Effect of insulin on short-circuit current and sodium transport across toad urinary bladder

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HERRERA, FRANCISCO C. Effect of insulin on short-circuit current and sodium transport across toad urinary bladder. Am. J. Physiol. 209(4): 819-824. 1965.—The effect of insulin on short-circuit current and on the sodium transport system of the toad bladder has been examined. The rate coefficients for sodium movements across the mucosal and serosal barriers of the bladder epithelium were studied by observing the approach to a steady value of the flux of Na⁺ across the bladder. Insulin added to the solutions bathing both surfaces of the bladder causes a marked increase in short-circuit current. A smaller effect may also be elicited by adding the hormone to either the serosal or the mucosal bathing media. Insulin causes an important increase in the rate coefficient for sodium movement from the cells to the serosal solution with no significant change in the rate coefficients for sodium movement across the mucosal surface of the epithelial cells. The results indicate that the action of insulin is the result of a stimulation of the active transport step at the serosal surface of the cells. Insulin does not appear to modify the permeability to sodium of the mucosal surface.

Insulin active transport

THE SHORT-CIRCUIT CURRENT is a measure of the net sodium transport across isolated amphibian skin and bladder (16, 12). Previous work has shown that insulin produced an increase in the short-circuit current across the isolated skin of the frog (7). This effect of insulin could be explained either by an increase in the permeability of the skin to sodium or by a direct effect of the hormone on the sodium-transporting system. The present experiments were carried out in order to choose between these two possibilities by studying in more detail the effect of insulin on the toad bladder, a simpler tissue similar to the skin in being capable of active ion transport (12).

METHODS

Symmetrical halves of the urinary bladder of Bufo marinus were used. Following the procedure described by Ussing and Zerahn (16), and Leaf, Anderson, and Page (12), each half-bladder was mounted as a diaphragm between two Lucite chambers filled with a physiological solution containing 115 mM NaCl; 0.5 mM CaCl₂; 2.4 mM NaHCO₃, and 2.0 mM KCl. The final pH was 8.3 and the measured osmolality 217 milliosmols/kg of water. The area of membrane exposed was 3.14 cm². The membranes were short circuited after mounting and allowed to equilibrate.

The insulin used in this study was pork insulin recrystallized 10 times and was kindly supplied by Dr. J. Schlichtkrull of the Novo Terapeutisk Laboratorium A/S, Copenhagen. A stock solution of insulin was prepared by dissolving 20 mg of the hormone in 1 ml of an appropriate vehicle made up of 115 mM NaCl dissolved in distilled water. The final pH of the stock solution was adjusted to 3.0 with HCl.

Effect of insulin on short-circuit current. It was necessary to find out whether insulin also increased short-circuit current in the toad bladder. Two groups of experiments were performed for this purpose. In the first group, once short-circuit current had stabilized, insulin was added to the solutions bathing both sides of the bladder half to a final concentration of 2.6 × 10⁻⁵ M. Since this involved the addition of 8 μl of insulin stock solution per milliliter of the bathing solutions, control experiments were performed by adding an equal amount of the same solvent but without insulin (115 mM NaCl, pH adjusted to 3.0 with HCl) to a half-bladder obtained from the same toad as the experiment half.

Effect of insulin on the parameters describing sodium transport. The effect of insulin on the parameters describing the sodium transport system was tested in another group of
experiments. The method employed has been described in detail by Curran, Herrera, and Flanigan (2). It allows the determination of the rate coefficients for sodium movement between the bathing media and the cells and vice versa. A three-compartment system is assumed, where compartment 1 is the mucosal bathing solution, compartment 2 corresponds to the transporting tissue, and compartment 3 is the serosal bathing solution.

The bladder halves were mounted as before between two Lucite chambers. The mucosal chamber contained 2.5 ml of physiological solution. The volume of the serosal chamber was 1.0 cm$^3$. In these experiments Na$^{22}$ was used as tracer and was added to the mucosal bathing solution.

Na$^{22}$ was obtained from the Radiochemical Centre, Amersham, England, as the chloride salt in neutral isotonic sodium chloride solution with a specific activity of 0.6 mc/mEq sodium.

The method involved the observation of the approach of tracer to a steady value in the serosal solution and the determination of the amount of tracer in the tissues at the end of the experiment. The former required the rapid and repeated sampling of the serosal solution immediately after isotope was added to the mucosal solution. During the experiment 14–16 samples were taken from the serosal chamber at 30-sec intervals followed by several samples at 2- to 10-min intervals. The specific activity of Na$^{22}$ in the serosal solution was kept very low during the experiment. The serosal chamber was washed out with four to five times its volume of fresh, nonradioactive solution. The sampling and replacing of the solution took less than 1 sec. In order to accomplish the rapid sampling of the serosal solution, the serosal compartment was arranged as a flow chamber. The physiological solution entered the chamber through a tube at the top and left through a tube at the bottom. The inflow tube was connected to a reservoir of fresh, nonradioactive physiological solution through a one-way valve incorporated into a T joint. The vertical arm of the T was connected in turn to a syringe. By means of the syringe, 5 ml of fresh, nonradioactive solution could be aspirated from the reservoir and rapidly flushed through the serosal chamber which was never empty during the experiment. With this method better than 99% of the radioactivity appearing in the serosal chamber was washed out into test tubes and the Na$^{22}$ counted in a scintillation counter. Near the end of the experiment a sample was withdrawn from the mucosal chamber, diluted, and counted. At the end of the experiment the bladder was removed from the chamber and rapidly blotted with filter paper. The exposed portion of the bladder was cut out and counted in a scintillation counter to determine the Na$^{22}$ present. In this series of experiments the total amount of Na$^{22}$ in the bladder at the end of the experiment was taken as equivalent to the total amount of Na$^{22}$ in the transporting cells ($P_{bc}$). No explicit assumption as to the nature of the transporting cells has been made. One or more epithelial cell types may be capable of active sodium transport. One may assess from the data of Choi (1) the proportion of the different cell types making up the epithelium of the toad bladder. Four types of cells have been described: granular cells, mitochondria-rich cells, mucous cells, and basal cells. By far the most numerous are the granular cells which constitute close to three-fourths of the epithelial cells. The mitochondria-rich cells exist in a proportion of one out of eight cells. The mucous cells also occur with a frequency of one out of eight cells. The basal cells are inconspicuous and rather small in size. The pool sizes determined in the present study would require a rather high sodium content in any of the cell types except the granular cells if only one cell type were capable of sodium transport.

This method allows the determination of $k_{12}$, the rate coefficient for sodium movement from the mucosal solution to the cells across the mucosal barrier of the cells; $k_{21}$, the rate coefficient for sodium movement from the cells to the mucosal solution; $k_{23}$, the rate coefficient for sodium movement from the cells to the serosal solution, which would represent the exchange rate for the sodium pump; and $S_{bc}$, the amount of sodium in the transporting system.

The rate of tracer appearance in the solution bathing the serosal surface is given by equation 7 of reference 2

$$\ln \left(1 - \frac{dP_S}{dt}/(dP_0/dt)_\beta\right) = -\lambda t \quad (r)$$

where $dP_S/dt$ is the change with time of the amount of
the bladder passes through the epithelial cells rather than
eters a figure for that in the mucosal bathing solution. From these param-
sodium in transit from the mucosal to serosal surfaces of
be obtained.
the serosal solution ascribable to active transport, may
Na22 in the mucosal solution. The latter method assumes
is the net flux of Na. Alternatively, $S_2$ may be determined
where $P_2W$ is the total mount of Na in the transporting tissue,
$S_2$, the total mount of Na in the transporting tissue,
may be determined by equation 11 of Curran et al. (2),
\[ S_1 = (k_{12}S_1 - \phi_w)/k_{11} \quad (2) \]
where $S_1$ is the total Na in the mucosal solution and $\phi_w$ is the net flux of Na. Alternatively, $S_2$ may be determined by the relation $P_2W/P_1$, where $P_1$ is the specific activity of Na22 in the mucosal solution. The latter method assumes that the specific activity of Na22 in the cells is equal to that in the mucosal bathing solution. From these parameters a figure for $\phi_w$, the flux of sodium from the cells to the serosal solution ascribable to active transport, may be obtained.

Frazier, Dempsey, and Leaf (6) have shown that the sodium in transit from the mucosal to serosal surfaces of the bladder passes through the epithelial cells rather than

![FIG. 2. Effect of addition of insulin first to serosal side and later to mucosal side is compared to effect obtained when insulin is added to both sides simultaneously. Experiment begins at the right-hand side and ends at the left because of direction of movement of chart. Upper record shows effect of insulin on short-circuit current when it is added first to serosal bathing solution and later to mucosal bathing solution. Lower record shows effect of insulin added simultaneously to both sides.]

following some intercellular channels. The contribution of Na22 in the extracellular space available from the mucosal solution as a source of error in the determination of $P_{2W}$ was negligible and was estimated as follows. Half-
bladders were mounted between two conventional Lucite chambers and short circuited. After an incubation period of 3 hr the half-bladders were quickly removed from the chambers, blotted with filter paper, and the wet weight determined by weighing on tared pieces of metal foil. The tissue was subsequently dried for 24 hr at 80°C and reweighed to obtain the dry weight of the bladders.

This method of handling yields reproducible values of water content of 81.2 ± 0.9% (mean ± standard error of the mean (SEM)) of wet weight. Inulin-C14 added to the mucosal solution came into diffusion equilibrium with 2.8 ± 0.6% of tissue water. This amounts to 0.31 ± 0.05 μl/cm2, which is equivalent to a layer of mucosal solution 3 μ in thickness and should not alter significantly the main conclusions derived from this study. The diffusion delay due to the connective tissue layer between the epithelium and the serosal surface (8, 9) should be relatively unimportant in the present instance. Frazier (5) and Essig and Leaf (4) have evaluated the electrical conductance and ionic permeability of the submucosa and of the serosal membrane and found them to be highly permeable. Electron microscopic studies from this laboratory indicate that the submucosal and serosal layers allow the penetration from the serosal solution of particles of thorium dioxide 100 Å in diameter. These layers are not more than 40 μ thick. From the data of

### Table 1. Effect of insulin added to solutions bathing both surfaces of bladder compared to effect obtained with solvent alone

<table>
<thead>
<tr>
<th>Experimental Half-Bladder Short-Circuit Current, μA/3.14 cm²</th>
<th>Control Half-Bladder Short-Circuit Current, μA/3.14 cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial After insulin Inulin effect</td>
<td>Initial After solvent Solvent effect</td>
</tr>
<tr>
<td>76 114 28</td>
<td>17 17 0</td>
</tr>
<tr>
<td>108 130 22</td>
<td>82 82 0</td>
</tr>
<tr>
<td>37 53 16</td>
<td>26 29 3</td>
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<tr>
<td>51 75 24</td>
<td>15 17 2</td>
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<tr>
<td>47 73 26</td>
<td>41 46 5</td>
</tr>
<tr>
<td>50 70 20</td>
<td>54 59 2</td>
</tr>
<tr>
<td>14 24 10</td>
<td>16 20 4</td>
</tr>
<tr>
<td>42 70 28</td>
<td>52 58 0</td>
</tr>
<tr>
<td>110 186 76</td>
<td>90 88 4</td>
</tr>
<tr>
<td>32 48 16</td>
<td>30 30 0</td>
</tr>
<tr>
<td>36 80 44</td>
<td>42 48 8</td>
</tr>
</tbody>
</table>

S.C.C. = short-circuit current. * Insulin effect was determined as the maximum value reached by short-circuit current after insulin addition.

Na22 in the serosal solution before isotope flux reaches a steady value and $<dP_1/dt>$ after a steady state has been achieved; $\lambda = k_{12} + k_{23}$.

The plot of $\log(1 - (dP_1/dt)/dP_1)$ against time obtained in a typical experiment is presented in Fig. 1. It may be seen that, as described by equation 1, a straight line is a good fit for the experimental points. The slopes of the lines obtained when the experimental data were plotted in the manner suggested by equation 1 were used to obtain $k_{12}$ and $k_{23}$. From the ratio $(dP_1/dt)_P P_{2W},$ the rate coefficient for Na movement from the cells to the serosal solution, $k_{23}$, is determined. The rate coefficient $k_{23}$ is considered to describe the active transport process between the cells and the solution at the serosal side. $S_2$, the total mount of Na in the transporting tissue, may be determined by equation 11 of Curran et al. (2),

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**TABLE 1. Effect of insulin added to solutions bathing both surfaces of bladder compared to effect obtained with solvent alone**

<table>
<thead>
<tr>
<th>Initial</th>
<th>After insulin</th>
<th>Inulin effect</th>
<th>Initial</th>
<th>After solvent</th>
<th>Solvent effect</th>
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<tbody>
<tr>
<td>76</td>
<td>114</td>
<td>28</td>
<td>17</td>
<td>17</td>
<td>0</td>
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<tr>
<td>108</td>
<td>130</td>
<td>22</td>
<td>82</td>
<td>82</td>
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<tr>
<td>37</td>
<td>53</td>
<td>16</td>
<td>26</td>
<td>29</td>
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<td>36</td>
<td>80</td>
<td>44</td>
<td>42</td>
<td>48±8</td>
<td>4</td>
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</table>

S.C.C. = short-circuit current. * Insulin effect was determined as the maximum value reached by short-circuit current after insulin addition.
Table 2. Effect of successive addition of insulin first to serosal bathing solution and later to mucosal bathing solution

<table>
<thead>
<tr>
<th>Initial</th>
<th>Insulin</th>
<th>Serosal</th>
<th>Insulin</th>
<th>Mucosal</th>
<th>Total effect</th>
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<tr>
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<td>7</td>
<td>80</td>
<td>17</td>
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<td>72</td>
<td>10</td>
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<tr>
<td>86</td>
<td>110</td>
<td>24</td>
<td>120</td>
<td>10</td>
<td>34</td>
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<tr>
<td>30</td>
<td>40</td>
<td>10</td>
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<td>86</td>
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<td>95</td>
<td>7</td>
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<tr>
<td>86</td>
<td>110</td>
<td>24</td>
<td>130</td>
<td>20</td>
<td>44</td>
</tr>
</tbody>
</table>

Mean effect

\[ \text{SEM} \]

\[ \begin{array}{c|c|c|c|c|c} \hline P & <0.01 & <0.001 & <0.001 \hline \end{array} \]

*Insulin effect was determined as the maximum value reached by short-circuit current after insulin addition.
† After a steady initial short-circuit current was obtained, insulin was added to serosal side to a concentration of 2.6 $\times 10^{-5}$ M.
‡ When short-circuit current stabilized after serosal addition of insulin, hormone was added to mucosal solution to a concentration of 2.6 $\times 10^{-5}$ M. See Fig. 1.

Essig (3) and assuming that the permeability coefficient for sodium is 10 times lower than that for potassium, it can be calculated, using the equation for diffusion in a plane sheet (10), that the time required for 99% equilibration of submucosal sodium with the very low specific activity of the serosal solution is 10 sec. It may be estimated on this basis that the error induced by Na22 entrapped in these layers is less than 1% of the total amount of Na22 in the bladder.

RESULTS

Effect of insulin on short-circuit current. Insulin added to the mucosal and serosal solution simultaneously caused a rise in current which reached a maximum after 30-60 min. Table 1 summarizes the results. The mean increment in current $\pm$ SEM due to insulin was 28 $\pm$ 5 $\mu$A/3.14 cm², which was statistically significant ($P < 0.001$). This represents a 54% increase relative to the mean initial short-circuit current.
The results of the addition of insulin first to the serosal and later to the mucosal solution are shown in Table 2. Figure 2 shows the results of a typical experiment. As may be seen in the figure, the serosal addition of insulin caused a slow rise in current which required 30-60 min to reach a plateau. The increase in current induced by insulin was 16 $\pm$ 4 $\mu$A/3.14 cm². A subsequent addition of insulin to the mucosal solution elicited a second increase in current of 12 $\pm$ 2 $\mu$A/3.14 cm². In this case the current reached a steady value within 15 min after addition of the hormone. As may be seen in Table 2 both the serosal and the mucosal increments in current are statistically significant. The sum of the partial increments, 28 + 4 $\mu$A/3.14 cm², is not different from the mean effect of 28 $\pm$ 5 $\mu$A/3.14 cm² obtained when insulin is added to both sides simultaneously. It is evident that the addition of insulin solvent had no effect on the current. Moreover, it should be mentioned that addition of insulin vehicle to the bathing solutions did not change their pH, which remained at 8.3. Therefore, the possibility that the action of insulin was due to an “acid effect” of the insulin solution (13) may be readily ruled out.

Effect of insulin on sodium transport system. To locate the site of action of the hormone, flux buildup experiments of Na22 were performed as described above. Experimental bladder halves were treated with the usual concentration of insulin on both sides and the corresponding bladder halves served as controls. The results of the flux buildup experiments are presented in Table 3. The rate coefficient for sodium movement from the cells to the serosal solution, $k_{bs}$, increased from a mean of 2.9 to one of 5.4 hr⁻¹ under the influence of the hormone. This represents a mean increase of 86%.

$S_2$, the amount of sodium in the transporting tissue, was calculated from the ratio $P_{22}/P_1$. The results are also shown in Table 3 and agree quite well with results obtained by using equation 1 of Curran et al. (2) quoted under METHODS. The mean values of $S_2$ in the insulin-treated bladders (1.06 $\mu$Eq) are somewhat lower than those obtained in the controls (1.52 $\mu$Eq). However, the difference is not statistically significant. It may well be that the difficulty in stretching all the bladders to the same extent when mounting may have acted against the appearance of a statistical significance in the difference between the sodium pools of the insulin treated and control bladders.
The flux of sodium from the cells to the serosal solution across the serosal barrier, $\phi_{bs}$, was calculated from the mean values of $S_2$ and $k_{bs}$ obtained in the insulin-treated bladders and in the controls. Under the influence of the hormone the values of $\phi_{bs}$ increased from 4.4 to 5.7 $\mu$Eq/hr per 3.14 cm². This increase in $\phi_{bs}$ induced by insulin, 1.3 $\mu$Eq/hr per 3.14 cm², was expected from the short-circuit current measurements. The average increase in short-circuit current caused by the hormone, 28 $\mu$A/3.14 cm², was equivalent to 1.1 $\mu$Eq/hr per 3.14 cm².

DISCUSSION

The results of the present study indicate that insulin increases short-circuit current and sodium transport across the isolated toad bladder. Assuming that the active transport process is located at the serosal barrier of the mucosal cells (6, 15), the effect of insulin may be explained in terms of a direct alteration of the active transport mechanism as represented by $k_{bs}$, the rate coefficient for sodium movement from the cells to the serosal solution. No effect of insulin could be demonstrated on the passive permeability to sodium of the mucosal barrier of the epithelial cells. Thus, the present results are at variance with those described for vasopressin, which has been shown to increase the permeability of the mucosal barrier to sodium (6).

Leaf et al. (11, 15) have suggested that increased so-
or of insulin stock solution in the concentrations used in the present study did not modify the pH of the bathing media. Moreover, the addition of vehicle without insulin hormone, has no effect from this side (1). The action of insulin from the mucosal side probably cannot be ascribed to an acid effect caused by the low pH of the insulin stock solution. Addition of vehicle without insulin had no effect on short-circuit current. An acid effect would not be compatible with the present findings. Acidification of the mucosal medium increases the permeability to sodium of the mucosal membrane of the epithelial cells of the bladder (13) which is at variance with the present results.

An insulinlike effect of vasopressin, possibly due to similarities in molecular configuration in the two hormones, has been found under certain conditions (14). In the present instance, although the increase in short-circuit current brought about by insulin superficially resembles the effect of vasopressin, it cannot be said to represent a vasopressinlike effect of insulin because of the different sites of action on sodium transport of the hormones. It may be added that vasopressin enhances the permeability of the bladder to water whereas insulin appears to have no effect (Leaf, personal communication).

Zierler (17, 18) has observed that the transmembrane potential in rat muscle fibers is augmented by insulin in the absence of added glucose. Similarly, the effect of insulin on short-circuit current and active sodium transport occurs in the absence of added glucose in the bathing media. This indicates that the effect of the hormone on sodium movement is independent of uptake of glucose from the bathing solutions.

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REFERENCES