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Serum induces the immediate opening of Ca^{2+}-activated channels in quiescent human fibroblasts

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Application of fetal calf serum to quiescent human fibroblasts produces an immediate (3 – 20 s delay) increase in membrane conductance which lasts about 20 – 30 s. This conductance is strongly outwardly-rectifying and has a reversal potential between – 45 and – 10 mV. The conductance increase may also be induced by application of the Ca^{2+} ionophore A23187 while it does not occur when intracellular K^+ is replaced by Cs^+. It is concluded that this early effect of serum is due to the opening of Ca^{2+}-activated channels. This permeability change will alter the membrane potential and thus possibly interact with other voltage-sensitive processes induced by serum growth factors.

Serum; Cell activation; Calcium mobilization; Membrane current; (Fibroblast)

1. INTRODUCTION

Quiescent cultures of human fibroblasts can be stimulated to enter DNA synthesis by serum growth factors. The initiation of DNA synthesis is a late event, however a number of processes take place immediately after stimulation, which include activation of tyrosine-specific protein kinases and increased breakdown of phosphoinositides with the generation of the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [1–4]. While DAG activates the protein kinase C, IP_3 leads to an elevation of intracellular Ca^{2+} upon interaction with intracellular stores [5]. In turn the transient rise in cytosolic Ca^{2+} may activate several cellular processes including the regulation of membrane ionic channels. There is also recent evidence [6] that IP_3 may directly open voltage-insensitive Ca^{2+} channels on the plasma membrane.

The stimulation of monovalent ion transport across the plasma membrane, such as the Na^+/H^+ antiport, is another consequence of interaction with growth factors. In addition, it has been reported that in human fibroblasts serum activates an unselective cationic conductance [7,8]. In neuroblastoma cells serum induces a first hyperpolarizing phase that was suggested to be due to K^+ conductance possibly mediated by Ca^{2+} [9,10]. More recently bradykinin has been shown to hyperpolarize the membrane of neuroblastoma hybrid cells [11–13] through the activation of a Ca^{2+}-dependent K^+ current. The possibility arises then of complex interactions between membrane potential and other membrane-associated processes.

By voltage-clamp recording we have explored the electrical responses of quiescent human fibroblasts to serum: we have found that serum produces an immediate increase in membrane permeability due to the opening of Ca^{2+}-activated channels.

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2. MATERIALS AND METHODS

The whole-cell variation of the patch-clamp technique was used throughout, details can be found in Peres et al. [14]. Once the whole-cell condition was established, each cell was held continuously at the selected V_h (usually +50, +40 or −80 mV).

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Current and voltage signals were continuously stored on a video cassette recorder via pulse code modulation (PCM). Traces were subsequently digitized and analyzed with a personal computer as previously described [14]. Human skin fibroblasts were used between the 8th and the 15th passage. Cells were plated in Dulbecco modified Eagle medium (DMEM, from Flow) with 10% fetal calf serum (FCS, from Flow). After 4–5 days the medium was substituted with DMEM containing 1% FCS and cells used 48 h later. Experiments were performed on suspended cells prepared as previously described [14] for Swiss 3T3 fibroblasts. The recording bath contained about 500 μl of solution; serum was added as a 40 μl drop which will give 8% serum concentration when fully mixed; the calcium ionophore was dissolved in dimethylsulphoxide (DMSO, concentration 5 mM) and 1 μl of this solution added to the bath (final concentration of ionophore 10 μM). The pipettes were filled with the following solution (in mM): 140 KCl, 2 $MgCl_2$, 10 Hepes-KOH, 1 EGTA, 2 ATP, 0.5 GTP, pH 7.3; when needed KCl and KOH were substituted with equal amounts of CsCl and CsOH. The external solution had the following composition (in mM): 116 NaCl, 5 KCl, 1.2 $MgCl_2$, 8 $CaCl_2$, 25 Hepes-NaOH, 6 glucose, pH 7.3. The temperature was between 33 and 36°C.

Voltage ramps of 310 ms in duration were applied at various times; ramps were of two kinds: from V_h = +50 (+40) mV, the potential was moved linearly with time to −80 (−90) mV and then stepped back to +50 (+40) mV (see inset in fig.2C); from V_h = −80 mV, the potential was moved linearly with time to +50 mV and then stepped back to −80 mV (see inset in fig.2A). In all cases the voltage range +40 to −80 mV was explored.

3. RESULTS

When quiescent human fibroblasts are exposed to serum, in about 70% of the cells (22/30 cells) an electrical response takes place which is strongly voltage-dependent: while in the range of voltages between −90 and −20 mV very little change in current can be seen under voltage-clamp, holding the cell at potentials more positive than about −20 mV reveals the occurrence, upon serum addition, of an outward current which increases steeply with potential.

This is shown in fig.1 where two fibroblasts were exposed to serum while being held at −80 mV holding potential (V_h, panel A) and at +50 mV (panel B), respectively. In fig.1B a clear response can be seen beginning about 5 s after serum addition: a fast and large outward current develops which lasts about 20 s together with an increase in

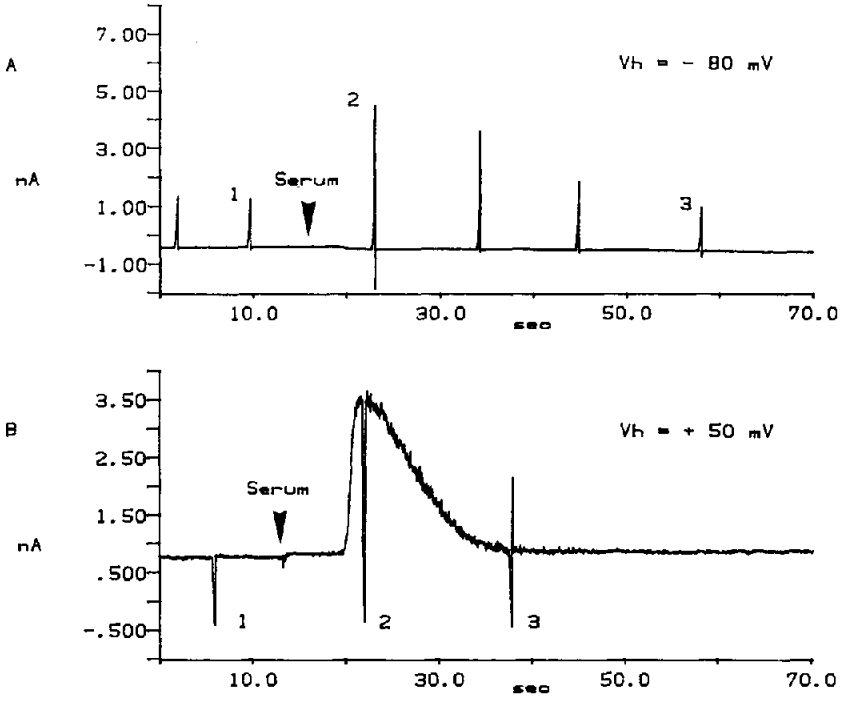

Fig.1. Effect of serum on two fibroblasts held at −80 (A) or +50 (B) mV. The rapid deflections on the traces are the currents in response to voltage ramps ranging from −80 to +50 mV (A) or from +50 to −80 mV (B). The amplitude of the current during the ramps is not quantitatively correct in this figure because of the slow conversion rate.

noise. In the cell of fig.1A, in contrast, only a very small downward deflection can be seen about 3 s after serum addition. However, in both cases the response is actually the same: this is shown by the current elicited in response to fast voltage ramps aimed to explore a wide voltage range during the response. The ramps appear as fast deflections in fig.1 and are plotted with greater detail in fig.2. Panels A and C of fig.2 plot three ramps each from fig.1A and B, respectively, on an expanded time scale. In panels B and D the ramps are plotted as $I-V$ curves: it can be seen that before the response (ramp 1 in both panels) the current-voltage relationship of the fibroblasts membrane is only slightly outwardly rectifying, while during the response (ramp 2 in both panels) the $I-V$ curves show a strong outward rectification. For this reason the detection of the reversal potential is difficult and subject to large variability as shown in the insets (E_{rev} = −35 mV in fig.2B and E_{rev} = −13 mV in fig.2D). As shown in fig.2A and C, the $I-V$ curves before and after the response are very similar.

This kind of response is reminiscent, with regard to both time course and voltage dependence, of those induced by Ca^{2+} injections in molluscan and vertebrate sympathetic neurones [15–17]. An increase in cytoplasmic free Ca^{2+} is known to occur shortly after addition of serum and other growth factors [5] and a recent report indicates that Ca^{2+}-activated K^+ channels are present in human fibroblasts [18].

We exposed some cells to the Ca^{2+} ionophore A23187 in order to increase cytoplasmic free Ca^{2+}. Fig.3 shows the typical response of a fibroblast to such treatment (this result was observed in 9/10 cells). The response (fig.3A) is very similar in amplitude and time course to those obtained in serum-treated cells; furthermore the $I-V$ curve at the peak of the response shows the same kind of rectification as that in response to serum (fig.3B).

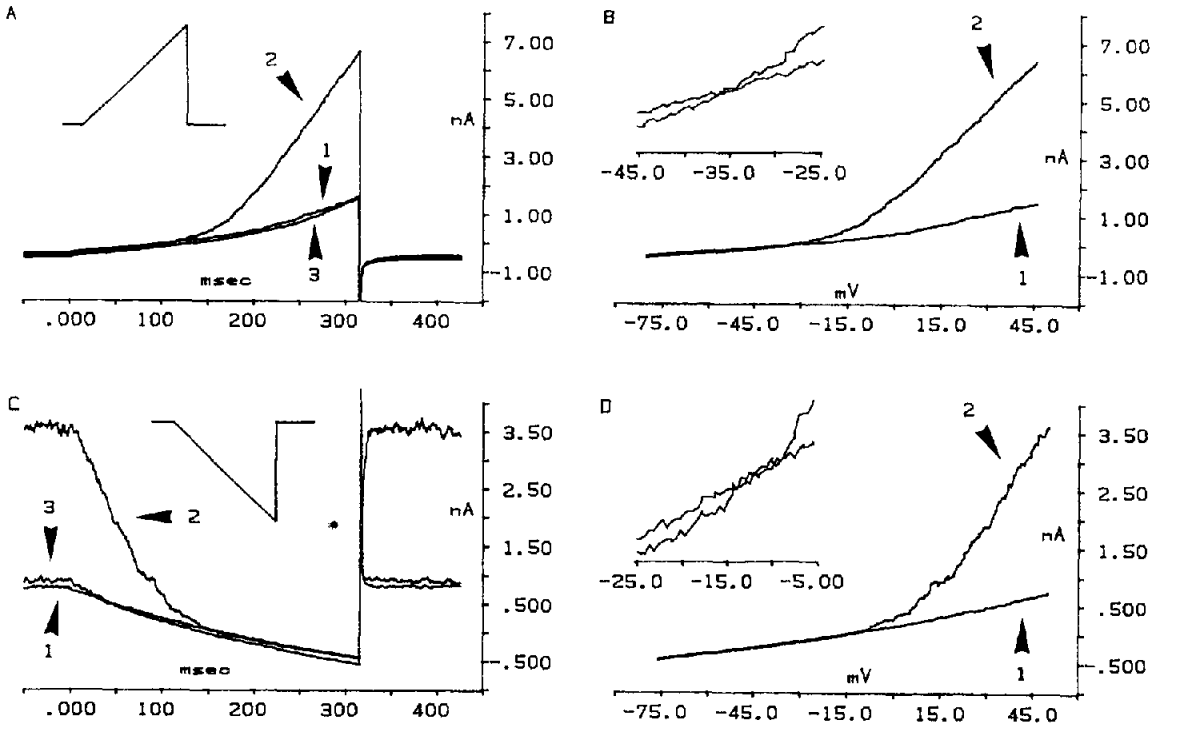

Fig.2. In A and C the currents in response to voltage ramps (shown in the insets) from fig.1A and B, respectively, are plotted on a faster time scale; numbers close to each trace correspond to the numbers in fig.1. B and D show the same currents as in A and C but plotted versus voltage. The insets show an enlargement (current scale × 10) of the crossing of the two $I-V$ curves which indicates the reversal potential of the serum-induced current. E_{rev} is at about −35 mV in B and at about −13 mV in D.

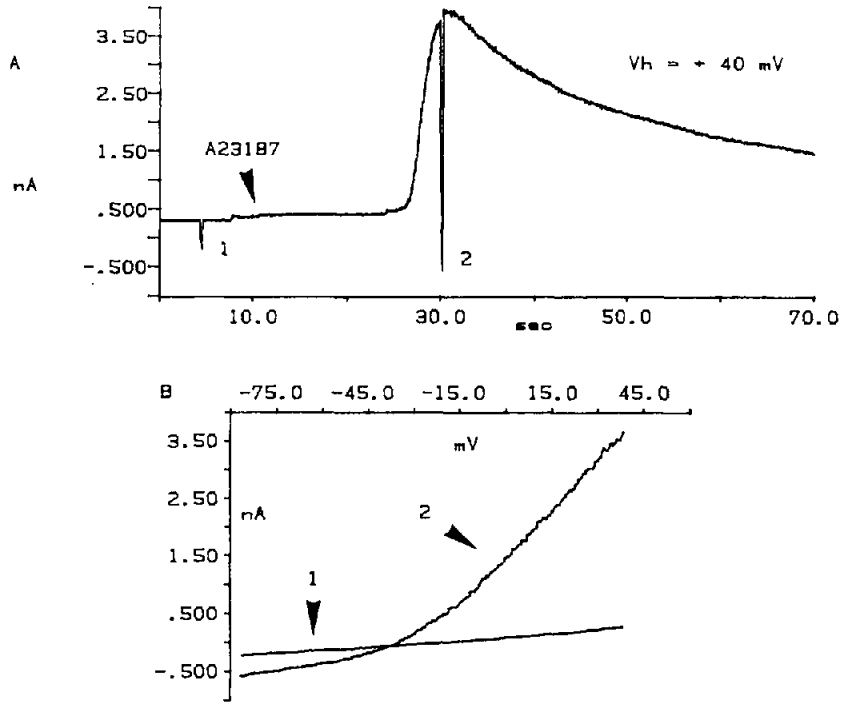

Fig.3. (A) The time course of the response induced by application of A23187 is very similar to that induced by serum. (B) The ramps from A are plotted against voltage to show the strong outward rectification of the A23187 response.

This finding then indicates that Ca^{2+} is involved in the response.

4. DISCUSSION

We have shown here that one of the early effects of serum in quiescent human fibroblasts is the Ca^{2+}-dependent activation of a membrane conductance. This finding fits pretty well with several previous observations about the chain of events brought about by growth factors and external messengers on a number of cell lines. Recently Brown and Higashida [12,13] have given a detailed report on the effects of bradykinin on neuroblastoma hybrid cells. In this model system bradykinin leads to the opening of voltage-independent, K^+ selective, channels through the production of inositol 1,4,5-trisphosphate and the subsequent mobilization of Ca^{2+} from intracellular stores.

In our case the identification of the ion(s) carrying the current is complicated by the strong rectification which prevents an accurate determination of the reversal potential (E_{rev}). In our case, anyway, E_{rev} is certainly more positive than the K^+ equilibrium potential (E_k), being between -45 and -10 mV. This may either mean that the current flows through a single type of unselective channel or that two different ions flow independently through their specific channels. A possibility that must be considered for future investigations is that Ca^{2+} may enter the cell via receptor-operated or second messenger-operated channels as appears to be the case in human T-lymphocytes [6], while K^+ may flow through a Ca^{2+}-activated, outwardly rectifying channel of the kind found in cultured nerve and skeletal muscle cells [19–21].

This kind of channel has a large conductance and is not particularly sensitive to Ca^{2+}, needing micromolar concentrations of Ca^{2+} to be activated [22]. This fact together with the Ca^{2+} buffering capacity of our intracellular solution may explain why in about 30% of the cells the response was not observed.

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