PN 1206

Effect of insulin on short-circuit current across isolated frog skin in the presence of calcium and magnesium

The short-circuit current is defined as the external current necessary to decrease the potential difference across the frog skin to zero. The short-circuit current corresponds to the net flux of actively transported ions across the skin[1].

In the present work the effect of insulin on short-circuit current in controls and in calcium- and magnesium-treated skins was studied. Paired experiments were made using symmetrical halves of abdominal skins of *Rana pipiens*. One skin half served as "control" for the other which will be called "experimental" half. Each skin half was mounted as a flat sheet between solutions contained in 2 lucite chambers. The short-circuit current was measured following the method of USSING AND ZERAHN[1]. The area of the skin exposed was 3.14 cm^2.

The solutions used are shown in Table I. Sodium concentration remained unchanged at 69.0 mM. Solution A, which was used as control, contained 44.8 mM choline and no calcium. In the experimental solutions B and C, choline was replaced by 22.4 mM of either calcium or magnesium.

TABLE I

COMPOSITION OF BATHING SOLUTIONS*

Solution	*Sodium (mM)*	*Choline (mM)*	*Calcium (mM)*	*Magnesium (mM)*
A	69.0	44.8	—	—
B	69.0	—	22.4	—
C	69.0	—	—	22.4

* Other ions present: potassium, 2.4 mM; chloride, 113.5 mM; bicarbonate, 2.4 mM.

To study the effect of insulin on calcium-treated skins, a series of 7 experiments was made. Initially, both sides of both skin halves were bathed in Solution A, which was calcium free. 0.4 mM EDTA (tetrasodium salt) was added to the solution bathing both sides of the control skin half, and only to the outer side of the experimental skin half. When a steady short-circuit current was obtained, the solution bathing the outer side of the experimental skin half was replaced with solution B, containing 22.4 mM calcium. The short-circuit current would fall after the addition of calcium. When a steady level was attained, insulin (glucagon-free) was added to both sides of both skins halves to obtain concentration of 4 units/ml solution. From results given in Table II, it may be seen that calcium reduced the short-circuit current to about a half of the control values and insulin restored it to about 3/4 of the control values. Insulin increased the short-circuit current only in the calcium-treated skin halves. In the calcium-free controls practically no change was observed. The effect of insulin on the calcium-treated skin halves and on the controls may be compared in terms of the fractional increase in the short-circuit current of the skin half. Fractional increase is defined as $I/I_0 - 1$, where I is the current in the presence of insulin and I_0 is the current in the absence of insulin. The fractional increase in current due to insulin is much greater in the calcium-treated skins than in the controls. The differences treated as paired data are statistically significant at the $P < 0.05$ level.

To study the effect of insulin on magnesium-treated skins, a series of 6 experiments was made in which the procedure described above was followed with the difference that Solution C (22.4 mM of magnesium) was used, instead of Solution B, as the solution bathing the outer side of the experimental skin half.

As may be seen in Table III, magnesium reduced the short-circuit current to about a fourth of the control values, and insulin restored it to about half the control values. A rise in short-circuit current was observed only in the magnesium-treated skin halves. In the magnesium-free controls, practically no change was observed. The effect of insulin on the magnesium-treated skin halves and on the controls were compared in terms of the fractional increase in current. The fractional increase in current due to insulin is much greater in the magnesium-treated skin halves than

TABLE II

EFFECT OF INSULIN ON THE SHORT-CIRCUIT CURRENT OF CALCIUM-TREATED AND CONTROL SKINS

Expt.	*Calcium-treated skins*			*Control skins**		
	I_0** (μA)	I*** (μA)	$I/I_0 - 1$ §	I_0 (μA)	I (μA)	$I/I_0 - 1$
1	42	72	0.71	126	120	−0.05
2	62	84	0.35	100	92	−0.08
3	34	60	0.79	134	150	0.12
4	120	130	0.08	200	185	−0.07
5	50	82	0.64	134	144	0.08
6	110	135	0.24	190	190	0.00
7	110	115	0.05	215	215	0.00

* 0.4 mM EDTA added to the solutions bathing both sides of the skin.
** Current in the absence of insulin.
*** Current after insulin addition.
§ $I/I_0 - 1$ is the insulin-induced fractional increase in current.

TABLE III

EFFECT OF INSULIN ON THE SHORT-CIRCUIT CURRENT OF MAGNESIUM-TREATED AND CONTROL SKINS

Expt.	*Magnesium-treated skins*			*Control skins**		
	I_0** (μA)	I*** (μA)	$I/I_0 - 1$ §	I_0 (μA)	I (μA)	$I/I_0 - 1$
1	34	70	1.06	280	280	0.00
2	56	80	0.43	275	250	−0.19
3	44	102	1.32	122	120	−0.02
4	18	52	1.89	61	73	0.19
5	26	42	0.62	38	33	−0.13
6	30	76	1.53	158	130	−0.18

* 0.4 mM EDTA added to the solutions bathing both sides of the skin.
** Current in the absence of insulin.
*** Current after insulin addition.
§ $I/I_0 - 1$ represents the insulin-induced fractional increase in current.

in the controls. The differences, treated as paired data, are statistically significant at the $P < 0.01$ level. It had been found sufficient to add insulin to the outside solution to obtain an effect.

Thus, insulin appears to increase the short-circuit current across frog skin only if calcium of magnesium are present in the solution bathing the outer side of the skin.

It has been reported that insulin increases the membrane potential of th... muscle of rat[2], that insulin and antidiuretic hormone share some metabolic action[3], and that insulin and lactate stimulate active sodium transport in muscle[4].

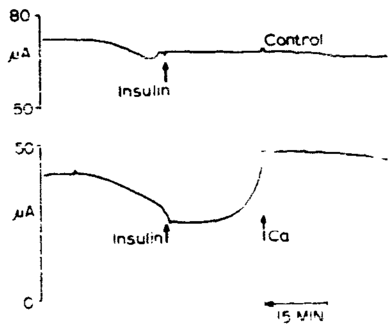

Fig. 1. Continuous record of short-circuit current during a typical experiment. The experiment begins at the right-hand side and ends at left due to the direction of movement of the chart. The upper record shows the current in the control experiment, without calcium or magnesium. Insulin produces only a slight increase in current. The lower record shows the effect of insulin on the short-circuit current after it has been partially inhibited by calcium. Insulin produces a large and maintained increase in the current.

The present experiments demonstrate an increase of the short-circuit current by insulin, in frog skins immersed in solutions without glucose or any other metabolic substrate. Further experiments are required to ascertain whether this action is on the active-transport mechanism or on the permeability of the skin.

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