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Pituitary Adenylate Cyclase-Activating Polypeptide Receptors Mediating Insulin Secretion in Rodent Pancreatic Islets Are Coupled to Adenylate Cyclase But Not to PLC

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a potentiator of glucose-induced insulin secretion. PACAP binds to a PACAP-specific receptor (PAC1) and to VPAC receptors (VPAC1 and VPAC2), which share high affinity for vasoactive intestinal polypeptide (VIP). In the present study, the molecular expression of PACAP receptor isoforms and the signaling pathways involved in the insulin secretory effect of PACAP were investigated in isolated rat and mouse pancreatic islets. mRNA encoding PAC1-short, -hop, and -very short variants, as well as VPAC1 and VPAC2, were expressed in pancreatic islets. PACAP and VIP were equipotent in potentiating glucose-induced insulin release. Both peptides were also equipotent in increasing cAMP production, but PACAP was more efficient than VIP. Unlike carbachol, PACAP and VIP had no effect on inositol phosphate production. In the PAC1-deficient mouse, the insulinotropic effect of PACAP was reduced, and its differential effect on cAMP production was abolished, whereas the effects of VIP remained unchanged. These results clearly show that the insulinotropic effect of PACAP involved both VPAC and PAC1. The PAC1 variants expressed in rat and mouse pancreatic islets seem to be coupled to adenylate cyclase but not to PLC. (*Endocrinology* 143: 1253–1259, 2002)

PITUITARY ADENYLATE CYCLASE-activating polypeptide (PACAP) belongs to the vasoactive intestinal polypeptide (VIP)/glucagon/secretin family of peptides (1). PACAP exists in 2 biologically active forms, PACAP-38 and PACAP-27, which consists of the 27 NH_2-terminal residues of PACAP-38 (2). PACAP receptors belong to the GTP-binding protein-coupled receptor family. Three receptors have been cloned: a PACAP-preferring receptor, PAC1, and 2 receptors with similar high affinity for PACAP and VIP, named VPAC1 and VPAC2 (see Refs. 3 and 4). All 3 receptors are positively coupled to adenylate cyclase (AC). In addition, PAC1 can also be coupled to PLC (5). Several PAC1 isoforms generated by alternative splicing have been identified. In the third intracellular loop of the receptor, the absence (short variant) or the presence of either one or two 28-amino acid cassettes (hip and hop) generates 6 isoforms with different coupling to AC and PLC (5). In the N-terminal extracellular domain, the deletion of a 21-amino acid cassette (very short variant) results in an increased affinity for PACAP-27 (6). Another PAC1 variant, designated PAC1-TM4, differs from the short variant by discrete sequences located in transmembrane domains II and IV and activates neither AC nor PLC but activates L-type Ca^{2+} channels (7). For VPAC1 and VPAC2, no variants have been reported, so far, and they are primarily coupled to AC (8, 9).

Glucose is the major stimulator of insulin secretion, but the amount of insulin secreted in response to sugar is modulated by various nutritional, neural, and hormonal factors. PACAP has been reported to potentiate glucose-induced insulin release *in vitro* in the isolated perfused rat (10–12) and mouse pancreas (13), in isolated islets (14, 15), as well as *in vivo* in mice (13, 16) and humans (17). In numerous studies, PACAP and VIP have been shown to be equipotent in stimulating insulin secretion in perfused rat pancreas, mouse islets, and insulinoma cells, suggesting that VPACs are involved in the insulinotropic effect of PACAP (12, 15, 18). Another report has indicated that PACAP was more potent than VIP in stimulating insulin release from rat islets, suggesting the involvement of PAC1 (14). We have recently shown that the insulin secretory response to PACAP was reduced in perfused pancreas of PAC1-deficient mice, confirming the involvement of PAC1 in the insulin secretory effect of the peptide (13). However, in pancreatic islets, the type of PACAP receptors expressed and the signal transduction mechanisms involved in the insulin secretory effect of PACAP remain unclear. Expression of mRNA for PAC1 and VPAC2 has been detected in insulin-secreting cell lines and in mouse and rat islets by *in situ* hybridization (15), and PAC1-like immunoreactivity has been reported in rat islet cells (19). PAC1-TM4 has also been reported to be the major PAC1 variant expressed in rat pancreatic β-cells (7). On the other hand, a recent study showed that PAC1-short and -hop variants were the major isoforms expressed in rat insulinoma cells and neonatal pancreatic islets (20). Concerning the signaling pathways involved in the insulin secretory effect of PACAP and VIP, studies have only been performed in insulin-producing cell lines. They have shown that the peptides increase insulin secretion mainly through the activation

Abbreviations: AC, Adenylate cyclase; IP, inositol phosphate; PACAP, pituitary adenylate cyclase-activating polypeptide.

of AC (18, 20, 21). This does not exclude that other signaling pathways are involved in native β-cells (18, 22).

The aim of the present study was to characterize PACAP receptors and the signaling pathways involved in the insulin secretory effects of PACAP and VIP in isolated rat and mouse pancreatic islets. To further ascertain the functional characterization of native PAC1 in pancreatic islets, the study was also performed in isolated islets of PAC1-deficient mice.

Materials and Methods

Animals

All experiments were performed with adult male Wistar rats (320–350 g) and adult female mice (25–30 g). The animals were fed *ad libitum*. The PAC1-deficient mice were generated by homologous recombination on a 129/Sv × C57BL/6J background, as previously described (13). Heterozygote mice were crossed to inbred 129T mice for 10 generations to transfer the mutation to a homogeneous genetic background. Homozygote crosses were then set up to yield homozygous mutants and wild-type mice.

Preparation of isolated pancreatic islets

Animals were anesthetized by ip injection of pentobarbitone (60 mg/kg). Pancreatic islets were isolated by collagenase digestion of the pancreas and hand-collected. They were used immediately after isolation, except for one experiment in which they were cultured for 48 h in RPMI-1640 medium (Life Technologies, Inc., Rockville, MD) containing 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FCS and 10 mM glucose.

Analysis of mouse and rat PACAP receptor mRNAs by RT-PCR

Total RNA was extracted using RNAWIZ solution (Ambion, Inc., Austin, TX) and according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed with 100 pmol of random primers and 200 U of MMLV reverse transcriptase (Promega Corp., Madison, WI). PCR amplification was then performed with specific primers. To identify PAC1, we used primers flanking the hip-hop region (upper, 5′-CATCCTTGTGCAGAAGCTGC-3′; lower, 5′-GGTGCTTGAAGTCCATAGTG-3′) or with the upper primer located in hip cassette (5′-ACAAATTTAAGACTGAGAGT-3′) or hop cassette (5′-TCCACCATTACTCTACGGCT-3′). We used primers flanking the N-terminal domain (upper, 5′-CTGCATCTTCAAGAAGGAGC-3′; lower, 5′-CACAAGCATCGAAGTAGTGG-3′). Primers used to identify mouse VPAC1 and VPAC2 mRNAs were based on those used by Rawlings *et al.* (23). For VPAC1, primers used were: upper, 5′-GGCCCCATCCTCATCTCCAT-3′; and lower, 5′-CCGCCTGCACCTCACCATTG-3′. For VPAC2, primers used were: upper, 5′-ATGGACAGCAACTCGCCTCTCTTTAG-3′; and lower, 5′-GAAGGAACCAACACATAACTCAAACAG-3′. For actin amplification, which served as a control of the integrity of RNA, primers used were: upper, 5′-GACTCCGGAGACGGGGTCAC-3′; and lower, 5′-CCAGGGAGGAAGAGGATGCG-3′. The PCR was run at 94 C for 5 min, followed by 35 cycles of 94 C for 30 sec, 55 C for 30 sec, 72 C for 1 min, and then a final cycle of 72 C for 10 min. To investigate the existence of the PAC1-TM4 splice variant, we used the PCR strategy previously described by Chatterjee *et al.* (7). Briefly, a first PCR with upper (5′-CTGCATCTTCAAGAAGGAGC-3′) and lower (5′-GCCCAGCCCAAGCTCAAA-3′) primers was followed by a nested PCR using: upper, (5′-ACTCCTACTGTGTCAGCATGG-3′); and lower, (5′-TTCCCTCTTGCTGACGTT-3′) primers. The expected size of the nested PCR product was 317 bp for PAC1-TM4.

Measurement of insulin release

After isolation, the islets were first preincubated for 90 min in a medium containing glucose (8.3 mM for rat or 10 mM for mouse islets). Batches of five islets were then incubated for 30 min in a medium containing glucose (8.3 mM for rat or 16.7 mM for mouse islets) with or without PACAP38 (Neosystem, Strasbourg, France) or VIP (Neosystem). At the end of the incubation, a portion of the medium was taken for measurement of insulin by RIA (12).

Measurement of cAMP levels

The islets were loaded with [2-^{3}H]adenine for 90 min in the presence of glucose (8.3 mM for rat or 10 mM for mouse islets). After washing, they were subdivided into batches of 15–20 islets and incubated in a medium containing glucose (8.3 mM for rat and 16.7 mM for mouse islets) for 30 min and then for 5 min with PACAP-38 or VIP in the presence of 1 mM isobutylmethylxanthine, an inhibitor of phosphodiesterase. The supernatant was removed, and the reaction was stopped by 5% trichloracetic acid at 4 C. After centrifugation, [2-^{3}H]cAMP was separated from [2-^{3}H]ATP sequentially through dowex and alumina columns and measured as previously described (24).

Measurement of inositol phosphate (IP) levels

The islets were preincubated for 2 h in a medium containing glucose (8.3 mM for rat and 10 mM for mouse islets) and myo-[^{3}H]inositol. After washing, they were subdivided in batches of 20–30 islets and incubated for 30 min in a medium containing glucose (8.3 mM for rat and 16.7 mM for mouse islets) and 10 mM LiCl to prevent IP degradation. The peptides were then added for a further 30 min. The medium was removed, and the reaction was stopped with 1 ml 5% perchloracetic acid at 4 C. Total IPs were extracted through dowex columns as previously described (25). IP production was also measured in cerebellar granular cells, which were isolated and cultured as described (26).

Data and statistical analysis

Data are presented as means ± SEM for the indicated number of experiments. The statistical significance was assessed by *t* test or by ANOVA, followed by the Newman-Keuls test in the case of multiple comparisons.

Results

The mRNAs encoding for the PACAP/VIP receptors were detected in mouse islets

RT-PCR with specific primers was performed to detect mRNAs encoding for PACAP/VIP receptor subtypes in wild-type and PAC1-deficient mouse pancreatic islets (Fig. 1). Mouse cerebellar neurons, used as positive control, expressed transcripts for VPAC1, VPAC2, and PAC1 variants. mRNAs encoding for PAC1, VPAC1, and VPAC2 were detected both in freshly isolated islets and in 48-h-cultured islets of wild-type mouse. To determine the expression of PAC1 variants, we used different primer pairs. Amplification with a primer pair external to the site of insertion of the hip/hop cassettes produced amplification products of 305 and 389 bp (Fig. 1, lane 1), indicating the existence of PAC1-short (without cassette) and PAC1 variants containing one cassette. A primer pair with the upper primer matching the beginning of the hip cassette produced very weak products (Fig. 1, lane 2), whereas a primer pair with upper primer matching the end of the hop cassette produced a clear amplification product of 259 bp (Fig. 1, lane 3). These results therefore indicated the predominance of the PAC1-short and -hop variants over the others in mouse islets. In addition, to look for the existence of N-terminal spliced variants, we used a primer pair external to the site of insertion of the 21-amino-acid cassette. Amplification generated 2 products, of 332 and 269 bp (Fig. 1, lane 4), indicating the expression of the PAC1-short and -very short variants. To investigate the existence of PAC1-TM4, we used the PCR strategy previously described by Chatterjee *et al.* (7). Amplification did not generate the ex-

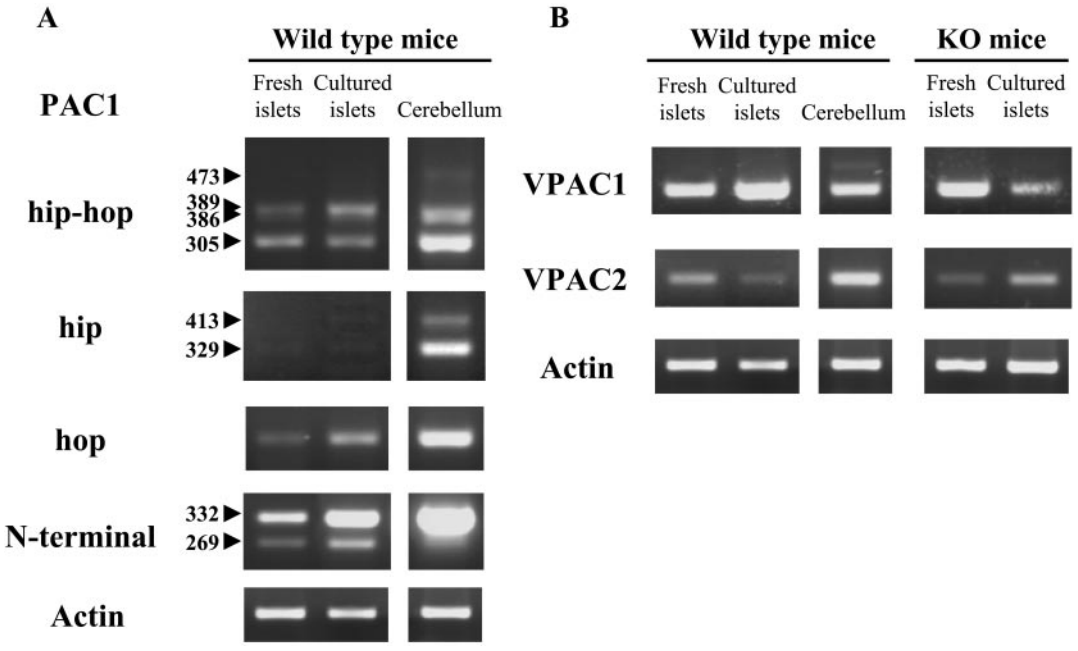

FIG. 1. RT-PCR analysis of PACAP/VIP receptor mRNAs in freshly isolated islets, 48-h-cultured islets, and cerebellum of wild-type mice and PAC1-deficient mice. Total RNA was reverse-transcribed and PCR amplified with primer pairs specific for PAC1 isoforms (A) and VPAC1 and VPAC2 (B). The amplification of actin served as a control of the integrity of RNA and as a standard of the extracted RNA quantity.

pected product of 317 bp either in islets or cerebellar neurons. PAC1-deficient mice exhibited signals for VPAC1 and VPAC2. We also performed the same experiments with adult rat islets and detected the same mRNA expression pattern (data not shown).

In isolated rat islets, PACAP-38 and VIP were equipotent in stimulating insulin secretion, whereas PACAP-38 was more efficient than VIP in increasing cAMP production

PACAP-38 and VIP (10 pM–100 nM) caused a concentration-dependent amplification of insulin release induced by 8.3 mM glucose (controls) (Fig. 2A). The peptides were equipotent and exhibited similar maximal responses (about 250% of controls). In the same concentration range, the peptides produced a concentration-dependent increase in islet cAMP production (Fig. 2B). However, from 10 nM, PACAP-38 induced higher cAMP responses than VIP. For both peptides, the maximal increase occurred at 100 nM and averaged 235 ± 8 and 187 ± 7% ($P < 0.01$), respectively, with PACAP-38 and VIP. At 100 nM, forskolin, an activator of AC, elicited similar increases to PACAP-38 on cAMP levels (229 ± 17%, Fig. 2B, *inset*).

In isolated wild-type mouse islets, PACAP-38 and VIP were equipotent in stimulating insulin secretion, but PACAP-38 was more efficient than VIP in increasing cAMP production

At 10 and 100 nM, PACAP-38 and VIP exhibited comparable amplification of insulin release induced by 16.7 mM of glucose (Fig. 3A). Similarly, as found in rat, PACAP-38 was more efficient than VIP in increasing cAMP production: 268 ± 22 *vs.* 170 ± 11% ($P < 0.001$), respectively, at 100 nM (Fig. 3B). At 100 nM, forskolin elicited a lower increase than PACAP on cAMP levels (216 ± 23% of control, Fig. 3B).

In isolated PAC1-deficient mouse islets, the effects of PACAP-38 on insulin secretion and cAMP production were greatly decreased

In PAC1-deficient mouse islets, the effects of PACAP-38 both on insulin release and on cAMP accumulation were reduced, whereas those of VIP remained unaffected (Fig. 4). In contrast to that recorded in wild-type mouse islets, PACAP-38 elicited a lower insulin response than that obtained with VIP: 163 ± 13 *vs.* 226 ± 15% ($P < 0.01$), respectively, at 100 nM. On the other hand, the PACAP effect on cAMP production was greatly reduced and was then similar to that of VIP (167 ± 11 and 169 ± 16%, respectively, at 100 nM). With forskolin, similar increases in cAMP levels were recorded in PAC1-deficient and wild-type mouse islets (212 ± 22 *vs.* 216 ± 23%, respectively).

PACAP-38 and VIP had no effect on IP production in rat and mouse pancreatic islets

Because PAC1-short and PAC1-hop isoforms, detected by RT-PCR in islets, were described to be coupled not only to AC but also to PLC, we investigated whether PACAP and VIP could stimulate IP production in rat and mouse islets. At 100 nM (not shown) and at 1 μM, PACAP-38 and VIP failed to increase IP production in rat or mouse islets (Table 1). In contrast, PACAP increased IP production in cerebellar granule cells, our positive control, which express PAC1-short and PAC1-hop isoforms (27). On the other hand, carbachol (100 μM), a muscarinic receptor agonist known to stimulate insulin secretion through activation of PLC, markedly increased IP production in pancreatic islet, as well as in cerebellar granule cells.

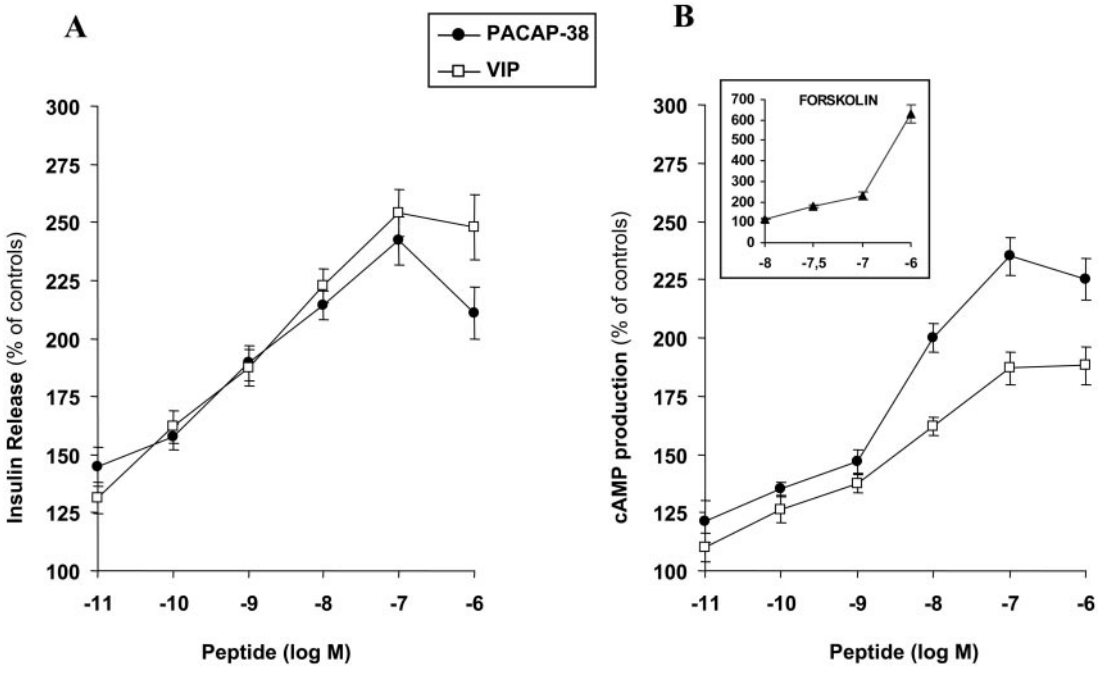

FIG. 2. Effects of PACAP-38 and VIP on insulin secretion (A) and cAMP production (B) from freshly isolated rat islets in the presence of 8.3 mM glucose. *Inset* in B, Effects of different concentrations of forskolin. Results are expressed as a percentage of controls (8.3 mM glucose alone). The control values were: for insulin release, 3.1 ± 0.2 ng/islet·30 min; and for cAMP accumulation ([^{3}H]cAMP/[^{3}H]ATP), 0.88 ± 0.03%. Data are the means ± SEM of at least six independent experiments, each performed in triplicate.

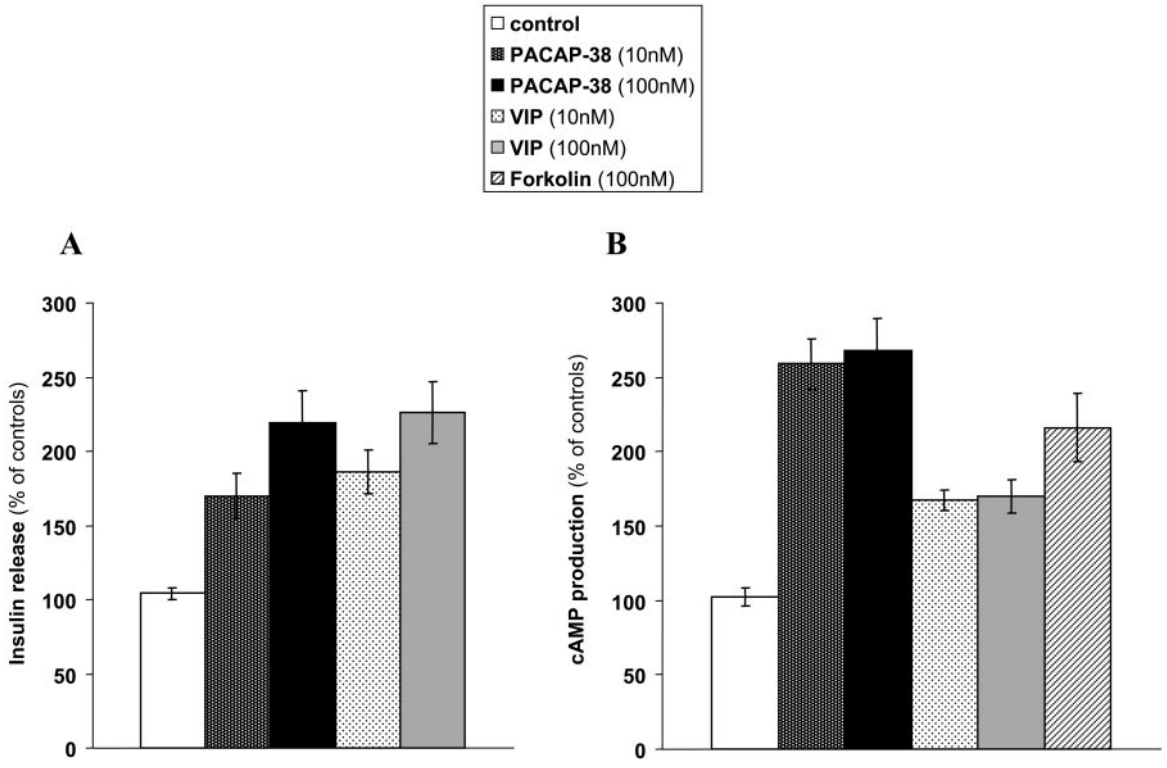

FIG. 3. Effects of PACAP-38 and VIP on insulin secretion (A) and cAMP production (B) from wild-type mouse islets in the presence of 16.7 mM glucose. Results are expressed as a percentage of controls (16.7 mM glucose alone). The control values were: for insulin release, 721 ± 42 pg/islet·30 min; and for cAMP accumulation ([^{3}H]cAMP/[^{3}H]ATP), 0.91 ± 0.09%. Data are the means ± SEM of at least eight determinations.

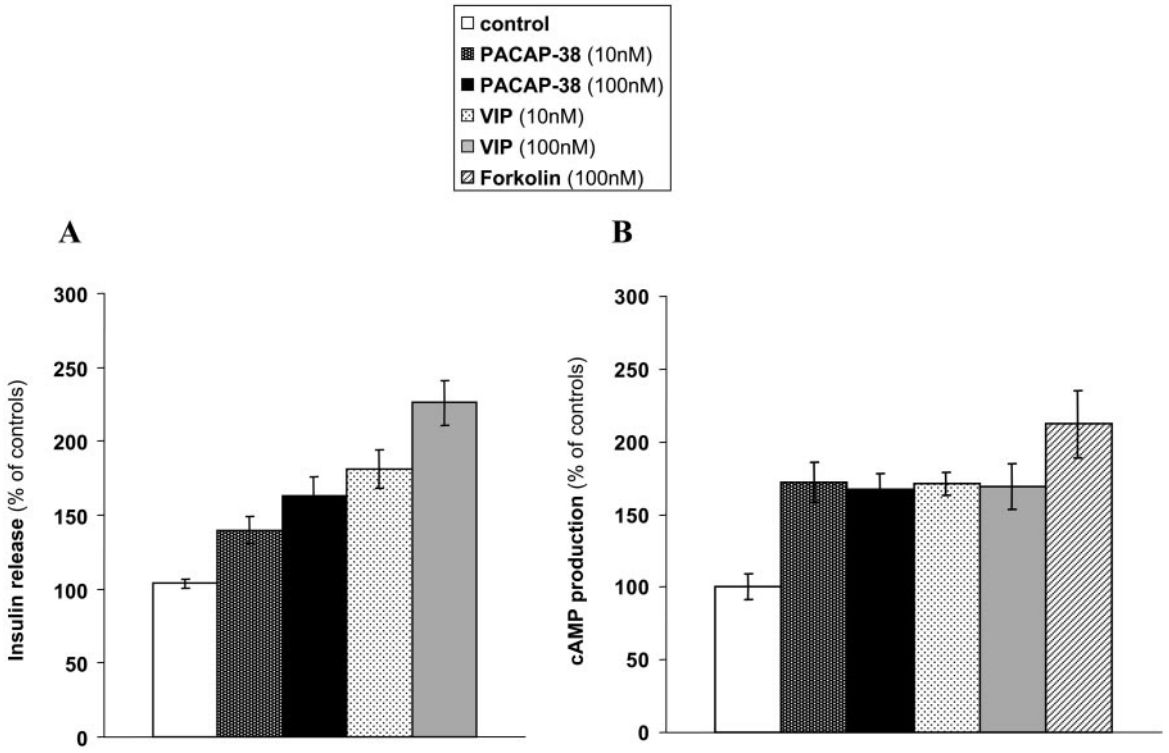

FIG. 4. Effects of PACAP-38 and VIP on insulin secretion (A) and cAMP production (B) from PAC1-deficient mouse islets in the presence of 16.7 mM glucose. Results are expressed as a percentage of controls (16.7 mM glucose alone). The control values were: for insulin release, 542 ± 31 pg/islet·30 min; and for cAMP accumulation ([^{3}H]cAMP/[^{3}H]ATP), 0.82 ± 0.12%. Data are the means ± SEM of at least six determinations.

TABLE 1. Effects of PACAP (1 μM), VIP (1 μM), and carbachol (100 μM) on inositol phosphate production from rat, wild-type and PAC1-deficient mouse pancreatic islets, and cerebellar granule mouse cells

	Pancreatic islets			Cellular neurons
	Rat	Mouse		
		Wild-type	PAC1 knockout	Mouse
Control	100 ± 5	100 ± 4	99 ± 5	100 ± 14
PACAP (1 μM)	102 ± 3	125 ± 12	103 ± 8	327 ± 4
VIP (1 μM)	99 ± 4	108 ± 9	118 ± 11	116 ± 3
Carbachol (100 μM)	211 ± 17.17	1038 ± 183	1096 ± 116	435 ± 7

Results are expressed as a percentage of controls (8.3 mM glucose for rat islets; 16.7 mM glucose for mouse islets). The control values were 490 ± 52 dpm/islet in rat; 49 ± 3 and 31 ± 3 dpm/islet, respectively, in wild-type and PAC1-knockout mice; and 7897 ± 1039 dpm/10^6 cells in mouse cerebellar neurons. Data are the means ± SEM of at least five experiments.

Discussion

The present study, performed in pancreatic isolated islets of both rat and mice, shows that: 1) islets express mRNA of three major isoforms of PAC1 and also VPAC1 and VPAC2; 2) PACAP and VIP are equipotent in increasing glucose-induced insulin secretion and cAMP production, but PACAP is more efficient than VIP on the latter; 3) both peptides fail to increase the production of IP; and 4) in PAC1-deficient mice, the effects of PACAP (but not those of VIP) are reduced, and the effect on cAMP production is then comparable with that of VIP. Thus, the insulin secretory effect of PACAP clearly involves both PAC1 and VPAC receptors and is mediated, in part, by the cAMP (but not by the IP) pathway.

The RT-PCR study showed that the mRNAs of the three cloned PACAP/VIP receptors were expressed in pancreatic islets. In the pancreas, islet cells have been shown to express both PAC1 and VPAC2 by *in situ* hybridization (15, 28) but not VPAC1, which was only associated with blood vessels (28). However, mRNA encoding for VPAC1 has been reported to be expressed not only in rat neonatal pancreatic islets but also in a β-cell line (20). On the other hand, nerve terminals have been reported to remain in freshly isolated islets but do not persist after culture (29, 30). Therefore, we looked at mRNA expression of PACAP/VIP receptors in islets cultured for 48 h to eliminate nerve fibers. The isoform expression pattern found was similar to that of freshly isolated islets, demonstrating the presence of PACAP/VIP receptors in islets.

Because PAC1 has been described to have many spliced variants that display differences in ligand affinity and coupling efficiency (5, 6), we looked more carefully at the PAC1 variants expressed in adult rodent islets. In the third intracellular loop splicing region, which determines the signaling pathway, the major PAC1 isoforms expressed were the

PAC1-short and -hop splice variants. This expression pattern, found in adult islets of both rat and mouse, is in agreement with that recently reported in a β-cell line and neonatal pancreatic islets of rat (20). In the N-terminal splicing region that determines ligand affinity, we detected the two PAC1-short and -very short isoforms without the 21-amino-acid domain that confers to PACAP-27 a comparable affinity to that of PACAP-38 (6). In rat neonatal pancreatic islets, only the mRNA of the PAC1-short isoform has been detected (20). However, in accordance with the expression of the very short variant, both forms of PACAP have been shown to be equipotent in stimulating insulin secretion in adult mouse and rat pancreatic islets (15). In neuronal tissues, an N-terminal spliced variant, characterized by an extensive deletion of 57 amino acids, has also been reported to display a selective (but low) affinity for PACAP (31). Nevertheless, we were unable to detect this variant in rat and mouse pancreatic islets (data not shown). The PAC1-TM4 variant, previously reported by Chatterjee *et al.* (7) to be expressed in rat pancreatic β-cells, has not been detected either in rat or mouse islets while using the primers and conditions described by these authors. Here, therefore, we cannot confirm the expression of the PAC1-TM4 variant in rodent pancreatic β-cells. This discrepancy requires further investigation.

We have shown that PACAP and VIP were equipotent in stimulating glucose-induced insulin release from rat and mouse islets, consistent with the implication of VPAC. Yada *et al.* (14) described a specific PACAP receptor which displayed an extraordinarily high affinity for PACAP (< pM) in rat pancreatic islets. Nevertheless, we detected no effect of PACAP, at 10^{-13} M, on insulin secretion (data not shown), in agreement with a previous study in rat and mouse islets (15). In PAC1-knockout mouse islets, the insulin response to PACAP-38 is reduced, confirming the role of PAC1 in the insulinotropic effect of PACAP, as we previously reported in perfused pancreas (13). However, the insulin response to PACAP was lower than that of VIP, which was not changed, compared with that observed in wild-type mice. Therefore, it seems that the expression of VPAC was intact in PAC1-deficient mouse islets but that PACAP was less efficient than VIP in stimulating insulin secretion by activating these VPAC. This may explain why, in wild-type mice, PACAP (which activates both PAC1 and VPAC) was not more efficient than VIP, which only activates VPAC.

Alternative splicing in the third intracellular loop, a region classically involved in the interaction with specific G proteins, determines second messenger coupling. In contrast to the PAC1-hip variant, which does not activate PLC, the PAC1-short and -hop variants are described to be well coupled to both AC and PLC (5). We show here, in the presence of the phosphodiesterase inhibitor IBMX, that PACAP and VIP were equipotent in increasing cAMP production, but PACAP was more efficient than VIP in both rat and mouse pancreatic islets. In PAC1-knockout mouse islets, the effect of PACAP on cAMP production was reduced and was then similar to that of VIP. This result clearly showed that PACAP stimulated AC in islets via both PAC1 and VPAC receptors. However, it must be noted that in wild-type mice, PACAP was more efficient than VIP on cAMP production, yet the peptides exhibited comparable insulin secretory responses. This did not seem to result from a saturation step in the final pathway leading from cAMP production to insulin secretion. Indeed, in PAC1-deficient mice, the impairment of the stimulatory action of PACAP on cAMP production clearly resulted in a reduction in the insulin secretory effect of the peptide. Moreover, in PAC1-deficient mice, VIP seemed to be more efficient than PACAP on insulin release, whereas the two peptides similarly increased cAMP levels. Taken together, these results suggest that, in the absence of PAC1, the apparent higher efficacy of VIP, compared with PACAP, may attributable to a specific signaling pathway engaged by VIP but not by PACAP. One possible explanation may be that VPACs exist in various agonist-specific active receptor states, according to the concept of *agonist trafficking of receptor signals* (32). In this case, the agonists (PACAP and VIP) may stabilize different receptor states, which do not elicit the same magnitude of response, and may select between several potential signaling pathways. Future investigations of VPAC-deleted mice are now required for a more complete understanding of signaling mechanisms that undergo the insulinotropic action of PACAP and VIP. On the other hand, neither PACAP nor VIP had any effect on IP production. These data are in agreement with previous results demonstrating a very weak (or no) increase in IP production by PACAP in insulinoma cell lines (20, 21) or by VIP in mouse islets (33). PAC1-short and -hop isoforms have been reported to be coupled to PLC when transiently expressed in porcine renal epithelial LLCPK1 cells or in *Xenopus* oocytes (5). We have seen a stimulation of IP production in rat cerebellar neurons, which expressed the PAC1-hop and PAC1-short variants (27). It is possible that PAC1 variants affect the intracellular transducing systems differently when expressed in neurons and islets. However, PAC1-short expressed in astrocytes has been reported to not activate the PLC pathway (34). These differences in PLC coupling between the different cells in which PAC1 is expressed could be attributable to a difference in receptor density. It must be noted that the potency of PACAP-38 to increase cAMP production has been shown to be much higher than its potency to stimulate IP production in LLCPK1 cells or neurons expressing PAC1 (5, 6). A lower receptor density in pancreatic islet cells might alter more drastically the potency of PACAP-38 to stimulate IP than cAMP production and therefore explain the apparent ineffectiveness of the peptide on the IP production. Moreover, PAC1 exhibits a different agonist pharmacology according to the effector pathway to which it is coupled. Indeed, whereas PACAP-38 exhibited a lower potency than PACAP-27 in increasing cAMP levels, it was at least 100-fold more potent in stimulating IP production (5). This may indicate different active receptor conformations that are preferentially coupled to Gs or Gq, respectively. This may also be explained by the *agonist trafficking of receptor signals* concept (32). In addition, a difference in the nature of the G proteins coupled to PLC in pancreatic β-cells and LLCPK1 or neurons might also explain a difference in the PLC coupling.

In summary, we have demonstrated here that the insulinotropic effect of PACAP in rodent pancreatic islets was mediated by PAC1 and VPAC receptors. The isoforms of PAC1 predominantly expressed in islets were PAC1-short, -hop, and -very short. Though these PAC1 variants were

described to be coupled to both AC and PLC, we showed that PAC1 activation stimulated the AC pathway (but not the PLC pathway) in rat and mouse pancreatic islets.

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