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Published in:
American Journal of Physiology - Endocrinology and Metabolism

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1998

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Filipsson, K., Pacini, G., Scheurink, AJW., & Ahren, B. (1998). PACAP stimulates insulin secretion but inhibits insulin sensitivity in mice. *American Journal of Physiology - Endocrinology and Metabolism*, 274(5), 834 - 842.

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G. Pacini, M. Ahren and B. Ahren

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A novel approach to assess insulin sensitivity reveals no increased insulin sensitivity in mice with a dominant-negative mutant hepatocyte nuclear factor-1{alpha}

B. Ahren and G. Pacini

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Diabetes, May 1, 2002; 51 (5): 1453-1460.

[Abstract] [Full Text] [PDF]

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R. N. Bergman, M. Ader, K. Huecking and G. Van Citters

Diabetes, February 1, 2002; 51 (90001): S212-220.

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PACAP stimulates insulin secretion but inhibits insulin sensitivity in mice

KARIN FILIPSSON,¹ GIOVANNI PACINI,² ANTON J. W. SCHEURINK,³ AND BO AHRÉN¹

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 Ahrén. 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 (AUC_{insulin}) was potentiated by 60% ($P < 0.001$). In contrast, PACAP-27 and PACAP-38 reduced the insulin sensitivity index (S_I) [$0.23 \pm 0.04 \cdot 10^{-4} \text{ min}^{-1}/(\text{pmol/l})$ for PACAP-27 and $0.29 \pm 0.06 \cdot 10^{-4} \text{ min}^{-1}/(\text{pmol/l})$ for PACAP-38 vs. $0.46 \pm 0.02 \cdot 10^{-4} \text{ min}^{-1}/(\text{pmol/l})$ 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 ($AUC_{\text{insulin}} \cdot S_I$) 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, intraorbital, capillary plexus in heparinized tubes, whereafter D-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 μ l/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 radioimmunochemically 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 12 pmol/l, 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 radioimmunochemically with the use of guinea pig antiglucagon antibodies 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_1 (insulin sensitivity index), which is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (8), and the parameter S_C , which is the glucose effectiveness, representing glucose disappearance per se from plasma without any change in dynamic insulin (1). The parameter S_C 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_1 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 trapezoid rule of data with basal levels subtracted.

Statistics. Means \pm SE are shown. Statistical analyses were performed with the SPSS for Windows system. Analysis of the normal distribution was performed with the Kolmogorov-Smirnov goodness-of-fit test. Statistical comparisons between groups were performed with the nonparametric Mann-Whitney *U*-test, since several of the parameters did not display normal distribution. Pearson's product moment correlation was used to estimate linear relationships between normally distributed variables. The coefficient of variation of estimated parameters was given by the percent ratio between the SD and the absolute values. The SD was obtained by the square root of the main diagonal of the covariance matrix calculated during the least square estimation procedure (30).

RESULTS

Circulating insulin and glucose after intravenous glucose or saline. In the control mice not given PACAP, the rapid intravenous injection of glucose (1 g/kg) raised plasma insulin levels from 438 ± 32 to $2,088 \pm 191$ pmol/l after 1 min ($n = 54$; $P < 0.001$; Fig. 1). Thereafter, plasma insulin levels rapidly returned toward baseline values. The plasma insulin level decline exhibited two phases, with a first phase during the first

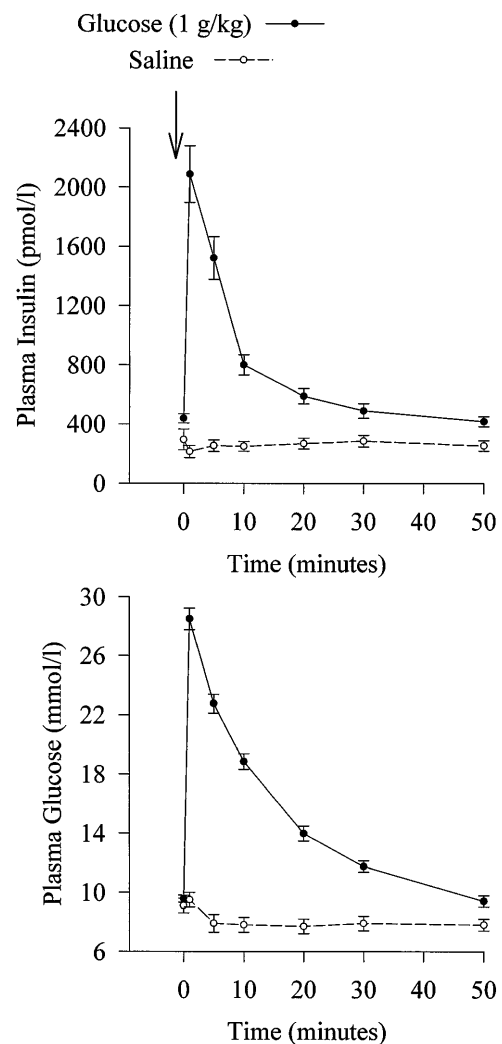


Fig. 1. Plasma insulin and glucose immediately before and at 1, 5, 10, 20, 30, and 50 min after iv injection of glucose (1 g/kg, $n = 54$) or saline ($n = 14$) in anesthetized mice. Means \pm SE are shown.

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, $P < 0.001$), whereafter 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} [$r = -0.06$, 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 $4.2 \pm 0.1\%/min$. The K_G value correlated to AIR ($r = 0.48$, $P < 0.001$) but not to AUC_{insulin} ($r = 0.001$, 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,

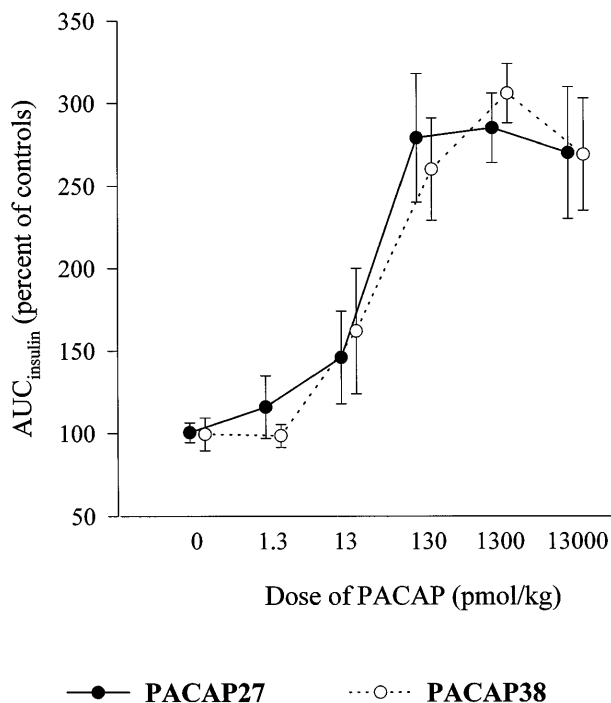
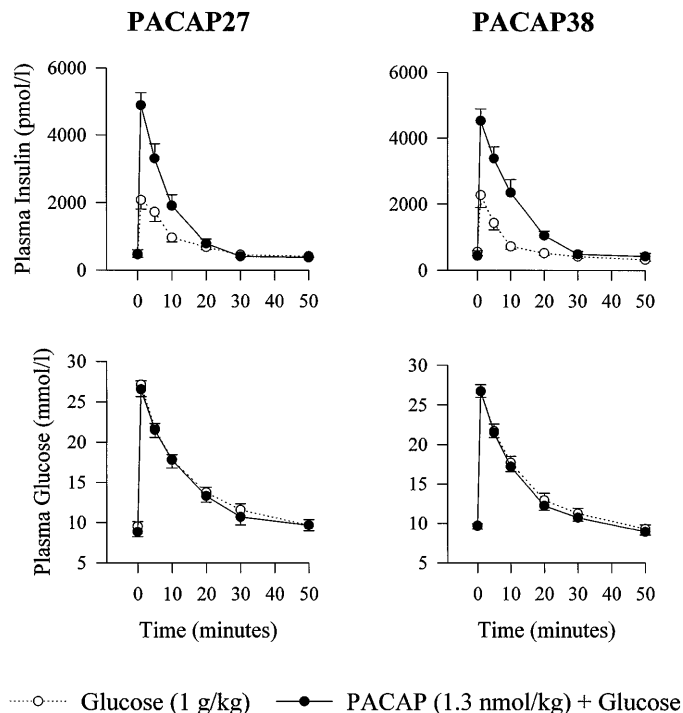


Fig. 2. Area under the curve for supra-basal plasma insulin levels during 50 min [on y-axis, shown as area under the curve for insulin (AUC_{insulin})] after iv administration of pituitary adenylate cyclase-activating polypeptide (PACAP)-27 or PACAP-38 at various dose levels together with glucose (1 g/kg) expressed as percent mean of the respective control animals run in the same experimental series. Groups without PACAP consisted of 24 animals each, group with PACAP-27 at 1.3 nmol/kg consisted of 16 animals, and group with PACAP-38 at 1.3 nmol/kg consisted of 24 animals, whereas the other groups consisted of 6–12 animals each. Means \pm SE are shown.



—○—○— Glucose (1 g/kg) —●—●— PACAP (1.3 nmol/kg) + Glucose

Fig. 3. Plasma insulin and glucose immediately before and at 1, 5, 10, 20, 30, and 50 min after iv injection of glucose (1 g/kg) alone or together with either PACAP-27 or PACAP-38 at 1.3 nmol/kg in anesthetized mice. Means \pm SE are shown. There were 16 mice in each of the two experimental groups with glucose \pm PACAP-27 and 24 mice in each of the two experimental groups with glucose \pm PACAP-38.

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 $4.2 \pm 0.1\%/min$ in controls vs. $3.9 \pm 0.2\%/min$ after glucose plus PACAP-27 and $4.3 \pm 0.2\%/min$ 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

Table 1. Baseline, nonfasted levels of glucose and insulin and parameters calculated from the seven-sample minimal model in control mice intravenously injected with glucose

Parameter	Means \pm SE	P Value for Test of Normality
Baseline plasma glucose, mmol/l	9.3 \pm 0.3	0.89
Baseline plasma insulin, pmol/l	438 \pm 46	0.02*
AIR, nmol/l	1.4 \pm 0.1	0.09
AUC _{insulin} , nmol/l in 50 min	39.8 \pm 2.5	0.15
AUC _{glucose} , mmol/l in 50 min	719 \pm 20	0.80
S _I , 10 ⁻⁴ min ⁻¹ /(pmol/l)	0.46 \pm 0.02	0.05*
S _G , min ⁻¹	0.042 \pm 0.004	0.42
GEZI, min ⁻¹	0.020 \pm 0.005	0.78
BIE, min ⁻¹	0.022 \pm 0.003	0.02*
GDI	1.9 \pm 0.2	0.01*

Means \pm 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 (AUC_{insulin}) and glucose (AUC_{glucose}); S_I, insulin sensitivity index; S_G, measure of glucose effectiveness. S_G 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_I times AUC_{insulin}. * $P < 0.05$.

AIR were both significantly increased by PACAP-27 and PACAP-38, whereas AUC_{glucose} was not altered by either of the two peptides. Furthermore, S_I was reduced both by PACAP-27 and PACAP-38, whereas S_G was not altered significantly. However, the two components of S_G 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_I and the increase in AUC_{insulin} 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 undertaken 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 \pm 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 2. Coefficients of variation of the estimated parameters, S_I and S_G, in the various sets of experiments

Experimental Series	Group	S _I	S _G
Series with PACAP-27	Controls ($n = 16$)	10.4 \pm 2.3 (1.7–35.3)	25.1 \pm 3.6 (7.4–61.0)
	PACAP-27 ($n = 16$)	15.0 \pm 5.1 (2.2–67.0)	30.8 \pm 1.7 (16.1–41.2)
Series with PACAP-38	Controls ($n = 24$)	24.5 \pm 4.3 (2.2–107.0)	24.3 \pm 3.6 (0.6–82.6)
	PACAP-38 ($n = 24$)	17.7 \pm 3.6 (2.2–88.7)	30.9 \pm 4.5 (5.6–101.2)

Values are means \pm SE in %; n , no. of mice. PACAP, pituitary adenylate cyclase-activating polypeptide. Nos. in parentheses are minimum and maximum values.

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 \pm 0.4 mmol/l in the absence of insulin injection vs. 17.3 \pm 0.6 mmol/l in the presence of insulin injection ($P = 0.002$), and the corresponding 20-min values were 12.3 \pm 0.4 vs. 7.1 \pm 0.6 mmol/l ($P < 0.001$). Furthermore, insulin increased the K_G value from 3.9 \pm 0.2%/min in controls to 6.1 \pm 0.2%/min ($P = 0.030$). The calculated half-life of circulating insulin for the first 5 min after the insulin injection was 2.5 \pm 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_{glucagon} during the 50 min was 981 \pm 86 pg/ml in 50 min after injection of PACAP-27 vs. 77 \pm 8 pg/ml in 50 min after administration of saline ($P < 0.001$). Similarly, the AUC_{insulin} was 4.9 \pm 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 glucagon 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) together 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 \pm 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 neuropeptide PACAP on the glucose homeostasis. The net action of PACAP is of interest not only physiologically 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, glucagon-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 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)

Parameter	Experimental Series with PACAP-27			Experimental Series with PACAP-38		
	Controls (n=16)	P	PACAP-27 (n=16)	Controls (n=24)	P	PACAP-38 (n=24)
Baseline plasma glucose, mmol/l	9.1 ± 0.7	NS	8.3 ± 0.7	9.6 ± 0.3	NS	9.8 ± 0.4
Baseline plasma insulin, pmol/l	432 ± 38	NS	469 ± 41	441 ± 92	NS	486 ± 134
AIR, pmol/l	1.6 ± 0.2	0.001	3.8 ± 0.4	1.3 ± 0.2	<0.001	3.3 ± 0.3
AUC _{insulin} , nmol/l in 50 min	39.4 ± 3.1	0.029	70.3 ± 10.1	40.0 ± 4.6	<0.001	64.6 ± 5.9
AUC _{glucose} , mmol/l in 50 min	681 ± 34	NS	682 ± 43	744 ± 38	NS	672 ± 33
S _I , 10 ⁻⁴ min ⁻¹ /(pmol/l)	0.48 ± 0.08	0.005	0.23 ± 0.04	0.68 ± 0.09	<0.001	0.29 ± 0.06
S _C , min ⁻¹	0.040 ± 0.006	NS	0.041 ± 0.005	0.043 ± 0.006	NS	0.057 ± 0.008
GEZI, min ⁻¹	0.020 ± 0.008	0.046	0.029 ± 0.006	0.019 ± 0.008	0.027	0.046 ± 0.008
BIE, min ⁻¹	0.020 ± 0.003	0.046	0.012 ± 0.002	0.024 ± 0.006	0.020	0.011 ± 0.003
GDI	1.7 ± 0.2	NS	1.2 ± 0.1	2.1 ± 0.2	NS	1.6 ± 0.3

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.

action of PACAP-27 and PACAP-38 is equipotent and equiefficient, which confirms previous results in other models (2, 21, 34, 35).

In our previous study on the influence of PACAP-38 on insulin secretion in unanesthetized mice, PACAP-38 did not increase plasma insulin, either under baseline conditions or in conjunction with glucose, and in combination with the cholinergic agonist carbachol, PACAP-38 actually inhibited insulin secretion (15). In contrast, in the present study, PACAP potently increased plasma insulin levels both under basal conditions and in the presence of glucose. Although a potentially diminished insulin elimination by PACAP has not been studied, such an effect is unlikely to explain the rapid and marked increase in plasma insulin after PACAP administration, since the magnitude of the increase far exceeds what is possible, even with a complete inhibition of insulin elimination. Therefore, a stimulated insulin secretion most likely explains the marked increase in plasma insulin executed by PACAP. The mechanism of this potent insulinotropic action of PACAP cannot be established from the results of this *in vivo* study. It could theoretically be due to indirect actions of PACAP through the liberation of other insulinotropic agents by the neuropeptide. It could also be due to a

compensatory response to a primary reduction in insulin sensitivity by PACAP. However, the most likely mechanism, considering the potent insulinotropic action of PACAP on isolated islets and insulin-producing tumor cells (2, 3, 10, 17, 22, 34, 35), is a direct action on the islets. Furthermore, PACAP-38 also increased circulating glucagon, which confirms our previous study in unanesthetized mice (15). This is most likely executed through stimulation of glucagon secretion from the pancreatic islets. In our previous study in unanesthetized mice, glucose could not inhibit the glucagon response to PACAP-38 (15), which is different from the abolishment by glucose of PACAP-induced glucagon secretion in the present study in anesthetized mice. It is likely that stress-related phenomena in unanesthetized mice also account for this difference. Hence, the present model in anesthetized animals seems to more reliably show the direct action of PACAP on islet function, when stress-related actions are circumvented. It should also be emphasized that our present result of potentiated glucose-stimulated insulin secretion and increased baseline glucagon secretion in response to PACAP in mice is identical to the results of our recent report on the action of PACAP-27 in humans (13).

The main new finding in this study is that, in spite of a marked insulinotropic 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 *t*_{1/2} 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-

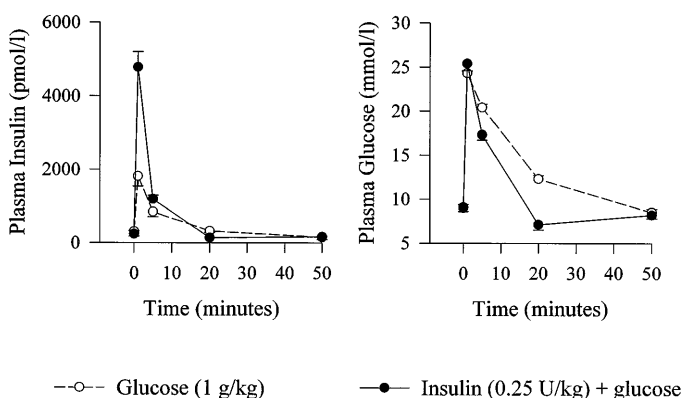


Fig. 4. Plasma insulin and glucose immediately before and at 1, 5, 20, and 50 min after iv injection of glucose (1 g/kg) alone or together with human insulin (0.25 U/kg) in anesthetized mice. Means ± SE are shown. There were 6 mice in each group.

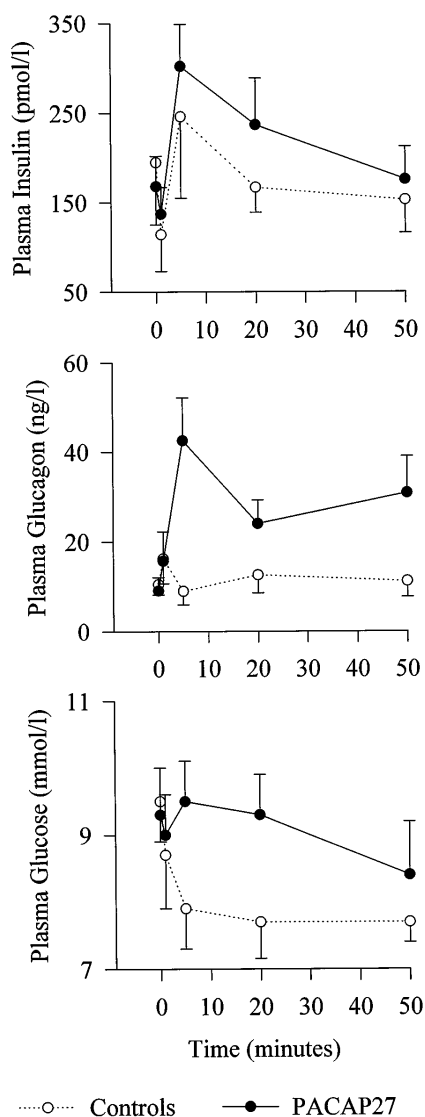


Fig. 5. Plasma insulin, glucagon, and glucose immediately before and at 1, 5, 20, and 50 min after iv injection of saline or PACAP-27 (1.3 nmol/kg) in anesthetized mice. Means \pm SE are shown. There were 8 mice in each group.

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-

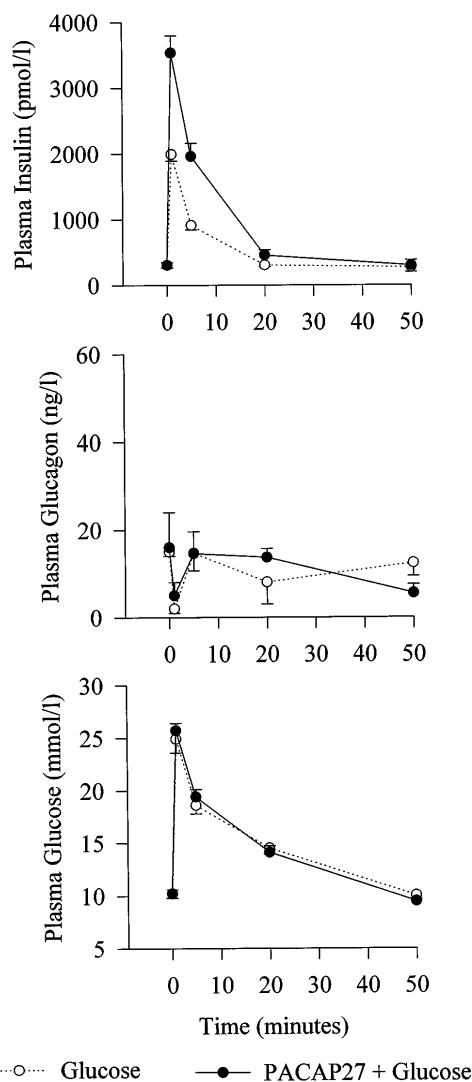


Fig. 6. Plasma insulin, glucagon, and glucose immediately before and at 1, 5, 20, and 50 min after iv injection of glucose (1 g/kg) without or with addition of PACAP-27 (1.3 nmol/kg) in anesthetized mice. Means \pm SE are shown. There were 6 mice in each group.

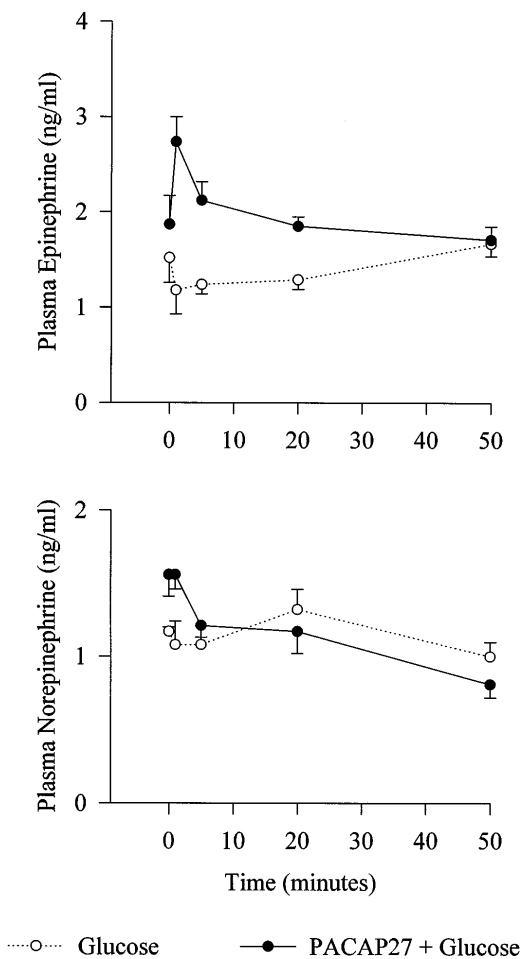


Fig. 7. Plasma epinephrine and norepinephrine immediately before and at 1, 5, 20, and 50 min after iv injection of glucose (1 g/kg) with addition of PACAP-27 (1.3 nmol/kg) in anesthetized mice. Means \pm SE are shown. There were 5 mice in each group.

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 quantify 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 >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 target 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 rationale for such development, because PACAP seems to be an extraordinary potent insulinotropic peptide.

We are grateful to Lena Kvist for active participation in the execution of the experiments and to Lilian Bengtsson and Ulrika Gustavsson for expert technical assistance in the determinations of insulin and glucose.

The study was supported by the Swedish Medical Research Council (Grant 14X-6834), Ernhold Lundström, Albert Pålsson, and Novo Nordisk Foundations, Swedish Diabetes Association, Malmö University Hospital, and the Faculty of Medicine, Lund University.

Address for reprint requests: B. Åhrén, Dept. of Medicine, Malmö Univ. Hospital, S-205 02 Malmö, Sweden.

Received 14 October 1997; accepted in final form 20 January 1998.

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