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Electrophysiology of a Clonal Osteoblast-Like Cell Line: Evidence for the Existence of a Ca^{2+}-Activated K^+ Conductance

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Summary. Intracellular microelectrode measurements were made on a well-characterized osteoblast-like clonal cell line isolated from a rat osteosarcoma. In serum-free medium, stable membrane potentials of -42 ± 9 mV (SD, $n = 190$) were recorded. Ion substitution experiments suggested that this membrane potential is primarily a Na^+/K^+ diffusion potential. Input resistance was correlated strongly with colony size, ranging from 49 ± 18 MΩ (SD, $n = 14$) for colonies of 1–3 cells, to 4 ± 4 MΩ (SD, $n = 164$) for colonies of 100 or more cells. These results are consistent with the existence of low resistance intercellular junctions.

Application of the carboxylic calcium ionophore A23187 by pressure microejection onto the cell surface resulted in a transient hyperpolarization and concomitant decrease in input resistance. Both these effects are consistent with an increased K^+ conductance. Ion substitution experiments demonstrated that the degree of hyperpolarization was dependent on the external concentration of both K^+ and Ca^{2+}. Quinine, a blocker of Ca^{2+}-activated K^+ channels, inhibited the ionophore-induced hyperpolarization in a dose-dependent manner. It was concluded that these cells exhibit a Ca^{2+}-activated K^+ conductance.

Key Words Ca^{2+}-activated K^+ conductance · osteoblasts · electrophysiology · membrane potential · A23187 · quinine

Introduction

The selective transport of ions across a cell membrane gives rise to electrical properties that can be measured using microelectrode techniques. Recent studies suggest that ion movements across the cell membrane are involved in the regulation of a variety of cellular functions, including proliferation (Kaplan, 1978; Moolenaar, Mummery, van der Saag & de Laat, 1981), endocytosis (Davies et al., 1980) and response to hormones (Zierler & Rogus, 1981).

This investigation was motivated by our interest in the regulation of bone cell function. As an *in vitro* osteoblast model, we chose a clonal rat osteosarcoma cell line (ROS 17/2) (Majeska, Rodan & Rodan, 1980). Extensive studies of the physiological and biochemical behavior of these cells have been made (reviewed by Rodan, Rodan & Majeska, 1982). The purpose of our investigation was twofold: first, to characterize the electrical properties of ROS 17/2 cells as a basis for further electrophysiological studies; second, to determine if these cells exhibit a Ca^{2+}-activated K^+ conductance. It has been established that Ca^{2+}-activated K^+ channels occur in a variety of cell types, including neurons, muscle cells, erythrocytes, glandular epithelial cells, and hepatocytes (reviewed by Lew & Ferreira, 1978; Meech, 1978; Putney, 1979), pancreatic β-cells (Atwater, Dawson, Ribalet & Rojas, 1979), macrophages (Oliveira-Castro & Dos Reis, 1981), lymphocytes (Tsien, Pozzan & Rink, 1982), and fibroblast-like cells (Okada, Tsuchiya & Yada, 1982). In excitable cells, several possible functions have been attributed to these channels, e.g., the adaptation of neurons to prolonged stimulation (Meech, 1978) and the generation of pacemaker activity (Gorman, Hermann & Thomas, 1981). In nonexcitable cells, however, the functions of Ca^{2+}-activated K^+ channels are unclear. The occurence of Ca^{2+}-activated K^+ channels in the osteoblast-like ROS 17/2 cells would allow us to follow changes in Ca^{2+} concentration within individual cells by monitoring the resulting changes in membrane potential. Such a system would be of great value in studying the role of intracellular calcium in various physiological (Rasmussen & Goodman, 1977) and pathological (Schanne, Kane, Young & Farber, 1979) processes, and perhaps in elucidating the function of Ca^{2+}-activated K^+ channels in nonexcitable cells.

In this report we describe the general electrophysiological characteristics of ROS 17/2 and the occurrence of a Ca^{2+}-activated K^+ conductance in these cells.

Materials and Methods

Cell Culture

Cells were grown in Lux T-75 flasks at 37°C in culture medium consisting of alpha minimal essential medium (α-MEM) supplemented with 15% fetal bovine serum, 100 μg/ml penicillin G, 50 μg/ml gentamicin, 0.3 μg/ml amphotericin B, and a 25 mM bicarbonate/5% CO_2 buffer. One to nine days prior to measurements, cells were seeded at a density of 2×10^3 cells/cm^2 into Falcon 60-mm culture dishes containing 5 ml of culture medium.

Electrophysiological Measurements

Thirty minutes before measurements, the culture medium was replaced with 5 ml of fresh medium (wash medium, *see* Materials) and the dish was returned to the incubator. Immediately prior to measurements, the wash medium was replaced with 10 ml of fresh medium (test medium, *see* Materials), which was then covered with paraffin oil to prevent evaporation. The dish was then placed on an inverted microscope stage equipped with a heater adjusted to maintain the culture at 37 ± 1° C.

Electrophysiological measurements were made using microelectrodes filled with 3 M KCl and having resistances of 30 to 100 MΩ. The microelectrode was coupled to a high impedance preamplifier (W.P.I., model 750), and the voltage signal was amplified and displayed on a strip chart recorder (Gould, Model 110).

A hydraulic micromanipulator was used to bring the microelectrode tip into contact with the cell surface, after which a momentary increase in the capacity compensation control usually resulted in penetration of the cell membrane. Approximately 50% of attempts resulted in membrane potential recordings which were judged to be acceptable on the basis of having minimal overshoot and being stable for periods lasting from several minutes to several hours.

Current-voltage curves were generated by injecting current pulses of up to ±1.2 nA and measuring the resulting voltage shifts. The voltage drop across the cell membrane was determined by subtracting the voltage drop across the electrode from the total voltage shift. Input resistance was measured with a standard injected current pulse of 0.1 nA.

Microejection System

An extracellular microejection system was used to apply ionophore solution to the cell surface during electrical recording. The system consisted of a glass micropipette coupled to a Picospritzer Model-2 pressure injection system (General Valve Corp.). Using a manual micromanipulator, the tip of the micropipette was positioned to a location 400 μm from the site of impalement. Reproducible volumes of ionophore solution were then ejected under pressure towards the cell surface. One or more pressure pulses of 70 kPa, each lasting 2 to 999 msec, were used. After each experiment, the micropipette was calibrated to determine the volume ejected per pulse.

This system enabled us to apply ionophore without interrupting the electrical recording, so that each cell acted as its own control for quantifying ionophore-induced effects. This eliminated from our data any variation attributable to differences between cells and any variation in tip or junction potentials which might arise from microelectrode removal and reinsertion (Nagel, 1981). This technique did not, however, allow precise control of the concentration of ionophore either spatially or temporally. In order to minimize this source of experimental error, in the ion substitution and quinine experiments, the same micropipette was used throughout a single experiment without any change in position. In addition, the responses of cells under control conditions were always recorded.

Materials

Calcium ionophore A23187 (Calbiochem—Behring Corp.) was dissolved in absolute ethanol at a concentration of 2×10^{-3} M, and aliquots were stored at −80°C. Immediately before use, the ionophore solution was diluted to 10^{-5} M in HEPES-buffered α-MEM. The normal wash medium and test medium were identical to the ionophore vehicle, i.e., α-MEM buffered with 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanosulfonic acid) pH 7.4, containing 0.5% absolute ethanol.

For the Na^+/K^+ ion substitution experiments, the wash medium was HEPES-buffered α-MEM. The test media and ionophore vehicles were HEPES-buffered α-MEM modified to give a final K^+ concentration of 1, 5, 25, and 125 mM. Corresponding Na^+ concentrations were 146, 142, 122, and 22 mM.

For the Ca^{2+} ion substitution experiments, the wash medium, test medium and ionophore vehicle all consisted of HEPES-buffered Ca^{2+}-free α-MEM plus 4 mM EGTA (ethyleneglycol-bis(β-aminoethyl ether)N,N'-tetraacetic acid, Sigma).

For the quinine experiments, a stock solution of 1 mM quinine monohydrochloride dihydrate (Aldrich) in normal test medium was prepared and stored in the dark at 4°C. Prior to measurements, wash and test media containing the desired concentration of quinine were prepared by diluting stock solution in normal test medium.

Results

Membrane Potential

Figure 1 is a typical membrane potential recording. Contact of the microelectrode with the cell surface resulted in a small positive potential of 1–2 mV. Upon penetration of the membrane, the potential immediately went negative and stabilized within 1–2 min. In normal medium the mean membrane potential was −42 ± 9 mV (SD, n = 190). There was no significant variation in the membrane potential with the age of the culture over a period of 1 to 9 days. However, the membrane potential was slightly dependent on colony size, i.e., cells in small colonies of 1–3 cells had a mean membrane potential of −35 ± 9 mV (SD, n = 11), which was significantly less than the overall mean. The mean membrane potentials of cells in larger colonies were not significantly different from the overall mean.

We investigated the ionic basis of the mem-

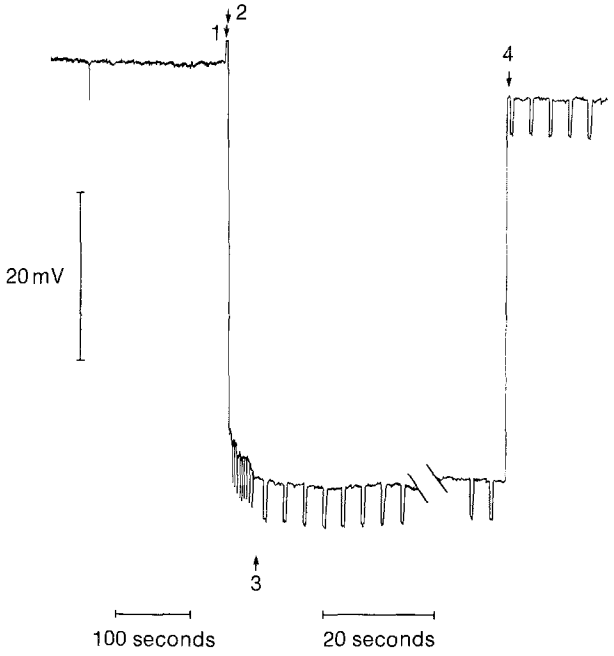

Fig. 1. A typical recording of membrane potential. Superimposed on the record are the responses to 0.1 nA hyperpolarizing current pulses of 0.5 sec duration used to monitor input resistance. (*1*) Contact with the cell surface resulted in a small positive potential. (*2*) Upon penetration of the membrane, the potential immediately went negative. In 50 sec the recording stabilized to the cell's membrane potential. (*3*) Time scale changed. (*4*) After approximately 30 min of recording, the electrode was withdrawn. The electrode resistance remained virtually unchanged, but the baseline had drifted slightly

brane potential by varying the extracellular concentration of K^+ and Na^+ while maintaining constant both the total ionic strength and the concentration of all other ions. The results are shown in Fig. 4. The uppermost data points (closed circles) are the mean membrane potentials in each medium. The Na^+/K^+ diffusion potentials for selected Na^+/K^+ permeability ratios are included in Fig. 4. These were calculated using the modified Goldman-Hodgkin-Katz equation (Hodgkin & Katz, 1949), assuming Cl^- is in equilibrium across the cell membrane. A range of values for the diffusion potential was calculated using ranges of 15 to 27 mM and 134 to 160 mM for the concentrations of intracellular Na^+ and K^+, respectively. These ranges are the means ± 1 SD of the intracellular Na^+ and K^+ concentrations in rat cells as tabulated by Moyer, Moyer and Waite (1982). It can be seen from Fig. 4 that the membrane potential approximates a Na^+/K^+ diffusion potential with a permeability ratio of slightly less than 0.2.

Table 1. The dependence of input resistance on colony size

Colony size (cells)	Input resistance (MΩ)
1–3	49 ± 18 (n = 14)
4–9	44 ± 23 (n = 5)
10–31	23 ± 12 (n = 24)
32–99	13 ± 8 (n = 17)
100 or more	4 ± 4 (n = 164)

Colony size is the number of cells in the colony which contained the impaled cell. Values of input resistance are means ± SD; n is the number of cells on which measurements were made.

Input Resistance

Measured values of input resistance were found to be correlated strongly with colony size (Table 1). Current-voltage relationships were essentially linear over the range ±1.2 nA. The resistivity of the membrane was not calculated because of difficulty in estimating the cell surface area. Although the cell surface appears smooth with phase contrast optics, scanning electron microscopy revealed that these cells are covered with numerous microvilli (S.J. Dixon & J.E. Aubin, *unpublished results*).

Effects of Calcium Ionophore A23187

Application of medium without ionophore to the cell surface had no effect on either membrane potential (Fig. 2*a*) or input resistance. When a small quantity (10^{-15} to 10^{-11} mol) of A23187 was applied, a rapid hyperpolarization and a concomitant decrease in input resistance resulted. Subsequently, both the membrane potential and input resistance gradually returned to their initial values (Fig. 2*b*).

Both the degree and duration of the electrophysiological effects were dependent on the quantity of A23187 applied to the cell surface. With increasing quantities of A23187, the degree of hyperpolarization increased to a maximum and then remained relatively constant (*see* Fig. 3). The duration of the hyperpolarization, however, continued to increase with increasing quantity of A23187 (data not shown). Decreases of up to 90% of the initial input resistance were observed, depending on the quantity of ionophore applied. Repeated application of a fixed quantity of A23187 did not always result in identical responses. Whereas the degree of hyperpolarization was often reduced, in some cases the

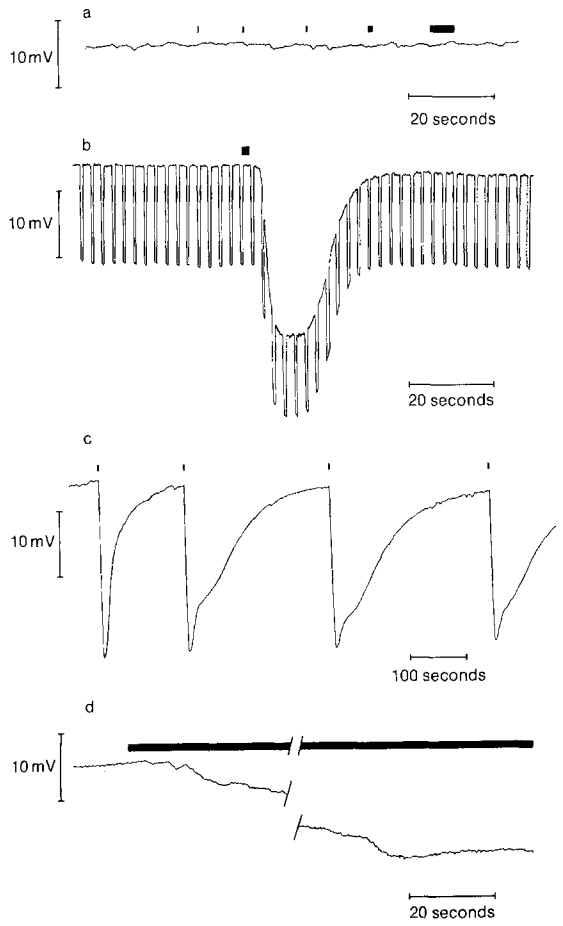

Fig. 2. Membrane potential recordings. (*a*): Application of medium without ionophore to the cell surface. Volumes of 4, 8, 16, 32 and 160 nl were applied at the times indicated by the bars. (*b*): 200×10^{-15} mol of A23187 in 20 nl of medium were applied to the cell surface. 0.1-nA current pulses revealed a decrease in input resistance during hyperpolarization. By subtracting the electrode resistance of 90 MΩ, the input resistance was found to decrease in this case from an unstimulated value of 60 to 30 MΩ at maximum hyperpolarization. (*c*): 80×10^{-15} mol of A23187 in 8 nl of vehicle were applied to the cell surface at the times indicated by the bars. Upon repeated application of ionophore, the degree of hyperpolarization decreased, whereas the duration of hyperpolarization increased. (*d*): A23187 was applied to the cell surface at a rate of approximately 200×10^{-15} mol/sec during the time indicated by the long horizontal bar. Approximately 1 min of the recording was deleted where indicated. Continuous application of ionophore resulted in a sustained hyperpolarization

duration of the hyperpolarization increased (Fig. 2*c*). If a cell was then not stimulated for several minutes allowing recovery to occur, that cell's response to an additional application of ionophore was similar to its initial response to ionophore.

Rapid application of very large quantities of

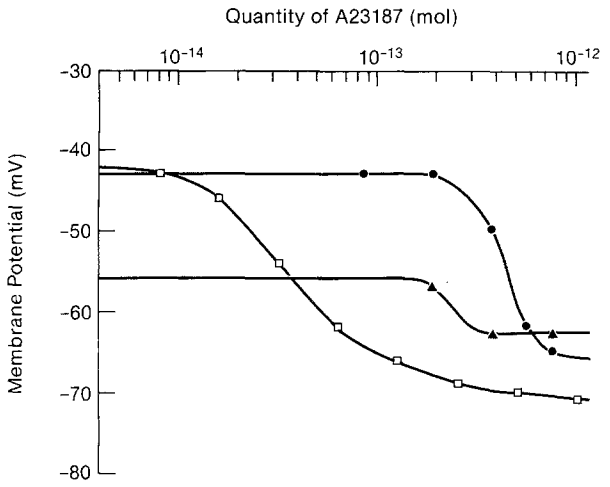

Fig. 3. The relationship between the maximum hyperpolarization and the quantity of A23187 applied is shown for three cells. Initial values are the unstimulated membrane potentials. Increasing quantities of A23187 were applied sequentially to each cell. Between applications of ionophore, sufficient time was allowed for the membrane potential to return to its initial value

A23187 to the cell surface was usually cytotoxic, as indicated by irreversible depolarization and morphological changes. However, it was occasionally possible to apply A23187 to the cell surface continuously for an extended period of time. This resulted in a sustained hyperpolarization (Fig. 2*d*).

The Dependence of A23187-Induced Hyperpolarization on the Extracellular K^+ Concentration

Fixed quantities of A23187 were applied to cells bathed in medium containing different concentrations of Na^+ and K^+. In Fig. 4, the maximum hyperpolarization is plotted against the log of the extracellular K^+ concentration for one such experiment. At low concentrations of K^+, a larger quantity (1.2×10^{-12} mol) of A23187 consistently resulted in larger hyperpolarizations than a smaller quantity (0.6×10^{-12} mol) of A23187. The maximum hyperpolarization clearly depended on the extracellular Na^+ and K^+ concentrations. Largest hyperpolarizations were obtained with an extracellular K^+ concentration of 1 mM. At 125 mM K^+, both large and small quantities of A23187 resulted in a small but significant depolarization.

Included in Fig. 4 is the expected range of the Na^+/K^+ diffusion potentials for selected Na^+/K^+ permeability ratios. A23187 has the effect of shifting the membrane potential towards a Na^+/K^+ diffusion

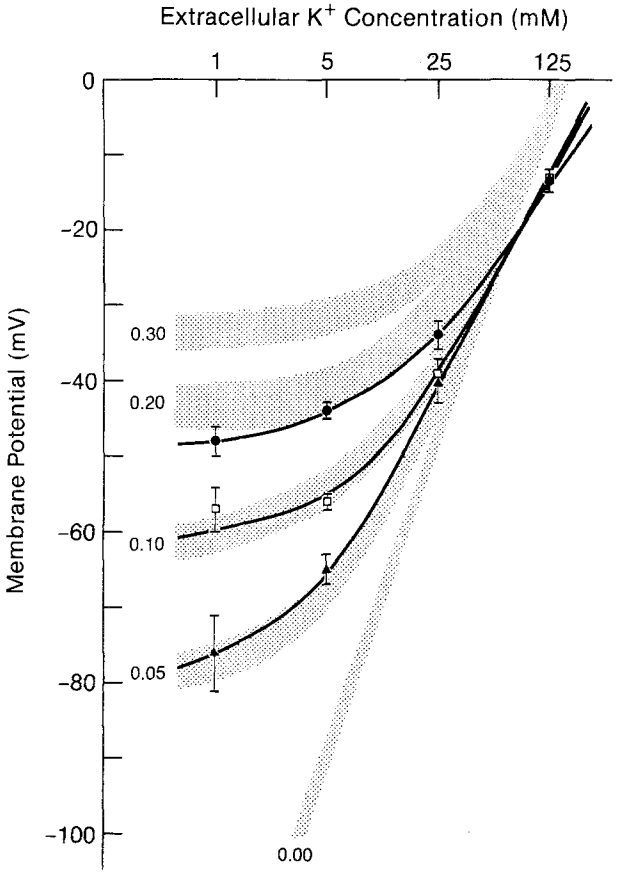

Fig. 4. The effect of the extracellular concentration of K^+ on the unstimulated membrane potential (●) and on the maximum hyperpolarization resulting from application of 0.6×10^{-12} mol (□) and 1.2×10^{-12} mol (▲) A23187. At each concentration, measurements were made on 2 to 4 cells. Values are means ± SEM. Each stippled curve represents the expected range of the Na^+/K^+ diffusion potential for the indicated Na^+/K^+ permeability ratio. These were calculated from the modified Goldman-Hodgkin-Katz equation using ranges of values for the intracellular Na^+ and K^+ concentrations (*see* text for complete details)

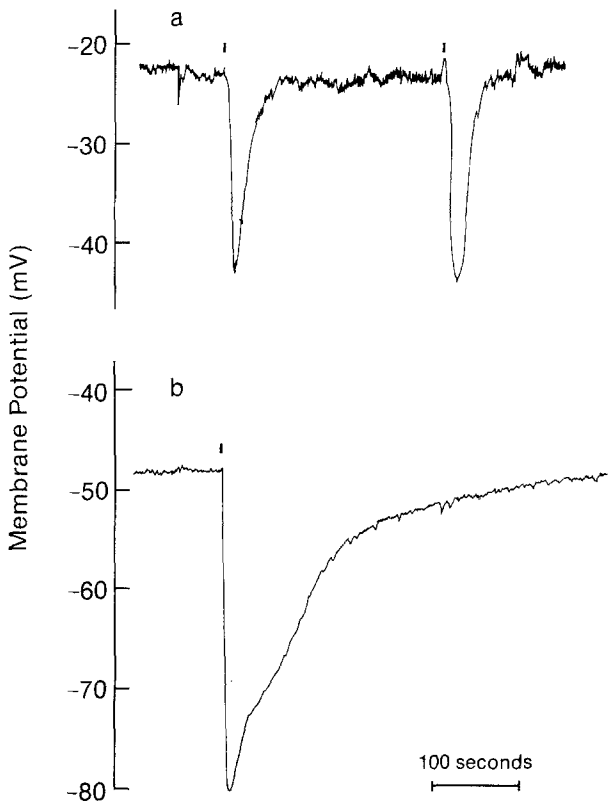

Fig. 5. 0.5×10^{-12} mol of A23187 were applied to the cell surface at the times indicated by the bars: (*a*) cell in calcium-free medium; (*b*) cell from an identical culture in normal medium

potential corresponding to a lower Na^+/K^+ permeability ratio. Changes in input resistance were unaffected by the concentrations of extracellular Na^+ and K^+.

The Dependence of A23187-Induced Hyperpolarization on Extracellular Ca^{2+}

Removal of Ca^{2+} from the extracellular medium resulted in cellular retraction, a depolarization of membrane potential (mean 20 ± 2 mV SD, $n = 4$), and an increase in input resistance. The hyperpolarization resulting from application of A23187 was reduced both in magnitude and duration. In addition, the onset of hyperpolarization was delayed slightly (*see* Fig. 5).

The Effect of Quinine on A23187-Induced Hyperpolarization

Two experimental protocols were used to test the effect of quinine on A23187-induced hyperpolarization. In one, A23187 was applied to the same cell first in control medium, then in medium containing 50 μM quinine and finally in medium containing 100 μM quinine (Fig. 6). The presence of quinine resulted in a dose-dependent inhibition of ionophore-induced hyperpolarization. Because it was possible that some of the reduction in hyperpolarization resulted from the repeated application of ionophore to the same cell, a second protocol was used in which identical cultures were bathed in either control medium or medium containing 100 μM quinine and then tested for response to A23187. Results are summarized in Table 2. Quinine inhibited not only the A23187-induced hyperpolarization but also the

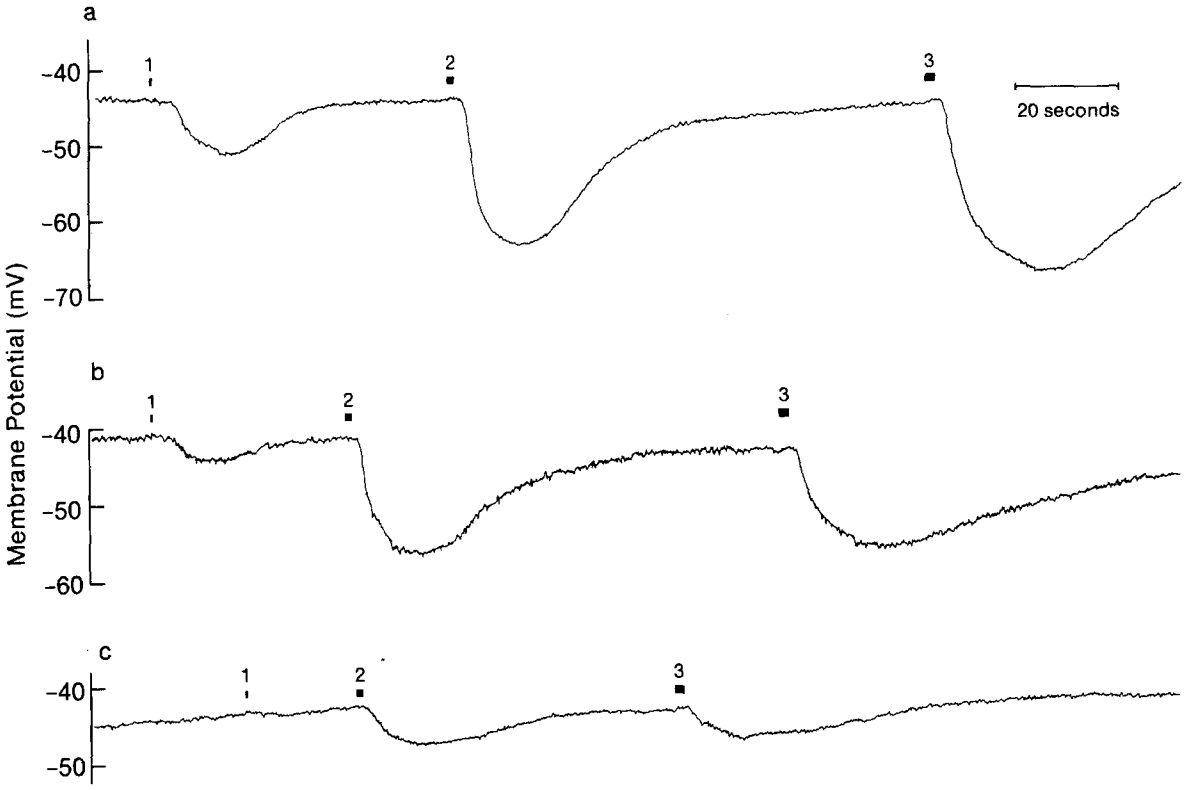

Fig. 6. The effects of quinine on A23187-induced hyperpolarization. Membrane potential data from a single cell are shown. Three quantities of A23187 were applied at the times indicated by the bars: (*1*) 0.4×10^{-12} mol, (*2*) 0.6×10^{-12} mol, and (*3*) 0.8×10^{-12} mol. Measurements were made in normal medium (*a*), and then repeated in medium containing 50 μM quinine (*b*), and 100 μM quinine (*c*)

concomitant decrease in input resistance. Quinine had no significant effect on the unstimulated membrane potential of these cells. A small transient depolarization was observed occasionally following an A23187-induced hyperpolarization in the presence of quinine.

Discussion

General Electrophysiological Characteristics

The mean membrane potential of ROS 17/2 cells in serum-free medium was -42 ± 9 mV (SD, $n = 190$). Cells within small colonies (1–3 cells) had smaller membrane potentials than cells within larger colonies. This may be a density-dependent effect as suggested by Cone (1974) for other cells types, or it may result from nonspecific leakage at the site of microelectrode puncture. Such leakage would have a minimal effect on the membrane potential of cells coupled by low resistance junctions to a large number of other cells, but would have a significant effect on the membrane potential of cells in small colonies. The mean value of the membrane potential (−42 mV) is comparable to those reported for other types of nonexcitable cells (Williams, 1970). The dependence of the membrane potential on the extracellular Na^+ and K^+ concentration is also seen in other types of nonexcitable cells and suggests that the membrane potential is primarily a Na^+/K^+ diffusion potential with a Na^+/K^+ permeability ratio much larger than that in excitable cells (Williams, 1970). We estimate that the Na^+/K^+ permeability ratio in these cells is approximately 0.2. However, two observations suggest that the membrane potential cannot be entirely explained in terms of a Na^+/K^+ diffusion potential. First, it is apparent from Fig. 4 that at high concentrations of extracellular K^+ the measured membrane potential is more negative than the expected Na^+/K^+ diffusion potential. Second, at an external K^+ concentration of 125 mM, application of A23187 induced a small depolarization of membrane potential (Fig. 4). If the ionophore-induced change in permeability results entirely from the activation of K^+ selective channels, then the depolarization implies that under these conditions the K^+ electrochemical gradient is

inwards. A negative membrane potential occurring with an inwardly-directed K^+ electrochemical gradient cannot result entirely from a Na^+/K^+ diffusion potential. In spite of these discrepancies, the strong dependence of membrane potential on the extracellular concentration of Na^+ and K^+ suggests to us that it is primarily a Na^+/K^+ diffusion potential. The discrepancies discussed above may have arisen from tip or junction potentials (Armstrong & Garcia-Diaz, 1981), a contribution to the membrane potential by electrogenic ion transport, or a contribution to the diffusion potential by an ion other than Na^+ or K^+.

The correlation of input resistance with colony size suggests the existence of low resistance intercellular junctions among the ROS 17/2 cells. For several other cell types in monolayer cultures, it has been shown that a lower input resistance is correlated with the existence of electrical coupling between cells (Hülser & Webb, 1973; Laerum, Hülser & Rajewsky, 1976), and that only for electrically coupled cells is input resistance dependent on cell density (Hülser & Webb, 1973). In our measurements, input resistance correlated strongly with colony size. The average colony size did increase with time in culture but, comparing colonies of equal size, we found no significant change in input resistance with time in culture. We also found that removal of calcium from the medium resulted in cellular retraction and an increase in input resistance. This increase in input resistance is consistent with the loss of low resistance coupling between cells. Rose and Loewenstein (1971) have reported the loss of intercellular coupling within isolated *Chironomus* salivary glands upon removal of Ca^{2+} from the extracellular medium. The occurrence of low resistance junctions between osteoblasts in isolated calvariae has been reported (Jeansonne et al., 1979), as has the occurrence of gap junction-like complexes between osteoblasts (Holtrop, 1975).

Evidence for the Existence of a Ca^{2+}-Activated K^+ Conductance

Our evidence for the existence of a Ca^{2+}-activated K^+ conductance in these cells is based on the following electrophysiological effects of A23187. Applied to the cell surface, A23187 induces a hyperpolarization and concomitant decrease in input resistance. At higher doses of ionophore, the hyperpolarization saturates near the K^+ reversal potential. Na^+/K^+ ion substitution demonstrates that A23187 causes a decrease in the Na^+/K^+ permeability ratio. The electrophysiological response to ionophore is partially dependent on extracellular Ca^{2+} and is inhibited by quinine. Each of these points is discussed in turn.

Table 2. The effect of quinine on A23187-induced hyperpolarization

Experiment number	Quantity of A23187 (mol)	Hyperpolarization[a] (10^2 mV · sec)	
		Control	100 μM quinine
1	1.0×10^{-12}	10 ± 7 ($n = 3$)	1.9 ± 0.4 ($n = 4$)
2	1.0×10^{-12}	17 ± 9 ($n = 2$)	1.7 ± 0.4 ($n = 2$)
3	3.7×10^{-12}	32 ($n = 1$)	0.6 ($n = 1$)

Values are means ± SD; n is the number of cells.
[a] A time integral of potential was used as a measure of hyperpolarization. This was determined by measuring the area on the strip chart enclosed by the hyperpolarization. Because of uncertainty in determining exactly at which point the potential returned to its initial value, we used the area enclosed by the voltage tracing at all values more negative than 10% of the maximum hyperpolarization.

A23187 is a carboxylic ionophore that catalyses electrically neutral ion exchange across biological membranes and is highly selective for divalent over monovalent ions (Pressman, 1976). In our experiments, application of A23187 would be expected to result in a large influx of extracellular Ca^{2+} across the cell membrane in exchange for intracellular H^+ and Mg^{2+}. Because these ion movements are electrically neutral, the ionophore-induced hyperpolarization and decrease in input resistance cannot result directly from the action of the ionophore itself. The hyperpolarization and decrease in input resistance are, however, consistent with an increase in K^+ conductance secondary to an increase in intracellular Ca^{2+} concentration, via Ca^{2+}-activated K^+ channels.

Large initial input resistances were observed only in cells in small colonies (Table 1). Significant decreases in total resistance (input resistance plus electrode resistance) were always observed following application of ionophore to these cells. Because of smaller initial input resistances in cells in large colonies, application of ionophore resulted in either a very slight decrease in total resistance or no significant change. It is also possible that application of ionophore A23187 induced a transient loss of intercellular electrical coupling as has been shown in *Chironomus* salivary glands (Rose & Loewenstein, 1976; Rose & Rick, 1978). However, in our system, the increase in resistance resulting from the loss of electrical coupling would be masked by a concomi-

tant decrease in resistance resulting from activation of Ca^{2+}-dependent K^+ channels.

The kinetics of the ionophore-induced electrophysiological changes are consistent with the kinetics of Ca^{2+}-activated K^+ channels in other cell types. The rapid onset of hyperpolarization is consistent with the rapid activation of Ca^{2+}-dependent K^+ channels by intracellular Ca^{2+} (Lew & Ferreira, 1978). The sustained hyperpolarization resulting from continuous application of A23187 is consistent with channel inactivation being independent of time (Brown & Lew, 1981). The lack of time-dependent inactivation implies that the repolarization of membrane potential following pulsatile application of A23187 (Fig. 2*b* and *c*) reflects the return of the intracellular calcium concentration to normal levels. Therefore the desensitization (Fig. 2*c*) and recovery of response to repeated applications of A23187 may in turn reflect properties of the intracellular Ca^{2+} buffering mechanisms. It has been demonstrated recently that in frog neurons having a Ca^{2+}-activated K^+ conductance the kinetics of Ca^{2+}-induced changes in membrane potential directly reflect the kinetics of the underlying changes in Ca^{2+} concentration (Smith, MacDermott & Weight, 1983).

The degree of hyperpolarization saturated with the application of larger quantities of ionophore (Fig. 3). This saturation is consistent with the activation of a K^+ conductance which cannot result in a hyperpolarization greater than the K^+ reversal potential (estimated to be between −87 and −92 mV). We observed, however, that saturation usually occurred at values less than the estimated reversal potential. These measurements were made by sequentially applying increasing quantities of A23187 to the cell surface. A reduction in hyperpolarization may have resulted from the same desensitization that occurred when fixed quantities of A23187 were applied repeatedly. Although the degree of hyperpolarization saturated with larger quantities of A23187, the duration of the hyperpolarization continued to increase. This observation is consistent with the saturation not resulting simply from the saturation of the cell membrane with ionophore.

The Na^+/K^+ ion substitution experiments confirmed that A23187 induces an increase in K^+ conductance. At various concentrations of extracellular K^+, A23187 induced a dose-dependent shift of membrane potential towards the K^+ reversal potential (Fig. 4). In the experiment illustrated in Fig. 4, application of 1.2×10^{-12} mol A23187 resulted in an approximately fourfold decrease in the Na^+/K^+ permeability ratio. Since the Ca^{2+}-activated K^+ channel is highly selective for K^+ over Na^+ (Simons, 1976; Pallotta, Magleby & Barrett, 1981), it would seem that a fourfold increase in K^+ permeability occurred. It is difficult, however, to quantify precisely the ionophore-induced change in K^+ permeability. In these experiments we manipulated the Na^+ and K^+ chemical potential gradients by varying the extracellular Na^+ and K^+ concentrations. However, it has been shown for erythrocytes that the Ca^{2+}-activated K^+ permeability is sensitive to the concentration of extracellular K^+ (Knauf et al., 1975). Recent studies have shown also that in other cell types, Ca^{2+}-activated K^+ channels are voltage sensitive (Gorman & Thomas, 1980; Hermann & Hartung, 1982*b*; Barrett, Magleby & Pallotta, 1982). The Ca^{2+}-activated K^+ channels in our cells may have a similar dependence on extracellular K^+ and transmembrane voltage. If significant, these effects would cause us to underestimate the ionophore-induced change in K^+ permeability.

Removal of Ca^{2+} from the extracellular medium resulted in a delayed and reduced hyperpolarization in response to ionophore. Although the relative degree of hyperpolarization was not reduced, the absolute degree and the duration of the hyperpolarization were significantly reduced (Fig. 5). These results are consistent with an ionophore-catalysed release of intracellular Ca^{2+} (Babcock, First & Lardy, 1976), resulting in reduced and delayed electrophysiological effects.

The final evidence for the existence of a Ca^{2+}-activated K^+ conductance in these cells is the inhibition of the ionophore-induced electrophysiological effects by quinine. Quinine blocks Ca^{2+}-activated K^+ channels in a variety of cell types [red cells (Armando-Hardy et al., 1975), barnacle photoreceptors (Hanani & Shaw, 1977), pancreatic β-cells (Atwater et al., 1979), hepatocytes (Burgess, Claret & Jenkinson, 1981), renal epithelial cells (Brown & Simmons, 1982), snail neurons (Hermann & Hartung, 1982*a*), and fibroblast-like cells (Okada et al., 1982)]. Inhibition by quinine is rapid and reversible (Lew & Ferreira, 1978). It has been shown in red blood cells that quinine directly blocks Ca^{2+}-activated K^+ channels without affecting A23187-induced Ca^{2+} influx (Reichstein & Rothstein, 1981). In our cells, the ionophore-induced electrophysiological effects were inhibited by approximately the same concentration of quinine that inhibits Ca^{2+}-activated K^+ channels in other cell types. Half maximal inhibition occurs with 150–200 μM quinine in barnacle photoreceptors (Hanani & Shaw, 1977), 100–200 μM in red cells (Lew & Ferreira, 1978), and 200 μM in hepatocytes (Burgess et al., 1981). The lack of a significant effect of quinine on the unstimulated membrane potential of our cells suggests that the Ca^{2+}-activated K^+ channels are inactive at basal levels of intracellular Ca^{2+}.

These results form the basis for using this system to study electrophysiological changes associated with cell function. In particular, by monitoring quinine-sensitive hyperpolarization we hope to study physiological changes in intracellular Ca^{2+} and perhaps elucidate the function of Ca^{2+}-activated K^+ channels in these cells.

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