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Serum-induced net K^+ influx performed by the diuretic-sensitive transport system in quiescent NIH 3T3 mouse fibroblasts

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In serum deprived NIH 3T3 mouse cells the diuretic-sensitive transport system performs K^+ self-exchange. The addition of serum which stimulates cell proliferation induces a net influx of K^+, carried out by the diuretic-sensitive transport system. Thus, serum growth factors appear to induce a change in the mechanism of action of the diuretic-sensitive transporter from K^+ self-exchange to an uphill transport pumping K^+ into the cell. I propose here that this uphill uptake of K^+ contributes to the increase of intracellular K^+ content, found in the early G_1 phase of the cell cycle.

Changes in monovalent ion fluxes appear to be the earliest observed phenomena in the sequence of events leading quiescent cells to proliferate, after being exposed to growth factors [1,2]. It was shown that increased Na^+ influx stimulates the Na^+/K^+ pump activity and subsequently increases the cellular level of K^+ [3,4]. The hypothesis that the increase in intracellular K^+, induced by growth factors at the early G_1 phase is a signal for proliferation is a focus of intensive research [1,2,5]. The increase in cellular K^+ following the addition of growth factors to quiescent cells was attributed to the stimulation of the Na^+/K^+ pump [4]. However, K^+ (and Na^+) is transported through the plasma cell membrane by several distinct transport systems, among them the ouabain-resistant diuretic-sensitive K^+ transporter [6].

A diuretic-sensitive K^+ transport system was characterized in reticulocyte cell membrane [7], and its activity was found to be coupled to protein synthesis via modulation of ATP/ADP ratio in the cell [8,9]. A similar ouabain-resistant diuretic-sensitive K^+ transport system was found in several other cell types, but the exact role of this transporter is not clear [6,10]. Recently, we have shown that release of NIH 3T3 cells from arrest at the early G_1 phase of the cell cycle is accompanied by a transient increase of diuretic-sensitive K^+ (Rb^+) influx concomitant with activation of the Na^+/K^+ pump [11,12]. In addition, we demonstrated that in nonsynchronized growing NIH 3T3 mouse cells, the diuretic-sensitive transport system performs a net flux of K^+ outwards, coupled to a net flux of Na^+ inwards, driven by the concentration gradients of these ions [13]. In order to understand the role of this transport system in early G_1 phase, we measured the unidirectional Rb^+ fluxes, first in the quiescent cells and then, after the addition of serum.

NIH 3T3 cells were arrested by serum starvation as described before [11]. Quiescent cultures were stimulated by adding medium containing 10% calf serum. The different components of $^{86}Rb^+$ influx, namely ouabain-sensitive, ouabain-resistant furosemide-sensitive, and diffusion were measured as described before; the furosemide-sensitive influx rate was determined from measurement of both, the saturable part of the ouabain-resistant influx [7], and its furosemide-sensitive part. The two methods gave comparable results, which were

consistent with the fact that about 100% of the saturable ouabain-resistant K^+ influx was inhibited by diuretics [7,13]. $^{86}Rb^+$ efflux rates in the presence and absence of furosemide were measured simultaneously with the influx, under physiological conditions as described before [13].

As can be seen in Table I, the total efflux and influx of Rb^+ were equal in the quiescent cultures, indicating that their K^+ content was in a steady state. The ratio of furosemide-sensitive $^{86}Rb^+$ influx to efflux is close to one, thus no net flux is carried out by the furosemide-sensitive transporter.

The results presented in Table I and Fig. 1 show the changes in different $^{86}Rb^+$ fluxes following the addition of serum to the quiescent cells. The furosemide-sensitive influx was increased by a factor of 4.8 within 2 min, while the furosemide-sensitive $^{86}Rb^+$ efflux was not changed. Consequently, the influx to efflux ratio increased from 0.86 to 4.46 within 2 min. Further, the influx remained higher than the efflux during the first 10 minutes following serum addition. Only after 25 min did the furosemide-sensitive $^{86}Rb^+$ efflux start to increase, while the influx decreased, bringing influx to efflux ratio close to one as in the quiescent cells (Table I, Fig. 1). To investigate whether this change in the net flux of K^+ carried out by the diuretic-sensitive transport system is specific to G_1 phase of the cell cycle, we measured the unidirectional Rb^+ fluxes first in isoleucine deprived cells, and then, after release of the cells from the arrest. Isoleucine deprivation was shown to arrest cells at the G_1 phase of the cell cycle, in the presence of serum [14]. Table II shows the changes in $^{86}Rb^+$ fluxes that followed the addition of isoleucine to isoleucine deprived cells. Measurements were made only 15 min after the release from the isoleucine arrest, since in previous work

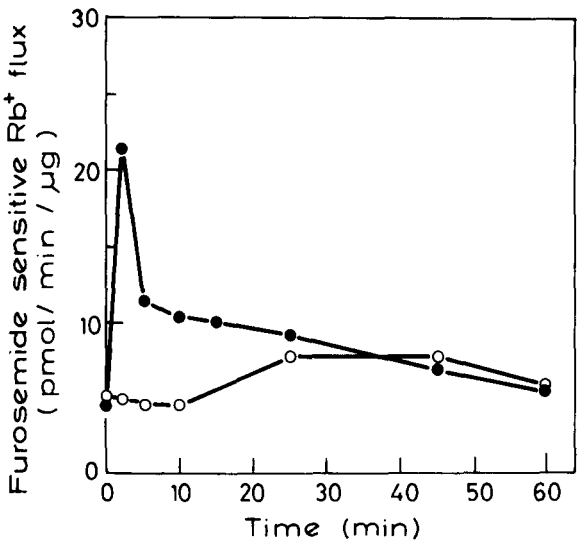

Fig. 1. Kinetics of furosemide-sensitive $^{86}Rb^+$ influx and efflux following the addition of serum to quiescent cells. Results were taken from Table I. Furosemide-sensitive $^{86}Rb^+$ influx (●——●) and efflux (○——○) were measured following the addition of serum to the cells as described above.

TABLE I

COMPARISON OF $^{86}Rb^+$ FLUXES FOLLOWING THE ADDITION OF SERUM TO SERUM-STARVED CELLS

Cells were arrested and $^{86}Rb^+$ fluxes were assayed as described in the text. The results presented here are averages of triplicate cultures ± S.D.

Serum addition (min)	$^{86}Rb^+$ influx rate (pmol/min/μg)			$^{86}Rb^+$ efflux rate (pmol/min/μg)			Furosemide-sensitive $^{86}Rb^+$ influx/ $^{86}Rb^+$ efflux
	Total	Ouabain-sensitive	Furosemide-sensitive	Total	Furosemide-resistant	Furosemide-sensitive	
0	9.10 ± 0.27	4.30 ± 0.25	4.47 ± 0.28	8.83 ± 0.73	3.69 ± 0.59	5.14	0.86
2	31.29 ± 0.96	9.24 ± 0.90	21.55 ± 0.28	11.11 ± 1.34	6.11 ± 0.58	4.83	4.46
5	19.00 ± 1.10	7.20 ± 1.10	11.40 ± 0.48	9.38 ± 1.05	5.01 ± 0.52	4.37	2.61
10	19.37 ± 0.53	8.49 ± 0.53	10.38 ± 0.53	9.15 ± 0.87	4.82 ± 0.40	4.33	2.40
15	21.51 ± 0.69	11.00 ± 0.69	10.00 ± 0.28	–	–	–	–
25	19.21 ± 1.17	9.50 ± 1.17	9.21 ± 0.49	14.45 ± 1.13	6.67 ± 0.60	7.77	1.19
45	15.73 ± 0.31	8.22 ± 0.31	6.79 ± 0.39	12.93 ± 1.79	5.04 ± 0.60	7.87	0.86
60	15.19 ± 0.29	9.06 ± 0.29	5.41 ± 0.44	11.52 ± 1.05	5.70 ± 0.58	5.82	0.93

TABLE II

COMPARISON OF $^{86}Rb^+$ FLUXES FOLLOWING THE ADDITION OF ISOLEUCINE TO ISOLEUCINE-DEPRIVED CELLS

Cells were arrested by isoleucine deprivation as described before [9]. Quiescent cultures were stimulated by adding complete medium with isoleucine. $^{86}Rb^+$ fluxes were assayed as described in the text.

Isoleucine addition (min)	$^{86}Rb^+$ influx rate (pmol/min/μg)				$^{86}Rb^+$ efflux rate (pmol/min/μg)			Furosemide-sensitive $^{86}Rb^+$ influx/$^{86}Rb^+$ efflux
	Total	Ouabain-sensitive	Furosemide-sensitive	Diffusion	Total	Furosemide-resistant	Furosemide-sensitive	
0	7.76 ± 0.25	4.26 ± 0.11	2.44 ± 0.08	0.45 ± 0.11	7.87 ± 0.53	2.58 ± 0.17	5.29	0.46
15	26.89 ± 1.10	14.03 ± 0.62	12.24 ± 0.53	0.62 ± 0.04	7.55 ± 0.54	1.18 ± 0.31	6.37	1.92
30	18.46 ± 0.81	11.74 ± 0.51	6.00 ± 0.25	0.72 ± 0.03	7.67 ± 0.53	1.60 ± 0.10	6.07	0.98

we showed that the kinetics of $^{86}Rb^+$ fluxes differed in cells after exit from arrest by isoleucine deprivation, as compared with serum starvation [12]. A 5-fold increase of the furosemide-sensitive $^{86}Rb^+$ influx was observed within 15 min, together with a small increase in the furosemide-sensitive efflux. As a result, the influx to efflux ratio changed from 0.46 (net K^+ efflux along its concentration gradient) to 1.92 (net K^+ influx against its concentration gradient) following isoleucine addition. After 30 min the influx started to decrease while the efflux did not change, bringing the influx to efflux ratio to one (Table II).

It was first shown by Rozengurt [15] and confirmed by other groups [4,11,16] that the addition of serum or combination of growth factors to quiescent 3T3 mouse fibroblasts, rapidly increased the rate of Rb^+ influx with no effect on Rb^+ (K^+) efflux. It has been proposed that monovalent cations play a critical role in regulating cell proliferation [1,2]. According to this model an increased rate of Na^+ entry into mitogen-treated cells, rapidly stimulates the Na^+/K^+ pump [1,2], and results in an increased cellular level of K^+ [4]. Furthermore, it has been suggested that the uphill uptake of K^+ may be an essential event for exit of cells from G_1/G_0 phase. In a previous work, we have shown that exit of NIH 3T3 mouse fibroblasts from G_1/G_0 phase of the cell cycle, resulted in a transient marked increase of the diuretic-sensitive Rb^+ influx followed by stimulation of the Na^+/K^+ pump [11,12]. Some authors attributed a similar increased influx to an increased K^+ self-exchange, with actually no increased net flux [4]. However, efflux measurements were not conducted simultaneously under these experimental conditions. By performing such simultaneous measurements, we showed previously that the furosemide-sensitive transport system carried out a net efflux of K^+ in non-synchronized growing 3T3 mouse fibroblasts [13]. In the present work, we show that the diuretic-sensitive transport system performs a self-exchange of K^+ with no net K^+ flux, in cells arrested by serum starvation, and a net efflux of K^+ in isoleucine-deprived cells. In addition, exit of both quiescent cell cultures from arrest is accompanied by a transient increase of the diuretic-sensitive K^+ influx, without increase of the K^+ efflux. As a result, during a short interval in the early G_1 phase, the diuretic-sensitive transport system carries out a net influx of K^+ against its concentration gradient, in the same direction as of the Na^+/K^+ pump. This K^+ influx is contributing to the increase of intracellular K^+ content found in G_1 phase after stimulation by serum [4]. Therefore, it appears that in the same cell cultures this transport system can perform K^+ self exchange (in serum starved cells), net efflux of K^+ (in growing cells and in isoleucine-deprived cells), and net influx of K^+ following exit of the quiescent cells from arrest. This work may resolve the conflicting results reported by several groups [13,17–19], as to the net flux of K^+ carried out by the diuretic-sensitive transport system, since differences in growth conditions could change the direction of the net flux performed by this system. However, the reversal of the net flux of K^+, in the same cells under different growth conditions, raises

the question as to what is the energy for the uphill uptake of K^+ by the diuretic-sensitive transport system.

We have shown in reticulocytes, that the diuretic-sensitive transport system was independent of metabolic energy [7]. This finding is consistent with the conclusion that the diuretic-sensitive transport system, in growing fibroblasts [13] and in reticulocytes [20], works as a facilitated diffusion, performing a net transport of cations according to their concentration gradient: K^+ outwards and Na^+ inwards. Nevertheless, based on our findings that following exit of cells from arrest in early G_1 this transport system performs net influx of K^+, the question remains as to what is the driving force for this net K^+ flux against its concentration gradient at this phase. It is possible that serum growth factors induce an increased Na^+ influx carried out by the diuretic-sensitive transport system itself, working as a cotransport of Na^+ and K^+ [10]. Thus, the potential energy of this Na^+ concentration gradient would be used by the same transporter to pump K^+ inwards against its electro-chemical potential gradient. This latter suggestion is substantiated by our recent finding that serum stimulates furosemide-sensitive Na^+ influx in NIH 3T3 cells (unpublished result). Alternatively, the diuretic-sensitive transport system could be coupled to another transporter of Na^+, such as the Na^+/H^+ amiloride-sensitive system which was found to be stimulated by addition of serum or combination of growth factors [21–23]. In conclusion, the reversal of the net K^+ flux reported here, implies the existence of a regulatory mechanism, able to change the kinetics of the transport system (from a facilitated diffusion to uphill transport of K^+ against its concentration gradient) as a consequence of interaction with the growth factors which trigger cell division.

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