BBA 75345

EFFECT OF VALINOMYCIN ON ISOLATED URINARY BLADDER OF *PSEUDEMYS SCRIPTA*

I. EFFECT ON ELECTRICAL PARAMETERS UNDER AEROBIC CONDITIONS

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(Received May 21st, 1969)

SUMMARY

1. Isolated hemibladders of the fresh-water turtle, *Pseudemys scripta*, were bathed on both surfaces by oxygenated, Na^+–Ringer solution (17 mM HCO_3^- buffer) and were maintained in a short-circuited state.

2. The cyclic dodecadepsipeptide antibiotic, valinomycin, was added to a final concentration of 1 μM to either the mucosal or serosal surface of one hemibladder while the paired hemibladder served as a time control.

3. Addition of valinomycin to the mucosa resulted in a 60 $\pm$ 15 % increase in total transbladder resistance (R_t), in a 60 $\pm$ 10 % decrease in short-circuiting current (I_{sc}) and in a 40 $\pm$ 10 % decrease in spontaneous open-circuited transbladder potential difference (PD_{oc}) for the valinomycin-treated hemibladders relative to the control hemibladders.

4. Half-maximal effect of valinomycin added to the mucosa was achieved 18 min after addition for I_{sc}; 24 min for PD_{oc}; and 30 min for R_t.

5. Addition of valinomycin to the serosa did not result in any change in electrical parameters.

INTRODUCTION

Transport properties of the isolated turtle bladder

Prepared *in vitro*, urinary bladders of *Pseudemys scripta* have been shown to transport Na^+ (refs. 1–7) and Cl^- (refs. 3, 4 and 8) actively from mucosa to serosa (m $\rightarrow$ s) against their respective electrochemical gradients and to acidify the mucosal medium by either removal of HCO_3^- (refs. 9 and 10) or possibly by secretion of H^+ (refs. 6 and 11).

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Effect of valinomycin on various systems

Various workers have shown that the cyclic dodecadepsipeptide antibiotic, valinomycin, produces a selective increase in permeability to K^+ relative to Na^+ in various lipid membrane systems[12–16] and in intact sheep erythrocytes[17].

Valinomycin (1–10 μM) did not have any effect on (Na^+-K^+)-stimulated, ouabain-inhibited ATPase activity of sheep erythrocytes[17].

McMURRAY AND BEGG[18] showed that valinomycin could stimulate adenosine triphosphatase activity and uncouple respiration from phosphorylation in isolated mitochondria. Consequently it was shown that addition of valinomycin to isolated mitochondria resulted in a net flux of K^+ into the mitochondria[19–21]. Other studies have been concerned with energetics of valinomycin-induced K^+ transport[22] into mitochondria and with various effects of valinomycin on oxidative phosphorylation[18,23,24].

Other references to valinomycin may be found in refs. 16 and 17.

The purpose of the present work is to find the effect of valinomycin on parameters associated with the transport of ions across an isolated epithelial membrane *viz.* across isolated urinary bladder of the fresh-water turtle, *P. scripta*. In subsequent papers, attempts will be made to separate the effects of valinomycin on transport mechanisms, series passive membrane and source of energy.

METHODS

Tissue preparation

Both male and female turtles (*P. scripta*), weighing 0.75–1.5 kg and possessing a carapace 15–20 cm wide, were obtained from the Lemberger Co. (Oshkosh, Wisc.).

The six experiments reported in Table I and Fig. 1 were performed during February and March and those in Table II and Figs. 2 and 3 during April.

Turtles were sacrificed by decapitation, and bladders were excised and mounted in a double-barreled chamber (modified after USSING AND ZERAHN[25]) by a technique described previously[8]. See ref. 8 for the structure and function of the Lucite chamber. Approx. 30 min elapsed between the time of sacrifice and the start of the experiment ($t = 0$).

The double-barreled chamber permitted two hemibladders to be used simultaneously as experimental and control halves which were chosen at random. Each side of each hemibladder was bathed by 12 ml of Na^+–Ringer solution. All bathing media were continuously mixed and circulated past the bladder. Each hemibladder had a surface area of 1.5 cm^2 exposed to the bathing solution, a dry wt. of 11.9 $\pm$ 4.5 mg and a tissue-water content of 98 $\pm$ 51 μl (values shown are the mean $\pm$ S.D.).

All experiments were performed at 24–26°.

Short-circuiting technique

Isolated bladders were maintained in the short-circuited state using the technique of USSING AND ZERAHN[25]. The short-circuiting current (I_{sc}) and transbladder potential difference (PD) were measured by techniques described previously[8]. Total membrane resistance (R_t) was measured as follows. Every 10 min short-circuiting current was interrupted for about 10 sec. During this time a calibrated pulse of current (ΔI) ranging between 10 and 100 μA was sent through the bladder. R_t was

taken as the increment in PD resulting from the sending of ΔI divided by ΔI. The PD during this interruption also provided evaluation of the spontaneous open-circuited PD (PD_{oc}). The series resistance of the solution (35 Ω) was not taken into account. The following sign reference was adopted: cations (*viz.*, Na^+) moving in the forward (m → s) direction contributed a positive moiety of I_{sc}; anions (*viz.*, Cl^- and HCO_3^-), a negative moiety. I_{sc} is positive when the bladder is bathed on both sides by Na^+–Ringer solution, for Na^+ transport is normally greater than anion transport in the short-circuited state.

An Esterline-Angus model E1102S potentiometric strip-chart recorder with a high input-impedance (100 kΩ) was used to measure PD (Esterline-Angus Co., Indianapolis, Ind.).

All values of I_{sc} are per hemibladder (*i.e.*, μA/1.5 cm^2), and values of R_t are also on the basis of a hemibladder (*i.e.*, k$\Omega \cdot$ 1.5 cm^2). PD_{oc}, an intensive parameter, is reported in units of mV.

Na^+–Ringer solution

Both surfaces of each hemibladder were bathed at all times by a Krebs-buffer system which had the following composition (in mM): Na^+, 101; K^+, 4.8; Ca^{2+}, 2.0; Mg^{2+}, 0.8; Cl^-, 92; HCO_3^-, 17; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; SO_4^{2-}, 0.80; CO_2, 0.33; and D-glucose, 11. Osmolality was 221 mosmoles/kg and I was 0.116. During the experiment, the isolated preparation was continuously replenished with O_2 by bubbling both serosal and mucosal media with a mixture of O_2–CO_2 (99:1, v/v) pre-saturated with water vapor. The pH varied between 7.4 and 7.8.

Chemical measurements of Na^+, Cl^-, K^+, HCO_3^-, pH, and osmolality were performed routinely on all stock Na^+–Ringer solutions by analytical techniques described previously[3,9,26]. Valinomycin, A grade (lot No. 860031), was obtained from Calbiochem, Los Angeles, Calif. A stock solution of valinomycin was made up to a final concentration of 0.12 mM in absolute ethanol. The molecular weight was assumed to be 1111. The addition of 0.1 ml of valinomycin stock to the serosal or mucosal bathing fluid (volume = 12 ml) of an experimental hemibladder resulted in a final concentration of valinomycin of approx. 1 μM. A simultaneous control addition of 0.1 ml of absolute ethanol was always made to the appropriate bathing solution of the control hemibladder.

RESULTS

Addition of valinomycin to serosa (Fig. 1 and Table I)

Fig. 1 shows the effect of adding valinomycin to the serosal bathing fluid on electrical parameters of six hemibladders. When t = 100 min, valinomycin dissolved in ethanol was added to a final concentration of 1 μM to the serosal bathing fluid of the experimental hemibladders (●), and ethanol was added to the control hemibladders (○). The magnitude of each electrical parameter when t = 100 min was taken as 100 % for each hemibladder. The top section of Table I (t = 100 min) shows that the electrical parameters (R_t, I_{sc}, and PD_{oc}) of the experimental and control groups were not significantly different at the time of addition; the bottom section (t = 180 min) shows that the addition of valinomycin to the serosal bathing fluid did not result in a significant change in any of the electrical parameters even after 80 min.

Addition of valinomycin to mucosa (Fig. 2 and Table II)

Fig. 2 shows the effect of adding valinomycin to the mucosal bathing fluid on electrical parameters of six hemibladders. When t = 100 min, valinomycin dissolved in ethanol was added to a final concentration of 1 μM to the mucosal bathing fluid of the experimental hemibladders (●), and ethanol was added to the control hemibladders (○). The magnitude of each electrical parameter when t = 100 min was taken as 100% for each hemibladder. The top section of Table II (t = 100 min) shows that the electrical parameters of the experimental and control groups were not significantly different at the time of addition. The middle and bottom sections (t = 160 min and t = 220 min, respectively) show that the addition of valinomycin to the mucosal side resulted in a significant difference in R_t and I_{sc} between the experi-

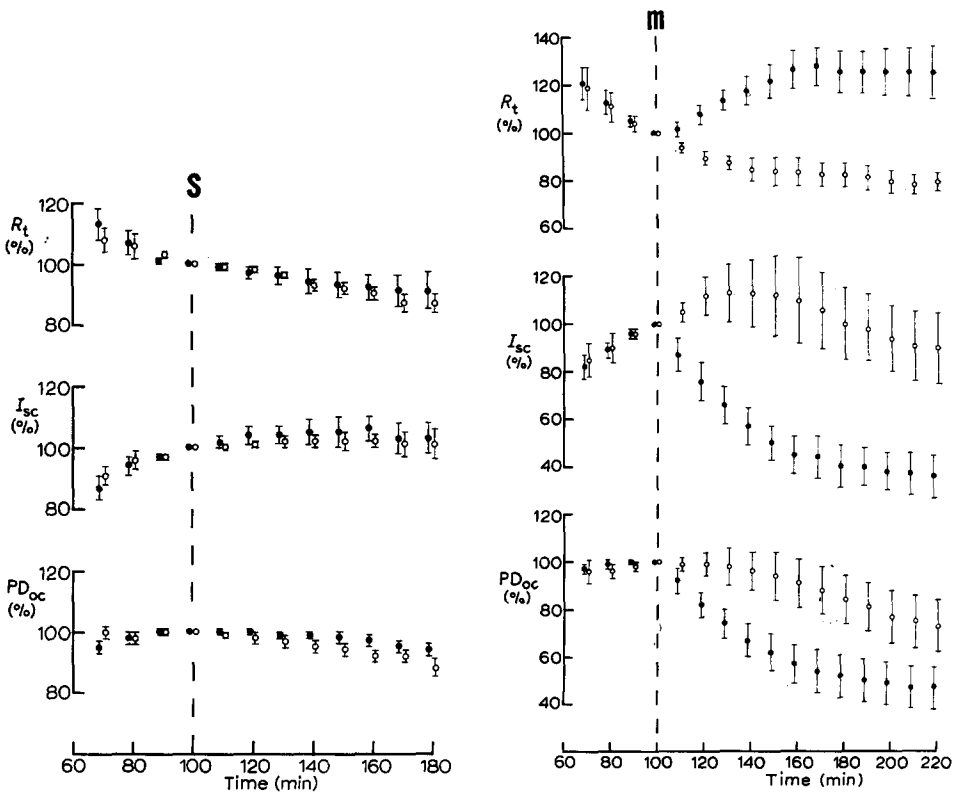

Fig. 1. The effect of 1 μM valinomycin (added to the serosa) on normalized electrical parameters. All values are with respect to values at the time of serosal addition (t = 100 min). Experimental group (●): valinomycin dissolved in ethanol was added to the serosa when t = 100 min (s ↓). Control group (○): requisite amount of ethanol was simultaneously added to serosal of paired hemibladder. All values are given as mean ± S.E. for the same six hemibladders represented in Table I.

Fig. 2. The effect of 1 μM valinomycin (added only to the mucosa) on normalized electrical parameters. All values are with respect to values at time of addition (t = 100 min). Experimental group (●): valinomycin dissolved in ethanol was added to the mucosa when t = 100 min (m ↓). Control group (○): requisite amount of ethanol was simultaneously added to mucosa of paired hemibladder. All values are given as mean ± S.E. for the same six hemibladders represented in Table II and Fig. 3.

TABLE I

EFFECT OF ADDITION OF VALINOMYCIN TO SEROSA ON ELECTRICAL PARAMETERS

All values are given as mean ± S.E. for the same six pairs of hemibladders represented in Fig. 1. Normalized values (%) are with respect to values at time of addition (t = 100 min). Unpaired data of the groups were compared by means of the t-test.

Time (*min*)	*Group*	*Valinomycin* (μM)		R_t	I_{sc}	PD_{oc}
		s	*m*			
100 (time of addition)	Exptl.	0	0	0.41 ± 0.03 kΩ	127 ± 19 μA	54 ± 6 mV
	Control	0	0	0.40 ± 0.03 kΩ	92 ± 16 μA	43 ± 6 mV
				$P > 0.7$	$P > 0.1$	$P > 0.2$
180 ($1^1/_3$ h after addition)	Exptl.	1	0	91 ± 6%	103 ± 5%	94 ± 2%
	Control	0	0	87 ± 3%	101 ± 5%	88 ± 3%
				$P > 0.5$	$P > 0.7$	$P > 0.1$

TABLE II

EFFECT OF ADDITION OF VALINOMYCIN TO MUCOSA ON ELECTRICAL PARAMETERS

All values are given as mean ± S.E. for the same six hemibladders represented in Figs. 2 and 3. Normalized values (%) are with respect to values at time of addition (t = 100 min). Unpaired data of the groups were compared by means of the t-test.

Time (*min*)	*Group*	*Valinomycin* (μM)		R_t	I_{sc}	PD_{oc}
		s	*m*			
100 (time of addition)	Exptl.	0	0	0.61 ± 0.11 kΩ	117 ± 24 μA	63 ± 6 mV
	Control	0	0	0.55 ± 0.06 kΩ	95 ± 16 μA	54 ± 5 mV
				$P > 0.6$	$P > 0.4$	$P > 0.2$
160 (1 h after addition)	Exptl.	0	1	127 ± 8%	45 ± 8%	57 ± 8%
	Control	0	0	84 ± 6%	110 ± 18%	91 ± 10%
				$P < 0.01$	$P < 0.01$	$P < 0.05$
220 (2 h after addition)	Exptl.	0	1	126 ± 11%	36 ± 9%	47 ± 9%
	Control	0	0	80 ± 4%	90 ± 15%	73 ± 11%
				$P < 0.01$	$P < 0.02$	$P > 0.1$

mental and control groups; and PD_{oc} for the experimental group was significantly less than that for the control group 1 h after addition; but 2 h after addition, PD_{oc} for the control group had decayed so that it was not significantly different from that of the experimental group.

Time-course of the effect of mucosal valinomycin on R_t, I_{sc} and PD_{oc} (Fig. 3)

In Fig. 2, the ratio of the value of each electrical parameter for the experimental group to the corresponding value for the control group was found for each time after the addition of valinomycin. This ratio of relative values was found to reach a maximum of 1.59 ± 0.14 when t = 110 min for R_t, a minimum of 0.40 ± 0.11 when t = 80 min for I_{sc} and a minimum of 0.61 ± 0.12 when t = 70 min for PD_{oc}

(values shown are mean ± S.E.)*. These extrema were defined as 100 % maximal effect and a relative value of 100 % (at time of addition) was defined as 0 % maximal effect (no effect). A simple linear transformation was used to convert relative values to re-normalized parameters. Fig. 3 shows a plot of the re-normalized relative values

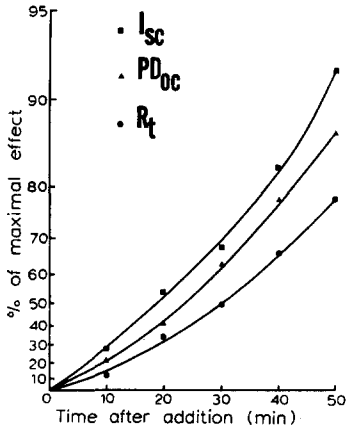

Fig. 3. Percent of maximal effect of 1 μM valinomycin (added to the mucosa) on electrical parameters as a function of time after addition. Relative values for each parameter were defined as the value for the experimental group divided by the corresponding value for the control group. All values were taken from Fig. 2. Minimal or maximal relative value achieved after addition of valinomycin to mucosa was defined as 100 % maximal effect, and a relative value of 100 % (at time of addition) was defined as 0 % maximal effect (no effect). A simple linear transformation converted relative values to percent of maximal effect. 100 % *minus* percent of maximal effect would be on a logarithmic scale.

(percent of maximal effect) *vs.* time after addition of valinomycin to the mucosal bathing fluid to a final concentration of 1 μM. Half-maximal effect of valinomycin added to the mucosa was achieved 18 min after addition for I_{sc}; 24 min for PD_{oc}; and 30 min for R_t.

DISCUSSION

Valinomycin can gain access to processes related to transport in urinary bladder of *P. scripta* only from the mucosal side.

The valinomycin-induced decrease in I_{sc} is compatible with a general decrease in transport of all ions, a specific decrease in Na^+ transport, a specific increase in either Cl^- or HCO_3^- transport or the opening of a new cationic path resulting in serosal to mucosal movement of cation (*e.g.*, valinomycin-mediated movement of K^+). The valinomycin-induced increase in R_t suggests that no new serosal to mucosal cationic paths were opened and existing anionic paths were not increased. Thus, the most probable interpretation of the decrease in I_{sc} is either a general decrease in transport of all ions or a specific decrease in Na^+ transport. Further experiments in

* Thus, addition of valinomycin to the mucosal bathing fluid to a final concentration of 1 μM resulted in a 59 ± 14 % increase in R_t, a 60 ± 11 % decrease in I_{sc} and a 39 ± 12 % decrease in PD_{oc} for the six valinomycin-treated hemibladders relative to the six hemibladders (values shown are mean ± S.E.).

which isotopes are used to trace individual ionic species are needed to explain unequivocally the decrease in I_{sc}.

Although the most obvious explanation for the action of valinomycin on electrical parameters is the direct action of valinomycin on a source of energy for transport (*e.g.*, on the mitochondria), a direct action of valinomycin on the pump or on a series membrane cannot be dismissed *a priori*. The data of the present study do not permit these effects to be separated. Work is currently in progress to determine the effect of valinomycin on the transbladder movement of specific ions including K^+ and on transport under anaerobic conditions.

ACKNOWLEDGMENTS

This study was supported, in part, by National Institutes of Health Research Grant AM 13037; in part, by National Science Foundation Research Grant GB-7764; and in part, by National Aeronautics and Space Administration Research Grant 33-171-(001).

The author is indebted to Miss Susan Bernstein for her technical assistance.

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