Reduced ionic conductance in turtle brain

CHRISTOPHER J. DOLL, PETER W. HOCHACHKA, AND PETER B. REINER
Department of Zoology, University of British Columbia, Vancouver V6T 1Z4; and Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada

Doll, Christopher J., Peter W. Hochachka, and Peter B. Reiner. Reduced ionic conductance in turtle brain. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R929–R933, 1993.—Whole cell recording techniques were employed to measure whole cell (G_w) and specific membrane (G_m) conductance in turtle and rat pyramidal neurons in slices. Results indicate that rat neurons are 4.2 times more conductive compared with turtle neurons at 25°C, which is accentuated by temperature, so that rat neurons at 37°C are 22 times more conductive than turtle neurons at 15°C. A conductance Q_{10} of 1.9 was measured for both turtle (15–25°C) and rat (25–35°C) pyramidal neurons. Conductance measurements of turtle pyramidal neurons over 6–9 h of anoxia indicate no statistical difference between G_m or G_w from normoxic control measures. These results indirectly support the concept of low ATP-dependent ion pump activity in the turtle brain as one mechanism for reduced energy expenditure in the normoxic state.

leakage channels; turtle pyramidal neurons; rat pyramidal neurons; anoxia; membrane conductance; channel arrest; ion pumping

THE TURTLE *Chrysemys picta* has been shown to survive forced submergence for over 6 mo at 3°C (31), suggesting a central nervous system (CNS) that is highly adapted to survive and function under conditions of anoxia. Considerable evidence in support of this contention has been acquired including intracellular (9) and extracellular (12) recording measurements, EEG recordings, and extracellular ion measures (29). In contrast, the mammalian brain shows degenerative signs within minutes for a similar insult (9, 29). The observation that ATP concentration ([ATP]) decreases to 25% of control values within minutes in the anoxic rat brain (26) while remaining unchanged for hours in the turtle brain (18, 20) has led to the hypothesis that [ATP] is directly or indirectly responsible for the functional collapse of the neuron (15, 19). Note that the term functional collapse denotes the rapid loss in neuronal membrane resistance that accompanies both anoxia and ischemia in the rat and ischemia in the turtle (8, 25). Although this collapse is a precursor to cell death, the exact relationship has yet to be determined.

If energy status is to be sustained in the turtle brain during anoxia, supply must meet demand. Three basic mechanisms in principle could aid in the maintenance of energy balance during anoxia, assuming the tissue is not substrate limited: *1*) enhanced glycolytic capability to aid in the supply of ATP to the cell, *2*) low normoxic metabolic rate to lessen the initial ATP demand of the brain, *3*) metabolic depression during anoxia to further accentuate a low normoxic metabolic rate.

With respect to *1*) and *2*) above, we have demonstrated that the turtle brain expresses twice the glycolytic capability of the rat brain while consuming only 1/12 to 1/24 the glucose under control (normoxic) conditions (30). Additionally, the metabolic rate of the turtle brain appears low compared with other ectotherms (22). With regard to *3*), significant anoxic metabolic depression has been estimated in both whole brain preparations (18, 20) and in slices (27).

Two of the three protective mechanisms outlined above concern reduced cellular metabolic rate. However, the question remains as to what metabolic processes are being suppressed in both normoxia and anoxia to account for lowered ATP turnover. It has been hypothesized that one mechanism that could reduce energy expenditure in turtle brain would be to decrease ATP-dependent ion pump activity by reducing background ion leakage (15).

The concept of regulating cell metabolism through regulation of ATP-dependent ion pump activity is not new (32) and has been forwarded as a possible mechanism for the action of thyroid hormone in controlling thermogenesis (17). This idea was later expanded as a possible explanation for endothermy (10). This hypothesis predicts that endotherms have leakier membranes and thus increased ATP-dependent ion pump activity serving as an indirect mechanism of heat production. A similar concept involving regulation of ion pumping by Ca^{2+}-ATPase is believed to account for thermogenesis in marlin heater tissue, a modified muscle that displays an expanded sarcoplasmic reticulum but has little contractile protein (4). Taken together, this evidence provides strong support for the idea that regulation of leakiness ("channel arrest") and hence, ion pump activity, could regulate ATP turnover in the cell.

This paper tests two predictions of channel arrest (16). First, do turtle cortical pyramidal neurons display lower passive ion leakage (conductance) compared with the rat cortical pyramidal neurons during normoxia, and second, are leakage channels in the turtle pyramidal neuron downregulated during long-term anoxia as a potential survival strategy?

MATERIALS AND METHODS

Turtles (*C. picta*), 150–300 g, were cold anesthetized before decapitation. The brain was rapidly dissected free and immersed in precooled oxygenated artificial cerebrospinal fluid (aCSF). The cortical tissue was then dissected free similarly as described in Ref. 7. Blocks of whole cortex (≈500 μm thick) were stored at room temperature (≈22°C) in a holding chamber for at least 60 min until their use in the recording chamber. Individual slices were transferred to a recording chamber where they were continuously superfused with aCSF at a flow rate of 1.5–2.0 ml/min.

Young Wistar rats (25–40 g) were anesthetized with halothane and decapitated, and the brain was rapidly removed and immersed in precooled oxygenated aCSF. After a few minutes of precooling, a block containing frontal-parietal cortex was dissected free, glued with cyanoacrylate to a mounting block, and sliced (400 μm thick) on a vibratome. Slices were stored at ≈22°C for at least 60 min before use.

Methods for the formation of whole cell seals in slices of both turtles and rats have been described in detail elsewhere (3). Whole cell patch recordings were carried out using Sutter 1.5 OD × 1.10 ID borosilicate nonfilament glass. Electrodes were pulled on Sutter model P-15 horizontal puller. Rat patch solution contained (in mM) 15 NaCl, 110 KOH (pH adjusted to 7.4, titrated with methanesulfonic acid), 10 Na-HEPES, 11 EGTA (dissolved in 29 mM KOH), 1 $CaCl_2$, 2 Mg-ATP, and 0.3 GTP, final pH = 7.4. Turtle patch solution was the rat patch solution diluted by 10%. The osmolarity of the patch solution was 320–330 mosM in rat; 290–300 mosM in turtle.

Data were acquired using an Axoclamp 2A amplifier connected to an Axolab 1100 interface, which also served to generate current commands using the pClamp set of programs. Data were also independently digitized at 49 kHz and stored on videotape for off-line analysis. Criteria for using a patched cell included the GΩ seal, positive-going action potentials, and electrode impedance ≤45 MΩ (most were <20 MΩ) at the completion of the experiment. Although some cells were held for up to 3 h, changes in cell conductance generally occurred within 30–45 min after breaking into the cell. All measurements were made within 10 min of obtaining the whole cell configuration.

Pyramidal neurons in the turtle were identified by location, action potential size, action potential duration, input resistance, and time constant, as previously discussed (7). Pyramidal neurons in the rat were identified by location (cortical layer) as well as action potential duration, frequency, and size, as discussed elsewhere (21).

The rat aCSF consisted of (in mM) 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 11 glucose, 25 $NaHCO_3$, and 0.03 phenol red as a pH indicator. The aCSF for the turtle was a modification from Connors and Kreigstein (7), and consisted of (in mM) 96.5 NaCl, 2.6 KCl, 2.5 $CaCl_2$, 2.0 $MgCl_2$, 2.0 NaH_2PO_4, 10 glucose, 26.5 $NaHCO_3$, and 0.03 phenol red as a pH indicator. Final pH of both solutions was 7.4 when saturated with 95% O_2-5% CO_2. To mimic anoxia, the oxygenated aCSF was switched to aCSF presaturated with 95% N_2-5% CO_2 during anoxia. No O_2 was detected using a radiometer and an O_2 electrode compared with a control (sodium dithionite solution) for the anoxic solution.

Whole cell conductance (G_w) and time constant (T_c) were calculated using depolarizing current pulses of 500 ms duration (turtle) or 200 ms duration (rat) sufficient to elicit 6.2 ± 0.9 mV change in membrane potential from the resting potential of the cell as previously described by Doll et al. (8). All measures were done on cells in a quiescent state to minimize leakage due to electrical activity. In brief, G_w was calculated from Ohm's law

$$V_m = I/G_w \quad (1)$$

where V_m is the change in membrane potential and I is the current. T_c was calculated by fitting the membrane charging curve to the equation

$$Y = A_0 + A_1 e^{-t/T_c} \quad (2)$$

where Y is the voltage at any given time t, A_0 is the offset, and A_1 is the maximum voltage, using the Clampfit feature of pClamp as described in Ref. 8. Specific membrane conductance (G_m) was calculated from the equation

$$T_c = C_m/G_m \quad (3)$$

where C_m is the capacitance of the membrane per unit area, which is assumed to be 1 $\mu F/cm^2$ for all biological membranes. All computer fits of membrane charging curves showed a 0.9900 least-squares residual R value or they were rejected.

The term Q_{10} is used to refer to the effect of a 10°C temperature change on the values being measured, and for the purpose of this paper, it is defined as

$$Q_{10} = \text{Value}(x + 10°C)/\text{Value}(x°C) \quad (4)$$

where x is the variable being tested.

Turtles were obtained from Lemberger (Oshkosh, WI), chemicals were purchased from Sigma Chemical (St. Louis, MO) and Fisher Scientific (Vancouver, British Columbia), data were analyzed using pClamp software (Axon Instruments, Burlingame, CA), statistics were carried out using Systat (Evanston, IL), and graphics were done using Cricket Graph III (Computer Associates, San Jose, CA).

RESULTS

Data presented are based on recordings of turtle (n = 30) and rat (n = 20) pyramidal neurons resulting in a total of 50 cells being tested for this paper. Each group represents 10 recordings. No neurons were repeatedly used for any group. Examples of raw data traces are illustrated by Fig. 1*A* (turtle, 25°C) and 1*B* (rat, 25°C). The relevant electrophysiological properties of these populations are detailed in Tables 1 and 2. To assess differences in ion leakage between turtle and rat cortical neurons, G_m was calculated for both species at 25°C. Conductance values indicate a 4.2-fold higher G_m in rat compared with turtle neurons. Comparison of conductance at more physiological temperatures (35°C, rat; 15°C, turtle) indicated a 17-fold increase in G_m for rat pyramidal neurons. Although not directly measured, G_m for the rat neurons at physiological temperature (37°C) was inferred from the measured conductance Q_{10} of 1.9 (25–35°C, Table 3). The calculated G_m at 37°C for the rat neuron was 53.58 $\mu S/cm^2$ indicating a 22-fold more conductive membrane for the rat pyramidal neurons compared with turtle pyramidal neurons (15°C). The conductance ratios between turtle and rat pyramidal neurons are summarized in Fig. 2.

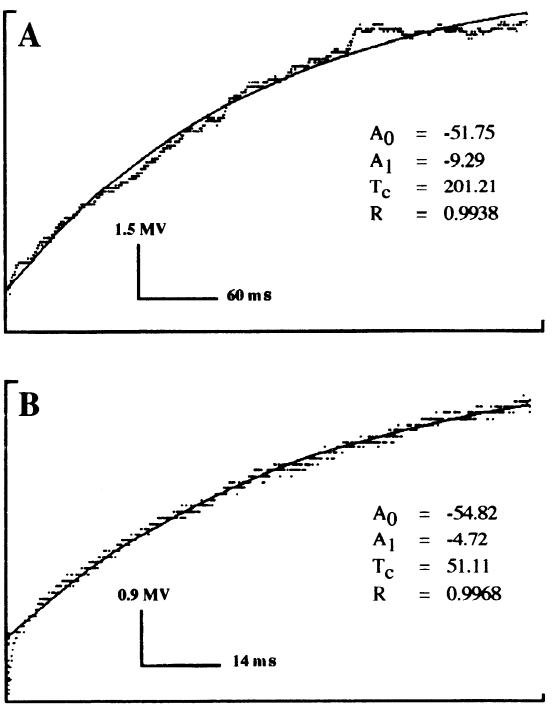

Fig. 1. Examples of membrane charging curves (dotted curves) with a computer-generated fit (solid curved lines). Time constants (T_c) were calculated from the computer-fit line using the Clampfit feature of pClamp. *A*: representative membrane charging curve for the turtle neuron at 25°C. *B*: representative membrane charging curve for the rat neuron at 25°C. For a description of the listed parameters, see *Eq. 2*.

Table 1. *Turtle cortical pyramidal cell values*

Parameter	n	Normoxia, 15°C	n	Normoxia, 25°C	n	Anoxia, 25°C
Membrane potential, mV	10	−75.0±1.0‡	10	−72.8±0.5‡	9	−74.0±2.5‡
G_w, ns	10	1.84±0.21*†	10	2.73±0.36†	10	2.35±0.21†
T_c, ms	10	423.5±28.4*†	10	200.7±19.3†	10	209.3±23.5†
G_m, μs/cm²	10	2.46±0.17*†	10	5.56±0.67†	10	5.37±0.61†

Values are means ± SE. For anoxia measurements, turtle cortical slices were held in an anoxic chamber at ≈22°C for 6-9 h before recording. * Significantly different ($P \leq 0.05$; Newman-Keuls test) from the control (25°C) measure. † Significantly different ($P \leq 0.05$; Newman-Keuls test) from rat (35 and 25°C) cortical values (Table 2). ‡ Significantly different ($P \leq 0.05$; Tukey's honestly significant difference test) from rat (35 and 25°C) cortical values (Table 2).

Table 2. *Rat cortical pyramidal cell values*

Parameters	n	25°C	n	35°C
Membrane potential, mV	8	−57.5±2.9‡	10	−55.4±1.3‡
G_w, ns	10	4.08±0.57†	10	7.56±1.00*†
T_c, ms	10	46.5±4.4†	10	25.0±2.0*†
G_m, μs/cm²	10	23.5±2.4†	10	42.4±3.5†

* Significantly different ($P \leq 0.05$; independent t test) from the 25°C value. † Significantly different ($P \leq 0.05$; Newman-Keuls test) from turtle (15 and 25°C) cortical values (Table 1). ‡ Significantly different ($P \leq 0.05$; Tukey's honestly significant difference test) from turtle (15 and 25°C) cortical values (Table 1).

Table 3. *Effect of temperature on membrane ion leakage*

	Turtle, 15-25°C	Rat, 25-35°C
Measured Q_{10}		
G_m	2.3	1.8
G_w	1.5	1.9
Average	1.9	1.9

Values obtained from Tables 1 and 2.

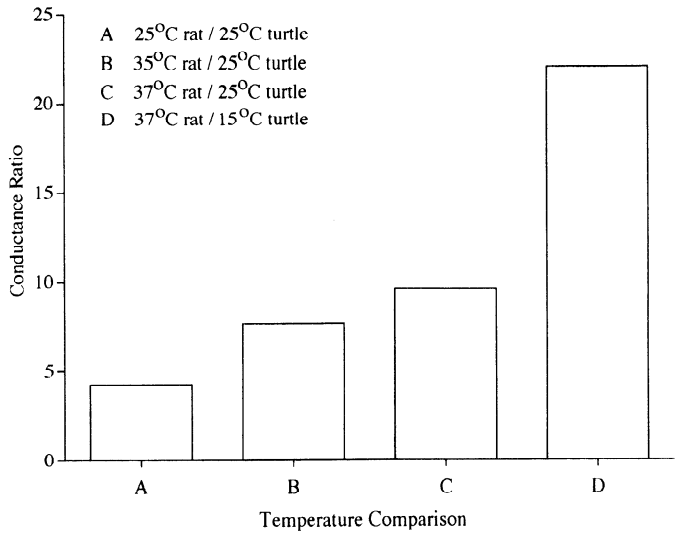

Fig. 2. Conductance ratios (G_m) for rat cortical pyramidal neurons vs. turtle cortical pyramidal neurons. Values are obtained from Tables 1 and 2 except for the rat 37°C value, which is calculated from the rat 35°C assuming a Q_{10} of 1.9 (Table 3).

To assess whether ion leakage further decreases with prolonged anoxia, turtle slices were incubated in anoxic aCSF for 6-9 h. Two separate and independent measures of conductance were done (G_w and G_m) for reasons discussed by Doll et al. (8). The results reported in Table 1 indicate no change in G_w or G_m with 6-9 h of anoxia, supporting and extending earlier results using intracellular recording techniques during short-term anoxia (8, 25). These results suggest no further downregulation of ion channel activity with anoxia.

Current evidence supports that anoxic survival of the turtle is highly temperature dependent (31). One question that remains unanswered is whether turtle neurons express a large Q_{10} value for ion conductance at lower temperatures. Table 3 summarizes the Q_{10} values for conductance in turtle and rat cortical neurons. Surprisingly, an average Q_{10} of 1.9 was measured for both turtle (15-25°C) and rat (25-35°C). These results indicate that Q_{10} for leakage channels may be conserved across various temperature regimes as well as species.

Interestingly, temperature did not significantly change membrane potential in either the rat or turtle cortical cell populations even though conductance of these cells is changed (Table 1 and 2). Additionally, one might suspect a change in membrane potential if ion channels were downregulated during anoxia. However, there were no significant changes in membrane potential with anoxia in turtle cortical neurons, supporting the findings of no change in either G_m or G_w with anoxia.

DISCUSSION

The channel arrest hypothesis is based on the premise that as the conductance of a cell (G_m) increases so must the rate of ion pumping if ion homeostasis and membrane potential are to be maintained. This prediction is experimentally supported (24, 28). The concept can be applied not only to anoxia, but also to normoxia. If one cell is more conductive than another during normoxia, then ion pumping due to this increased conductance may be increased to maintain ion homeostasis (16). Based on G_m, our results support reduced passive ion leakage during normoxia as one mechanism used by the turtle neuron to reduce ATP-dependent ion pump activity (Fig. 2).

It is tempting to estimate the energy (ATP) savings achievable by this lower leakage; however, this is not yet possible. First, the specific passive ion conductance contributing to this savings is not known. Results presented here are slightly higher than results reported for nondiving reptiles using tracer methods (4.2 vs. 3.6) (11). Earlier studies on turtle and rat cortex indicated that rat expressed 2.3 times more activity for Na^+-K^+-ATPase supporting the differences in G_m reported here. Second, the exact energy expenditure due to ion leakage independent

of electrical activity is not known. Studies on canine brains in vivo suggest that ion pumping due to passive ion leakage consumed <20% of resting ATP turnover (1).

The calculations for G_m are based on two assumptions. First, both biological membranes display a 1 $\mu F/cm^2$ capacitance. Careful studies of both ectotherms and endotherms from a variety of cell types yield this value (5), suggesting that C_m may be a biophysical constant (14). Because C_m is a function of the membrane composition, and because membrane structure is fairly conserved, it is reasonable that C_m would also be conserved. Secondly, the assumption of a Q_{10} of 1.9 for the rat brain between 35 and 37°C is made. We have measured the Q_{10} for membrane conductance in the turtle neuron between the temperatures of 15–25°C and for the rat neuron between 25 and 35°C (Table 3), and have extrapolated the latter values to 37°C.

The Q_{10} for both tissues is approximately the same (1.9; Table 3), which is similar to the whole body metabolic Q_{10} for both species for these temperature ranges (13). The conservation of the conductance Q_{10} between animals and temperatures suggests that the Q_{10} for ion channels may be conserved across channel types as well as animals. Single-channel studies from a variety of channel types from both ectotherms and endotherms report Q_{10} values ranging from 1.0 to 2.5 with most values between 1.3 and 1.6, close to the aqueous diffusion Q_{10} of 1.3 (14). However, conservation of the conductance Q_{10} may hold true only for leakage channels. The Q_{10} for this study is an average between G_m and G_w. The average is used because these measures, although calculated independently of each other, should change both qualitatively and quantitatively in parallel with temperature. This parallelism appears to be true for the rat cortical neuron but quantitatively deviates for the turtle neuron. Several reasons could possibly explain this deviation since these two measures are calculated independently of each other. Changes in capacitance, cell size, soma to dendritic conductance, and bleb formation (23) may all influence G_w differently from G_m with respect to Q_{10}.

In addition to large inherent leakage differences between rat and turtle membranes, the channel arrest hypothesis predicts a decrease in G_w and G_m with anoxia. Previously, using microelectrode intracellular recording procedures in turtle cortical slices, we reported that membrane resistance (whole cell and specific membrane) of the turtle pyramidal neuron does not increase in response to short-term anoxia (≤180 min) (8). However, data from Chih et al. (2) suggests that K^+ leakage in vivo is suppressed by anoxia (6). Recent cortical slice data indicates that $[Ca]_i$ accumulation during long-term anoxia (180 min) is slower compared with short-term anoxia (5 min) after the addition of iodoacetate (glycolytic inhibitor). However, specific leakage processes were not measured in either of these studies, and thus the lowered leakage observed could be caused from a reduction in any leakage process including electrical activity. Because of the above observations as well as limitations of the microelectrode technique, we measured whole cell conductance using patch clamping techniques. The advancement of whole cell patch clamp techniques in slices provides a way of easily comparing populations of cells while ensuring that membrane electrode seals are not significantly contributing to the apparent cell conductance due to the GΩ seal formation between electrode and cell membrane. The values obtained for T_c and G_w are greater than results reported previously using microelectrode intracellular recording techniques for the turtle pyramidal neurons (7–9). Thus ion leakage around the microelectrode may significantly contribute to the values of T_c and G_w, masking possible conductance changes. However, results reported here for long-term anoxia (6–9 h) support earlier observations using microelectrode techniques and short-term anoxia (≤180 min) in that no downregulation of leakage channels is measurable with anoxia in the turtle brain.

Both turtle and rat pyramidal neurons have inwardly rectifying K^+ channels (7, 21). Thus one method for changing cell conductance is to change membrane potential. As a result, channel arrest could be achieved by simply depolarizing the cell. However, no noticeable change in membrane potential occurred during anoxia in turtle neurons (Table 1), supporting earlier results with intracellular recording and short-term anoxia (8).

The energy conservation mechanism predicted by the channel arrest hypothesis is not directly due to ion channels closing but rather to a decrease in ATP-dependent ion pump activity resulting from downregulation of ion channels. We have tested one leakage process (leak channels). However, ion leakage can result from a variety of other processes. Current evidence supports both a reduction in ion leakage with anoxia in the turtle brain (2, 6) and electrical activity (12, 29). Our results support that the turtle brain may be spending less energy on maintaining ion homeostasis in the normoxic state compared with the rat but that further downregulation with anoxia is not measurable. Accumulating evidence supports downregulation of electrical activity as a more likely scenario for metabolic depression in the turtle brain.

We thank Andy Laycock for technical assistance.

This work was funded by operating grants from the Natural Sciences and Engineering Research council of Canada and the British Columbia Health Care Research Foundation. C. J. Doll is the recipient of a Medical Research Council Studentship, and P. B. Reiner is a Scholar of the Medical Research Council.

Address for reprint requests: C. J. Doll, Dept. of Zoology, Univ. of British Columbia, 6270 University Blvd., Vancouver, British Columbia V6T 1Z4 Canada.

Received 10 February 1993; accepted in final form 7 May 1993.

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