**Mechanisms of the Stimulation of Insulin Release by Arginine-Vasopressin in Normal Mouse Islets**

Zhi Yong Gao, Gisela Drews, Myriam Nenquin, Tim D. Plant, and Jean-Claude Henquin

From the Unité de Diabetologie et Nutrition, University of Louvain Faculty of Medicine, UCL 54.74, B-1200 Brussels, Belgium and the I. Physiologisches Institut, University of Saarland, D-6650 Homburg, Federal Republic of Germany

The mechanisms by which arginine-vasopressin (AVP) affects pancreatic B-cell function were studied in normal mouse islets. AVP produced a dose-dependent (0.1–1000 nM; EC50 ≈ 1–2 nM) amplification of glucose-induced insulin release. This amplification was of slow onset and reversibility. AVP was ineffective when the concentration of glucose was <7 mM, but was still very effective in 30 mM glucose. The increase in insulin release produced by AVP was accompanied by small accelerations of 86Rb and 46Ca efflux from islet cells. Omission of extracellular Ca2+ accentuated the effect of AVP on 86Rb efflux, attenuated that on 46Ca efflux, and abolished that on release. Under no condition did AVP inhibit 86Rb efflux. AVP did not significantly affect AMP levels, but increased inositol phosphate levels in islet cells, even in the absence of extracellular Ca2+. AVP did not affect the membrane potential in unstimulated B-cells and augmented glucose-induced electrical activity only slightly. This was not due to a direct action on ATP-sensitive K+ channels or from the insulin-secreting cell line RINm5F. Two additional effects of AVP are also unclear. An increase in cAMP levels was observed in rat islets stimulated by AVP (13). This could be suggestive of the existence of a V2-type receptor (16), but cannot explain the inhibition of insulin release that was concomitantly observed.

An increase in total inositol phosphates was observed in RINm5F cells stimulated by AVP (15). However, this effect, which is suggestive of the existence of a V1-type receptor (16), was not considered to be important for the small increase in insulin release produced by AVP. Two additional effects of AVP in RINm5F cells have been reported very recently (17). It mobilized intracellular Ca2+ and depolarized the plasma membrane by closing ATP-sensitive K+ channels. The latter observation is extremely interesting because of the central role of these channels in the control of B-cell function.

The present study was an attempt to characterize the effects of AVP on pancreatic B-cells from normal mice. Evidence will be presented that AVP potently amplifies glucose-induced insulin release by mechanisms that involve stimulation of phosphoinositide metabolism rather than changes in AMP levels or in B-cell membrane potential.

**Regulation of Pancreatic B-Cell Function**

The mechanisms by which AVP exerts glycogenolytic and gluconeogenic actions on the liver (1, 2). These effects are, at least partly, due to a receptor-mediated hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate with subsequent mobilization of intracellular Ca2+ by inositol 1,4,5-trisphosphate, and activation of protein kinase C by diacylglycerol (3, 4).

On the other hand, the effects of AVP on the endocrine pancreas, in particular on insulin release, are controversial. Intravenous or intramuscular injection of AVP was found to cause a moderate increase in plasma insulin levels in man (5), rabbits (6), and rats (7). However, in other in vivo studies, AVP was found to be ineffective in dogs (8, 9), neonatal sheep (10), and man (11, 12). Contradictory results have also been obtained in vitro. It has been reported that AVP inhibits (13) or does not affect (7) insulin release from isolated rat islets, but stimulates it slightly from the perfused rat pancreas (14) or from the insulin-secreting cell line RINm5F (15).

The cellular pathways involved in these effects of AVP are also unclear. An increase in cAMP levels was observed in rat islets stimulated by AVP (13). This could be suggestive of the existence of a V2-type receptor (16), but cannot explain the inhibition of insulin release that was concomitantly observed. An increase in total inositol phosphates was observed in RINm5F cells stimulated by AVP (15). However, this effect, which is suggestive of the existence of a V1-type receptor (16), was not considered to be important for the small increase in insulin release produced by AVP. Two additional effects of AVP in RINm5F cells have been reported very recently (17).

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**EXPERIMENTAL PROCEDURES**

**Measurements of Insulin Release and Ionic Fluxes**—All experiments were performed with islets isolated by collagenase digestion of the pancreas of fed female NMRI mice (25–30 g), killed by decapitation. The medium used was a bicarbonate-buffered solution which contained 120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, and 24 mM NaHCO3. It was gassed with O2/CO2 (94:6) to maintain pH 7.4 and was supplemented with bovine serum albumin (1 mg/ml). Ca2+-free solutions were prepared by replacing CaCl2 with MgCl2.

For the experiments in which only insulin release was measured, islets were first preincubated for 90 min in a medium containing 15 mM glucose. Batches of 25 islets were then placed in parallel chambers and perfused at 37°C at a flow rate of 1.3 ml/min (18). Each effluent fraction, collected at 2 min intervals, insulin was measured...
were subdivided into three to four batches of 40-50 islets. Each batch was efflux, islets were first loaded with the tracers during 90 min of incubation in a medium containing 15 mM glucose. Batches of 25-30 islets were then perifused in the same system as above. During the experiments of 82Rb efflux, a portion of each effluent fraction was taken for measurement of insulin.

Measurement of Inositol Phosphates—Immediately after isolation, islets were loaded with [2-3H]inositol during 2 h of incubation in 100 μL of medium containing 15 mM glucose and supplemented with 20 μCi of the tracer. After being washed five times with a medium supplemented with 1 μM nonradioactive inositol, the islets were subdivided into three to four batches of 40-50 islets. Each batch of islets was then incubated in a medium containing 15 mM glucose, 1 mM inositol, and 5 mM LiCl with or without AVP or acetylcholine (ACh). The incubation was stopped by the addition of 3 ml of CHCl3/CH30H/concentrated HCl (200:100:1, v/v) and 100 μl of EDTA (10 mM). The tubes were then vortexed for 30 s and centrifuged for 10 min at 1500 x g to separate the lipid and water phases. Free inositol and inositol phosphates were separated by anion exchange chromatography. The aqueous phase was applied to a column containing 1.5 ml of Dowex 1-x8, 100-200 mesh, formate form. The different fractions were eluted stepwise by addition of ammonium formate solutions of increasing ionic strengths (20).

Measurement of cAMP—After isolation, the islets were first preincubated for 60 min in a medium containing 15 mM glucose. Batches of 12 islets were then incubated in a medium containing 10 or 15 mM glucose, with or without AVP or forskolin. At the end of the incubation, the tubes were chilled on ice and the islets were transferred into acetate buffer. Islet cAMP concentration was then determined by radioimmunoassay with a commercially available kit (Du Pont-New England Nuclear) after acetylation of samples (21). Portions of the incubation medium were saved for insulin measurement.

Recordings with Intracellular Microelectrodes—A piece of mouse pancreas was fixed in a perfusion chamber and a few islets were partially microdissected by hand. The membrane potential of a single cell within the islet was continuously measured with a high resistance electrode (22). B-cells were identified by the typical electrical activity that they display in the presence of 10–15 mM glucose. The temperature was 37 °C, and the perfusion medium was similar to that described above except for the absence of albumin.

Patch-Clamp Experiments—Patch-clamp measurements were performed in single mouse B-cells cultured for 1-2 days (23). B-cells were identified by the presence of ATP-sensitive K+ channels in the cell-attached mode or by the development of the ATP-sensitive current in whole cell. ATP-sensitive currents are not present in A-cells (24), and other cell types compose only a very small proportion of the population.

ATP sensitive K+ currents were measured at room temperature (20–22 °C) in the whole cell and outside-out modes of the patch-clamp technique. The method of measurement in the whole cell mode has been described previously (25, 26). For measurements from outside-out patches, the pipette was withdrawn from the cell, excising a patch of the membrane with a patch pipette and ATP-sensitive K+ current under whole cell conditions. Currents were measured at a potential of 0 mV. Recordings were made in plastic Petri dishes reduced in volume to about 0.6 ml. The dish was continuously perfused at a rate of about 2 ml/min by an "extracellular" solution containing: 135 mM NaCl, 5.6 mM KCl, 2.6 mM CaCl2, 1.2 mM MgCl2, 0.5 mM glucose, 10 mM HEPES, titrated to pH 7.4 with NaOH. Pipettes were filled with an "intracellular" solution containing: 130 mM KCl, 4 mM MgCl2, 2 mM CaCl2, 10 mM EGTA, 0.65 or 0.3 mM Na2ATP, 0.1 mM Na3GTP, and 20 mM HEPES, titrated to pH 7.15 with KOH.

Materials—Arginine-vasopressin was obtained from Peninsula Laboratories (Belmont, CA). The same batch was used for all experiments. Acetylcholine chloride and GTP were from Sigma, forskolin from Calbiochem-Behring, Dowex 1 X8 from Bausch (Buchs, Switzerland), formic acid, ammonium formate, and sodium tetraborate were from Janssen Chimica (Beerse, Belgium), EGTA from Serva (Heidelberg, Federal Republic of Germany), ATP and HEPES from Boehringer, tolbutamide from Hoechst AG (Frankfurt/Main, FRG), and all other reagents were from Merck A.G. (Darmstadt, FRG). Radiochemicals were purchased from the Radiochemical Center (Amer sham, Bucks, United Kingdom).

Presentation of Results—Certain electrophysiological experiments are illustrated by recordings which are representative of the indicated number of experiments. Otherwise, results are presented as means ± S.E. for the indicated number of experiments (different animals or islet preparations) or batches of islets. The statistical significance of differences between means was assessed by Student’s t test or by analysis of variance followed by Dunnett’s test when more than two groups were compared. Differences were considered significant at p < 0.05.

RESULTS

Effects of AVP on Insulin Release—The time course and dose dependency of the effects of AVP on insulin release were studied with perfused islets. Addition of 1 or 100 nM AVP to a medium containing 10 mM glucose and 2.5 mM Ca2+ was followed by a progressive increase in insulin release (Fig. 1). The rate of release typically started to plateau after 20–30 min of stimulation with AVP. This was confirmed in experiments in which the stimulation was prolonged for 60 min (results not shown). The effect of AVP was slowly reversible on withdrawal of AVP from the medium (Fig. 1).

Fig. 2 shows the dose dependency of the increase in insulin release brought about by AVP in the presence of 10 or 15 mM glucose in the perfusion medium. The effect of 0.1 nM AVP was not statistically significant by analysis of variance. However, this low concentration of AVP consistently increased insulin release in the presence of 15 mM glucose. The half maximal increase was observed at about 1–2 nM AVP, and the maximal response was produced or almost produced by 100 nM AVP. This concentration was, therefore, used in all further experiments.

Fig. 3 shows the sigmoidal relationship between the concentra-
Vasopressin Stimulation of Insulin Release

FIG. 3. Effects of AVP on insulin release from mouse islets perfused with a medium containing various concentrations of glucose. The experimental protocol was similar to that illustrated in Fig. 1. Steady state insulin release is given as the average rate of release between 64 and 70 min in the presence or in the absence of 100 nM AVP. Values are means ± S.E. for five to nine experiments.

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FIG. 4. Effects of AVP on $^{45}$Ca efflux, $^{86}$Rb efflux, and insulin release from mouse islets perfused with a medium containing 15 mM glucose (G) and 2.5 mM Ca$^{2+}$. AVP (100 nM) was added between 40 and 70 min. Control experiments without AVP are shown by the broken lines. Insulin release was measured in the experiments of $^{86}$Rb efflux. Values are means ± S.E. for five to six experiments.

Effects of AVP on Ionic Fluxes—AVP (100 nM) accelerated $^{45}$Ca and $^{86}$Rb efflux from islets perfused with a medium containing 15 mM glucose and 2.5 mM Ca$^{2+}$. These effects were of rapid onset but of small amplitude. They did not display the slowly rising pattern of the stimulation of insulin release (Fig. 4).

When the medium did not contain Ca$^{2+}$, AVP (100 nM) was without effect on the low rate of insulin release but still increased $^{45}$Ca and $^{86}$Rb efflux (Fig. 5). The stimulation of $^{45}$Ca efflux was similar to that seen in the presence of Ca$^{2+}$ during the first minutes of stimulation, but subsequently faded out. In contrast, the acceleration of $^{86}$Rb efflux was clearly larger than in the presence of Ca$^{2+}$.

Experiments were also made in the absence of glucose. Under these conditions, the rate of $^{86}$Rb efflux decreases with time. This decrease was unaffected by 100 nM AVP: 2.91 ± 0.06% per min at 40 min, 2.00 ± 0.09%/min at 70 min in control islets, and 2.10 ± 0.04%/min at 70 min in islets stimulated by AVP since 40 min ($n = 5$). AVP was also without effect on $^{86}$Rb efflux in the presence of a nonstimulatory concentration of glucose (3 mM) (data not shown).

Effects of AVP on the Membrane Potential of B-cells—When islets were perifused with a medium containing 3 mM glucose, the membrane potential of B-cells was high and stable (~60.8 ± 2.2 mV). It was not affected by addition of 100 nM AVP for 10 min (~60.3 ± 2.2 mV; $n = 4$).

In the presence of 15 mM glucose, B-cells were depolarized and exhibited a rhythmic electrical activity consisting of slow waves of the membrane potential with Ca$^{2+}$ spikes superimposed on the plateau (Fig. 6). This electrical activity was barely affected by 100 nM AVP. The only obvious change in the representative recording shown in Fig. 6 was a shortening of the intervals between the slow waves. The lower panels summarize a quantification of the effects of AVP on the electrical activity produced by 10 and 15 mM glucose. The duration of the slow waves with spikes was not modified by AVP, but that of the intervals decreased, this decrease being significant ($p < 0.01$) only in 15 mM glucose. The frequency of the slow waves was unaffected. The net effect on the electrical activity was a small but significant ($p < 0.02$)
The interruption corresponds to an interval of 11 min. The lower panel, continuation of the recording in the middle panel showing the effect of 5 \( \mu M \) tolbutamide. AVP (100 \( \mu M \)) was added to the bathing solution as indicated. Following removal of AVP, the current decreased slowly to a new steady state (not shown). Effects of AVP on \( cAMP \) Levels—In the presence of either 10 or 15 mM glucose, islet \( cAMP \) concentrations tended to be slightly higher (nonsignificant) after incubation with, than without, AVP and were increased 2.5-fold by a low concentration of forskolin (Table II). AVP produced its usual effect on insulin release, which was markedly increased by forskolin.

**DISCUSSION**

The present study shows that AVP produces a glucose-, calcium-, and concentration-dependent (EC\(_{50} \approx 1-2 \) mM) increase in the plateau phase (percentage of time with spike activity) (Fig. 6).

**Effects of AVP on ATP-sensitive \( \mathbf{K^+} \) Channels—ATP-sensitive \( \mathbf{K^+} \) currents recorded in the whole cell mode are illustrated in the upper panel of Fig. 7. Currents increased as the cell equilibrated with the pipette solution and attained a steady level after about 5 min. Addition of 100 \( \mu M \) AVP to the bath did not affect the ATP-sensitive \( \mathbf{K^+} \) current. After 10 min, the average current change in response to a potential step from \(-70 \) to \(-60 \) mV was \( 96.5 \pm 3.2\% \) (\( n = 5 \)) of the current before addition of AVP. This contrasts with the rapid inhibition (by \( 88.8 \pm 1.1\% \); \( n = 5 \)) of the current produced by 10 \( \mu M \) tolbutamide (Fig. 7).

The effect of 100 \( \mu M \) AVP was also tested on excised outside-out patches. The patch illustrated in Fig. 7 contained at least nine active ATP-sensitive \( \mathbf{K^+} \) channels. At 0 mV, the single channel current was approximately 1 pA. AVP did not inhibit channel activity; if anything, there was a slight increase. In eight other patches, no effect of AVP was observed. In contrast, single channel activity was markedly and reversibly reduced by 5 \( \mu M \) tolbutamide (Fig. 7, lower panel).

**Effects of AVP on Inositol Phosphate Levels**—Inositol phosphate levels were measured in islets which were preincubated with [\( ^2H \)]myo-inositol to label their phosphoinositides, and then stimulated by AVP or \( ACh \) in a medium containing 5 mM LiCl (Table I). An increase in IT, IP, and IP3 was measured in islets incubated for 2 min with 100 \( \mu M \) AVP. Similar and much larger changes were produced by 1 and 100 \( \mu M \) \( ACh \), respectively. After 30 min of incubation, the increases in IP3 and IP6 caused by AVP were not more pronounced, whereas the accumulation of IP6 was much larger. \( ACh \) produced a concentration-dependent increase in the three inositol phosphates. In the absence of Ca\(^{2+}\), AVP and \( ACh \) still increased IP6 and IP6 levels, but no effect on IP6 levels was detected. In control experiments, it was verified that 5 mM LiCl did not prevent the increase in insulin release brought about by either AVP or \( ACH \) in the presence of Ca\(^{2+}\) (not shown).
Incubations in 15 mM glucose e.g. purinergic agonists (27, 28), producing opposite effects in seem to hold for AVP. Thus, AVP concentrations (0.2-20 nM) release from rat islets (13). There are rare examples of agents, increased insulin release from the tumoral RINm5F cell line, but this effect was weak and required a high concentration agents also depolarize the membrane if the decrease in K’ on ATP-sensitive K’ currents studied in the whole cell con-
tivative K’ channels in normal B-cells for several reasons. Firstly, sensitive K’ channels of B-cells (29-32), AVP is, as yet, the indeed exclude the hypothesis that AVP inhibits ATP-sensi-
tars) may inhibit insulin release partly by opening ATP-
somatostatin, and catecholamines (acting on cup-adrenocep-
depolarization in insulin-secreting RINm5F cells by directly AVP neither decreased 86Rb efflux from islet cells nor depo-
larized the B-cell membrane when the perifusion medium
contained no or only 3 mM glucose. Under these conditions, the effects of AVP on insulin release, strong evidence indica-
tes that other mechanisms play a major role. The combi-
nation of 10 mM glucose and 100 nM AVP induced a similar rate of insulin release as did 15 mM glucose, although the electrical activity was lower. Even more strikingly, addition of AVP to a medium containing 15 mM glucose, increased insulin release to levels similar to or higher than in 30 mM glucose alone, although the plateau phase of electrical activity was only 65% compared with 100% in 30 mM glucose (37).

The mechanisms by which AVP causes this small increase in electrical activity are not clear. We have discussed above the evidence against the role of a blockade of ATP-sensitive K’ channels. This is further supported by two observations. Firstly, AVP slightly decreased the duration of the intervals but did not affect that of the slow waves. These slow waves with spikes correspond to the periods of Ca2+ influx in B-cells, and their duration is increased by a rise in the concent-
gration of glucose or by the addition of tolbutamide (41), two conditions known to inhibit ATP-sensitive K’ channels. Sec-
ondly, AVP accelerated 45Ca efflux paradoxically more in the absence than in the presence of extracellular Ca2+. However, this hypothesis seems to be valid only for tumoral cells. We can indeed exclude the hypothesis that AVP inhibits ATP-sensitive K’ channels in normal B-cells for several reasons. Firstly, AVP neither decreased 86Rb efflux from islet cells nor depo-
larized the B-cell membrane when the perfusate medium contained no or only 3 mM glucose. Under these conditions, many ATP-sensitive K’ channels are open (33-35), and all agents known to close them decrease 86Rb efflux (36). These agents also depolarize the membrane if the decrease in K’ permeability is sufficient (37). Secondly, AVP had no effect on ATP-sensitive K’ currents studied in the whole cell config-
uration of the patch-clamp technique, nor on ATP-sensitive K’ channels studied in outside-out patches. This lack of effect contrasted sharply with the blockade produced by tol-
butamide. The discrepancy between our results in normal B-
cells and those obtained in the tumoral RINm5F cells is not readily explained, but is not without precedent. In the RINm5F cells used by Martin et al. (17), activators of protein kinase C were found to inhibit ATP-sensitive K’ channels and to depolarize the plasma membrane (38). This was not at all the case in normal mouse B-cells (39). It is thus possible that AVP closes ATP-sensitive K’ channels in RINm5F cells by virtue of its ability to increase diacylglycerol production.

In RINm5F cells, AVP caused a biphasic change in cytosolic free Ca2+ as measured by the fura-2 technique (17). A short-
lived, large increase was followed by a smaller, sustained rise. The initial increase was ascribable to the combination of Ca2+ influx (reflected by Ca2+ spikes) and mobilization of cellular Ca2+, whereas the sustained rise was apparently only due to an influx of Ca2+ (although no Ca2+ spikes were seen), since it was abolished in a Ca2+-free medium. The relationships between these changes in Ca2+ concentration and insulin release are unclear since the latter was not measured. In another study, published in abstract form (40), AVP was found to cause a transient release of insulin from RINm5F cells, but the dependence of this release on extracellular Ca2+ was not reported.

Our results show that the increase in insulin release pro-
duced by AVP in normal B-cells requires the presence of extracellular Ca2+. However, this does not necessarily imply that the effects of AVP involve an increase in Ca2+ influx. Two observations are compatible with a small increase. Firstly, during steady state stimulation by AVP, the efflux of Ca from islet cells was accelerated slightly more in the presence than in the absence of extracellular Ca2+. Secondly, glucose-induced electrical activity, that is known to reflect Ca2+ influx in B-cells (37), was slightly augmented by AVP. Although this small increase in Ca2+ influx may contribute to the effects of AVP on insulin release, strong evidence indicates that other mechanisms play a major role. The combi-
nation of 10 mM glucose and 100 nM AVP induced a similar rate of insulin release as did 15 mM glucose, although the electrical activity was lower. Even more strikingly, addition of AVP to a medium containing 15 mM glucose, increased insulin release to levels similar to or higher than in 30 mM glucose alone, although the plateau phase of electrical activity was only 65% compared with 100% in 30 mM glucose (37).

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gration of glucose or by the addition of tolbutamide (41), two conditions known to inhibit ATP-sensitive K’ channels. Sec-
ondly, AVP accelerated 45Ca efflux paradoxically more in the absence than in the presence of extracellular Ca2+, whereas glucose and tolbutamide decrease 86Rb efflux in the absence of Ca2+ (42, 43). The steady-state effects of AVP on the membrane potential of B-cells resemble those produced by activators of protein kinase C (39, 44) and, to a lesser extent, by the activator of adenylate cyclase forskolin (45). On the other hand, they clearly differ from the marked changes that m uscarinic agonists produce by increasing Na’ permeability of the membrane (46). The most conservative explanation is that the marginal effects of AVP on the membrane potential are mediated by an activation of protein kinase C (see below). Unfortunately, the effects of AVP in normal B-cells are probably too small to be resolved by patch-clamp techniques. AVP consistently caused a small acceleration of the Ca2+ efflux from islets perifused with a Ca2+-free medium. This suggests that AVP mobilizes intracellular Ca2+ in mouse B-cells as it does in RINm5F cells (17). That no insulin release occurred simultaneously is not surprising given the small magnitude of this Ca2+ mobilization in B-cells. Previous experiments using various concentrations of ACh have shown that the relation-

### Table II

**Comparison of the effects of AVP and forskolin on cAMP levels and insulin release by mouse islets**

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>n</th>
<th>cAMP concentration</th>
<th>Insulin release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubations in 10 mM glucose</td>
<td></td>
<td>f mol/islet</td>
<td>ng/islet/60 min</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>29.1 ± 1.5</td>
<td>1.06 ± 0.08</td>
</tr>
<tr>
<td>AVP, 100 nM</td>
<td>20</td>
<td>25.3 ± 1.8</td>
<td>1.66 ± 0.16*</td>
</tr>
<tr>
<td>Forskolin, 100 nM</td>
<td>12</td>
<td>56.2 ± 3.4*</td>
<td>2.39 ± 0.18*</td>
</tr>
<tr>
<td>Incubations in 15 mM glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>30.6 ± 1.4</td>
<td>4.45 ± 0.35</td>
</tr>
<tr>
<td>AVP, 100 nM</td>
<td>20</td>
<td>35.0 ± 1.8</td>
<td>6.36 ± 0.43*</td>
</tr>
<tr>
<td>Forskolin, 100 nM</td>
<td>10</td>
<td>70.2 ± 4.4*</td>
<td>9.15 ± 0.60*</td>
</tr>
</tbody>
</table>

* p < 0.01 versus controls.
ship between insulin release and Ca\(^{2+}\) mobilization is nonlinear (47). Release increases only when Ca\(^{2+}\) mobilization exceeds a certain threshold attained with 10 \(\mu\)M ACh. This threshold is not reached during stimulation by 100 nM AVP, whose effect is even smaller than that of 1 \(\mu\)M ACh (43, 47). Although the small effect of AVP on intracellular Ca\(^{2+}\) stores does not lead to insulin release in the absence of extracellular Ca\(^{2+}\), it may contribute to the amplification of release that occurs when Ca\(^{2+}\) influx is stimulated by glucose.

In other cells, the mobilization of cellular Ca\(^{2+}\) triggered by AVP is mediated by inositol 1,4,5-trisphosphate generated by hydrolysis of phosphatidylinositol bisphosphate (48-50). It has also been shown that 1 \(\mu\)M AVP increased total inositol phosphates in RINm5F cells (15). We measured inositol phosphates in islets incubated in the presence of LiCl, after checking that this did not affect the action of AVP on insulin release. AVP was found to increase the levels of IP\(_1\), IP\(_2\), and IP\(_3\). The changes in IP\(_1\) and IP\(_2\) were still observed in the absence of extracellular Ca\(^{2+}\), which suggests that it is not the consequence of Ca\(^{2+}\) influx. The effects of 100 nM AVP were similar to or smaller than those of 1 \(\mu\)M ACh. This supports the explanation proposed above for the lack of effect of AVP on insulin release in the absence of Ca\(^{2+}\).

Diacylglycerol production and protein kinase C activation by AVP were not directly measured, but it is reasonable to assume that they occurred concomitantly with the production of inositol phosphates (51). Moreover, the observation that AVP also increased phosphatidylcholine metabolism in RINm5F cells (15) raises the possibility that phospholipids other than the phosphoinositides might be a source of diacylglycerol during AVP stimulation (52, 53). The proposal that an activation of protein kinase C is involved in the increase in insulin release brought about by AVP is entirely compatible with several observations: the lack of effect of AVP in the absence of a stimulatory concentration of glucose, its marked efficacy in the presence of a maximally effective concentration of glucose, and to or smaller than those of 1 \(\mu\)M ACh. This supports the unique role of AVP in B-cells.

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