J. Membrane Biol. 124, 63–71 (1991)
002226319100132V

The Journal of
Membrane Biology

Activation of Voltage-Sensitive Ca^{2+} Currents by Vasopressin in an Insulin-Secreting Cell Line

P. Thorn and O.H. Petersen
The MRC Secretory Control Research Group, The Physiological Laboratory, University of Liverpool, Liverpool, L69 3BX, England

Summary. The effect of vasopressin on voltage-sensitive Ca^{2+} currents in the rat insulinoma cell line RINm5F has been investigated in patch-clamp whole-cell and single-channel current recording experiments. In the whole-cell recording configuration the dominant inward current in the presence of tetrodotoxin was noninactivating and had a high voltage threshold. This current was much enhanced when external Ca^{2+} was replaced by Ba^{2+} and was blocked by 1 μM nifedipine. It can therefore be classified as an L-current. Vasopressin enhanced the L-current without changing the voltage threshold of activation or the voltage at which the peak current was observed. Vasopressin effects were seen at concentrations as low as 0.01 nM, and the maximal effect was observed at about 1 nM. In higher concentrations the vasopressin effects were weaker, with effects at 50 nM of about the same magnitude as at 0.01 nM. In single-channel current recording experiments carried out with the cell-attached configuration there were no effects on single L-channel currents when vasopressin was added to the bath solution, but in experiments in which vasopressin (5 nM) was infused into the patch pipette a marked increase in the apparent channel open state probability was observed. We conclude that vasopressin, a peptide that is known to markedly enhance glucose-evoked insulin secretion, stimulates opening of the voltage-sensitive Ca^{2+} channels in insulin-secreting cells.

Key Words Ca^{2+} channels · vasopressin · single-channel currents · whole-cell current · insulin secreting cell

Introduction

Insulin is secreted in a calcium-dependent manner from the pancreatic β cells of the islets of Langerhans (Wollheim & Sharp, 1981). Two pathways by which carbohydrates induce insulin secretion have been described. Firstly carbohydrate metabolism evokes closure of the ATP-sensitive K^+ channels (K^+_{ATP}) causing depolarization (Ashcroft, Harrison & Ashcroft, 1984; Dunne et al., 1986; Ashcroft, Ashcroft & Harrison, 1988; Rorsman et al., 1990). Cell depolarization in turn promotes the opening of voltage-sensitive Ca^{2+} channels in the membrane and allows Ca^{2+} influx (Petersen & Findlay, 1987; Pralong, Bartley & Wollheim, 1990). Secondly it has been shown that carbohydrates enhance voltage-dependent Ca^{2+} channel opening by lowering the voltage-activation threshold (Velasco, Petersen & Petersen, 1988; Smith, Rorsmann & Ashcroft, 1989; Petersen, 1990). Thus by two complementary mechanisms carbohydrate secretagogues are able to raise the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and evoke insulin secretion.

In the insulin secreting cell line RINm5F the K^+_{ATP} channel is modulated by neuropeptides such as galanin which promotes (Dunne et al., 1989) and vasopressin which inhibits channel opening (Martin et al., 1989). In this way these peptides may act as important secondary regulators of insulin secretion. Vasopressin has been shown to be present in rat and human pancreas by immunological techniques (Amico, Finn & Haldar, 1988), causes insulin release in the HIT cell line (Richardson et al., 1990) and markedly enhances glucose-induced insulin secretion in normal mouse pancreatic islets (Gao et al., 1990). In view of the functionally coordinated control of K^+ and Ca^{2+} channels in neurones by certain transmitters (Miller, 1990), our aim in this work was to investigate whether vasopressin can regulate the voltage-sensitive Ca^{2+} currents under conditions of complete voltage control in the RINm5F cells.

Some of the findings presented here have been communicated to a meeting of The Physiological Society (Thorn & Petersen, 1991).

Materials and Methods

Tissue Culture

Experiments were carried out on the clonal insulin secreting cell line RINm5F. The cells were maintained in a CO_2 incubator at

37°C in RPMI 1640 with 10% fetal calf serum solution as described previously (Dunne et al., 1989). The cells were plated on Falcon 3001 dishes and used within three days.

Solutions

The standard extracellular medium contained (in mM): 140 NaCl, 4.7 KCl, 1.13 $MgCl_2$, 11 glucose, 1 μM TTX (Sigma) 10 HEPES-NaOH, pH 7.2. Ca^{2+} or Ba^{2+} were added to this solution as indicated in the text. The standard intracellular media (pipette solution in whole cell experiments) contained (in mM): 140 KCl, 10 NaCl, 1.13 $MgCl_2$, 2–5 EGTA, 2 ATP, 10 HEPES-KOH, pH 7.2. To investigate Ca^{2+} currents we used a pipette solution containing (in mM): 140 CsCl, 10 NaCl, 1.13 $MgCl_2$, 2–5 EGTA, 2 ATP, 10 HEPES-NaOH, pH 7.2. Vasopressin (Sigma) was stored frozen in solution and used at dilutions as indicated as was the vasopressin antagonist B-mercapto-B-B-cyclopentamethyleneпropionyl O-Me-Tyr^2,Arg^8 Vasopressin (Sigma). Nifedipine was made up in DMSO (final concentration 0.01% DMSO). For patch-clamp single-channel current recording experiments, the bath (extracellular) solution was the standard intracellular solution with the addition of 11 mM glucose and the pipette solution contained (in mM): 100 $BaCl_2$, 10 TEA (Sigma), 11 glucose, 1 μM TTX, 10 HEPES-NaOH, pH 7.2. In some initial experiments BAY K8644 (Calbiochem) 0.1 μM in methanol (final concentration 0.001% methanol) was present.

Experimental Protocol

Experiments were all carried out at room temperature (20–25°C). Pipettes of between 2–10 MΩ were pulled from microhematocrit (Assistent) tubes, and coated with Sylgard (Dow-Corning) in single channel experiments. Seals of >10 GΩ were formed on the cell membrane and gentle suction or voltage pulses often formed whole cell recordings as assessed by an increase in capacitance and noise. Equilibration between the cell and pipette contents was usually rapid (less than 10 sec) judged by the stability of the current amplitudes recorded after formation of a whole cell. Any cells that showed slow changes in current amplitude or shape were rejected. Series resistance was 7.9 ± 2.1 MΩ (mean ± SE; n = 38) and cell capacitance 16.9 ± 0.79 pF (mean ± SE; n = 38) measured by the compensation circuitry of the LIST EPC7. Series resistance was routinely compensated when using the LIST EPC7, but this was not possible in experiments with the LIST EPC5. In practice, when using uncompensated recordings we rejected any that showed unusual current artifacts. Even at the maximum current recorded in these experiments of 500 pA an uncompensated series resistance of 7.9 MΩ (mean) would only produce a voltage error of 3.95 mV.

Currents were recorded using LIST EPC5 or EPC7 amplifiers. Whole-cell currents were filtered at 3 kHz (Kemo) and sampled at 10 kHz (Cambridge Electronic Design C.E.D. software). Whole-cell records were leak subtracted by averaging, scaling and subtracting the current response to 30 mV hyperpolarizing steps. Single-channel currents were filtered at 1–3 kHz stored on FM tape recorder (Racal Store 4), replayed and sampled at 3–10 kHz (C.E.D.). Depolarizing steps were applied to the cells under software control at a frequency of 0.1–0.2 Hz. Idealized current traces were obtained from computerized threshold analysis of the data using a threshold of 50% of the single channel amplitude. The apparent open-state probability was determined as the sum of all channel open times divided by the sum of the open and close times. The results from test conditions were expressed as a percentage of the relevant control period.

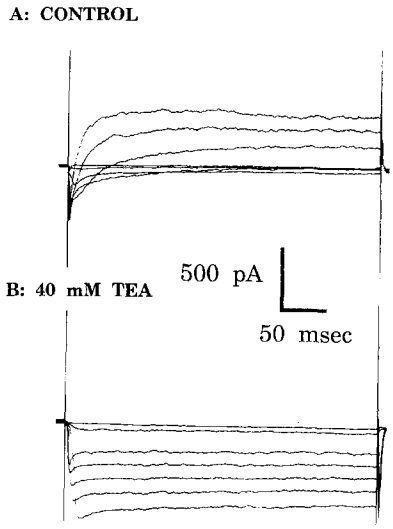

Fig. 1. (*A*) Whole-cell current recording from a holding potential of −80 mV stepping for 350 msec to potentials of −40, −30, −20, −10, 0, 10 and 20 mV. The cell was bathed in 2.5 mM Ca^{2+} solution with 1 μM TTX. The pipette contained K^+-rich solutions with 1 mM EGTA, 1 mM ATP, pH 7.2. Currents were low-pass filtered at 1 kHz and sampled at 3 kHz. An inward transient current develops at less depolarized steps; stronger depolarizations produce a slowly developing sustained outward currents after a brief inward current transient. (*B*) Whole-cell current recording from the same cell as in *A* under the same voltage protocol. The bathing solution was changed to one containing 25 mM Ca^{2+} and 40 mM TEA. The effect of these two changes in the bathing solution was to increase the amplitude of the inward and block the outward currents. It is also clear that the inward current is sustained and not transient.

Bath solution changes through polythene tubes (Portex I.D. 0.5 mm) positioned in close proximity to the cells were rapid as judged by the effects of inorganic compounds such as Cd^{2+} which took less than 30 sec to affect the currents. Intrapipette perfusion in the single channel current recording experiments were performed by positioning a fine polythene tube inside the pipette down to the first taper of the pipette tip (3 mm from pipette tip). After forming a seal the suction tube was left open to the atmosphere; in this way pressure injection of solution down the perfusion tube did not result in any increase in pressure in the pipette.

This paper presents results obtained from more than 165 successful whole-cell patch-clamp experiments on the insulin secreting cell line RINm5F. Ca^{2+} currents were seen in most cells, although there was a considerable variation in the peak current amplitude from cell to cell.

Results

Isolation of the Inward Current

Figure 1 illustrates a typical example of the current records obtained from leak subtracted, whole-cell voltage-clamp experiments when voltage jumps to positive potentials were carried out from a holding

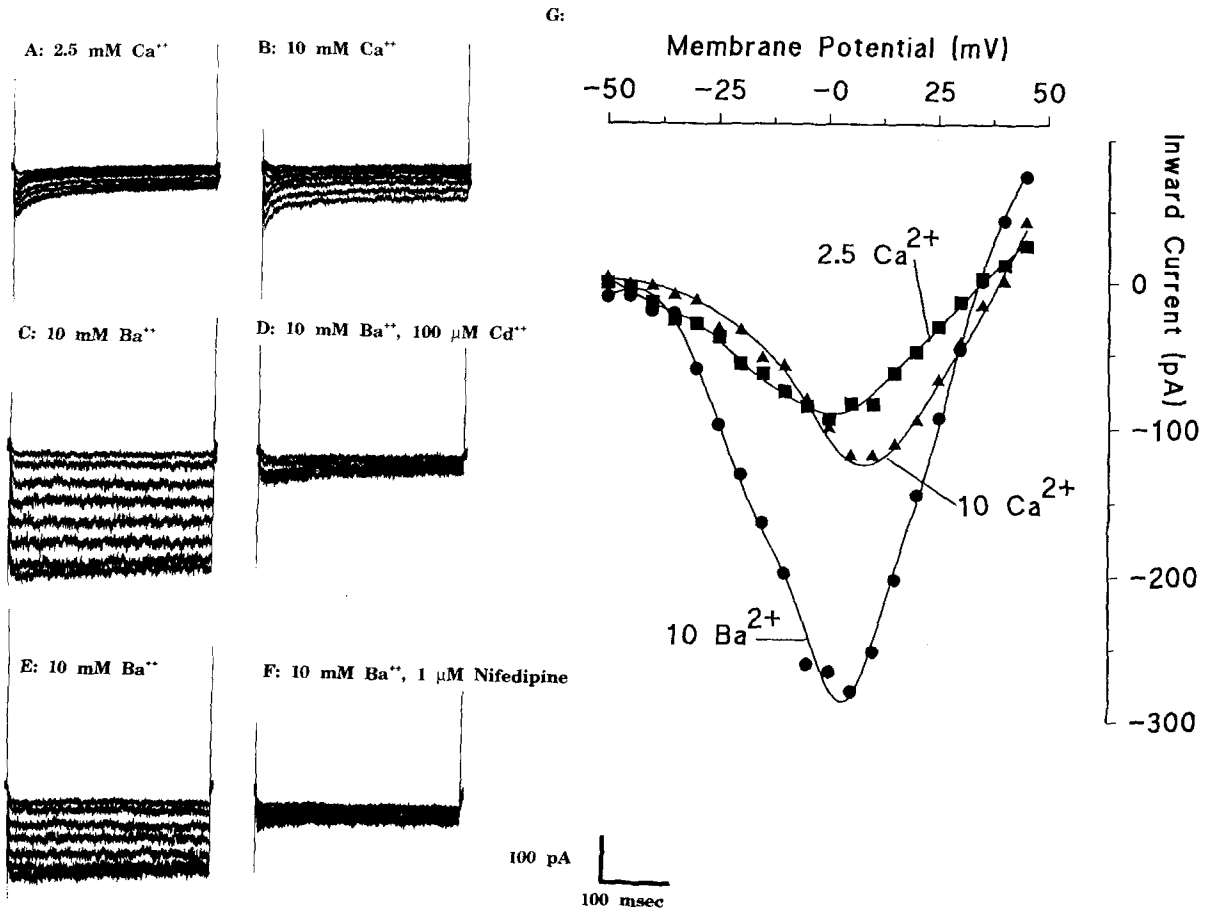

Fig. 2. (*A*) Whole-cell current records obtained from a cell bathed in 2.5 mM Ca^{2+} with a holding potential of −80 mV and steps to −35, −30, −25, −20, −15, −10 and −5 mV. Cs^{2+} solution was in the pipette. A sustained inward current was observed reaching a peak amplitude at −10 mV. (*B*) Current records from the same cell with the bathing solution changed to one containing 10 mM Ca^{2+} and held under the same voltage protocol as in *A*. The current increased in amplitude; no apparent change in current inactivation was observed. (*C*) Current records from the same cell with 10 mM Ba^{2+}. Due to the hyperpolarizing shift in the current/voltage relationship the step potentials illustrated in the figure are to −45, −40, −35, −30, −25 and −20 mV; the holding potential remains at −80 mV. The inward current shows a dramatic increase in amplitude. This was a very rapid effect occurring seconds after introducing the Ba^{2+} solution. (*D*) Again current records from the same cell bathed in 10 mM Ba^{2+} and under the same voltage protocol as in *C*, but now Cd^{2+} (0.1 mM) was added to the bathing solution. An immediate, rapid, reversible (*see E*), total block of the inward current was observed. (*E*) Current records in the same cell under the same conditions and voltage protocol as in *C* and *D*, illustrating almost complete recovery of the current after removal of Cd^{2+}. (*F*) Current records after application of 1 μM nifedipine in 10 mM Ba^{2+} solution. This experiment demonstrates a block of the Ba^{2+} current that was not reversed after prolonged washing. (*G*) Current/voltage relationships illustrating the currents for the full range of voltage steps applied. The squares were obtained in 2.5 mM Ca^{2+}, the triangles in 10 mM Ca^{2+} and the circles in 10 mM Ba^{2+}.

potential of −80 mV. The currents in Fig. 1*A* show a voltage-dependent outward current that increases in amplitude at more positive step potentials. The addition of TEA to the bathing solution blocked the outward current (14 applications, 20–40 mM TEA in nine cells) and reveals an inward current (Fig. 1*B*). The inward current activates at a membrane potential of −35 mV, and when the step potentials are made more positive the current increases in amplitude to a peak at 0 mV. Larger depolarizing steps to more positive potentials elicit smaller inward currents due to a decrease in the driving force for inward current flow.

The outward current blocked by TEA was presumed to be a K^+ current. Further experiments on the inward current were all carried out with Cs^+ replacing K^+ in the pipette solution (intracellular) to block K^+ currents. All bathing solutions also contained 1 μM TTX to block any Na^+ component of the inward current.

Selectivity of the Inward Current

Figure 2*A* illustrates an example of the type of experiment used to determine the ionic selectivity of the pathway for the inward current. All records in

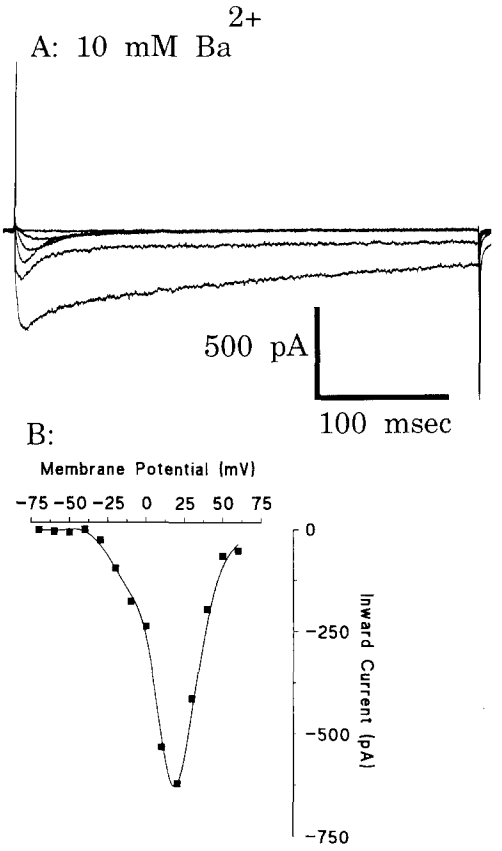

Fig. 3. (*A*) Whole-cell leak subtracted current records from a cell clamped at −80 mV and stepped to a series of potentials (−40, −30, −20, −10, 0, and 10 mV). The cell was bathed in 10 mM Ba^{2+} solution. The records show that at the smaller depolarizations a transient current is elicited that decays back to the zero current level within 100 msec. At stronger depolarizations a sustained inward current is elicited. (*B*) The current/voltage relationship obtained from the experiment shown in *A* shows a shoulder at depolarizations between −30 and 0 mV and a peak current at 10 mV.

Fig. 2 were obtained from the same cell at a holding potential of −80 mV. Voltage steps of 300 msec duration were applied to potentials between −50 and +15 mV. The voltage steps are to −35, −30, −25, −20, −15 and −10 mV in the Ca^{2+} solutions and −45, −40, −35, −25 and −20 mV in the Ba^{2+} solutions. The cell was initially bathed in a solution containing 2.5 mM Ca^{2+} (Fig. 2*A*). A solution change to 10 mM Ca^{2+} (Fig. 2*B*) caused an immediate increase in current amplitude at all the voltage steps. The bathing fluid Ca^{2+} was replaced by 10 mM Ba^{2+} (Fig. 2*C*), and a dramatic increase in peak current amplitude was observed as well as an apparent absence of an initial transient current at the start of the pulse. Application of 0.1 mM Cd^{2+} to the Ba^{2+} solution (Fig. 2*D*) reversibly (Fig. 2*E*) abolished (four cells, six applications) the inward currents. Finally, application of nifedipine (1 μM) in 10 mM Ba^{2+} solution irreversibly abolished the Ba^{2+} currents (Fig. 2*F*). The increased amplitude of the inward current with Ba^{2+} as the charge carrier and the block by Cd^{2+} (0.1 mM) both indicate that we are dealing with an inward current through Ca^{2+} channels.

Two Components of the Inward Ca^{2+} (Ba^{2+}) Current

Figure 3*A* illustrates leak subtracted inward currents obtained with Cs^{+} in the pipette and 10 mM Ba^{2+} in the extracellular medium (n = 105). This particular current record differs from the one shown in Fig. 2*C* and was chosen to illustrate the presence of a transient component to the inward current. The transient current was found in about 10% of all cells studied. From a holding potential of −80 mV depolarizing steps to −40, −30, −20, −10, 0 and 10 mV are shown. Steps to potentials less negative than a threshold of −45 mV elicited an inward current. Depolarizations to more positive potentials (−30 to −20 mV) led to an increase in the peak inward current amplitude. The currents rise to a peak within 30 msec and then decay over the length of the depolarizing pulse to the zero current level.

Depolarization to potentials more positive than −20 mV elicited a sustained component to the inward current that did not significantly decay over the 350 msec period of the depolarizing pulse. Figure 3*B* shows the leak subtracted current-voltage graph obtained from the experiment shown in Fig. 3*A* for the full range of step potentials applied to the cell. The peak current amplitude is seen to increase up to a maximum at +20 mV and then to decline at more positive potentials. The current-voltage graph also shows a small "shoulder" over the range −25 to 0 mV. The data lead us to believe that the inward current is made up of two components: a low threshold transient current that is activated at steps more depolarized than −45 mV and a high threshold sustained current that has a threshold of activation at more depolarized levels than the transient current and reaches a peak at +20 mV.

Since the transient current was only seen in a minority of the cells investigated we did not study its ionic dependency or pharmacology.

Effect of Vasopressin on the Whole Cell Ca^{2+} (Ba^{2+}) Current

The effect on the Ba^{2+} current of applying vasopressin to the bath was investigated. Figure 4*A* shows a set of leak subtracted currents obtained from a cell

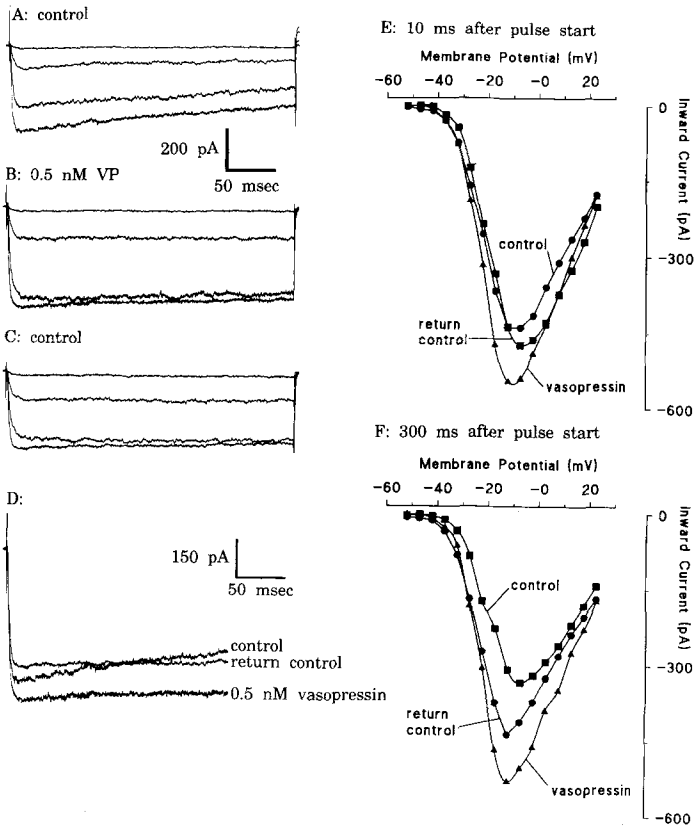

Fig. 4. (*A*) Leak subtracted whole-cell currents during steps to −35, −25, −15 and −5 mV from a holding potential of −80 mV. 10 mM Ba^{2+} solution. (*B*) Currents from the same cell as in *A*, with the same voltage protocol, 2 min after application of 0.5 nM vasopressin to the bathing solution. The currents are increased in amplitude. (*C*) Currents from the same cell as in *B* and *A*, 5 min after returning to the control 10 mM Ba^{2+} solution. The current amplitudes are comparable to those in *A*. (*D*) Superimposed currents (at −5 mV) obtained from the above three experiments. Note the different scale bar for this illustration. The effect of vasopressin on the Ba^{2+} current is shown. (*E*) Current/voltage graph from the previously illustrated currents showing the full set of voltage data obtained 10 msec after the start of the pulse. Filled squares represent the points obtained in control 10 mM Ba^{2+} solution. Filled triangles are the points obtained in 0.5 nM vasopressin and filled circles are the points after return to control solution. The graph shows that vasopressin increases the current amplitude. (*F*) Current/voltage graph as in *D* only obtained 300 msec after the start of the depolarizing pulse. The effect of 0.5 nM vasopressin on the current amplitude is greater than that obtained at the beginning of the pulse.

in the 10 mM Ba^{2+} solution before application of vasopressin. Figure 4*B* illustrates currents obtained under the same voltage protocol after the addition of 0.5 nM vasopressin to the bath. The current amplitude was seen to increase within 2 min of perfusion with vasopressin. Figure 4*C* shows currents under the same voltage protocol after returning to the 10 mM Ba^{2+} solution. For clarity Fig. 4*D* superimposes the currents obtained at −5 mV before, during vasopressin stimulation and after return to control from the same experiment (note the different scale). Vasopressin appears to reduce channel inactivation in this experiment. In most experiments an inactivating component to the inward current was not seen. Current-voltage relationships were measured 10 msec (Fig. 4*E*) and 300 msec (Fig. 4*F*) after the start of the depolarizing pulse. A larger effect of vasopressin was observed 300 msec after the start of the pulse, indicating an effect on the sustained inward current. The records shown in Fig. 4 are untypical in the sense that the effect of vasopressin was only partially reversible in most cells. We therefore also carried out experiments using the vasopressin (V_{1a}) antagonist, B-mercapto-B-B-cyclopentamethylenepropionyl O-Me-Tyr^2, Arg^8 Vasopressin. At concentrations of 100 to 300 nM a reversible inhibition of the Ba^{2+} current evoked by vasopressin was observed (8 out of 10 applications in seven cells) as illustrated in Fig. 5.

The effects of a wide range of vasopressin concentrations were tested to obtain a dose-response curve. Figure 6 shows a log graph of the percentage increase in the peak current amplitude after the application of vasopressin at different concentrations. The peak inward current was taken from the step pulse that elicited the maximum current. The graph indicates that the threshold for the vasopressin effect is at around 0.01 nM and that the current increase reaches a maximum at about 1 nM.

Vasopressin Activation of Single-Channel Currents

To further investigate the nature of the action of vasopressin, single-channel current recording was carried out. Cell-attached patches (n = 46) were studied with 100 mM Ba^{2+} solution in the pipette and a 140 mM K^+ solution in the bath to zero the cell resting membrane potential. In early experiments 0.1 μM Bay K 8644 was present in the patch pipette. This increased channel open probability, but was not found to be necessary when studying the effects of vasopressin. Single-channel currents were re-

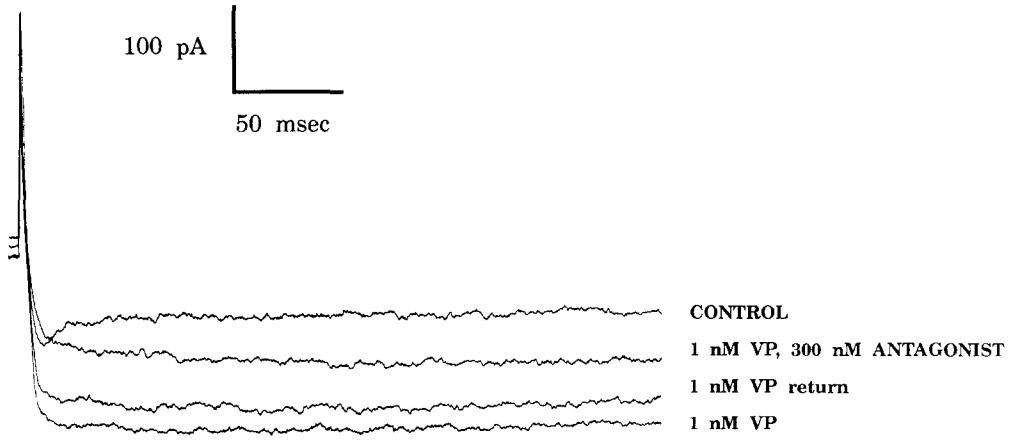

Fig. 5. Leak-subtracted current records obtained from a single cell stepped to −10 mV from a holding potential of −80 mV. The control (10 mM Ba^{2+}) solution, shows a small current. Addition of 1 nM vasopressin increases the amplitude of the current; application of 1 nM vasopressin plus 300 nM of the vasopressin antagonist causes a reduction in current amplitude that is seen to reverse when the antagonist is removed.

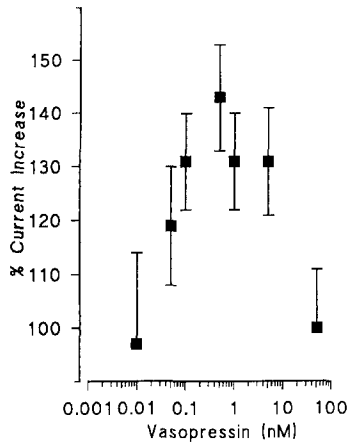

Fig. 6. A log-dose response graph drawn from a series of experiments conducted in the same manner as those shown in Fig. 4. The points represent the percentage increase in the peak inward current amplitude after application of vasopressin. The points are the means from 5 different cells, except at 0.01 nM where three cells were used. The standard error of the mean is also shown.

ded either with the 300 msec step protocol used in the whole-cell experiments or simply by adjusting the holding potential. Single-channel conductances of between 22 and 27 pS were obtained. All showed voltage sensitivity with few channel openings at a membrane potential of −45 mV and an increase in the probability of opening as the patch is depolarized (Fig. 7). The voltage-activated inward currents must be due to opening of L-type Ca^{2+} channels since Ba^{2+} is the only current carrier and Na^+ channels were blocked by TTX. The magnitude of the single-channel conductance (22–27 pS), the lack of inactivation during long depolarizing pulses and the activation by Bay K 8644 are all consistent with this view. Vasopressin (1–5 nM) was applied to the bath while recording single-channel currents in the cell-attached configuration. Bay K 8644 was not present in any of these experiments. In a total of 17 experiments on 17 cells no difference in the degree of channel opening was observed after the bath application of vasopressin. In a second series of experiments cell-attached patches were again used, but in this case vasopressin was applied to the extracellular surface of the membrane patch via a perfusion tube placed inside the patch pipette. The system employed allowed for only one change of solution, and therefore reversibility of any effect could not be tested. In control experiments it was shown that the exchange of the Ba^{2+} pipette solution with exactly the same solution had no effect on channel opening. However, when the Ba^{2+} pipette solution was changed for a Ba^{2+} solution containing 5 nM vasopressin, an increase in the frequency of channel openings (five out of seven cells) was observed (Fig. 8). Channel openings at a holding potential of −25 mV were analyzed in five experiments. In the presence of 5 nM vasopressin the apparent channel open probability increased by 1187% ± 679% (n = 5, mean ± SE). Multiple channel openings were observed in all experiments. Therefore, kinetic analysis of channel open and closed times was not possible. However, it appeared that the open times were not markedly affected by addition of 5 nM vasopressin. This is similar to the effect of fuel secretagogues which did not affect Ca^{2+} channel open times in mouse beta cells and RINm5F cells but

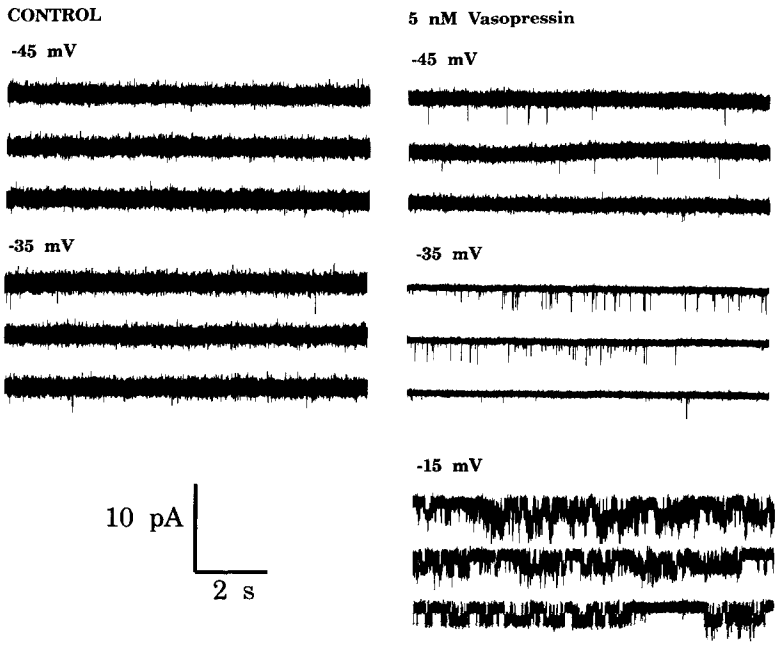

Fig. 7. Single-channel currents recorded from a cell-attached patch held at the indicated trans-patch potentials. The traces to the left were obtained before the infusion of vasopressin into the pipette solution and those to the right after start of vasopressin stimulation. The current records for each potential are continuous. Downward channel openings are observed at all potentials in the presence of vasopressin, with up to two channels open at a potential of −15 mV. Brief flickers of channel openings are observed at a holding potential of −35 mV in control solution, whereas no openings at −45 mV in the control solution were seen.

markedly reduced the long shut times (Velasco et al., 1988; Smith et al., 1989). The records shown in Fig. 7 indicate the very infrequent openings at potentials of −35 mV and the absence of openings at −45 mV before application of vasopressin. Also shown in Fig. 7 are current records obtained at a holding potential of −15 mV in the presence of vasopressin (the noise level was too great to show channel openings before vasopressin). These records clearly demonstrate the voltage dependence of the channel openings.

Discussion

This report demonstrates modulation of the voltage-sensitive Ca^{2+} current in the insulin secreting cell line RINm5F by vasopressin. Vasopressin was found to increase, in some cells reversibly, the Ca^{2+} current amplitude in whole-cell experiments. In all our experiments glucose (11 mM) was present. At the single-channel level vasopressin applied to the extracellular side of a cell-attached patch was shown to dramatically increase the apparent open state probability of the channels. Our recordings (Figs. 7 and 8) showing an increased rate of channel openings do not allow us to conclude whether vasopressin evokes an increase in open state probability or an increase in the number of functioning channels. The effect of vasopressin was more impressive in the intact cells (single-channel recording in cell-attached configuration) than in the internally perfused cell (whole-cell recording configuration). This

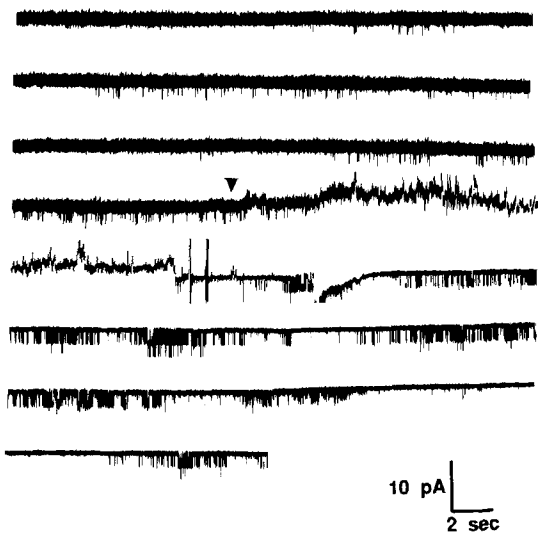

Fig. 8. Single-channel current records obtained from a cell-attached patch held at a membrane potential of −25 mV. The bathing solution contained 140 mM K^+ to zero the cell resting potential. The pipette solution contained 100 mM Ba^{2+}. The traces are a continuous record obtained over a period when vasopressin (5 nM) was infused (triangle at middle of third trace) into the pipette solution in contact with the extracellular side of the membrane patch. Downward deflections representing channel openings are observed infrequently before addition of vasopressin. Fluctuations and artifacts as a result of switching on the pipette perfusion are observed followed by an increase in the frequency of channel openings. The noise level decreases after the vasopressin was added. This was because the solution was present in the side arm of the pipette holder and acting as an aerial. Addition of the vasopressin solution filled the pipette, and the emptying of the side arm reduced the noise.

could be due to the loss of one or several intracellular cytoplasmic components after the formation of a whole cell leading to Ca^{2+} channel "run-down" (Belles et al., 1988).

The biphasic dose-response curve for the effect of vasopressin on the peak Ca^{2+} current amplitude could indicate the involvement of multiple receptors or possibly receptor desensitization. Under whole-cell current clamp in RINm5F cells an initial transient depolarization after application of vasopressin was followed by a sustained smaller depolarization (Martin et al., 1989). This could also be interpreted as evidence for two receptor subtypes. In addition, although the dose-response relationship was not studied in any detail, a smaller depolarizing response was observed with 100 nM vasopressin than with 10 nM (Martin et al., 1989). The electrophysiological dose-response relationship presented here broadly coincides with that for vasopressin-induced insulin release from HIT cells (Richardson et al., 1990). They report inhibition of insulin secretion at vasopressin concentrations of 1 to 10 pM and then at higher concentrations, up to 100 nM, a steady increase in insulin secretion. In normal mouse pancreatic islets 0.1 nM vasopressin significantly augments glucose (10 or 15 mM)-evoked insulin secretion, and the maximal effect is obtained at a vasopressin concentration of 100 nM (Gao et al., 1990).

Our experiments demonstrate a weak antagonism of the vasopressin-induced Ca^{2+} current increase by a V1 antagonist, used at 300 times the concentration of vasopressin. This might suggest a V1 receptor subtype such as proposed by Richardson et al. (1990) for the HIT cell. A V1 receptor classification is also supported by experiments in which vasopressin-induced insulin secretion in RINm5F cells was antagonized by a V1 antagonist (Li, Ullrich & Wollheim, 1988). It was also shown that vasopressin promoted the formation of inositol 1,4,5-trisphosphate and diacylglycerol (Li, Ullrich & Wollheim, 1988), but did not stimulate cyclic AMP production (Li et al., 1990).

Substance P has been shown to enhance voltage-sensitive Ca^{2+} currents in the pancreatic acinar cell line AR42J. Activation of protein kinase C by a phorbol ester or a cell-permeable diacylglycerol analogue was also shown to evoke nifedipine-sensitive Ca^{2+} influx, suggesting that Substance P stimulated opening of Ca^{2+} channels via protein kinase C-mediated phosphorylation (Gallacher et al., 1990). In the heart cyclic AMP-mediated phosphorylation is the major mechanism by which beta-adrenergic receptors control the degree of channel opening (Reuter, 1983). If activation of the diacylglycerol pathway was important for Ca^{2+} channel modulation in the RINm5F cells we would expect to have observed a Ca^{2+} current increase with the application of vasopressin to the bath solution when recording from a cell-attached patch. This was not the case, and preliminary experiments using phorbol 1,2-myristate 13-acetate (PMA) (100 nM) applied to the bath while recording whole-cell currents failed to demonstrate any effect on the Ca^{2+} current. PMA can in some preparations including the RINm5F cells inhibit Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (DiVirgilio et al., 1986). In cell-attached recording of single-channel Ca^{2+} (Ba^{2+}) currents from RINm5F cells PMA (100 nM) had no effect on channel opening, whereas the diacylglycerol analogue 1,2 didecanoylglycerol markedly increased the open state probability (Velasco & Petersen, 1989).

Vasopressin may activate the Ca^{2+} channels via a G-protein link. The vasopressin effect on K^+_{ATP} channels has been suggested to be mediated through a G protein (Martin et al., 1989) and the same G protein could possibly directly regulate the Ca^{2+} channel. In cardiac L-channels evidence suggests a direct channel activating effect of G_s (Yatani et al., 1987). In normal mouse β cells vasopressin at 100 nM had only a very small effect on closing the K_{ATP} channel (Gao et al., 1990) in contrast to the clear vasopressin block at 10 nM of the K^+_{ATP} channel in RINm5F cells (Martin et al., 1989). Gao et al. (1990) did not directly record Ca^{2+} currents, but the changes in the action potentials observed during application of vasopressin are not inconsistent with our findings.

Two types of voltage-sensitive single-channel Ca^{2+} (Ba^{2+}) currents have been reported in the RINm5F cell line (Velasco, 1987), but the L-channel currents are by far dominant (Velasco et al., 1988). Rorsman, Arkhammar & Berggren (1986) have found a transient TTX-sensitive inward Na^+ current and a sustained inward Ca^{2+} current in whole-cell recording experiments on RINm5F cells. The previously described large transient inward currents in the RINm5F cells characterized as Ca^{2+} currents (Findlay & Dunne, 1985) are now thought to be Na^+ currents (Rorsman et al., 1986). The whole-cell experiments in this paper indicate a transient low threshold Ca^{2+} current in a minority of the cells and a sustained high threshold Ca^{2+} current seen in all cases. The sustained current is blocked by Cd^{2+} and nifedipine and is most like the L-type current (Nowycky, Fox & Tsien, 1986). Two types of Ca^{2+} current, transient and sustained, have been identified in rat beta cells (Hiriart & Matteson, 1988) and the hamster-derived HIT cell line (Satin & Cook, 1988). Only sustained Ca^{2+} currents have been found in mouse beta cells (Rorsman, Ashcroft

& Trube, 1988). It appears from our work that it is the L-type Ca^{2+} channel that is modulated by vasopressin.

The vasopressin-induced modulation of the Ca^{2+} current described in this paper provides a pathway for elevating intracellular Ca^{2+} levels and inducing insulin release. Depolarization by vasopressin-induced K_{ATP} channel closure (Martin et al., 1989) would open the voltage-sensitive Ca^{2+} channels. This effect would be augmented by the vasopressin action on the Ca^{2+} channels amplifying the intracellular Ca^{2+} signal.

We thank Alan Higgins for effective technical assistance. This work was supported by an MRC Programme Grant.

References

Amico, J.A., Finn, F.M., Haldar, J. 1988. Oxytocin and Vasopressin are present in human and rat pancreas. *Am. J. Med. Sci.* **296:**303–307

Ashcroft, F.M., Ashcroft, S.J.H., Harrison, D.E. 1988. Properties of single potassium channels modulated by glucose in rat pancreatic B-cells. *J. Physiol.* **400:**501–527

Ashcroft, F.M., Harrison, D.E., Ashcroft, S.J.H. 1984. Glucose induces closure of single potassium channels in isolated rat pancreatic B-cells. *Nature* **312:**446–448

Belles, B., Malecot, C.O., Hescheler, J., Trautwein, W. 1988. Run-down of the Ca current during long whole-cell recordings in guinea pig heart cells: Role of phosphorylation and intracellular calcium. *Pfluegers Arch.* **411:**353–360

DiVirgilio, F., Pozzan, R., Wollheim, C.B., Vicentini, L.M., Meldolesi, J. 1986. Tumour promoter phorbol myristate acetate inhibits Ca^{2+} influx through voltage-gated Ca^{2+} channels in two secretory cell lines, PC12 and RINm5F. *J. Biol. Chem.* **261:**32–35

Dunne, M.J., Bullett, M.J., Li, G., Wollheim, C.B., Petersen, O.H. 1989. Galanin activates nucleotide-dependent K^+ channels in insulin secreting cells via a pertussis toxin sensitive G-protein. *EMBO J.* **8:**412–420

Dunne, M.J., Findlay, I., Petersen, O.H., Wollheim, C.B. 1986. ATP-sensitive K^+ channels in an insulin secreting cell line are inhibited by D-glyceraldehyde and activated by membrane permeabilization. *J. Membrane Biol.* **93:**271–279

Findlay, I., Dunne, M.J. 1985. Voltage-activated Ca^{2+} currents in insulin secreting cells. *FEBS Lett.* **189:**281–285

Gallacher, D.V., Hanley, M.R., Petersen, O.H., Roberts, M.L., Squire-Pollard, L.G., Yule, D.I. 1990. Substance P and bombesin elevate cytosolic Ca^{2+} by different molecular mechanisms in rat pancreatic acinar cell line. *J. Physiol.* **426:**193–207

Gao, Z.-Y., Drews, G., Nenquin, M., Plant, T., Henquin, J.-C. 1990. Mechanisms of the stimulation of insulin release by arginine-vasopressin in normal mouse islets. *J. Biol. Chem.* **265:**15724–15730

Hiriart, M., Matteson, D.R. 1988. Na channels and two types of Ca channels in rat pancreatic B cells identified with the reverse haemolytic plaque assay. *J. Gen. Physiol.* **91:**617–639

Li, G., Regazzi, R., Ullrich, S., Pralong, W.-F., Wollheim, C.B. 1990. Potentiation of stimulus-induced insulin secretion in protein kinase C-deficient RINm5F cells. *Biochem. J.* **272:**637–645

Li, G.D., Ullrich, S., Wollheim, C.B. 1988. Inositol phosphate generation and insulin secretion by vasopressin in RINm5F cells. *Diabetologia* **31:**514*a*

Martin, S.C., Yule, D.I., Dunne, M.J., Gallacher, D.V., Petersen, O.H. 1989. Vasopressin directly closes ATP-sensitive potassium channels evoking membrane depolarization and an increase in the free intracellular Ca^{2+} concentration in insulin-secreting cells. *EMBO J.* **8:**3595–3599

Miller, R.J. 1990. Receptor-mediated regulation of calcium channels and neurotransmitter release. *FASEB J.* **4:**3291–3299

Nowycky, M.C., Fox, A.P., Tsien, R.W. 1986. Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature* **316:**440–443

Petersen, O.H. 1990. Control of insulin secretion in pancreatic β-cells. *News Physiol. Sci.* **5:**254–258

Petersen, O.H., Findlay, I. 1987. Electrophysiology of the pancreas. *Physiol. Rev.* **67:**1054–1116

Pralong, W.F., Bartley, C., Wollheim, C.B. 1990. Single islet beta-cell stimulation by nutrients: Relationship between pyridine nucleotides, cytosolic Ca^{2+} and secretion. *EMBO J.* **9:**53–60

Reuter, H. 1983. Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* **301:**569–574

Richardson, S.B., Eyler, N., Twente, S., Monaco, M., Altszuler, N., Gibson, M. 1990. Effects of vasopressin on insulin secretion and inositol phosphate production in a hamster beta cell line (HIT). *Endocrinology* **126:**1047–1052

Rorsman, P., Arkhammar, P., Berggren, P.-O. 1986. Voltage-activated Na^+ currents and their suppression by phorbol ester in clonal insulin-producing RINm5F cells. *Am. J. Physiol.* **251:**C912–C919

Rorsman, P., Ashcroft, F.M., Trube, G. 1988. Single Ca channel currents in mouse pancreatic β-cells. *Pfluegers Arch.* **412:**597–603

Rorsman, P., Berggren, P.-O., Bokvist, K., Efendic, S. 1990. ATP-regulated K^+ channels and diabetes mellitus. *News Physiol. Sci.* **5:**143–147

Satin, L.S., Cook, D.L. 1988. Evidence for two calcium currents in insulin-secreting cells. *Pfluegers Arch.* **411:**401–409

Smith, P.A., Rorsman, P., Ashcroft, F.M. 1989. Modulation of dihydropyridine sensitive Ca^{2+} channels by glucose metabolism in mouse pancreatic B-cells. *Nature* **342:**550–553

Thorn, P., Petersen, O.H. 1991. Calcium channel modulation by vasopressin in insulin-secreting RINm5F cells. *J. Physiol.* **434:**93P

Velasco, J.M. 1987. Calcium channels in insulin-secreting RINm5F cell line. *J. Physiol.* **398:**15P

Velasco, J.M., Petersen, O.H. 1989. The effect of a cell-permeable diacylglycerol analogue on single Ca^{2+} (Ba^{2+}) channel currents in the insulin-secreting cell line RINm5F. *Q. J. Exp. Phys.* **74:**367–370

Velasco, J.M., Petersen, J.U.H., Petersen, O.H. 1988. Single-channel Ba^{2+} currents in insulin-secreting cells are activated by glyceraldehyde stimulation. *FEBS Lett.* **231:**366–370

Wollheim, C.B., Sharp, G.W.G. 1981. Regulation of insulin release by calcium. *Physiol. Rev.* **61:**914–973

Yatani, A., Codina, J., Imoto, Y., Reeves, J.P., Birnbaumer, L., Brown, A.M. 1987. A G-protein directly regulates mammalian cardiac calcium channels. *Science* **238:**1288–1292

Received 14 February 1991; revised 23 April 1991