Protein acylation in the inhibition of insulin secretion by norepinephrine, somatostatin, galanin, and PGE₂

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Cheng, Haiying, Susanne G. Straub, and Geoffrey W. G. Sharp. Protein acylation in the inhibition of insulin secretion by norepinephrine, somatostatin, galanin, and PGE₂. Am J Physiol Endocrinol Metab 285: E287–E294, 2003.—The major physiological inhibitors of insulin secretion, norepinephrine, somatostatin, galanin, and PGE₂, act via specific receptors that activate pertussis toxin (PTX)-sensitive G proteins. Four inhibitory mechanisms are known: 1) activation of ATP-sensitive K⁺ channels and repolarization of the β-cell; 2) inhibition of L-type Ca²⁺ channels; 3) decreased activity of adenyl cyclase; and 4) inhibition of exocytosis at a “distal” site in stimulus-secretion coupling. We have examined the underlying mechanisms of inhibition at this distal site. In rat pancreatic islets, 2-bromopalmitate, cerulenin, and polyunsaturated fatty acids, all of which suppress protein acyltransferase activity, blocked the distal inhibitory effects of norepinephrine in a concentration-dependent manner. In contrast, control compounds such as palmitate, 16-hydroxypalmitate, and etomoxir, which do not block protein acylation, had no effect. Furthermore, 2-bromopalmitate also blocked the distal inhibitory actions of somatostatin, galanin, and prostaglandin E₂. Importantly, neither 2-bromopalmitate nor cerulenin affected the action of norepinephrine to decrease cAMP production. We also examined the effects of norepinephrine, 2-bromopalmitate, and cerulenin on palmitate metabolism. Palmitate oxidation and its incorporation into lipids seemed not to contribute to the effects of 2-bromopalmitate and cerulenin on norepinephrine action. These data suggest that protein acylation mediates the distal inhibitory effect on insulin secretion. We propose that the inhibitors of insulin secretion, acting via PTX-sensitive G proteins, activate a specific protein acyltransferase, causing the acylation of a protein or proteins critical to exocytosis. This particular acylation and subsequent disruption of the essential and precise interactions involved in core complex formation would block exocytosis.

rat pancreatic islets; β-cell; signaling; pertussis toxin; G proteins

INSULIN SECRETION is a complex process regulated by nutrients, such as glucose and amino acids, and hormonal and neural factors that provide stimulatory and inhibitory influences on the pancreatic β-cells. The major physiological inhibitors, such as norepinephrine (NE), somatostatin, prostaglandin E₂ (PGE₂), and galanin, can inhibit insulin secretion that is stimulated by all of the nutrient and modulatory pathways. They interact with specific G protein-linked receptors at the plasma membrane, which activate pertussis toxin (PTX)-sensitive Gᵢ and Gₒ proteins that mediate the inhibition of insulin secretion (17, 27, 30).

The inhibitory pathways act in contradictory fashion on many of the same sites as the stimulatory pathways (30). For example, 1) inhibitors activate the ATP-sensitive K (Kₐtp) channel, repolarize or hyperpolarize the cell membrane, and thus inhibit the action of glucose that depolarizes the membrane via closure of this channel; 2) inhibitors decrease the activity of the L-type Ca²⁺ channel by two means, indirectly by the activation of the Kₐtp channel and hyperpolarization, and directly by inhibiting the channel itself; 3) inhibitors decrease the activity of adenyl cyclase and lower cAMP levels, thus reducing the effect of cAMP to potentiate insulin secretion. However, the most dominant inhibitory mechanism (4) is at a “distal” site in stimulus-secretion coupling. This distal site is at a crucial, late step in exocytosis, beyond the elevation of intracellular Ca²⁺ and beyond the potentiating actions of cAMP and diacylglycerol. Information on the G protein interactions with these four major inhibitory sites is limited. It was reported that activation of Kₐtp channels was due to βγ-subunits of G₁ and/or G₆ proteins (43). The α-subunits of G₂-2 and G₂-3 mediate the decreased activity of adenyl cyclase in the β-cell (18).

In this study, we have taken advantage of two well-known inhibitors of protein acylation, 2-bromopalmi- tate (2-BrP) (8, 20, 36, 39) and cerulenin (11, 29, 31, 41), to investigate the distal inhibitory mechanisms of insulin secretion. By mediating protein-membrane and protein-protein interactions, protein acylation is thought to be important for cell signaling, e.g., in the T cell (25, 26, 34, 39) and β-cell (6, 31, 41, 44). We found that inhibitors of protein acylation blocked the distal inhibitory effects of NE, somatostatin, galanin, and PGE₂. The data suggest that the distal mechanism of inhibition of insulin secretion involves the acylation of a protein or proteins that are critical to the normal process of exocytosis.

MATERIALS AND METHODS

Isolation of pancreatic islets. Male Sprague-Dawley rats (250–400 g) were used. Immediately after CO₂ asphyxiation,
the pancreata were removed, and the islets were isolated by a collagenase digestion technique (16). Krebs-Ringer bicarbonate HEPES buffer (KRBB) containing (in mM) 129 NaCl, 5 NaHCO3, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 2.5 CaCl2, 2.8 glucose, 10 HEPES, and 0.1% BSA at pH 7.4 was used for isolation and selection of the islets. All procedures were approved by the Institutional Animal Care and Use Committee at Cornell University.

Measurements of insulin release. Insulin release was measured under static incubation conditions at 37°C with batches of five size-matched islets per tube. When Ca2+ was present in the buffer, islets were preincubated in regular KRBB buffer for 60 min. The islets were then incubated in the presence and absence of test agents (as we will describe) for 15 or 60 min. Under Ca2+-free conditions, the islets were washed three times with Ca2+-free KRBB buffer containing 1 mM EGTA (Ca2+-free KRBB-EGTA buffer) and preincubated in Ca2+-free KRBB-EGTA buffer for 60 min. They were then incubated in fresh Ca2+-free KRBB-EGTA buffer in the presence and absence of test agents for 60 min. At the end of the incubations, samples were taken and kept at −20°C until radioimmunoassayed for insulin using a charcoal separation method. Insulin secretion was expressed as fractional release, the percentage of the insulin content of the islets that was released over 15 or 60 min (15, 41). When exogenous free fatty acids (FFAs) and their analogs were used, the concentrations of the FFAs were estimated from the molar ratios of the fatty acids and FFA-free BSA, as reported in the literature (16, 18). In the last series of experiments (on the effects of arachidonic and eicosapentaenoic acids), the FFA-free BSA concentration was changed from 0.5 to 0.4%, because the new batch of BSA appeared to bind 2-BrP differently from the old batch, and the complete blockade of NE action by 2-BrP was obtained at 0.4%.

Palmitate oxidation measurement. Palmitate oxidation was measured as previously reported with minor modifications (5, 41). Briefly, groups of 15 islets (in duplicate) were incubated at 37°C for 1 h in 200 μl of KRBB buffer, containing 0.2 μCi [1-14C]palmitate, 0.8 mM L-carnitine, and other components as indicated. At the end of the incubation, islet metabolism was terminated by adding 200 μl of 0.5 M HCl to the incubation mixture. 14CO2 was collected overnight in 300 μl of 1 M NaOH and measured by liquid scintillation spectrometry. One batch of islets was used for each experiment, so that all of the determinations were paired.

Measurement of palmitate incorporation into lipids. This procedure was performed as previously described with minor modification (1, 5). Briefly, groups of 30–50 islets were incubated at 37°C for 1 h in 200 μl of KRBB buffer, containing 0.2 μCi [1-14C]palmitate, 0.8 mM L-carnitine, and other components as indicated. At the end of the incubations, the incubation media were removed, and the islets were washed twice with 1 ml of ice-cold PBS. Two hundred microliters of 0.2 M NaCl were added to the islet pellet, and the mixture was immediately frozen in liquid N2. After the islet pellet was thawed, 750 μl of chloroform-methanol (2:1) and 50 μl of 0.1 N KOH were added. After vigorous vortexing for 3 min, the phases were separated by centrifugation at 2,000 g for 20 min. The top aqueous layer was removed, and the bottom lipid-soluble layer was washed once with 200 μl of methanol-water-chloroform (48:47:3). Two hundred microliters of the lipid-soluble phase were added to 5 ml of BioSafe2 scintillation mixture, and incorporation of radiolabel into lipids was quantified by liquid scintillation spectrometry.

Measurement of cAMP. This measurement was performed as previously described with minor modification (9, 13, 40). Briefly, groups of 20 islets were preincubated at 37°C for 1 h in 200 μl of KRBB buffer, in the presence or absence of cerulenin as indicated. The islets were then incubated for 15 min in 200 μl of KRBB containing 5.6 mM Glc, 50 μM IBMX, 6 μM forskolin (Fsk), and 10 μM propranolol, with or without testing reagents as indicated. The incubation was terminated by addition of 300 μl of 0.2 N HCl. The tubes were then placed in boiling water for 15 min with occasional vortexing. cAMP was determined by the Biotrak cAMP enzymeimmunoassay (EIA) system.

Materials. Glucose, 2-BrP, palmitate, 16-hydroxy palmitate (16-OH palmitate), 2-hydroxy myristate (2-OH myristate), FFA-free BSA, NE, forskolin, 12-O-tetradecanoylphorbol 13-acetate (TPA), L-carnitine, somatostatin, galanin, and PGE2 were obtained from Sigma (St. Louis, MO). Cerulenin was purchased from Fluka (Milwaukee, WI). [1-14C]palmitate was obtained from New England Nuclear (Boston, MA). The Biotrak cAMP EIA system was from Amersham Pharmacia Biotech (Piscataway, NJ).

Statistical methods. All data are shown as means ± SE. Statistical significance was evaluated by two-way ANOVA or t-tests as appropriate. Differences were considered significant at P < 0.05.

RESULTS

Effects of 2-BrP, palmitate, 16-OH palmitate, and 2-OH myristate on the inhibition of insulin secretion by NE. To "isolate" the distal inhibitory effect of NE and distinguish it from the effects of NE on the KATP and Ca2+ channels, the first studies were performed in the absence of extracellular Ca2+. In addition, a supermaximally effective concentration of Fsk was used (20) to eliminate any effect of NE via adenyl cyclase. The results of the experiments are shown in Fig. 1. Under these Ca2+-free conditions, 11.1 mM glucose stimulated insulin secretion 2.6-fold (0.68 ± 0.12 vs. 2.43 ± 0.05). In the absence of extracellular Ca2+, palmitate, 16-OH palmitate, and 2-OH myristate had no effect on the inhibition of insulin secretion by NE.
0.38% of content/h; Δ = 1.75 ± 0.35; P < 0.01) in the presence of 6 μM Fsk and 100 nM TPA, similar to data reported previously (14, 15). This glucose-stimulated insulin secretion was not altered by 20 μM 2-BrP (control, 2.43 ± 0.38 vs. 2-BrP, 2.49 ± 0.11% of content/h; Δ = –0.06 ± 0.04; P = 0.21) and was completely inhibited by 10 μM NE (2.43 ± 0.38 vs. 0.84 ± 0.24% of content/h; Δ = 1.59 ± 0.17; P < 0.01). However, in the presence of 20 μM 2-BrP, glucose-stimulated insulin secretion was unaffected by NE (control, 2.43 ± 0.38 vs. NE, 2.33 ± 0.38% of content/h; Δ = –0.10 ± 0.16; P = 0.57). The effect of 2-BrP to block the action of NE action was concentration dependent, with complete blockade at 20 μM.

Experiments were next carried out under normal conditions in the presence of Ca2+. As shown in Fig. 2, insulin secretion was stimulated by 11.1 mM glucose, and this was not affected by 20 μM 2-BrP (control, 1.98 ± 0.41 vs. 2-BrP, 2.12 ± 0.47% of content/h; Δ = –0.14 ± 0.37; P = 0.73), a finding that is consistent with previous studies (32, 45). NE inhibited glucose-stimulated insulin secretion completely (1.98 ± 0.41 vs. 0.58 ± 0.09% of content/h; Δ = 1.40 ± 0.10; P < 0.001) in the absence of 2-BrP but incompletely (1.98 ± 0.41 vs. 1.01 ± 0.17% of content/h; Δ = 0.97 ± 0.06; P < 0.05) in the presence of 2-BrP. Thus the blocking effect of 2-BrP on the inhibitory action of NE in the presence of extracellular Ca2+ was less than that in the absence of extracellular Ca2+. Under Ca2+-free conditions, the only functional inhibitory mechanism for NE to inhibit insulin secretion is at the distal site, and this is completely blocked by 2-BrP. In the presence of extracellular Ca2+, the inhibitory effect of NE seen when the islets are exposed to 2-BrP could be exerted via activation of the KATP channels, reduced Ca2+ influx, and inhibition of adenylyl cyclase. These combined data suggest that only the distal inhibitory action of NE is blocked by 2-BrP.

To exclude the possibility that the action of 2-BrP was due to nonspecific effects rather than inhibition of protein acylation, we examined the effects of palmitate and 16-OH palmitate under the same Ca2+-free conditions as we tested 2-BrP. These two compounds are structurally similar to 2-BrP but do not block protein acylation (39) as does 2-BrP. Insulin secretion was not changed by either 20 μM palmitate (control, 2.64 ± 0.49 vs. palmitate, 2.72 ± 0.33% of content/h; Δ = –0.08 ± 0.39; P = 0.85) or 20 μM 16-OH palmitate (control, 2.29 ± 0.26 vs. 16-OH palmitate, 2.48 ± 0.38% of content/h; Δ = –0.19 ± 0.15; P = 0.21). It was inhibited equally by NE in the absence and presence of 20 μM palmitate (control, 0.74 ± 0.16 vs. palmitate, 0.51 ± 0.06% of content/h; Δ = 0.23 ± 0.11; P = 0.12), and in the absence and presence of 20 μM 16-OH palmitate (control, 0.78 ± 0.11 vs. 16-OH palmitate, 0.92 ± 0.12% of content/h; Δ = –0.14 ± 0.17; P = 0.31).

To distinguish between the two most common forms of protein acylation, palmitoylation and myristoylation, we examined the effect of 2-OH myristate, which blocks myristoylation but not palmitoylation (19). Under Ca2+-free conditions, insulin secretion was stimulated by 11.1 mM glucose in the presence of TPA and Fsk and was completely inhibited by NE (2.23 ± 0.37 vs. 0.79 ± 0.10% of content/h; Δ = 1.44 ± 0.43; P < 0.05). In the presence of 20 μM 2-OH myristate, glucose-stimulated secretion was not altered by 2-OH myristate (2.23 ± 0.37 vs. 2-OH myristate, 2.58 ± 0.39% of content/h; Δ = –0.35 ± 0.40; P = 0.44) and was still completely inhibited by NE (2.58 ± 0.39 vs. 0.93 ± 0.18% of content/h; Δ = 1.65 ± 0.51; P < 0.05). Hence, NE inhibited glucose-stimulated insulin secretion to a similar extent in the absence and presence of 20 μM 2-OH myristate (0.79 ± 0.10 vs. 0.93 ± 0.18% of content/h; Δ = –0.14 ± 0.11; P = 0.23). Therefore, myristoylation does not appear to be involved in the mechanism of inhibition.

Effect of cerulenin to block the inhibition of insulin secretion by NE. The effects of cerulenin, a well-known inhibitor of protein acylation, are shown in Fig. 3. In this series of experiments, insulin secretion was evoked by a depolarizing concentration of 50 mM KCl in the presence of TPA and Fsk. We used KCl because nutrient-stimulated, but not non-nutrient-stimulated, insulin secretion is inhibited by cerulenin (41). Fsk was used to keep intracellular cAMP at supermaximal levels and, like TPA, to maximize the secretory response. In these experiments, cerulenin was included only in the preincubation period. In the absence of cerulenin, NE inhibited the stimulated insulin secretion by 96% (P < 0.001). Cerulenin blocked the inhibitory action of NE in a concentration-dependent manner. The IC50 for cerulenin to block the action of NE was 10 μg/ml (45 μM), and complete blockade was seen at 100 μg/ml (450 μM). In separate experiments, basal insulin secretion in the presence of 2.8 mM glucose was not significantly affected by 100 μg/ml cerulenin (control,
0.40 ± 0.08 vs. cerulenin, 0.48 ± 0.10% of content/15 min; Δ = −0.08 ± 0.04; P = 0.16), nor was insulin secretion stimulated by the combination of KCl, TPA, and forskolin (control, 0.96 ± 0.23 vs. cerulenin, 1.14 ± 0.25% of content/15 min; Δ = −0.18 ± 0.32; P = 0.63). The fact that 100 μg/ml cerulenin did not alter either basal or KCl-stimulated insulin secretion suggests that cerulenin affects neither the islet’s ability to secrete insulin nor its membrane integrity to permit leakage of insulin. Under these experimental conditions, in the presence of 50 mM KCl and 6 μM Fsk, the effects of NE on the K<sub>A<sub>TPP</sub></sub> channel to hyperpolarize the cell and to lower cAMP levels are bypassed. Therefore, the data presented so far with 2-BrP and cerulenin, two structurally different inhibitors of protein acylation, strongly suggest that protein acylation is involved in the distal inhibitory action of NE.

Effects of 2-BrP on the inhibition of insulin secretion by somatostatin, galanin, and PGE<sub>2</sub> under Ca<sup>2+</sup>-free conditions. Three other physiological inhibitors of insulin secretion, somatostatin, galanin, and PGE<sub>2</sub>, were tested to see whether the effect of 2-BrP was specific for NE or whether it blocked the effects of other inhibitors also. As seen in Fig. 4, under Ca<sup>2+</sup>-free conditions, 1 μM somatostatin, 100 nM galanin, and 10 μM PGE<sub>2</sub> inhibited glucose-stimulated insulin secretion by 73% (P < 0.01), 61% (P < 0.01), and 66% (P < 0.01), respectively. In contrast, in the presence of 20 μM 2-BrP, these inhibitors had no effect on insulin secretion; their inhibitory actions were completely blocked. Thus the effect of 2-BrP is a general effect against several inhibitors.

Effects of NE, 2-BrP, and cerulenin on palmitate metabolism. It has been reported that NE reduced the oxidation of palmitate as well as the incorporation of glucose into phospholipids and neutral lipids in the presence of palmitate through an α<sub>2</sub>-adrenergic mechanism (35). 2-BrP and cerulenin have also been reported to affect lipid metabolism (11, 17). Consequently, we examined the effects of NE, 2-BrP, and cerulenin on lipid metabolism to determine whether a change in metabolism was a contributory factor in the inhibitory effects of 2-BrP and cerulenin on the action of NE. As expected, and as shown in Table 1, palmitate oxidation was significantly lower in the presence of 11.1 mM glucose than in the presence of 2.8 mM glucose (0.38 ± 0.03 vs. 0.51 ± 0.04 pmol·islet<sup>−1</sup>·h<sup>−1</sup>; Δ = −0.13 ± 0.02; P < 0.01). Palmitate oxidation was not significantly affected by NE at either 2.8 mM or 11.1 mM glucose. Similarly, NE did not significantly change [1-<sup>14</sup>C]palmitate incorporation into cellular lipids at either 2.8 mM or 11.1 mM glucose. Glucose at 11.1 mM increased [1-<sup>14</sup>C]palmitate incorporation into lipid by 20% (P < 0.001) relative to incorporation in the presence of 2.8 mM glucose. Furthermore, both 2-BrP and cerulenin decreased 11.1 mM glucose-induced palmitate oxidation by 66% in the absence and presence of NE. However, the effects of 2-BrP and cerulenin on [1-<sup>14</sup>C]palmitate incorporation were different. Cerulenin had no effect, whereas 2-BrP increased 11.1 mM glucose-induced palmitate esterification.

Effects of NE, 2-BrP, and cerulenin on cAMP production. The data so far demonstrated that the distal site of NE action was blocked by 2-BrP and cerulenin.
However, it was not clear whether 2-BrP and cerulenin acted at any of the other sites of NE action. Therefore, we examined their effects on cAMP production of islets. As shown in Table 2, 10 μM NE significantly decreased Fsk-stimulated cAMP production from 580 ± 57 to 275 ± 52 fmol·islet⁻¹·15 min⁻¹, a 53% inhibition (P < 0.01). More importantly, in the presence of 20 μM 2-BrP or 100 μg/ml cerulenin, NE was still able to suppress cAMP production (Table 2). This demonstrates that neither 2-BrP nor cerulenin blocks the binding of NE to the α2-adrenergic receptor, receptor activation of related G proteins, and G protein inhibition of adenylyl cyclase.

Effect of 2-BrP on the inhibition of insulin secretion by high concentrations of NE. On the basis of these data on cAMP production, the displacement of NE from the α2-adrenergic receptor appears not to contribute to the blocking effect of 2-BrP on NE action. To strengthen this point, we further tested the effect of 2-BrP in the presence of a very high NE concentration (100 μM) under Ca²⁺ -free conditions. As shown in Fig. 5, insulin secretion was stimulated by 11.1 mM glucose in the presence of TPA and Fsk, and this was not affected by 2-BrP (control, 3.65 ± 0.45 vs. 2-BrP, 3.18 ± 0.35% of content/h; Δ = -0.47 ± 0.34; P = 0.27). This stimulated insulin secretion was completely inhibited by NE at both 10 μM (3.65 ± 0.45 vs. 0.73 ± 0.08% of content/h; Δ = 2.92 ± 0.52; P < 0.01) and 100 μM (3.65 ± 0.45 vs. 0.86 ± 0.19% of content/h; Δ = 2.78 ± 0.62; P < 0.01). However, in the presence of 2-BrP, insulin secretion was not affected by NE at either 10 μM (3.65 ± 0.45 vs. 2.89 ± 0.21% of content/h; Δ = 0.76 ± 0.48; P = 0.40) or 100 μM (3.65 ± 0.45 vs. 3.44 ± 0.62% of content/h; Δ = 0.21 ± 0.99; P = 0.73). Therefore, increasing the NE concentration, even to 100 μM, did not reinstate its inhibitory action on insulin secretion.

Effects of etomoxir and polyunsaturated fatty acids on the inhibition of insulin secretion. 2-BrP has been used extensively to suppress carnitine palmitoyltransferase I (CPT I) activity. To determine whether the effects of 2-BrP to block the inhibitory effect of NE could be related to this suppression of CPT I activity, we examined the effects of another CPT I inhibitor, etomoxir (45). In paired experiments shown in Fig. 5, etomoxir was used under exactly the same conditions as was 2-BrP. In contrast to 2-BrP, insulin secretion was inhibited by NE to a similar extent in the absence and presence of etomoxir (0.73 ± 0.08 vs. 1.06 ± 0.17% of content/h; Δ = -0.33 ± 0.14; P = 0.10). Hence, inhibition of CPT I activity is not a contributory factor in the effects of 2-BrP on NE action.

In further control experiments, we asked whether polyunsaturated fatty acids, such as arachidonic acid and eicosapentaenoic acid, could mimic the effect of 2-BrP on NE-mediated inhibition of insulin secretion. Both arachidonic acid and eicosapentaenoic acid interfere with T cell signal transduction, an action that is thought to be due to the inhibition of protein palmitoylation (39). Under Ca²⁺ -free conditions, NE failed to inhibit insulin secretion in the presence of 0.5 mM arachidonic acid or eicosapentaenoic acid (data not shown).

DISCUSSION

In the work presented here, 2-BrP and cerulenin, both of which inhibit protein acyl transferase (39, 41), blocked the distal effect of NE to inhibit insulin secre-
protein acylation. In contrast, control compounds such as palmitate, 16-OH palmitate, and etomoxir (which do not block protein acylation) had no effect on the action of NE. These data suggest that protein acylation is involved in the distal inhibitory action of NE. Furthermore, 2-BrP also blocked the distal inhibitory actions of somatostatin, galanin, and PGE2. Therefore, protein acylation appears to be generally involved in the distal inhibition of insulin secretion and is not specific to a particular inhibitor.

Inhibition of protein acylation by 2-BrP and cerulenin is considered responsible for the effects shown in this study. The reasons for this follow. 1) If the suppression of CPT I activity (23) were the reason for 2-BrP to block the inhibitory action of NE, etomoxir would be expected to mimic this effect, because etomoxir is also a CPT I inhibitor. Instead, etomoxir had no effect on the action of NE. Additionally, cerulenin is not a CPT I inhibitor. Furthermore, whereas both 20 μM 2-BrP and 100 μg/ml cerulenin inhibited palmitate oxidation of 11.1 mM glucose, only 2-BrP significantly increased 11.1 mM glucose-induced palmitate incorporation into lipids. Hence, the different influences on palmitate metabolism by 2-BrP and cerulenin suggest that palmitate metabolism has no significant role in the effects of 2-BrP and cerulenin on the action of NE.

2) The similarities between the concentration-response characteristics of cerulenin to block the action of NE in this study and the concentration-response characteristics for the inhibition of protein acylation in other reports (11, 29, 41) suggest that cerulenin is functioning under our conditions as an inhibitor of protein acylation. 3) The short incubation time of our experimental conditions precludes the long-term effects of cerulenin and 2-BrP. These include the effects of cerulenin on apoptosis, cell growth, DNA, RNA, and protein synthesis (10, 11, 21, 22, 29) and the effects of 2-BrP on the levels of insulin and uncoupling protein-2 mRNA (3, 38).

Because of the fast, reversible nature of inhibition of insulin secretion by most physiological inhibitors, the rapidly reversible palmitoylation reaction would be more important for modulating this function (7) than the relatively slower myristoylation reaction. This is in accord with our observation that 2-OH myristate, which blocks myristoylation but not palmitoylation (19), had no influence on the inhibitory action of NE.

There is indirect evidence to indicate that NE inactivates long-chain acyl-CoA synthetase and other enzymes of glycerolipid synthesis (35). However, in the present study, we did not find any effects of NE on palmitate oxidation. Nor did we find any effects on esterification at low or high concentrations of glucose. Moreover, 2-BrP was still able to increase [1-14C]palmitate incorporation into cellular lipid at 11.1 mM glucose even in the presence of NE. Thus our data provide no evidence for an effect of NE on palmitate metabolism under our experimental conditions.

With respect to the other actions of NE in the β-cell, neither 2-BrP nor cerulenin affected the action of NE to decrease cAMP production. This indicates that inhibitors of protein acylation do not block the interaction of NE at the α2-adrenergic receptor, related G protein activation, or inhibition of adenylyl cyclase. This further excludes the possibility that the effects of 2-BrP and cerulenin are due to nonspecific effects.

In a study using capacitance measurements in mouse pancreatic β-cells, activation of calcineurin was proposed as the distal mechanism for NE, galanin, and somatostatin to inhibit insulin secretion (24). Calcineurin is a Ca2+- and calmodulin-dependent protein phosphatase (42). However, NE-induced inhibition of insulin secretion was unaffected by blockade of calcineurin in INS-1 (12), βHC-9, and HIT cell lines (L. M. Shen and G. W. G. Sharp, unpublished observations). Moreover, under stringent Ca2+-free conditions that presumably exclude the Ca2+-dependent activity of calcineurin, NE completely blocks stimulated insulin secretion (13). Additionally, under these Ca2+-free conditions, 2-BrP still effectively blocked the distal inhibitory effects of NE, somatostatin, galanin, and PGE2. Calcineurin does not appear to be involved in the mechanism of the distal inhibition of insulin secretion. PTX-sensitive Gi and Go proteins can exert their inhibitory effects on insulin secretion via two possible mechanisms: 1) direct action of the G1 and G9 subunits (α and/or βγ) on proteins critical for exocytosis; 2) activation of an enzyme or protein mediator of the inhibition by the α- and/or β3-subunits. Relevant to the latter, our data suggest that distal inhibition of insulin secre-
tion is achieved by activation of a specific protein acyltransferase.

It is known that, regarding inhibition of insulin secretion, $\alpha_2$-adrenergic receptor, $G_{\alpha}\alpha$ and $G_{\alpha}$, SNAP-25, and synaptotagmin are palmitoylated, whereas $G_{\alpha}\alpha$ and $G_{\alpha}$ are myristoylated. $G_{\alpha}\alpha$-specific palmitoyltransferase (to H. Cheng).

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DISCLOSURES

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