Theophylline: Potentiation of Arginine-induced Somatostatin Release from the Isolated Rat Pancreas

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SUMMARY

Somatostatin's release from the isolated rat pancreas was studied using a perfusion technique. Arginine at a concentration of 19 mM produced a biphasic increase in somatostatin release from the perfused rat pancreas. Both first and second phases of somatostatin's increase are significantly higher in the presence of 1 mM theophylline than in the absence of the drug. These results indicate the possible inclusion of the adenylate cyclase–cyclic AMP system in the regulatory mechanism of rat pancreatic somatostatin secretion. DIABETES 28:457–459, May 1979.

The dynamics of somatostatin's release have been demonstrated in the perfused canine pancreas.[1,2] Patton et al.[1] demonstrated that arginine enhances the release of somatostatin as well as that of insulin and glucagon. On the other hand, a possible role of the adenylate cyclase–cyclic AMP system in the release of pancreatic somatostatin using a static incubation method has been suggested.[3,4] The present study was undertaken to investigate the release of immunoreactive somatostatin from the perfused rat pancreas and to examine the effect of theophylline on arginine-induced somatostatin secretion.

MATERIALS AND METHODS

Wistar strain, male rats, weighing 300–350 g, were used in all experiments. Perfusions of rat pancreas were performed by the procedure described by Goto et al.[5] with minor modifications. (The preparation includes the attached segment of duodenum.) All perfusions were accomplished with Krebs-Ringer bicarbonate buffer (KRB) containing 0.25% bovine serum albumin and 3.8% dextran (mean mol. wt., 70,000). The medium was gassed with 95% O_2–5% CO_2 and maintained at pH 7.4 and PaO_2 350 mm Hg. The flow rate was kept constant at 2.1–2.2 ml/min. The test material was introduced through a side arm to give an appropriate concentration. In experiment 1, the pancreas was perfused with 5.5 mM glucose for 60 min. In experiment 2, 5.5 mM glucose was perfused for the same duration, except L-arginine hydrochloride was added through a side arm from minutes 35 to 55 of perfusion to provide a final concentration of 19 mM. In experiment 3, theophylline (Nakarai Co., Japan) was added to the medium at a final concentration of 1 mM from minute 20 to the end of perfusion, the other protocol of experiment 2 remaining the same. The effluent from the portal vein was collected at the times indicated in Figures 1 and 2 in chilled tubes containing a bacitracin-Trasylol mixture (2×10^{-5}M and 1000 U/ml, respectively), frozen immediately, and stored at −20°C until assayed.

Assay procedure. Immunoreactive somatostatin was measured by a specific radioimmunoassay using antiserum RA-823, which is specific for somatostatin, as previously described.[6] The assay is capable of detecting as little as 10 pg/ml, and serial dilutions of perfusate somatostatin were parallel to a standard curve. Immunoreactive insulin was measured by the radioimmunoassay with the polyethylene glycol method described previously.[7] Immunoreactive glucagon was determined by radioimmunoassay with a talcum adsorption technique[8] using antiserum 30K. The same concentration of the bacitracin-Trasylol mixture used for sampling was also present in each radioimmunoassay standard solution.

N-tyrosyl somatostatin was kindly supplied by Drs. D. H. Coy and A. Arimura, VA Medical Center, New Orleans. Antiserum 30K was purchased from the Diabetes Research Fund of the University of Texas Health Science Center, Dallas, Texas. Statistical differences were determined with Student's *t* test.

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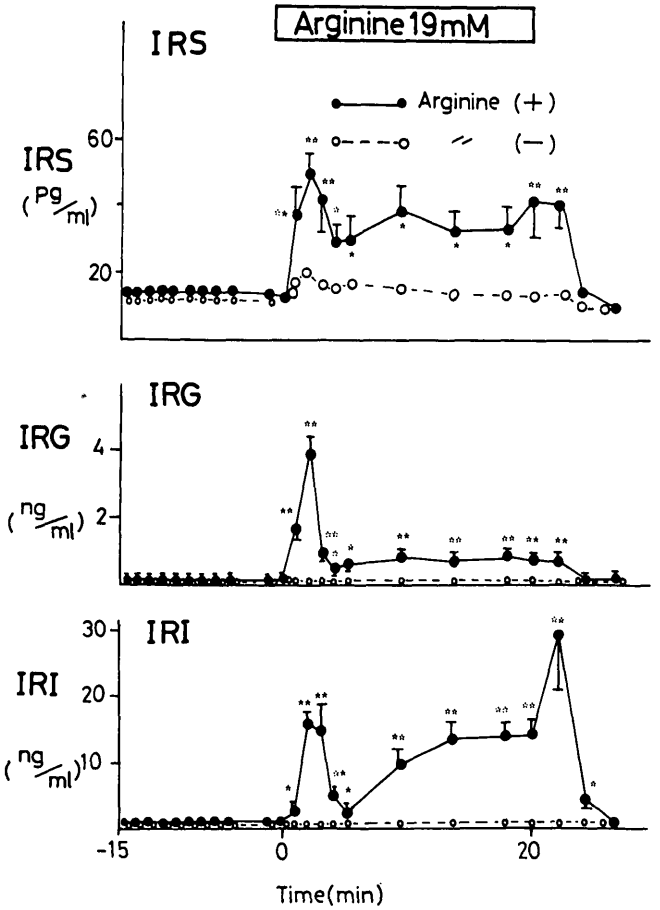


FIGURE 1. Somatostatin (IRS), glucagon (IRG), and insulin (IRI) release in the control (only 5.5 mM glucose) (○, n = 4) and in response to arginine with 5.5 mM glucose (●, n = 6). *P < 0.05; **P < 0.01.

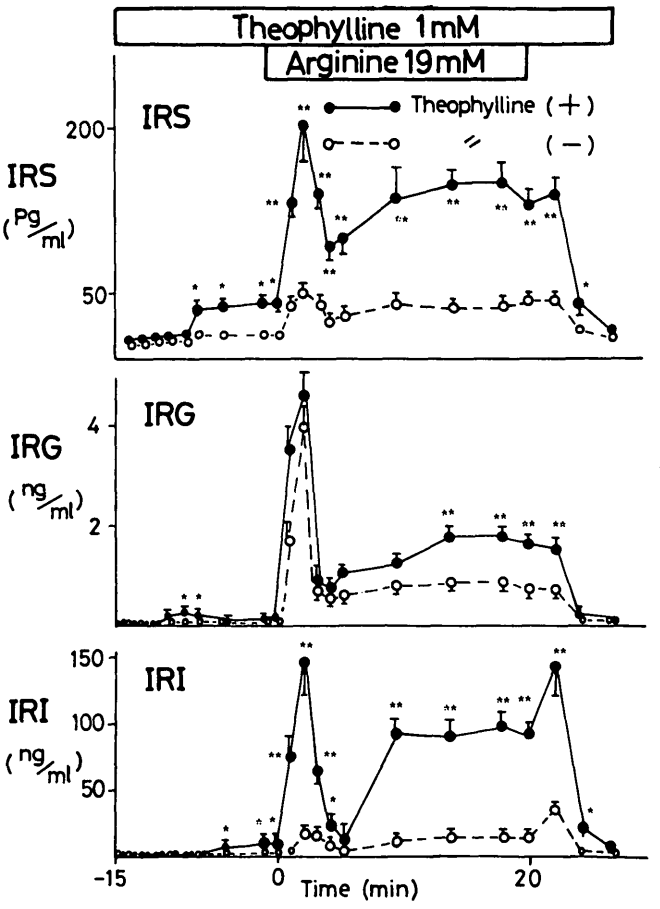
RESULTS

In experiment 1, basal somatostatin concentrations ranged from 10 to 18 pg/ml and were not significantly changed during the infusion of 5.5 mM glucose. A constant basal level of glucagon and insulin release was also observed during the perfusion with 5.5 mM glucose alone (Figure 1). As shown in Figure 1, infusion of arginine (19 mM) caused biphasic somatostatin release, the mean first peak value of which was 50 ± 7 pg/ml (±SE) and the second peak was 42 ± 10 pg/ml at the end of the arginine infusion. These were significantly higher than those with glucose alone ($P < 0.01$). After cessation of arginine, somatostatin concentrations decreased to prestimulation levels. Glucagon and insulin release in response to arginine was also biphasic. Before arginine infusion, somatostatin concentrations were considerably higher in the presence of theophylline (experiment 3) than they were in the absence of the drug (experiment 2), as is shown in Figure 2. Somatostatin responses to arginine were markedly exaggerated in the presence of theophylline. The mean first and second peak values during arginine infusion in the presence of theophylline were 215 ± 35 pg/ml and 152 ± 18 pg/ml, which were significantly higher than corresponding values in the absence of theophylline ($P < 0.05$). Both first and second phases of insulin response to arginine were also significantly exaggerated in the presence of theophylline. However, only the second phase of glucagon level was significantly higher in the presence of theophylline than in the absence of the drug (Figure 2).

DISCUSSION

The present study has clearly demonstrated that immunoreactive somatostatin is released from isolated perfused rat pancreas as well as perfused dog pancreas,[1,2] indicating that theophylline potentiates arginine-induced somatostatin secretion. Patton et al.[1] reported that arginine enhances the release of somatostatin in dogs. In the present experiment, we confirmed this observation and demonstrated further that theophylline augments both first and second phases of somatostatin secretion induced by arginine. The mechanism by which theophylline enhances somatostatin secretion, though not clear at present, deserves consideration. There seems to be two possible explanations of this phenomenon. The mechanism for the enhanced somatostatin concentration caused by theophylline might be influenced by elevated glucagon and insulin. This seems unlikely, however, because infusions of large amounts of exogenous insulin have no acute effect on D-cell secretion.[9] In addition, the first phase of glucagon secretion is not enhanced by theophylline in the present experiment, although a high dose of glucagon is accompanied by a prompt increase in somatostatin release.[2,9] On the other hand, theophylline is known to increase the cyclic AMP content of isolated pancreatic

FIGURE 2. Somatostatin (IRS), glucagon (IRG), and insulin (IRI) release induced by arginine with 5.5 mM glucose in the presence of 1 mM theophylline (●, n = 6) and in the absence of theophylline (○, n = 6). *P < 0.05; **P < 0.01.



islets,[10] and recent studies[3,4] have suggested a possible role for the adenylate cyclase cyclic AMP system in the release of somatostatin from isolated rat pancreatic islets. It is likely, therefore, that theophylline raises intracellular cyclic AMP concentrations of D-cells by antagonizing the action of phosphodiesterase, resulting in augmentation of somatostatin secretion from isolated perfused rat pancreas.

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