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Vasoactive Intestinal Polypeptide Enhances Hormone Content and Insulin Release in Cultured Fetal Rat Islets[1] (42597)

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Abstract. The effect of porcine vasoactive intestinal polypeptide (VIP) on development of the biphasic insulin release response in cultured fetal rat islets was investigated. Fetal islets, 21.5 days gestational age, were cultured for 7 days in RPMI 1640 culture medium containing either 2.8 or 11.1 m*M* glucose and subsequently challenged with 16.7 m*M* glucose in a perifusion system. Islets were exposed to VIP at a final concentration of 13.2 n*M* by adding the peptide to the perifusion buffer (acute exposure) or by adding it to the culture medium throughout the culture period (chronic exposure). Islet hormone and DNA contents were also quantitated at the end of the culture period. Acute exposure to VIP resulted in no alterations of the insulin release pattern after culture in the presence of either glucose concentration. However, chronic treatment of islets with 13.2 n*M* VIP in the presence of 2.8 m*M* glucose resulted in significant increases in the maximum rate of insulin release during the first phase and the total amount of insulin release during both phases. Similarly, islets cultured in the presence of 11.1 m*M* glucose and 13.2 n*M* VIP demonstrated enhanced biphasic insulin release patterns with increased maximum rate and total amount of release during both phases. The presence of VIP and 2.8 m*M* glucose increased islet glucagon and somatostatin contents, but islet DNA and insulin contents remained unchanged. These findings indicate that VIP plays a significant role in the *in vitro* development of the biphasic insulin release pattern and may be a factor controlling the maturation of the fetal islet *in vivo*.

Adult rat pancreatic islets respond to an acute glucose challenge with a biphasic insulin release pattern (1). Fetal and neonatal pancreatic islets have a quantitative deficiency in their biphasic insulin release response to glucose *in vivo* (2) and *in vitro* (3). Near-term fetal islets display only a slight first-phase release, whereas neonatal islets have a partial biphasic response characterized by an unsustained second-phase release. From these findings it appears that the perinatal period is important to the maturation of stimulus-secretion coupling in the B cell.

Recently Dudek *et al.* (4) studied the insulin secretory dynamics of cultured fetal rat islets and reported that elevated glucose concentrations partially enhanced the functional maturation of the fetal islets after 7 days in culture. Since fetal islets are not exposed under normal conditions to the elevated glucose levels used in the above study, other factors must influence the transition from the fetal and/or neonatal secretory response to that of the adult. In view of the incomplete effects of glucose on islet maturation, it should be noted that digestive and neural reflexes are initiated during the neonatal period, suggesting possible effects by gut hormones and/or neuropeptides. One such neuropeptide, vasoactive intestinal peptide (VIP), has been immunolocalized in nerves of rat islets (5–7) and has been found to stimulate insulin and glucagon release from both the adult (8, 9) and newborn rat pancreas (10). It was therefore decided to investigate the effects of VIP on the glucose-induced insulin release pattern of cultured fetal rat islets.

Materials and Methods. *Animals.* Time-pregnant Sprague–Dawley rats were sacrificed by cervical dislocation on Day 21.5 of gestation. The gestation period for this strain is 22–22.5 days with an accuracy of ±12 hr. The fetuses were removed and sacrificed by decapitation. Male Sprague–Dawley rats, body weight 250–300 g, were also sacrificed by decapitation. All animals were allowed free access to food and water until sacrifice.

Islet tissue culture. The tissue culture pro-

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cedure for fetal islets followed that of Hellerström *et al.* (11). Fetal pancreases were partially digested with collagenase (Sigma Chemical Co., St. Louis, MO) and cultured in RPMI 1640 culture medium (GIBCO, Grand Island, NY) containing either 2.8 or 11.1 m*M* glucose, 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT), 20 m*M* Hepes, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. For chronic experiments, 13.2 n*M* porcine VIP (Sigma Chemical Co.) was present in the culture media throughout the 7-day culture period. This amount of the peptide was chosen on the basis of the findings of Bataille *et al.*, who reported that 6.6–33 n*M* of VIP enhanced glucose-stimulated insulin release from perifused neonatal islets (10). The media were changed daily.

Adult islets were isolated by the collagenase method of Lacy and Kostianovsky (12) and otherwise cultured by the same procedure as the fetal islets except that VIP was not included in the culture media.

Islet perifusion. The perifusions were performed as described by Dudek *et al.* (4). At the end of the culture period, the islets were washed three times with a modified Krebs–Ringer bicarbonate (KRB) buffer, pH 7.4, containing 0.5 mg/ml fatty acid-free bovine serum albumin (BSA) (Sigma chemical Co.) and 10 m*M* 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes). Groups of 50 fetal islets were placed on polyester membranes (5-μm pore size, 13-mm diameter; Nuclepore Corp., Pleasanton, CA) and perifused in plastic chambers (0.95-ml volume; Millipore Corp., Bedford, MA). Twenty-five islets were placed on successive filters until each filter contained 50 islets in order to assure that a randomized sampling procedure had been followed. Adult islets were similarly treated for perifusion except that each chamber contained 30 islets. After 30 min of stabilizing perifusion with KRB containing 2.8 m*M* glucose, the islets were stimulated with a constant square wave of KRB containing 16.7 m*M* glucose (plus 13.2 n*M* VIP for the acute experiments). The perifusion was performed with a flow rate of 1 ml/min and the gas phase was 95% O_2 and 5% CO_2. Perifusate samples were collected at designated time points and kept frozen at −20°C until insulin assays were performed.

DNA and hormone assays. After 7 days in culture, 50 islets were removed, washed three times with Hanks' balanced salt solution, and sonicated prior to performing the assay procedures. DNA was assayed by the fluorometric method of Labarca and Paigen (13) using calf thymus DNA (Sigma Chemical Co.) for standard. Insulin was measured by the radioimmunoassay method of Morgan and Lazarow (14) with crystalline rat insulin (Novo Research Laboratory, Copenhagen, Denmark) for the standard. Glucagon was measured by the method of McEvoy *et al.* (15) using antiserum 20C (provided by Dr. R. C. McEvoy, Mount Sinai School of Medicine) and porcine pancreatic glucagon (Novo Research Laboratory) for the standard. Somatostatin was assayed (16) using synthetic cyclic somatostatin (Calbiochem Biochemicals, San Diego, CA) as standard and rabbit antisomatostatin serum R10 (provided by Dr. J. K. Stewart, Virginia Commonwealth University).

Expression and analysis of data. Four parameters were analyzed from the perifusion data: (i) maximum rate (highest peak) of insulin release during the first 7 min (first phase) of 16.7 m*M* glucose stimulation expressed as picograms IRI per islet per minute; (ii) maximum rate of insulin release during 8–30 min of stimulation (second phase); (iii) total amount of insulin release, expressed as picograms IRI per islet, during the first phase; and (iv) total amount of insulin release during the second phase. Hormone and DNA contents were expressed as nanograms hormone or DNA per islet. Data were analyzed using the unpaired Student *t* test and differences with $P \leq 0.05$ were considered statistically significant.

Results. Although developed for the cultured fetal islets (11), the procedures described here are also suitable for adult rat islets. Adult islets, cultured in the presence of 11.1 mM glucose for 7 days, display two phases of insulin release when challenged with a square wave of 16.7 m*M* glucose (Table I). This becomes evident when comparing the secretory parameters of maximum rate and total amount of insulin release during the previously described phase periods (1). Islets cultured in low glucose-containing medium lost their ability to respond to an acute glucose challenge. Adult islets therefore retain freshly isolated secretory patterns in this culture system, thus allowing us to make valid comparisons between cultured adult and fetal islets.

When the cultured fetal islets were chal-

TABLE I. INSULIN SECRETION FROM ADULT ISLETS AFTER 7 DAYS OF CULTURE IN 2.8 OR 11.1 m*M* GLUCOSE

	First phase		Second phase	
	2.8 m*M* glucose	11.1 m*M* glucose	2.8 m*M* glucose	11.1 m*M* glucose
Maximum rate (ng/islet/min)	0.018 ± 0.008 (3)	0.18 ± 0.02[a] (3)	0.016 ± 0.006 (3)	0.44 ± 0.10[b] (3)
Total release (ng/islet)	0.095 ± 0.006 (3)	0.67 ± 0.08[a] (3)	0.273 ± 0.10 (3)	6.75 ± 1.35[a] (3)

Note. Groups of 30 islets were challenged with a square wave of 16.7 m*M* glucose in a perifusion system. Data are expressed as the mean ± SEM for the number of observations in parentheses.
[a] $P \leq 0.01$, as compared to 2.8 m*M* glucose.
[b] $P \leq 0.05$, as compared to 2.8 m*M* glucose.

lenged with glucose in the perifusion system, they responded in a manner similar to that described previously (4). Islets cultured in the presence of high glucose secreted insulin in a biphasic manner with a significantly increased first phase and a partially developed, sustained second phase, in comparison to islets cultured in the presence of low glucose. Elevation of glucose in the culture medium enhanced both the maximum rate and the total amount of insulin release during both phases with the exception of the maximum rate for the second phase.

The addition of 13.2 n*M* VIP to the perifusion medium (acute exposure) resulted in no changes in the response by the islets to a glucose challenge, regardless of the glucose concentration during the prior culture period (data not shown). In striking contrast, chronic exposure to VIP with both low and high glucose throughout the culture period resulted in a significant enhancement of the insulin release response during both phases (Tables II and III). Since VIP exerted a tremendous enhancement of insulin release in the presence of 11.1 m*M* glucose, this lends further support to previous findings (3) that glucose does play a partial role in the development of insulin secretory dynamics.

The results in Table IV demonstrate the VIP-induced increase in islet glucagon and somatostatin contents in the presence of low glucose, paralleling those observed in the presence of 11.1 m*M* glucose alone. However, no differences were observed when VIP was added to media containing 11.1 m*M* glucose. In view of the insulin secretory data, it is especially noteworthy that VIP had no effect on islet insulin content in the presence of either glucose concentration. Islet DNA values were unaffected by glucose concentration or VIP, indicating that hyperplasia cannot account for the hormone content data.

Discussion. The autonomic innervation of

TABLE II. INSULIN SECRETION FROM FETAL ISLETS AFTER 7 DAYS OF CULTURE IN 2.8 m*M* GLUCOSE ± VIP

	First phase		Second phase	
	2.8 m*M* glucose	2.8 m*M* glucose + VIP	2.8 m*M* glucose	2.8 m*M* glucose + VIP
Maximum rate (pg/islet/min)	5.24 ± 0.73 (5)	11.09 ± 1.47[a] (7)	13.68 ± 4.72 (5)	20.86 ± 7.52 (7)
Total release (pg/islet)	40.16 ± 7.47 (5)	74.31 ± 12.76[b] (7)	80.22 ± 13.13 (5)	129.46 ± 15.87[b] (7)

Note. Groups of 50 islets were challenged with a square wave of 16.7 m*M* glucose in a perifusion system. Data are expressed as the mean ± SEM for the number of observations in parentheses.
[a] $P \leq 0.01$, as compared to 2.8 m*M* glucose.
[b] $P \leq 0.05$, as compared to 2.8 m*M* glucose.

TABLE III. INSULIN SECRETION FROM FETAL ISLETS AFTER 7 DAYS OF CULTURE IN 11.1 m*M* GLUCOSE ± VIP

	First phase		Second phase	
	11.1 m*M* glucose	11.1 m*M* glucose + VIP	11.1 m*M* glucose	11.1 m*M* glucose + VIP
Maximum rate (pg/islet/min)	25.40 ± 6.51 (6)	54.15 ± 4.93[a] (8)	20.07 ± 4.09 (6)	43.25 ± 3.69[a] (8)
Total release (pg/islet)	110.70 ± 17.50 (6)	225.80 ± 17.48[a] (8)	262.40 ± 33.39 (6)	632.95 ± 98.19[a] (8)

Note. Data are expressed as the mean ± SEM for the number of observations in parentheses.
[a] $P \leq 0.01$, as compared to 11.1 m*M* glucose.

the islets is not only cholinergic and adrenergic but peptidergic as well (17–19). Therefore, certain neuropeptides are valid candidates as factors affecting the functional maturation of the fetal islet. VIP has been localized in nerve terminals of the pancreatic islets of several species, including rats and humans (4, 5), and within intrapancreatic ganglia (5). VIP has also been shown to be insulinotropic both *in vivo* (20) and *in vitro* in the rat (8–10). Based on these findings, the present study was undertaken to ascertain the role of VIP in the development of the glucose-induced biphasic insulin release response.

In this study, VIP significantly enhanced insulin secretory dynamics in fetal islets cultured for 7 days, irrespective of the glucose concentration in the culture media. Previous findings (21) have demonstrated that 21.5-day fetal islets display a monophasic release pattern in response to a glucose challenge. Our data indicate that culturing fetal islets in the presence of VIP enhances both phases of release. However, since this enhanced release is still diminished with respect to that of the adult, VIP may represent only one of numerous extra-islet factors involved in the functional development of the islet organ. Nevertheless, the fact that this peptide, a nonnutrient, enhances glucose sensitivity of the B cell is especially significant.

This study confirms our earlier observations (4) that elevated glucose levels resulted in increased islet insulin content *in vitro*. Furthermore, islet glucagon and somatostatin contents were also increased when cultured in the presence of elevated glucose. The presence of VIP in the culture media did not affect the islet insulin content, suggesting that the overall VIP effect is on the secretory mechanism rather than on biosynthesis or storage of insulin. Glucagon and somatostatin contents also remained unchanged when VIP was present with 11.1 m*M* glucose. But the presence of VIP with 2.8 m*M* glucose increased islet glucagon and somatostatin to levels comparable to those levels found when islets were cultured in the presence of high glucose alone. While it is tempting to speculate that the increased contents of these two hormones could affect insulin release, this is uncertain since we did not analyze glucagon or somatostatin secretion.

TABLE IV. HORMONE AND DNA CONTENT IN FETAL ISLETS AFTER 7 DAYS OF CULTURE WITH AND WITHOUT VIP

Ng/islet	2.8 m*M* glucose	2.8 m*M* glucose + VIP	11.1 m*M* glucose	11.1 m*M* glucose + VIP
Insulin	6.098 ± 1.157 (9)	8.085 ± 1.958 (11)	20.683 ± 1.878[a] (13)	22.171 ± 1.762 (14)
Glucagon	0.116 ± 0.015 (10)	0.332 ± 0.044[a] (12)	0.292 ± 0.049[a] (13)	0.438 ± 0.062 (15)
Somatostatin	0.007 ± 0.001 (10)	0.013 ± 0.002[b] (13)	0.017 ± 0.003[a] (13)	0.018 ± 0.002 (15)
DNA	17.060 ± 3.045 (10)	15.792 ± 2.405 (12)	17.786 ± 2.061 (13)	20.035 ± 1.752 (15)

Note. Groups of 50 islets were removed from the culture media, sonicated, and assayed for hormone and DNA contents. Data are expressed as means ± SEM for the number of observations in parentheses.
[a] $P \leq 0.01$, as compared to 2.8 m*M* glucose.
[b] $P \leq 0.05$, as compared to 2.8 m*M* glucose.

Another surprising finding was that the islet DNA content remained unchanged regardless of the concentration of glucose or the presence of VIP in the culture media. This conflicts with our earlier data (4) which revealed that elevation of the glucose concentration in the culture medium resulted in an increase in the islet DNA content. The discrepancy between these two studies is probably best explained by the use of a different lot of fetal calf serum. Regardless, our findings on the enhanced secretory function of the β cell due to the presence of VIP, or glucose for that matter, cannot be explained on the basis of β-cell hyperplasia.

In conclusion, VIP enhances glucose-stimulated insulin secretion in cultured fetal rat islets without increasing islet insulin content. Since VIP only causes the islet to respond in an incomplete manner, this peptide may be only one of several possible endogenous substances to exert such effects.

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