Br. J. Pharmac. (1976), **58**, 17–25

FURTHER STUDIES ON THE MODE OF ACTION OF ISOPRENALINE ON GASTRIC SECRETION IN THE CONSCIOUS RAT

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1 The effect of isoprenaline on gastric secretion evoked by various means has been studied in conscious rats provided with Pavlov and Heidenhain pouches.

2 Interdigestive acid secretion in the Pavlov pouch was reduced by isoprenaline, whereas pepsin secretion was unaltered.

3 Central vagal stimulation effected by 2-deoxy-D-glucose injection evoked a gastric secretory response that was substantially reduced by isoprenaline.

4 2-Deoxy-D-glucose increased the mobilization of gastric mucosal histamine, an effect that was prevented by isoprenaline.

5 Isoprenaline infusion alone induced a slight increase in histamine mobilization and also a considerable elevation of immunoreactive serum gastrin concentration.

6 The secretory response to food in the Pavlov pouch was almost abolished by isoprenaline.

7 Although the acid response to histamine in the Heidenhain pouch was susceptible to isoprenaline inhibition, that to methacholine was not.

8 Pepsin secretion in the Heidenhain pouch preparation stimulated by histamine or methacholine seemed to be enhanced by isoprenaline.

Introduction

In the rat, mobilization of gastric mucosal histamine may be closely related to the excitation of the parietal cell. Gastrin mobilizes histamine in a dose-dependent way from cells in close proximity to the parietal cells, in amounts sufficient to stimulate hydrochloric acid secretion (Kahlson, Rosengren, Svahn & Thunberg, 1964; Johansson, Lundell, Rosengren & Svensson, 1972; Lundell, 1974a, b). Consequently, the mode of action of gastrin in stimulating HCl secretion has been suggested to be by means of histamine mobilization. This suggestion has been further strengthened by the results obtained with histamine H_2-receptor antagonists on gastrin-induced secretion (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973; Lundell, 1973; 1975a).

It has recently been demonstrated in this laboratory that isoprenaline inhibits pentagastrin-induced acid secretion in the rat and also restrains the increase in mucosal histamine mobilization, otherwise seen on pentagastrin infusion (Lundell & Svensson, 1974). These effects of isoprenaline were almost completely prevented by propranolol, indicating involvement of a β-adrenoceptor.

In the present study, the effect of isoprenaline was investigated on vagally-induced gastric secretion and histamine mobilization. Although vagus nerve stimulation is followed by both an increased mobilization of the amine and a direct activation of the parietal cells, the role of mobilized histamine for the resulting secretory response is far from settled (Rosengren & Svensson, 1969; Hakanson & Liedberg, 1970; Johansson *et al.*, 1972; Lundell, 1974b; 1975a). Furthermore, the effect of isoprenaline was studied on histamine and methacholine-induced gastric secretion, since these agents are considered to excite acid secretion without alterations in the mobilization of mucosal histamine. In order to obtain further information on the mode of action of isoprenaline, the serum concentrations of gastrin and glucose were determined in some relevant conditions.

Methods

Determination of gastric secretion

Female rats of the Sprague-Dawley strain, weighing about 250 g, and fed on a standard pellet diet, were

used. All operations were done under ether anaesthesia. The rats were provided with Heidenhain (vagally denervated) pouches in the main according to Alphin & Lin (1959) or Pavlov (innervated) pouches as described by Svensson (1970). At least one month was allowed for postoperative recovery. Rats were fasted for 18 h before experiments except when the secretory response to food was studied when the fasting period was 24 hours. During the experiments the rats were kept unanaesthetized in Bollman cages. Gastric juice was collected in 30 min samples by the use of a perfusion technique (Svensson, 1970) and the amount of HCl secreted was determined by titration against 0.1 M NaOH with phenol red as an indicator. The pepsin output was measured by a modification of the method of Hunt (1948). The amount of HCl is expressed in μEq/30 min and pepsin output in μg/30 min, with the corresponding activity of a commercial crystalline preparation of pepsin (Lot 95B – 1270, Sigma Chemical Co) used as a standard as proposed by Bitsch (1966). Throughout an experiment, a constant infusion of 0.9% w/v NaCl solution (saline), into a catheter inserted in a tail or neck vein, was maintained by a motor-driven syringe. After interdigestive secretion had been collected for 1–2 h, drugs were added to the infusate. In the feeding experiments, food was allowed for 30 min, when the rats consumed 2–3 g of the standard pellet diet. Isoprenaline infusion did not apparently influence the amount of food ingested.

The following drugs were used: methacholine chloride (Fluka AG), 2-deoxy-D-glucose (Sigma Chemical Co), (±)-isoprenaline sulphate, (±)-propranolol hydrochloride (Inderal, ICI) and histamine dihydrochloride (E. Merck AG – Darmstadt). The doses of all agents are expressed in terms of the salt, except for histamine which is given in terms of the base.

Determinations of content and formation of mucosal histamine

Observations were made on intact rats fasted for 18 h and injected intravenously with 2-deoxy-D-glucose alone or in combination with either isoprenaline or isoprenaline plus propranolol. The effect of isoprenaline infusion alone was also studied. The animals were killed by a blow on the head and exsanguinated. The stomach was opened along the lesser curvature, washed with saline, pinned flat and blotted with gauze; the oxyntic gland area was removed by scraping with a scalpel. After mincing, the mucosa from one stomach was divided in two portions, one of which was used for determinations of histamine content, the other for formation. Histamine content was determined by a modification of the method of Feldberg & Talesnik (1953) as described in detail by Lundell (1974a).

The rate of histamine formation, i.e. histidine decarboxylase activity, was determined by a method devised by Kobayashi (1963), as adapted for use in this laboratory (Henningsson, Lundell & Rosengren, 1974). The minced mucosa was incubated for 3 h at 37°C under N_2 in beakers containing 100 mg tissue, 40 μg [^{14}C-carboxyl]-L-histidine (sp. act. 104.9 μCi/mmol), 1 mM disodium edetate (EDTA), 0.5 mM glutathione, 10 μM pyridoxal-5-phosphate, 0.1M sodium phosphate buffer pH 7.4 and 0.2% w/v glucose, the total volume made up to 3 ml. In the blanks 10 mM semicarbazide was added. The incubation was stopped by gentle tipping of 1 ml 2 M citric acid from a side arm into the incubation mixture. Adequate $^{14}CO_2$ trapping into the Hyamine 10–x (0.1 mmol) on the filter paper, placed within the incubation vessel, was achieved by continuing mechanical shaking for another 45 minutes. The filter paper was placed in a counting vessel containing 10 ml of Bray (1960) scintillation solution and counted in a liquid scintillation spectrometer.

Determinations of serum gastrin

Serum gastrin was determined in intact rats fasted for 18 hours. A polyethylene tube was inserted in a tail vein for administration of isoprenaline alone or combined with propranolol. For collection of blood, another polyethylene tube was inserted in the external jugular vein and the tip was passed into the superior caval vein. These procedures required ether anaesthesia for about 10 min, after which a period of at least 3 h was allowed for recovery. The tube for collection of blood was kept patent by intermittent flushing with a heparin solution (10 iu/ml). During the experiment, blood was drawn at 30 min intervals (usually 0.5 ml). After centrifugation, serum was decanted and stored at −20°C until analysis. The immunoreactive gastrin concentration was determined by a specific radioimmunoassay (Nilsson, 1975). The antiserum employed in the present study (2604-8; Rehfeld, Stadil & Vikelsøe, 1974) was used in a final dilution of 1:120.000. Mono-iodinated ^{125}I synthetic human gastrin I was employed as tracer. To elevate the extent to which the assay measured the amount of the hormone in the actual species, rat serum was diluted and tested over a 10-fold range of concentration. The immunoreactive rat gastrin could not be distinguished from the porcine gastrin I standard. However, since rat gastrin was not used as a reference standard, the values relate to porcine gastrin I standard.

Determinations of serum glucose

Blood was drawn from an external jugular vein at 30 min intervals. Saline and isoprenaline were infused into a tail vein. Serum was decanted and glucose con-

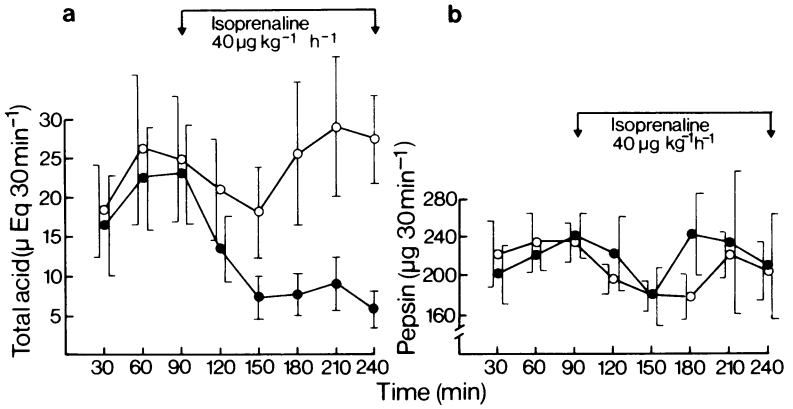

Figure 1 Effect of isoprenaline infusion (40 μg kg^{-1} h^{-1}) on interdigestive secretion (●) of (a) acid and (b) pepsin. Each point represents the mean of one determination in each of six Pavlov pouch rats. The vertical lines show s.e. mean. (○) = Control values.

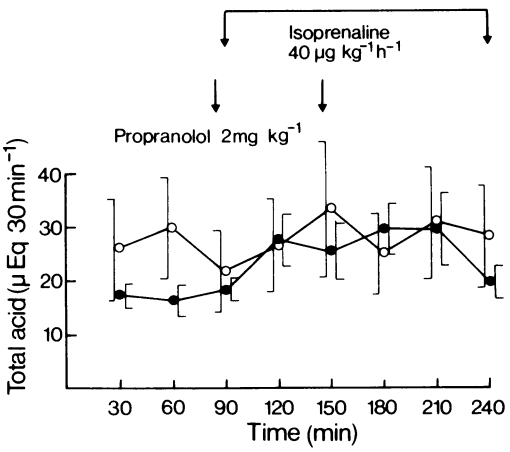

Figure 2 Effect of combined intravenous administration of isoprenaline (40 μg kg^{-1} h^{-1}) and propranolol (2 mg/kg) on interdigestive acid secretion (●). Propranolol was given as indicated by the arrows. Each point represents the mean of one determination in each of six Pavlov pouch rats. The vertical lines show s.e. mean. (○) = Control values.

centration determined by the glucose-oxidase technique.

P-values were obtained by using Student's *t*-test for paired or unpaired values.

Results

The effect of isoprenaline on interdigestive secretion

Interdigestive acid and pepsin secretion (i.e. during saline infusion) were investigated in six Pavlov pouch rats for 4 hours. Interdigestive secretion of acid was about 25 μEq/30 min and that of pepsin about 220 μg/30 minutes. After 1 h of isoprenaline infusion (40 μg kg^{-1} h^{-1}) acid secretion was reduced to a value of about 30% of the control level whereas the pepsin output was unaltered (Figure 1a and b). The pronounced effect os the β-adrenoceptor agonist on interdigestive acid secretion was completely abolished by propranolol (2 mg/kg) (Figure 2).

Inhibition by isoprenaline of the secretory response to 2-deoxy-D-glucose

Central vagal excitation effected by 2-deoxy-D-glucose injection (100 mg/kg) was followed by a rapid increase in acid secretion with a peak of 83.3 ± 25.80 (s.e. mean) μEq/30 min after 60 min (Figure 3a). Isoprenaline infusion (40 μg kg^{-1} h^{-1}) substantially reduced the response to 2-deoxy-D-glucose, attaining a value of 34.7 ± 11.32 μEq/30 min after 60 minutes. This reduction is highly significant ($P < 0.01$). Propranolol completely antagonized the effect of isoprenaline.

Pepsin secretion in response to 2-deoxy-D-glucose rose rapidly to a peak of 654 ± 80.9 μg/30 min after 60 min after which the secretory rate diminished (Figure 3b). Isoprenaline reduced the peak response to a value about 55% less than with 2-deoxy-D-glucose alone. Propranolol also blocked this inhibitory effect of isoprenaline (Figure 3b). In another series of experiments, isoprenaline infusion (40 μg kg^{-1} h^{-1}) started 1 h before injection of 2-deoxy-D-glucose (100 mg/kg). The effect of isoprenaline was the same as described above. A background infusion of histamine (1000 μg/h), which started 30 min before 2-deoxy-D-glucose injection did not significantly diminish the inhibitory effect of isoprenaline.

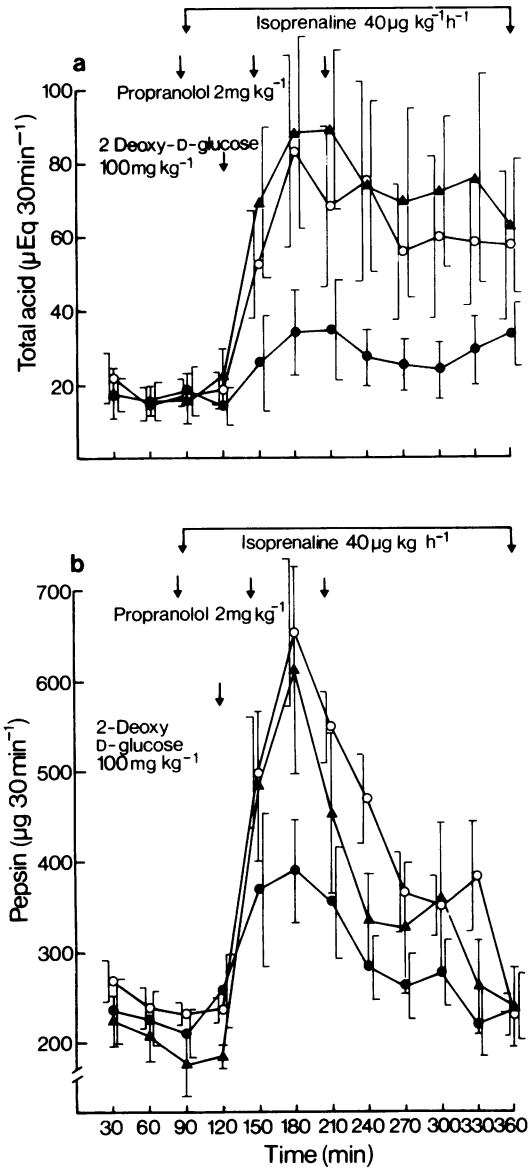

Figure 3 (a) The acid and (b) pepsin secretory effects of 2-deoxy-D-glucose (100 mg/kg) injection alone (○) or combined with isoprenaline (40 μg kg^{-1} h^{-1}) (●). Included is the effect of 2-deoxy-D-glucose combined with both isoprenaline and propranolol (2 mg/kg) (▲). Propranolol was given as indicated by the arrows. Each point represents the mean of one determination in each of six Pavlov pouch rats. The vertical lines show s.e. mean.

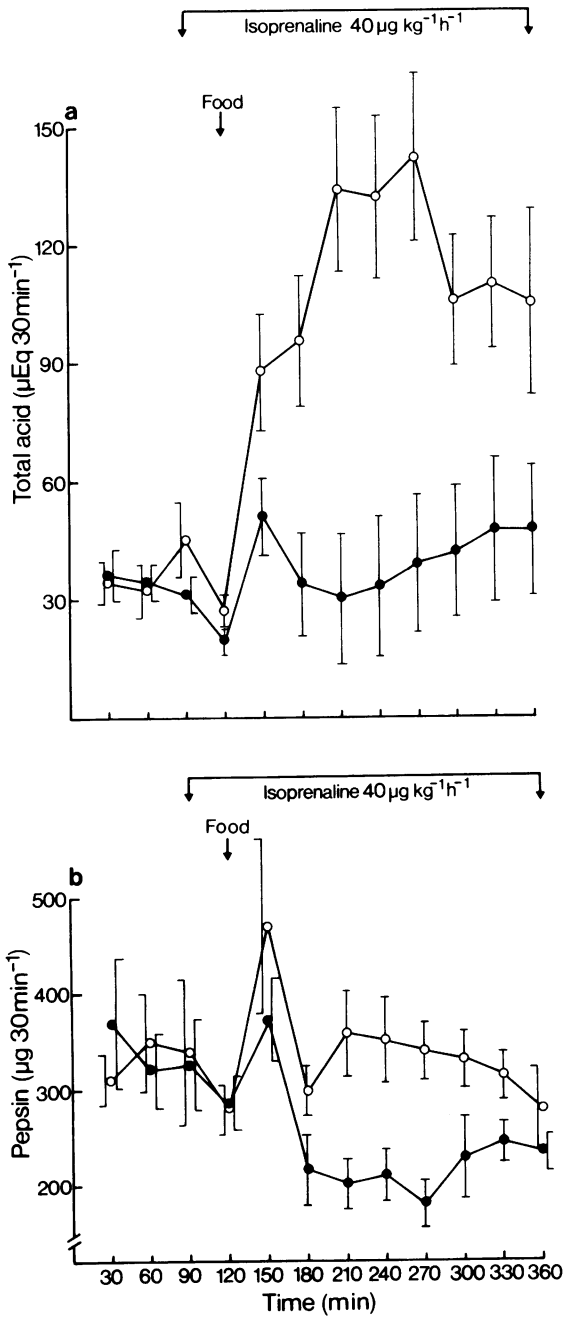

Figure 4 (a) Acid and (b) pepsin secretion in response to food alone (○) or combined with isoprenaline infusion (40 μg kg^{-1} h^{-1}) (●). Each point represents the mean of one determination in each of six Pavlov pouch rats. The vertical lines show s.e. mean.

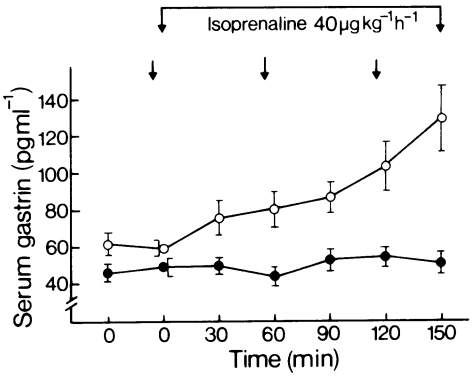

Figure 5 Serum gastrin level (pg ml^{-1}) during isoprenaline infusion (40 μg kg^{-1} h^{-1}) alone (○) or combined with propranolol (2 mg kg^{-1}) injection (●). Propranolol was injected as indicated by the arrows. Each point represents the mean of one determination in each of six rats. The vertical lines show s.e. mean.

Isoprenaline inhibition of the feeding response

Isoprenaline infusion almost abolished the response to feeding in six Pavlov pouch rats and only an initial response of about 50 μEq/30 min was recorded (Figure 4a). Feeding induced a pepsin secretory response that lasted only for 30 minutes. During isoprenaline infusion, a small initial pepsin response was noted, after which the response was significantly lower than in the controls ($P < 0.01$) (Figure 4b).

Inhibition of the mobilization of mucosal histamine by isoprenaline

Histamine mobilization was estimated by concomitant determination of mucosal histamine content and formation. 2-Deoxy-D-glucose (100 mg/kg) reduced histamine content from 83.8 ± 7.10 to 57.4 ± 3.58 μg/g and increased the rate of formation from 6.6 ± 1.00 to 17.7 ± 1.37 μg g^{-1} 3 h^{-1}. During isoprenaline infusion, vagal stimulation induced only a slight increase in histamine formation to 9.0 ± 1.41 μg g^{-1} 3 h^{-1} and no effect was noted on histamine content. Propranolol reversed the inhibitory effect of isoprenaline on 2-deoxy-D-glucose-induced histamine mobilization (Table 1). It should be noted that isoprenaline alone increased the rate of histamine formation and slightly reduced histamine content (Table 1).

Isoprenaline-induced alterations in serum gastrin and glucose

Isoprenaline infusion (40 μg kg^{-1} h^{-1}) induced a progressive increase in immunoreactive serum gastrin concentration from about 60 pg/ml in the fasting state to 130.8 ± 17.95 pg/ml after 2.5 h of infusion. Propranolol (2 mg/kg) administered at hourly intervals prevented the increase (Figure 5). The serum concentration of glucose in the fasting state was about 80 mg/100 ml and increased on isoprenaline infusion to a peak value of 115.1 ± 10.25 mg/100 ml after 60 minutes. On prolonged isoprenaline infusion, serum glucose concentration returned to control levels.

The effect of isoprenaline on histamine-induced secretion

The effect of isoprenaline (20 μg kg^{-1} h^{-1}) on histamine-induced (1000 μg/h) gastric secretion was investigated in rats provided with Heidenhain pouches. This dose of isoprenaline was chosen since it effectively reduced the acid secretory response to pentagastrin (Lundell & Svensson, 1974). In six rats, isoprenaline given 30 min before and maintained during histamine infusion, inhibited acid secretion by about 40% (Figure 6a). In contrast, the stimulatory effect of histamine on pepsin secreting cells was slightly augmented by isoprenaline (Figure 6b).

In another group of six Heidenhain pouch rats, the effect of isoprenaline was studied on a plateau secretion induced by histamine. Histamine infusion failed to elicit a stable plateau secretion, instead the secretory response faded with time of infusion. Isoprenaline (20 μg kg^{-1} h^{-1}) reduced the acid response, a reduction that reached statistical significance 1 and 1.5 h after the start of isoprenaline

Table 1 Histamine formation (μg g^{-1} 3 h^{-1}) and content (μg g^{-1}) 2 h after intravenous injection of 2-deoxy-D-glucose (2DG, 100 mg/kg) alone and in combination with isoprenaline (Iso, 40 μg kg^{-1} h^{-1}) or isoprenaline plus propranolol (Prop, 2 mg/kg)

	Controls	*2DG*	*2DG + Iso*	*2DG + Iso + Prop*	*Iso*
Histmine formation	6.6 ± 1.00	17.7 ± 1.37	9.0 ± 1.41	17.3 ± 1.82	9.4 ± 2.90
Histamine content	83.8 ± 7.10	57.4 ± 3.58	90.0 ± 13.40	58.3 ± 7.03	75.2 ± 11.45

Isoprenaline infusion started 30 min before the injection of 2-deoxy-D-glucose. Propranolol was injected 10 min before the start of isoprenaline infusion and the injection was repeated at hourly intervals. The controls were given saline only. The mean and s.e. mean are calculated from determinations on six rats in each group.

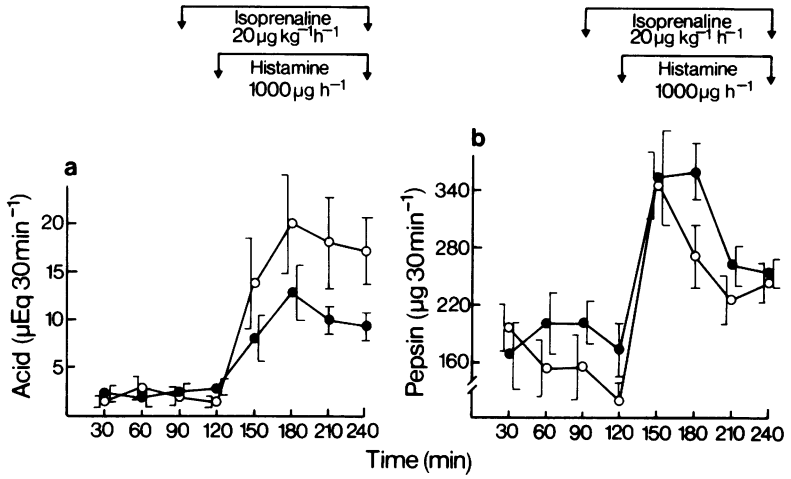

Figure 6 (a) Acid and (b) pepsin secretion in response to histamine infusion (1000 μg h^{-1}) alone (O) or combined with isoprenaline (20 μg kg^{-1} h^{-1}) (●). Each point represents the mean of one determination in each of six Heidenhain pouch rats. The vertical lines show s.e. mean.

infusion ($P < 0.05$) (Figure 7). Propranolol blocked the effect of isoprenaline (Figure 7), but the β-receptor antagonist did not influence the acid secretory response to histamine alone.

The stimulant effect of histamine on pepsin secretion was slight on prolonged infusion. Neither isoprenaline nor propranolol affected the pepsin secretory response.

The effect of isoprenaline on methacholine-induced secretion

The secretory response to methacholine alone (1 μg/h) or in combination with isoprenaline (20 μg kg^{-1} h^{-1}) was studied in six Heidenhain pouch rats. Methacholine induced an acid secretory response of about 15 μEq/30 min with a tendency to increase with time of infusion. Isoprenaline infusion did not significantly inhibit the secretory response (Figure 8a).

The potent stimulation of pepsin secretion by methacholine is illustrated in Figure 8b. Isoprenaline augmented this effect of methacholine, an increase that reached statistical significance ($P < 0.05$) during the last 90 min of isoprenaline administration.

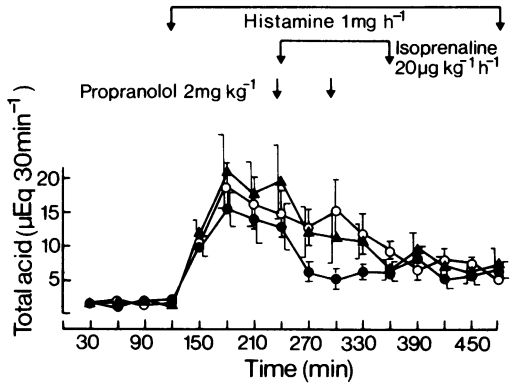

Figure 7 Acid secretion in response to histamine (1 mg h^{-1}) alone (O) or combined either with isoprenaline (20 μg kg^{-1} h^{-1}) (●) or isoprenaline plus propranolol (2 mg kg^{-11}) (▲). Propranolol was injected as indicated by the arrows. Each point represents the mean of one determination in each of six Heidenhain pouch rats. The vertical lines show s.e. mean.

Discussion

Isoprenaline is known to inhibit histamine release from sensitized lung tissue (Assem & Schild, 1969) and leucocytes (Lichtenstein & Margolis, 1968). In the leucocytes, the β-receptor agonist also restrains the rate of histamine formation (Assem, 1974). Similar effects of isoprenaline have been noted on histamine release and formation in the rat gastric mucosa upon stimulation with pentagastrin (Lundell & Svensson, 1974). Since isoprenaline also inhibited the acid secretory response to pentagastrin, Lundell & Svensson (1974) tentatively suggested that isoprenaline inhibited acid secretion by restraining histamine mobilization. Curwain, Holton, McIsaac & Spencer (1974) reached a similar conclusion from their studies in the dog.

In dogs, cholinergically-induced acid secretion is extremely susceptible to inhibition by sympathomimetic amines (Harries, 1957; Pradhan &

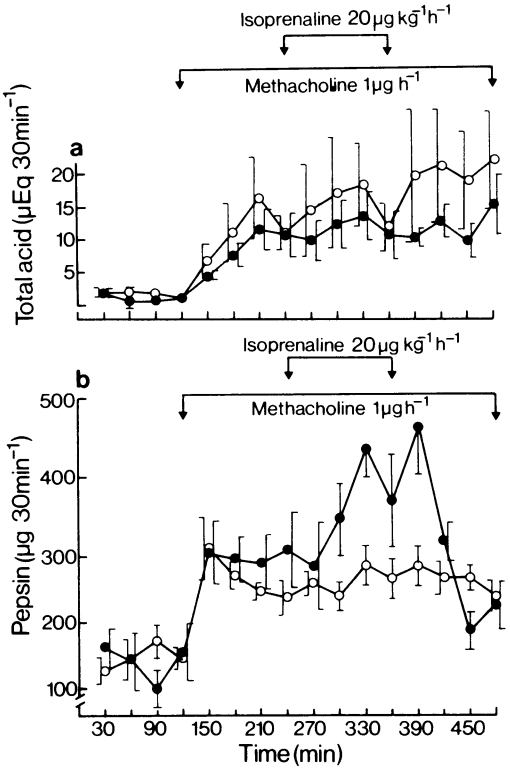

Figure 8 (a) Acid and (b) pepsin secretion in response to methacholine (1 μg h^{-1}) alone (O) or combined with isoprenaline (20 μg kg^{-1} h^{-1}) (●). Each point represents the mean of one determination in each of six Heidenhain pouch rats. The vertical lines show s.e. mean.

Wingate, 1962). In the present study, isoprenaline infusion almost abolished acid secretion in the Pavlov pouch induced by feeding and substantially reduced the response to 2-deoxy-D-glucose. These observations raise the question whether the mechanism behind the effect of the β-receptor agonist is a reduction in the mobilization of gastric mucosal histamine.

2-Deoxy-D-glucose injection is known to be followed by an increase in the vagal tone above that prevailing in the interdigestive state, allegedly by stimulation of nuclei in the hypothalamus (Colin-Jones & Himsworth, 1970). Central vagal excitation effected in this way elicited a reduction in mucosal histamine content and a substantial increase in the rate of formation of the amine. In the presence of the antrum, 100 mg/kg of 2-deoxy-D-glucose evokes an immediate increase in serum gastrin (Lundell & Nilsson, unpublished observations). In the absence of the antrum, vagal excitation does not alter the serum gastrin level but is still effective in increasing the rate of mucosal histamine formation (Rosengren & Svensson, 1969; Lundell, 1975a). Accordingly, enhanced gastrin secretion seems to be only partly responsible for the observed effects of vagal stimulation on mucosal histamine mobilization. From the present results it is obvious that isoprenaline almost completely prevented the combined effect of the vagus nerve and endogenous gastrin on the mobilization of mucosal histamine.

Interdigestive acid secretion is sustained by both antral gastrin release and vagus nerve activity. In the rat, the latter mechanism appears to be predominant (Svensson, 1970; Lundell, 1975b). Isoprenaline strongly inhibited interdigestive secretion. Surprisingly, after 2.5 h of isoprenaline infusion, mucosal histamine content was slightly decreased and the rate of formation increased. However, it should be pointed out that isoprenaline induced a progressive increase in serum gastrin concentration, thus providing a potent stimulus for histamine mobilization, which was apparently counterbalanced by the β-receptor agonist (see also Hayes, Ardill, Kennedy, Shanks & Buchanan, 1972; Stadil & Rehfeld, 1973). However, these results are irreconcilable with a primary site of secretory inhibition by isoprenaline on histamine mobilization. Furthermore, exogenous histamine failed to prevent the inhibitory effect of β-adrenoceptor stimulation on the 2-deoxy-D-glucose response.

In dogs, isoprenaline has little effect on histamine-induced secretion but inhibits pentagastrin-induced secretion and increases the ratio of gastric mucosal blood flow to acid secretion (Curwain & Holton, 1972). In the rat Heidenhain pouch, histamine-induced secretion was inhibited by isoprenaline infusion, although isoprenaline was more effective when secretion was stimulated by pentagastrin (Lundell & Svensson, 1974). Since no corresponding effect was noted on methacholine-induced secretion, isoprenaline may interact with the histamine H_2-receptor on the parietal cell. However, such interaction has not been demonstrated on other target organs having H_2-receptors (Black *et al.*, 1973; Pöch, Kukovetz & Scholz, 1973). Nevertheless, isoprenaline has been reported to inhibit histamine-induced acid secretion in the bull frog isolated mucosa (Thorpe, Frusco, Bass & Hug, 1971), indicating a direct effect on the parietal cell.

The secretory responses studied here in the Pavlov pouch are predominantly governed by vagus nerve activity. Another mechanism, besides those discussed above, contributing to the mode of inhibition of isoprenaline may be a central or peripheral depression of vagal activity. Although it has been reported that an artificial elevation of blood glucose concentration inhibits the secretory response to 2-deoxy-D-glucose (Hirschowitz & Sachs, 1965; Himsworth & Colin-Jones, 1969), it is unlikely that the rather small increase in serum glucose that we observed on

isoprenaline infusion would diminish the secretory rate during the interdigestive state and upon feeding or 2-deoxy-D-glucose injection. Catecholamines and sympathetic nerve stimulation are known to inhibit cholinergically-induced excitatory motor responses in the gastric and intestinal wall. This effect seems to be at least partly attributable to an inhibited acetylcholine release from the cholinergic neurones (Jansson & Martinsson, 1966; Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970). The present finding that isoprenaline inhibited vagally- and histamine-induced secretory responses may be reconcilable with restrained acetylcholine release, since it has been shown that the secretory response to histamine is enhanced by cholinergic stimulation and is inhibited by atropine (Johansson, Lundell & Svensson, 1971; Johansson *et al.*, 1972). However, it should be recalled that catecholamines seem to prevent acetylcholine release from cholinergic neurones by an α-receptor mechanism (Paton & Vizi, 1969; Kosterlitz *et al.*, 1970).

In the Pavlov pouch, pepsin secretion is excited only by cholinergic stimulants (Svensson, 1970; Johansson *et al.*, 1972). The denervated peptic cells are responsive also to histamine and gastrin. In the rat Heidenhain pouch, isoprenaline enhanced the pepsin secretory response to all stimuli so far investigated (see also Lundell & Svensson, 1974). By contrast, in the innervated pouch pepsin secretion stimulated by 2-deoxy-D-glucose was inhibited by isoprenaline. These results contrast with those obtained in dogs provided with gastric fistulae (Grechishkin, 1970). It would thus appear that in the rat denervation profoundly alters the secretory behaviour of the peptic cells to β-adrenoceptor stimulation.

This work was supported by grants from the Swedish Medical Research Council, the Medical Faculty, University of Lund and Leo Ltd, Helsingborg Research Foundation.

References

ALPHIN, R.S. & LIN, T.M. (1959). Preparation of chronic denervated gastric pouches in the rat. *Am. J. Physiol.*, **197**, 257–259.

ASSEM, E.S.K. (1974). Effect of sympathomimetic amines on the histamine forming capacity of human leucocytes. *Br. J. Pharmac.*, **52**, 213–218.

ASSEM, E.S.K. & SCHILD, H.O. (1969). Inhibition by sympathomimetic amines of histamine release induced by antigen in passively sensitized human lung. *Nature, Lond.*, **224**, 1028–1029.

BITSCH, V. (1966). A modification of the Hunt method for estimating peptic activity in gastric juice. *Scand. J. clin. Lab. Invest.*, **18**, 357–358.

BLACK, J.W., DUNCAN, W.A.M., EMMETT, J.C., GANELLIN, C.R., HESSELBO, T., PARSONS, M.E. & WYLLIE, J.H. (1973). Metiamide – an orally active histamine H_2-receptor antagonist. *Agents and Actions*, **3**, 133–137.

BRAY, G.A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.*, **1**, 279–285.

COLIN-JONES, D.G. & HIMSWORTH, R.L. (1970). The location of the chemoreceptor controlling gastric acid secretion during hypoglycaemia. *J. Physiol., Lond.*, **206**, 397–409.

CURWAIN, B.P. & HOLTON, P. (1972). The effects of isoprenaline and noradrenaline on pentagastrin-stimulated gastric acid secretion and mucosal blood flow in the dog. *Br. J. Pharmac.*, **46**, 225–233.

CURWAIN, B.P., HOLTON, P., McISACC, R.L. & SPENCER, J. (1974). Can the actions of adrenoceptor agonists and antagonists on pentagastrin-induced gastric secretion be due to their effects on histamine formation? *Br. J. Pharmac.*, **51**, 217–223.

FELDBERG, W. & TELESNIK, J. (1953). Reduction of tissue histamine by compound 48/80. *J. Physiol., Lond.*, **120**, 550–568.

GRECHISHKIN, L.L. (1970). Catecholamines and 5-hydroxytryptamine effect on the gastric secretion. *Farmakologija i Toksikologija*, **30**, 612–615.

HAKANSON, R. & LIEDBERG, G. (1970). The role of endogenous gastrin in the activation of gastric histidine decarboxylase in the rat. Effect of antrectomy and vagal denervation. *Eur. J. Pharmac.*, **12**, 94–103.

HARRIES, E.H.L. (1957). The mode of action of sympathomimetic amines in inhibiting gastric secretion. *J. Physiol., Lond.*, **138**, 48–50P.

HAYES, J.R., ARDILL, J., KENNEDY, T.L., SHANKS, R.G. & BUCHANAN, K.D. (1972). Stimulation of gastrin release by catecholamines. *Lancet*, **i**, 819–821.

HENNINGSSON, S., LUNDELL, L. & ROSENGREN, E. (1974). Comparative study of two isotopic methods determining histamine formation *in vitro*. *Biochem. Pharmac.*, **23**, 2671–2678.

HIMSWORTH, R.L. & COLIN-JONES, D.G. (1969). Factors which determine the gastric secretory response to 2-deoxy-D-glucose. *Gut*, **10**, 1015–1019.

HIRSCHOWITZ, B.I. & SACHS, G. (1965). Vagal gastric secretory stimulation by 2-deoxy-D-glucose. *Am. J. Physiol.*, **209**, 452–460.

HUNT, J.N. (1948). A method for estimating peptic activity in gastric contents. *Biochem. J.*, **42**, 104–109.

JANSSON, G. & MARTINSSON, J. (1966). Studies on the ganglionic site of action of sympathetic outflow to the stomach. *Acta physiol. scand.*, **68**, 184–192.

JOHANSSON, I., LUNDELL, L., ROSENGREN, E. & SVENSSON, S.E. (1972). Gastric secretion and its facilitation as related to gastric mucosal histamine. *J. Physiol., Lond.*, **226**, 431–452.

JOHANSSON, I., LUNDELL, L. & SVENSSON, S.E. (1971). Inhibition of gastric secretion by atropine in conscious rats. *J. Physiol., Lond.*, **217**, 723–735.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol.; Lond.*, **174**, 400–416.

KOBAYASHI, Y. (1963). Determination of histidine decarboxylase activity by liquid scintillation counting of $^{14}CO_2$. *Analyt. Biochem.*, **5**, 284–290.

KOSTERLITZ, H.W., LYDON, R.J. & WATT, A.J. (1970). The

effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.*, **39**, 398–413.

LICHTENSTEIN, L.M. & MARGOLIS, S. (1968). Histamine release *in vitro*: Inhibition by catecholamines and methylxanthines. *Science*, **161**, 902–903.

LUNDELL, L. (1973). Mobilization of gastric mucosal histamine and gastric secretion after H_2-receptor blockade. *Br. J. Pharmac.*, **49**, 715–717.

LUNDELL, L. (1974a). Histamine metabolism of the gastric mucosa following antrectomy. *J. Physiol., Lond.*, **241**, 437–451.

LUNDELL, L. (1974b). Acid secretion and mobilization of gastric mucosal histamine on combined cholinergic and pentagastrin stimulation. *Agents and Actions*, **4**, 211–216.

LUNDELL, L. (1975a). Elucidation by a H_2-receptor antagonist of the significance of mucosal histamine mobilization in exciting acid secretion. *J. Physiol., Lond.*, **244**, 365–383.

LUNDELL, L. (1975b). Alterations in secretory patterns following antrectomy in rats with Pavlov pouches. *J. Physiol., Lond.*, **245**, 737–754.

LUNDELL, L. & SVENSSON, S.E. (1974). Implication of gastric mucosal histamine in inhibition by isoprenaline of pentagastrin-induced gastric secretion. *Br. J. Pharmac.*, **52**, 69–75.

NILSSON, G. (1975). Increased plasma gastrin levels in connection with inhibition of gastric acid responses to sham feeding following bulbar perfusion with acid in dogs. *Scand. J. Gastroenterol.*, **10**, 273–277.

PATON, W.D.M. & VIZI, E.S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmac.*, **35**, 10–28.

PÖCH, KUKOVETZ, W.R. & SCHOLZ, N. (1973). Specific inhibition by Burimamide of histamine effects on myocardial contraction and cyclic AMP. *Naunyn-Schmiedebergs Arch. Pharmak.*, **280**, 223–228.

PRADHAN, S.N. & WINGATE, H.N. (1962). Effects of adrenergic agents on gastric secretion in dogs. *Archs int. Pharmacodyn. Thér.*, **140**, 399–408.

REHFELD, J.F., STADIL, F. & VIKELSØE, J. (1974). Immunoreactive gastrin components in human serum. *Gut*, **15**, 102–111.

ROSENGREN, E. & SVENSSON, S.E. (1969). The role of the antrum and the vagus nerve in the formation of gastric mucosal histamine. *J. Physiol., Lond.*, **205**, 275–288.

STADIL, F. & REHFELD, J.F. (1973). Release of gastrin by epinephrine in man. *Gastroenterology*, **65**, 210–215.

SVENSSON, S.E. (1970). The secretory pattern of three stomach preparations in the rat. *J. Physiol., Lond.*, **207**, 329–350.

THORPE, C.D., FRUSCO, R.A., BASS, P. & HUG, Jr. C.C. (1971). Effects of catecholamines on acid secretion by bullfrog isolated gastric mucosa. *Surg. Forum*, **12**, 317–319.

(Received July 8, 1975.
Revised April 4, 1976.)