Immunological blocking of exogenous and endogenous secretin in the dog[1]

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Summary. In mongrel dogs with chronic gastric and duodenal fistula the biological activity of secretin on exocrine pancreatic secretion could be blocked by preincubating the secretin injected with rabbit antisecretin antibody. In addition, the activity of endogenous secretin released by acid was markedly reduced by application of antibody. Since no such effect was observed after testmeal stimulation, secretin is most probably not released in a significant amount by the liquid meal used in this experiment.

Acidification of the duodenum is unanimously recognized to stimulate secretin release from the gut, as well as pancreatic volume and bicarbonate secretion[3]. However, there is continuing controversy as to the physiological significance of secretin in the response to a mixed meal. Insufficient sensitivity and nonspecific interference in the secretin radioimmunoassay limit the interpretation of plasma concentrations measured in peripheral blood. To elucidate the physiological role of secretin in response to a meal, other approaches are required.

We therefore investigated: 1. whether exocrine pancreatic secretion in response to exogenous secretin can be inhibited by incubating the secretin injected with various concentrations of antisecretin antibody. 2. The effect of passive immunisation with antisecretin antibodies on exocrine pancreatic secretion in response to testmeal and intraduodenal acid stimulation.

In a mongrel dog, weighing 23 kg, with chronic gastric and duodenal fistula, pure pancreatic juice was collected in 3 15-min-samples following injection of 0.05 µg/kg b.wt synthetic secretin 'Roche'[4,5] incubated with saline for 30 min at room temperature before injection. The volume was fixed at 2 ml for all experiments. This procedure was performed 4 times to establish baseline data on pancreatic volume, bicarbonate, and protein secretion. Incubation of secretin with undiluted normal rabbit serum did not alter the pancreatic secretory response (table). The same experiments were then performed after incubating the secretin with an anti-secretin anti-serum (No.21) before injection. The antibody was produced in a rabbit with synthetic secretin coupled to BSA[6]. 2 ml of undiluted anti-serum were able to bind 2.5 µg synthetic secretin in vitro as shown by the identical binding (80%) of labelled secretin with or without addition of 2.5 µg of secretin.

The table contains the results for exocrine pancreatic secretion obtained with injection of secretin preincubated with the antiserum undiluted as well as diluted 1:4, 1:8, and 1:16. The data show that with undiluted antiserum the pancreatic response to exogenous secretin was reduced to a level that did not differ from fasting secretion. Inhibition was less marked with dilutions 1:4 and 1:8. At a dilution of 1:16 no effect was noted.

The effect of anti-secretin antibody on the endogenous release of secretin in response to acid and to a meal was then examined. In another mongrel dog, weighing 25 kg, with a similar fistula in the stomach and duodenum, pure pancreatic juice was collected during either intraduodenal infusion of 40 ml 0.1 M HCl administered within 5 min or intragastric application of a liquid meal at a rate of 5 ml/kg b.wt[7]. Volume, bicarbonate, and protein output were determined[7]. The concentration of antibodies in the peripheral blood was followed by determining the capacity of the plasma to bind I^{125}-secretin in vitro. The experiments were performed 3 times before, and on days 1, 3, and 7 after i.v.

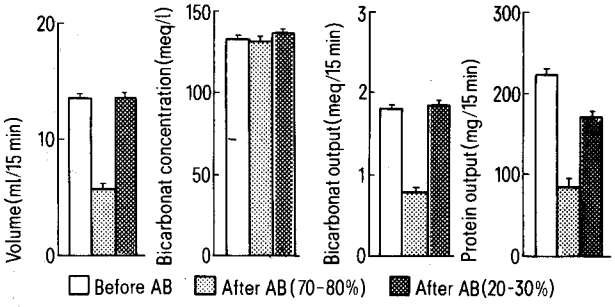

Fig. 1. Pancreatic secretory response to intraduodenal acid before and after administration of antisecretin antibody (AB). Bars represent mean ± SEM.

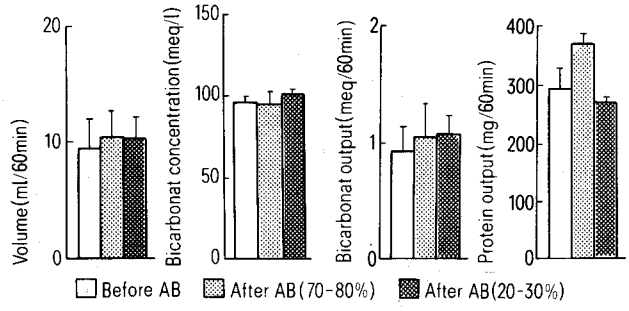

Fig. 2. Pancreatic secretory response to testmeal before and after administration of antisecretin antibody. Bars represent ± SEM.

Pancreatic secretory response to exogenous secretin incubated with saline and rabbit antisecretin antiserum

Secretin 0.05 µg/kg reincubated with	Number of trial	Volume[a] ml/15 min	Bicarbonate output meq/15 min	Protein output mg/15 min
NaCl	4	15.0 ± 0.54[b]	2.04 ± 0.07	67.1 ± 17.8
Normal rabbit serum undiluted	3	16.0 ± 0	2.14 ± 0.3	147.2 ± 23.3
Secretin-AB diluted 1:16	1	15.5	2.15	102.3
Secretin-AB diluted 1:8	1	6.25	0.74	79.4
Secretin-AB diluted 1:4	1	7	0.87	74.2
Secretin-AB undiluted	1	1	0.07	35.9
Fasting secretion	4	0.47 ± 0.2	0.022 ± 0.01	28.14 ± 2.94

[a] The first 15 min-samples after secretion injection were used for comparison. [b] Mean ± SEM.

administration of 2 ml of the anti-secretin antibody (No. 21) undiluted at high antibody concentrations, and on days 27 and 70 at low concentrations.

Anti-secretin antibody significantly diminished HCl-stimulated volume by 61.4% ($p < 0.01$), bicarbonate by 62% ($p < 0.001$), and protein output by 63% ($p < 0.005$) respectively (figure 1). All parameters returned to pretreatment levels, when the antibody 'concentration' was 30% (day 27) or less (day 70) of the immediate post injection value.

In contrast, pancreatic secretion following a test meal did not show any significant changes at any time after antibody administration (figure 2).

The data show that the secretin antibody used blocks the biological activity of exogenous and endogenous secretin. The pancreatic response to intraduodenal acid was reduced by over 60%. The residual pancreatic secretion could be due to pancreozymin[8,9] and peptide hormones such as VIP[10]. Alternatively, it is possible that the antibodies used did not completely block the biological activity of secretin.

The observation that there was no reduction of pancreatic response to a mixed liquid meal after antibody injection suggests that secretin is not involved in stimulating pancreatic secretion after the type of meal used in this experiment.

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Regularly firing neurones in the rat suprachiasmatic nucleus

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Summary. The spontaneous discharge of some suprachiasmatic neurones in vivo and in vitro was found to exhibit a very constant interspike interval. In vivo these cells were comparatively rare and appeared to be mutually coupled. These findings are discussed in relation to coupled oscillator theories of circadian rhythm generation.

In mammals the suprachiasmatic nuclei of the hypothalamus (SCN) are generally thought to be involved in the photic entrainment of circadian rhythms. Moreover, they play an important role in the generation of selfsustained circadian oscillations[1]. Although gradually more insight is being obtained with respect to the physiology of entrainment[2,3] little is known about the putative oscillating mechanism of the SCN. In the present paper, we report the presence of regularly firing neurones in the rat SCN in vitro and in vivo. This finding will be discussed in relation to Pavlidis' coupled oscillator model of circadian rhythm generation[4].

Materials and methods. Hypothalamic slices were prepared from 16 albino rats which were decapitated after light halothane anaesthesia. From each brain 1 or 2 thin transverse slices (approx. 400 µm thick) containing the optic chiasm and SCN were dissected out. Within 12 min after decapitation, these slices were incubated in an in vitro incubation chamber[5]. The slice was bathed from below in a glucose/saline bicarbonate buffered medium, while the upper surface was exposed to a warm, humid atmosphere of 95% O_2 and 5% CO_2. The medium was equilibrated with this gas mixture to give a pH of approximately 7.3 at 37 °C. Flow rates varied between 1 and 2 $ml \cdot min^{-1}$. The composition of the incubation medium was: NaCl: 124 mM; KCl: 5 mM; KH_2PO_4: 1.24 mM; $MgSO_4$: 1.3 mM; $CaCl_2$: 1 mM; $NaHCO_3$: 26 mM; glucose: 10 mM. The temperature in the chamber was regulated at 37 ± 0.1 °C. Glass micropipettes either filled with Wood's metal and platinized at the tip or filled with 3 M KCl were positioned in the SCN. In addition single units were recorded in 42 anaesthetized rats using conventional recording techniques[2]. Power spectra of spike trains were computed according to the method of French and Holden[6]. Recording sites in both in vivo and in vitro experiments were verified histologically to be within the SCN by means of electrolytic or pontamine sky blue marking.

Results and discussion. The slices could be kept in good condition for recording for over 8 h after decapitation. Action potentials were recorded reliably for extended periods of time from 98 spontaneously active SCN units in vitro. 82 of the neurones had stationary randomly varying firing rates or showed a bursing spike pattern.

A remarkable observation was the presence of 16 regularly firing SCN cells (RFC's) exhibiting a very constant interspike interval (figure 1 A and C). These patterns do not represent an injury discharge as the firing rates were lower than those commonly observed in injured cells, while the frequency remained stable over extended periods of time. Moreover, the behaviour of these cells did not change when the electrode was slowly moved away from the cell and back. The interval distributions of all RFC's were Gaussian and typically had small coefficients of variation (range: 0.05–0.2, e.g. figure 1, B). Only cells with variation coefficients smaller than 0.2 were classified as regular. These small coefficients are only slightly larger than those found for molluscan beating neurones[7]. At present, however, it is unclear whether the RFC's in our sample are endowed with a similar intrinsic pacemaker mechanism as beating pace-