Biochimica et Biophysica Acta, 394 (1975) 438–448

BBA 76991

A COMPARISON OF THE EFFECTS OF OUABAIN AND 2-DEOXY-D-GLUCOSE ON THE THERMODYNAMIC VARIABLES OF THE FROG SKIN

ALBERT OWEN, S. ROY CAPLAN and ALVIN ESSIG

Biophysical Laboratory, Harvard Medical School, Cambridge, Mass. (U.S.A.), Laboratory of Membranes and Bioregulation, Weizmann Institute of Science, Rehovot (Israel) and the Department of Physiology, Boston University School of Medicine, Boston, Mass. (U.S.A.)

(Received December 31st, 1974)

SUMMARY

Previous studies support the validity of a linear thermodynamic formalism relating the rates of active Na^+ transport and oxygen consumption J_r to the electrical potential difference $\Delta\Psi$ and the affinity A (negative free energy) of the metabolic driving reaction. The formulation was further tested in paired control and experimental hemiskins by the use of two inhibitors of Na^+ transport. Ouabain, a specific inhibitor of the Na^+ pump, might be expected to diminish the dependence of J_r on $\Delta\Psi$ without affecting A, whereas 2-deoxy-D-glucose, a competitive inhibitor of glucose metabolism, should be expected to diminish A. Both inhibitors were used at concentrations adequate to depress Na^+ transport (i.e. short-circuit current I_0) to some 50% of control level. Measurements were made of I_0 and $dJ_r/d(\Delta\Psi)$, and the apparent value of the affinity A_{app} was calculated according to the thermodynamic formulation. Ouabain depressed $dJ_r/d(\Delta\Psi)$ without affecting A_{app} whereas 2-deoxy-D-glucose depressed A_{app} without affecting $dJ_r/d(\Delta\Psi)$. The demonstration of these effects indicates the utility of the formalism.

INTRODUCTION

The energetics of active Na^+ transport have been analysed in some detail by means of a formalism based on non-equilibrium thermodynamics [1, 2]. In recent years, experimental results have accumulated, supporting the validity of this formulation [3–7] and suggesting the possibility of determining the affinity (in essence the negative Gibbs free energy) of the metabolic reaction driving active Na^+ transport. In principle, the affinity is measurable from determinations of the chemical potentials of the various species taking part in the reaction. In practice, however, such measurements in biological systems are fraught with experimental difficulties and ambiguities.

The non-equilibrium thermodynamic treatment predicts that under appropriate conditions the affinity should be calculable, in a formal way, from macro-

scopic physiological measurements. Previous publications have reported such calculations [5, 6], the resulting estimate of the affinity being tentatively designated the "apparent affinity" [6]. It has not proved feasible as yet to compare the calculated apparent affinity with any values obtained from biochemical determinations because of the problems alluded to above. It is possible, however, to look for correlations and consistencies between the effects of certain agents on the thermodynamic variables and the supposed biochemical effects of these agents. While it is difficult to establish a quantitative agreement between the apparent affinity and the true affinity, the question of whether or not the apparent affinity behaves like an energetic variable can be investigated.

The present study reports the results of a comparison of the effects on the thermodynamic parameters of two inhibitors of active Na^+ transport which have very different biochemical mechanisms of action. Ouabain is a specific inhibitor of the sodium pump [8], while 2-deoxy-D-glucose is an inhibitor of carbohydrate metabolism and ATP production [9]. As will be shown, these two agents exhibit quite different effects on the thermodynamic variables.

MATERIALS AND METHODS

Frogs *(Rana pipiens)* were obtained from Carolina Biological Supply Co., Burlington, North Carolina ("X-jumbo" variety, 10–12 cm in length). The animals were kept at room temperature without feeding for a period not longer than 2 weeks. Tap water, in which the frogs could immerse themselves, was available in the holding tank.

Prior to experiments the animals were doubly pithed and then cleansed by gentle rubbing under tap water. The abdominal skin was cut along the midline and resected, providing two paired hemiskins. The hemiskins were washed twice with 30 ml of sterile Ringer's solution before mounting in modified Ussing-Zerahn lucite chambers of 7.1 cm^2 cross-sectional area for short-circuit current and oxygen consumption measurements as described previously [4–6]. The Ringer's solution had the following composition; 110 mM NaCl, 2.4 mM $KHCO_3$, 1.0 mM $CaCl_2$ (212 mosM, pH 8.2). Stock concentrated glucose and 2-deoxy-D-glucose solutions were isosmotic with the sodium Ringer's solution. The concentration of glucose and 2-deoxy-D-glucose in the chambers was brought to the values designated in the text by the addition of the appropriate volume of these stock solutions. All solutions were sterilized by filtration through sterile, disposable membrane filtering units with a 0.2 μm pore size (Falcon Plastics, Oxnard, Calif.). All glassware and dissection equipment was scrubbed with absolute ethanol and kept in a dry oven at 110 °C overnight to ensure cleanliness. The lucite chamber and pump system was cleansed by scrubbing with absolute ethanol and air dried. All solutions contained 80 μg/ml of gentamycin sulfate (Schering) to control bacterial growth.

Measurements of the dependence of rate of oxygen consumption on the potential difference $dJ_r/d(\Delta\Psi)$

The methods of measuring $dJ_r/d(\Delta\Psi)$ have been described previously [5, 6]. Minor differences between those procedures and the ones used here will be mentioned. First, the skins were subjected to preconditioning potential perturbations

of −80 mV and +80 mV for 6 min at each potential. Only those skins in which short-circuit current returned to within 10% of its initial value were studied further. Second, the sequence of potential perturbations used for determining $dJ_r/d(\Delta\Psi)$ was different, being −80, +80, −40, −40, −80, −80, −40, −40, 0 and 0 mV. This range is smaller than the range explored by Vieira et al. [5] and larger than that used by Saito et al. [6].

Experimental protocols

(1) Studies with ouabain. Measurements of short-circuit current I_0, rate of O_2 consumption J_r, and $dJ_r/d(\Delta\Psi)$ were made in the control and experimental hemiskins during a control period prior to the addition of ouabain, and again commencing 2.5 h after the addition of ouabain to the inside solution of the experimental hemiskin. This period was necessary to ensure that I_0 in the experimental hemiskin had reached a steady value after ouabain addition. The final concentration of ouabain was 10^{-7} M (see Results). The glucose concentration in the inside solution of both the control and experimental hemiskins was 1 mM.

*(2) Studies with 2-deoxy-*D*-glucose.* The protocol of the 2-deoxy-D-glucose experiments was essentially analogous to that of the ouabain experiments, except that the time between the first and second set of measurements was 1.0 h and the final concentration of 2-deoxy-D-glucose in the inside solution was 16 mM.

Analysis of the data

The thermodynamic equations appropriate for the experimental conditions used in the present study will be briefly reviewed. For identical solutions at each membrane surface the equations are [2]:

$$J_{Na} = L_{Na}(-F\Delta\Psi) + L_{Na,r} \cdot A \tag{1}$$

$$J_r = L_{Na,r}(-F\Delta\Psi) + L_r \cdot A, \tag{2}$$

where J_{Na} and J_r are the rates of net sodium flow and metabolism (here O_2 consumption), respectively. The L values are phenomenological coefficients, F is the Faraday constant, $\Delta\Psi$ is the electrical potential difference across the membrane ($\Psi_{inside} - \Psi_{outside}$), and A is the affinity of the metabolic driving reaction. Previous demonstrations of a linear relationship between J_r and $\Delta\Psi$ in the frog skin [5, 6] suggest that the values of L_r and A are constant during brief perturbations of $\Delta\Psi$. The value of A can then be estimated by:

$$A_{app} = -I_0/\{dJ_r/d(\Delta\Psi)\}, \tag{3}$$

where $I_0 = F(J_{Na})_{\Delta\Psi=0}$. The value of A calculated from Eqn 3 has been designated as the apparent affinity.

The data from paired hemiskins taken from the same animal were normalized according to the following formula:

$$R(x) = [(x)_t/(x)_{t=0}]_e/[(x)_t/(x)_{t=0}]_c, \tag{4}$$

where x stands for one of the measured variables (I_o, J_{ro}, $dJ_r/d(\Delta\Psi)$, A_{app}), e means the experimental hemiskin, c the control hemiskin, and $t = 0$ indicates the time prior to treatment with either ouabain or 2-deoxy-D-glucose. Thus the control hemiskins serve as controls correcting for spontaneous variations with time.

The significance of observations was evaluated by standard statistical means [10]. The results are expressed as the mean value±the standard error of the mean. Values of $P > 0.05$ were considered not significant (n.s.) and are not stated.

RESULTS

(1) Effects of ouabain

Previous studies have shown that high concentrations of ouabain (approx. 10^{-3} M) completely abolish both short-circuit current and the response of oxygen consumption to perturbation of $\Delta\Psi$ [5, 6]. Under such circumstances, the value of the affinity calculated according to Eqn 3 is indeterminate. In order to determine the effect of ouabain on A_{app} it is necessary to employ concentrations of ouabain which produce submaximal effects. We observed (Fig. 1) that 10^{-7} M ouabain in the inside solution of the frog skin produced after 2.5 h a quasi-steady state in which the normalized short-circuit current I_0 was approx. 50% of the initial value. We therefore chose this concentration for our study of nine skins.

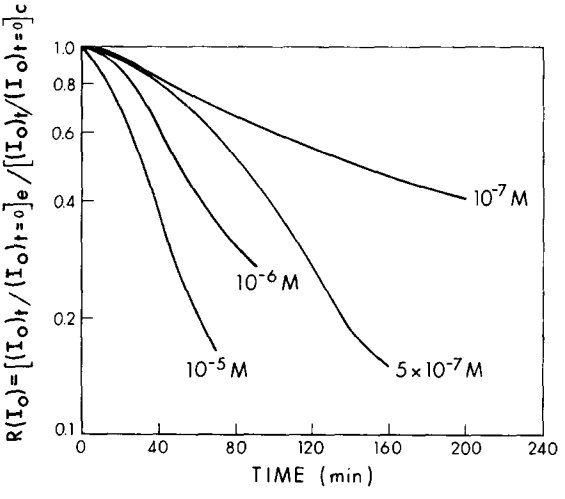

Fig. 1. Effect of various concentrations of ouabain on the short-circuit current I_0. At time $t = 0$, ouabain was added in the indicated concentrations to the solution bathing the inner surface of the experimental hemiskin. $I_0(t)$ was normalized as described in Materials and Methods. Measurements were made at 2-min intervals. Average values from measurements on three skins were used in the curve for 10^{-7} M ouabain. Average values from two skins were used for each of the other concentrations.

Table I shows the effects of 10^{-7} M ouabain on the thermodynamic variables. Each experiment was carried out on paired hemiskins c and e derived from the same animal. Group c were used as control tissues, group e as experimental tissues. Following the completion of initial measurements the inside surfaces of the group e hemiskins were exposed to 10^{-7} M ouabain. All inside solutions (both group c and group e) contained 1 mM glucose. Measurements in paired tissues were made between 1.5 and 0 h prior to, and 2.5 to 4 h after the administration of ouabain and are indicated by $t=0$ and 2.5 h, respectively.

TABLE I

EFFECT OF 10^{-7} M OUABAIN ON THE THERMODYNAMIC VARIABLES

	Time (h)	I_o ($\mu A \cdot cm^{-2}$)		J_{ro} (pmol $\cdot s^{-1} \cdot cm^{-2}$)		$dJ_r/d(\Delta\Psi)$ (pmol $\cdot s^{-1} \cdot cm^{-2} \cdot mV^{-1}$)		A_{app} (kcal $\cdot mol^{-1}$)	
		0	2.5	0	2.5	0	2.5	0	2.5
c	Mean	26.2	17.5	65.8	56.2	0.252	0.216	29.5	18.3
	S.E.	3.6	3.0	2.9	5.6	0.017	0.014	4.4	2.0
e	Mean	33.4	8.0	73.8	53.9	0.260	0.129	30.6	16.1
	S.E.	4.3	1.9	9.1	2.6	0.019	0.011	3.6	1.5

(a) Short-circuit current I_o. Columns 3 and 4 of Table I show the mean effect on I_0. Initial values of I_0 in paired hemiskins differed insignificantly. I_0 decreased significantly between the first and second periods of measurement in both sets of tissues (P (c_I, c_{II}) < 0.01, $P(e_I, e_{II}) < 0.001$), but to a greater extent in the treated than in the untreated control tissues ($P(c_{II}, e_{II}) < 0.01$). The difference of behavior of the control and experimental tissues was highly significant according to the paired t-test ($P(\Delta c, \Delta e) < 0.001$).

(b) O_2 *consumption at short-circuit* J_{ro}. Columns 5 and 6 show the effect on the mean value of J_{ro}. The initial values in paired hemiskins differed insignificantly. J_{ro} declined with time both in the absence and presence of ouabain, but the difference of behaviour of control and experimental tissues according to the paired t-test was significant ($P(\Delta c, \Delta e) < 0.001$). The fractional decrease in J_{ro} was not as great as the decrease in I_0. Presumably this difference reflects a component of "basal" oxygen consumption insensitive to ouabain.

(c) Dependence of O_2 *consumption on the electrical potential* $dJ_r/d(\Delta\Psi)$. Fig. 2 shows the result of measurement of $dJ_r/d(\Delta\Psi)$ in a representative experiment. $dJ_r/d(\Delta\Psi)$ in the experimental hemiskin decreased substantially after treatment with 10^{-7} M ouabain, while in the control hemiskin it decreased only slightly. Columns 7 and 8 of Table I show the mean values. Initial values in paired tissues differed insignificantly. The final value of $-dJ_r/d(\Delta\Psi)$ in experimental tissues was less than before treatment ($P(e_I, e_{II}) < 0.001$) and less than the simultaneous value in untreated control tissues ($P(c_{II}, e_{II}) < 0.001$). The difference of behavior of control and experimental tissues was highly significant according to the paired t-test ($P(\Delta c, \Delta e) < 0.001$).

(d) Apparent affinity of the metabolic reaction A_{app}. Mean values of A_{app} calculated from Eqn 3 are shown in columns 9 and 10 of Table I. Initial values in paired hemiskins differed insignificantly. A_{app} decreased significantly between the first and second measurements in both the control and experimental tissues ($P(c_I, c_{II}) < 0.01$, $P(e_I, e_{II}) < 0.01$). The difference of behavior of control and experimental tissues according to the paired t-test was not significant. Thus 10^{-7} M ouabain appears to change I_0 and $dJ_r/d(\Delta\Psi)$ without significant effect on A_{app}.

*(2) Effects of 2-deoxy-*D*-glucose*

Fig. 3 shows a representative example of the effect of 2-deoxy-D-glucose on I_0. The current begins to fall within a few minutes and reaches a new steady-state

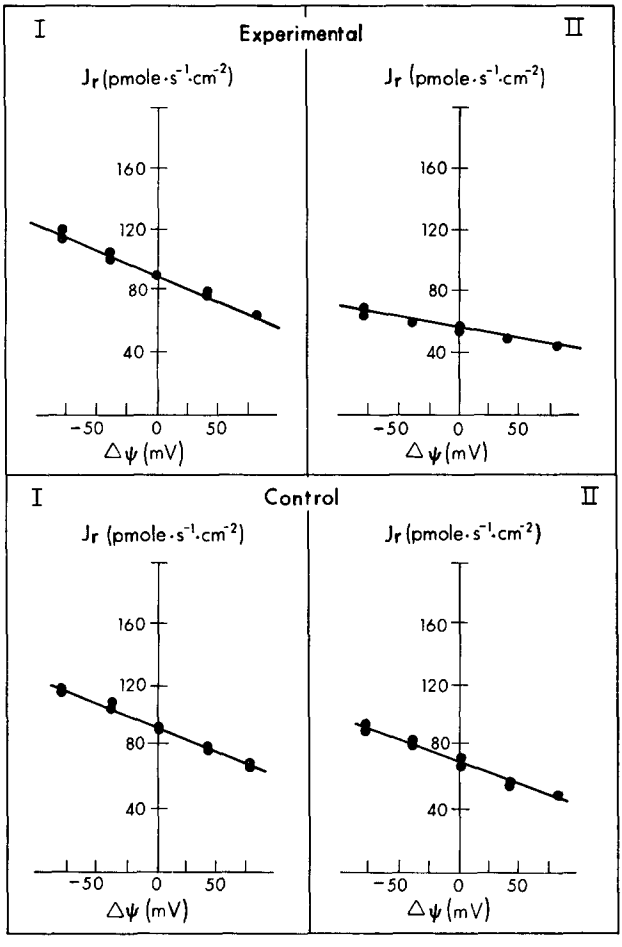

Fig. 2. Representative example of the effect of ouabain on the dependence of the rate of O_2 consumption on the electrical potential difference $\Delta\psi$. Following the completion of initial measurements (series I) the inside surface of the experimental hemiskin was exposed to 10^{-7} M ouabain and the measurements were repeated (series II).

level within 40 min. The time course of inhibition of I_0 was not a sensitive function of the concentration of 2-deoxy-D-glucose. The degree of inhibition produced by a given concentration, defined as $1-R(I_0)$ (see Materials and Methods, Eqn 4), was inversely related to the concentration of glucose in the Ringer's solution [11]. The concentrations of glucose (1 mM) and 2-deoxy-D-glucose (16 mM) were chosen for convenience of measurement of the various quantities in the study of 10 skins reported below.

Table II shows the mean effects of 16 mM 2-deoxy-D-glucose on the thermodynamic variables. As above, each experiment was carried out on paired hemiskins from a single animal. Following the completion of initial measurements, the inside surfaces of the group e hemiskins were exposed to 16 mM 2-deoxy-D-glucose. All inside solutions (both group c and group e) contained 1 mM glucose. Measurements

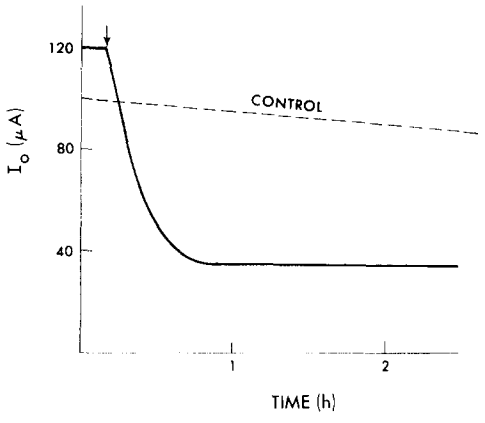

Fig. 3. Representative example of the effect of 2-deoxy-D-glucose on the short-circuit current I_0. Control (- - -) and experimental (—) hemiskins were obtained from the same animal. At the time indicated by the arrow the inside surface of the experimental hemiskin was exposed to 16 mM 2-deoxy-D-glucose, without change in solution osmolality. The inside solutions (both control and experimental) contained 1 mM glucose. No manipulations were performed on the control hemiskin.

in paired tissues were made between 1.5 and 0 h prior to, and 1.0 and 2.5 h after, administration of 2-deoxy-D-glucose, and are indicated by t 0 and 1 h, respectively.

(a) Short-circuit current I_0. Columns 3 and 4 of Table II show the mean effect on I_0. Initial values of I_0 in paired hemiskins differed insignificantly. I_0 in the control hemiskins decreased slightly between the first and second measurements, but not significantly. The value in experimental tissues following treatment was less than before treatment ($P(e_I, e_{II}) < 0.001$), and less than the simultaneous value in untreated control tissues ($P(c_{II}, e_{II}) < 0.005$). The difference of behavior of the control and experimental tissues was highly significant according to the paired t-test ($P(\Delta c, \Delta e) < 0.001$).

(b) O_2 *consumption at short-circuit* J_{ro}. Columns 5 and 6 of Table II show the effect of 2-deoxy-D-glucose on J_{ro}. The initial values in paired hemiskins differed insignificantly. J_{ro} appeared to decrease between the first and second measurements in experimental tissues ($P(e_I, e_{II}) < 0.02$): however, the difference of behavior of control and experimental tissues according to the paired t-test was insignificant.

TABLE II

EFFECT OF 16 mM 2-DEOXY-D-GLUCOSE ON THE THERMODYNAMIC VARIABLES

	Time (h)	I_o ($\mu A \cdot cm^{-2}$)		J_{ro} ($pmol \cdot s^{-1} \cdot cm^{-2}$)		$-dJ_c/d(\Delta\psi)$ ($pmol \cdot s^{-1} \cdot cm^{-1} \cdot mV^{-1}$)		A_{app} ($kcal \cdot mol^{-1}$)	
		0	1.0	0	1.0	0	1.0	0	1.0
c	Mean	14.2	11.2	56.1	51.9	0.188	0.164	18.2	17.0
	S.E.	1.6	1.1	2.9	2.8	0.019	0.014	1.2	1.9
e	Mean	18.2	8.0	58.1	48.4	0.196	0.169	24.9	11.8
	S.E.	2.1	1.1	3.8	2.1	0.019	0.007	2.2	1.8

Presumably the failure to demonstrate a significant difference reflects the imprecision of measurement at low rates of metabolism. In one hemiskin which had the highest values of I_0 and J_{ro}, J_{ro} declined from 85.3 to 42.4 pmol · s $^{-1}$ · cm $^{-2}$ in the presence of 2-deoxy-D-glucose while the corresponding drop in I_0 was from 47.7 to 5.4 μA · cm $^{-2}$.

(c) Dependence of O_2 consumption on the electrical potential difference $dJ_r/d(\Delta\Psi)$. As previously with control tissues and tissues treated with ouabain, linear relationships between the rate of oxygen consumption and the electrical potential difference were observed consistently in hemiskins treated with 16 mM 2-deoxy-D-glucose. Fig. 4 shows a representative experiment. The slope of the regression line, $dJ_r/d(\Delta\Psi)$, was not significantly affected by the addition of 2-deoxy-D-glucose to the experimental hemiskin. Note also that the intercept on the ordinate (J_{ro}) drops only slightly after treatment.

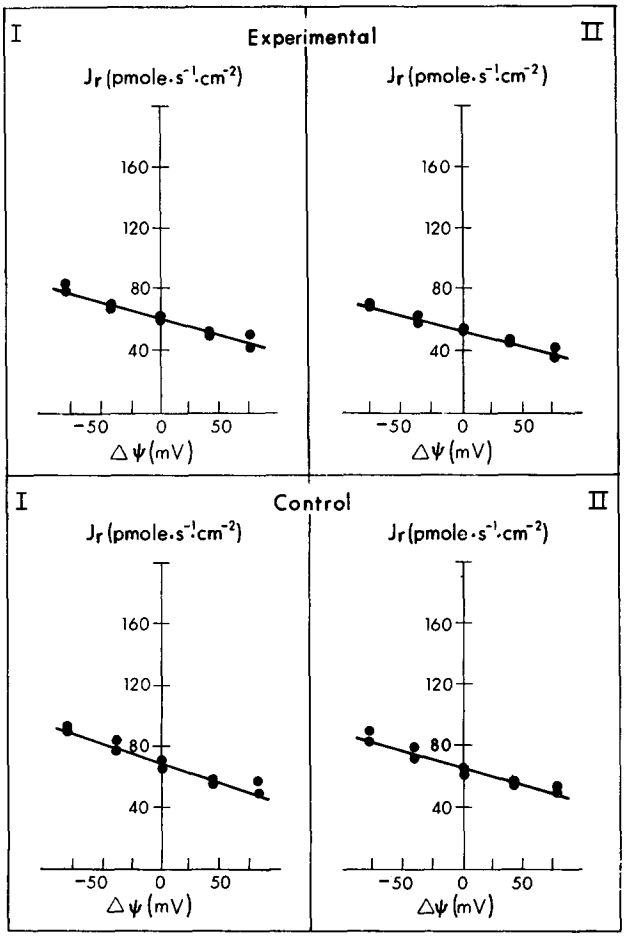

Fig. 4. Representative example of the effect of 2-deoxy-D-glucose on the dependence of the rate of O_2 consumption J_r on the electrical potential difference $\Delta\psi$. Following the completion of initial measurements (series I) the inside surface of the experimental hemiskin was exposed to 16 mM 2-deoxy-D-glucose and the measurements were repeated (series II).

Columns 7 and 8 show the mean results for the 10 skins studied. $-dJ_r/d(\Delta\Psi)$ dropped slightly in both the treated and control hemiskins. However, neither this decline nor the difference between the treated and untreated hemiskins was significant. These results should be contrasted with those observed after treatment with ouabain (Table I).

(d) Apparent affinity of the metabolic reaction A_{app}. Columns 9 and 10 of Table II show the mean values of A_{app}. Initial values in paired tissues in this instance differed significantly ($P(c_I, e_I) < 0.005$). The value in experimental tissues following treatment was less than before treatment ($P(e_I, e_{II}) < 0.001$) and less than the simultaneous value in untreated control tissues ($P(c_{II}, e_{II}) < 0.025$). The difference of behavior of control and experimental tissues was highly significant according to the paired t-test ($P(\Delta c, \Delta e) < 0.001$).

*(3) Comparison of the effects of ouabain and 2-deoxy-*D*-glucose*

The results of the experiments with ouabain and 2-deoxy-D-glucose were compared by recasting the data in the form of a ratio according to Eqn 4. Table III shows the results. The concentrations chosen for use in these studies were sufficient to reduce I_0 by about 50 %. Neither agent reduced J_{ro} to the same degree as I_0. However, the relative decrease in J_{ro} produced by ouabain treatment was demonstrably significant, while that produced by 2-deoxy-D-glucose was not. The contrast in the effects of ouabain and 2-deoxy-D-glucose on the thermodynamic variables can be seen in the last two columns of the table. Ouabain lowers $-dJ_r/d(\Delta\Psi)$, while 2-deoxy-D-glucose has no significant effect on this parameter. Ouabain, on the other hand, has no significant effect on A_{app}, while 2-deoxy-D-glucose markedly decreases A_{app}. The difference in effects can be interpreted in terms of the presumed difference in biochemical modes of action of the two agents.

TABLE III

COMPARISON OF EFFECTS OF OUABAIN AND 2-DEOXY-D-GLUCOSE

P represents the probability that the given quantity differs from unity.

		$R(I_0)$	$R(J_{ro})$	$R(dJ_r/d(\Delta\psi))$	$R(A_{app})$
Ouabain (10^{-7} M)	Mean	0.455	0.837	0.542	0.855
[$n = 9$]	S.E.	0.032	0.033	0.040	0.094
	P	< 0.001	< 0.005	< 0.001	n.s.
2-Deoxy-D-glucose	Mean	0.580	0.921	1.095	0.531
(16 mM)	S.E.	0.049	0.041	0.090	0.066
[$n = 10$]	P	< 0.001	n.s.	n.s.	< 0.001

DISCUSSION

The linear non-equilibrium thermodynamic analysis of active transport is potentially capable of providing useful information not readily available by other means. Various aspects of the linear model have been demonstrated experimentally. Evidence has been presented for linearity of the rate of active Na^+ transport with electrical potential in toad bladder [3] and of oxygen consumption with electrical

potential in frog skin [5]. Recently Danisi and Vieira [7] demonstrated linearity of the rates of both active Na^+ transport and oxygen consumption with the chemical potential difference across toad skins. A linear relationship has also been demonstrated between short-circuit current and the associated rate of oxygen consumption in frog skin when both these parameters vary either spontaneously or as a result of pharmacological treatment. Beauwens and Al-Awqati [12] have noted similar relationships between short-circuit current and the rate of CO_2 production in toad bladder.

The results cited are consistent with the validity of a linear non-equilibrium thermodynamic approach, but the experimental techniques presently available do not permit a systematic study of the effects of variation of the affinity. Nevertheless, certain observations do suggest that the affinity evaluated according to Eqn 3 does indeed reflect the substrate-product ratio in the metabolic pool sustaining transport. Thus Vieira et al. [5] observed in frog skins that, following a long period of enhanced transport at depolarizing potentials, on return to the short-circuited state both I_o and J_{ro} were transiently lower than in the control state. Depression of transport by hyperpolarization produces a converse effect. Danisi and Vieira [7] have recently presented evidence that in toad skin such a "memory effect" is attributable to changes in the affinity rather than the phenomenological coefficients. Apparently sufficiently extensive perturbations of $\Delta\Psi$ alter the rate of metabolism associated with transport so as to change appreciably the substrate and/or product concentrations in the metabolic pool.

Saito et al. [6] carried out studies in frog skin with amiloride, a diuretic which depresses active Na^+ transport by interfering with the passive entry process. Following 1 h exposure to amiloride there was no effect on A_{app}. After 4 h exposure, however, it was possible to demonstrate the well-recognized "overshoot" of short-circuit current on removal of the drug, and to show that this was associated with an increase in A_{app} over control levels [6]. Again these phenomena are interpretable in terms of changes in the concentrations of substrates and/or products in some critical metabolic pool.

The present study provides further evidence bearing on the nature of the thermodynamic affinity and the phenomenological coefficients. Since ouabain inhibits the $(Na^+ + K^+)$- ATPase generally identified with the sodium pump, it would be expected to decrease the phenomenological coefficients $L_{Na,r}$ and L_r relating transport and metabolism, respectively, to the thermodynamic affinity, but it should have no immediate effect on A_{app} itself. This was in fact observed. In a previous study employing high concentrations of ouabain, the dependence of oxygen consumption on $\Delta\Psi$ was completely abolished, i.e. $L_{Na,r} = 0$[5,6]. In the present study employing lower concentrations of ouabain, although $L_{Na,r}$ remained finite it was substantially less than in the absence of ouabain. Similarly, L_r was reduced below the control level. In contrast, A_{app} was unaffected by ouabain with the dosages and periods of exposure studied here. The effects of substantial depression of transport for extended periods would depend on the interaction between the pump at the inner surface and permeability factors at the outer surface. These have not yet been investigated.

In contrast to ouabain, the metabolic inhibitor 2-deoxy-D-glucose might be expected to exert its primary effect on the thermodynamic affinity and might or

might not be expected to affect the phenomenological coefficients. 2-deoxy-D-glucose interferes with cellular energy metabolism in three principal ways: competition with glucose for cell uptake; competition with glucose for phosphorylation by hexokinase; and blockage by 2-deoxy-D-glucose 6-phosphate of the isomerization of glucose 6-phosphate to fructose 6-phosphate [9]. Since 2-deoxy-D-glucose 6-phosphate is not metabolized, each of these effects should promote depletion of ATP. Survey experiments have shown that 2-deoxy-D-glucose is capable of decreasing I_0 in the absence of glucose in the bathing media, indicating that interference with glucose uptake is not the sole factor bringing about inhibition [11]. We have no information concerning the relative contribution of the other two factors mentioned above. Whatever the precise mechanisms of action of 2-deoxy-D-glucose, the present study has shown that it did indeed depress A_{app} but was without effect on $L_{Na,r}$. Its effects on L_{Na} and L_r remain to be determined.

The finding that ouabain affects only a phenomenological coefficient while 2-deoxy-D-glucose affects the thermodynamic driving force of active Na^+ transport further supports the applicability of the linear non-equilibrium thermodynamic formulation.

ACKNOWLEDGEMENTS

This study was supported by grants from the U.S.P.H.S. (HL 14322 to the Harvard-M.I.T. Program in Health Sciences and Technology, HE 13648, AM 17817) and grants from the N.S.F. (GB 24697, GB 40704).

REFERENCES

1 Kedem, O. (1961) in Membrane Transport and Metabolism (Kleinzeller, A. and Kotyk, A., eds), pp. 87–93, Academic Press, New York
2 Essig, A. and Caplan, S. R. (1968) Biophys. J. 8, 1434–1457
3 Saito, T., Lief, P. D. and Essig, A. (1974) Am. J. Physiol. 226, 1265–1271
4 Vieira, F. L., Caplan, S. R. and Essig, A. (1972) J. Gen. Physiol. 59, 60–76
5 Vieira, F. L., Caplan, S. R. and Essig, A. (1972) J. Gen. Physiol. 59, 77–91
6 Saito, T., Essig, A. and Caplan, S. R. (1973) Biochim. Biophys. Acta 318, 371–382
7 Danisi, G. and Vieira, F. L. (1974) J. Gen. Physiol. 64, 372–391
8 Glynn, I. M. (1964) Pharmacol. Rev. 16, 381–407
9 Webb, J. L. (1966) Enzyme and Metabolic Inhibitors Vol. II, p. 386, Academic Press, New York
10 Snedecor, G. W. and Cochran, W. G. (1968) Statistical Methods, Iowa State University Press, Ames, Iowa
11 Owen, A. (1974) Ph. D. Thesis, Harvard University, Cambridge
12 Beauwens, R. and Al-Awqati, Q. (1974) Abstr. Am. Soc. Nephrol. 7, 7