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ACTION OF ALDOSTERONE ON FROG SKIN IN THE PRESENCE AND ABSENCE OF IN VITRO MOLTING EFFECTS

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SUMMARY

The molting which occurs in frog skin following exposure to high concentrations of aldosterone interferes with the interpretation of physiological measurements. Exposure of skins from frogs maintained in standard smooth tanks to $5 \cdot 10^{-7}$ M aldosterone caused within a few hours erratic responses in short-circuit current I_0 and conductance κ followed by sustained stimulation of I_0 and κ: 10^{-8} M aldosterone caused only stimulation of I_0 and κ. Storage of frogs in "rough tanks" eliminated in vitro molting on exposure to $5 \cdot 10^{-7}$ M aldosterone. I_0 and κ were then superimposable for 3 h, after which I_0 increased far more rapidly than κ. These results are consistent with an early effect on permeability of the active pathway and later effects on metabolism, either a direct effect on the pump or enhanced interaction between transport and metabolism.

INTRODUCTION

The mode of action of aldosterone in salt regulation has been studied in a number of tissues, of which the most effective as physiological models are anuran bladder and skin. In recent years there has been much discussion as to whether the effect of aldosterone on active sodium transport is attributable primarily to permeability or energetic factors [1–11]. It would be desirable to evaluate both factors concurrently in the same tissue over an extended period. The frog skin would seem to be an appropriate model since the techniques for carrying out such a comprehensive study in this tissue have already been established [12]. However, there is a serious problem in this regard since aldosterone is known to induce in vitro molting in anuran skins [13–15]. This phenomenon is associated with irregular patterns of inhibition and stimulation of transport.

In this report, we examine the long-term response of conductance and short-

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circuit current when aldosterone is added to the solutions bathing frog skin. Manipulations of environmental factors will be described which eliminated in vitro molting, permitting the observation of hormonal effects which had previously been obscured.

MATERIALS AND METHODS

Frogs (*Rana pipiens*), obtained from the Carolina Biological Supply Co., Burlington, North Carolina, were kept at room temperature in tanks under two different conditions. One group, designated S, was kept in smooth bottom glass or slate tanks containing a pan of tap water. The other group, designated R, was kept in rough bottom tanks which contained commercial aquarium gravel and tap water. Paired hemiskins were mounted in modified Ussing-Zerahn lucite chambers of 7.1 cm^2 cross-sectional area and bathed in glucose-Ringer solution [11]. The electrical potential difference ($\Delta\psi$) was regulated by an automatic voltage clamp and the current (I) recorded continuously [12]. The total conductance (κ) was evaluated according to the method of Saito and Essig [10]. D-Aldosterone (Sigma) was dissolved in methanol and stored as stock solutions of 0.6 and 0.012 mg/ml.

Data for a particular piece of tissue were always expressed as the measurement at time t related to that at time $= 0$, or $x_t/x_{t=0}$. The experiments were analyzed by a double normalization procedure, comparing effects in paired experimental (e) and control (c) hemiskins according to the following equation:

$$R(x) = [x_t/x_{t=0}]_e/[x_t/x_{t=0}]_c. \tag{1}$$

The data are expressed as the geometric mean and standard error of the mean (Snedecor and Cochran [16]). Data in Fig. 1 were compared according to the rank order non-parametric analysis.

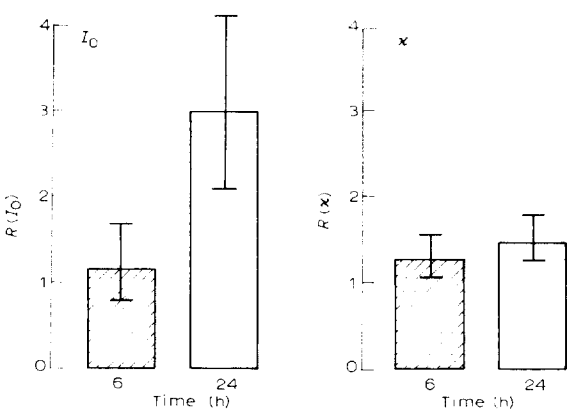

Fig. 1. Effect of $5 \cdot 10^{-7}$ M aldosterone on the short-circuit I_0 and the conductance κ in eight S-group skins. The data are expressed as mean $R(x) = [x_t/x_{t=0}]_e/[x_t/x_{t=0}]_c \pm$ S.E. where e and c refer to experimental and control tissues, respectively. Observations were made at 6 and 24 h after the addition of aldosterone. Values after 6 h were not significantly different from 1 while those after 24 h showed significant increases for both $I_0 (P < 0.02)$ and $\kappa (P < 0.05)$.

RESULTS

(1) *Effect of $5 \cdot 10^{-7}$ M aldosterone on S-group skins*

Skins from frogs kept in smooth bottom aquaria, referred to as S-group frogs, showed irregular responses of short-circuit current and conductance during the first 6 h following the administration of $5 \cdot 10^{-7}$ M aldosterone. These findings were similar to those previously described by Nielsen [13], who attributed them to in vitro molting.

Short-circuit current I_0 and conductance κ were measured in eight skins at 6 and 24 h after the addition of hormone. The results are shown in Fig. 1 in terms of the quantities $R(I_0)$ and $R(\kappa)$ (see Materials and Methods, Eqn 1). During the first steady-state period following the inhibition-stimulation sequence induced by aldosterone, no change from pre-hormone levels was observed. However, after 24 h I_0 had increased to 2.9 times the original level and κ to 1.5 times the original level. (Concurrent studies demonstrated the enhancement of the thermodynamic affinity following overnight exposure of frog skins to $5 \cdot 10^{-7}$ M aldosterone, as previously described by Saito et al. [11].)

(2) *Effect of $1 \cdot 10^{-8}$ M aldosterone on S-group skins*

When skins were exposed to $1 \cdot 10^{-8}$ M aldosterone the early inhibition-activation sequence seen with $5 \cdot 10^{-7}$ M aldosterone was replaced by a long-term progressive increase of current. In one of these experiments, following 14 h of observation the concentration of aldosterone was brought to $5 \cdot 10^{-7}$ M in both the experimental ($1 \cdot 10^{-8}$ M aldosterone) and control hemiskins. This treatment induced in both hemiskins the characteristic inhibition-stimulation sequence previously seen with exposure to the higher level of hormone alone. These results suggest that in experiments employing only high levels of hormone there are two discrete effects, one the stimulation of active sodium transport, the other an irregular influence on conductance and current, presumably attributable to in vitro molting. Since the two effects are superimposed, it is unclear just when the stimulation of transport begins, or to what extent it might be obscured by the induction of molting. The elimination of in vitro molting should provide a simpler system in which to study the hormonal stimulation of active transport, hopefully in pure form. One approach would be to use only low levels of hormone. However, the potential for in vitro molting would remain. Furthermore, the use of the lower level of hormone may produce only submaximal stimulation of transport.

(3) *Behavior of S-group and R-group skins*

In the course of attempts to improve the conditions in the holding tanks in which the frogs were kept we tested the effects of covering the tank bottoms with gravel. It was then noticed that animals kept in these rough gravel bed tanks (R-group frogs) molted in vivo far more frequently than frogs kept in smooth bottom tanks. Furthermore, we observed that the skins of R-group frogs failed to molt following the administration of aldosterone in vitro, and failed to show the early fluctuation of current and conductance noted with S-group frogs.

Further investigation showed that the initial short-circuit currents were not affected by the pre-experimental conditions. Initial mean values of I_0 in S-group and R-group skins were essentially the same. However, after a period of 16 h the mean I_0

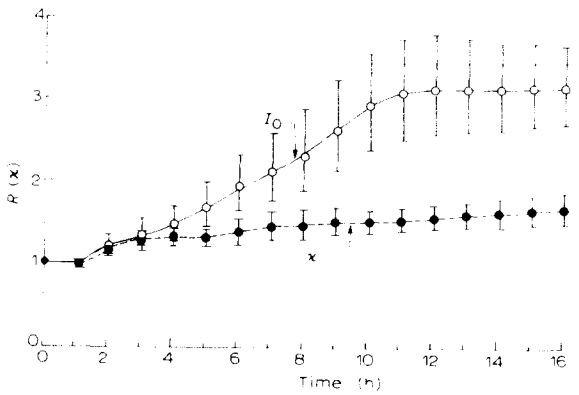

Fig. 2. Effect of $5 \cdot 10^{-7}$ M aldosterone on short-circuit current I_0 and conductance κ in eight R-group skins. The aldosterone was added to both the outer and inner bathing solutions. The data are expressed as mean $R(x) = [x_t/x_{t=0}]_e/[x_t/x_{t=0}]_c \pm$ S.E. where e and c refer to experimental and control tissues, respectively.

in R-group skins remained above 90 % of the initial level, while that of S-group skins had declined to below 40 % of the initial value.

(4) *Effect of* $5 \cdot 10^{-7}$ *M aldosterone on R-group skins*

Fig. 2 shows the effect of $5 \cdot 10^{-7}$ M aldosterone on skins from R-group frogs. I_0 remained constant for the first 60–90 min, and then increased until 12 h after the addition of hormone. Subsequently, it leveled off to a plateau which was maintained for at least the next 4 h. The conductance closely paralleled I_0 for the first 3 h, but then $R(I_0)$ and $R(\kappa)$ diverged. After 16 h I_0 had increased to 320 % and κ to 170 % of the initial values. Our studies show that the frog skin can maintain elevated current in the presence of aldosterone for at least 24 h.

DISCUSSION

Although the frog skin has served as a convenient model system for the study of transport processes, its relative histological complexity interferes with the interpretation of physiological measurements. The molting which occurs following exposure to high concentrations of aldosterone is a case in point. To the extent that this phenomenon causes erratic behavior of current and conductance, it is not possible to define precisely the specific effects of aldosterone on the fundamental transport process.

The mechanism by which a gravel environment eliminates the effect of in vitro molting is not clear. Gravel probably represents the "irritating stimulus" reported by Wilder [17] who claims that a localized irritation induces molting in the intact animal. Hviid Larsen [18] also found that preconditioning toads to a "terrestrial environment" led to a drastic reduction in the frequency of in vitro molting with aldosterone. Abrasion by gravel did not damage the tissue since R-group skins showed initial short-circuit currents similar to S-group skins and were well maintained for extended periods*.

* All of the present studies were carried out during the months October to May. A few skins studied during the summer failed to show the characteristic response to the gravel environment noted in "winter" frogs; however, there also appeared to be little or no stimulation of I_0 by aldosterone beyond that attributable to molting.

Elimination of the molting phenomenon by means of the above-described techniques would permit meaningful concurrent studies of permeability and energetic factors over extended periods. In the present study the use of gravel tanks permitted the demonstration of a period in which short-circuit current and conductance changed hand-in-hand, followed by a period in which the short-circuit current rapidly increased in association with only minimal changes in conductance. The close parallelism of I_0 and κ in the first period suggests an effect of aldosterone on the permeability of the active pathway, as has been demonstrated using isotopic techniques in the toad bladder [10]. The observations in the second period are consistent with enhancement of the thermodynamic affinity, as has been demonstrated earlier in frog skin following 14–18 h exposure to aldosterone [11]. The latter results are also consistent with a direct effect on the sodium pump, with enhancement of the interaction between metabolism and transport. The evidence obtained to date is compatible with any of the three mechanisms (permease theory, pump theory or metabolic theory) cited by various workers to explain the action of the aldosterone-induced protein [19]. The further application of the present technique in association with thermodynamic studies of the time course of oxidative metabolism should serve to characterize the precise significance of these different factors.

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