Short communications

Effect of a sulphated glycopeptide on rat gastric acid secretion stimulated by histamine, bethanechol, pentagastrin and dibutyryl cyclic AMP

R. NIADA AND G. PRINO

Crinos Biological Research Laboratories, 22079 Villa Guardia, Italy

The antisecretory activity of a semisynthetic sulphated glycopeptide (GLPS) was studied in rats in which the secretion rates of gastric acid were determined on the perfused stomach preparation. GLPS at 1 mg/kg i.v. reduced the hypersecretory effect of dibutyryl cyclic AMP, histamine, pentagastrin, bethanechol but not of theophylline.

A semisynthetic sulphated glycopeptide (GLPS) has been described as an antiulcer agent with antipeptic and antisecretory properties (Prino, Paglialunga, Nardi & Lietti, 1971; Prino, Lietti & Paglialunga, 1971; Prino, Lietti & Allegra, 1972; Prino, Paglialunga, Lietti & Niada, 1972). Experiments in rats and guinea-pigs show that GLPS administered orally or intravenously diminishes the secretory volume and total acid output both in normal and histamine-stimulated animals. However, the mechanism of action of GLPS on gastric secretion is not known.

The experiments reported here examine the effect of GLPS on rat gastric acid secretion stimulated by bethanechol, histamine, pentagastrin and dibutyryl cyclic AMP.

Methods.—Male Sprague-Dawley rats (Morini, Italia) were used. Gastric secretion was stimulated by histamine dihydrochloride (BDH), bethanechol chloride (Urecholine, Merck), pentagastrin (Gastrodiagnost, Merck), N^6-2′*O*-dibutyryl-adenosine-3′,5′-monophosphate (DB-cAMP, Boehringer) or theophylline (Biosintex). Sulphated glycopeptide, sodium salt, (GLPS); mol. wt. 175,000) was obtained from commercial batch no. 164 (prepared by Crinos Chemical Laboratories). GLPS was prepared according to Bertellini, Butti, Piantanida, Prino, Riva, Rossi & Rossi (1971) by polysulphonation of a naturally occurring glycopeptide isolated from swine duodenal mucosa and characterized by the presence of sialic acid, hexosamines, hexoses and a polypeptide residue. The secretion rates of gastric acid were determined on the perfused stomach preparation of Ghosh & Schild (1958). The H^+ content of consecutive samples of perfusate solution (collected at 10-min intervals) was determined by titration with Radiometer titration equipment. In the first series of experiments, GLPS and stimulants were administered via a cannulated jugular vein and the stomach was perfused with 0·9% w/v NaCl solution (saline) at the rate of 0·9 ml/min as described by Mantegazza & Piccinini (1962). In the second series, theophylline and DB-cAMP were added to the perfusing medium (Shaw & Ramwell, 1968): the rate of perfusion was of 0·15 ml/minute.

Results.—Figure 1 summarizes the results of several experiments in which GLPS was injected i.v. at a dose of 1 mg/kg. When gastric acid secretion was induced by the intravenous administration of either histamine or bethanechol, GLPS was given 30 min beforehand. The control acid output was $11{\cdot}2 \pm 1{\cdot}9$ μmol/10 min for histamine and $14{\cdot}0 \pm 2{\cdot}3$ μmol/10 min for bethanechol: GLPS reduced these responses to $6{\cdot}44 \pm 0{\cdot}5$ and to $8{\cdot}1 \pm 0{\cdot}8$ μmol/10 min, respectively. When acid secretion was induced by the i.v. administration of pentagastrin or DB-cAMP, GLPS was given just before the injection of the stimulants. Control acid output was $11{\cdot}4 \pm 0{\cdot}6$ μmol/10 min for pentagastrin and $11{\cdot}7 \pm 1{\cdot}9$ μmol/10 min for DB-cAMP: GLPS reduced these responses to $6{\cdot}1 \pm 0{\cdot}4$ and to $5{\cdot}6 \pm 0{\cdot}9$ μmol/10 min, respectively.

The ability of GLPS to inhibit acid secretion induced by DB-cAMP suggested that a study of theophylline, which presumably stimulates secretion by inhibition of phosphodiesterase, would be worthwhile; the method described by Shaw & Ramwell (1968) was used. With this method, theophylline or DB-cAMP added to the liquid perfusing the stomach produced a lasting increase in acid secretion. The intravenous administration of GLPS just before starting the gastric perfusion inhibited the effect of DB-cAMP but not that of theophylline. Similar results were obtained when GLPS was added to the

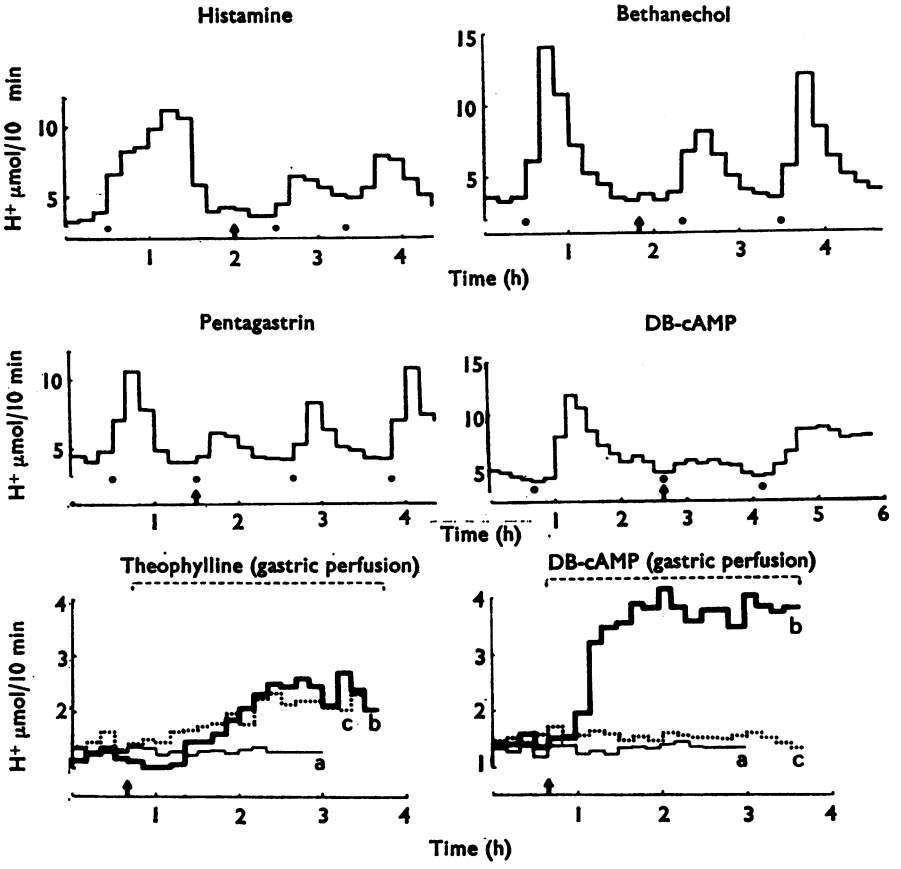

FIG. 1. Perfused rat stomach. Activity of semisynthetic sulphated glycopeptide (GLPS) given at a dose of 1 mg/kg i.v. on the gastric hypersecretion stimulated by the i.v. administration of the following substances: histamine 500 μg/kg, pentagastrin 2·5 μg/kg, DB-cAMP 20 mg/kg and stimulated by a gastric perfusion either with 4×10^{-3}M theophylline or 10^{-3}M DB-cAMP. Mean values of 5 rats per group. In the graphs referring to gastric perfusion, line a represents controls; line b the stimulant alone; line c the stimulant and GLPS. ●=Stimulant i.v. ↑=GLPS i.v. ----=Stimulant (gastric perfusion).

perfusion medium along with these two stimulants.

Discussion.—Antisecretory activity of GLPS has been shown when gastric secretion was stimulated by bethanechol, histamine, pentagastrin and dibutyryl cyclic AMP but not when it was stimulated by theophylline. The relationships among these stimulators of acid secretion are still undefined and so interpretation of the results is difficult. However, some authors (Bersimbaev, Argutinskaya & Salganik, 1971; Domschke, Classen & Demling, 1972) have shown that the injection of pentagastrin or histamine to rats, in doses which stimulate acid secretion, increase adenyl cyclase activity in gastric tissues. Recently, it has been shown in the dog (Bieck, 1972) that when histamine or pentagastrin are infused, the amount of cAMP in gastric juice is increased in a dose-dependent manner and that this increase in cAMP precedes the secretion of hydrochloric acid. The phosphodiesterase-inhibitor, theophylline, potentiates the effect of histamine on both the acid secretion and the output of cAMP. The stimulatory action of DB-cAMP and of theophylline on gastric secretion has also been demonstrated *in vivo* by a technique involving perfusion of the rat gastric mucosal surface with solutions containing these agents (Shaw & Ramwell, 1968). When DB-cAMP was administered intravenously it also enhanced rat gastric secretion

(Jawaharlal & Berti, 1972; Whittle, 1972). There is, therefore, some evidence linking cyclic AMP to the process of acid secretion.

If theophylline stimulates acid secretion by permitting the accumulation of endogenously-produced cAMP and if bethanechol, histamine and pentagastrin stimulate secretion by increasing the rate of cyclic AMP formation, then GLPS might block the effect of these agonists by acting at some point earlier than adenyl cyclase in the chain of events. The weakness of this interpretation is that DB-cAMP is also antagonized by GLPS. Unfortunately there is insufficient information at present to understand how DB-cAMP or cAMP acts when exogenously supplied to rats. Until this has been answered the mechanism of the inhibitory action of GLPS must remain unknown.

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