INHIBITION OF SODIUM PUMP AND INCREASE OF PASSIVE FLUXES BY X-RAYS IN HUMAN ERYTHROCYTES

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Received July 6, 1962

X-irradiation induces an increase of sodium and a decrease of potassium content in erythrocytes (1, 2, 3). This effect is true also for a number of other cells and tissues (4 to 9 and others) and it can be assumed that it is a general phenomenon of the x-irradiated cell, providing a large enough dose of x-rays is given.

The mechanism by which x-rays produce this effect, however, is not known and therefore the following experiments were performed to investigate the changes of active and passive fluxes of sodium across the erythrocyte membrane after x-irradiation.

Human blood freshly drawn (coagulation inhibited by heparin) was centrifuged at 500 x g for 5 min. and plasma and buffy coat were removed by suction. The red cells were resuspended in potassium-free Ringer (KCl was replaced by NaCl and a sodium phosphate buffer was used) containing 0.2 gm% of glucose and washed twice. After each washing the top most layer of cells was carefully removed to eliminate white cells. Finally, the packed red cells were divided in two parts, one of which was x-irradiated by a Dermopan machine with a Berillium window operating at 50 Kv, 25 mA with 1 mm of Al as a filter. The dose rate was 12,5 rads/sec.. The thickness of the x-irradiated suspension was 0.3 cm.. About 1 ml aliquots of

normal and x-irradiated packed cells were incubated in flasks containing 25 ml of prewarmed Ringer-phosphate free of or containing 10 mM potassium, with 0.2% glucose and Na^{22} as a tracer, in a water bath at 37°C and shaken by a motor during the period of incubation. Samples from the flasks were withdrawn immediately after the addition of erythrocytes to the Ringer solutions (0 time) and, thereafter at measured intervals of about 20 min.. Immediately after withdrawal from the flasks the red cell suspension samples were centrifuged for 3 min. at 500 x g and washed twice with ice cold sodium free choline

Table 1

Sodium uptake and sodium net change in x-irradiated human erythrocytes

No of exp.	additions to the potassium-free Ringer	x-ray dose	sodium uptake	sodium net change
		rad	mM/l cells/ 20 min.	mM/l cells/ h.
(14)	10 mM K	0	0.70±0.012	-1.51±0.032
	none	0	1.13±0.008	+0.89±0.027
(14)	10 mM K	890	0.77±0.010	-1.09±0.027
	none	890	1.23±0.013	+1.01±0.033
(12)	10 mM K	4450	1.03±0.022	-0.50±0.016
	none	4450	1.63±0.018	+1.70±0.037

arithmetic mean ± standard error of mean

Ringer-phosphate (NaCl was replaced by choline chloride) and once with ice cold isotonic choline chloride solution as rapidly as possible, according to GLYNN (10). After the washings were performed the erythrocytes were lysed and the haemolysates were divided in aliquots for measurement of radioactivity, haemoglobin

and sodium. Na^{22} radioactivity was measured by a DS 202 scintillation well detector connected with a 192 B Scaler and a 1810 radiation analyzer (Nuclear Chicago). Haemoglobin was determined as oxyhaemoglobin and the volume of cells was obtained by comparison with a standard haemolysate obtained by lysing a known amount of cells packed by centrifuging at 1500 x g for 1 h, in a known amount of water. Sodium was determined by flame photometry according to the common rules.

The sodium influx (m_i) was calculated from the initial slopes of the activity-time curves which are of the form (10) :

$$\text{activity} = \frac{m_i}{k}\left(1-e^{-kt}\right)$$

where m_i is the influx and k is proportional to efflux. By measuring net sodium changes contemporaneously the sodium efflux (m_e) was also obtained

$$m_e = m_i - \left(\frac{d\ Na_i}{dt}\right)$$

where Na_i is the intracellular concentration of sodium. GLYNN (10) showed that in absence of potassium in the external medium the active transport of sodium, i.e. the sodium pump, is abolished and only the passive fluxes occur. The quota of active efflux of sodium, therefore, can be calculated as following

$$m_{e(active)} = \left(m_e\left[K_o\right] = 10\ mM\right) - \left(m_e\left[K_o\right] = 0\right)$$

where $\left[K_o\right] = 10$ mM and $\left[K_o\right] = 0$ indicate the presence of 10 mM potassium or the absence of potassium in the external medium.

From the results shown in the tables it can be concluded that the increase of sodium content in erythrocytes after 890 rads depends upon decrease of the active extrusion of sodium (sodium pump), the passive fluxes being only slightly influenced. After 4450 rads not only a larger decrease of the sodium pump occurs but an unbalanced increase of both passive

Table 2

Active and passive fluxes in x-irradiated human erythrocytes calculated from data in Table 1

x-ray dose	active efflux of sodium (sodium-pump)	Δ	passive efflux of sodium	Δ	passive influx of sodium	Δ
rad	mM/l cells/ h.	%	mM/l cells/ h.	%	mM/l cells/ h.	%
0	1.1		2.5		3.4	
890	0.6	-45	2.7	+ 8	3.7	+ 9
4450	0.3	-73	3.2	+28	4.9	+44

flux rates, with influx prevailing over efflux, is also induced, which contributes to the abnormal high level of sodium in the x-irradiated cells.

The higher radiosensitivity of the sodium pump in comparison with passive fluxes in addition to the qualitatively different effect of x-irradiation on active and passive fluxes led to suggest that the substrate of x-ray action is different in the two cases. This is comprehensible if it is born in mind that the sodium pump is operated by metabolism while passive fluxes follow the electrochemical gradient and will flow undisturbed until physico-chemical damages to the membrane occur.

Experiments under way indicate that the inhibition of the sodium pump by x-rays does not depend upon inhibition of the cellular system fournishing energy to the ion pump but upon interference of x-irradiations with the biochemical mechanism of the pump in the membrane.

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