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Pituitary Adenylate Cyclase Activating Polypeptide Enhances Glucose-Evoked Insulin Secretion in the Canine Pancreas *In Vivo*

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ABSTRACT

Objective To study a local effect of pituitary adenylate cyclase activating polypeptide ($PACAP_{1-27}$) on glucose-evoked insulin release under *in vivo* conditions.
Intervention Glucose and $PACAP_{1-27}$ were locally infused to the pancreas via the superior pancreaticoduodenal artery without interrupting the blood supply.
Main outcome measures Plasma insulin and glucose concentrations were determined in samples obtained from the superior pancreaticoduodenal vein and the aorta. Superior pancreaticoduodenal venous blood flow was measured to compute the net output of insulin.
Results $PACAP_{1-27}$ (0.005-5 μg) increased the basal insulin secretion by about 15 folds in a dose-dependent manner. Local infusion of either glucose (5%) or $PACAP_{1-27}$ (0.05 μg) resulted in a significant increase in the basal insulin output to about 300 $\mu U \cdot min^{-1}g^{-1}$, which was highly reproducible upon the second administration of the same dose with an interval of 30 min. When $PACAP_{1-27}$ was simultaneously given during glucose infusion, the increased insulin output due to glucose was further enhanced to about 600 $\mu U \cdot min^{-1}g^{-1}$. The net increase in $PACAP_{1-27}$-induced insulin output in the presence of glucose was significantly greater than that obtained with $PACAP_{1-27}$ alone. There exists a strong and highly significant correlation between changes in glucose level and those in insulin output when both glucose and $PACAP_{1-27}$ were administered simultaneously.
Conclusion The results indicate that $PACAP_{1-27}$ directly enhances the glucose-evoked insulin secretion in the endocrine pancreas in anesthetized dogs. The study suggests that PACAP may play a local facilitating role in insulin secretion in response to glucose loading.

INTRODUCTION

In addition to the classic sympathetic and parasympathetic innervation, the mammalian pancreas receives a complex peptidergic neural supply and has distinct innervation sites depending on different neuropeptides [1]. A number of neuropeptides, including pituitary adenylate cyclase activating polypeptide (PACAP), have been postulated as putative neurotransmitters or cotransmitters playing a role of controlling the endocrine pancreatic functions [2].
PACAP is a neuropeptide with 38 amino acid residues ($PACAP_{1-38}$) and the N-terminal amidated 27-residues ($PACAP_{1-27}$), originally isolated from the ovine hypothalamus [3]. Both $PACAP_{1-38}$ and $PACAP_{1-27}$ show high affinity for PACAP specific (PAC_1) receptor in membranes from various tissues including the endocrine pancreas [4, 5]. $PACAP_{1-27}$ stimulates the secretion of both insulin and glucagon from the isolated, perfused rat pancreas [6]. It has been postulated that PACAP is a novel pancreatic peptide of neural and islet origin and may play a role of intrinsic stimulator of glucose-induced insulin secretion in the isolated rat islets [2]. These observations suggest that endogenous PACAP

may serve as a neurotransmitter or modulator controlling either directly or indirectly the secretion of insulin in response to glucose [7]. However, most of these observations have been derived from *in vitro* studies in the isolated, perfused pancreas or the perifused dispersed islet cells, leaving a question whether PACAP actually modulates insulin release induced by more physiological means *in vivo*. Information obtained from *in vivo* studies may provide further insight into human pancreatic pathophysiology. The present study was therefore aimed to investigate whether exogenous $PACAP_{1-27}$ can modulate insulin secretion induced by local glucose loading in the pancreas in anesthetized dogs.

METHODS

Preparation of Animals

Adult mongrel dogs, fasted overnight but allowed free access to water, were anesthetized with pentobarbital sodium (30 mg/kg i.v., followed by 4 mg/kg as needed). Artificial respiration (room air) was maintained through an endotracheal tube connected to a Harvard pump (model 607, Harvard, South Natick, MA, USA). The rectal temperature of each dog was monitored and kept constant at 37.5±0.5 °C by means of a thermoregulator (model 74; Yellow Spring Instruments, Yellow Spring, OH, USA) connected to a heating pad. Both femoral arteries were cannulated; the right femoral artery was used to measure aortic pressure, and the left femoral artery was used to obtain aortic blood samples.

Preparation of Pancreas for Local Intra-Arterial Drug Administration and Extracorporeal Venous Circuit

Following a median laparotomy, the superior pancreaticoduodenal (SPD) artery was dissected free from the surrounding tissues. Fine polyethylene tubing (PE-50) was inserted into the SPD artery through an adjacent small branch so that the SPD arterial blood flow remained unobstructed. Local drug administrations to the pancreas were made through this catheter. The volume of this catheter was fixed to be 0.5 mL and 0.25 mL in the first and second series of experiments, respectively, and the catheter was connected to an infusion pump (model 55-2226, Harvard, South Natick, MA, USA).
Soft and flexible silicon tubing (3 mm i.d.) was inserted in a retrograde manner into the SPD vein, so that a major part of venous blood draining the pancreas can be obtained [8, 9]. Venous blood from the pancreas was directly drained passing through an electromagnetic flow probe into a small reservoir through this catheter, from which pancreatic venous blood was sampled. Venous blood volume in the reservoir was kept as small as possible with an automatic blood level controller, and continuously returned to the dog by a perfusion pump (Masterflex model 7016-52; Cole-Parmer Instrument, Chicago, IL, USA) through a silicon tubing (3 mm i.d.) inserted into the portal vein [9]. After all surgical procedures were completed, sodium heparin (200 U/kg i.v.) was administered, followed by 100 U/kg every hour thereafter. The dog was then allowed a stabilization period of about 60 min. Blood volume withdrawn for sample collections was replaced with intravenous injection of the same volume of saline (0.9%) in addition to its continuous infusion at a slow rate during the whole period of the surgery and the experiment.

Measured Parameters

Pancreatic venous blood flow, mean aortic pressure and heart rate were recorded by means of a polygraph system (model RM-6000, Nihon-Kohden, Tokyo, Japan). The SPD venous blood flow was measured with an electro-magnetic flow probe (3 mm, i.d., model FF-030T, Nihon-Kohden, Tokyo, Japan) connected to the SPD venous catheter [9]. SPD venous and aortic blood (1.5 mL of each) was simultaneously sampled into two separate chilled tubes. Plasma concentrations of glucose and immunoreactive insulin were

determined by means of a glucometer (model 2300 STAT Plus-D, Yellow Springs Instruments, Yellow Spring, OH, USA) and a radioimmunoassay kit (IRI 07-260102, ICN Pharmaceuticals, Costa Mesa, CA, USA), respectively. Blood was immediately centrifuged at 4 °C for 5 min at 14,000 rpm with a refrigerated centrifuge (model 5402, Eppendorf, Hamburg, Germany). An aliquot (20 µL) of plasma was used for glucose determination, and the remaining plasma was stored at -80 °C until insulin was assayed. Hematocrit was measured in all pancreatic venous blood samples. At the end of each experiment, the pancreas was removed and weighed.

For evaluating insulin secretion, the net output of insulin from the pancreas was calculated as follows.

Net output of insulin ($\mu U \cdot min^{-1} \cdot g^{-1}$ of pancreas) = ($[INS]_{SPDV}$ - $[INS]_{AO}$) H $[BF]_{SPDV}$ H (1 - $[Hct]_{SPDV}$) / wet weight of pancreas,

where $[INS]_{SPDV}$ and $[INS]_{AO}$ is plasma immunoreactive insulin concentration in superior pancreaticoduodenal venous and aortic blood, respectively. $[BF]_{SPDV}$ and $[Hct]_{SPDV}$ is superior pancreaticoduodenal venous blood flow and its hematocrit, respectively.

Experimental Protocols

The present study consisted of two series of experiments. The first series involved four dogs (28.4±1.8 kg) and served to observe dose-related insulin response to $PACAP_{1-27}$, and, thereby, to determine the dose used for the second series of experiments. This group received $PACAP_{1-27}$ (Sigma Chemical, St. Louis, MO, U.S.A.) with four different concentrations of 0.01, 0.1, 1.0 and 10 $\mu g \cdot mL^{-1}$, or 0.00318, 0.0318, 0.318 and 3.18 µM, respectively. Each solution was locally infused at a rate of 0.5 $mL \cdot min^{-1}$ for precisely 1 min. A total dose delivered to the pancreas during each infusion was therefore 0.005, 0.05, 0.5 and 5 µg. The dead volume of the pancreatic arterial catheter (0.5 mL) was taken into account in relation to the infusion rate. After taking the initial control sample, simultaneously from the SPD vein and the aorta, saline was infused for 1 min and samples were obtained 1, 3 and 5 min after the onset of infusion. This procedure was repeated every 15 min for the doses of $PACAP_{1-27}$. The sample obtained at 15 min after the onset of each infusion served as control for the subsequent intervention.

The second series consisted of four groups. The first group (37.6±3.7 kg, n=7) served as control receiving only a vehicle (saline 0.9%, pH 7.38) to ensure the stability of the basal insulin secretion during a given experimental period of about 70 min. In this group, the two blocs containing identical procedures with local administrations of the vehicle to the pancreas were repeated with an interval of 30 min. The sequence of vehicle (VH) infusions could be shown as “VHVH - VHVH”.

In the second group (31.1±2.5 kg, n=7), the reproducibility of insulin responses to glucose was tested with an interval of 30 min. Following the initial control sample, the isotonic glucose solution (5%) was locally infused to the pancreas at a rate of 0.25 $mL \cdot min^{-1}$ for 4 min. At the third minute, immediately after taking the second control sample, the vehicle was added to the glucose infusion line at the same infusion rate. Thus, glucose and the vehicle were simultaneously conveyed to the pancreas. A sample was obtained during this simultaneous infusion period, followed by sample collections 1, 2, 4, 9, 14 and 29 min after the secession of the infusions. The sample obtained at 29 min served as control for the second bloc. The glucose and vehicle administrations as well as the sequence of sample collections in the second bloc followed exactly the same procedures described for the first bloc. The sequence of glucose (GL) and vehicle (VH) administrations could be indicated as “GLVH - GLVH”.

The third group (25.0±1.6 kg, n=7) served to ensure the reproducibility of insulin responses to $PACAP_{1-27}$ with an interval of 30 min. In the first bloc, the vehicle was administered to the pancreas at a rate of 0.25 $mL \cdot min^{-1}$ for 4 min. At the third minute, $PACAP_{1-27}$ (0.05 µg with 0.2 $\mu g \cdot mL^{-1}$ or 0.0636 µM) was added to

the vehicle infusion line at the same rate. The vehicle and $PACAP_{1-27}$ were thus simultaneously given to the pancreas during the last minute of infusion. The same procedures were repeated in the second bloc. The timing of sample collections was exactly the same as that for the first and second groups. The sequence of the vehicle and $PACAP_{1-27}$ infusion could be described as "VHPACAP - VHPACAP".

In the fourth group (27.7±2.3 kg, n=7), the effect of $PACAP_{1-27}$ on insulin secretion was compared in the absence and presence of glucose with an interval of 30 min. In the first bloc, the procedures for the infusion of vehicle and $PACAP_{1-27}$ (0.05 μg) as well as the sampling sequence were exactly the same as those described above. In the second bloc, glucose was infused to the pancreas as described for the second group. At the third minute, $PACAP_{1-27}$ (0.05 μg) was added to the glucose infusion line following the same protocol described for the third group. Both glucose and $PACAP_{1-27}$ were thus simultaneously delivered to the pancreas during the last minute of the infusion period in the second bloc. The sequence of drug administrations could be indicated as "VHPACAP - GLPACAP".

ETHICS

The local animal research committee at the Université de Montréal has approved the experimental protocol. The animals used in this study have been cared for and used in accordance with the principles of the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care.

STATISTICS

The statistical evaluations were carried out using a statistical software package (SigmaStat for Windows, Version 2.03, SPSS, Chicago, IL, USA). Differences over a given experimental period within the same subjects were assessed by the analysis of variance for repeated measures followed by multiple comparisons with the Student-Newman-Keuls test. The net insulin responses to $PACAP_{1-27}$ in the absence and the presence of glucose in the same subjects were analyzed using the paired t-test. When applicable, a preliminary logarithmic transformation was used to satisfy the condition of a normal distribution of variance [10]. All results are expressed as means±SE, and P values less than 0.05 were considered statistically significant.

Table 1. Initial values for plasma concentrations of glucose and insulin in pancreatic venous and aortic blood, pancreatic venous blood flow and hematocrit, mean aortic pressure, heart rate, and postmortem wet weight of pancreas in anesthetized dogs.

Parameters	Series 1	Series 2			
		Group 1	Group 2	Group 3	Group 4
	(n=4)	(n=7)	(n=7)	(n=7)	(n=7)
$[GL]_{SPDV}$ (mg/dL)	n.a.	120.6±1.9	122.3±6.9	115.8±4.3	131.0±10.8
$[GL]_{AO}$ (mg/dL)	n.a.	119.9±3.1	127.5±7.6	112.8±2.4	129.7±10.5
$[INS]_{SPDV}$ (μU/mL)	112.8±26.8	196.2±26.6	250.2±27.0	275.3±99.7	250.5±70.2
$[INS]_{AO}$ (μU/mL)	7.5±2.7	16.5±3.5	23.7±2.0	25.3±11.9	21.6±5.4
$[BF]_{SPDV}$ (mL/min)	33.0±3.7	52.0±4.6	54.7±6.0	50.1±5.7	55.9±2.9
$[Hct]_{SPDV}$ (%)	43.6±1.7	48.5±2.5	46.9±1.3	46.5±1.5	45.1±2.7
MAP (mmHg)	125.5±7.6	137.4±3.5	133.9±6.4	129.2±5.0	126.5±6.1
HR (beats/min)	132.0±4.7	165.9±5.9	160.7±7.0	144.4±7.5	142.6±7.6
PCR (g)	58.5±3.7	71.5±4.2	56.5±4.3	50.3±4.3	57.6±2.9

Values are means±SE.

AO: aortic; BF: blood flow; GL: glucose; Hct: hematocrit; HR: heart rate; INS: insulin; MAP: mean aortic pressure; n: number of dogs tested; n.a.: data not available; PCR: wet weight of postmortem pancreas; SPDV: superior pancreaticoduodenal venous.

RESULTS

Basal Values for the Measured Parameters

Initial resting values for plasma concentrations of glucose and insulin in SPD venous and aortic blood as well as the basal data for SPD venous blood flow and hematocrit, mean aortic pressure, heart rate and postmortem wet weight of the pancreas are summarized in Table 1. These initial values were not significantly different between groups. Plasma concentrations of glucose in aortic blood, mean aortic pressure, heart rate and hematocrit remained relatively stable during a given period of experiment, and observed variations in those parameters within the same subjects were not statistically significant.

Insulin Responses to Various Doses of $PACAP_{1-27}$

The local administrations of $PACAP_{1-27}$ to the pancreas resulted in significant increases in both SPD venous insulin concentration and blood flow as well as pancreatic insulin output following a dose-dependent manner (Figure 1). Within the dose range tested, the first statistically significant increase in both insulin concentration and output was obtained with the dose of 0.05 µg. This dose of $PACAP_{1-27}$ was, therefore, selected for the subsequent series of experiments. At all doses tested, the maximum response of both insulin concentration and output were observed 1 min after the onset of the net infusion of $PACAP_{1-27}$ and returned to the corresponding pre-infusion control level by 3 min (Figure 1). Plasma concentration of insulin in aortic blood and the other measured parameters did not change significantly at any dose tested.

Effect of Glucose on the Basal Secretion of Insulin

In the control group receiving the vehicle alone (VHVH - VHVH), plasma concentrations of insulin and glucose in SPD venous and aortic blood as well as insulin output did not change significantly and remained relatively stable during the whole period of experiment. The other measured parameters also remained stable and did not change significantly during the experiment.

In the group receiving glucose followed by the vehicle (GLVH - GLVH), the local administration of the isotonic glucose solution into the SPD artery resulted in an immediate and significant increase in glucose concentration in SPD venous blood (Figure 2A) and in the output of insulin (Figure 2B). Both responses were highly reproducible upon repetition of glucose infusion with an interval of 30 min (Figures 2A and 2B), and returned to their corresponding pre-infusion control levels by 5 min after the secession of glucose administration. SPD venous blood flow remained unchanged, while insulin concentration in aortic blood increased slightly.

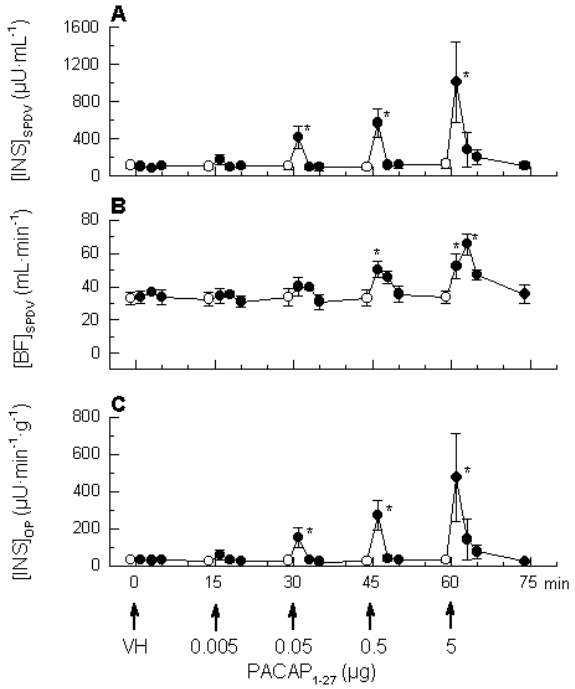

Figure 1. Effect of various doses of $PACAP_{1-27}$ infused into the superior pancreaticoduodenal (SPD) artery on A) plasma insulin concentration in SPD venous blood ($[INS]_{SPDV}$), B) SPD venous blood flow ($[BF]_{SPDV}$), and C) insulin output ($[INS]_{OP}$). Arrows indicate the time of administration of either the vehicle (VH, saline) or different doses of $PACAP_{1-27}$. Open circles represent control values observed immediately before the administration of the vehicle or $PACAP_{1-27}$.
* $P \leq 0.036$ *vs.* corresponding control value (n=4).

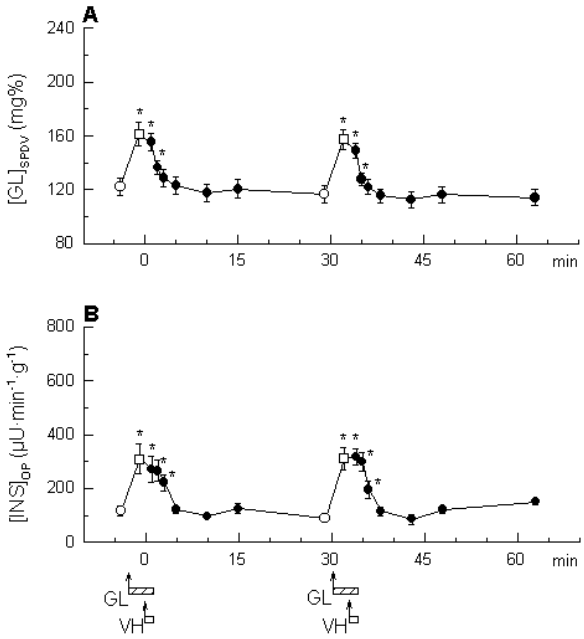

Figure 2. Effect of glucose infused with the isotonic solution (5%) into the superior pancreaticoduodenal (SPD) artery on A) plasma glucose concentration in SPD venous blood ($[GL]_{SPDV}$) and B) insulin output ($[INS]_{OP}$). Arrows indicate the onset of administration of either glucose (GL) or the vehicle (VH, saline). Horizontal bars attached to the arrows indicate the infusion period of glucose (bars filled with up-right diagonal lines) and vehicle (open bars). Open circles represent control values observed immediately before the administration of glucose, and open squares indicate those before the administration of the vehicle.
* P≤0.016 *vs.* corresponding controls taken before the administration of glucose (n=7).

Effect of $PACAP_{1-27}$ on the Basal Secretion of Insulin

In the group receiving the vehicle followed by 0.05 μg of $PACAP_{1-27}$ (VHPACAP - VHPACAP), insulin output increased significantly during the infusion of $PACAP_{1-27}$ (Figure 3B), while the basal glucose level in SPD venous blood remained unchanged (Figure 3A). The insulin response to $PACAP_{1-27}$ returned to its pre-infusion control level by 2 min after discontinuing the infusion. The increase in insulin output was highly reproducible in response to the second infusion of $PACAP_{1-27}$ with an interval of 30 min (Figure 3B). Thus, the net insulin response to $PACAP_{1-27}$ remained unchanged under basal conditions. SPD venous insulin concentration significantly increased in a similar way as observed in the insulin output, while aortic insulin concentration changed variably. SPD venous blood flow slightly increased during the infusion of $PACAP_{1-27}$.

Effect of $PACAP_{1-27}$ on the Glucose-Evoked Insulin Secretion

In the group receiving the vehicle followed by 0.05 μg of $PACAP_{1-27}$ and, 30 min later, the isotonic glucose solution followed by the same dose of $PACAP_{1-27}$ (VHPACAP - GLPACAP), the local administration of $PACAP_{1-27}$ in the absence of the glucose infusion significantly increased the basal insulin output (Figure 4B). The basal glucose concentration in SPD venous blood remained unchanged (Figure 4A). Thirty minutes later, glucose was locally infused resulting in significant increases in the basal SPD venous glucose concentration and the basal insulin

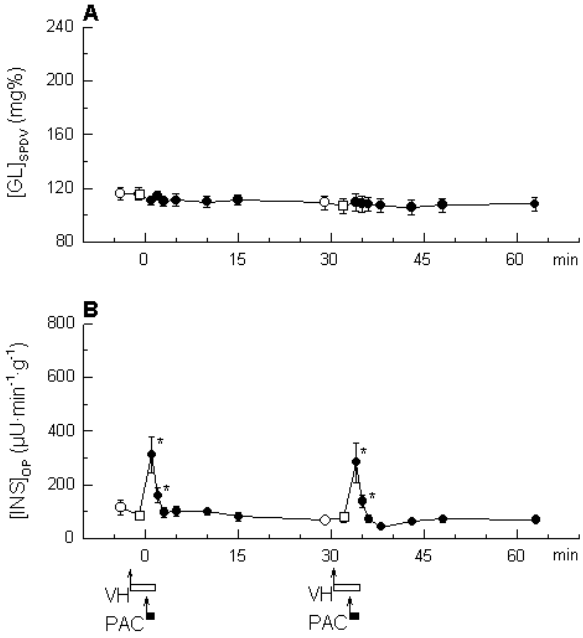

Figure 3. Effect of $PACAP_{1-27}$ infused into the superior pancreaticoduodenal (SPD) artery on A) plasma glucose concentration in SPD venous blood ($[GL]_{SPDV}$) and B) insulin output ($[INS]_{OP}$). Arrows indicate the onset of administration of either the vehicle (VH, saline) or $PACAP_{1-27}$ (PAC, 0.05 μg). Horizontal open bars and filled bars attached to the arrows indicate the infusion period of vehicle and $PACAP_{1-27}$, respectively. Open circles represent control values observed immediately before the administration of the vehicle, and open squares indicate those before the administration of $PACAP_{1-27}$.
* P≤0.044 *vs.* corresponding controls taken before the administration of the vehicle (n=7).

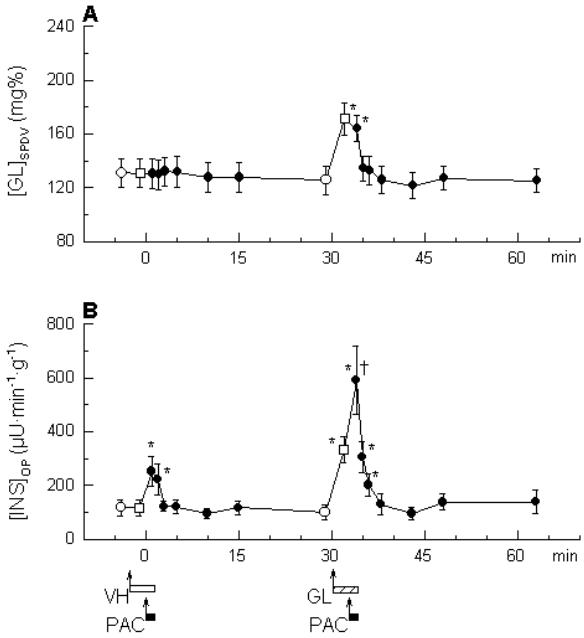

Figure 4. Effect of $PACAP_{1-27}$ infused into the superior pancreaticoduodenal (SPD) artery in the absence (first bloc) and the presence of glucose infusion (second bloc) on A) plasma glucose concentration in SPD venous blood ($[GL]_{SPDV}$) and B) insulin output ($[INS]_{OP}$). Arrows indicate the onset of administration either of the vehicle (VH, saline), glucose (GL, with 5% solution) or $PACAP_{1-27}$ (PAC, 0.05 μg). Horizontal bars attached to the arrows indicate the infusion period of vehicle (an open bar), glucose (a bar filled with up-right diagonal lines) and $PACAP_{1-27}$ (filled bars). Open circles represent control values observed immediately before the administration of the vehicle or glucose, and open squares indicate those before the administration of $PACAP_{1-27}$.
* P≤0.038 *vs.* corresponding controls taken before the administration of the vehicle or glucose (n=7).
† P=0.002 *vs.* the peak insulin response to $PACAP_{1-27}$ administered at minute 0 (n=7).

output (Figures 4A and 4B). This glucose-evoked increase in the basal insulin output was further enhanced by the addition of $PACAP_{1-27}$ (Figure 4B). At this point, the SPD venous glucose concentration remained elevated as the glucose infusion continued (Figure 4A). The net increase in insulin output induced by $PACAP_{1-27}$ during the glucose infusion was significantly greater than that observed in the absence of glucose (Figure 5). After discontinuing the infusion of both glucose and $PACAP_{1-27}$, the increased SPD venous glucose concentration and insulin output declined rapidly and returned to their pre-infusion control levels by about 5 min. Plasma insulin concentration in SPD venous blood increased significantly following a pattern similar to that observed in the response of insulin output. Insulin concentration in aortic blood also increased significantly, but only during the simultaneous infusion of glucose and $PACAP_{1-27}$. Aortic glucose concentration remained unchanged during the whole period of experiment.

Correlations

Correlations between changes in SPD venous glucose concentration and those in insulin output were sought in the absence and the presence of the local $PACAP_{1-27}$ infusion to the pancreas. In the group receiving glucose alone, a relatively weak correlation (r^2=0.795) was obtained. In the group receiving both glucose and $PACAP_{1-27}$, a strong and highly significant correlation (r^2=0.972) could be demonstrated (Figure 6).

DISCUSSION

The present study indicates that the local administration of $PACAP_{1-27}$ to the pancreas increased the basal insulin secretion in a dose-dependent manner. The insulin response to 0.05 μg of $PACAP_{1-27}$ was highly reproducible upon the repeated administration with an interval of 30 min. The net increase in

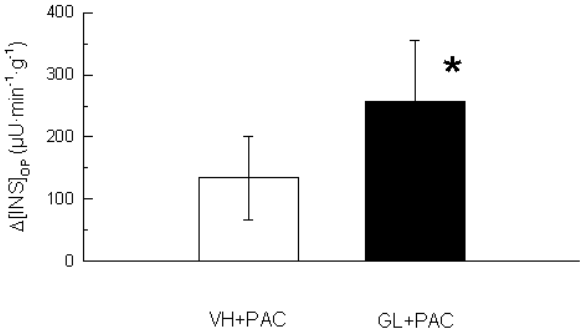

Figure 5. Net increase in insulin output ($\Delta[INS]_{OP}$) in response to $PACAP_{1-27}$ (0.05 μg) in the group receiving the vehicle and $PACAP_{1-27}$ (VH+PAC) in the first bloc and glucose (5%) and $PACAP_{1-27}$ (GL+PAC) in the second bloc. Open columns indicate the data obtained without glucose infusion, and the filled column represents the data with combined administration of glucose with $PACAP_{1-27}$.
* P=0.033 (n=7).

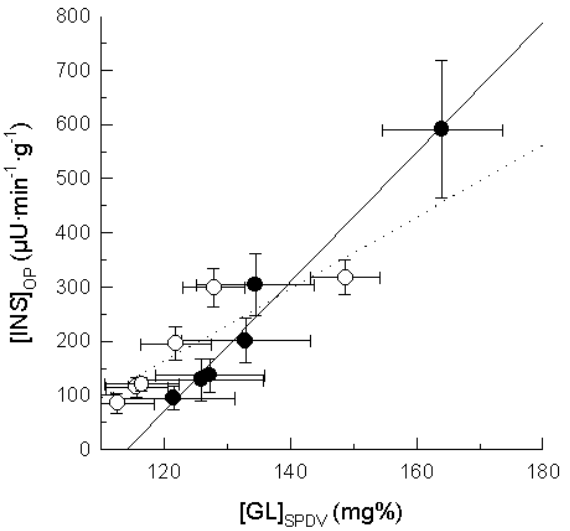

Figure 6. Correlation between changes in glucose concentration in superior pancreatico-duodenal (SPD) venous blood ($[GL]_{SPDV}$) and those in insulin output ($[INS]_{OP}$). Filled circles represent the values obtained under conditions in which glucose (5%) was simultaneously infused with $PACAP_{1-27}$ (0.05 μg) into the SPD artery. Open circles indicate the values obtained in response to the glucose infusion along with the vehicle. In both cases, the data represent those observed during the infusion of $PACAP_{1-27}$ or the vehicle and 1, 2, 4, 9, and 14 min after discontinuing the combined infusion of either glucose with $PACAP_{1-27}$ or glucose with the vehicle. The original data are shown in the second bloc in Figures 2 and 4. The correlation obtained in the presence (filled circles) and absence (open circles) of $PACAP_{1-27}$ is defined by the equation $y=-1362.459+11.955x$, ($r^2=0.972$, $P=0.0003$ for the regression indicated by a solid line, n=6); and $y=-633.809+6.644x$, ($r^2=0.795$, $P=0.017$ for the regression indicated by a dotted line, n=6), respectively.

insulin output in response to $PACAP_{1-27}$ during the increased basal glucose concentration was significantly greater than that observed under the normal resting glucose level. A better correlation could be established between changes in SPD venous glucose concentration and those in insulin output when $PACAP_{1-27}$ was given in the presence of glucose.

It has been shown that $PACAP_{1-27}$ significantly stimulates insulin release from the isolated perfused rat [6] and porcine pancreas [7]. In the present study, local infusion of $PACAP_{1-27}$ into the SPD artery resulted in the increase in insulin output in a dose-dependent manner. This increase in insulin output may have resulted, most probably, from the direct action of $PACAP_{1-27}$ on endocrine beta-cells and not from non-specific indirect effects such as local hemodynamic changes or those in plasma glucose concentration. Indeed, plasma insulin concentration in SPD venous blood also increased significantly despite the simultaneous increase in pancreatic venous blood flow. In addition, the basal resting glucose concentrations in both aortic and SPD venous blood remained unchanged in dogs receiving $PACAP_{1-27}$ alone. These observations are consistent with the view that, in anesthetized dogs, the observed insulinotropic effect of $PACAP_{1-27}$ results from its direct action in the endocrine pancreas.

It has been postulated that the stimulatory effect of PACAP on the basal secretion of insulin *in vitro* appears to be dependent on the glucose concentration in the perfusate. In the isolated perfused rat pancreas, $PACAP_{1-27}$ at 0.1 nM stimulated insulin release under a perfusate glucose concentration of 5.5 mM, whereas the ten times higher concentration (1 nM) of $PACAP_{1-27}$ did not when the perfusate glucose concentration was half (2.8 mM) of the control [11]. The similar glucose-dependent secretagogue effect of $PACAP_{1-27}$ has been observed in the perifused rat islets [2, 12] and in dispersed porcine islet cells [13].

In the present study, the insulin response to 0.05 μg of $PACAP_{1-27}$ under the basal glucose concentration (approximately 130 mg/dL as measured in SPD venous blood) was significantly potentiated by about 130% when the basal glucose concentration was raised to approximately 170 mg/dL. This increase in insulin response to $PACAP_{1-27}$ did not result from a simple base-line increase due to glucose, because the net insulin response was also significantly enhanced. Moreover, the coefficient of determination obtained between variations of the SPD venous glucose concentration and those of the insulin output could be considerably improved when both $PACAP_{1-27}$ and glucose were simultaneously administered. These present findings *in vivo* are consistent with the view that the PACAP-

induced insulin release may depend on the basal plasma glucose concentration. Nevertheless, a mechanism by which a stronger correlation between SPD glucose concentration and insulin output could be obtained in the group receiving both glucose and $PACAP_{1-27}$ remains unexplained in the present study, as the addition of $PACAP_{1-27}$ did not further increase the SPD glucose concentration. One possible explanation will be an interaction between $PACAP_{1-27}$ and certain intracellular factors activated by the already-elevated glucose concentration (see below), resulting in the enhanced insulin output that may have somehow improved the correlation coefficient. Thus, the glucose-dependency of PACAP-induced insulin secretion remains obscure *in vivo* at present. Similarly, the pancreatic reactivity to $PACAP_{1-27}$ also remains uncertain when the pancreas is exposed to a moderate to severe hypoglycemia under *in vivo* conditions.

The potentiating effect of $PACAP_{1-27}$ on the glucose-evoked insulin release observed in the present study may have resulted, most probably, from their direct actions in the endocrine pancreas as previously indicated by many similar observations in vitro. Although it is beyond the scope of the present study to define the precise underlying mechanisms, a most probable mechanism could be an interaction of $PACAP_{1-27}$ with the direct effect of glucose at the level of beta-cells. In the consensus model of glucose-evoked insulin secretion, the main trigger initiating insulin exocytosis involves the opening of voltage-sensitive Ca^{2+} channels resulting in an increase in the cytosolic Ca^{2+} concentration [14, 15]. As PACAP also increases cytosolic Ca^{2+} concentration by increasing Ca^{2+} influx through the activation of voltage sensitive L-type Ca^{2+} channel in pancreatic beta-cells [16], it is plausible that $PACAP_{1-27}$ stimulates Ca^{2+} influx through its own Ca^{2+} channel activating effect in addition to the glucose-induced increase in cytosolic Ca^{2+} concentration. In this context, the nature of the effect of $PACAP_{1-27}$ for enhancing insulin secretion in the presence of glucose would be additive rather than synergistic. Another possibility is that $PACAP_{1-27}$ could also facilitate the formation, in beta-cell mitochondria, of glucose-derived glutamate that has been postulated to play a role of second messenger in glucose-evoked, sustained insulin secretion [17].

Alternatively, however, the activation of reflex via the hepatic glucoreceptor vagal afferent - pancreatic vagal efferent pathway cannot completely be ruled out in the present study. In the model used in this study, glucose was locally infused into the SPD artery, and SPD venous blood that still contained high glucose concentration was returned to the portal vein. It is, therefore, conceivable that, during glucose infusion into the SPD artery, hepatic glucoreceptor - vagal afferent inputs to the central nervous system would be activated followed by a stimulation of vagal efferent outputs, resulting in the release of neural acetylcholine and PACAP in the pancreas. Indeed, the vagus mediated neural release of endogenous PACAP has been shown in the isolated, perfused pig pancreas [7]. Hence, the locally administered exogenous $PACAP_{1-27}$ could also interact with endogenous acetylcholine and/or PACAP released at the vicinity of pancreatic beta-cells, resulting in the enhanced insulin secretion. This possibility is compatible with a recent finding that the insulin response to gastric glucose gavage was significantly reduced by $PACAP_{6-27}$, a PAC_1 receptor antagonist, in anesthetized mice [18]. The latter observation strongly suggests that endogenous PACAP is actually released and stimulates insulin secretion in response to postprandial hyperglycemia under normal physiological conditions. It is further conceivable that endogenous PACAP may thus be functionally implicated in facilitating the secretion of insulin in situations where glucose-stimulated insulin secretion needs to be further enhanced or otherwise becomes deficient.

In conclusion, the present study was to investigate if exogenous $PACAP_{1-27}$ could locally modulate glucose-evoked insulin secretion in the canine pancreas *in vivo*. The insulinotropic effect of $PACAP_{1-27}$ observed

under resting plasma glucose concentration was significantly enhanced when the plasma glucose concentration in the pancreas was locally increased by about 30% as measured in SPD venous blood. The net increase in $PACAP_{1-27}$-induced insulin output in the presence of increased local glucose concentration was significantly greater than that observed under the normal resting glucose level. A better correlation could be established between SPD glucose concentration and insulin output when both $PACAP_{1-27}$ and glucose were simultaneously administered, as compared to that obtained under normal resting glucose level. These results are compatible with the view that PACAP may play a role of facilitating the release of insulin during glucose loading.

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Abbreviations $[BF]_{SPDV}$: superior pancreaticoduodenal venous blood flow; GL: glucose; $[Hct]_{SPDV}$: hematocrit of superior pancreaticoduodenal venous blood; $[INS]_{AO}$: plasma immunoreactive insulin concentration in aortic blood; $[INS]_{SPDV}$: plasma immunoreactive insulin concentration in superior pancreaticoduodenal venous blood; $PACAP_{1-27}$: pituitary adenylate cyclase activating polypeptide with the N-terminal amidated 27 amino acid residues; PAC_1: PACAP specific receptor; SPD: superior pancreaticoduodenal; VH: vehicle

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