whereby the fishing industry could be provided with the locations of stationary or transient upwelling areas based upon examination of satellite photographic data.

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24 June 1969

Cyclic Guanosine Monophosphate: Effects on Short-Circuit Current and Water Permeability

Abstract. *In the isolated toad bladder, cyclic guanosine monophosphate induces an increase in short-circuit current similar to that produced by cyclic adenosine monophosphate. In contrast, cyclic guanosine monophosphate has no effect on water permeability in this organ. This finding raises the possibility that different and independent intracellular secondary messengers may exist.*

3′5′-Adenosine monophosphate (cyclic AMP) has been well established as an intracellular messenger mediating a variety of hormonal effects (*1*). In both the toad bladder (*2*) and the frog skin (*3*), cyclic AMP appears to mediate the increases in sodium transport and water permeability which occur in the presence of vasopressin. The observations that cyclic AMP mimics the hydroosmotic effect of vasopressin in isolated renal tubules (*4*) and that cyclic AMP is present in urine support the thesis that the physiological effects of vasopressin in the kidney are also mediated by cyclic AMP. Urine contains 3′5′-guanosine monophosphate (cyclic GMP) as well as cyclic AMP (*5*). To explore the significance of this latter finding, we have studied the effect of cyclic GMP and other cyclic nucleotides on sodium transport and water permeability in the isolated toad bladder.

Urinary bladders were excised from large toads (*Bufo marinus*) obtained from the Pet Farm, Miami, Florida, during February and March. Short-circuit current, which represents net sodium flux in this organ, was measured (*6*) with a 2-cm^2 segment of bladder between two halves of a Lucite chamber. Osmotic water flow was measured by the method of Bentley (*7*). Unless otherwise specified, the Ringer solution (final *p*H 7.4) contained (mmole/liter): sodium, 111; potassium, 2.5; calcium, 1.4; magnesium, 2.0; chloride, 119; phosphate, 1.0; and glucose, 10. Substances tested were dissolved in Ringer solution and added to the solution bathing the serosal surface of the bladder (*8*). A final nucleotide concentration of 2 mmole/liter was used.

The effects on the short-circuit current of equimolar concentrations of cyclic AMP and cyclic GMP, the short-guanosine monophosphate), cyclic CMP (3′5′-cytidine monophosphate), and cyclic TMP (3′5′-thymidine monophosphate) are shown in Table 1. With both cyclic AMP and cyclic GMP, the short-circuit current increased after a lag of 3 to 5 minutes, reached a peak value within 15 minutes, and then generally declined gradually toward base-line values (Fig. 1). With both nucleotides the transmembrane potential increased much less than the short-circuit current, implying that there was a fall in electrical resistance. In marked contrast, 5′-GMP (a noncyclic derivative of cyclic GMP), cyclic CMP, and cyclic TMP did not increase the current.

The effects of cyclic AMP and cyclic GMP on osmotic water flow across isolated toad bladder sacs were also determined (Table 2). Addition of cyclic AMP to the serosal bathing solution gave the expected increase in net water loss from the hypotonic mucosal solution. The response was greater at a concentration of 5 m*M* than at 2 m*M* cyclic AMP. The same concentrations of cyclic GMP had no effect on net water flow. Cyclic TMP was also ineffective.

Thus cyclic GMP reproduced the well-documented effects of cyclic AMP on short-circuit current but not on water permeability. The effects of vasopressin on osmotic water flow and short-circuit current have been separated previously. Certain analogs of vaso-

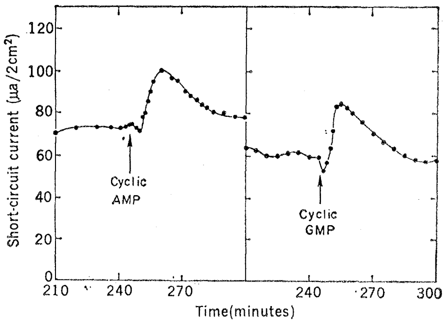

Fig. 1. Effect of 2 m*M* cyclic AMP and 2 m*M* cyclic GMP on short-circuit current across the isolated urinary bladder of the toad.

Table 1. Effects of cyclic nucleotides and 5′ GMP on short-circuit current across isolated toad bladder membranes. Results are expressed as the mean ± S.E.M. of the percentage increase (+) or decrease (−) in short-circuit current, at the peak of the response. Each nucleotide was present at a final concentration of 2 mmole/liter. Half of the experiments were performed in a modified Ringer solution with a final *p*H of 8.0 obtained by substituting 1.0 m*M* sodium phosphate for 2.5 m*M* tris(hydroxymethyl)aminomethane-hydrochloride. The mean ± S.E.M. base-line short-circuit current for the 34 experiments was 61 ± 4.1 μa/2 cm^2.

Nucleotide	Experiments (No.)	Short-circuit current (percent change)
Cyclic AMP	10	+ 33 ± 5.9
Cyclic GMP	11	+28 ± 4.2*
5′-GMP	5	+ 4 ± 0.7
Cyclic CMP	4	− 2 ± 1.7
Cyclic TMP	4	−13 ± 1.1

* Significantly different from 0 ($P < .005$) but not different from the change produced by cyclic AMP ($P > .4$).

Table 2. Effects of cyclic AMP and cyclic GMP on net water flow across isolated toad bladder sacs. Each hemibladder, with the mucosa inside, was filled with 2.5 ml of hypotonic Ringer solution (22 m*M* sodium chloride) and immersed in 20 ml of regular Ringer solution (110 m*M* sodium chloride). After the weight loss during three control periods of 15 minutes was measured, the sacs were transferred in 20 ml of the same Ringer solution in which cyclic nucleotides had been added. After equilibration for 15 minutes, the weight loss by the bladder sac was measured at 15-minute intervals during the next 45 minutes. Values in the table are the means of each set of three periods.

Experiment	Nucleotide concentration (m*M*)	Weight loss (mg/min)	
		Cyclic AMP	Cyclic GMP
Experiment 1			
Before nucleotide		0.9	1.1
After nucleotide	2	6.4	1.3
Experiment 2			
Before nucleotide		1.1	1.2
After nucleotide	5	9.5	1.1

pressin increase permeability to water but do not change sodium transport (*9*). If the secondary messenger concept is correct, the dissociation with vasopressin analogs of effects on short-circuit current and water permeability implies independent and different secondary messengers. Calcium ions, on the other hand, appear to diminish the increase in water permeability induced by vasopressin but not the increase in short-circuit current (*10*). The different sensitivity of the toad bladder to cyclic AMP and cyclic GMP as manifested by the water flow experiments raises the possibility that vasopressin may give rise to its separate effects by generating these two cyclic nucleotides.

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Silicon Oxynitride Stability

Abstract. *Silicon oxynitride occurs as a mineral (sinoite) in meteorites. Equilibrium measurements are reported which provide information on the approximate ratios of pressures of oxygen to nitrogen required for existence of this phase under equilibrium conditions at elevated temperatures. This ratio is approximately* 10^{-15} *at 1400° to 1500°C.*

Silicon oxynitride was first reported about 10 years ago by Forgeng and Decker (*1*) who obtained this phase when air was admitted to the reaction chamber during a study of the formation of silicon nitride. Subsequently, silicon oxynitride has been studied as a possible refractory material at elevated temperatures (*2*) and as a film on silicon substrates (*3*). The main impetus for the interest in silicon oxynitride in recent years, however, has come from the discovery of this compound as a mineral (sinoite) in the Jah deh Kot Lalu meteorite (*4*) and subsequently in four additional meteorites of the enstatite chondrite type (*5*). The appearance of this mineral in these meteorites, which are reported to bear textural characteristics suggesting equilibrium assemblages (*5*), provides a possible clue to the conditions of the environment in which these materials were formed. We report here data which serve to establish the order of magnitude of such equilibrium relations.

The approximate stability of silicon oxynitride, Si_2N_2O, was determined by two methods. The first method involved the reaction of SiO_2 with a gas phase of known oxygen and nitrogen pressures in an open system according to the reaction

$$2SiO_{2(s)} + N_{2(g)} = Si_2N_2O_{(s)} + 3/2\ O_{2(g)} \qquad (1)$$

For this reaction, the change in standard free energy ($\Delta G°$) is expressed as

$$\Delta G°_{(\mathrm{Eq.\ 1})} = \Delta G°_{Si_2N_2O} - 2\Delta G°_{SiO_2} = -2.303\ RT \log_{10} \frac{P_{O_2}{}^{3/2}{}_{\mathrm{equil}}}{P_{N_2\,\mathrm{equil}}} \qquad (1a)$$

This equilibrium was studied experimentally by passing gas mixtures of H_2O, H_2, and N_2 over silica at 1400° and 1500°C and determining, by x-ray diffraction, the presence or absence of Si_2N_2O in the reaction product. The partial pressure of nitrogen in the gas mixture was kept constant at approximately 0.5 atm, and the desired partial pressures of water were obtained by passing hydrogen gas through a saturator filled with a saturated sodium chloride solution held at selected temperatures in the range of −15° to −7°C. The oxygen pressure of the gas phase can then be calculated from the known equilibrium constant for the reaction

$$2H_2O_{(g)} = 2H_{2(g)} + O_{2(g)} \qquad (2)$$

As the temperature of the sodium chloride solution was reduced, thereby decreasing the H_2O pressure and hence the O_2 pressure, a point was reached where Si_2N_2O appears as a phase. The temperature of the solution was then increased by a few degrees to increase slightly the H_2O, and thereby the O_2, pressure. This causes the Si_2N_2O phase to disappear, and establishes the reversibility of the reaction. Initial attempts to study the reaction between the H_2O–H_2–N_2 gas mixture and crystalline SiO_2 at 1400° and 1500°C were unsuccessful, because of the sluggishness of the reaction. In later, successful experiments, a more reactive SiO_2 of unit activity was obtained by using a silica-saturated CaO–SiO_2 liquid for the equilibration experiments at 1500°C, and a silica-saturated CaO–MgO–SiO_2 liquid for the equilibration experiments at 1400°C. The silicate liquids were contained in silica crucibles in a vertical Pt-wound furnace. Temperatures were measured with a Pt versus 90 percent Pt 10 percent Rh thermocouple, and are judged to be accurate to ± 5°C (Table 1).

In an open system, only relatively small amounts of Si_2N_2O were formed. It is suspected that this may be related to loss of silicon monoxide (SiO) at the relatively high temperatures and low oxygen pressures of the experiments. To minimize or circumvent these difficulties, and to check the data obtained in the open system, a second experimental method was developed which involved the study of an alloy-oxide plus oxynitride-gas equilibrium in a closed system. This method provided silicon, oxygen, and nitrogen in highly reactive forms in a gas phase without appreciable loss of materials by volatilization, and still provided means of controlling both the nitrogen and oxygen pressures of the system. Briefly, the method was as follows. Within a silica

Table 1. Data for the equilibrium of Eq. 1.

Parameter	Values	
	1400°C	1500°C
P_{N_2}(atm)	0.5	0.5
P_{O_2}(calculated, atm)	$10^{-15.8}$	$10^{-14.8}$
$\frac{P_{O_2}}{P_{N_2}}$	$10^{-15.5}$	$10^{-14.5}$
$\Delta G°_{(\mathrm{Eq.\ 1})}$(kcal)	179.5	177.6
$\Delta G°_{Si_2N_2O}$(kcal)	−107.5	−102.4