Glucose Dependency of Arginine Vasopressin–Induced Insulin and Glucagon Release From the Perfused Rat Pancreas

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The purpose of this study was to investigate the glucose dependency of arginine vasopressin (AVP)-induced insulin, glucagon, and somatostatin release from the perfused rat pancreas. AVP (30 or 300 pmol/L) was tested in the presence of a glucose concentration of 0, 1.4, 5.5 (basal level), or 20 mmol/L. The rates of insulin release at 0 and 1.4 mmol/L glucose were approximately 70% to 80% and 60% to 70% less, respectively, than that at the baseline level. AVP (30 or 300 pmol/L) failed to change insulin release at 0 and 1.4 mmol/L glucose. At the basal glucose level, AVP (300 pmol/L) induced a biphasic insulin release, a peak followed by a sustained phase. In addition, the combination of glucose (20 mmol/L) and AVP (300 pmol/L) induced a higher insulin peak and sustained phase than 20 mmol/L glucose alone. The rates of glucagon release at 0 and 1.4 mmol/L glucose were about 3- and 2-fold more, respectively, than that at the baseline level. At 0 and 1.4 mmol/L glucose, both 30 and 300 pmol/L AVP caused a higher glucagon peak and sustained phase than 0 and 1.4 mmol/L glucose alone. At the basal glucose level, AVP (30 or 300 pmol/L) induced a biphasic glucagon release, a peak followed by a sustained phase. The rate of glucagon release at 20 mmol/L glucose was approximately 60% to 70% less than that at the baseline level. When AVP (300 pmol/L) was administered in 20 mmol/L glucose, it induced a transient glucagon peak, which was 2.4-fold of the baseline level. At all glucose concentrations tested, AVP (30 or 300 pmol/L) failed to change somatostatin release. These results suggested that (1) hypoglycemia directly increases glucagon and decreases insulin release; (2) AVP induces insulin and glucagon release by a direct action on β and α cells, respectively; (3) AVP induces insulin and glucagon release in a glucose-dependent manner—the higher the glucose concentration, the greater the enhancement of AVP-induced insulin release, whereas the lower the glucose concentration, the higher the enhancement of AVP-induced glucagon release; and (4) α cells are more sensitive to AVP than β cells in hormone release.

ARGININE VASOPRESSIN (AVP), a neurohypophysial nonapeptide hormone, is synthesized in supraoptic and paraventricular nuclei of the hypothalamus. After being synthesized, it is stored in neurosecretory granules and released from the posterior pituitary gland.[1] AVP is also found in extrapituitary tissues including adrenal gland[2] and pancreas.[3] AVP exerts a number of physiologic roles in mammals; it plays a major role in regulating body fluid volume, osmolality, and maintenance of blood pressure. In addition, AVP induces glycogenolysis,[4] proliferation of the pituitary gland[5] and vascular smooth muscle cells,[6] vasoconstriction,[7] and release of corticotropin (ACTH),[8] catecholamine,[9] insulin,[10,11] and glucagon.[12,13]

AVP stimulates insulin and glucagon release via the activation of both phospholipase C (PLC)-dependent and -independent pathways.[11-15] V_{1B} receptors mediate AVP-induced insulin release in the perfused rat pancreas and clonal β cells RINm5F,[16] and glucagon release in the perfused rat pancreas and clonal α cells InR1G9.[12,13] AVP induces insulin[11] and glucagon[17] release through Ca^{2+}–dependent and -independent pathways. For the Ca^{2+}-dependent pathway, G_q protein activates PLC, which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). Diacylglycerol activates protein kinase C and IP_3 induces Ca^{2+} release from the endoplasmic reticulum, which leads to Ca^{2+} influx. Both Ca^{2+} release and Ca^{2+} influx contribute to AVP-induced insulin and glucagon release.[11,17]

AVP may regulate glucose metabolism by its direct glycogenolytic and gluconeogenic effects on the liver,[4] and by modulating insulin and glucagon release from the endocrine pancreas. The fact that AVP is present in the pancreas[3] suggests that AVP may exert a local control on the endocrine pancreas. The effects of AVP on the endocrine pancreas, insulin release in particular, are controversial. AVP caused a concentration-dependent stimulation of glucagon release but failed to influence insulin release from isolated rat islets in medium containing 5.6 mmol/L glucose.[18] In isolated mouse islets, AVP failed to change insulin release at ≤7 mmol/L glucose.[19,20] This implies that AVP is not a primary insulin secretagogue, but is an enhancer of glucose-induced insulin secretion. In the perfused rat pancreas, AVP in 5.5 mmol/L glucose caused a small insulin release.[21] In contrary, AVP inhibited glucose-induced insulin release in rat islets[22] and in clonal β cells HIT-T15.[23] AVP (1 to 100 nmol/L) failed to stimulate somatostatin release in the absence of extracellular glucose; however, AVP increased somatostatin release in the presence of glucose (3 to 30 mmol/L).[20] Since there are discrepancies in the effects of AVP on the endocrine pancreas, insulin release in particular, the present study was designed to study the direct effect of AVP (30 or 300 pmol/L) on insulin, glucagon, and somatostatin release from the perfused rat pancreas in the presence of various glucose concentrations (0, 1.4, 5.5, and 20 mmol/L). We also investigated the direct effects of various glucose concentrations on insulin, glucagon, and somatostatin release.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (350 to 450 g) were used. The rats were anesthetized with pentobarbital sodium (60 mg/kg, intraperitoneally), and were maintained at 37°C on a hot plate during the experiment. Pancreatic perfusion was performed as previously described.[24] Briefly, after cannulation of the celiac artery, the rat pancreas was immediately perfused with Krebs-Ringer bicarbonate (KRB) solution supplemented with 20 mmol/L HEPES, 5.5 mmol/L glucose, 1% dextran, and 0.2% bovine serum albumin as a basal medium. The KRB was maintained at pH 7.4 and continuously aerated with 95% O_2 -5% CO_2. The rats were euthanized by the induction of pneumothorax immediately following the cannulation of the portal vein and the beginning of the flow.

Experimental Design

The first 20 minutes of perfusion was considered as an equilibration period. Subsequently, the effluent fluid was collected every minute from the cannula in the portal vein. The flow rate was set at 1 mL/min. After a baseline period of 12 minutes, the medium containing glucose (0, 1.4, 5.5, or 20 mmol/L) was administered for 30 minutes with or without AVP (30 or 300 pmol/L). This was followed by a washout period during which the basal medium was administered for 10 minutes. AVP concentrations of 30 and 300 pmol/L were chosen because in our preliminary experiments, AVP (3 pmol/L) caused a small increase in glucagon release and failed to increase insulin release. The effluent fluids were kept at 4°C and assayed within 6 hours for insulin, glucagon, and somatostatin using radioimmunoassys as previously described.[12,25,26]

Test Agents

AVP was purchased from Sigma Chemical Co (St Louis, MO), and dissolved in distilled water to make a stock solution (100 μmol/L). This solution was further diluted with KRB (basal medium) to attain appropriate AVP concentrations. Glucagon and rat insulin standards were donated by Eli Lilly laboratories (Indianapolis, IN). Insulin antibody was donated by Dr V. Leclercq-Meyer of the Free University of Brussels, Belgium. Glucagon antibody was donated by Dr Joseph Dunbar of Wayne State University (Detroit, MI). Somatostatin antibody was donated by Dr Y.N. Kenny Kwok of the University of British Columbia, Canada. ^{125}I-glucagon was purchased from Linco Research (St Charles, MO). Somatostatin standard was purchased from Sigma Chemical Co.

Data Expression and Statistical Analysis

The effluent concentrations of insulin, glucagon, and somatostatin were expressed as a percentage of the baseline level (mean of last 5 baseline values) in mean ± SE. The area under the curve (AUC) values for the different concentrations of glucose with or without AVP treatment period of 30 minutes were calculated using the Transforms and Regressions (Sigma Plot 5.0; SPSS Inc, Chicago, IL). One-way analysis of variance (ANOVA) was used to determine the effect of glucose concentrations on insulin and glucagon AUCs. Two-way ANOVA was used to determine the effect of AVP and glucose concentrations on insulin and glucagon AUCs. Individual mean comparisons were calculated using the F test. The significance level was set at $P < .05$.

RESULTS

Effects of Glucose on Insulin and Glucagon Release

Insulin release remained constant in the basal glucose control group (5.5 mmol/L) throughout the perfusion period (Fig 1A). In the absence of extracellular glucose or the presence of a low glucose concentration (1.4 mmol/L), insulin release was approximately 70% to 80% and 60% to 70% less than that at the baseline level, respectively (Fig 1A). Calculating the AUCs indicated that both the 0- and 1.4-mmol/L glucose groups had a significantly lower insulin release than the basal group. The higher concentration of glucose (20 mmol/L) induced a biphasic insulin release. The first transient insulin release peak was about 5-fold of the baseline level, followed by a sustained increase that was about 9-fold of the baseline level (Fig 1A). By calculating the AUCs for the 20-mmol/L glucose group and

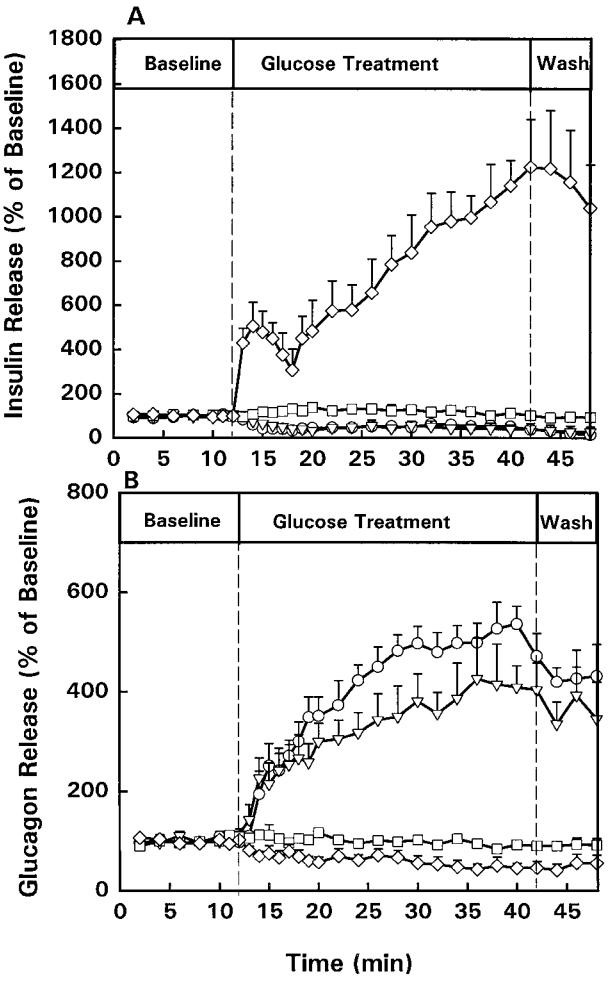

Fig 1. Effect of glucose (0, 1.4, 5.5, and 20 mmol/L) on (A) insulin and (B) glucagon release from the perfused rat pancreas. In this and all other figures, basal glucose (5.5 mmol/L) was administered during the equilibration period of 20 minutes preceded time 0 followed by another 12 minutes of the baseline period. Various concentrations of glucose were given for 30 minutes (glucose treatment), followed by 8 minutes of the washout period (wash). By calculating the areas under the curves for glucose (0, 1.4, and 20 mmol/L) and comparing them with those of the control group (5.5 mmol/L glucose), both 0 and 1.4 mmol/L glucose significantly ($P < .05$) decreased insulin release, whereas 20 mmol/L glucose significantly increased insulin release. In contrast, both 0 and 1.4 mmol/L glucose significantly increased glucagon release, whereas 20 mmol/L glucose significantly decreased glucagon release. Values are the mean ± SE (n = 4). (□) Basal control (5.5 mmol/L glucose); (○) 0 glucose; (▽) 1.4 mmol/L glucose; (◇) 20 mmol/L glucose. Range of baseline insulin and glucagon concentrations of effluents were 1,045 to 2,507 and 50 to 68 pg/mL, respectively.

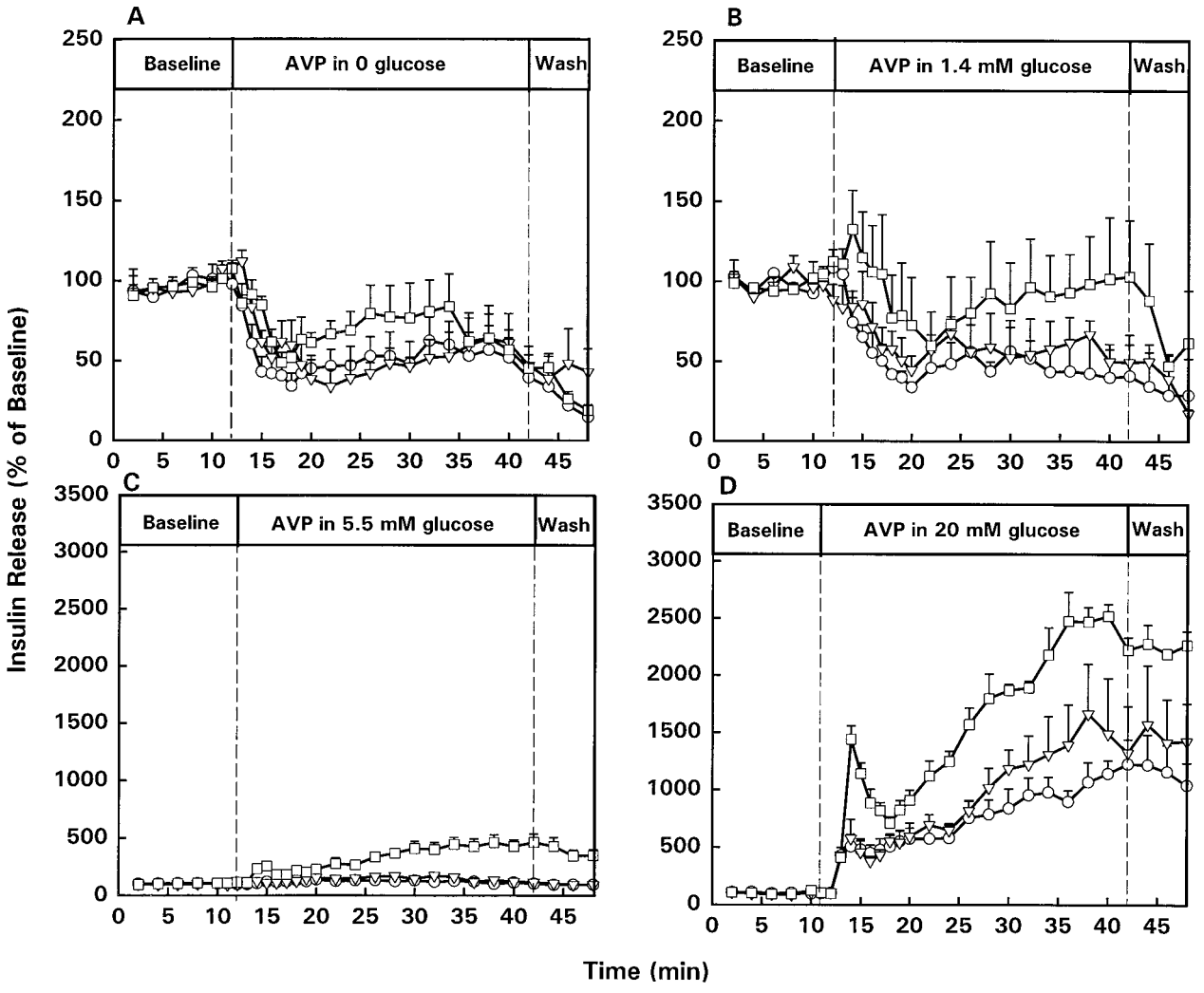

Fig 2. Effect of AVP (30 and 300 pmol/L) on insulin release from the perfused rat pancreas. The treatment protocol was the same as in Fig 1. AVP was given for 30 minutes. Glucose concentrations were (A) 0, (B) 1.4, (C) 5.5, and (D) 20 mmol/L. By calculating the AUCs for AVP and comparing them with those of the control group, 30 pmol/L AVP did not significantly change insulin release in all tested glucose concentrations, whereas 300 pmol/L AVP significantly ($P < .05$) increased insulin release at 5.5 and 20 mmol/L glucose. Values are the mean ± SE (n = 4). (▽) Control (glucose without AVP); (□) AVP (30 pmol/L); (○) AVP (300 pmol/L). Range of baseline insulin concentrations of effluents was 1,045 to 3,137 pg/mL. The *F* ratios are as follows: AVP concentration, $P < .0001$; glucose concentration, $P < .0001$; AVP concentration × glucose concentration, $P < .0001$.

comparing them with those of the basal glucose group, 20 mmol/L glucose significantly increased insulin release.

Glucagon release remained constant in the basal glucose group throughout the perfusion period (Fig 1B). In the absence of extracellular glucose or in the presence of 1.4 mmol/L glucose, glucagon release was approximately 3- and 2-fold higher than the baseline level, respectively (Fig 1B). Relative to the level at the basal glucose concentration, glucagon release was significantly higher at the low glucose concentrations (0 and 1.4 mmol/L). The higher concentration of glucose (20 mmol/L) significantly inhibited glucagon release, which was about 60% to 70% less than the baseline level (Fig 1B). Relative to the level at the basal glucose concentration, glucagon release was significantly lower at the high glucose concentration (20 mmol/L).

Effects of AVP on Insulin Release

At the basal glucose concentration, AVP (30 pmol/L) failed to change insulin release, while AVP (300 pmol/L) induced a biphasic insulin release; a peak followed by a sustained phase (Fig 2C). By calculating the AUCs for the AVP (300 pmol/L) group and comparing them with those of the basal glucose group, AVP (300 pmol/L) significantly increased insulin release. Neither 30 nor 300 pmol/L of AVP significantly increased insulin release at 0 or 1.4 mmol/L glucose (Fig 2A and B).

AVP (300 pmol/L) at 20 mmol/L glucose increased insulin release with a peak and a sustained phase of approximately 15- and 25-fold of the baseline level, respectively (Fig 2D). By calculating the AUCs for AVP (300 pmol/L) in the 20-mmol/L

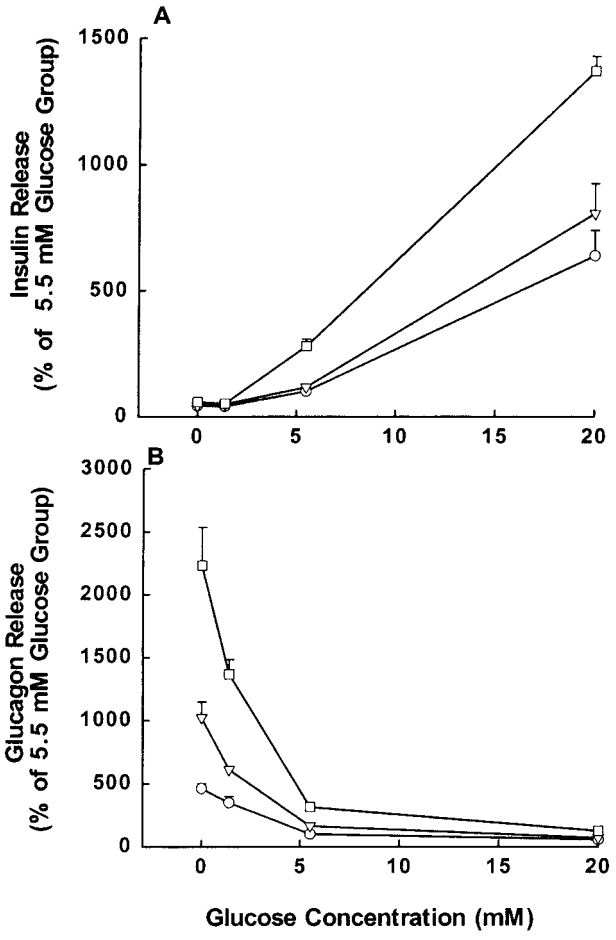

Fig 3. Schematic representation of data plotted showing the glucose dependency. Values are the mean ± SE (n = 4), obtained by calculating AUCs of the 30-minute (A) insulin or (B) glucagon release and expressed as a percentage of basal glucose (5.5 mmol/L) control group. (○) 0 AVP; (▽) AVP (30 pmol/L); (□) AVP (300 pmol/L).

glucose group and comparing them with those of the 20-mmol/L glucose alone group, AVP (300 pmol/L) in 20 mmol/L glucose significantly had a higher insulin release than glucose alone. The insulin release induced by AVP (30 pmol/L) at 20 mmol/L glucose was not significantly different from that induced by 20 mmol/L glucose alone (Fig 2D). A summary of AVP (30 and 300 pmol/L)-induced insulin release in various glucose concentrations is shown in Fig 3A, which demonstrates that the insulinotropic effect of AVP is glucose-dependent.

Effects of AVP on Glucagon Release

At the basal glucose level, both 30 and 300 pmol/L of AVP increased glucagon release; a peak followed by a sustained phase (Fig 4C). AVP 30 pmol/L increased glucagon release with a peak and a sustained phase of approximately 2- and 0.5-fold of the baseline level, respectively, whereas AVP 300 pmol/L caused a peak of approximately 5- and a sustained phase of about 3-fold of the baseline level, respectively (Fig 4C). By calculating the AUCs for the AVP (30 and 300 pmol/L) groups and comparing them with those of the basal glucose group, both 30 and 300 pmol/L AVP significantly increased glucagon release.

In the absence of extracellular glucose, AVP (30 pmol/L) increased glucagon release with a peak and a sustained phase of about 5- and 10-fold of the baseline level, respectively, whereas AVP (300 pmol/L) caused a peak and a sustained phase of about 11- and 35-fold of the baseline level, respectively (Fig 4A and B). In the presence of 1.4 mmol/L glucose, AVP (30 pmol/L) increased glucagon release with a peak and a sustained phase of approximately 5- and 7-fold of the baseline level, respectively, whereas AVP (300 pmol/L) caused a peak and a sustained phase of approximatly 5- and 21-fold of the baseline level, respectively (Fig 4A and B). By calculating the AUCs for AVP (30 and 300 pmol/L) in the 0- or 1.4-mmol/L glucose groups and comparing them with those of the 0- or 1.4-mmol/L glucose groups, both 30 and 300 pmol/L AVP had significantly higher glucagon release than 0 or 1.4 mmol/L glucose alone.

The high concentration of glucose (20 mmol/L) significantly inhibited glucagon release, under this condition, AVP (30 pmol/L) failed to change glucagon release (Fig 4D). However, AVP (300 pmol/L) at 20 mmol/L glucose induced a significant glucagon release for 5 minutes when compared to the glucose alone group (Fig 4D). A summary of AVP (30 and 300 pmol/L)-induced glucagon release in various glucose concentrations is shown in Fig 3B, which demonstrates that the glucagonotropic effect of AVP is glucose-dependent.

Neither glucose alone (0, 1.4, 5.5, or 20 mmol/L) nor AVP (30 or 300 pmol/L) in glucose (0, 1.4, 5.5, or 20 mmol/L) changed somatostatin release from the perfused pancreas (data not shown).

DISCUSSION

In the present study, AVP evoked both the release of insulin and glucagon from the perfused rat pancreas in a glucose concentration-dependent manner. AVP (30 and 300 pmol/L) failed to induce insulin release at the glucose concentrations of 0 and 1.4 mmol/L. However, in the presence of basal glucose level (5.5 mmol/L), AVP (300 pmol/L) increased insulin release and induced a higher increase in insulin release at 20 mmol/L than at basal glucose level. These finding suggested that the higher the glucose concentration, the greater the enhancement of AVP-induced insulin release. In the mouse islets, AVP (100 nmol/L) in the presence of 0, 3, or 7 mmol/L glucose failed to induce insulin release,[20] while at higher glucose concentrations (10 to 30 mmol/L), AVP (1 to 100 nmol/L) enhanced the glucose-induced insulin release.[20] These investigators concluded that AVP was not an initiator of insulin release but only enhanced glucose-induced insulin release. In contrast, our study demonstrated that lower concentration of AVP (300 pmol/L) not only enhanced glucose-induced insulin release, but also initiated insulin release at the basal glucose level. The discrepancy between these findings may be attributed to the use of 2 different preparations. In our study we used the perfused pancreas, which requires a much less invasive procedure than the use of isolated islets. In addition, Gao et al[20] preincubated the mouse islets for 60 minutes to permit the recovery from the isolation procedure. This period of incubation may not be sufficient, because a minimum incubation of 24 to 48 hours is

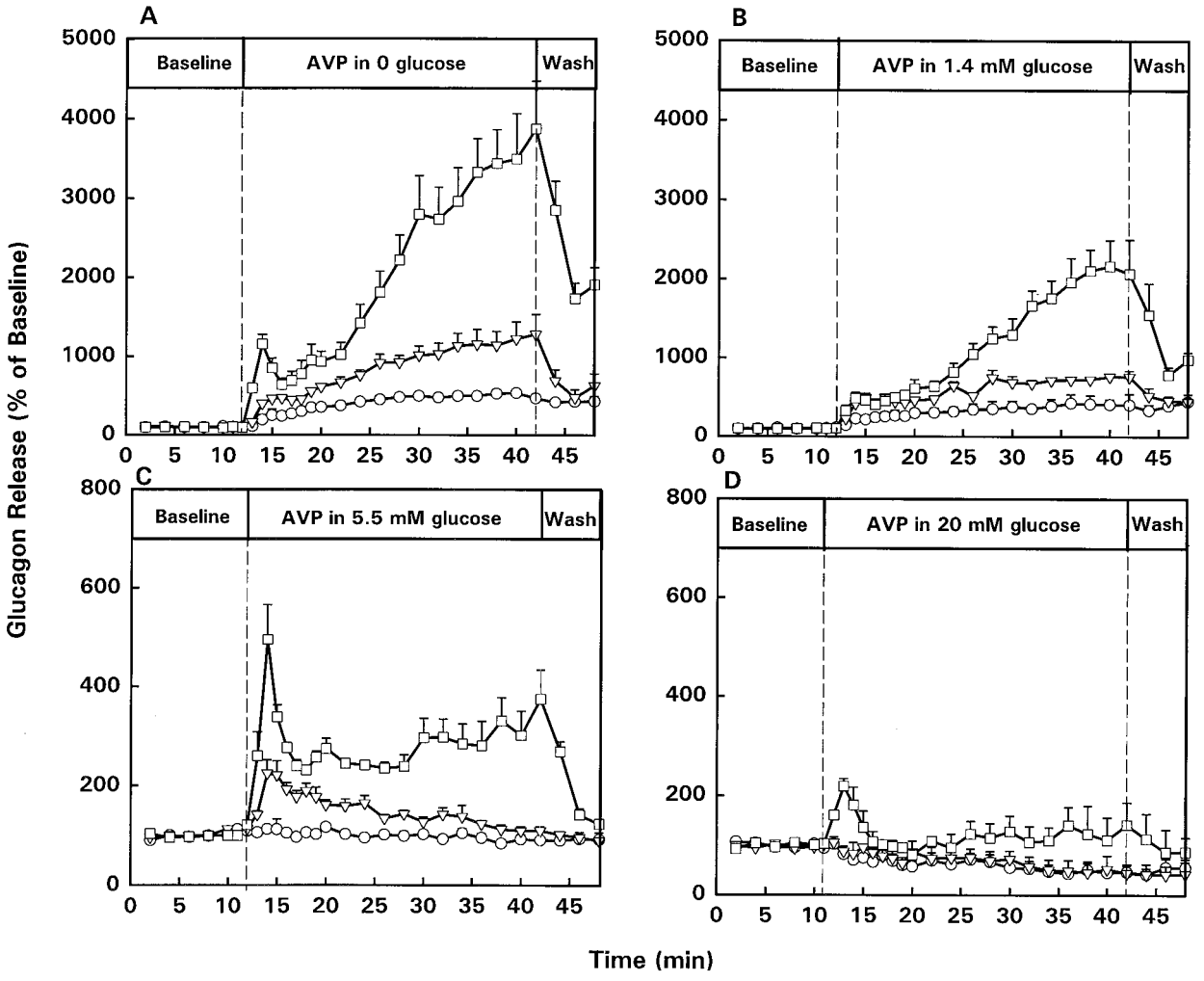

Fig 4. Effect of AVP (30 and 300 pmol/L) on glucagon release from the perfused rat pancreas. The treatment protocol was the same as in Fig 1. AVP was given for 30 minutes. Glucose concentrations were (A) 0, (B) 1.4, (C) 5.5, and (D) 20 mmol/L. By calculating the AUCs for AVP and comparing them with those of the control group, both 30 and 300 pmol/L AVP significantly ($P < .05$) increased glucagon release at 0, 1.4, and 5.5 mmol/L glucose, whereas only 300 pmol/L AVP significantly increased glucagon release at 20 mmol/L glucose. Values are the mean ± SE (n = 4). (○) Control (glucose without AVP); (▽) AVP (30 pmol/L); (□) AVP (300 pmol/L). Range of baseline glucagon concentrations of effluents was 50 to 75 pg/mL. The *F* ratios are as follows: AVP concentration, $P < .0001$; glucose concentration, $P < .0001$; AVP concentration × glucose concentration, $P < .0001$.

essential for receptor recovery.[27] This may also explain why in the mouse islets high concentrations of AVP were used to demonstrate AVP's effects and why AVP failed to initiate insulin release.

We also investigated the effects of different glucose concentrations on insulin and glucagon release from the perfused rat pancreas. The rate of insulin release in the presence of 0 and 1.4 mmol/L glucose was approximately 70% to 80% and 60% to 70% less, respectively, than the baseline level. The basal glucose concentration (5.5 mmol/L) maintained a constant level of insulin through out the perfusion period, whereas a higher concentration of glucose (20 mmol/L) induced a biphasic release pattern; a transient peak followed by a sustained increase with a greater magnitude. Similar findings have been reported in the perfused rat pancreata[28,29] and human islets.[30,31] On the other hand, the rates of glucagon release in the presence of 0 and 1.4 mmol/L glucose were about 3- and 2-fold more, respectively, than that at the basal glucose level. During hypoglycemic stress, glucagon release is increased and insulin release is decreased. Increased glucagon release is the main counterregulatory factor in the recovery from hypoglycemia.[32,33] However, the mechanism underlying hypoglycemia-induced glucagon release is uncertain. A number of studies suggest that the increase in release is due to the activation of autonomic nervous system.[34-37] In contrast, our results suggested that the low glucose level directly increases glucagon release, because these experiments were performed in the perfused pancreas where there was no input from the autonomic nervous system. This finding is consistent with what have been found in the isolated islets[37] and perfused pancreata.[38] The

basal glucose concentration maintained a constant level of glucagon through out the perfusion period, whereas a higher concentration of glucose (20 mmol/L) inhibited glucagon release, which was approximately 60% to 70% less than the baseline level.

AVP (30 and 300 pmol/L) increased glucagon release at 0, 1.4, or 5.5 mmol/L glucose, whereas only 300 pmol/L AVP increased glucagon release at 20 mmol/L glucose. Thus, this increase was in a glucose concentration-dependent manner; the lower the glucose concentration, the greater the enhancement of AVP-induced glucagon release. In experiments using mouse islets, as expected, AVP induced a large increase in glucagon release at 0 glucose and less glucagon release at glucose concentrations of 3 to 7 mmol/L.[20] Surprisingly, in mouse islets at higher glucose concentrations (15 to 20 mmol/L), AVP increased glucagon release to the same extent as at 0 glucose.[20] We failed to observe such changes in the perfused rat pancreas. AVP (3 to 30 pmol/L) increased glucagon but not insulin release from the perfused rat pancreas (unpublished data, January 1999). These findings suggested that α-cells are more sensitive to AVP than β cells in hormone release. Our findings are consistent with those obtained from the perfused rat pancreas[21] and isolated rat islets,[18] but are different from those obtained from mouse islets.[20] In addition, these findings indicated that AVP may physiologically increase glucagon release, since AVP increased glucagon release at similar plasma concentrations of AVP ($\leq$30 pmol/L),[39] while it failed to increase insulin release at these concentrations.

AVP increased insulin and glucagon release but did not affect somatostatin release. This precludes that somatostatin may influence AVP- or glucose-induced insulin and glucagon release. The findings that AVP induced a large increase in glucagon release at low glucose concentrations without changing insulin release supports the notion that AVP has a direct effect on α cells. In addition, the higher glucose concentration (20 mmol/L) inhibited glucagon release, which was approximately 60% to 70% less than the baseline level. Under these conditions, AVP (30 pmol/L) failed to change glucagon release, whereas AVP (300 pmol/L) only induced a transient glucagon release. On the other hand, AVP (300 pmol/L) induced a large increase in insulin release, which supports the notion that AVP also has a direct effect on β cells.

In conclusion, AVP may increase insulin and glucagon release by a direct action on β and α cells, respectively. These increases are glucose-dependent; the higher the glucose concentration, the greater the enhancement of AVP induced insulin release. In contrast, the lower the glucose concentration, the higher the enhancement of AVP induced glucagon release. AVP not only can enhance glucose-induced insulin release, but also can initiate insulin release. Alpha cells are much more sensitive to AVP than β cells in hormone release. Furthermore, our results confirmed the previous findings that hypoglycemia directly increases glucagon and decreases insulin release.

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