جریانهای یونی کانالهای پتاسیمی و کلسیمی در سلولهای ایزوله شده عضله صاف

سمینال وزیکول خوکچه و مهار این جریانها بوسیله Gilbenclamide

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چکیده:

سلولهای عضله صاف سمینال وزیکول به هنگام تحریک عصبی و یا سلولی تولید پتانسیل فعال می‌کنند (۱) ولی نوع کانالهای یونی که در ایجاد این فعالیتهای الکتریکی دخیل هستند تاکنون توصیف نشده‌اند. در این گزارش نوع و فارماکولوژی کانالهای یونی در روی سلولهای ایزوله شده عضله صاف سمینال وزیکول خوکچه با استفاده از تکنیک *Whole-cell voltage-clamp* مطالعه شده‌است. دو نوع جریان پتاسیمی بسمت خارج و یک نوع جریان کلسیمی بسمت داخل شناسائی گردیدند. اولین جریان پتاسیمی بسمت خارج جریانی زودگذر است که کاملاً از جریان ممتد وابسته به *Ca* قابل تمایز است. جریانهای بسمت خارج از روی خواص آنها مانند وابستگی به ولتاژ، وابستگی به *Ca* و حساسیت آنها نسبت به بلوکه شدن با *TEA* و *3,4-diaminopyridine* متمایز گردیده‌اند. جریان بسمت داخل هم از روی خواص وابسته به ولتاژ، غیرفعال شدن کند آن و بلوکه شدن بوسیله *Cd* و *nifedipine* شناسائی گردید. از آنجائیکه *glibenclamide* موجب *relaxation* عضله صاف سمینال وزیکول می‌شود اثر *glibenclamide* بر روی این جریانهای یونی نیز مطالعه گردید. *glibenclamide (500óM)* نه تنها هردو جریان زودگذر و ممتد بسمت خارج را مهار کرد بلکه جریان کلسیمی بسمت داخل را نیز مهار کرد(IC_{50} *60_M* = این آزمایشات نشان می‌دهد که حداقل سه نوع کانال یونی بر روی غشا سلولهای صاف سمینال وزیکول حضور فعال دارند. اینها عبارتند از :

L-type Ca channel, Ca-activated k channel, A-Type K channel خواص مهارکنندگی جریان کلسیمی بوسیله *glibenclamide* مکانیسمی است که می‌تواند خواص رفع انقباضی این دارو را توجیه کند.

کلید واژه‌ها: ۱ـ کانالهای پتاسیمی ۲ـ کانالهای کلسیمی ۳ـ کلی بتکامید ۴ـ خوکچه هندی ۵ـ وزیکول سمینال

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K CHANNEL AND CA CHANNEL CURRENTS AND THEIR INHIBITION BY GLIBENCLAMIDE IN SMOOTH MUSCLE CELLS ISOLATED FROM GUINEA-PIG SEMINAL VESICLE

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ABSTRACT

Smooth muscle cells of seminal vesicle exhibit excitatory junction patential on nerve stimulation and can fire evoked, action potential (1). However, the type of ion channels that underlie this electrical activity have not been described. I have investigated the type and pharmacology of ion channel in freshly isolated smooth muscle cells from the guinea-pig seminal vesicle using whole-cell patch-clamp technique. Two types of outward, k-current, and one type of inward Ca-Current are characterised. The first outward current had a transient appearance and was clearly distinguishable from the Ca-sensitive sustained current. The outward currents were classified on the basis of their voltage- and Ca-dependent and their sensitivities to block by TEA and 3,4- diaminopyridine. The inward current was also voltage-dependent, showed slow inactivation and was abolished by Cd and inhibited by nifedipine. The action of glibenclamide was also investigated because this compound had a surprising relaxant effect on the seminal vesicle. Glibenclamide (500μM) inhibited both the transient and sustained current indicating an IC50 of about 60μM. These experiments suggest that at least three types of ion channel are present in seminal vesicle smooth muscle: A-Type K channel, Ca-activiated K channel and L-type Ca channel. The observed inhibitory effect of glibenclamide on Ca channel current may be a mechanism underlying its relaxant effect on the whole tissue.

Key Words : *1) Potssium Channel* *2) Calcium Channel* *3) Glibenclamide* *4) Guinea-Pig* *5) Seminal Vesicle*

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INTRODUCTION

The mammalian seminal vesicle is a bind-ended sac, its wall consisting of mucosal and smooth muscle cell layers. The smooth muscle layers are arranged in an inner circular and an outer longitudinal layer, circular layer being the major type and longitudinal layer is absent in the distal end [1]. These smooth muscle cells are densely innervated: they receive sympatethic fibres of the hypogasteric nerve and parasympathetic fibres [7]. Intracellular microelectrode recordings indicate that the smooth muscle cells have a membrane potential of - 50 mV, can fire evoked action potentials, and exhibit excitatory junction potentials on verve stimulation [5] However, the types of ion channel that underlie this electrical activity have not been dscribed. I have investigated the voltage- and Ca^{2+} -dependence and pharmacology of ion channels in freshly isolated smooth muscle cells from the guiea-pig seminal vesicle.

METHODS

Smooth muscle cells were isolated from guinea-pig seminal vesicle Ca^{2+}-free media, using an enzyme mixture of collagenase (0.2-0.35%) and protease (0.15-0.25%) followed by mechanical agitation. Whole-cell voltage-clamp (see Fig. 1) of single cells was obtained by using a patch-clamp amplifier and borosilicate glass recording pipettes of 1-4 MΩ. Command voltage were genetated and signals captured on line using a PC and Data Acquisition Board (National Instruments). Signals were filtered at 1 KHz and digitised at 2 KHz. Cells were placed in a 0.1 ml recording chamber and different solutions were bath-applied by switching the inflow to another reservoir. The flow rate was 2 ml/min and 80% exchange occurred within about 30s. All recording were made at room temperature. Bath solution (mM): NaCl 135, KCl 5, $MgCl_2$ 1.2, HEAPS 10, Na_2 ATP 3, EGTA 0.2 or 10 and KCL 130 for K-current or CsCl 130 for Ca-current recording respectively. All solutions were titrated to pH 7.4. Glibenclamide and nifedipine were dissolved in dimethylsulphoxide (DMSO); the final bath concentration of DMSO was 0.01% for glibenclamide and 0.001% for nifedipine.

RESULTS

When the KCL pipette solution was used and the holding potential (HP) was -60 mV two major types of outward cutward current were elicited by square depolarisation pulses to positive of -20 mV. Figure 1 shows the initial transient outward current. This current reached a peak within 5±1 ms (mean±s.e. mean, n=6) and decayed completely in 106± 9 ms (n=6) at test potential of +60mV. Tetraethylammonium (TEA; 1 & 10mM) had no effect on initial transient current (Fig. 2A) but 3,4- diaminopyridine

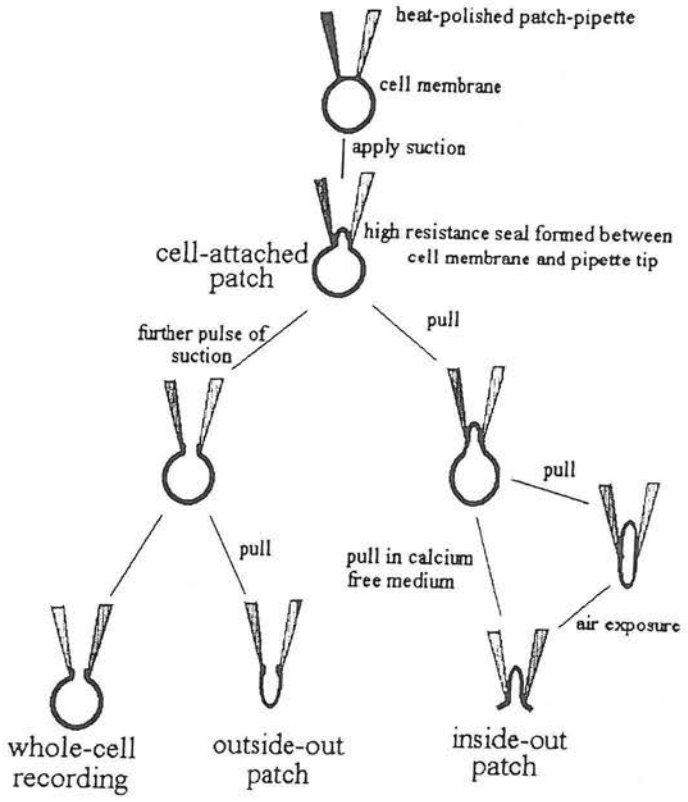

Figure 1. Multiple configuration of the patch-clamping technique. A patch-pipette with a fire polished tip is pressed against the cell membrane. Mild suction is applied to the patch-pipette and this draws a patch of cell membrance into the tip and a high resistance (109W) seal is formed between the membrance and the glass pipette. This configuration is called the cell attached-patch. A further pulse of suction may break the cell membrane within the pipette tip so that whole-cell recording can be achieved. If the pulse of suction is followd by pulling the patch-pipette away from the cell, an outside-out patch is formed. If the patch-pipette is pulled away from the cell, in the cell attached-patch configuration, an inside-out patch is formed. The pull may have to be followed by exposure of the patch to air to prevent vesicle formation. The patch-clamp electrode is connected to a feedback amplifier so that the potential across the patch may be clamped and transpatch current monitored.

(3,4-DAP, 1mM) reversibly blocked the initial transient current (Fig. 2B). With 0.2 mM EGTA in the recording pipette a large sustained current was observed on depolarisation. TEA (1 & 10 mM) reversibly blocked this current (Fig. 3A). With 10 mM EGTA in the recording pipette the sustained (TEA-sensitive) current was virtually absent (Fig. 3B). When the CsCl pipette solution and Ca^{2+} bath solution were used depolarisation to positive of +30 mV elicited a voltage-dependent inward current that reached its peak in 68±8 ms at test potential of +20 mV (n=10). When Ca^{2+} was

exchanged for Ba^{2+} the inward current was much larger (see Fig. 4) and the time to peak was reduced to 17±2 ms (n=10). The Ba-current was abolished by Cd^{2+} (100 μM) (Fig 4). The voltage-dependent inward current was concentration- dependently inhibited by nifedipine (Fig. 5). Glibenclamide inhibited both the sustained and initial transient current (Fig 6). Glibenclamide (100 μM) reversibly also inhibited the inward Ba-current (Fig 7).

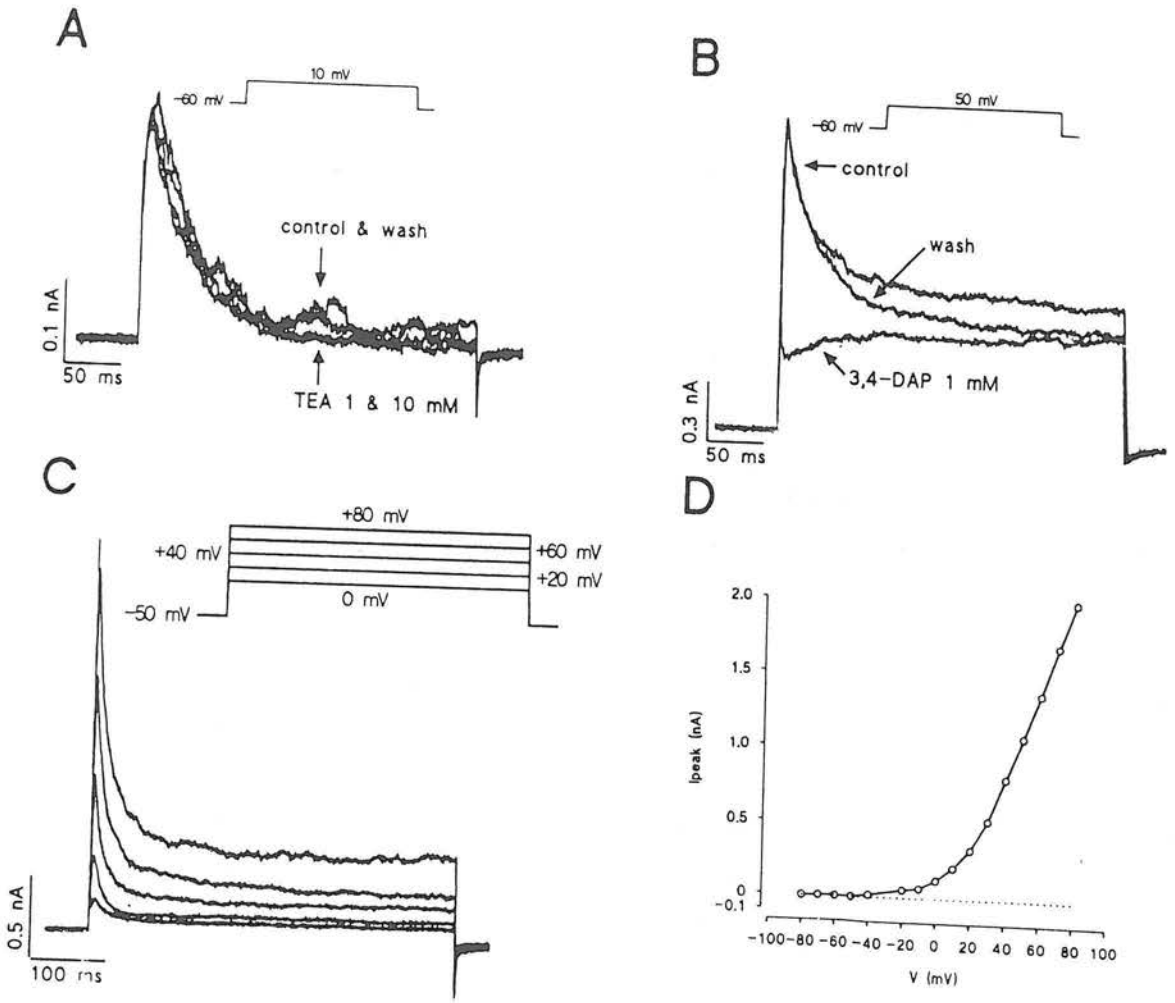

Figure 2. Initial transient outward current (with 10 mM EGTA in the recording pipettte). A, TEA(1 & 10 mM) had no effect on this current. B, 3,4-DAP (1 mM) reversibly blocked the initial transient current. C, original currecnt record at various test potentials in the presence of 1 mM TEA. D, the current/votage (I/V) relationship for peak initial current using a holding potential of -50 mV.

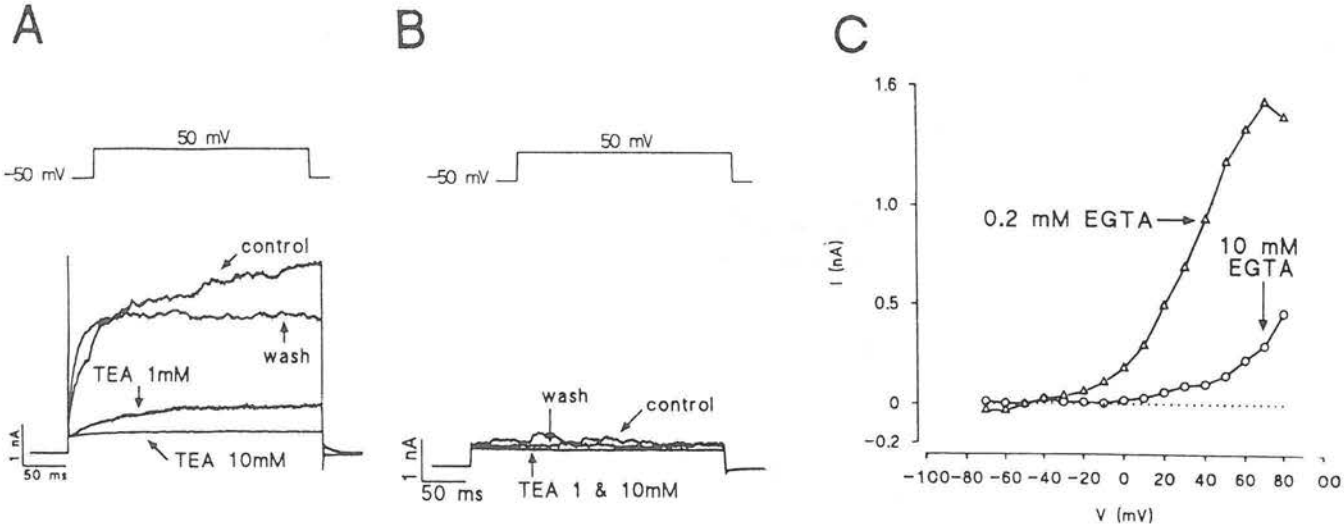

Figure 3. Sustained outward current (with 0.2 mM EGTA in the recording pipette). A, TEA (1 & 10 mM) reversibly blocked this current. B, With 10 mM EGTA in the recording pipette the sustained (TEA-sensitive) current was virtually absent. C, TEA-sensitive currents obtained by subtracting the mean current before and after addition of 1 mM TEA when there was 0.2 mM or 10 mM EGTA in the recording pipette solution (n=6 for each).

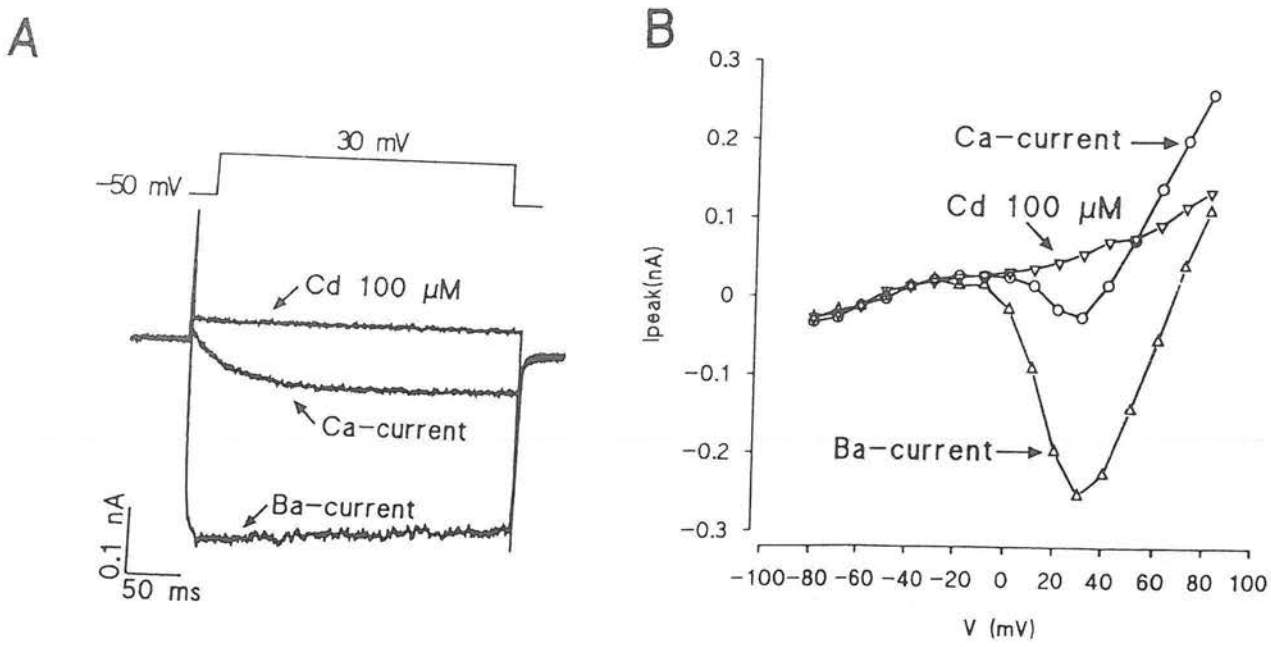

Figure 4. The inward Ca channel current recorded with CsCl solution. A, original record of Ca-Current and Ba-current and the blockade of Ba-current by Cd2+ , Ba2+ and Ba2+ plus Cd2+ bath solutions, showing inward Ca-current and Ba-current (n=5 for each).

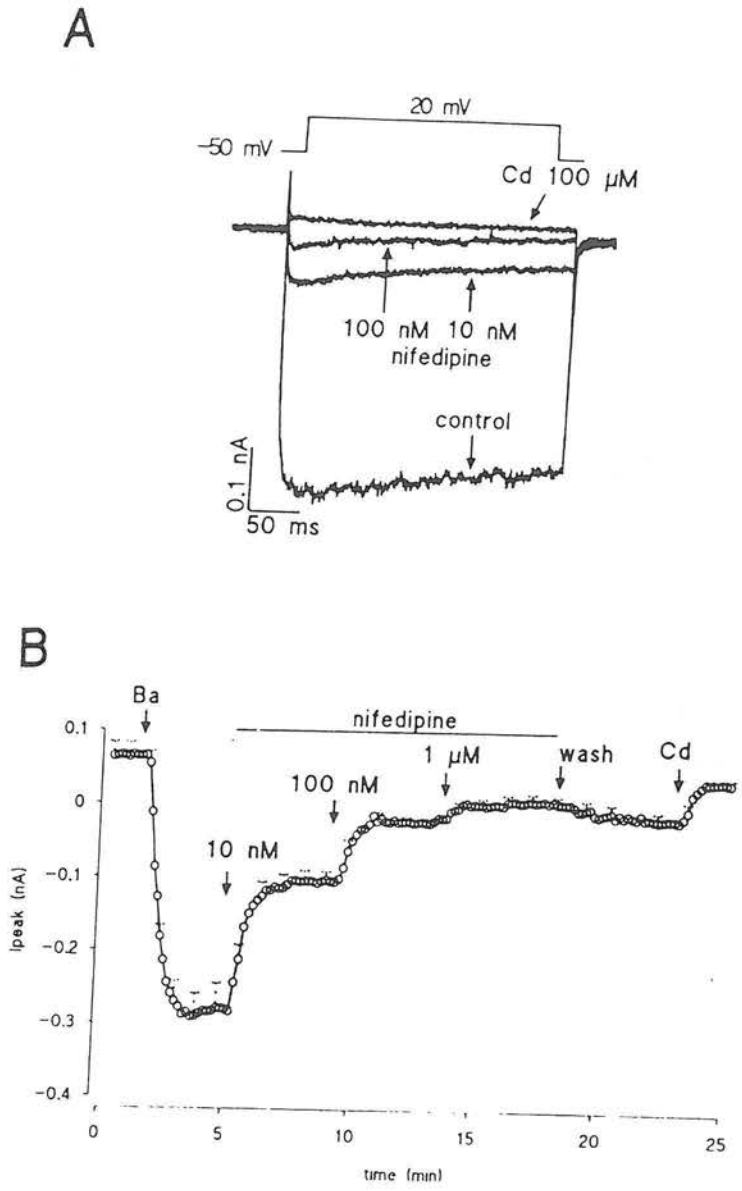

Figure 5. Effect of nifedipine on the inward current. A, orginal record showing the effect of nifedipine on Ba-current (HP -50 mV). B, mean current amplitudes (±s.e.mean for every 6th point) elicited by stepping to +20 mV every 10 s(n=6). Current amplitudes were measured relative to the holding current at -50 mV. The bath solution was changed from Ca2+ - to BA2+ -containing solution as indicated by ¨Ba¨.

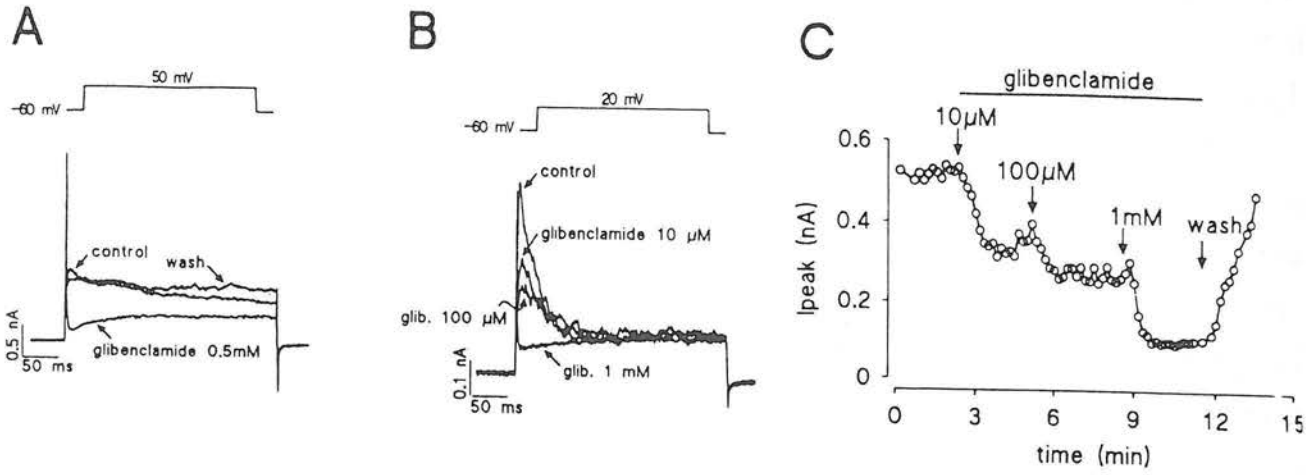

Figure 6. Effect of glibenclamide on the transient and sustained outward currents. A, glibenclamide reversibly inhibited the sustained outward current

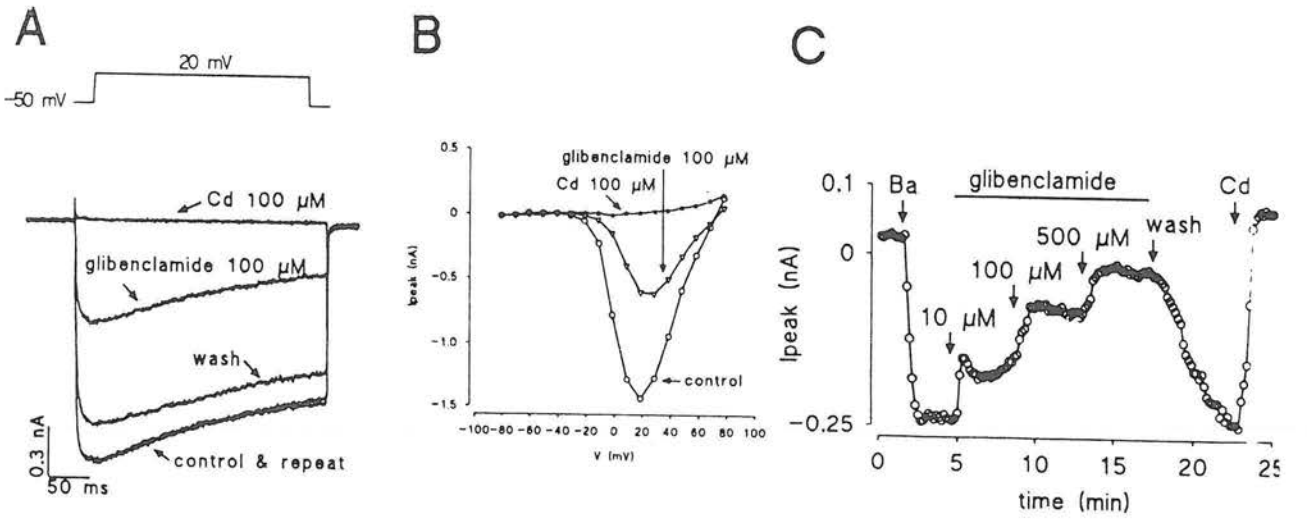

Figure 7. Effect of glibenclamide on the Ca channel current. A, original records showing that glibenclamide reversibly inhibited the Ba-current. B, the I/V curve for Ba-current and the effect of glibenclamide. C, concentration-dependent inhibition of Ba-current by glibenclamide. Ba-current was elicited by stepping to +20 mV from the holing potential of - 50 mV every 10 s.

DISCUSSION & CONCLUSION

In these experiments ionic current were recorded from single smooth muslce cells isolated from guinea-pig seminal vesicle. Two types of voltage-dependent outward currents are described. The first one which could be elicited depolarisation was present at holding potential more negative than resting membrane potential(-50 mV) and had a transient appearance with activation threshold of around - 20 mV. The charge carrier seemed to be K^+ and the current was blocked by 3,4-DAP but not TEA and did not appear to be Ca^{2+}-dependent. The characteristic of this current is very similar to the current carried by A type K channels[2], The second voltage-dependent outward current on the other hand was sustained for the duration of a depolarising pulse and unlike initial transient current its availability did not seem to be dependent on holding potential. The sustained current was the major outward current in smooth muscle cells of guinea-pig seminal vesicle. The reduction in current amplitude with 10 mM EGTA in the pipette solution clearly indicate that this current was carried by Ca^{2+}-activated K channels. the blockade of the sustained current by TEA at 1 μM concentration suggest that it was carried by large conductance Ca^{2+}-activated K channel but not apamine sensitive channels[3]. With the outward current, particularly those recorded with 10 mM EGTA in the recording pipette, a small inward current could be detected following leak subtraction. Further experiments were carried out to identify the nature of ion channel underlying this inward current.The inward current was studied by using a CsCl pipette solution to block K-current. The inward current was activated in a voltage-dependent manner with an activation threshold around-30 mV; reaching its maximum amplitude at about +20 mV. The inward current was slowly inactivated and blocked by nifedipine. When Ba^{2+} replaced Ca^{2+} as a charged-carrier the inward current activated faster and the current amplitude became bigger but otherwise had a similar characteristic to the Ca current and blocked by Cd^{2+} and nifedipine in a concentration-dependent manner with an IC_{50} about 7 nM for nifedipine. The features of the inward current indicate that it is carried by Ca channels and since it had slow inactivation and was dihydropyridine sensitive it is very likely that the Ca channel were of the L-type. The action of the sulphonylurea glibenclamide[4] was also investigated because this compound has a surprising relaxant effect on the seminal vesicle [6] It was found that glibenclamide had a pronounced inhibitory effect on both the transient outward current and the inward Ba-current.

These experiments suggest that at least

two types of K channel and one type of Ca channel are present in seminal vesicle smooth muscle cells: A-type K channels which inactivate rapidly, are blocked by 3,4-DAP and underlie the initial transient outward current; large conductance Ca^{2+}-activated K channels which are blocked by millimolar concentration of TEA and underlie the sustained outward current; L-type Ca channels which inactivate slowly and are blocked by nanomolar concentrations of nifedipine. The inhibitory effect of glibenclamide on the Ca-channel current may be a mechanism underlying its relaxant effect on the whole tissue.

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