IN VIVO EFFECTS OF PANCREOZYMIN, SECRETIN, VASOACTIVE INTESTINAL POLYPEPTIDE AND PILOCARPINE ON THE LEVELS OF CYCLIC AMP AND CYCLIC GMP IN THE RAT PANCREAS

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1. Introduction

Pancreozymin (PZ) and muscarinic cholinergic agents powerfully stimulate the secretion of pancreatic hydrolases. Their mode of action is at the present time unclear, though transmembranous ionic movements involving primarily Ca^{2+} [1,2], and cyclic AMP [3–5] have been implicated. However, the fact that calcium is involved [6] in 'stimulus-secretion coupling' does not prove that this ion is a necessary mediator of emiocytosis. A pancreatic adenylatecyclase can be stimulated by pancreozymin [5,7,8] yet the status of cyclic AMP as a second messenger of the secretagogues is ambiguous. The occurrence in the rat pancreas of phosphodiesterase activity capable of hydrolyzing physiological concentrations of cyclic GMP [9] and of cyclic GMP-dependent protein kinase [10] suggests that cyclic GMP may intervene in pancreatic secretion. It was decided therefore to compare the in vivo time course of cyclic AMP and cyclic GMP levels following administration of pancreozymin, pilocarpine, secretin and vasoactive intestinal polypeptide (VIP).

2. Materials and methods

Male Wistar rats weighing 150–200 g were maintained on a standard chow. In a first series of experiments designed to investigate amylase secretion in response to pancreozymin and pilocarpine, the animals were starved 24 hr and their pancreato–biliary duct was cannulated [11] under diethyl ether anesthesia. The animals were kept warm while the juice was collected over ice (fig. 1).

A second series of experiments were undertaken to ascertain the time course of cyclic nucleotide levels. Animals receiving intravenous injections of pancreozymin, secretin or VIP were fed ad libitum and were anesthetized with Nembutal. Animals receiving pilocarpine subcutaneously were not anesthetized and were starved 24 hr. All of the animals were sacrificed by decapitation. The pancreas was dissected out as rapidly as possible, plunged into 5% CCl_3COOH and frozen at −70°C. Later, the tissue was homogenized and centrifuged. Proteins in the precipitate were determined according to the method of Lowry et al. [14], using bovine albumin as a standard. The supernatant was acidified with HCl and CCl_3COOH was eliminated by 5 successive extractions with diethyl ether. The residue obtained after lyophilization was dissolved in an appropriate volume of water. Cyclic AMP was estimated by the protein binding assay of Gilman [15]. This method was valid in the 0.5–10.0 pmoles range. In order to assay cyclic GMP, the volume of supernatant which remained was loaded on Dowex AG1-X8 formate (0.7 × 3 cm) (Bio-Rad Lab., Richmond, Va., USA) and eluted, successively, with H_2((5 ml), 3 N formic acid (9 ml) and 4.5 N formic acid (12 ml). The last fraction, containing all of the cyclic GMP, was lyophilized. The cyclic nucleotide was determined by the radioimmunoassay of Steiner et

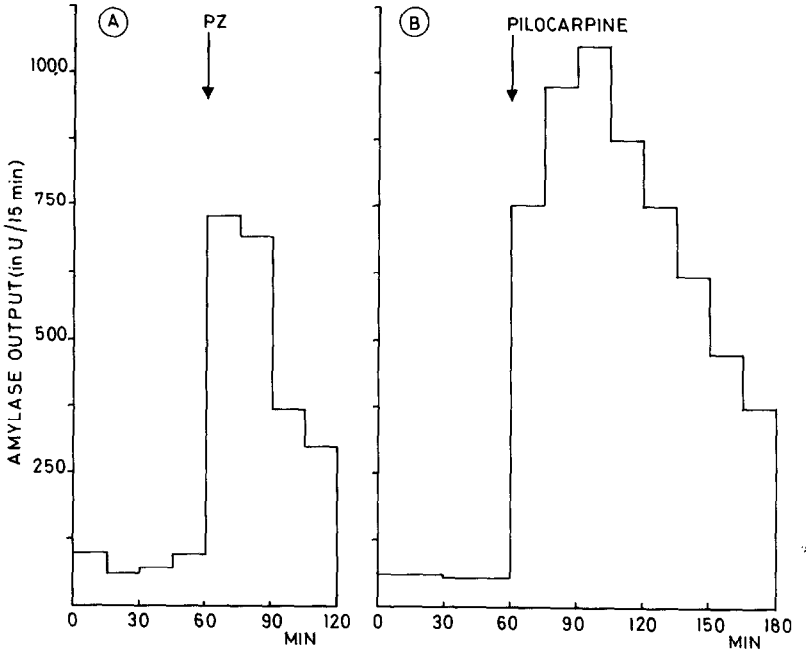

Fig. 1. (A) Secretion of amylase in response to pancreozymin. The pancreatobiliary juice was collected under diethyl ether anesthesia. The first two 30-min samples allowed a study of basal output in U/15 min. At time 60 min, seven rats received a single administration of pancreozymin (0.33 μg in 0.2 ml 0.9% NaCl corresponding to 5.5 Clin. U/kg) through a saphenous vein. The response was estimated on four 15-min samples. α-Amylase was estimated by the saccharogenic method of Noelting and Bernfeld [12] as automated by Vandermeers et al. [13]. One unit is defined as the amount of amylase which liberates a reducing power equivalent to 1 μmole of maltose/min at 25°C. (B) Secretory response following the injection of pilocarpine. The general conditions are the same as in A. The flow of amylase was estimated on two 30-min samples of juice, before, and eight 15-min samples after a subcutaneous injection of pilocarpine (30 mg/kg body weight). Each value represents the mean of 7 experiments.

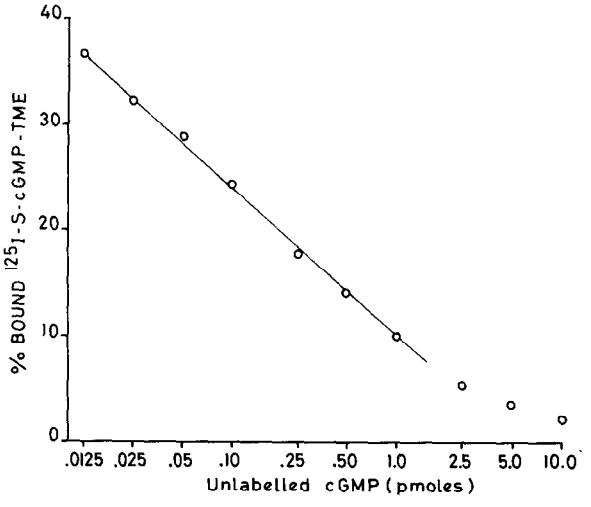

Fig. 2. Cyclic GMP standard curve obtained with a slight modification of the method of Steiner et al. [16]. ^{125}I-S-cGMP–TME (2000 cpm) was incubated for 3 hr at 4°C with the anti-cGMP serum and unlabelled cyclic GMP (0–10 pmoles) in 0.05 M acetate buffer (pH 6.2). The amount of antibody allowed the binding of 45% of the radioactivity in the absence of cold cyclic GMP. Free and bound antigen were separated by filtration on Millipore HAWP 0.45 μ and the radioactivity retained on the filter was estimated with a γ counter. All determinations were made in duplicate. A linear relationship was observed between the % of radioactivity remaining on the filter and the amount of unlabelled cyclic GMP added (in log doses). The antiserum and the antigen (^{125}I-S-cGMP–TME) were from Collaborative Research Inc. (Waltham, Mass., USA).

al. [16], slightly modified. Fig. 2 illustrates a typical standard curve used for the determination of cyclic GMP in the 0.02–2.0 pmole range. Portions of representative samples of cyclic GMP purified by column

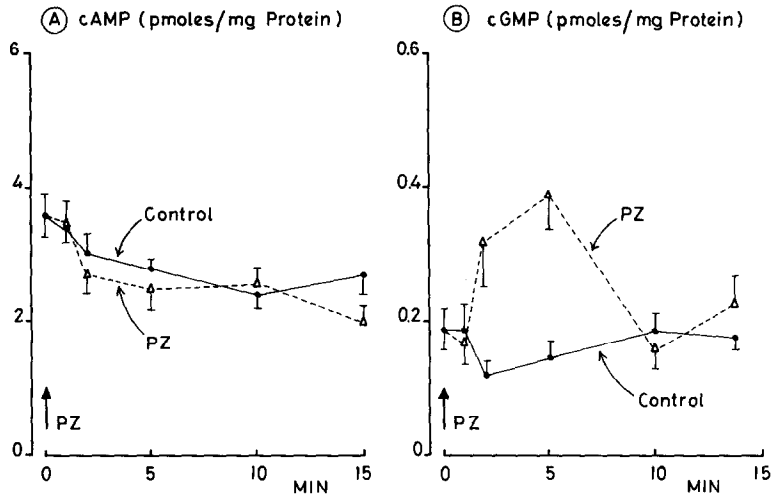

Fig. 3. Comparison of the levels of cyclic AMP (A) and cyclic GMP (B) in response to pancreozymin. The animals were anesthetized with Nembutal (40 mg/kg, administered intraperitoneally; Abbot Laboratories, Chicago, USA). Approximately 15 min later, they were injected through a saphenous vein with pancreozymin (0.33 μg in 0.2 ml 0.9% NaCl i.e. 5.5 Clin. U/kg: △) or with saline (●). Batches of six animals were killed at intervals of 1 min, 2 min, 5 min, 10 min and 15 min after the injection. Each value represents mean of 6 determinations ± SEM.

chromatography were incubated with cyclic 3′,5′-phosphodiesterase before assay. The degradation of the assayable cyclic GMP was essentially complete.

Pilocarpine hydrochloride was obtained from Federa (Brussels, Belgium), pancreozymin, secretin and vasoactive intestinal polypeptide (VIP) were obtained from the GIH Research Unit of the Karolinska Institute (Stockholm, Sweden).

3. Results and discussion

The average in vivo pancreatic concentration of cyclic AMP was 6.5 pmoles/mg protein (1.3×10^{-6} M), assuming uniform distribution (fig. 4). A Nembutal anesthesia of approximately 30 min duration caused a progressive decrease in cyclic AMP to 3.0 pmoles/mg protein (fig. 3). Pentobarbital anesthesia is known

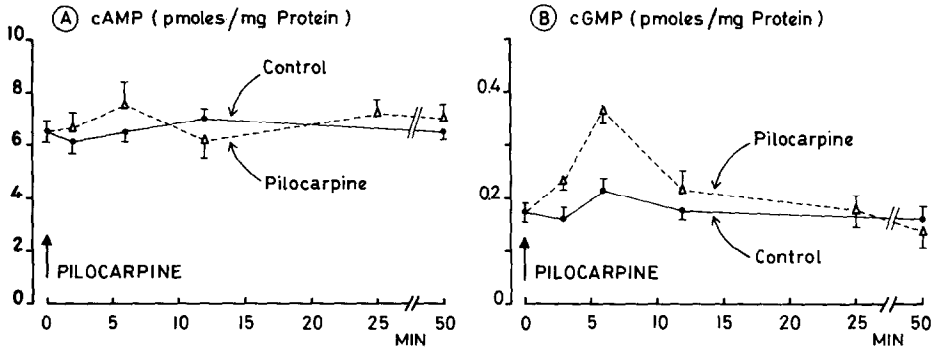

Fig. 4. Comparison of the levels of cyclic AMP (A) and cyclic GMP (B) in response to pilocarpine. The animals were conditioned for one week, being gently handled and receiving a daily subcutaneous injection of saline in order to minimize the stress due to injection when they received pilocarpine (△: 30 mg/kg subcutaneously.) The controls (●) were also conditioned and were injected with saline only. Batches of six animals were killed at intervals of 3, 6, 12, 24 and 50 min after the injection. Each value represents mean of 6 determinations ± SEM.

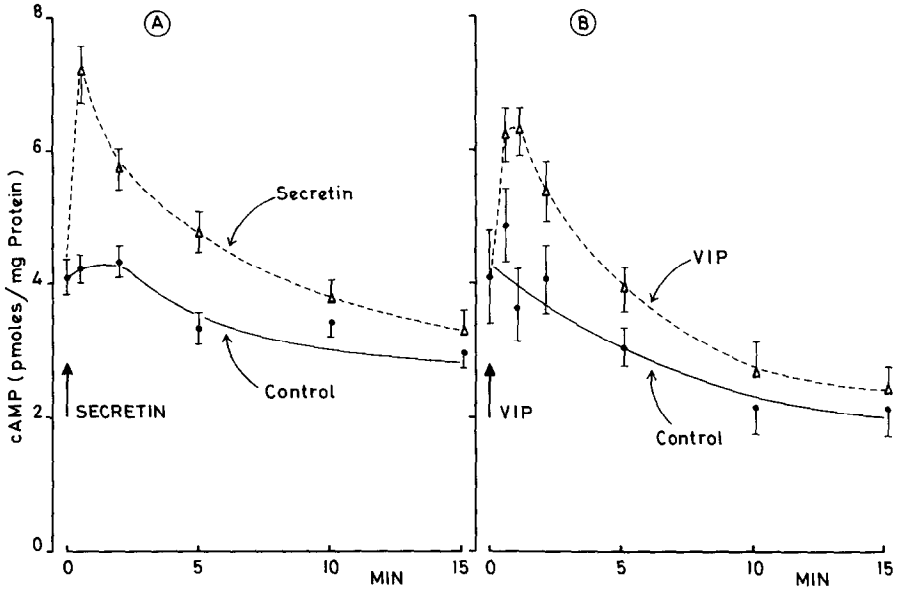

Fig. 5. Time course of variations in the levels of cyclic AMP in response to secretin (A) and VIP (B). Same general conditions as in fig. 3. The animals were anesthetized with Nembutal and were injected intravenously with secretin (2.75 μg/kg) or VIP (27.5 μg/kg), in 0.2 ml 0.9% NaCl (△). The controls (●) were injected with saline only. Each value represents mean of 6 determinations ± SEM.

to increase certain amino acid levels [17] and to depress protein synthesis [18] in the rat pancreas. All these effects may be due to inhibition of oxidative phosphorylation, reduction of visceral blood flow and/or to hypothermia. The average pancreatic cyclic GMP concentration was 0.18 pmoles/mg protein, in vivo, only 1/36 the average cyclic AMP concentration. This control concentration was unaffected by Nembutal.

The intravenous injection of pancreozymin caused a rapid increase in amylase secretion. Amylase output remained high at 30 min following injection, and declined to control values after 60 min (fig. 1A). The levels of cyclic AMP remained unchanged during the 15 min period following administration of pancreozymin (fig. 3A).

Figs. 1B and 4B illustrate the secretory response and variations in the levels of cyclic AMP following the administration of pilocarpine. A dose capable of provoking a sustained hypersecretion of amylase caused no apparent changes in the levels of cyclic AMP. Our data are at variance with the data of Case et al. [4] who reported a rapid rise (within 1 min) of cyclic AMP following the administration of pancreozymin or acetylcholine in perfused cat pancreas. However, there was no temporal relationship between cyclic AMP levels and protein secretion in their experimental preparation.

This stability of cyclic AMP we observed following the injection of these secretagogues was in marked contrast with the transient doubling of cyclic AMP following the intravenous injection of secretin (fig. 5A) and VIP (fig. 5B), two hormones which elicit primarily the secretion of water and bicarbonate. Cyclic AMP levels showed a significant increase as early as the first time interval (30 to 60 sec), and after reaching a peak, declined exponentially to the control value in 10–15 min. Our results support the role of cyclic AMP as second messenger of secretin [19]. They suggest also that the hormonal receptor sites of secretin and VIP are connected to the same adenylate cyclase [20].

Variations in pancreatic levels of cyclic GMP following the administration of pancreozymin or pilocarpine are shown in figs. 3B and 4B. The level of cyclic GMP rose rapidly and markedly in the pancreas within 1 to 3 min after injection depending on the mode of administration (intravenous or subcutaneous). These

variations in the concentration of cyclic nucleotide were of shorter duration than the hypersecretory response: the levels of cyclic GMP exhibited a maximum 2-fold increase at 5–6 min and decreased sharply thereafter, declining to control levels after about 10–15 min. An elevation of cyclic GMP in response to acetylcholine has been observed in a number of tissues [21–24]. Some polypeptide hormones such as insulin in adipose cells [24] and the α and β subunits of human chorionic gonadotropin in the ovary [25] exert similar effects. In the exocrine pancreas, it has been shown [26] that the stimulation of amylase secretion is associated with an increase in protein phosphorylation. This may be due in part to the rise of cyclic GMP. Indeed the pancreas contains a cyclic GMP-dependent protein kinase [10]. To substantiate a role for cyclic GMP as a second messenger of both pancreozymin and cholinergic agents in acinar cells, it will be necessary to demonstrate the existence of a membranous guanylate cyclase responsive to both the polypeptide hormone and to the neurotransmitter.

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