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PACAP stimulates insulin secretion but inhibits insulin sensitivity in mice

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1Department of Medicine, Lund University, SE-205 02 Malmö, Sweden; 2Institute of Systems Science and Biomedical Engineering (LADSEB-Consiglio Nazionale delle Ricerche), I-35127 Padua, Italy; and 3Department of Animal Physiology, University of Groningen, 9750 Haren, The Netherlands

Filipsson, Karin, Giovanni Pacini, Anton J. W. Scheurink, and Bo Ahren. PACAP stimulates insulin secretion but inhibits insulin sensitivity in mice. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E834–E842, 1998.—Although pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates insulin secretion, its net influence on glucose homeostasis in vivo has not been established. We therefore examined the action of PACAP-27 and PACAP-38 on insulin secretion, insulin sensitivity, and glucose disposal as derived from the minimal model of glucose disappearance during an intravenous glucose tolerance test in anesthetized mice. PACAP-27 and PACAP-38 markedly and equipotently potentiated glucose-stimulated insulin secretion, with a half-maximal effect at 33 pmol/kg. After PACAP-27 or PACAP-38 (1.3 nmol/kg), the acute (1–5 min) insulin response was 3.8 ± 0.4 nmol/l (PACAP-27) and 3.3 ± 0.3 nmol/l (PACAP-38), respectively, vs. 1.4 ± 0.1 nmol/l after glucose alone (P < 0.001), and the total area under the curve for insulin (AUCinsulin) was potentiated by 60% (P < 0.001). In contrast, PACAP-27 and PACAP-38 reduced the insulin sensitivity index (S_i) [0.23 ± 0.04 10⁻⁴ min⁻¹/(pmol/l) for PACAP-27 and 0.29 ± 0.06 10⁻⁴ min⁻¹/(pmol/l) for PACAP-38 vs. 0.46 ± 0.02 10⁻⁴ min⁻¹/(pmol/l) for controls (P < 0.01)]. Furthermore, PACAP-27 or PACAP-38 did not affect glucose elimination determined as glucose half-time or the glucose elimination rate after glucose injection or the area under the curve for glucose. Moreover, glucose effectiveness and the global disposition index (AUCinsulin times S_i) were not affected by PACAP-27 or PACAP-38. Finally, when given together with glucose, PACAP-27 did not alter plasma glucagon or norepinephrine levels but significantly increased plasma epinephrine levels. We conclude that PACAP, besides its marked stimulation of insulin secretion, also inhibits insulin sensitivity in mice, the latter possibly explained by increased epinephrine. This complex action explains why the peptide does not enhance glucose disposal.

pituitary adenylate cyclase-activating polypeptide; glucose effectiveness; minimal model

THE NEUROPEPTIDE pituitary adenylate cyclase-activating polypeptide (PACAP) was originally isolated from the ovine hypothalamus (26) and has later been shown to be expressed in several tissues throughout the body (6). The peptide exists in two forms: PACAP-38 and PACAP-27, of which the latter is equivalent to PACAP-38-(1–27) (27). In the pancreas, PACAP has been localized to intrapancreatic nerve ganglia and to single nerves in the exocrine parenchyma, around blood vessels, and in conjunction with islets (2, 15), and one study has also indicated that PACAP occurs in islet endocrine cells (34). Furthermore, PACAP receptors have been demonstrated in the pancreas and in insulin-producing cells (2, 6, 17, 33). Moreover, PACAP has been demonstrated to stimulate insulin secretion in vivo in humans and dogs (13, 21), in vitro in the perfused rat pancreas (10, 35), and in insulin-producing cells (2, 3, 17, 22, 34), mainly through activating adenylate cyclase (3, 22). Hence, PACAP is a pancreatic neuropeptide with a stimulatory action on insulin secretion. However, the potential involvement of PACAP in glucose homeostasis is not established, since the peptide has also been demonstrated to stimulate the secretion of glucagon (10, 13, 15, 29, 35) and catecholamines (6, 21) as well as to induce glycogenolysis (29, 36), the net effect being hyperglycemia. Also, both the stimulation of glucagon and catecholamines and the hyperglycemia induced through stimulation of glycogenolysis might indirectly influence insulin secretion and the glucose disposal rate. Therefore, the integrated influence of PACAP on the glucose homeostasis might be complex.

The aim of the present study was to examine in more detail the in vivo influence of PACAP on insulin secretion, insulin sensitivity, and glucose disposal after an intravenous glucose challenge in anesthetized mice. To that end, we exploited the minimal model of glucose disappearance for glucose and insulin data analysis after an intravenous glucose tolerance test (8, 14) with seven samples over 50 min in mice. This method allowed estimation of acute insulin secretion, total insulin secretion, insulin sensitivity (the ability of insulin to enhance glucose disappearance and to inhibit glucose production), glucose effectiveness (glucose disappearance per se at basal insulin without any change in insulin), and the glucose elimination rate. We also examined the influence of PACAP on glucagon secretion and on plasma levels of norepinephrine and epinephrine, and, finally, we compared the influences of PACAP-27 with those of PACAP-38.

METHODS

Animals. Nonfasted NMRI mice (Bomholdtgaard Breeding and Research Center, Ry, Denmark), weighing 20–25 g, were used throughout the study. The animals were fed a standard pellet diet and tap water ad libitum.

Intravenous glucose tolerance test. The mice were anesthetized with an intraperitoneal injection of midazolam (Dormicum, 0.4 mg/mouse; Hoffmann-La Roche, Basel, Switzerland) and a combination of fluanison (0.9 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm; Janssen, Beerse, Belgium). Thereafter, a blood sample was taken from the retrobulbar, intraborital, capillary plexus in heparinized tubes, whereafter b-glucose (1 g/kg; British Drug Houses, Poole, UK) was injected rapidly intravenously either alone or together with
synthetic ovine PACAP-27 or synthetic ovine PACAP-38 at various dose levels (both peptides from Peninsula Europe Laboratories, Merseyside, UK). In two other experimental series, human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was injected intravenously (0.25 U/kg) together with glucose, or PACAP-27 (1.3 nmol/kg) was injected intravenously under baseline conditions (i.e., without any concomitant glucose injection; controls were given saline). The volume load was 10 µl/g body wt. New blood samples were taken after 1, 5, 10, 20, 30, and 50 min. In the experiments on circulating glucagon and catecholamines, synthetic ovine PACAP-27 was injected intravenously at a dose level of 1.3 nmol/kg alone or together with glucose (1 g/kg), and blood was sampled immediately before and at 1, 5, 20, and 50 min after the injection. The samples for glucose and insulin were taken in heparinized tubes, the samples for glucagon were taken in chilled tubes containing aprotinin, and the samples for catecholamines were taken in chilled tubes containing heparin and EDTA. After immediate centrifugation at 4°C, plasma was separated and stored at −20°C or −80°C until analysis.

Analysis. Plasma insulin was determined radiimmunologically with the use of a guinea pig anti-rat insulin antibody, 125I-labeled porcine insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). Free and bound radioactivity was separated by use of an anti-immunoglobulin G (IgG) (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 7.5 pg/ml, and the coefficient of variation is <3% at both low and high levels. Plasma glucose was determined with the glucose oxidase method. Plasma glucagon was determined radiimmunologically with the use of a guinea pig antilucagon antibody specific for pancreatic glucagon, 125I-labeled glucagon as tracer, and glucagon standard (Linco). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 7.5 pg/ml, and the coefficient of variation is <9% at both low and high levels. Plasma catecholamines were determined by liquid chromatography in combination with electrochemical detection, as previously described (28).

Data analysis. Insulin and glucose data from the seven-sample intravenous glucose tolerance test were analyzed with the minimal model technique (9). The model assumes a first-order nonlinear insulin controlled kinetic and accounts for the effect of insulin and glucose itself on glucose disappearance after exogenous glucose injection. It provides the parameter $S_0$ (insulin sensitivity index), which is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (8), and the parameter $S_G$, which is the glucose effectiveness per se from plasma without any change in dynamic insulin (1). The parameter $S_G$ is composed of a glucose disposal action of basal insulin, the parameter $BIE$ (basal insulin effect), and the parameter $GEZI$ (glucose effectiveness at zero insulin), which is the glucose disposal without any influence of insulin (19). Acute insulin secretion (AIR) was calculated as the mean of suprabasal 1- and 5-min insulin levels, and the areas under the curve for insulin (AUC$_{insulin}$) and glucose (AUC$_{glucose}$) were assessed using the trapezoidal rule of suprabasal values. Finally, we also calculated a unitless index called GDI (global disposition index) by multiplying $S_I$ times AUC$_{insulin}$. The glucose elimination rate after the glucose injection ($K_G$) was calculated using the half-time ($t_{1/2}$) for minutes 1–20 after glucose injection after logarithmic transformation of the individual plasma glucose values. In the experiment on basal levels, AUC$_{insulin}$ the AUC for glucagon (AUC$_{glucagon}$), and AUC$_{glucose}$ were calculated using the trapezoidal rule of data with basal levels subtracted.
10 min (t_{1/2} 4.9 ± 0.5 min) and a second phase after the first 10 min (t_{1/2} 24.7 ± 2.7 min). Also, plasma glucose levels peaked at 1 min after the intravenous glucose injection (28.5 ± 0.7 vs. 9.6 ± 0.3 mmol/l at baseline, P < 0.001), whereas plasma glucose levels declined with an elimination half-life of suprabasal glucose of 9.7 ± 0.8 min for the first 10 min after glucose injection (Fig. 1). The half-life of circulating glucose correlated significantly and inversely to AIR \( (r = -0.30, P = 0.024) \) but not to the AUC_{insulin} \( (r = -0.06, \text{not significant (NS)}) \). However, AUC_{insulin} correlated significantly to the plasma glucose level at 50 min after glucose injection \( (r = -0.41, P < 0.001) \). The glucose elimination rate determined as the K_G value was 4.2 ± 0.1%/min. The K_G value correlated to AIR \( (r = 0.48, P < 0.001) \) but not to AUC_{insulin} \( (r = 0.001, \text{NS}) \). Figure 1 also shows that the single injection of saline did not significantly increase circulating insulin or glucose during the 50-min study period.

Effects of PACAP-27 and PACAP-38 on glucose-stimulated insulin secretion. When PACAP-27 or PACAP-38 was administered intravenously at various dose levels together with glucose at 1 g/kg, it was found that the two peptides equipotently and equiefficiently potentiated glucose-stimulated insulin secretion (Fig. 2). The half-maximal response for the two peptides was obtained at a dose level of ~33 pmol/kg. At the maximally effective dose for both peptides, 130 pmol/kg, insulin secretion, as determined by the AUC_{insulin}, was approximately twofold potentiated \( (P < 0.001) \).

Effects of PACAP-27 and PACAP-38 on glucose disappearance. Both PACAP-27 and PACAP-38 at 1.3 nmol/kg potentiated the glucose-induced increase in plasma insulin levels (Fig. 3). Thus plasma insulin levels in the 1-min sample were increased more than twofold by either of the peptides \( (P < 0.001) \). However, in spite of the marked increase in plasma insulin after administration of PACAP together with glucose, glucose elimination rate was not altered, as is evident from Fig. 3, bottom, showing an almost identical reduction in circulating glucose after the 1-min peak. This is also evident from the calculation of the K_G value, which was 4.2 ± 0.1%/min in controls vs. 3.9 ± 0.2%/min after glucose plus PACAP-27 and 4.3 ± 0.2%/min after glucose plus PACAP-38 (NS).

The data for circulating insulin and glucose were used for the estimation of the parameters of the minimal model. Table 1 shows the mean and SE for all control mice injected with glucose alone \( (n = 54) \). In the Kolmogorov-Smirnov goodness-of-fit test, baseline insulin, S_i, BIE, and GDI did not show a normal distribution \( (P < 0.05) \), whereas the other parameters did \( (P > 0.05) \). The accuracy of parameter estimation was evaluated with the coefficients of variation of the estimates, the values of which for the single groups of experiments are reported in Table 2. Table 3 shows the parameters estimated from the minimal model injected with PACAP-27 and PACAP-38. It is seen that AUC_{insulin} and
the increase in AUC insulin compensated each other.

Figure 3 shows elimination in mice, a separate experiment was under-
albeit marked, was insufficient to increase glucose
insulin after injection of PACAP-27 or PACAP-38,
and glucose
and PACAP-38 increased GEZI but inhibited BIE. The
however, in contrast to the failure of PACAP-27 or
PACAP-38 to augment glucose elimination, insulin
clearly increased glucose elimination. Thus the 5-min
plasma glucose values were 20.4 ± 0.4 mmol/l in the
absence of insulin injection vs. 17.3 ± 0.6 mmol/l in the
presence of insulin injection (P = 0.002), and the
corresponding 20-min values were 12.3 ± 0.4 vs. 7.1 ±
0.6 mmol/l (P < 0.001). Furthermore, insulin increased
the Kc value from 3.9 ± 0.2%/min in controls to 6.1 ±
0.2%/min (P = 0.030). The calculated half-life of circu-
lating insulin for the first 5 min after the insulin
injection was 2.5 ± 0.1 min, which is lower than that
previously reported in humans and pigs (25, 32).

Effects of PACAP-27 on glucagon levels. When
PACAP-27 was injected intravenously under baseline
conditions (1.3 nmol/kg), plasma glucagon levels were
increased concomitantly with an increase in insulin
and glucose levels (Fig. 5). Thus the AUC glucose,
during the 50 min was 981 ± 86 pg/ml in 50 min after injection
of PACAP-27 vs. 77 ± 8 pg/ml in 50 min after administra-
tion of saline (P < 0.001). Similarly, the AUC insulin
was 4.9 ± 0.5 nmol/l in 50 min after PACAP-27 vs.
−1.0 ± 0.2 nmol/l in 50 min in controls (P < 0.001), and
the AUC glucose was −13 ± 2 mmol/l in 50 min after
PACAP-27 vs. −79 ± 7 mmol/l in 50 min in controls
(P < 0.001). In contrast, when PACAP was injected
together with glucose, no significant change in gluca-
gon levels was evident when compared with animals
injected with glucose alone (Fig. 6).

Effects of PACAP-27 on catecholamine levels. When
PACAP-27 was injected intravenously (1.3 nmol/kg) in-
teraction with glucose, plasma epinephrine levels were
significantly elevated at both 1 min (P = 0.004) and 5
min (P = 0.013) after injection when compared with
animals injected with glucose alone (Fig. 7, top). The
change in plasma epinephrine at 1 min after injection
was 0.87 ± 0.34 ng/ml in animals injected with
PACAP-27 plus glucose vs. −0.39 ± 0.22 ng/ml in
animals injected with glucose alone (P = 0.039). In
contrast, plasma norepinephrine was not significantly
altered in any of the two groups of animals after the
injection (Fig. 7, bottom).

**DISCUSSION**

We have examined the net influences of PACAP-27
and PACAP-38 on insulin secretion, insulin sensitivity,
and glucose disposal in normal mice to study the
integrated action of the pancreatic insulinotropic neu-
ropeptide PACAP on the glucose homeostasis. The
net action of PACAP is of interest not only physiologi-
but also since it may be suggested that agents which
increase the formation of cAMP may be of potential
interest in the treatment of diabetes. For example,
glucon-like peptide 1, which raises cAMP, has been
considered as a new regimen in the treatment of type 2
diabetes (16). Our present results confirm previous
studies in dogs and humans that PACAP stimulates
insulin secretion, which also confirms several previous
studies in vitro verifying that PACAP exerts its action
directly on the insulin-producing cells (2, 3, 10, 13, 17,
21, 22, 34, 35). We also show that the insulinotropic

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SE</th>
<th>P Value for Test of Normality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline plasma glucose, mmol/l</td>
<td>9.3 ± 0.3</td>
<td>0.89</td>
</tr>
<tr>
<td>Baseline plasma insulin, pmol/l</td>
<td>438 ± 46</td>
<td>0.02*</td>
</tr>
<tr>
<td>AIR, nmol/l</td>
<td>1.4 ± 0.1</td>
<td>0.09</td>
</tr>
<tr>
<td>AUCinsulin, nmol/l in 50 min</td>
<td>398 ± 2.5</td>
<td>0.15</td>
</tr>
<tr>
<td>AUCglucose, nmol/l in 50 min</td>
<td>719 ± 20</td>
<td>0.80</td>
</tr>
<tr>
<td>SG, 10⁻⁴ min⁻¹/(pmol/l)</td>
<td>0.46 ± 0.02</td>
<td>0.05*</td>
</tr>
<tr>
<td>SGR, min⁻¹</td>
<td>0.042 ± 0.004</td>
<td>0.42</td>
</tr>
<tr>
<td>GDI</td>
<td>0.020 ± 0.005</td>
<td>0.78</td>
</tr>
<tr>
<td>GEZI, min⁻¹</td>
<td>0.022 ± 0.003</td>
<td>0.02*</td>
</tr>
<tr>
<td>BIE, min⁻¹</td>
<td>1.9 ± 0.2</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

Means ± SE and the P value for test of normality of the distribution
of the parameters according to the Kolmogorov-Smirnov goodness
of fit; n = 54 mice. Glucose was administered in a dose of 1 g/kg. AIR, acute
insulin response to glucose (1–5 min suprabasal circulating insulin); AUC, area under the curve for the entire 50-min period for insulin (AUCinsulin) and glucose (AUCglucose); S, insulin sensitivity index; SG, measure of glucose effectiveness. SGR consists of the parameters GEZI (glucose effectiveness at 0 insulin) and BIE (basal insulin effect). GDI is the unitless global disposition index, which is calculated by multiplying S, times AUCinsulin. *P < 0.05.

AIR were both significantly increased by PACAP-27
and PACAP-38, whereas AUCglucose was not altered
by either of the two peptides. Furthermore, S, was reduced
both by PACAP-27 and PACAP-38, whereas SG was not
altered significantly. However, the two components of
SG were both significantly altered, since both PACAP-27
and PACAP-38 increased GEZI but inhibited BIE. The
unitless GDI, finally, was not significantly affected by
PACAP-27 or PACAP-38, since the reduction in S, and the increase in AUCinsulin compensated each other.

Effects of intravenous insulin on circulating insulin
and glucose. To examine whether the increase in plasma
insulin after injection of PACAP-27 or PACAP-38,
albeit marked, was insufficient to increase glucose
elimination in mice, a separate experiment was under-
taken in which insulin was injected intravenously (0.25
U/kg) together with glucose (1 g/kg). Figure 4 shows that
this insulin injection increased plasma insulin to
4,783 ± 416 pmol/l, i.e., by the same degree as injection
of PACAP-27 or PACAP-38 at 1.3 nmol/kg (cf. Fig. 3).

<table>
<thead>
<tr>
<th>Experimental Series</th>
<th>Group</th>
<th>S,</th>
<th>SGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series with PACAP-27</td>
<td>Controls</td>
<td>10.4 ± 2.3</td>
<td>25.1 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>(n = 16)</td>
<td>(1.7–35.3)</td>
<td>(7.4–61.0)</td>
</tr>
<tr>
<td></td>
<td>PACAP-27</td>
<td>15.0 ± 5.1</td>
<td>30.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>(n = 16)</td>
<td>(2.2–67.0)</td>
<td>(16.1–41.2)</td>
</tr>
<tr>
<td>Series with PACAP-38</td>
<td>Controls</td>
<td>24.5 ± 4.3</td>
<td>24.3 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>(n = 24)</td>
<td>(2.2–107.0)</td>
<td>(0.6–82.6)</td>
</tr>
<tr>
<td></td>
<td>PACAP-38</td>
<td>17.7 ± 3.6</td>
<td>30.9 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>(n = 24)</td>
<td>(2.2–88.7)</td>
<td>(5.6–101.2)</td>
</tr>
</tbody>
</table>

Values are means ± SE in %, n, no. of mice. PACAP, pituitary
adrenaline cyclic-activating polypeptide. Nos. in parentheses are
minimum and maximum values.
Table 3. Baseline, nonfasted levels of glucose and insulin and parameters calculated from the seven-sample minimal model in mice intravenously injected with glucose (1 g/kg) either alone or together with PACAP-27 (1.3 nmol/kg) or PACAP-38 (1.3 nmol/kg)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental Series with PACAP-27</th>
<th></th>
<th>Experimental Series with PACAP-38</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 16)</td>
<td>P</td>
<td>PACAP-27 (n = 16)</td>
</tr>
<tr>
<td>Baseline plasma glucose, mmol/l</td>
<td>9.1 ± 0.7</td>
<td>NS</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td>Baseline plasma insulin, pmol/l</td>
<td>432 ± 38</td>
<td>0.001</td>
<td>469 ± 41</td>
</tr>
<tr>
<td>AIR, pmol/l</td>
<td>1.6 ± 0.2</td>
<td>0.004</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>AUC_insulin, nmol/l in 50 min</td>
<td>39.4 ± 3.1</td>
<td>0.029</td>
<td>70.3 ± 10.1</td>
</tr>
<tr>
<td>AUC_glucose, mmol/l in 50 min</td>
<td>681 ± 34</td>
<td>NS</td>
<td>682 ± 43</td>
</tr>
<tr>
<td>S1, 10^{-4} min^{-1}/pmol/l</td>
<td>0.48 ± 0.08</td>
<td>0.005</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>S2, min^{-1}</td>
<td>0.040 ± 0.006</td>
<td>NS</td>
<td>0.041 ± 0.005</td>
</tr>
<tr>
<td>G2ζ, min^{-1}</td>
<td>0.020 ± 0.008</td>
<td>0.046</td>
<td>0.029 ± 0.006</td>
</tr>
<tr>
<td>BIE, min^{-1}</td>
<td>0.020 ± 0.003</td>
<td>0.046</td>
<td>0.012 ± 0.002</td>
</tr>
<tr>
<td>GDI</td>
<td>1.7 ± 0.2</td>
<td>NS</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. P, probability level of random difference between mice injected with PACAP-27 or PACAP-38 and their respective controls as determined by Mann-Whitney U-test. NS, not significant.

The main new finding in this study is that, in spite of a marked insulinoetric action of PACAP, the glucose disposal was not augmented. This was evident by calculating the area under the curve for plasma glucose levels during the entire 50-min test as well as the glucose elimination rate for the 20 min after glucose administration and the t½ of glucose after glucose administration. We interpret this finding to be due to the marked (by ~50%) inhibition of insulin sensitivity induced by PACAP, as evident from the minimal model data of the insulin sensitivity index. The potentiated insulin secretion and the inhibited insulin sensitivity thus together compensated each other, the consequence of which was the unaltered glucose disposal. Consequently, also the global disposition index, which quantifies the capacity to compensate a change in insulin secretion or insulin sensitivity with a comparable in-
verse change in the other parameter, was not significantly altered by PACAP.

The reason for the marked reduction in insulin sensitivity by PACAP is at present not clear. Several hypothetical explanations might be offered, however. One hypothesis is that the rapid and marked increase in plasma insulin had compensatorily reduced insulin sensitivity and thereby prevented its own action to enhance glucose elimination. This in turn would suggest that the reduction in glucose levels during an intravenous glucose tolerance test is largely independent of insulin. Previous reports in rats have indeed indicated this to be the case. Thus at 3 h after prolonged hyperglycemia in rats, the glucose elimination after an intravenous glucose challenge has been found to be unaltered in spite of an exaggerated insulin response (23), and, furthermore, cholinergic antagonism by atropine has been found to markedly inhibit glucose-stimulated insulin secretion in rats preinfused for 48 h with glucose without altering the glucose disposal rate (7). To examine this intriguing possibility, we administered insulin intravenously at the dose of 0.25 U/kg together with glucose. This yielded a circulating insulin level of approximately the same degree as that seen after PACAP plus glucose. However, we found that insulin under this condition clearly augmented glucose elimination. This therefore shows that a rapid reduction in insulin sensitivity simply due to the rapidly increasing circulating levels of insulin is not the explanation for the failure of PACAP to augment glucose disposal. Another hypothesis, which more likely would explain the reduced insulin sensitivity by PACAP, is that other insulin-antagonistic actions counteracting the insulin effect have evolved after PACAP administration. Such insulin-antagonistic actions could be either PACAP itself, if PACAP directly reduces or counteracts the action of insulin. It could also, however, be due to other factors, the secretion of which would be stimu-
lated by PACAP and which in vivo could prevent the action of insulin. An antagonistic action of PACAP itself on glucose elimination would be supported by data that mice injected with PACAP had elevated glucose levels when compared with mice injected with saline (cf. Fig. 5) and by previous reports that PACAP stimulates the hepatic glucose delivery (29, 36). However, although this is a possibility under baseline conditions, it is unlikely to be a mechanism after a glucose challenge, since glucose inhibits liver glucose delivery (11). The other possible factors that could counteract the glucose reduction after PACAP administration are glucagon and catecholamines. Thus previous reports have demonstrated that PACAP stimulates glucagon secretion from the pancreas (10, 13, 15, 21, 29, 35) and epinephrine secretion from the adrenals (6, 21). To test these possibilities, we also determined plasma glucagon and catecholamines after administration of PACAP-27 to mice. We found that, although PACAP, as expected from results of previous studies (10, 13, 15, 21, 29, 35), increased baseline glucagon levels, the peptide did not increase plasma glucagon in the presence of elevated glucose levels, since plasma glucagon levels after the combined injection of PACAP-27 and glucose were not significantly different from those after injection of glucose alone. This is interpreted to indicate that glucose inhibited PACAP-induced glucagon secretion. Therefore, the mechanism of the impaired insulin sensitivity by PACAP under in vivo conditions in mice is probably not explained by glucagon. In contrast, plasma epinephrine levels, but not norepinephrine levels, were elevated after the combined injection of PACAP-27 and glucose, which might support a role for epinephrine. Although not directly examined in this study, such an action of epinephrine could be executed through activation of the β-adrenoceptors and subsequent formation of CAMP, which is a well-known mechanism whereby catecholamines induce insulin resistance (24). However, the mechanism of this action remains to be fully established.

To analyze the data, we have exploited the minimal model approach to experiments in mice. The frequently sampled intravenous glucose tolerance test with minimal model analysis was originally developed to quantitate glucose disposal in dogs and humans after a single intravenous glucose injection (8, 14). The method provides parameters characterizing insulin and glucose action on glucose homeostasis (8). This model has provided a useful and powerful tool for the understanding of glucose disposal under a variety of conditions, such as impaired glucose tolerance and type 2 diabetes, and has also been used in pharmacological studies to examine influences of exogenously administered substances, such as somatostatin and glucagon-like peptide 1 (5, 8, 9, 12, 14, 19). To the best of our knowledge, the minimal model has been used in small animals (rats) only a few times (14). No particular constraint should exist that restrains the use of this technique also in mice, being the only arguments about the length of the experiment and the limited number of samples (31). The model does not, however, seem to have problems related to the length of the study period, since, by the end of the experiments, both insulin and glucose have returned to preinjection levels, and thus the entire dynamics of the test are taken into account. In fact, the low dispersion of the estimates along with the coefficients of variation demonstrated a precise and accurate assessment of insulin secretion, insulin sensitivity, and glucose disposal in mice. This seven-sample technique therefore offers promises to be of importance in future physiological and pharmacological studies in mice, as well as in phenotypic characterization of animal models of interest for glucose homeostasis and diabetes, although this technique needs to be validated against other methods for determination of insulin secretion and insulin sensitivity in mice.

We have previously shown in unanesthetized mice that an intravenous injection of glucose elicits a marked and rapid increase in circulating insulin with a peak level within the first 2 min and a return to baseline levels within 6 min (4). The elevation of circulating insulin was more sustained in the anesthetized animals, which is interpreted as a lower degree of stress exhibited in these animals, reducing increase in catecholamines with their inhibiting action on insulin.
secretion. The seven-sample technique in anesthetized mice therefore does not seem to be accompanied by any significant degree of stress, which also is supported by our finding that there was no increase in basal glucose in the saline-injected controls.

Glucose elimination is not governed only by insulin-dependent actions. Thus insulin-independent mechanisms also contribute substantially to the glucose disposal after glucose administration, mainly by insulin-independent glucose uptake in the brain and in skeletal muscle and by suppression of liver glucose output during hyperglycemia (1, 11). The glucose effectiveness calculated by the minimal model quantifies both of these processes (11), and it has previously been found that glucose effectiveness accounts for \( \geq 20\% \) of the variance in glucose elimination in healthy subjects (20). This is the first time the minimal model is applied to mice; therefore, there is no previous history that allows comparisons to deeply evaluate the role of glucose effectiveness. Although it is generally agreed that the minimal model yields reliable measurements of insulin sensitivity, it is still the subject of controversy how to interpret glucose effectiveness in terms of true physiological implications and limitations (11). Nonetheless, there is a general consensus on its important role in the comprehension of the mechanisms involved in glucose disappearance. In the present study, we found that the glucose effectiveness was not significantly affected by PACAP. Therefore, the global glucose effectiveness is not compensatorily increased when insulin sensitivity is reduced by PACAP, which underlines earlier observations that insulin sensitivity and glucose effectiveness can change independently of each other (18). The global glucose effectiveness is composed of glucose effectiveness at basal insulin and at zero insulin. Interestingly, PACAP had significant effects on both of these parameters. Thus the peptide inhibited glucose effectiveness at basal insulin, which is a reflection of its inhibitory influence on insulin sensitivity. In contrast, PACAP increased glucose effectiveness at zero insulin. This latter effect is similar to glucagon-like peptide 1 in a previous study in humans (12). However, in contrast to PACAP, glucagon-like peptide 1 had no influence on glucose effectiveness at basal insulin and therefore increased global glucose effectiveness (12). The mechanism of an increase in glucose effectiveness at zero insulin by PACAP might reside in stimulation of glucose uptake in tissues like the skeletal muscle or inhibition of liver glucose production. The molecular mechanism of action by PACAP or the site of the action cannot be determined by the minimal model, however, and remains therefore to be established directly. In addition, it is worth pointing out that, despite being reported in many studies, the basal insulin effect and glucose effectiveness at zero insulin are still the subject of debate concerning their true physiological meaning. For this reason, our conclusions in this respect must be interpreted as study hypotheses, since it is not possible to date to draw unquestionable conclusions from the analysis of the components of glucose effectiveness.

Based on this integrative study on the development of a seven-sample technique in mice and the influence of PACAP on insulin secretion, insulin sensitivity, and glucose disposal, we conclude that 1) PACAP stimulates insulin secretion in a concentration-dependent manner, 2) the insulinotropic actions of PACAP-27 and PACAP-38 are equipotent, 3) PACAP inhibits insulin sensitivity without altering the glucose effectiveness, 4) PACAP stimulates glucagon secretion under basal conditions but not after administration of glucose, 5) PACAP-27 increases circulating levels of epinephrine but not of norepinephrine, 6) the net effect of PACAP during an intravenous glucose tolerance test is an unaltered glucose disposal in spite of a marked insulinotropic action, and 7) the minimal model of the seven-sample technique is a reliable model in anesthetized mice. The study therefore is consistent with and supports the view that PACAP is involved in the regulation of insulin secretion but that additional complex actions are exerted on the net glucose homeostasis. In contrast, PACAP does not seem to be a good reagent as a new reagent in the treatment of diabetes. However, B cell-specific PACAP receptor agonists, devoid of peripheral influences counteracting the action of insulin, might offer a good rational for such development, because PACAP seems to be an extraordinary potent insulinotropic peptide.

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