Calcium Transport Essay, Research Paper

Calcium Transport in SF-9 and Bull Frog Ganglion Cells Kenny Yu University of Toronto Faculty of Pharmacy 19 Russell Street Toronto, Ontario M5S 2S2 PHM499 Research Project Supervisors: Dr. P. S. Pennefather, Dr. S. M. Ross Calcium transport study of SF-9 lepidopteran cells and bull frog sympathetic ganglion cells Kenny Yu Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario M5S 2S2 ABSTRACT The intracellular calcium level and the calcium efflux of the bull-frog sympathetic ganglion cells (BSG) and the SF-9 lepidopteran ovarian cells were investigated using a calcium-sensitive fluorescence probe fura-2. It was found that the intracellular calcium levels were 58.2 and 44.7 nM for the BSG cells and SF-9 cells respectively. The calcium effluxes following zero calcium solution were 2.02 and 1.33 fmole+cm-2+s-1 for the BSG cells and SF-9 cells. The calcium effluxes following sodium orthovanadate (Na2VO4) in zero calcium solution were 6.00 and 0.80 fmole+cm-2+s-1 for the BSG cells and the SF-9 cells. The SF-9 cells also lost the ability to extrude intracellular calcium after 2-3 applications of Na2VO4 while the BSG cells showed no apparent lost of calcium extruding abilities for up to 4 applications of Na2VO4.

INTRODUCTION Spodoptera frugiperda clone 9 (SF-9) cells are a cultured insect cell line derived from the butterfly ovarian tissue. SF-9 cells are used by molecular biologists for the studies of gene expression and protein processing (Luckow and Summers, 1988). However, there is not much known about these cells’ basic biophysiology. Since calcium is involved in many cells’ activities such as acting as a secondary messenger, it is important for cells to control their intracellular calcium level. This study was aimed toward looking at the some of the basic properties of the SF-9 cells such as resting calcium concentration and rate of calcium extrusion after being calcium level being raised by an ionophore 4-bromo-A23187. The effect of sodium orthovanadate (an active transport inhibitor) on calcium extrusion was also looked at. Microspectrofluorescence techniques and the calcium-sensitive probe fura-2 were used to measure the intracellular calcium concentration of these cells. In addition, the BSG cells were used to compare with the SF-9 cells for the parameters that were studied. It was found that the SF-9 cells appeared to have a calcium concentration similar to the BSG cells. Moreover, the calcium extrusion rates of both cell types with no Na2VO4 added seemed to the same. However, due to insufficient data, the effects of Na2VO4 could not be statistically analyzed. From the data available, it suggested that the BSG cells’ rate of calcium extrusion was enhanced by the Na2VO4 and was greater than the SF-9 cells. It was more important to note that the calcium extruding capabilities of the SF-9 cell seemed to impaired after two to three applications of Na2VO4 but it had apparent effects on the BSG cells even up to 4 applications. After obtaining these basic parameters, many questions raised such as how does the SF-9 cells extrude their calcium and why the Na2VO4 affected the calcium efflux for the SF-9 cells but not the BSG cells? The SF-9 cells may have a calcium pump or exchanger to extrude their calcium and they may be very sensitive to the ATP (adenosine 3′-triphosphate) supply. This was apparently different from the BSG cells’ since their calcium extrusion were not affected by the Na2VO4.. It may be useful to find the mechanism(s) of the actions of Na2VO4 on the SF-9 cells because it may find possible applications in agriculture such as pest control.

MATERIALS AND METHODS Chemicals and solutions 4-bromo-A23187 and Fura-2/AM were purchased from Molecular Probes (Eugene, OR). Na2VO4 was purchased from Alomone Lab (Jerusalem, Israel). Dimethyl sulfoxide (DMSO) was obtained from J. T. Baker Inc. (Phillipsburg, NJ). All other reagents were obtained from Sigma (St. Louis, MO). The normal Ringer’s solution (NRS) contained (mM): 125 NaCl, 5.0 KCl, 2.0 CaCl2, 1.0 MgSO4, 10.0 glucose, 10.0 N-[2-hydroxyethyl] piperazine-N’-[2-ethanesulfonic acid] (HEPES). The calcium free Ringer solution (0CaNRS) is the same as the NRS except CaCl2 was substituted with 2.0 mM ethylene glycol-bis(b-aminoehtyl) ether N,N,N’,N’-tetraacetic acid (EGTA). Fura-2/AM solution was prepared as follows: a stock solution of 1mM fura-2/AM in DMSO was diluted 1:500 in NRS containing 2% bovine albumin. It was then sonicated for 10 minutes. It was then kept frozen until the day of the experiment. 20 mM 4-bromo-A23187 solution was prepared by diluting a stock of 5mM 4-bromo-A23187 in DMSO 1:250 with NRS. Na2VO4 solution (VO4NRS) contained 100 mM. Na2VO4 in 0CaNRS. All experiments were performed at room temperature, 22-26 .C. The above solutions were adjusted to pH 7.3 with NaOH. Cells BSG cells were obtained as described by Kuffler and Sejnowski (1983). BSG cells were plated and incubated at 3-10 .C for up to 4 days before the experiments. The cells were plated on custom made 3.5 cm plastic culture dishes. A circular hole about half the diameter of the dishes were cut out in the middle and fitted with a piece of aclar. The aclar dishes were then treated with poly-d-lysine for one hour before plating. SF-9 cells (non-transfected) were cultured as described by Summers and Smith (1987). The SF-9 cells were plated and incubated (at 37 .C) on the custom made dishes as used for the BSG cells one day prior to the experiments. They were not kept for more than two days to avoid overgrowth of cells that might cause difficulties in experimental measurement. Each dish contained approximately 100 ml of cell suspension. To load the cells with fura-2/AM, 100 ml of fura-2/AM /BSA solution was added for 30 minutes. Intracellular calcium measurements Fura-2 is a fluorescence indicator of calcium that is used to determine the free intracellular calcium concentration. Fura-2/AM was used in the experiments instead of fura-2. Fura-2/AM is an ester moiety of fura-2 which has the advantages of being permeable to the cell membrane (where fura-2 is not permeable to the cell membrane to any great extent) and is subsequently broken down into fura-2 intracellularly by esterase. The apparatus included a fluorescence microscope unit and a spectrofluorometer system. The fluorescence microscope unit consisted of a 75 W Xenon arc lamp and a Zeiss inverted microscope with a Zeiss Neofluor 63X objective. In addition, a pipette was placed close to the sample cells (within 5mm) for perfusion. The pipette delivered the solutions at a rate of 2-3 ml/min and could switch between the solutions from five different solutions simultaneously. This would allow rapid switching of solutions and improved the speed of responses The PTI Deltascan 4000 microscope system (Photon Technology International Inc., South Brunswick, NJ) was used to make fluorescence measurements. Emitted fluorescence signal was detected by the photomultiplier tube (PMT) and recorded via a NEC 286 microcomputer. The software used was PTI Instrument Control Program from Photon Technology International Inc. (South Brunswick, NJ). The experimental methods of calcium measurements used in the experiments were similar to the one described by Schwartz et al. (1991). In brief, intracellular free calcium concentration can be determined through the following formula (Grynkiewicz et al. 1985): [Ca2+]i = Kd.(Fmin/Fmax).(R-Rmin)/(Rmax-R) where Kd is the effective dissociation constant for the Ca2+-fura-2 complex, Fmin and Fmax are the fluorescence intensities at l=380nm obtained from calcium-free fura-2 sample and calcium-bound fura-2 sample respectively, R is the fluorescence intensities ratio obtained with excitation at 340 and 380nm (R = F340/F380), Rmin and Rmax are the F340/F380 ratios of the calcium-free and calcium-saturated fura-2 sample respectively. One average size cell from each dish was randomly selected for the measurement. NRS was initially perfused to wash out the fura-2/AM in the cell suspension. When the intracellular calcium level was stabilized, it was switched to 2-bromo-A23187 to raise the intracellular calcium concentration. 0CaNRS was used to decrease the calcium concentration when the calcium level reached over 200 nM. Once the calcium concentration was decreased and stabilized with the 0CaNRS, 4-bromo-A23187 was added again and the whole procedure was repeated for two to four times. Then 4-bromo-A23187 was used once again to bring the intracellular calcium concentration up, VO4NRS now was used instead of 0CaNRS to lower the calcium concentration. This procedure was used for two to four cycles or until the cell showed no response and unable to lower the calcium concentration to the previous resting level. Statistical Analysis Statistical analysis was performed with using The Student Edition of Minitab release 8 (Minitab Inc., 1991).

Results It was found that the intracellular calcium concentration in the SF-9 cells was 44.7 x 8.3 nM (mean x S.E., n = 8) in NRS. The calcium concentration in the BSG cells was found to be 58.2 x 9.0 nM (n = 4). Student’s t test did not indicate a significant difference between the intracellular calcium concentration of the SF-9 and the BSG cells (P = 0.31). The rates of active transport of calcium out of the cells following 0CaNRS were also calculated. They were determined by performing a linear regression on the linear portion (ranging from 20 – 50 seconds) of the decline following the maximum calcium concentration. It was found that the rates of calcium depletion (DC/Dt) of BSG and SF-9 cells were 3.92 x 0.81 nM/s (mean x S.E., n = 10) and 4.12 x 0.81 nM/s (n = 7) respectively. However, the BSG cells and the SF-9 cells were generally in different sizes in which the SF-9 cells (about 15-20 mm in diameter) were usually smaller in sizes relative to the BSG cells (about 25-40 mm in diameter). It is therefore important to take into the account of the size of the cells for the analysis of the calcium flux. The calcium flux (J) out of the cell can be determined by adjusting the rates of calcium depletion with the volume to area ratio of the cells (assuming the cells were spherical in shape). The flux can be found by: J = -DC/Dt +V/S where J is the flux, -DC/Dt is the rate of calcium depletion and V/S is the volume to surface area of the cell (V/S can be further simplified to r/3 where r is the radius of the cell). The calculated calcium efflux of the BSG cells and the SF-9 cells were 2.02 x 0.44 fmole+cm-2+s-1 (n = 10) and 1.33 x 0.26 fmole+cm-2+s-1 (n = 7) respectively (table 1). There was no significant difference between the two efflux values (P = 0.2) shown by t-test. Similarly, the rates of calcium depletion of the BSG cells and the SF-9 cells following VO4NRS were 9.24 x 0.22 nM/s (n=2) and 2.46 x 0.75 nM/s (n=3) respectively. The adjusted calcium efflux of the BSG cells and the SF-9 cells were 6.00 x 0.14 fmole+cm-2+s-1 (n = 2) and 0.80 x 0.24 fmole+cm-2+s-1 (n = 3) respectively (table 2). In addition, it was observed that SF-9 cells lost the ability to extrude the calcium after two to three cycles of VO4NRS applications (Figure 1). On the other hand, the BSG cells did not appear to lose their abilities to extrude the calcium after up to three to four VO4NRS applications (Figure. 2). Table 1 Rate of Calcium depletion of BSG and SF-9 cells after the addition of 0CaNRS BSG rate of calcium depletion (nMs-1) BSG calcium efflux (fmole+cm-2+s-1) SF-9 rate of calcium depletion (nMs-1) SF-9 calcium efflux (fmole+cm-2+s-1) 2.23 1.01 4.67 1.51 0.54 0.24 4.10 1.33 4.36 1.98 3.19 1.03 8.58 3.89 7.74 2.51 5.88 2.67 5.55 1.80 1.28 5.81 2.01 0.65 5.28 2.40 1.56 0.50 7.02 4.55 2.22 1.44 2.27 1.47 Intracellular calcium concentration of a single sample cell was raised using 4-bromo-A23187 and was subsequently lowered by introducing 0CaNRS. These data represented the rates of decline (DC/Dt) of the initial linear portion after the maximum calcium concentration. Table 2 Rate of Calcium depletion of BSG and SF-9 cells after the addition of VO4NRS BSG rate of calcium depletion (nMs-1) BSG calcium efflux (fmole+cm-2+s-1) SF-9 rate of calcium depletion (nMs-1) SF-9 calcium efflux (fmole+cm-2+s-1) 9.02 5.85 1.05 0.34 9.47 6.14 3.59 1.16 2.74 0.89 Similar to Table 1 except VO4NRS was used instead of 0CaNRS to lower the calcium concentration. Figure 1. Intracellular calcium concentration of a SF-9 cell A time course calcium recording of a single SF-9 cell (19 mm) with the successive applications of 4-bromo-A23187, NRS, 0CaNRS and VO4NRS. It was noted that after 2 applications of VO4NRS, the cell was impaired in its ability to extrude calcium. Abbreviations: A, 4-bromo-A23187; N, NRS; 0, 0CaNRS; V, VO4NRS. Figure 2. Intracellular calcium concentration of a BSG cell In contrast to the SF-9 cell in Figure 1, the BSG cell (39 mm) still maintained its ability to extrude (or decrease) calcium after three applications of VO4NRS even at a high calcium concentration. Abbreviations: same as in Figure 1.

DISCUSSION In the beginning of the experiment, both the transfected and non-transfected SF-9 cells were used although only non-transfected SF-9 cells were reported here. It was found that the transfected cells had unusual low calcium concentration (less than 20 nM, results are not included in this report). However, it was later found that the cells were not very successfully transfected. T-test did not show any significant difference between the calcium levels in the BSG cells and the SF-9 cells which leads to the question of whether the transfecting process would cause certain biophysiological changes in the cells which led to low intracellular calcium concentrations. Moreover, it was learned during the experiment that it was not necessary to apply 4-bromo-A23187 every cycle to raise the calcium level. It was only necessary to apply once in the beginning of the experiment to raise the calcium concentration. NRS was then used to raise the calcium concentration in the subsequent cycles. This is probably due to the high lipidphilicity of the 4-bromo-A23187 which enable it to partition into the cell membrane and the internal organelles. Hence the one application of 4-bromo-A23187 would allow it to partition and remain in the cell membrane and acted as an ionophore without the necessity of further subsequent addition. The effects of the NRS at raising calcium concentration appeared to be similar to 4-bromo-A23187’s. This technique was more economical and also reduced the effects of DMSO (which was used to dissolve 4-bromo-A23187) on the cells. A general discussion on of ionophores can be found in an article by Pressman (1976). A more specific topics of 4-bromo-A23187 on use with fluorescent probes and its action on calcium can be refered to Deber (1985) and Reed and Lardy (1972). The calcium efflux after VO4NRS for the BSG cells appeared to be greater than the SF-9 cells’ (see result section). But there were insufficient data to perform a reliable statistical test to prove such view. Vanadate is referred to an active transport inhibitor. It acts as a phosphate substitute for ATP and thus stops or slows the ATP production. Without ATP, active transport cannot be carried out. In the case of calcium, the addition of VO4NRS would cause the cells not able to extrude the calcium out after the application of 4-bromo-A23187. It was indeed what was observed for the SF-9 cell (Figure 1). It was noted that the calcium concentration remained at a high level and became unstable after 2 applications of VO4NRS. It suggested that the calcium mobilization in the SF-9 cells was closely linked to the ATP production. Without ATP, the SF-9 cells were unable to regulate their intracellular level in a normal manner. However, the BSG cells showed different responses to VO4NRS (Figure 2) compared to the SF-9 cells. After 3 applications of VO4NRS, the BSG cell was still able to extrude calcium, despite the abnormal high calcium concentration after the third VO4NRS application. This result was not anticipated because the BSG cells had higher calcium effluxes relative to the SF-9 cells, hence calcium extrusion of the BSG cells were more dependent on the ATP production. One possible explanation would be that the BSG cells had excess organelles to store calcium instead of extruding it. Since the SF-9 cells are commonly used for gene expressions, it is important to know the basic biophysiology of these cells. However, there is still a lot unknown about these cells. By studying these cells in greater details, it will improve our understanding of the calcium transport system. Also, it may be useful for the molecular biologists to improve the techniques of gene expressions using the SF-9 cells.

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