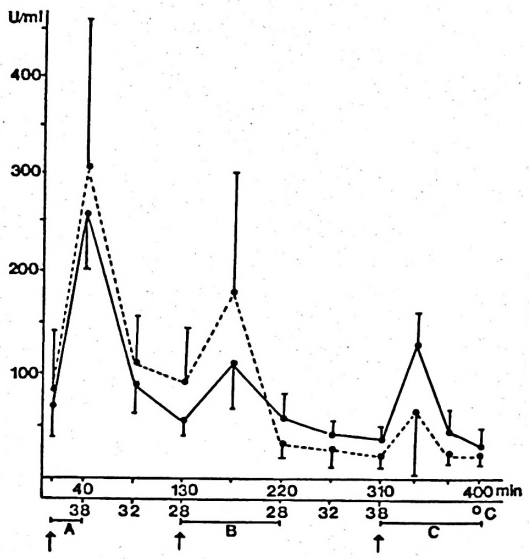


Fig. 5. *Effect of bethanechol on trypsin secretion.* Legend as in fig. 2.

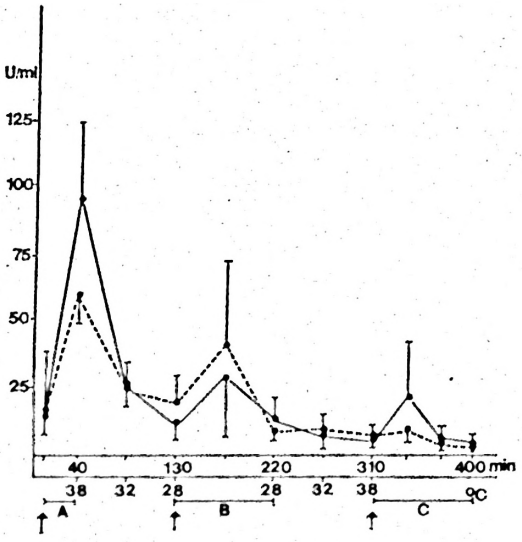


Fig. 4. *Effect of bethanechol on chymotrypsin secretion.* Legend as in fig. 2.

Discussion

In the light of the results it seems clear that hypothermia does not directly affect the action of secretin on hydroelectrolytic secretion in the rabbit pancreas. LUPIANI *et al.* (11), in similar experimental circumstances, concluded that hypothermia depresses the sensitivity of the pancreas to secretin. However, these authors did not perform parallel experiments with animals under continued normothermia (controls); in this experiment, although it is true that in the animals subjected to temperature changes the response to secretin administered at hypothermia was appreciably lower than that observed when the hormone was injected in the initial period of normothermia, it was also clear that in the animals maintained at normal temperatures the response to the hormone was significantly greater at the start of the experimental period than

during later stages, when the animals reached hypothermia or, where this was the case, the time it took the animals to reach normothermia again. Accordingly, the determinant of the intensity of the response to secretin is not the variation in body temperature but rather the time during which the animals are subjected to great stress. The physical exhaustion of the animals during the experimental period may, therefore, be responsible for a certain deterioration in the mode of action of the hormone. The inverse relationship in Cl^- concentrations with respect to those of HCO^-_3 found after the secretin injections has been described in other experimental conditions (14, 16).

The cholinergic influences on enzyme secretion are clear from the results obtained. Control of the enzyme fraction in the rabbit, and in many other species has been shown to be governed by the parasympathetic nervous system, as well as by CCK-PZ (10). In this study bethanechol was chosen as a cholinergic agonist in view of its relatievely unimportant vascular effects. Recently, BEGLINGER *et al.* (1) have reported effects in total protein secretion in dog comparable to those obtained in this experiment in rabbit at similar doses. The action of bethanechol is similar in the control animals and in those subjected to temperature changes and hence hypothermia does not modify the mechanism of action of this drug at all. De DIOS *et al.* (4) have demonstrated that hypothermia does not affect enzyme secretion by the pancreas; this situation is maintained under the influence of physiological stimulants of this fraction. A parallel secretion of enzymes is conserved, as has been pointed out in other situations (4, 7, 8).

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Resumen

Se estudia el efecto de la secretina y del betanecol sobre la secreción pancreática exocrina en conejos sometidos a cambios de temperatura: desde 38°C ± 1 se baja la temperatura corporal a 28°C ± 1 (hipotermia) y posteriormente se vuelve a las condiciones de normotermia. La hipotermia no deprime la acción de la secretina sobre la secreción de flujo, CO_3H^- y Cl^-. La acción del betanecol sobre la secreción enzimática tampoco se ve afectada por los cambios de temperatura corporal.

Palabras clave: Secreción pancreática exocrina, Secretina, Betanecol, Hipotermia.

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