Short Communication


Activation Energy in the Mammalian Brain Slice as Determined by Oxygen Micro-electrode Measurements

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Summary The activation energies were calculated from oxygen consumption rates obtained from oxygen partial pressure ($P_0$) profiles in olfactory cortical slices of guinea pig in vitro, assuming that the Krogh coefficient varied linearly over the range of investigated temperatures (19–39°C).

The experiment reported in the present paper is designed to determine the activation energy ($E_a$) of the chemical reaction for oxygen in a brain slice (ca. 500 μm) from $P_0$ profiles measured with a oxygen micro-electrode (Whalen et al., 1967). The techniques of brain slice preparation, obtained from guinea pig olfactory cortex, and the method of maintenance were the same as those described in previous reports (Fujii, 1977; Fujii et al., 1970). In brief, the brain slice was incubated at constant temperature by the “gas-blow and medium-flow method” which allowed continuous stable recordings of electrical activity during the perfusion of the medium (Krebs-Ringer's solution). The temperature of the brain slice could be controlled within the range from 19 to 39°C. The oxygen consumption of the brain slice at any given temperature was determined from the $P_0$ profile measured with the oxygen micro-electrode. The $P_0$ profile was obtained by lowering the electrode in 50μm steps perpendicular to the tissue surface with a hydraulic micro drive (David Kopf Instruments). The tissue surface was located as precisely as possible by viewing the electrode tip through a microscope (40×) and the penetration was observed as the electrode was inserted into the slice. Values deeper than 400 μm were excluded from analysis since the electrode was not penetrating smoothly at greater depths. Most $P_0$ profiles were obtained by inward penetrations from the tissue surface. Since the oxygen micro-electrode is temperature-sensitive, no values

Received for publication September 5, 1980

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were obtained during temperature changes. \( P_{O_2} \) profiles were measured only during periods of constant temperature from areas with constant oxygen supply at the surface. The electrode was calibrated in the flowing solution above the slice, which was equilibrated with a known concentration of oxygen. The \( P_{O_2} \) profile was obtained only after the current signal was confirmed to be stable for at least 1 min at each location in the tissue. The time response of the oxygen micro-electrode is typically less than 100 msec (Whalen et al., 1973). A zero \( P_{O_2} \) calibration was obtained by penetrating a thick piece of cortex in the calibration well. An additional check on zero values was obtained in the preparation during brief anoxic exposures. One disadvantage of the experimental set-up was the necessity to open the polarizing circuit in order to move the electrode into the calibrating well. However, repolarization was usually fairly rapid and the electrode stabilized within a few minutes. Electrode poisoning with prolonged use at high \( P_{O_2} \) values was common, requiring frequent calibrations. A more detailed discussion of the oxygen micro-electrode and calibration procedures has been published (Whalen et al., 1973).

The tissue slice is assumed to be homogeneous and isothermal, consuming oxygen at a rate, \( Q \). The chemical reaction is assumed to be a zero-order reaction, independent of \( P_{O_2} \) to 0 mm Hg in order to simplify the equations describing

![Fig. 1](image)

**Fig. 1.** \( P_{O_2} \) profiles for Case A (no anoxic area) and B (anoxic area) in the slices. Experimental data are shown by open circles. In both cases, \( a=6.5 \times 10^{-3} \) mm Hg/\( \mu \)m².
the mass transport of oxygen through the tissue. No attempt was made to define a “critical \( P_{O_2} \)” (Jonsis, 1964), although at low \( P_{O_2} \) the experimental data did not appear to be adequately described by a zero-order chemical reaction rate. More complicated models for oxygen consumption have been described for the profile at low \( P_{O_2} \) (Buerk and Saeidel, 1978). Two types of \( P_{O_2} \) profiles are possible as shown in Fig. 1, depending on oxygen consumption, the Krogh coefficient, surface \( P_{O_2} \) values and the tissue thickness (usually less than 500 \( \mu m \)). The steady-state mass transport equation (Fick’s second law) for oxygen is

\[
\frac{d^2P_{O_2}}{dx^2} = \frac{Q}{DS},
\]

for a flat sheet of tissue, where \( Q = \) oxygen consumption rate, \( D = \) oxygen diffusion coefficient and \( S = \) oxygen solubility coefficient. Equation 1 has a general solution \( (P_{O_2} > 0) \) given by the parabolic equation

\[
P_{O_2} = ax^2 + bx + c,
\]

where \( a = Q/2DS \) and \( DS \) (diffusion coefficient multiplied by the solubility coefficient) is the Krogh coefficient. The constants \( b \) and \( c \) can be found from the boundary conditions, which can be solved for two cases. When there are no anoxic areas in the slice (Case A), the boundary conditions are \( P_{O_2} = P_o \) at \( x = 0 \) and \( P_{O_2} = P_L \) at \( x = L \). The \( P_{O_2} \) profile is given by

\[
P_{O_2} = ax^2 - \left( aL + \frac{P_o - P_L}{L} \right)x + P_o,
\]

and is shown as the solid curve through the experimental \( P_{O_2} \) profile (circles) in Fig. 1A. When there is an anoxic center in the slice (Case B), the boundary conditions are \( P_{O_2} = P_o \) at \( x = 0 \) and \( P_{O_2} = 0 \) at the critical depth \( x = X_{cr} \). The condition that the oxygen flux \( dP_{O_2}/dx = 0 \) at \( x = X_{cr} \) has been used to obtain the \( P_{O_2} \) profile given by

\[
P_{O_2} = ax^2 - 2aX_{cr}x + P_o,
\]

which is shown as the solid curve through the experimental \( P_{O_2} \) profile (circles) in Fig. 1B. The critical depth is given by

\[
X_{cr} = \sqrt{2DSP_0/Q},
\]

and is marked by the arrow in Fig. 1B. Either Eq. 3 or 4 was fitted to the experimental \( P_{O_2} \) profiles using a nonlinear regression analysis. No \( P_{O_2} \) values below 5 mm Hg were used. The average value for the ratio \( Q/DS \) from six animals was \((1.99 \pm 0.18) \times 10^{-4} \) mm Hg/\( \mu m^2 \) \((T = 36.5^\circ C)\). Results from a similar study of cat cerebral cortex at 37°C produced a higher value \((5.3 \pm 0.6) \times 10^{-4} \) mm Hg/\( \mu m^2 \) (Ganfield et al., 1970). Using the value for the Krogh coefficient \( DS = 1.29 \times 10^{-5} \) ml O\(_2\)/cm·min·atm calculated by Ganfield et al. (1970), the oxygen consumption per cubic centimeter of tissue from this study was \( Q = (3.38 \pm 0.31) \times 10^{-2} \) ml O\(_2\)/min at \( T = 36.5^\circ C \).
If we make the approximation that the Krogh coefficient $DS$ varies linearly with temperature, then $(DS)_2/(DS)_1 = T_2/T_1$ for any two temperatures. We may assume that the oxygen consumption rate $Q$ is related to temperature by the Arrhenius equation

$$Q = Q_0 e^{-E_a/RT}$$

where $E_a$ is the energy of activation for the chemical reaction. One can show that the ratios $a_1 = Q_1/(2(DS)_1)$ and $a_2 = Q_2/(2(DS)_2)$ obtained from fitting the $P_{O_2}$ profiles at temperatures $T_1$ and $T_2$ may be expressed as

$$\frac{a_2T_2}{a_1T_1} = \frac{Q_2}{Q_1}$$

Using the Arrhenius equation, we obtained the following linear transformation by taking the natural logarithm

$$\ln \frac{Q_2}{Q_1} = \frac{E_a(T_2-T_1)}{RT_1T_2}$$

The energy of activation $E_a$ can be determined from Eq. 8, without determining values for the Krogh coefficients. Some error is introduced by the assumption that the Krogh coefficient varies linearly with temperature, but this error is small over the temperature range investigated (19 to 39°C). Activation energies calculated from four experiments are summarized in Table 1. Among them, the best fit of the theoretical relationship was obtained with data taken over the greatest temperature range from 19 to 38°C, although this value for $E_a$ was the lowest one we found. We do not have sufficient data as yet to explain the wide range in values for $E_a$, although the higher values may be more characteristic of normal tissue and the lower values could reflect cellular damage due to anoxic exposure during the surgical procedure to remove the tissue slice.

The numerical values of the activation energies calculated from these experiments are based on oxygen consumption rates at different temperatures determined from $P_{O_2}$ profile measurements in slice of guinea pig olfactory cortex in vitro. Oxygen molecules which diffuse into the slice from the perfusing medium around it must pass through many barriers such as the cytoplasmic membrane of neurons, oxygen-carrying systems in the cytoplasm, the membrane of mitochondria and other membranes before reaching the reaction site of oxidative phosphorylation in

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>Temperature range (°C)</th>
<th>$E_a \pm$ S.E. (kcal/mol)</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>316</td>
<td>19–38</td>
<td>$13.0 \pm 0.7$</td>
<td>0.97</td>
</tr>
<tr>
<td>321</td>
<td>30–36.5</td>
<td>$23.8 \pm 4.3$</td>
<td>0.64</td>
</tr>
<tr>
<td>323</td>
<td>33–39</td>
<td>$36.0 \pm 5.3$</td>
<td>0.91</td>
</tr>
<tr>
<td>326</td>
<td>30–36.5</td>
<td>$28.5 \pm 5.1$</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*Table 1. Linear regression analysis of transformed oxygen consumption data.*
the mitochondria or other subcellular organelles. Of course, the distribution of $P_{O_2}$ in vivo is quite different, depending on the blood flow patterns in the microcirculation, but the minimum $P_{O_2}$ values in tissue will depend on the basic properties $Q$, $D$, and $S$ and the blood $P_{O_2}$ values in the capillaries. More experiments are needed to clarify the temperature dependence of the oxygen consumption rate and its relationship to electrical activities in mammalian brain tissues. In particular, the time-dependent behaviour of the oxygen consumption rate in individual experiments and possible irreversible effects due to anoxic exposure should be examined in greater detail. However, this report has demonstrated that the activation energy for the chemical reaction for oxygen in brain tissue can be obtained by analyzing $P_{O_2}$ profiles measured with oxygen micro-electrodes using the “gas-blow and medium-flow method” at different temperatures.

This research was performed by the support of the Ministry of Education, Science and Culture of Japan and the United States Public Health Service (Grant Nos. HL 12703 and HL 11906).

REFERENCES


