Intracellular Distribution of Free Potassium in *Chironomus* Salivary Glands

Abstract. *Potassium activities have been measured in the nucleus and cytoplasm of* Chironomus *salivary gland cells, using potassium-selective electrodes. The data provide the first rigorous evidence that potassium is at electrochemical equilibrium across the nuclear membrane. In addition, no difference in potassium chemical activity was found between nucleus and cytoplasm.*

The physiological functions of the nuclear membrane have not been extensively studied because of the limited size and accessibility of most cell nuclei. In this report we focus on one aspect of the problem: whether this membrane supports an ionic environment different from that of the rest of the cytoplasm.

Compartmentalization of ions in the nucleus of the salivary gland cells of dipteran larvae is of special interest. Loewenstein and co-workers (*1*) have measured a substantial resistance of the nuclear membrane in salivary gland cells of *Chironomus thummi* and *Drosophila flavorepleta*. This datum supports the concept that ionic concentrations may be different in the nucleus and cytoplasm.

We therefore decided to probe the *Chironomus* salivary gland cell with K^+-selective microelectrodes in order to determine directly the difference in K^+ activity across the nuclear membrane.

Open-tipped micropipets, drawn from ordinary glass tubing (Corning 7740), were characterized by resistances of 10 to 20 megohms and tip potentials of less than 5 mv when filled with 3*M* KCl. Similar pipets, whose inner surfaces were coated with dichlorodimethylsilane and filled with a liquid K^+ ion exchanger (Corning 477317), had resistances of 5×10^9 to 15×10^9 ohms when backfilled with 0.5*M* KCl. Silver–silver chloride pellets permitted electrical coupling between the microelectrodes and preamplifiers, which had input impedances greater that 10^{13} ohms.

Pulses of constant current were provided by a pulse generator in series with a 100-megohm resistor and the current-passing pipet. Current was calculated from the voltage deflection measured across another series resistor.

All voltage signals were displayed singly or differentially on a storage oscilloscope. In addition, the voltage outputs from the K^+-selective electrode and one micropipet were simultaneously monitored on a paper chart recorder.

The K^+ microelectrodes were calibrated in standard solutions before each experiment. Over the range of Na^+ and K^+ concentrations from 0 to 200 m*M*, the microelectrode response could be described by the empirical equation

$$E = E_0 + 59 \log[a_K + (k_{Na})a_{Na}]$$

where E is the voltage output, E_0 is a constant characterizing the reference state, a_K and a_{Na} are the chemical activities of K^+ and Na^+, respectively, and $k_{Na} = 0.017 \pm 0.003$ (mean ± standard error of the mean). Since the electrodes are more than 50 times more selective for K^+ than for Na^+, no correction for intracellular Na^+ was applied to the data.

Larvae of *Chironomus (chironomus) attenuatus* (Walker) in the fourth instar phase were collected from a local pond. Salivary glands were excised from the larvae and bathed in insect saline solution (*2*).

The glands were observed at a magnification of 250 with a Leitz compound microscope fitted with Nomarski optics. This system enhances contrast over a very shallow depth of field, facilitating accurate localization of the electrode tips relative to the nucleus.

The experiment was done with three electrodes in the same cell. One open-tipped micropipet was placed in the nucleus and the other in the cytoplasm. The difference in electrical potential across the nuclear membrane was measured as the voltage difference between the K^+ electrode and the appropriate micropipet.

The position of the K^+ electrode was rigorously verified electrically by passing current (1×10^{-7} to 3×10^{-7} amp for 1 to 3 seconds) from the micropipet in the nucleus to a grounded electrode in the bathing saline. The voltage difference between the K^+ electrode and the micropipet in the cytoplasm was monitored. When the K^+ electrode was in the nucleus, a change in the voltage difference was observed, coinciding with the current pulse. The change was proportional to the resistance of the nuclear membrane. When the K^+ electrode was in the cytoplasm, no change in the measured voltage difference was seen.

Table 1. Electrochemical and electrical potential differences across the plasma and nuclear membranes and estimated intracellular K^+ distribution. The voltage outputs from the intracellular K^+ microelectrode and the intracellular open-tipped micropipets with respect to the external indifferent electrode are E and V, respectively. The concentration of free K^+, C_K, is calculated from the measured chemical activity, assuming that the intracellular activity coefficient equals that of the external medium, 0.77 (*13*). Each value is the mean ± standard error of the mean for the data obtained from 17 experiments.

Location	E (mv)	V (mv)	C_K (m*M*)
Cytoplasmic	51 ± 3	−28 ± 3	135 ± 11
Intranuclear	52 ± 2	−30 ± 3	146 ± 12
Difference	−0.5 ± 0.4	1 ± 1	−11 ± 7

The procedure for passing electrical current was also necessary to determine if the nuclear membrane had been damaged by the microelectrodes. Cellular impalement with the three-electrode system was considered successful only if the resistance of the nuclear membrane was at least 5 kilohms and the rest potential across the plasma membrane was greater than 10 mv. During a successful impalement, the resting potentials of the nucleus and cytoplasm remained constant while the K^+ electrode was moved between the two compartments. The steady-state voltage outputs from both microelectrodes and micropipets remained constant during the course of the resistance measurements and returned to their initial values when withdrawn from the cells. All cells studied were transparent and nonswollen. Glands could usually be used for 2 hours after isolation.

The mean value of the measured resistances of the nuclear membranes was 13 kilohms. Assuming a mean nuclear diameter of 70 μm, the membrane resistivity was then 2.0 ohm cm^2. This agrees well with the data of Loewenstein and co-workers (*1*), who measured resistivities of 2 and 1.5 ohm cm^2 for the nuclear membranes of *C. thummi* and *D. flavorepleta*, respectively.

Our results from 17 cells in 12 glands are presented in Table 1. There was no significant difference across the nuclear membrane in either the electrochemical activity of K^+ or the electrical potential. The simplest and most direct conclusion to be drawn is that there is also no significant difference in a_K across the nuclear membrane.

Apart from the artifact of tip potential (*3*), measurements of electrical potentials using irreversible electrodes, such as our standard open-tipped micropipets, are subject to an intrinsic ambiguity: spatial inhomogeneities of electrical potential may arise from local suspension and Donnan effects generated by charged macromolecules within the cell (*4*). Since measurements with open-tipped micropipets are used in our system to calculate a_K, the conclusion that a_K is constant across the nuclear membrane is subject to this ambiguity.

However, there is no direct evidence that these effects are quantitatively significant in biological cells. Furthermore, such effects would have had to offset within ± 1 mv the putative difference in a_K between

nucleus and cytoplasm. We think that the simplest interpretation of the data is that free K^+ is, indeed, uniformly distributed across the nuclear membrane.

Similar results were obtained by Starodubov and Kurella (*5*) for the salivary glands of *Drosophila*. However, the significance of that study was limited by two technical difficulties. First, the K^+ electrode they used (*6*) was an order of magnitude less sensitive for K^+ over Na^+ than the electrodes used in our study. Second, they used two rather than three electrodes, so that the nuclear membrane resistance was not measured. Thus, the intranuclear position of their electrodes was not rigorously established, and possible damage to the nuclear membrane on impalement was not excluded.

Kroeger *et al.* (*7*) attempted to measure K^+ chemically in samples of nucleoplasm and cytoplasm extracted from *Chironomus thummi* salivary glands with tungsten needles. They stated that the ionic concentrations changed during development, but that the nuclear and cytoplasmic concentrations closely tracked one another, which is consistent with our data.

In other tissues, estimated ratios of nuclear to cytoplasmic K^+ concentrations have ranged from 0.84 to 2.4 (*8*). These values depend not only on the tissue studied and the technique utilized, but also on whether the K^+ concentrations were calculated as millimoles per kilogram of water or millimoles per kilogram dry weight.

Our study differs from those cited above in that ionic activity, rather than concentration, was measured. The two parameters need not be related in a simple manner, insofar as the amount of ion binding and the activity coefficient for K^+ may be different in nucleus and cytoplasm.

The data reported here bear on the possible specific physiological roles that have been suggested for intranuclear ions. Kroeger and Lezzi (*9*) proposed that gene activity may be regulated in part by the nuclear Na^+/K^+ ratio. Allfrey *et al.* (*10*) found that entry of amino acids into the isolated nucleus was facilitated by a high Na^+ concentration in the medium and suggested that the Na^+ content of the nucleus in vivo must be high, possibly because of direct ionic pathways between the nucleus and the extracellular fluid. Siebert *et al.* (*11*) proposed a similar model based on studies of the flux of radioactive ions into rat liver nuclei and cytoplasm.

The nuclear membrane might at least partially control nuclear ionic activities. This concept was supported by the finding of Ito and Loewenstein (*12*) that ecdysone, the insect moulting hormone, induced changes in the permeability of the nuclear envelope in *Chironomus* salivary glands.

In the case of K^+, however, our data demonstrate an ionic equilibrium across the nuclear membrane. This suggests that nuclear K^+ activity is controlled not by the nuclear membrane, but by the plasma membrane, or alternatively, by the putative nuclear-extracellular pathway.

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References and Notes

2. One solution, used in 11 experiments, consisted of (millimoles per liter): NaCl, 100; KCl, 5; $MgCl_2$, 15; $CaCl_2$, 10; NaH_2PO_4, 2; Na_2HPO_4, 2.85; and glucose, 12; the *p*H was 6.5 to 6.6. The other solution, used for six experiments, consisted of (millimoles per liter): NaCl, 135; KCl, 5; $MgCl_2$, 3; $CaCl_2$, 2; NaH_2PO_4, 3; Na_2HPO_4, 4.2; glucose, 12; the *p*H was 6.5 to 6.6. Similar results were obtained with the two solutions.
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14. We thank S. S. Roback for classifying, the *Chironomus* used; G. Ellis for advice concerning the optical system; and I. Silver, W. McD. Armstrong, and G. Gerstein for helpful discussions concerning various aspects of the ion-selective microelectrodes. Supported in part by grants from the National Science Foundation (BMS73-01261 A01), American Heart Association (71-847), and American Cancer Society (institutional grant IN-38-0). L.G.P. holds a University Fellowship and M.M.C. is an Established Investigator of the American Heart Association (70-148).

29 January 1975

BCG Inhibition of Melanoma: Specific?

Faraci and Schour studied the effect of pretreatment with bacillus Calmette-Guérin (BCG) on tumor growth (*1*): "Three different malignant tumors—a melanoma (S-91), a mammary carcinoma, and a methylcholanthrene-induced sarcoma—were used in syngeneic BALB/c mice." Pretreatment of BALB/c mice with BCG inhibited growth of the S-91 melanoma, but did not inhibit growth of the mammary carcinoma or sarcoma. In vitro studies were extended in another article (*2*). The results obtained were interpreted as evidence ". . . of a specific BCG-induced protection against malignant melanoma in an inbred strain of laboratory mice."

The S-91 melanoma arose in a DBA/1J mouse (*3, 4*). The DBA/1J and BALB/c strains differ at the major murine histocompatibility locus ($H\text{-}2^q$ and $H\text{-}2^d$, respectively), as well as at minor histocompatibility loci (*3*). Although S-91 is transplantable in allogeneic BALB/c mice, it would be expected to express some of the histocompatibility characteristics of the DBA/1J strain. Specificity is a relative term. From the data reported, it is not possible to determine whether the resistance observed by Faraci and Schour should be interpreted as melanoma specific, tumor-line specific, or strain specific.

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31 July 1974