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Fibroblast growth factor induces a transient net K^+ influx carried by the bumetanide-sensitive transporter in quiescent BALB/c 3T3 fibroblasts

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The bumetanide-sensitive transport system performed a net efflux of K^+ in serum deprived quiescent cells The addition of partially purified fibroblast growth factor (FGF) to G0/G1 phase 3T3 fibroblasts induced a transient net influx of K^+, carried out by the bumetanide-sensitive transport system for 2–6 minutes. The stimulation of the bumetanide-sensitive K^+ influx by FGF was followed by stimulation of the ouabain-sensitive K^+ influx. In addition, both the bumetanide-sensitive and the ouabain-sensitive K^+ influxes were found to be similarly stimulated when the G0/G1 3T3 cells were treated with insulin. These results suggest that growth factors such as FGF and insulin induce a change in the action of the bumetanide-sensitive transporter from performing net K^+ efflux along its concentration gradient to an uphill transport pumping of K^+ into the cell. We propose, therefore, that the bumetanide-sensitive transporter contributes to the increase in the intracellular K^+ (and probable Na^+) stimulated by growth factors such as FGF and insulin in early G1 phase of the cell cycle.

Changes in monovalent ion fluxes are among the earliest phenomena in the sequence of events leading quiescent cells to proliferate after exposure to growth factors [1,2] It has not been conclusively established that the changes in intracellular, K^+, Na^+, Ca^{2+} or H^+ concentrations induced by growth factors at the early G0/G1 phase are signals for proliferation The diuretic-sensitive K^+ transport system has been extensively studied in a variety of cells [3–10] Recently it has been shown that release of mouse fibroblasts from G0/G1 phase of the cell cycle by serum growth factors is accompanied by a transient increase of the diuretic-sensitive K^+ influx and by activation of the Na^+/K^+ pump [7–9,11–14] In addition, in nonsynchronized growing mouse cells the bumetanide-sensitive transport system performs a net efflux of K^+ coupled to a net influx of Na^+, driven by the concentration gradient of these ions [10] Recently we demonstrated that serum induced a net influx of K^+ by the bumetanide-sensitive transport system which appeared to be driven by Na^+ influx, carried out by the bumetanide-sensitive transporter itself or by another Na^+ transporting system [14]

To study the role of the bumetanide-sensitive Rb^+ transporter during the early G1 phase of the cell cycle, we have studied the effect of fibroblast growth factor (FGF) on net bumetanide-sensitive K^+ fluxes FGF is known to stimulate G0/G1 phase quiescent cells (for review see Ref 15) Our results show that in G0/G1 phase cells FGF stimulates bumetanide-sensitive and ouabain-sensitive K^+ fluxes in a similar manner to serum

BALB/c 3T3 cells were arrested by serum starvation and stimulated by the addition of

medium containing increasing FGF concentrations In all experiments Hepes buffer (20 mM, pH 7 0) was included in the medium to prevent pH changes which might affect ion fluxes Ouabain-sensitive and bumetanide-sensitive components of Rb^+ influx were measured as described before for 2 min assay time [7,10], except that 10 μM bumetanide was used instead of furosemide Rb^+ influx in the presence of ouabain and bumetanide was subtracted from Rb^+ influx in the presence of ouabain, to give the bumetanide-sensitive Rb^+ influx As could be seen in Fig 1 addition of FGF above 200 ng/ml stimulated Rb^+ influxes in the quiescent cells Both the ouabain-sensitive and the bumetanide-sensitive Rb^+ influxes were stimulated by FGF to a similar extent, and saturation of both activities was observed above 1 μg/ml of the FGF preparation It should be noted that relatively high concentrations of FGF were needed to maximally stimulate the Rb^+ fluxes in the quiescent cells Similarly, high concentrations of FGF were needed to induce ornithine decarboxylase enzyme activity in these cells, a marker of mid G1 phase [16] (data not shown)

To investigate whether FGF stimulates net influx of K^+ into the cells, we measured simultaneously the kinetics of Rb^+ influx and efflux

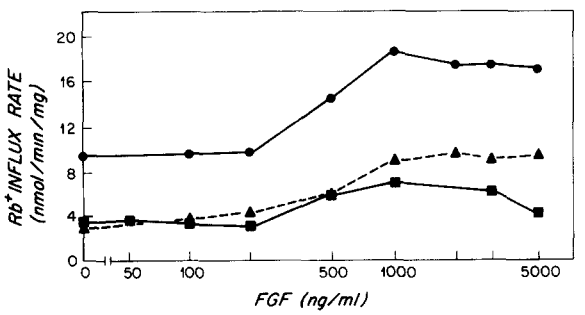

Fig 1 Dose response of FGF on ouabain-sensitive and bumetanide-sensitive Rb^+ influxes FGF was partially purified from bovine brain according to Gospodarowicz et al [20] and kindly given to us by Dr Israel Vlodavski, Hadassah University Hospital, Jerusalem, Israel Quiescent BALB/c 3T3 cells were serum deprived as described before [7] The quiescent cells were stimulated by the addition of fresh medium containing the indicated FGF concentrations and 20 mM Hepes-Tris (pH 7 0) After 5 min the medium was removed and Rb^+ influxes were measured for 5 min as described in the text ●——●, total Rb^+ influx, ■——■, ouabain-sensitive Rb^+ influx, ▲- - - - -▲, bumetanide-sensitive Rb^+ influx

Quiescent BALB/c 3T3 cells were stimulated by the addition of medium containing 2 μg/ml FGF Rb^+ influx and efflux were measured simultaneously following FGF addition, as described before, except that 10 μM bumetanide replaced the furosemide In Figs 2, 3 and 4 the different components of Rb^+ flux, following cell stimulation by FGF, are presented Rb^+ influx rate measurements were conducted for 2 min and the results are averages of triplicate cultures As shown in Fig 2, the total influx and efflux of $K^+(Rb^+)$ were equal in the quiescent cultures, indicating that their K^+ content was at a steady state Addition of FGF to the quiescent cells markedly increased the rate of total Rb^+ influx, reaching a maximum at 4 min of FGF addition In contrast, total Rb^+ efflux was only slightly stimulated during this period As a result total Rb^+ influx was higher than total K^+ efflux during the 30 min following addition of FGF These kinetics of K^+ influx are similar to those observed with serum growth factors [3,7,11,17–19]

The ouabain-sensitive and bumetanide-sensitive Rb^+ influxes were stimulated with different kinetics following FGF addition Whereas, bumetanide-sensitive Rb^+ influx reached a maximum 4 minutes following FGF addition, and dropped to a level lower than that of quiescent cells, the ouabain-sensitive Rb^+ influx reached maximum only 10 min after FGF addition, and remained high (Fig 3) In order to study whether FGF could stimulate the bumetanide-sensitive trans-

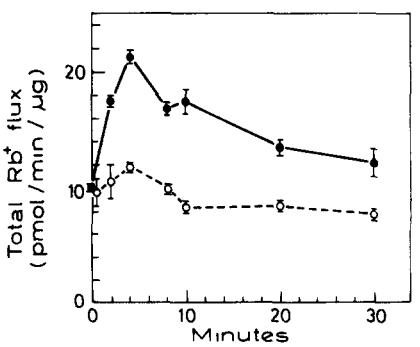

Fig 2 The effect of FGF on total Rb^+ influx and efflux Quiescent cells were released by the addition of medium with FGF (2 μg/ml) At the indicated intervals total Rb^+ influx (●——●) and total Rb^+ efflux (O- - - - -O) were measured as described before [7,10]

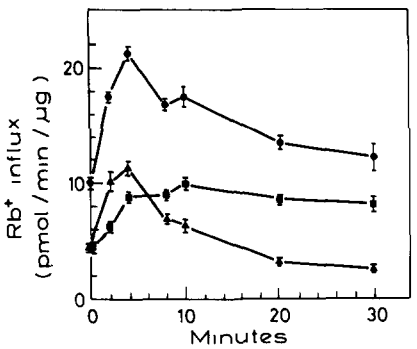

Fig 3 Ouabain-sensitive and bumetanide-sensitive Rb^+ influxes following addition of FGF to serum starved cells The results presented in Fig 2 for the total Rb^+ influx (●———●) bumetanide-sensitive (▲———▲) and the ouabain-sensitive (■———■) components of Rb^+ influx, were measured as reported before [7]

porter to perform net influx of K^+ as was found with serum [14], we measured unidirectional $K^+(Rb^+)$ fluxes in the quiescent cells, and following FGF addition As Fig 4 illustrates, a 3-fold increase in the bumetanide-sensitive Rb^+ influx was observed within 4 min of FGF addition, however, under the same conditions there was only a slight (25%) increase of the bumetanide-sensitive Rb^+ efflux As a result, following FGF addition the bumetanide-sensitive influx to efflux ratio changed from 0 65 (net Rb^+ efflux along its concentration gradient) to 1 32 (net K^+ influx against its concentration gradient) The bumetanide-sensitive Rb^+ influx is higher than the bumetanide-sensitive Rb^+ efflux for a short time following FGF addition to the quiescent cells, and after 10 min there is again net bumetanide-sensitive efflux of K^+ as in the quiescent cells

To study whether these effects on ouabain-sensitive and bumetanide-sensitive Rb^+ influxes are uniquely stimulated by FGF, we measured the effect of insulin on the two fluxes in the quiescent cells In agreement with previous reports insulin stimulated both ouabain-sensitive [19] and bumetanide-sensitive [13] Rb^+ influx within the first few minutes The kinetic of ouabain-sensitive and bumetanide-sensitive Rb^+ influxes stimulated by FGF and insulin are similar (Figs 3 and 5) In cells stimulated by either of the growth factors the bumetanide-sensitive Rb^+ influx reached a maximum after approx 4 min and subsequently declined By contrast, the ouabain-sensitive Rb^+ influx remained relatively high and constant during the 30 min following FGF or insulin addition (Figs 3 and 5) Thus it seems that insulin like FGF induces a change in the activity of the

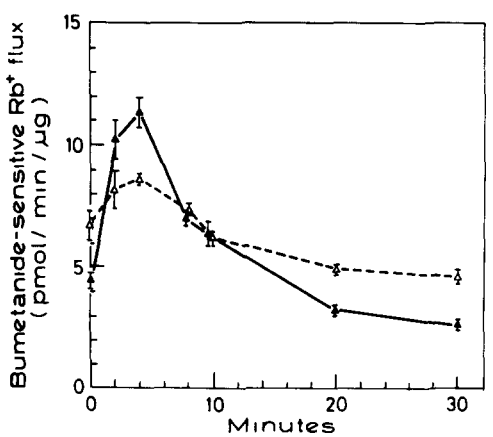

Fig 4 Bumetanide-sensitive Rb^+ influx and efflux following the addition of FGF to quiescent cells Results were taken from Fig 3 for the bumetanide-sensitive Rb^+ influx (▲———▲) Rb^+ efflux rates in the presence and absence of 10 μM bumetanide were measured simultaneously as described before [10,14] △- - - - -△, bumetanide-sensitive Rb^+ efflux

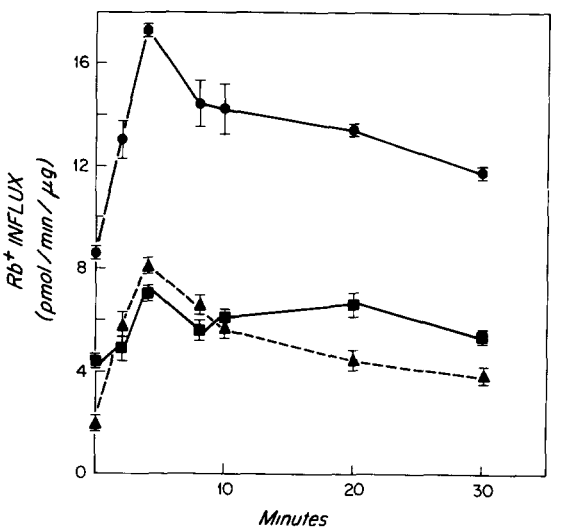

Fig 5 Rb^+ influxes following the addition of insulin to serum starved cells Quiescent BALB/c 3T3 cells were released by the addition of fresh medium containing 20 mM Hepes-Tris (pH 7 0) and insulin (10 μg/ml) At the indicated intervals total Rb^+ influx (●———●), bumetanide-sensitive (▲- - - — — -▲) and ouabain-sensitive (■———■) components of Rb^+ influx were measured as described before [7]

bumetanide-sensitive transporter to carry net influx of K^+, concomitant by stimulation of the ouabain-sensitive K^+ influx Both K^+ transporters contribute to the increase in K^+ content at the early G0/G1 phase of the cell cycle

In this study we examined the effect of FGF on two different K^+ fluxes in G0/G1 BALB/c 3T3 fibroblasts We found that FGF stimulates both the ouabain-sensitive and the bumetanide-sensitive K^+ influxes against its concentration gradient The role of the bumetanide-sensitive transporter is not known Although its activity has been shown to be regulated by growth factors [7,9,11–14], it is not known whether its stimulation by serum growth factors is necessary for cell exit from the G0/G1 phase [12,13]

The bumetanide-sensitive transporter performs a cotransport of K^+ and Na^+ [5,6] We have shown previously that this transporter performs net efflux of K^+ in growing cells [10] as well as in quiescent cells arrested by isoleucine deprivation [14] We also demonstrated that serum factors induce the bumetanide-sensitive to carry net influx of K^+ uphill the concentration gradient, possibly driven by Na^+ influx [14,22] Recently is has been shown that insulin which by itself is a poor mitogen in eukariotic cells stimulates the bumetanide-sensitive K^+ influx [12] The question arose whether a competence growth factors [21] such as FGF which appears to stimulate early events in cell exit from G0/G1 arrest, would also stimulate the bumetanide-sensitive transporter The finding that FGF like serum induces the bumetanide-sensitive transporter to perform net influx of K^+ suggests that this uphill uptake of K^+ could be cotransported with Na^+ This cotransport may in turn contribute to the stimulation of the Na^+/K^+ pump, shown to be regulated by intracellular Na^+ [1,2] The kinetics of the K^+ influxes stimulated by FGF are consistent with this hypothesis, as the bumetanide-sensitive net influx of K^+ is transient and followed by a slower stimulation of the Na^+/K^+ pump Similar kinetics of K^+ influxes were found when the quiescent NIH 3T3 cells were stimulated by serum [7] The possibility that the Na^+/K^+ pump stimulation preceded the bumetanide-sensitive transporter stimulation could not be ruled out theoretically However, in our study with FGF described in Fig 3 (see also Fig 1 in Ref 7 and Table I in Ref 14) the bumetanide-sensitive K^+ influx stimulation preceded the Na^+/K^+ pump stimulation It is possible, however, that with other purified growth factors, or in other cell types, the pump stimulation would create a potential gradient of Na^+ which would drive the bumetanide-sensitive transporter known to be regulated by electrochemical potential of Na^+ and K^+ [6,10] The finding that the bumetanide-sensitive transporter is poorly stimulated by EGF or FGF in human fibroblasts [13] may be a consequence of inadequate arrest as cells were serum deprived for only four hours

In conclusion, our observation of reversal of the net K^+ flux induced by FGF reported here, concomitant with the stimulation of ouabain-sensitive transporters Direct measurements of the bumetanide-sensitive Na^+ influx stimulated by the different growth factors would be needed in order to substantiate this proposal

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