Mechanosensitivity of voltage-gated calcium currents in rat anterior pituitary cells

Shlomo Ben-Tabou, Efrat Keller and Itzhak Nussinovitch*

Department of Anatomy and Embryology, Hebrew University, Hadassah Medical School, PO Box 1172, Jerusalem 91010, Israel

1. Sensitivity of voltage-activated calcium currents to flow-induced mechanical stress was examined in enriched populations of rat anterior pituitary somatotrophs. Voltage-activated calcium currents were recorded with the whole-cell configuration of the patch-clamp technique. Pituitary cells were exposed to flow (from pipettes) which was produced by a hydrostatic pressure of about 3 cmH_2O.

2. In 92 % of the cells studied ($n = 87$ cells) flow reduced the amplitude of both low voltage-activated (LVA) and high voltage-activated (HVA) calcium currents. These effects of flow on calcium currents did not result from changes in either seal resistance or leak conductance of the cell and were dependent on the magnitude of flow.

3. The effect of flow is selective. We found that LVA calcium currents were substantially more sensitive to flow than HVA calcium currents. Under constant flow conditions, LVA calcium currents were reduced by 57·6 ± 29·6 % (s.d.), whereas HVA currents (recorded from the same cells) were reduced by only 17·8 ± 15·9 % (s.d.).

4. The effects of flow on calcium currents were associated with effects on their related calcium tail currents. Slowly deactivating calcium tail currents were reduced by 75·3 ± 25·6 % (s.d.), whereas rapidly deactivating calcium tail currents were reduced by 29·1 ± 14·4 % (s.d.).

5. The effect of flow on calcium currents was not associated with any significant shift in the activation curves of the calcium currents (voltage range −60 to +30 mV), suggesting that the effect of flow is not voltage dependent.

6. The effect of flow is not dependent on activation of calcium currents during the exposure to flow. Calcium currents which were evoked immediately after cessation of the exposure to flow were reduced in amplitude and recovered to control values.

7. Possible mechanisms underlying the flow effect and possible physiological relevance of the effect on pituitary cells are discussed.

Voltage-gated calcium channels play a key role in regulation of many cellular functions, such as muscle contraction, neurotransmitter release and hormone secretion (for review see Hille, 1992). In anterior pituitary somatotrophs, it has been suggested that voltage-gated calcium channels are involved in regulation of growth hormone secretion (Holl, Thorner & Leong, 1988; Mason & Rawlings, 1988; Nussinovitch, 1988, 1989; Chen, Zhang, Vincent & Israel, 1990; Rawlings, Hoyland & Mason, 1991; Lussier, French, Moor & Kraicer, 1991). Two types of voltage-activated calcium currents have been described in somatotrophs (DeRiemer & Sakmann, 1986; Lewis, Goodman, St John & Barker, 1988; Chen *et al.* 1990) similar to the low voltage-activated (LVA or T-type) and high voltage-activated (HVA) calcium currents, which were described in pituitary tumour cells (Armstrong & Matteson, 1985), cardiac cells (Nilius, Hess, Lansman & Tsien, 1985) and neuronal cells (Carbone & Lux, 1984; Nowycky, Fox & Tsien, 1985).

In control experiments, designed for the study of chemical regulation of calcium channels in pituitary cells, we applied physiological solution, identical to the extracellular solution, to the cell under study. We discovered that voltage-gated calcium currents in somatotrophs were very sensitive to the stream of physiological solution, which was produced by very low hydrostatic pressures (3 cmH_2O) and directed (from pipettes) to the surface membrane of the cell under study.

Several reports in recent years have shown that shear forces induced by flow can alter the gating or activate ion channels. In vascular endothelial cells flow-induced shear forces activated an inward-rectifying potassium

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*To whom correspondence should be addressed.

permeability (Olesen, Clapham & Davies, 1988) and a cationic channel, which was found to be more permeable to calcium than to sodium (Schwarz, Callewaert, Droogmans & Nilius, 1992a; Schwarz, Droogmans & Nilius, 1992b). In skeletal muscle inside-out patches, flow (directed to the inside part of the membrane) reduced the open probability of inward-rectifying potassium channels (Burton & Hutter, 1990). The mechanisms underlying these effects of flow on endothelial cells and muscle cells are not known. However, it has been demonstrated, in endothelial cells, that flow-induced shear forces can regulate cellular functions that are associated with regulation of vascular tone (Nollert, Diamond & McIntire, 1991).

Since calcium channels in pituitary cells are suggested to be involved in regulation of hormone secretion, it was of interest to examine further the effects of flow on calcium currents in these cells. A preliminary account of this work in an abstract form has appeared elsewhere (Nussinovitch, Ben-Tabou & Keller, 1993) .

METHODS

Cell culture

Anterior pituitary glands were obtained after decapitation of four male rats (Sabra strain 250–300 g). Anterior pituitary cells were obtained by enzymatic dispersion of the anterior pituitary glands using a method similar to that previously described (Nussinovitch, 1988). The dissociation medium (F-12, Biological Industries, Beth-Haemek, Israel) contained: 0·1 % trypsin (Difco 1/250), 0·06 % collagenase (type II), 0·05 % hyaluronidase (type IV), 200 units DNase (type I), 0·25 % bovine serum albumin (BSA) and the antibiotic kanamycin sulphate (2·5 mM). All the enzymes (except trypsin) were obtained from Sigma (St Louis, MO, USA). After 90 min of incubation at 37 °C, the cell suspension was washed and treated with 0·02 % lima bean trypsin inhibitor (LBTI) to stop the enzymatic activity of trypsin. The next step was to load the cell suspension on top of a Percoll (Sigma, USA) discontinuous gradient, in order to obtain enriched populations of growth hormone-secreting cells (somatotrophs). This method, known as 'density gradient centrifugation', was previously used to obtain somatotrophs (DeRiemer & Sakmann, 1986; Nussinovitch, 1989). Aliquots of 100 μl from the cell suspension were plated in the centre of 35 mm plastic Petri dishes (Falcon) and stored in the incubator (37 °C, 5–6 % CO_2) so that cells would attach to the plastic dish. Two hours later, 2 ml of Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Israel) supplemented with 10 % fetal bovine serum (Biological Industries) were added to the culture dish. Cells were kept in the incubator for 1–9 days before the electrophysiological experiment.

Electrophysiological recording and analysis

Calcium currents were recorded (amplifier: Axopatch 1C, Axon Instruments, Foster city, CA, USA) with the whole-cell mode of the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) at room temperature. Patch-electrodes were pulled from 1·6 mm (o.d.) borosilicate glass (Hilgenberg GmbH, Malsfeld, Germany) on a two-stage puller (L/M-3P-A, List Electronics, Germany) and their resistance was in the range of 4–10 MΩ when filled with the 'intracellular

solutions' (see below). Holding potential in all the experiments was −80 mV. Low voltage-activated calcium currents were activated by 50 mV voltage steps and HVA calcium currents by 80 mV voltage steps, except when a different protocol is mentioned. The interval between test voltage pulses was always 10 s and pulse duration usually 200 ms. Membrane currents were filtered with a four-pole low-pass Bessel filter with a cut-off frequency (−3 dB) of 2 kHz and sampled (at 2·5–3·3 kHz, or at 33·3 kHz in tail current experiments, see Fig. 5) with an A–D converter board (Labmaster, Scientific solutions, USA) and stored on-line on the hard drive of an AT–286 microcomputer. Capacitative currents and series resistance were compensated using potentiometers provided with the amplifier. Linear leak currents (and residual capacitative currents) were digitally subtracted after extrapolation of leak currents, which were recorded in response to 20 mV hyperpolarization pulses.

Solutions

The extracellular solution contained (mM): 150 NaCl, 5 KCl, 10 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 Hepes and 1·5 μM TTX. In some of the experiments NaCl and $CaCl_2$ were replaced with equimolar concentrations of TEACl and $BaCl_2$ respectively. The pipette 'intracellular' solution consisted of (mM): 150 N-methyl-glucamine chloride (NMG-Cl), 11 EGTA, 10 Hepes, 2 MgATP, and 80 μM GTP; actual final calcium concentration less than 1 nM. In several experiments (Fig. 7) EGTA concentration was reduced to 1·1 mM and 0·55 mM calcium was added to the patch pipette. All the chemicals for the intracellular solution were from Sigma, USA.

Estimation of flow rate and flow-induced shear force

Flow pipettes (with tip diameter of 2–6 μm) were pulled from 1·6 mm (o.d.) glass tubes. Sensitivity of voltage-gated calcium currents to flow was tested by exposing pituitary cells to gravity flow from pipettes placed near the surface of the cells under study (see Fig. 1A). Flow of solution from these pipettes was evidenced by the movement of small tissue debris in the vicinity of the flow pipette (this movement could be stopped by applying negative pressure to the pipette). A hydrostatic pressure of about 3 cmH_2O ($4 \sin(45)$) was produced at the tip of the flow pipettes by 4 cm of control solution in the pipettes, which were held at an angle of 45 deg. The solutions in the flow pipettes were always identical to the extracellular solutions and kept at room temperature. Flow rate (Q) from the tip of the pipette was estimated to be 0·6 × 10⁻⁸ ml s⁻¹ (or linear velocity of 0·3 mm s⁻¹) by using Poiseuille's law for tip radius of 2·5 μm, tip length of 1 mm and a constant hydrostatic pressure of 3 cmH_2O. The shear force (τ) at the tip of the flow pipette was calculated to be 3·77 dyn cm⁻² by using the equation (Olesen et al. 1988; Schwarz et al. 1992b):

$$\tau = 4\eta Q/\pi r^2,$$

where η is fluid viscosity (7·7 × 10⁻³ Poise) and r is tip radius of the flow pipette (2·5 μm). However, the actual shear force generated by the stream of flow was smaller, since the pipettes were usually located 50–100 μm from the cell surface.

RESULTS

Effect of flow on calcium currents

Sensitivity to flow was observed in 92 % of the anterior pituitary cells studied ($n = 87$ cells). Figure 1B shows a typical effect of flow on T-type calcium currents. Outflow

from the pipette reduced the amplitude of these T-type calcium currents by 75 % and removal of the pipette resulted in slow recovery of the T-type currents to control values. A typical effect of flow on HVA currents (with barium as charge carrier) is illustrated in Fig. 2*A*. In this experiment, the flow from the pipette reversibly reduced the amplitude of these HVA barium currents by 60 % and removal of the flow pipette resulted in rapid recovery to control values.

These effects of flow on calcium currents could be either genuine effects on calcium channels or experimental artifacts, due to reversible changes in either seal resistance or in leak currents through the cell membrane. Figure 2*B* shows that the latter is not the case. Flow reversibly reduced barium currents, which were activated by a voltage ramp (from −100 to 0 mV), without affecting the preceding leak currents. Likewise, flow had no effect on the amplitude of leak currents that were produced by 20 mV hyperpolarizations (Fig. 2*C*, same cell as in Fig. 2*A* and *B*). Similar results were obtained from all the cells in which we tested the effects of flow on leak currents (*n* = 9 cells). In additional experiments we found that flow had no effect on

leak currents that were revealed after blocking of HVA calcium currents with 2 mM cadmium (*n* = 4 cells, not shown).

In order to examine whether the effects of flow on calcium currents were actually initiated and dependent on the flow of extracellular solution from pipettes, we used different methods to control the outflow of solution from the flow pipettes. We found that the response to flow was reduced or abolished by negative pressure, which was applied (with a threaded syringe) to the flow pipettes (Fig. 3*A*, *n* = 11 cells). In addition, the magnitude of the response to flow was reduced by increasing the distance of the flow pipette from the cell (for example, Fig. 3*B*, *n* = 7 cells). These results show that outflow of solution from the application pipettes was responsible for the effects of flow on voltage-activated calcium currents. The flow-induced shear forces needed to produce these effects on calcium currents were less than 3·77 dyn cm⁻² (see Methods), similar to the flow-induced shear forces needed to affect ionic permeabilities in endothelial cells (Olesen *et al.* 1988; Schwarz *et al.* 1992*a*, *b*).
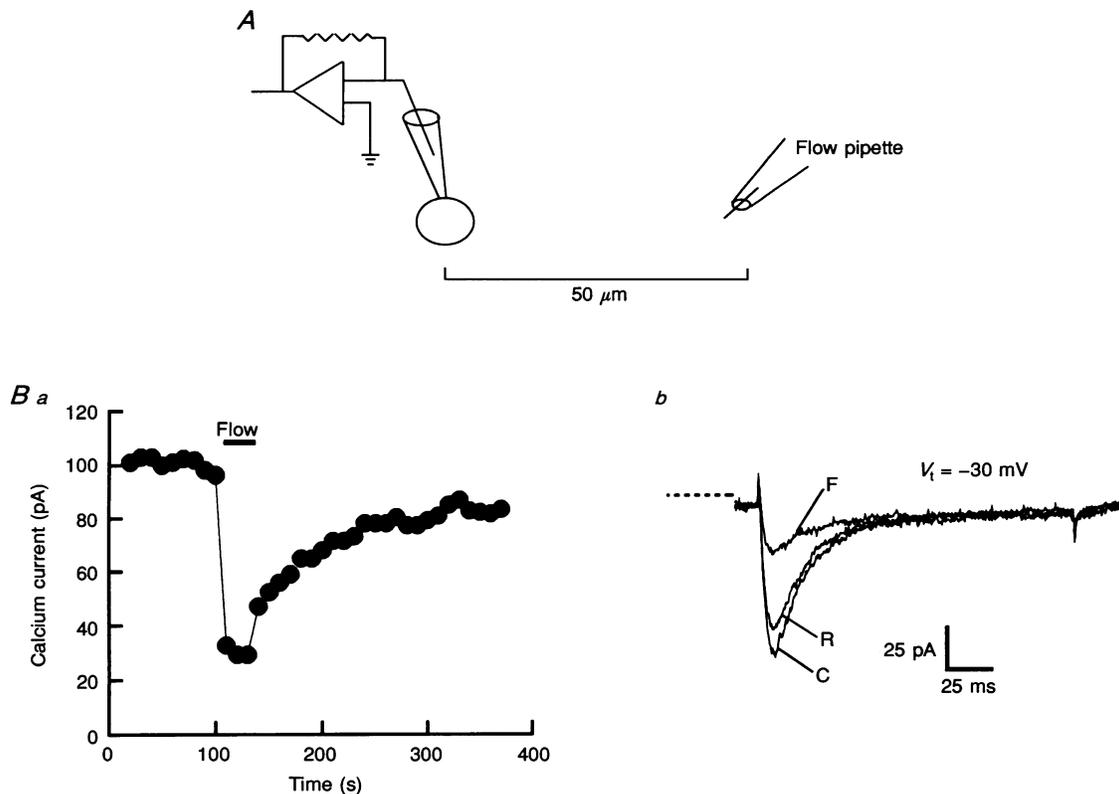

Figure 1. The effect of flow on LVA (T-type) calcium currents

A, scheme showing a typical set-up of a flow experiment. The size of the cell is 9 μm, the distance of the flow pipette from the cell is 50 μm and tip diameter of the flow pipette is 3 μm. *Ba*, time course of the flow effect on T-type calcium currents. Flow (tip 2 μm, distance 50 μm) reduced these LVA currents by 75 % of their control value. *Bb*, T-type calcium current traces from the experiment illustrated in *Ba*. (C, control; F, flow; R, recovery). Zero current level in this figure (and in the following figures) is represented as a dashed line to the left of the current traces.

## Differential sensitivity of LVA and HVA calcium currents to flow

LVA calcium currents in pituitary cells were found to be substantially more sensitive to flow than HVA calcium currents. Flow reduced LVA calcium currents in all the cells studied, whereas HVA calcium currents were reduced

in 85% of the cells studied. Under constant conditions, flow (distance 100 μm) reduced LVA calcium currents by 63·9 ± 19·5% (S.D.; $n = 15$ cells), whereas HVA calcium currents were reduced by 40·2 ± 31·9% (S.D.; $n = 37$ cells). This difference was found to be statistically significant (Student's independent t test, $P \leqslant 0·0092$). Differential sensitivity was also demonstrated by exposing LVA and
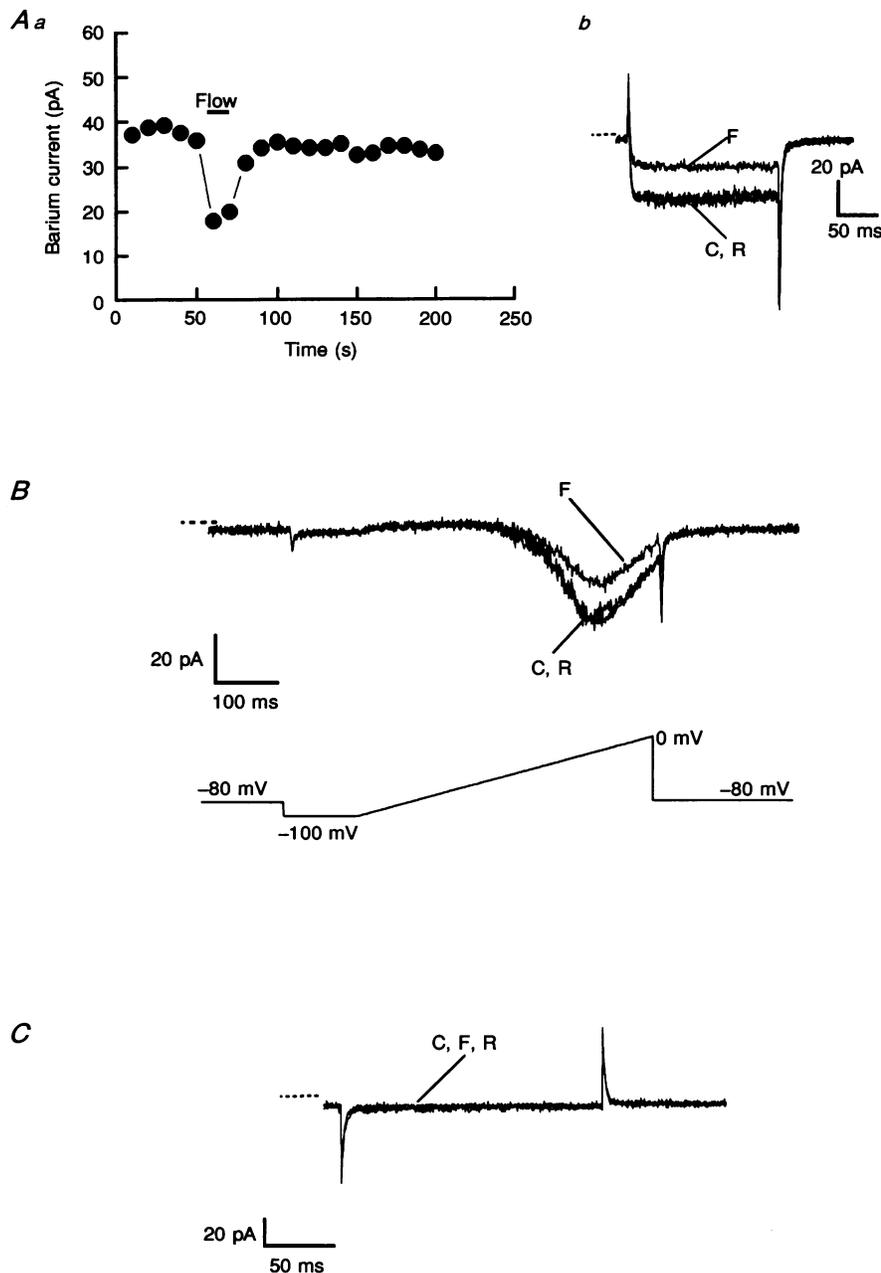

Figure 2. The effect of flow on HVA barium currents
A a, time course of the flow effect on HVA barium currents which were activated by 70 mV voltage steps. Flow (tip 4 μm, distance 100 μm) reduced these currents by 60% of their control value. *A b*, HVA current traces from the experiment illustrated in *A a* (C, control; F, flow; R, recovery). *B*, HVA barium currents which were activated by voltage ramps (shown below the current). Flow reduced these currents by 45% without affecting the leak currents preceding the voltage ramp (same flow as in *A*). *C*, flow had no effect on leak currents which were produced by 20 mV hyperpolarizing pulses (same flow as in *A*). All the current traces in this figure (*A*, *B* and *C*) are from the same cell.
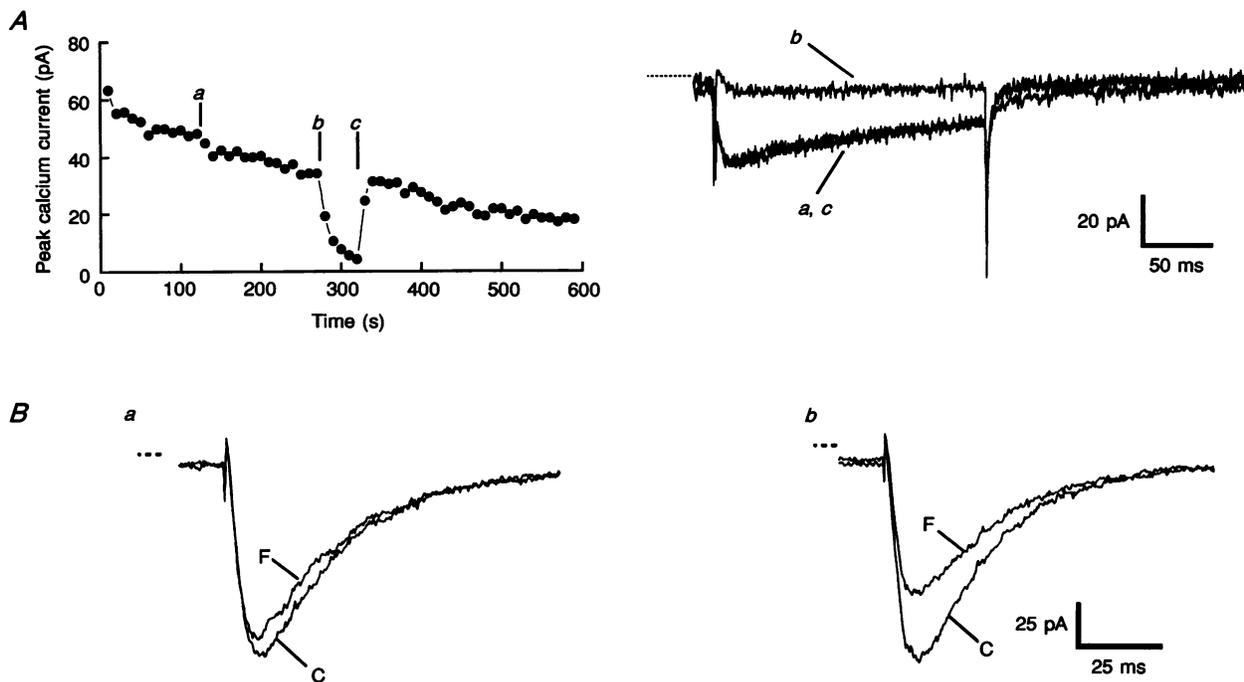
Figure 3. The effect of flow depends on its magnitude

A, effect of flow (regulated by negative pressure) on HVA calcium currents (test potential, $V_t = 0$ mV). *A a*, flow pipette with negative pressure placed near the cell; *b*, negative pressure released; *c*, negative pressure imposed. The inset shows individual current traces from the same experiment (the fast inward current preceding the slow inward calcium currents is a voltage-activated sodium current, which was not affected by flow; TTX was not added to the bath solution in this experiment). *B*, effect of flow from different distances on T-type calcium currents ($V_t = -30$ mV). The flow pipette (tip 3 μm) was first placed 200 μm (*B a*) and then 100 μm (*B b*) from the surface of the cell. T-type currents were reduced by 10 and 36 %, respectively.
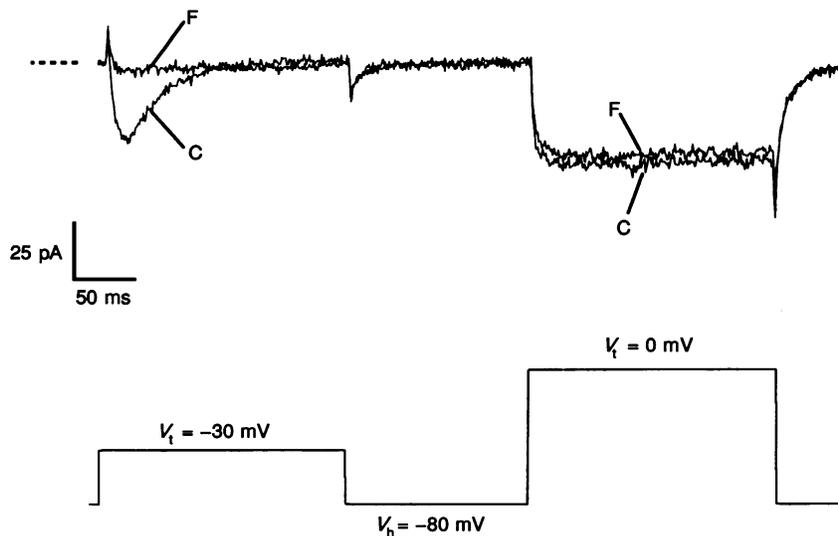

Figure 4. Differential sensitivity of LVA and HVA currents to flow

Differential sensitivity was tested with double pulse experiments. The first pulse evoked a T-type calcium current and the second pulse (initiated 150 ms after the first) evoked an HVA calcium current. Flow (tip 5 μm, distance 50 μm) reduced the T-type current by 90 % and the HVA calcium current by only 10 %. V_h, holding potential; V_t, test potential.

HVA calcium currents, both recorded from the same cell, to flow (Fig. 4). In these experiments ($n = 11$ cells), LVA calcium currents were reduced by $57\cdot6 \pm 29\cdot6\%$ (s.d.), whereas HVA currents were reduced by only $17\cdot8 \pm 15\cdot9\%$ (s.d.; Student's paired t test; $P \leqslant 0\cdot00064$). These effects of flow on HVA calcium currents were found to be statistically different from zero effect (Student's paired t test; $P \leqslant 0\cdot0099$). Differential sensitivity of LVA and HVA calcium currents to flow was also displayed in the time course of recovery from the flow effect (see for example

Figs $1B$ and $2A$). Under constant conditions of flow (exposure 10–30 s, distance 100 μm) HVA currents recovered to 50% of their control values in $31\cdot7 \pm 28\cdot8$ s (s.d.; $n = 12$ cells), whereas LVA currents recovered to 50% of their control values in 267 ± 183 s (s.d.; $n = 5$ cells). The flow-induced reduction in the size of both LVA and HVA calcium channel currents was reversible (Figs 1, 2, 3, 5 and 8) and was therefore not a consequence of calcium current run-down.
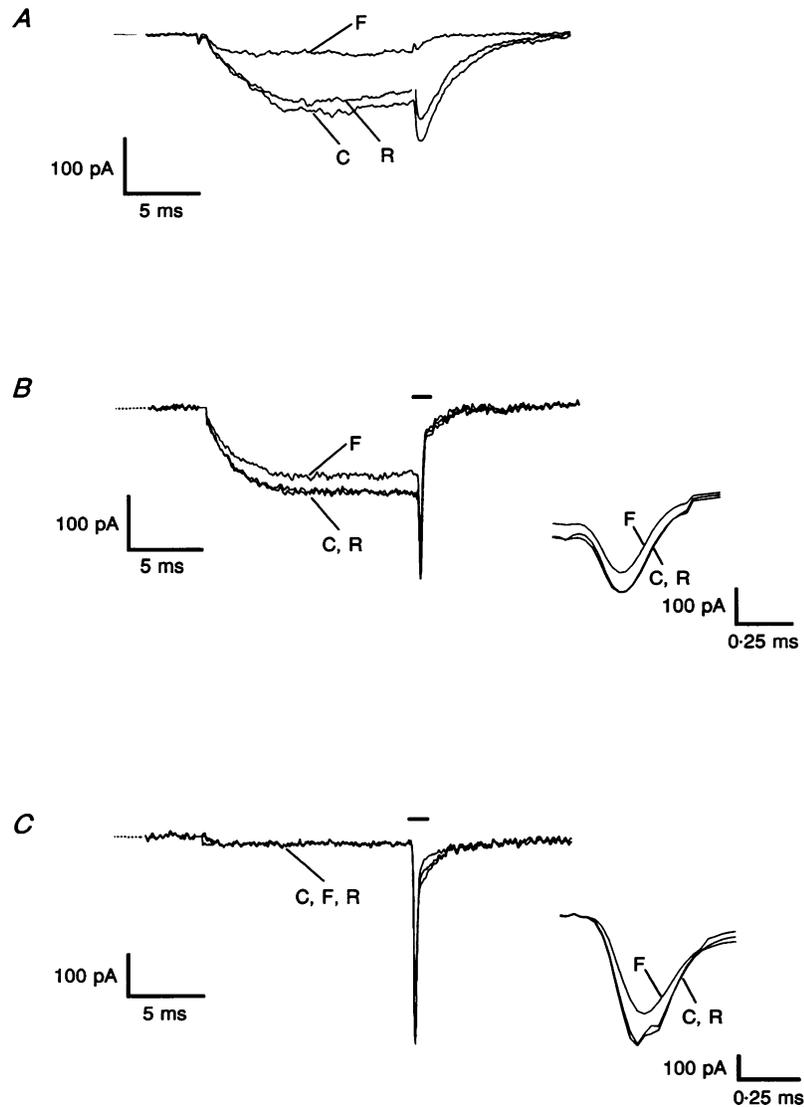
Figure 5. The effect of flow on calcium tail currents

A, flow (tip 4 μm, distance 100 μm) reduced the amplitude of both the LVA calcium current and the related slowly deactivating calcium tail current, by 90% ($V_t = -30$ mV). B, flow (tip 3 μm, distance 100 μm) reduced, by 25%, the amplitude of both the HVA calcium current and its related fast deactivating calcium tail current ($V_t = 0$ mV). C, The same cell as in B. A voltage step to +50 mV activated a small calcium current and a large fast deactivating calcium tail current. Flow (same pipette as in B) reduced the tail current by 25%. Horizontal bars in B and C represent the parts of the traces that are expanded and illustrated as insets. In these experiments we used short (15 ms) voltage steps and increased the sampling rate to $33\cdot3$ kHz. Leakage and capacitative current components were averaged from 10 hyperpolarizing pulses (15 ms, 20 mV) and then digitally extrapolated and subtracted from the whole-cell calcium currents.

Effect of flow on calcium tail currents

Further evidence for the mechanosensitivity of calcium channels in somatotrophs was provided by testing the response of the calcium tail currents to flow. Since flow reduced both LVA and HVA calcium currents, it was expected that their related tail currents would be reduced as well. We found that, as previously described in pituitary tumour cells (Armstrong & Matteson, 1985), LVA calcium currents in somatotrophs were associated with slowly deactivating tail currents, whereas HVA calcium currents were associated with fast deactivating tail currents (Fig. 5A and B). Exposure to flow reversibly reduced the slowly deactivating calcium tail currents by $75\cdot3 \pm 25\cdot6\%$ (S.D.; $n = 3$ cells, Fig. 5A) and the fast deactivating calcium tail currents by $29\cdot1 \pm 14\cdot4\%$ (S.D.; $n = 7$ experiments, 6 cells, Fig. 5B). In one of these experiments (shown in Fig. 5C) flow reversibly reduced (by 25%) the large fast deactivating calcium tail current which was activated by a test potential ($V_t = +50$ mV) close to the reversal potential of the calcium currents (Fig. 5C).

Voltage independence of the flow effect

We questioned the voltage dependence of the flow effect on calcium currents by examining the effects of flow on current–voltage (I–V) plots of both peak and sustained calcium currents. Figure 6A and D shows I–V plots of sustained and peak calcium currents respectively, each before and after exposure to flow. As can be seen in Fig. 6B and E the effect of flow on the sustained and peak calcium current was not associated with any significant shift in the activation curves.

The magnitude of the flow effect on sustained calcium currents, which represent mainly activation of HVA currents (-20 mV $\leqslant V_t \leqslant 30$ mV), was a 30% reduction, which was independent of the magnitude of the initiating voltage steps (Fig. 6C). The peak calcium currents are composed of both LVA and HVA calcium currents, which show differential sensitivity to flow (Fig. 4) and which dominate at different voltage ranges, so they are expected to show, and actually do show, a shift in the flow-induced effect between these two voltage ranges (Fig. 6F). Peak
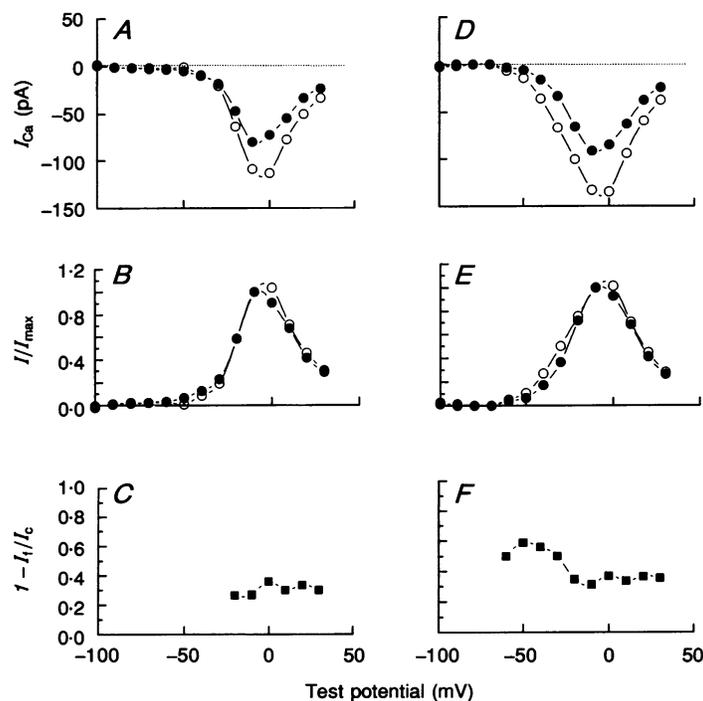

Figure 6. Voltage dependence of the flow effect

A, I–V plot of sustained calcium currents (I_{Ca}) in control conditions ($\bigcirc$) and after exposure to flow ($\bullet$). B, activation curve for sustained calcium currents in control conditions ($\bigcirc$) and after exposure to flow ($\bullet$). C, the magnitude of the flow effect on sustained calcium currents at different test potentials. I_c, control calcium current; I_f, calcium current after exposure to flow. D, E and F, same as in A, B and C, respectively, for peak currents, which were measured from the same current traces. The voltage dependence of the peak currents (F) is due to differential sensitivity to flow and difference in voltage range for activation of LVA and HVA currents. I–V plots were generated by 200 ms voltage steps to different test potentials with 10 mV increments (voltage range, -100 to $+30$ mV; $V_h = -80$ mV). Each current trace was measured twice, once at the peak of the current and again at 180–200 ms of the pulse (referred to as sustained).
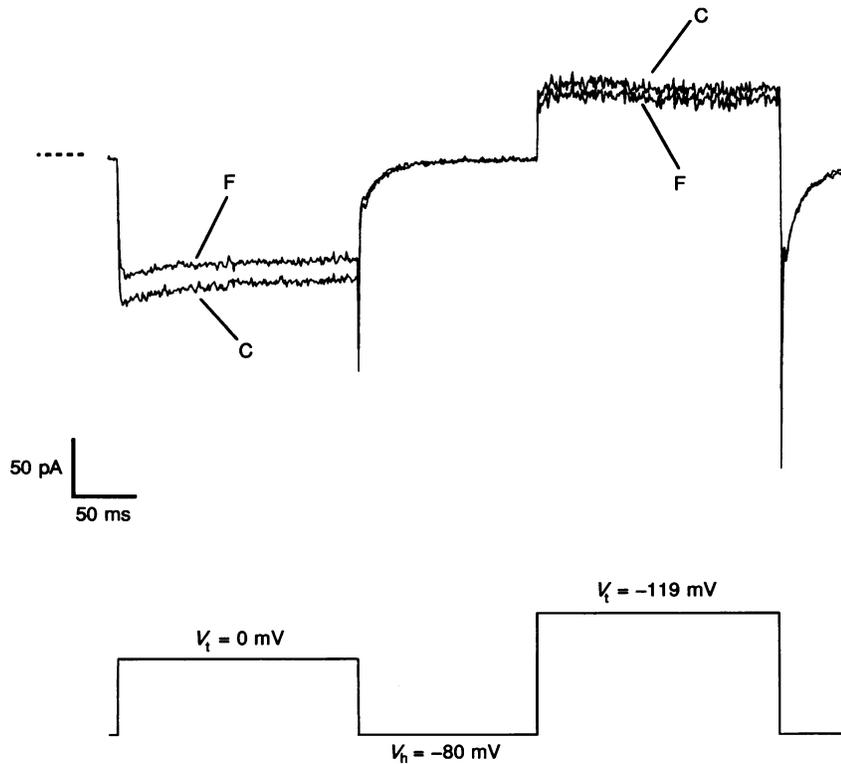
Figure 7. Effects of flow on outward calcium currents

Double pulse protocols were used to activate inward and outward calcium currents. The first test pulse elicited an inward calcium current; the second test pulse, which was above the reversal potential for calcium currents in this cell (60 mV), elicited an outward calcium current. Flow (tip 3·5 μm, distance 50 μm) reduced both the inward and outward calcium currents. In these experiments, EGTA was reduced from 11 to 1·1 mM and calcium (0·55 mM) was added to the patch pipette. Under these conditions, calcium currents after leak subtraction reversed at 50·5 ± 10·7 mV (S.D.; $n = 15$ cells).
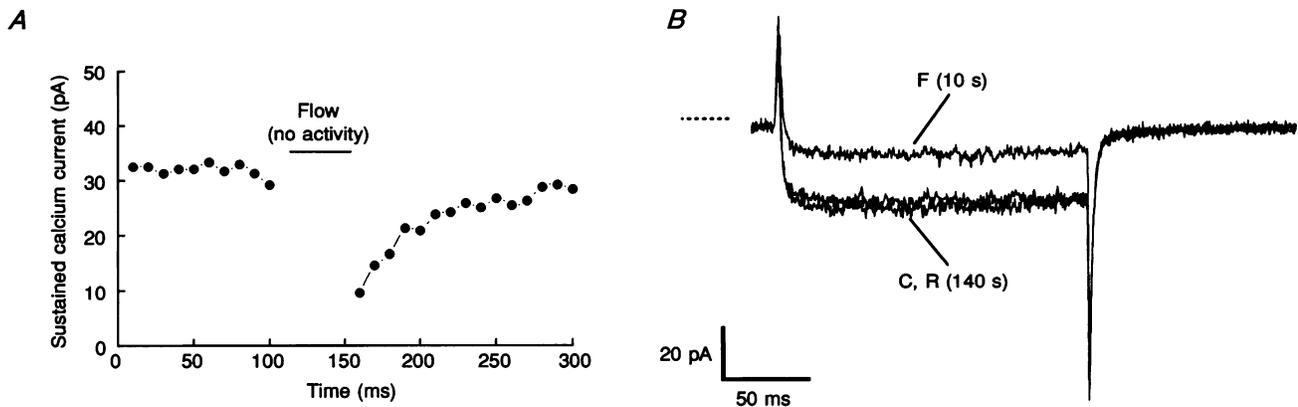
Figure 8. The effect of flow does not depend on activation of calcium currents

A, exposure to flow (tip 3 μm, distance 100 μm) during a period of 40 s in which calcium currents were not activated ($V_h = -80$ mV) resulted in reduction of the HVA calcium currents which were activated after this period ($V_t = 0$ mV). *B*, HVA calcium current traces from the experiment illustrated in *A*. C, control before exposure to flow; F, 10 s after the end of the flow period; R, 140 s after the end of the flow period.

calcium currents that represent mainly LVA currents (-60 mV $\leqslant V_t \leqslant -30$ mV) were reduced by 60%, while peak currents that represent mainly HVA currents (-20 mV $\leqslant V_t \leqslant 30$ mV) were reduced by 35% (Fig. 6F). The effects of flow were not voltage dependent in four additional similar experiments.

Effect of flow on outward calcium currents

Since flow reduced the influx of calcium through voltage-sensitive channels it is expected that flow will also reduce the efflux of calcium through the same channels (Bean, Nowycky & Tsien, 1984; Hess, Lansman & Tsien, 1984). Figure 7 shows calcium currents which were activated by a double pulse protocol; the first test pulse ($V_t = 0$ mV) elicited an inward calcium current and the second test pulse ($V_t = 119$ mV) elicited an outward calcium current. Flow reduced both the inward and outward calcium currents by 19 and 15% respectively. In similar experiments ($n = 6$ cells), inward calcium currents were reduced by $24 \cdot 8 \pm 17 \cdot 3$% (s.d.) and outward calcium currents by $45 \cdot 4 \pm 31 \cdot 4$% (s.d.). This difference in the averages was found to be statistically insignificant (paired t test; $P \leqslant 0 \cdot 159$).

The effect of flow does not depend on activation of calcium currents

In order to find whether or not the effect of flow depends on activation of calcium channels, we exposed cells to flow while holding the membrane potential at -80 mV. In these experiments (Fig. 8), calcium currents which were evoked *after* termination of flow were reduced in amplitude in comparison to control current (recorded before flow application) and recovered slowly to control values ($n = 4$ cells). These results suggest that flow can reduce calcium currents by acting on calcium channels in either the closed or the opened state.

DISCUSSION

The present study demonstrates that voltage-activated calcium currents in anterior pituitary cells are sensitive to flow of physiological solution from pipettes placed near the cells under study. Gravity flow from these pipettes was produced by very low hydrostatic pressure (3 cmH$_2$O) and the estimated shear force induced by this flow was smaller than $3 \cdot 77$ dyn cm^{-2} (see Methods). Similar flow-induced shear forces were needed to activate an inward rectifying potassium permeability (Olesen *et al.* 1988) and a cationic permeability (Schwarz *et al.* 1992b) in endothelial cells. The estimated linear flow from the pipette in our experiments ($0 \cdot 3$ mm s^{-1}, see Methods) is smaller, by one order of magnitude, than the flow needed to alter the gating of inward potassium rectifier channels in inside-out patches of skeletal muscle (Burton & Hutter, 1990). Our study

shows that the effect of flow did not result from reversible changes in either seal resistance or leak conductance (see Fig. 2). In addition, the effect of flow did not result from activation of outward potassium or inward chloride currents. Potassium currents were blocked (see Methods) and the calculated reversal potential for chloride current (-4 mV) is very close to the test potentials ($V_t = 0$ mV) which were used to activate HVA currents. As for LVA currents, they were activated at a test potential ($V_t = -30$ mV) which would elicit an outward chloride current, which tends to increase rather than decrease these currents. Moreover, LVA currents were selectively reduced by flow with no effect on the leak currents observed immediately after their inactivation (see Figs 1, 3 and 4). Additional support for a selective effect of flow on calcium channels came from the effects of flow on LVA and HVA calcium tail currents (Fig. 5) and from the effect of flow on reversed calcium currents (Fig. 7).

Possible mechanisms of the flow effect

The mechanism of the flow-induced response is not yet known. However, it is reasonable to speculate that the effects of flow can be explained either by a direct action on the calcium channel or, alternatively, by an indirect action due to deformation of the cell membrane (Burton & Hutter, 1990). The effects of flow may also result from wash-away of chemicals (such as ATP and GTP) leaking from the recording pipette into the outer mouth of the calcium channel. However, since the effects of flow were observed in the presence of both ATP and GTP in the flow pipette and, in other experiments, in the absence of GTP in the recording pipette (S. Ben-Tabou, unpublished observations) we favour this wash-away hypothesis less.

Flow-induced deformation of the cell membrane might be associated with displacement of charged molecules, on the outer surface of the membrane, in the vicinity of the calcium channel or with displacement of membrane-attached cytoskeletal components (Guharay & Sachs, 1984; Ruknudin, Song & Sachs, 1991; Sokabe, Sachs & Jing, 1991), which might be connected to the channel protein itself (Srinivasan, Elmer, Davis, Bennett & Angelides, 1988; Steiner, Walke & Bennett, 1989).

Morris (1990) classified mechanosensitive ion channels into three major subtypes, based upon their mode of activation: stretch-sensitive, displacement-sensitive and shear stress- (or flow-) sensitive channels. The mechano-sensitivity of voltage-gated calcium currents observed in the present study might be related to the group of shear stress-sensitive channels. However, since each of these three mechanosensitive channel subtypes might be associated with deformation of the cell membrane, this classification does not provide us with new insight about the mechanism of the flow effect. In this regard, it is of

interest that stretch-activated cationic channels in endothelial cells (Lansman, Hallam & Rink, 1987) were found to be similar in their properties to shear stress-activated cationic currents in the same cells (Schwarz *et al.* 1992*b*).

Possible role of the flow effect

At this stage, it is still not known whether the mechanical sensitivity of voltage-activated calcium channels in pituitary somatotrophs plays a functional role in either regulation of hormone secretion or any other cellular function. However, since influx of calcium plays a key role in regulation of growth hormone secretion (Holl *et al.* 1988; Rawlings *et al.* 1991; Lussier *et al.* 1991), it is tempting to speculate that a flow-dependent mechanism might play an important regulatory role in this secretion during different physiological and pathological conditions of blood flow through the gland. The anterior pituitary is highly vascular and receives a very high rate of blood flow (Page, 1982; Lees, Lynch, Richards, Lovick, Perry & Pickard, 1992) through a venous portal system, which divides into a dense network of sinusoidal capillaries (for illustrations see Bergland & Page, 1978; Wheater, Burkitt & Daniels, 1987). Adjacent to these sinusoidal capillaries, somatotrophs (and other pituitary cells) can be found, secreting their hormones into the perivascular or intercellular spaces (Shimada & Tosaka-Shimada, 1989). Changes in blood flow rates through the anterior lobe may result in increased intracapillary pressure and in changes in the flow rate of fluid through the perivascular and intercellular space which, in turn, may affect the calcium channels situated in the external membrane of the somatotrophs. According to this hypothesis, the somatotrophs can sense and respond to changes in pressure or fluid flow through the interstitial space. Mechanical regulation of calcium influx may also act as a protective mechanism to prevent excessive hormone secretion in pathological conditions such as pituitary tumour (adenoma), which might be associated with increased mechanical pressure on the pituitary cell membrane.

Mechanosensitivity of calcium currents in pituitary cells may also play a role in other cellular functions, such as volume regulation and control of cell size and growth (for review see Sachs, 1991; McCarty & O'Neil, 1992).

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