The present study demonstrates that a highly purified insulin receptor preparation retains kinase activity and that the β-subunit of the insulin receptor is phosphorylated by this kinase activity and has an ATP binding site. The results suggest, therefore, that the insulin receptor is itself a kinase as well as a substrate for phosphorylation. Prior studies have indicated that the α subunit of the insulin receptor (the 135K band) is predominantly labeled when [^{125}I]insulin is cross-linked to the receptor (*1*). These results, together with the data in the present study, suggest that the insulin receptor is composed of two subunits with separate functions: an α subunit that binds insulin and a β subunit with kinase activity.

The addition of insulin to either whole cells or solubilized plasma membranes increases the extent of phosphorylation of the β subunit of the insulin receptor (*3, 4*). The simplest explanation for these results would be for insulin to stimulate the kinase activity of the receptor which, in turn, phosphorylates itself as well as other proteins. These phosphorylations could then initiate a cascade of reactions resulting in the various effects of insulin. The increased kinase activity of the insulin receptor may not, however, be sufficient to induce all the effects of insulin on cells. In analogy, the epidermal growth factor (EGF) receptor has also been shown to be a protein kinase (*11*). Most interesting was the finding that although cyanogen bromide–cleaved EGF was capable of inducing the phosphorylation of the EGF receptor and some of the short-term effects of EGF, it was not capable of stimulating cell division (*12*). Thus, it remains to be determined whether the kinase activity of the insulin receptor is both necessary and sufficient to generate all of the multiple effects of insulin.

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Prostaglandins Mediate Inhibition of Gastric Acid Secretion by Somatostatin in the Rat

Abstract. *Somatostatin, a tetradecapeptide with potent inhibitory actions on gastric acid secretion, potentiated carbamylcholine-induced synthesis and release of prostaglandin E_2 from isolated perfused rat stomachs. The ability of somatostatin to inhibit acid secretion was blocked by indomethacin, an inhibitor of prostaglandin synthesis. These results suggest that prostaglandins mediate gastric acid inhibition by somatostatin in the rat.*

Somatostatin, a tetradecapeptide initially isolated from ovine hypothalamus, occurs in virtually all areas of the mammalian gut (*1*). One of its many actions is inhibition of gastric acid secretion (*1–3*). This action of somatostatin may be physiological since it has been observed with doses comparable to those released into plasma after meal ingestion (*3*). Since the acid-inhibitory activity is independent of the peptide's ability to inhibit release of the gastric secretagogue gastrin (*3*), somatostatin is thought to inhibit gastric parietal cells directly. However, attempts to demonstrate a direct inhibitory action of somatostatin on isolated parietal cell preparations have met with little success (*4*). Because somatostatin inhibits adenosine 3′,5′-monophosphate (cyclic AMP) production in gastric glands (*5*) and because prostaglandins are thought to inhibit gastric acid secretion by inhibiting parietal cell adenylate cyclase (*6*), we examined the possibility that somatostatin may inhibit acid secretion by promoting the synthesis and release of prostaglandins.

Isolated stomachs from anesthetized male Sprague-Dawley rats (150 to 250 g) were perfused through the aortic remnant with Krebs-Ringer buffer containing 0.2 percent human serum albumin and 3 percent dextran T-40 (Pharmacia Fine Chemicals) as described (*7*). The perfusate was oxygenated with 95 percent O_2 and 5 percent CO_2 throughout the experiments and the temperature was constantly monitored and maintained at 37°C. Portal vein effluent was collected in ice-chilled containers at 2-minute intervals for subsequent measurement of prostaglandin E_2 by radioimmunoassay (*8*). As shown in Fig. 1A, the procedures used for perfusion were as follows: after a 30-minute stabilization period a 10-minute baseline was obtained, then 10-minute pulses of stimulants were administered followed in each instance by a 10-minute washout with unmodified perfusate.

Initial studies indicated that somatostatin (somatostatin-14, Peninsula Laboratories) by itself had no ability to stimulate prostaglandin release (Fig. 1B). Because the preparation was extrinsically denervated and because of preliminary evidence that cholinergic agonists stimulate prostaglandin synthesis, we reasoned that resting vagal tone might be required for somatostatin to influence prostaglandin production and release. When we applied increasing concentrations of somatostatin (10^{-10} to 10^{-8} *M*) to our preparations, we observed a dose-dependent potentiation of the effect of carbamylcholine (10^{-6} *M*) to release prostaglandins (Fig. 1, A and B). This biphasic stimulatory effect was inhibited completely by atropine (10^{-7} *M*), indicating that muscarinic receptors must be activated for somatostatin to act on prostaglandin production. Inhibition of pros-

taglandin synthesis by prior treatment of rats with indomethacin (5 mg/kg, intraperitoneally) 1 hour before gastric resection and perfusion diminished the potentiating effect of somatostatin. Pentagastrin in doses as high as $10^{-4}M$ had no effect on prostaglandin synthesis in our preparations.

We then determined whether the ability of somatostatin to potentiate the synthesis and secretion of prostaglandins is a mechanism by which it inhibits acid secretion. We used urethane-anesthetized (1.25 g/kg, intraperitoneally) rats in which the esophagus and pylorus had been ligated and the stomach penetrated with a double-lumen cannula. The gastric lumen was flushed with two 5-ml boluses of $0.15M$ NaCl and one 5-ml bolus of air at the end of each 10-minute period. Acid output was determined by titration of the flushed perfusate with $0.05N$ NaOH and use of an automatic titrator (Radiometer, Copenhagen, Denmark).

Control rats had virtually no basal acid secretion, but on stimulation with bethanechol (100 μg/kg-hour, intravenously) acid secretion gradually increased to a plateau by 1 hour (Fig. 2). The stimulated acid secretion was inhibited in a dose-dependent fashion by somatostatin infusion (2 or 4 μg per kilogram per hour, intravenously), and upon cessation of peptide infusion, acid secretion resumed to reach plateau values. Treatment of rats with indomethacin (10 mg/kg, intraperitoneally) 1 hour prior to study had two effects: (i) basal acid output in response to bethanechol was enhanced, and (ii) somatostatin's ability to inhibit acid secretion was abolished. Although indomethacin may have some nonspecific effects, these results suggest that endogenous prostaglandins exert a tonic inhibitory effect on acid secretion in the anesthetized rat and that somatostatin's ability to inhibit acid secretion in this

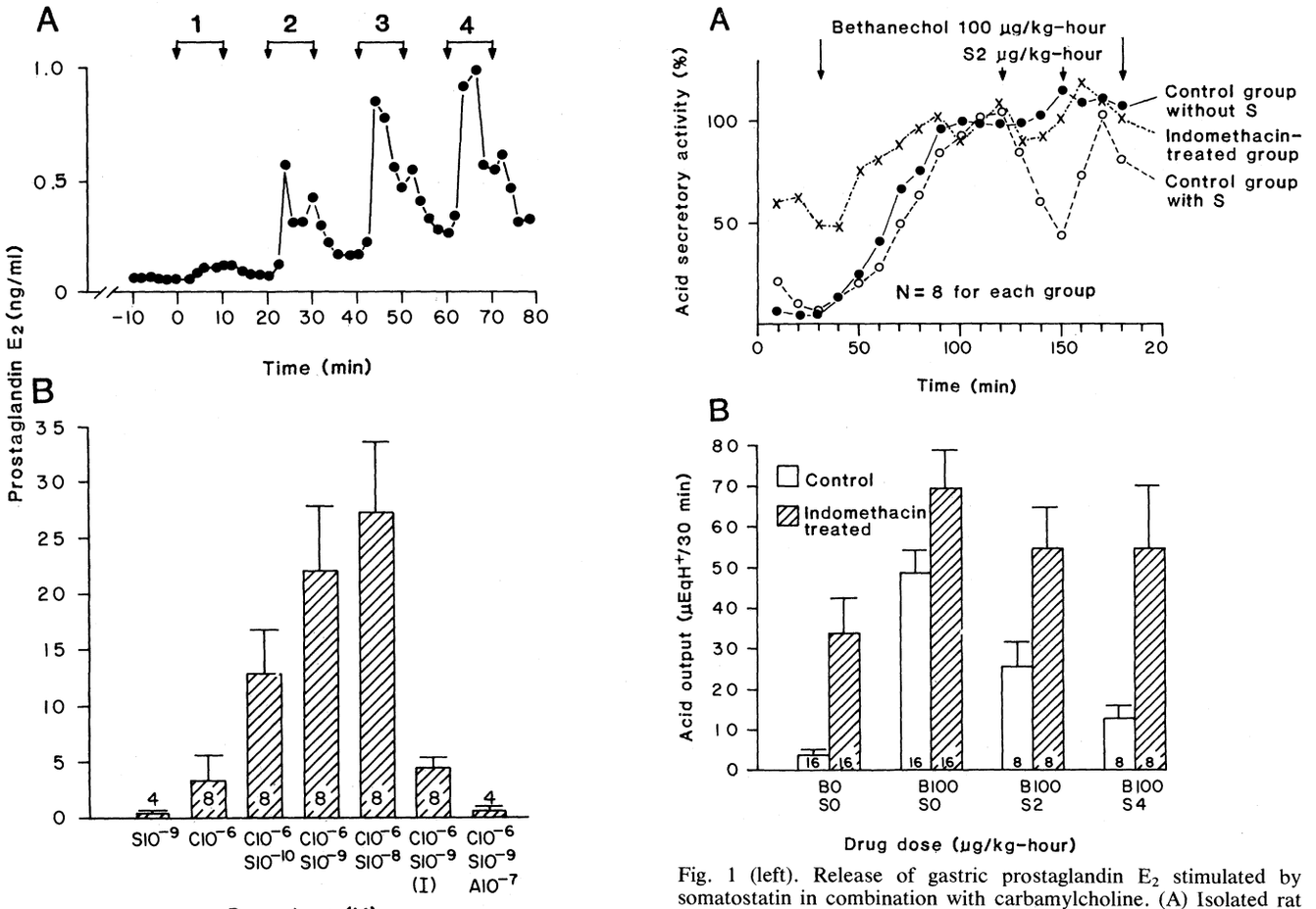

Fig. 1 (left). Release of gastric prostaglandin E_2 stimulated by somatostatin in combination with carbamylcholine. (A) Isolated rat stomachs ($N = 4$) perfused via the aorta with oxygenated Krebs-Ringer buffer were given 10-minute pulses of carbamylcholine ($10^{-6}M$) alone, or, sequentially, in combination with increasing doses of somatostatin (1, none; 2, $10^{-10}M$; 3, $10^{-9}M$; 4, $10^{-8}M$). Prostaglandin E_2 in the portal vein affluent was quantified by radioimmunoassay. Somatostatin caused a dose-dependent biphasic potentiation of prostaglandin E_2 release elicited by carbamylcholine. (B) The integrated prostaglandin responses (mean ± standard error) of isolated rat stomachs to 10-minute pulses of various agents is depicted on this slide. Comparisons were made by analysis of variance followed by linear contrast. For the number of animals in each treatment regimen, see the bottom of each bar. Somatostatin (*S*) alone caused no significant release of prostaglandin E_2 but potentiated, in a dose-dependent fashion ($P < .05$), the release caused by carbamylcholine (*C*) alone. Atropine (*A*) virtually abolished the release of prostaglandin E_2 stimulated by carbamylcholine and somatostatin ($P < .01$). Prior treatment of the rats with indomethacin (*I*) significantly diminished ($P < .01$) the potentiating effect of somatostatin, but did not abolish prostaglandin release. Fig. 2 (right). Effect of indomethacin on inhibition of gastric acid secretion by somatostatin. (A) Urethane-anesthetized rats with gastric fistulas received intravenous injections of bethanechol, and then gastric acid output was determined by titration. Acid output was expressed as the percentage of mean acid output obtained for the three 10-minute collection periods just prior to somatostatin (*S*) infusion (90 to 120 minutes). Somatostatin infusion inhibited bethanechol-stimulated acid secretion. Prior treatment of animals with indomethacin (10 mg/kg) resulted in increased basal acid secretion and loss of somatostatin's inhibitory effect. (B) Integrated basal and stimulated acid outputs (mean ± standard error) were compared for control and indomethacin-treated rats. Data were examined by analysis of variance followed by linear contrast. The number of animals for each treatment regimen is indicated at the bottom of each bar. Indomethacin significantly enhanced basal acid output ($P < .01$), but the apparent increase in bethanechol (*B*)-stimulated acid output was not significant. The ability of somatostatin (*S*) to diminish acid output in a dose-dependent fashion ($P < .01$) was abolished by indomethacin ($P < .05$).

model is dependent on prostaglandin generation.

Prostaglandins are known to be potent inhibitors of acid secretion (*9*), and have been shown to protect gastric mucosa from ulcer and erosion formation (*10*). In the complex model developed by Soll (*11*) with isolated canine parietal cells, acid secretion is regulated by mutually potentiating gastrin, histamine, and cholinergic receptors. Prostaglandins appear to act by inhibiting histamine-stimulated cyclic AMP production through inhibition of the enzyme adenylate cyclase (*6*). While the actions of cholinergic agonists alone are not mediated directly by cyclic AMP, they are always acting in concert with histamine in vivo. The potentiating interactions between the two secretagogues is blocked by prostaglandins (*6*). Our observations that prostaglandin synthesis and release from the stomach is enhanced by somatostatin (in the presence of carbamylcholine) and that somatostatin's ability to inhibit bethanechol-stimulated acid secretion is blocked by indomethacin suggest that somatostatin action on gastric secretion is mediated by prostaglandins. Although we did not measure gastric cyclic AMP in our experiments, Gespach *et al.* (*5*) have reported that cyclic AMP production in rat gastric glands is markedly inhibited in a noncompetitive fashion by somatostatin. This is consistent with our hypothesis regarding the actions of somatostatin. Since prostaglandins, in turn, stimulate somatostatin secretion (*12*) it is possible that their release further amplifies the acid inhibitory action of somatostatin. The implication of our results regarding a potential prostaglandin-mediated cytoprotective action of somatostatin requires further investigation.

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Protein Kinase Injection Reduces Voltage-Dependent Potassium Currents

Abstract. *Intracellular iontophoretic injection of the catalytic subunit of cyclic adenosine monophosphate–dependent protein kinase increased input resistance and decreased a delayed voltage-dependent K^+ current of the type B photoreceptor in the nudibranch* Hermissenda crassicornis *to a greater extent than an early, rapidly inactivating K^+ current (I_A). This injection also enhanced the long-lasting depolarization of type B cells after a light step. These findings suggest the involvement of cyclic adenosine monophosphate–dependent phosphorylation in the differential regulation of photoreceptor K^+ currents particularly during illumination. On the other hand, conditioning-induced changes in I_A may also be regulated by a different type of phosphorylation (for example, Ca^{2+}-dependent).*

Numerous studies have suggested a role for protein phosphorylation in many types of neuronal processes, including synaptic transmission, regulation of membrane permeability, phototransduction, and learning (*1*). Recently a change in the level of phosphate incorporation into a specific phosphoprotein band (*2*) has been detected in the eyes of the nudibranch *Hermissenda crassicornis* after training with paired light and rotation (*3, 4*) but not after control procedures. Type B photoreceptors (of which there are three in each eye) also showed biophysical changes both before and after isolation from the central nervous system (*5, 6*) during acquisition and retention of the learned behavior specifically produced by this associative training (*4*). An early voltage-dependent K^+ current (I_A) was reduced and inactivated more rapidly in associatively trained than in control type B cells (*6*). Approximately 60 percent of the resting input resistance of the type B soma membrane is due to this I_A conductance (*7, 8*). The reduction of I_A produced by training, therefore, can account for an increased input resistance and, at least partially, an enhanced long-lasting depolarization (LLD) to a light response also found for conditioned cells (*5, 9*). Since type B cell impulses affect interneuron and motoneuron impulse activity via known synaptic pathways (*10*), these biophysical changes, shown to be intrinsic to the type B photoreceptor soma membrane, can play a causal role in producing the associatively learned behavior. Sustained illumination, unlike conditioning, dramatically reduced a delayed voltage-dependent K^+ current (I_B), but had no effect on I_A when the cell was voltage-clamped at −60 mV (*6*).

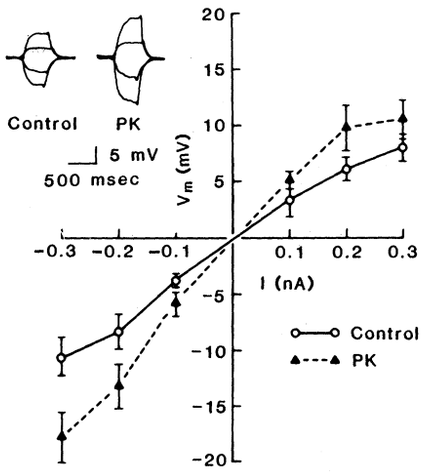

Fig. 1. Effects of iontophoretic protein kinase (*PK*) injection on dark membrane effective input resistance in type B photoreceptors. Symbols: ○, control injection of carrier solution (for each point, mean ± standard error, $N = 12$); ▲, *PK* values ($N = 10$). Inset shows representative voltage responses to current injection (±0.1 and ±0.2 nA).

In order to investigate the possible relationship of protein phosphorylation to these type B photoreceptor K^+ conductances, we have injected, under cur-