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# Dose-Response Effects of VIP on the Rabbit Exocrine Pancreatic Secretion. Comparison with PACAP-27 Actions

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A dose-response study of the effects of vasoactive intestinal peptide (VIP) on the exocrine pancreatic secretion of the rabbit has been made. Furthermore, the actions of VIP and pituitary adenylate cyclase activating peptide (PACAP) on the exocrine pancreatic secretion were compared at a similar molar dose. After the infusion of VIP a linear dose-response relationship for pancreatic flow rate and bicarbonate output, up to the dose of 4 µg/kg, was observed. VIP acts as a partial agonist of secretin, the rabbit pancreas being less sensitive to VIP compared with other mammals. Moreover, VIP did not significantly stimulate the pancreatic protein output. PACAP stimulated the hydroelectrolyte fraction of the exocrine pancreatic secretion in a similar manner to that of VIP. Unlike what was observed with VIP, PACAP, on the same molar basis, significantly stimulated the protein and amylase outputs. Furthermore, PACAP releases VIP, so that the action of PACAP on the hydroelectrolyte fraction may be partially mediated by VIP; on the other hand, VIP is not involved in the effect of PACAP on the pancreatic enzyme secretion of this species.

Key words: Exocrine pancreas, PACAP, Rabbit, VIP.

Vasoactive intestinal peptide (VIP) is considered to be a partial agonist of secretin actions on exocrine pancreas (25). The stimulatory effect of VIP on exocrine pancreatic secretion, mainly the hydroelectrolyte fraction, has been reported in man (7), rats (5) dogs (16), cats (14, 25) and pigs (13). In the rabbit, VIP is certainly involved in the stimulation of pancreatic electrolyte secretion since after a dose-response study on intraduodenally administered HCl (8) significant statistical

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correlations between portal VIP levels and both pancreatic flow rate and bicarbonate output were found.

In mammals the effect of VIP on the hydroelectrolyte fraction is smaller than that produced by secretin and only in turkey a similar stimulatory action for secretin and VIP has been reported (6). Most researchers support a neural origin for VIP (10) though the possibility of an endocrine source for this peptide should not be discarded (23).

Regarding pituitary adenylate cyclase activating peptide (PACAP), a peptide originally isolated from ovine hypothalamus (18), it is present in two forms: PACAP-38 (18) and an N-terminal fragment PACAP-27 (17), which shows a close sequence homology with VIP. PACAP stimulates the amylase and protein secretions of rat exocrine pancreas in a dose-dependent manner (20). Moreover, stimulation of amylase secretion by PACAP-27 was greater than that induced with the same dose of PACAP-38 or VIP (1, 20). Furthermore, similar results to that reported in rat have been recently shown in dog (22), the conclusion being reached that PACAP is a VIP-like weak stimulant of pancreatic fluid and bicarbonate secretion, but a potent stimulant of pancreatic protein secretion, and that PACAP-27 is more potent than PACAP-38 on the pancreatic exocrine secretion. Finally, since its presence has been demonstrated along the enteric nervous system (28) PACAP has been suggested to be another gut-regulatory peptide.

In the present work we have studied the action of increased doses of VIP on the exocrine pancreatic secretion of the rabbit. Furthermore, the effects of the same dose of VIP and PACAP-27 on the exocrine pancreas of the rabbit have been compared; to do so, the VIP dose that produced the maximal increase in pancreatic secretion was chosen.

## Materials and Methods

*Animals and surgical technique.*– Male adult New Zealand rabbits weighing 2-2.5 kg were fasted for 24 h with free access to water. Coprophagy was prevented by a collar. Rabbits were anaesthetized with sodium pentobarbital (30 mg/kg b. w., i.v.), and the surgical method was carried out according to a procedure previously described (8). Moreover, the carotid artery was cannulated for measuring systemic blood pressure by means of a transducer connected to a polygraph.

Animals remained unconscious throughout the procedure and a heating pad was used to maintain the body temperature at 38 ± 1 °C as measured by a rectal thermometer. The rabbits were sacrificed at the end of the experiment by a high i.v. dose of sodium pentobarbital.

*Experimental design.*– After an equilibration period of 30 min, pure pancreatic juice was collected in preweighed Eppendorf tubes in a 20 min basal period (S1), a 10 min stimulation period (S2) and five 10 min post-stimulation periods (S3 to S7). In the basal and post-stimulation periods animals were intravenously infused with 0.9 % NaCl containing 0.2 % bovine serum albumin (BSA, Sigma). In the stimulation period animals were intravenously infused with VIP (Sigma) at the doses of 1, 2, 4 or 6 µg/kg or with PACAP-27 (Peninsula) at the dose of 4 µg/kg, both in saline with 0.2 % BSA. Only one dose of either peptide was infused in each rabbit. In a control group of animals the infusion solution during this period was the same as in the basal and post-stimulation ones. Intravenous infusion was performed with a peristaltic pump, at a flow rate of 0.1 ml/min.

Portal venous blood samples were drawn 5 min before ending the basal period (S1), and in the middle of each 10 min

period: stimulation (S2) and post-stimulation (S3 to S7), the volume withdrawn being replaced by injecting the same volume of dextran-saline (Braun). Blood samples, that were collected in heparinized tubes containing 500 kallikrein inhibitory units (KIU)/ml of aprotinin (Trasylol, Bayer), were immediately centrifuged and the plasma stored at –70 °C.

Throughout the experimental protocol the duodenum was perfused with phosphate buffered saline at pH 7.4, by means of a peristaltic pump, at a flow rate of 2 ml/min.

*Analytical methods.*– The flow rate of pancreatic juice was determined by weighing the samples on an electronic balance, assuming the density of the juice to be 1 g/ml. Bicarbonate concentrations were determined by measuring the total $CO_2$ content with a Natelson microgasometer (Scientific Industries). Total protein concentrations were measured by the Coomassie Blue binding method of BRADFORD (2). Amylase activity in the pancreatic juice samples was analyzed according to the method of HICKSON (12).

The concentration of VIP in plasma samples was measured by a specific radioimmunoassay developed in our laboratory and described in detail previously (9).

*Data Analysis.*– Values of each parameter in each period (mean ± SEM) are expressed as percentage of basal values, which were considered equal to 100 %. Comparison of the data was performed, as indicated, by means of a two-factor ANOVA, or a one-factor ANOVA followed by the Scheffé's test; $p < 0.05$ was considered statistically significant.

## Results

The basal values of each parameter analyzed in pancreatic juice and in portal plasma are shown in table I.

Table I. *Basal values of the parameters analyzed in pancreatic juice and in portal plasma.* Values are means ± SEM (n = 36).

| | |
|---|---|
| Flow rate (µl/min) | 7.40 ± 0.43 |
| Bicarbonate output (nmol/min) | 502.1 ± 47.5 |
| Protein output (µg/min) | 54.55 ± 6.15 |
| Amylase output (mU/min) | 114.9 ± 10.0 |
| Portal plasma ir-VIP (fmol/ml) | 11.9 ± 0.9 |

Pancreatic hydroelectrolyte secretion (flow rate and bicarbonate output) remained unchanged throughout the experimental time in the control group of animals. On the other hand, the enzyme secretion (protein and amylase outputs) progressively diminished up to 50 % of the basal values.

Results obtained in the VIP dose-response studies are shown in figure 1. As a consequence of VIP administration, the hydroelectrolyte fraction of pancreatic secretion increased in a dose-dependent way, being maximally stimulated by the 4 µg/kg dose; with the following dose, 6 µg/kg, a lower response was attained (fig. 1A). The stimulation observed in the enzymatic fraction of pancreatic secretion was clearly lower than that of the hydroelectrolyte one; a maximal response of 166 ± 30 % was obtained for protein output with the dose of 6 µg/kg, whereas in amylase output the maximal response, 190 ± 39 % , appeared with the 4 µg/kg dose (fig. 1B).

As a consequence of VIP intravenous infusion, and as expected, portal plasma levels of ir-VIP increased in a dose-dependent way, the maximal values being attained after the administration of the VIP dose of 6 µg/kg (fig. 1C).

Figure 2A shows the results obtained in the pancreatic juice flow rate in the 4 µg/kg VIP and 4 µg/kg PACAP infused rabbits. Two-way ANOVA demonstrated the existence of significant differences among the values of the samples, but not

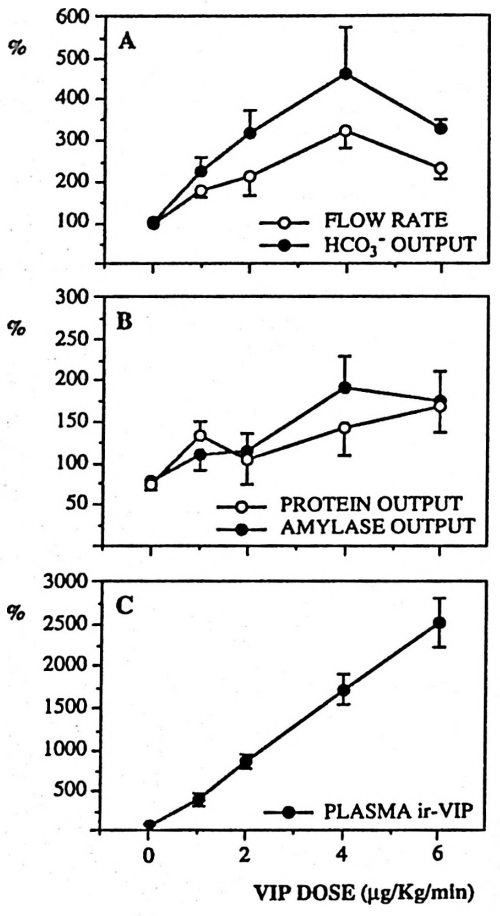


Fig. 1.- *Parameters analysed in pancreatic juice and in portal plasma of control animals (not VIP infused) and rabbits infused with graded (1, 2, 4 and 6 µg/kg) doses of VIP.*

Values (mean ± SEM) are expressed as percent of peak values (S2 sample) referred to the basal values (= 100 %) (n = 6). A: flow rate and bicarbonate output; B: protein and amylase outputs; C: plasma ir-VIP.

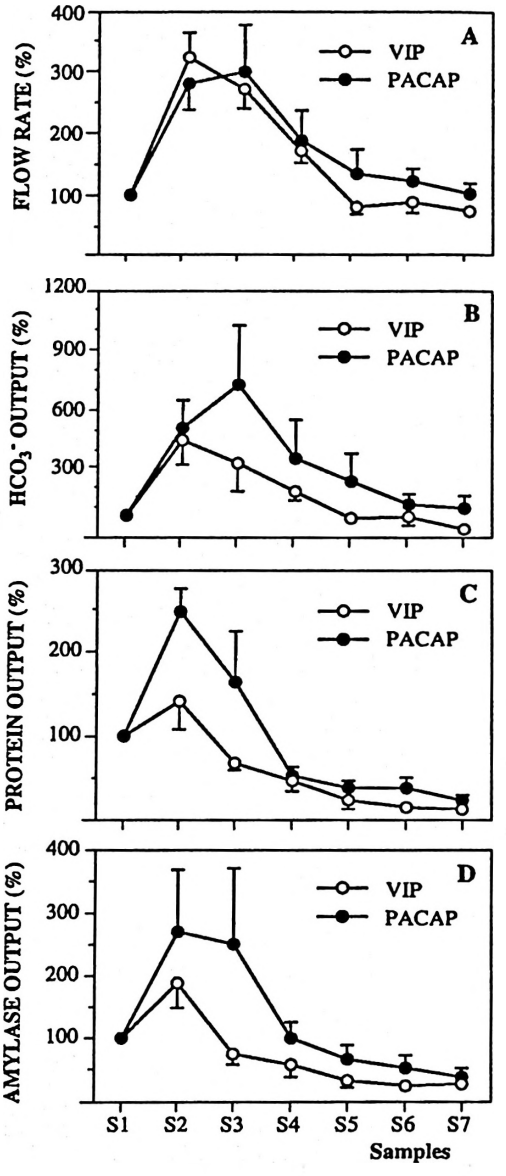


Fig. 2. *Pancreatic juice flow rate (A), bicarbonate output (B), protein output (C) and amylase output (D) in rabbits infused during S2 with 4 µg/kg of VIP or PACAP.*

Values (mean ± SEM) are expressed as percent of basal (n = 6). Two-way ANOVA test showed significant differences among the samples ($p < 0.001$, for all the four parameters) and between the effects of VIP and PACAP ($p < 0.05$ for bicarbonate output, $p<0.01$ for protein output and $p < 0.05$ for amylase output).

between the effects of VIP and PACAP. In bicarbonate output (fig. 2B), there were significant differences among the samples and between the effects of VIP and PACAP, the stimulation induced by the latter being greater than that induced by the former. In both flow rate and in bicarbonate output the maximal response to

VIP stimulation was attained in the stimulation sample (S2), whereas PACAP elicited its maximal stimulation action on the first post-stimulation sample (S3).

Total protein and amylase output increased after the administration of VIP or PACAP, the stimulation induced by this latter peptide being greater and more lasting than that of VIP (figs. 2C and 2D). ANOVA test showed significant differences among the samples and between the effects of VIP and PACAP.

After the infusion of PACAP, at the dose of 4 µg/kg, an increase could be observed in portal plasma ir-VIP levels (real basal values of 10.57 ± 3.04 fmol/ml), that reached maximal values (30.47 ± 9.32 fmol/ml) in the first post-stimulation sample (S3), returning to basal values in the following samples (fig. 3). These differences are statistically significant after performing a one-way ANOVA. Previously, the inexistence of crossed immunoreactivity between VIP and PACAP had been verified in our VIP assay. As shown in figure 4, crossed immunoreactivity appeared only at PACAP concentrations above 1 nM,

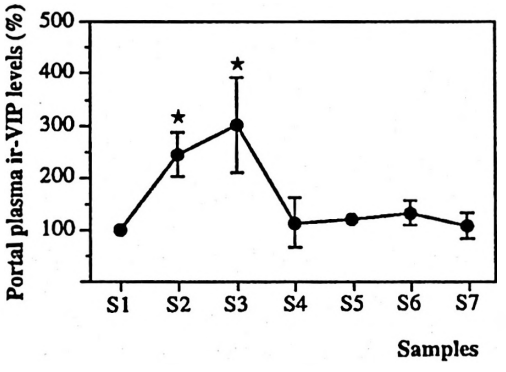


Fig. 3. *Portal plasma ir-VIP levels in rabbits infused during S2 with 4 µg/kg of PACAP.*
Values (mean ± SEM) are expressed as percent of basal (n = 6). One-way ANOVA test showed significant differences among the samples ($p < 0.01$). Results of Scheffé's test are shown as: * $p < 0.05$ (compared with the basal values).

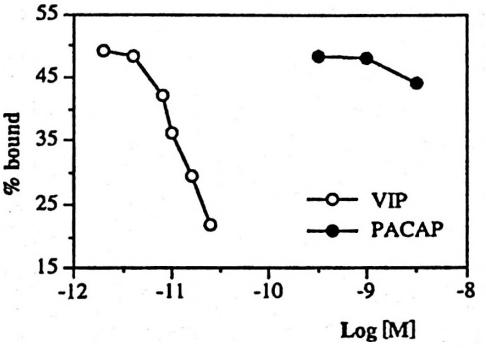


Fig. 4. *Percentage of [125I]-VIP bound to the antibody used in the VIP radioimmunoassay, in the presence of graded concentrations of VIP or PACAP.*
Crossed immunoreactivity between the two peptides appeared only at PACAP concentrations above 1 nM.

clearly greater than that which could be expected to exist in the plasma after the infusion of PACAP *in vivo*.

Mean arterial pressure decreased in a similar fashion after the infusion of 4 µg/kg of both peptides: the mean reduction in arterial pressure was 20.4 ± 3.4 mmHg for VIP and 17.9 ± 4.8 mmHg for PACAP.

## Discussion

Our results show that, in the rabbit, VIP stimulates the hydroelectrolyte fraction of the exocrine pancreatic secretion in a dose-dependent way. Furthermore, the novel peptide PACAP-27 is as effective as VIP in the stimulation of the flow rate, slightly more effective in the bicarbonate secretion, while it stimulates the enzymatic fraction of the exocrine pancreatic secretion to a greater extent than VIP at the same dose.

The effects of stimulant peptides of the exocrine pancreatic secretion have been studied while the duodenum was simultaneously perfused with phosphate buffered

saline. The reason for this procedure is that, as previously reported (19), the basal protein output from the pancreas is greater in rabbits subjected to perfusion of the duodenum than in control ones (not intraduodenally perfused), which points to the existence of an inhibitory factor in the duodenum of these animals, that is eliminated by the washing out procedure; the presence of this factor should be avoided when studying the effects of a stimulant agent. Furthermore, it has also been reported (8) that this procedure does not stimulate the release of secretin or VIP. In the control animals it has been observed that while the hydroelectrolyte secretion remained unchanged along the experimental time, the protein output progressively diminished up to values of 50 % compared with basal. This is in agreement with results previously reported for this species where the same experimental design had been used (8).

After the infusion of VIP a linear dose-response relationship could be observed for pancreatic flow rate up to the dose of 4 µg/kg; this parameter reached a maximum value of 321 % over the basal. The same occurred with the bicarbonate output, although in this case a 461 % of basal values was attained. Therefore, VIP is an effective stimulant for the hydroelectrolyte pancreatic secretion of the rabbit. However, on the basis of previous reports on the effects of secretin in this species (3, 21, 26, 27) it is evident that VIP acts as a partial agonist of secretin, with smaller efficacy. This coincides with the role ascribed to VIP in other mammals such as rats (6), dogs, (15), cats (25) and humans (7). Furthermore, the rabbit pancreas is less sensitive to VIP compared with what is described in other mammal species since a minor effect on hydroelectrolyte secretion was seen after higher doses of this peptide.

Concerning the effects of VIP on protein secretion, our results show an increase in the S2 sample, which, however, did not reach statistical significance; this points to a washout effect derived from the increase in the pancreatic flow rate. Our results are in agreement with those found by many authors in other species (7, 15, 25) though it has been described that VIP stimulates the pancreatic protein secretion in sheep (11) and also the amylase secretion in rat pancreatic acini (29). The effects of VIP on the pancreatic protein output of the rabbit can be considered similar to those produced by secretin, which is not able to significantly stimulate the enzyme secretion of this species (3, 4).

The infusion of PACAP increased the plasma levels of VIP which means that PACAP releases VIP. A possible interference of PACAP with VIP assay can be discarded, since, as it is shown in the Results section, there is a very low crossed immunoreactivity between PACAP and VIP antibodies. If our results of the dose-response curve for VIP are taken into account, the concentrations of VIP after PACAP infusion are not high enough to justify the increase observed in the pancreatic flow rate and bicarbonate output. Furthermore, the maximum effects of PACAP were observed in the S3 sample of both the pancreatic flow rate and bicarbonate output, unlike VIP, whose maximum stimulant action was elicited in the S2 sample. Therefore, the action of PACAP on the hydroelectrolyte fraction may be partially mediated by VIP.

PACAP infusion increased the pancreatic protein and amylase outputs, these increases being significantly higher than those observed after the infusion of VIP at a very similar molar concentration (given that both peptides have similar molecular weights, 3326 for VIP and 3146 for PACAP-27). Therefore, PACAP is an effective stimulant agent of the pancreatic

protein secretion in the rabbit which is in agreement with what is described in other mammals like rats (1, 20) and dogs (22). The possible mediation of VIP on the stimulation of the protein secretion by PACAP can be discarded, since concentrations of VIP much higher than those observed after PACAP infusion were only weakly able to stimulate the protein output.

Specific binding sites for PACAP-27 have been reported to exist in the acinar AR4-2J cell line derived from rat pancreas (24), but no specific receptors have yet been found for PACAP-27 in the rabbit pancreas and, as it has been suggested by NARUSE *et al.* (22) for the dog, the protein response to PACAP may be, at least in part, mediated by a cholinergic mechanism. Further experiments should be done to elucidate whether this cholinergic involvement is specific for the dog or is a general mechanism of action of this peptide.

On the other hand, the administration of VIP or PACAP has cardiovascular effects, with a marked reduction in mean arterial pressure, and therefore part of their stimulatory effect on the pancreatic secretion could be elicited by a vasodilation in the pancreas, with an increase in pancreatic blood flow. However, given that both peptides reduce arterial pressure in a similar way, it seems improbable that the differences observed in their actions could be a consequence of cardiovascular events.

In summary, PACAP-27 stimulates both the hydroelectrolyte and enzyme fractions of the exocrine pancreatic secretion, the increase of the hydroelectrolyte fraction probably being partially mediated by VIP. Therefore PACAP can be considered to be a new peptide which might have an important role as a modulator of the exocrine pancreatic secretion according to previous results obtained in rat (1, 20) and dog (22) and according to our findings in rabbit. Finally, though according to SUNDLER *et al.* (28) PACAP may be added to the list of the gut neurohormonal peptides, it is necessary to do more experiments to clarify its role in the physiology of the exocrine pancreas.

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A. M. RODRÍGUEZ-LÓPEZ, I. DE DIOS, L. J. GARCÍA, M. A. LÓPEZ y J. J. CALVO. *Estudio dosis-respuesta de los efectos del VIP sobre la secreción pancreática exocrina en el conejo. Comparación con las acciones del PACAP-27.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (1), 29-36, 1995.

Se estudia la dosis-respuesta de los efectos del VIP sobre la secreción pancreática exocrina en el conejo, y se comparan las acciones del VIP y el PACAP, a similar dosis molar, sobre la secreción pancreática exocrina. Tras la infusión de VIP se observa una relación dosis-respuesta de tipo lineal, hasta los 4 µg/kg, para el flujo de jugo pancreático y para la producción de bicarbonato. El VIP actúa como un agonista parcial de la secretina, siendo el páncreas de conejo menos sensible al VIP que el de otros mamíferos. Además, el VIP no estimula de forma significativa la producción de proteínas pancreáticas. El PACAP-27 produce una estimulación de la fracción hidroelectrolítica de la secreción pancreática similar a la del VIP, y estimula de forma significativa la producción de proteínas y de amilasas; también provoca liberación de VIP, de forma que la acción del PACAP-27 sobre la fracción hidroelectrolítica puede estar mediada parcialmente por el VIP; sin embargo, el VIP no está involucrado en el efecto del PACAP-27 sobre la secreción de enzimas pancreáticos en esta especie.

Palabras clave: Conejo, Páncreas exocrino, PACAP, VIP.

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