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

Haiying Cheng, Susanne G. Straub, and Geoffrey W. G. Sharp

Department of Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Submitted 10 December 2002; accepted in final form 1 April 2003

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. First published April 8, 2003; 10.1152/ajpendo.00535.2002.—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.

Address for reprint requests and other correspondence: G. W. G. Sharp, Dept. of Molecular Medicine, College of Veterinary Medicine, Cornell Univ., Ithaca, NY 14853-6401 (E-mail: gws2@cornell.edu).

http://www.ajpendo.org 0193-1849/03 $5.00 Copyright © 2003 the American Physiological Society
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 radiolabeled insulin was measured. 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, the palmitate 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 cerululin 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 enzyme immunoassay (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). Cerululin 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 K ATP and Ca²⁺ channels, the first studies were performed in the absence of extracellular Ca²⁺. In addition, a supramaximally 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 ±
0.38% 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 con-
tent/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 com-
pletely blocked by 2-BrP. In the presence of extracel-
ular Ca2+, the inhibitory effect of NE seen when the
islets are exposed to 2-BrP could be exerted via activa-
tion 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 condi-
tions 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 myristoyla-
tion, we examined the effect of 2-OH myristate, which
blocks myristoylation but not palmitoylation (19).
Under Ca2+-free conditions, insulin secretion was stimu-
lated 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, glu-
cose-stimulated secretion was not altered by 2-OMy-
ristate (control, 2.23 ± 0.37 vs. 2-OMyristate, 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 secre-
tion 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 lev-
els and, like TPA, to maximize the secretory response.
In these experiments, cerulenin was included only in
the 60-min incubation period. Values are means ± SE,
n = 4. *P < 0.05.

Fig. 2. Effects of 2-BrP on the inhibition of
insulin secretion by NE under normal condi-
tions with Ca2+ present. Insulin secretion
was evoked by 11.1 mM glucose. 2-BrP (20
μM) and NE were present in the 60-min incu-
bation period. Values are means ± SE, n = 4. *P < 0.05.
Fig. 3. Concentration dependence of effect of NE on inhibition of insulin secretion by NE. Insulin secretion was evoked by 50 mM KCl in the presence of 100 nM TPA and 6 μM Fsk for 15 min. Cerulenin was included only in the 60-min preincubation period and was not present in the incubation period. Values are means ± SE; n = 7.

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 KATP 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 PGE2 under Ca2+-free conditions. Three other physiological inhibitors of insulin secretion, somatostatin, galanin, and PGE2, 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 Ca2+-free conditions, 1 μM somatostatin, 100 nM galanin, and 10 μM PGE2 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.

Fig. 4. Effects of 2-BrP on inhibition of insulin secretion by somatostatin, galanin, and PGE2 under Ca2+-free conditions. Insulin secretion was evoked by 11.1 mM glucose in the presence of 20 μM Fsk and 100 nM TPA. 2-BrP (20 μM) was present in the 60-min incubation period. 1 μM Somatostatin, 100 nM galanin, and 10 μM PGE2 were present during the last 10 min of the 60-min preincubation and in the 60-min test incubation. Values are means ± SE; n = 6. *P < 0.01.

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 α2-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−1·h−1; 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 [1-14C]palmitate incorporation into cellular lipids at either 2.8 mM or 11.1 mM glucose. Glucose at 11.1 mM increased [1-14C]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 [1-14C]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.
Table 1. Effects of norepinephrine, 2-BrP, and cerulenin on palmitate oxidation and incorporation into lipids

<table>
<thead>
<tr>
<th></th>
<th>NE (−)</th>
<th>NE (+)</th>
<th>Δ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>14CO₂ Production</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8 mM glucose</td>
<td>0.51 ± 0.04 (7)</td>
<td>0.47 ± 0.07 (6)</td>
<td>−0.04 ± 0.02</td>
<td>0.67a</td>
</tr>
<tr>
<td>11.1 mM glucose</td>
<td>0.38 ± 0.03 (7)</td>
<td>0.41 ± 0.05 (7)</td>
<td>−0.03 ± 0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>11.1 mM glucose + 20 μM 2-BrP</td>
<td>0.13 ± 0.02 (7)</td>
<td>0.13 ± 0.02 (7)</td>
<td>0.00 ± 0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>11.1 mM glucose + 100 μg/ml cerulenin</td>
<td>0.13 ± 0.04 (6)</td>
<td>0.14 ± 0.04 (6)</td>
<td>−0.01 ± 0.02</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>14Cpalmitate Incorporation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8 mM glucose</td>
<td>0.80 ± 0.08 (7)</td>
<td>0.81 ± 0.08 (5)</td>
<td>0.01 ± 0.02</td>
<td>0.92a</td>
</tr>
<tr>
<td>11.1 mM glucose</td>
<td>0.96 ± 0.07 (7)</td>
<td>0.89 ± 0.07 (7)</td>
<td>0.07 ± 0.06</td>
<td>0.97</td>
</tr>
<tr>
<td>11.1 mM glucose + 20 μM 2-BrP</td>
<td>1.24 ± 0.07 (7)</td>
<td>1.14 ± 0.07 (7)</td>
<td>0.10 ± 0.05</td>
<td>0.34</td>
</tr>
<tr>
<td>11.1 mM glucose + 100 μg/ml cerulenin</td>
<td>0.93 ± 0.07 (5)</td>
<td>0.92 ± 0.07 (5)</td>
<td>0.01 ± 0.07</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Values (expressed as pmol-islet⁻¹·h⁻¹) are means ± SE, with no. of determinations in parentheses. NE, norepinephrine; 2-BrP, 2-bromopalmitate. Statistical analysis was by paired or unpaired (*) Student’s t-test. For palmitate oxidation: 2.8 mM glucose vs. 11.1 mM glucose, P < 0.01; 11.1 mM glucose vs. 11.1 mM glucose + 20 μM 2-BrP, P < 0.0001; 11.1 mM glucose vs. 11.1 mM glucose + 100 μg/ml cerulenin, P < 0.001. For [1-14C]palmitate incorporation into cellular lipid: 2.8 mM glucose vs. 11.1 mM glucose, P < 0.01; 11.1 mM glucose vs. 11.1 mM glucose + 20 μM 2-BrP, P < 0.005; 11.1 mM glucose + NE vs. 11.1 mM glucose + 20 μM 2-BrP + NE, P < 0.01; 11.1 mM glucose vs. 11.1 mM glucose + 100 μg/ml cerulenin, P = 0.76.

Table 2. Effects of norepinephrine, 2-BrP, and cerulenin on cAMP production

<table>
<thead>
<tr>
<th></th>
<th>NE (−)</th>
<th>NE (+)</th>
<th>Δ</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cAMP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(fmol-islet⁻¹·15 min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>580 ± 57</td>
<td>275 ± 52</td>
<td>305 ± 40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>+20 μM 2-BrP</td>
<td>607 ± 53</td>
<td>315 ± 40</td>
<td>292 ± 83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>+100 μg/ml cerulenin</td>
<td>743 ± 73</td>
<td>512 ± 94</td>
<td>231 ± 60</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. Control, incubation of rat islets with 5.6 mM glucose, 50 μM IBMX, and 6 μM forskolin (Fsk). Basal cAMP content without Fsk or IBMX averaged 21 ± 6 fmol-islet⁻¹·15 min⁻¹. No comparison between corresponding values in the absence and presence of 2-BrP or cerulenin was significant. The same batch of islets was used for all conditions in each experiment.

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 NE 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 adenyl 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 the 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-
Fig. 5. Effects of 2-BrP or etomoxir on inhibition of insulin secretion by NE under Ca\(^{2+}\)-free conditions. Insulin secretion was evoked by 11.1 mM glucose in the presence of 6 \(\mu\)M forskolin and 100 nM TPA. 2-BrP, etomoxir, and NE were present in the 60-min incubation period. Values are means ± SE; \(n = 4\). *\(P < 0.01\).

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 \(\mu\)M 2-BrP and 100 \(\mu\)g/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-\(^{14}\)C]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 \(\beta\)-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 \(\alpha_{2}\)-adrenergic receptor, related G protein activation, or inhibition of adenyl 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 \(\beta\)-cells, activation of calcineurin was proposed as the distal mechanism for NE, galanin, and somatostatin to inhibit insulin secretion (24). Calcineurin is a Ca\(^{2+}\) - and calmodulin-dependent protein phosphatase (42). However, NE-induced inhibition of insulin secretion was unaffected by blockade of calcineurin in INS-1 (12), \(\beta\)HC-9, and HIT cell lines (L. M. Shen and G. W. G. Sharp, unpublished observations). Moreover, under stringent Ca\(^{2+}\)-free conditions that presumably exclude the Ca\(^{2+}\)-dependent activity of calcineurin, NE completely blocks stimulated insulin secretion (13). Additionally, under these Ca\(^{2+}\)-free conditions, 2-BrP still effectively blocked the distal inhibitory effects of NE, somatostatin, galanin, and PGE\(_{2}\). Calcineurin does not appear to be involved in the mechanism of the distal inhibition of insulin secretion. PTX-sensitive \(\alpha_{i}\) and \(\alpha_{o}\) proteins can exert their inhibitory effects on insulin secretion via two possible mechanisms: 1) direct action of the \(\alpha_{i}\) and \(\alpha_{o}\) subunits (\(\alpha_{i}\) and/or \(\beta_{\gamma}\)) on proteins critical for exocytosis; 2) activation of an enzyme or protein mediator of the inhibition by the \(\alpha_{i}\) and/or \(\beta_{\gamma}\)-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, α2-adrenergic receptor, Gα1 and Gαα, SNAP-25, and synaptotagmin are palmitoylated, whereas Gαβ1 and Gαo are myristoylated (2, 4, 28, 33, 37). Finally, on the basis of our finding that inhibitors of protein acylation blocked the distal site of inhibitors of insulin secretion, we propose that the inhibitors of insulin secretion, acting via PTX-sensitive G proteins, activate a specific protein acyltransferase that results in acylation of a protein or proteins critical to exocytosis. This particular acylation and subsequent disruption of essential and precise interactions of the exocytotic machinery would block insulin secretion. It should be noted that protein acyltransferase would be a novel target for G protein activation.

DISCLOSURES

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-42063 and a New York State Bridge Grant (to G. W. G. Sharp), by a Career Development Award from the Juvenile Diabetes Association (to S. G. Straub), and by a Predoctoral Fellowship from the Pharmaceutical and Research Manufacturers Association (to H. Cheng).

REFERENCES


