Radiation-Induced Ouabain-Insensitive K⁺ Exchange of Erythrocytes under the Quasi-Physiological Conditions

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Erythrocytes irradiated with 60Co γ-rays took up more extracellular ⁴²K⁺ and released more intracellular ⁴²K⁺ during and immediately after irradiation at 37°C than did unirradiated control. The result of K⁺ transport was contrasted with the well-known K⁺ loss on prolonged incubation. However, total K⁺ and ATP levels in irradiated cells did not change significantly under the same conditions. Such radiation-induced K⁺ exchange was insensitive to 1 mM ouabain, an inhibitor of active transport, but sensitive to 20 mM NaF, an inhibitor of glycolysis. These results suggest that this K⁺ exchange is associated with glycolysis, but not with ouabain-sensitive ATPase.

1. INTRODUCTION

Radiation is known to damage erythrocytes causing loss of intracellular K⁺ and taking up of extracellular Na⁺ (1, 2). Passive and/or active transport system could be considered the target of the damage (1, 2). But the passive transport system seems to participate in the process of damage, because the inhibition of active transport by ouabain (3) or low temperature (4) does not decrease the damage. However, the detail of its mechanism has not yet been clarified. Moreover, little has been investigated on the damage of transport systems immediately after irradiation under the physiological conditions (e.g., in vivo) probably because no greater damage to K⁺ transport system occurred under the conditions even after prolonged post-irradiation incubation. Accordingly, the dynamic inward and outward transports of K⁺ in irradiated erythrocytes were examined under quasi-physiological conditions that are similar to in vivo conditions with respect to the medium and temperature.
2.1. Preparation of erythrocytes

Guinea-pig erythrocytes were prepared according to the method of Myers and Bide (4) with a slight modification. Fresh heparinized blood was centrifuged and the cells were then washed twice with modified Krebs-Ringer solution (pH 7.2) containing 130 mM NaCl, 4.4 mM KCl, 1.2 mM CaCl₂, 1.1 mM MgSO₄, 5.5 mM glucose, 4.8 mM Na₂HPO₄, 1.2 mM KH₂PO₄ and 20% calf serum. The washed erythrocytes were finally resuspended in the same solution supplemented with 0.001% penicillin and 0.007% streptomycin to a haematocrit (Ht) of 20%. These conditions at 37°C are called quasi-physiological conditions.

2.2. Irradiation of cells

Cells were irradiated with ⁶⁰Co γ-rays at the dose rates of approximately 350 rad/min. Irradiation and subsequent incubation were carried out at 37°C.

2.3. Determination of K⁺ and ATP contents in erythrocytes

The cell suspensions were centrifuged at 1,000 x g for 5 min, and the pellet was washed twice with isotonic choline chloride solution. After the haemolysis with 0.01% LiCl and subsequent centrifugation, K⁺ ions in the supernatant, diluted appropriately, were determined with a Hitachi flame photometer (5).

The ATP content in the cells, i.e., in the supernatant obtained above, were determined with the aid of hexokinase and glucose-6-phosphate dehydrogenase. For each mole of ATP, 1 mole of NADPH is formed: this product was determined by measurement of the absorbance at 340 nm (6).

2.4. Measurement of ⁴²K incorporation into cells

For measurement of K⁺ incorporation into cells, ⁴²KC₁ (Japan Atomic Energy Research Institute) was added to the cell suspension immediately after irradiation, and each 2 ml aliquot of the mixture was taken at appropriate times, followed by washing of the cells with isotonic choline chloride. The radioactivity of ⁴²K in cells was determined in a Packard auto-gamma scintillation spectrometer. Specific activity of ⁴²K varied from ca. 60 — 400 μCi/ml.

2.5. Measurement of ⁴²K release from cells

Cells were incubated with shaking for 6 hr at 37°C in the medium containing ⁴²KC₁, and then washed twice with the same medium excluding ⁴²KC₁. The cells were irradiated with ⁶⁰Co γ-rays, and each 2 ml aliquot, taken after appropriate incubation times, was centrifuged at 1,000 x g for 5 min. The radioactivity of the supernatant was determined and the value was used as an index of K⁺ release from cells.
2.6. Inhibition of energy metabolism

Inhibitors, ouabain and NaF, were added to cell suspensions at the concentrations of 1 mM and 20 mM, respectively, 10 min before irradiation and then shaken gently at 37°C.

3. RESULTS

3.1. Increase in the incorporation of extracellular K+ into irradiated cells

After exposure with 10 krad of 60Co γ-rays, the cells were incubated with 42KC1 at 37°C. As shown in Fig. 1, both irradiated and unirradiated cells rapidly took up 42K from the medium, but the former did more than the latter. However, the difference in 42K uptake between irradiated and unirradiated cells disappeared at 10 hr of incubation (data not shown).

3.2. Sensitivity of K+ uptake to inhibitors of energy metabolism

To examine whether the energy metabolism is involved in the induced K+
uptake, the cells that had been treated with 1 mM ouabain, an inhibitor of active transport, or 20 mM NaF, an inhibitor of glycolysis, were irradiated with 10 krad, and then incubated with $^{42}$KCl. As Fig. 1 shows, $^{42}$K uptake into cells was greatly decreased by ouabain. The resultant ouabain-insensitive $^{42}$K uptakes in irradiated and unirradiated cells represented about 30 and 15% of the total $^{42}$K uptakes, respectively. But the net radiation-induced $^{42}$K uptake was not changed by ouabain. On the other hand, 20 mM NaF depressed this radiation effect as shown in Fig. 1. Only radiation-induced $K^+$ uptake was inhibited in the presence of 20 mM NaF, but $K^+$ uptake in unirradiated cells was not changed by NaF at all. Therefore, these results indicate that the radiation-induced $^{42}$K uptake is closely related to an activity of glycolysis, but not to the ouabain-sensitive Na$^+$-K$^+$ ATPase.

3.3. Increase in the release of intracellular $^{42}$K from irradiated cells

For examination of whether intracellular $K^+$ is rapidly released on radiation, the cells containing $^{42}$K were prepared by incubating cells with $^{42}$KCl at 37°C for 6 hr. After washed, these cells were irradiated with 10 krad and then incubated for appropriate times at 37°C. The $^{42}$K loss in the cells is shown in Fig. 2. Irradiation of the cells with 10 krad led to a significant increase in the release of intracellular $^{42}$K, as shown in the graph. The data indicate that the radiation-induced release of $^{42}$K is closely related to the activities of glycolysis and the Na$^+$-K$^+$ ATPase.

Fig. 2. Effects of 1 mM ouabain (□, ■) on $^{42}$K release from the irradiated (10 krad) (■, ■) and unirradiated (○, □) cells (2 ml, Ht = 0.2) into which $^{42}$K had been incorporated.
ated cells released intracellular $^{42}$K more than unirradiated ones even immediately after irradiation. The result indicates that radiation causes not only incorporation but also release of K+, that is, a rapid K+ exchange in cells.

The effect of ouabain on $^{42}$K release from cells was also investigated. Treatment of the cells with 1 mM ouabain depressed their $^{42}$K loss, but did not affect the radiation-induced K+ exchange (Fig. 2).

3.4. Time course of pulse-labelled cells with $^{42}$K

The K+ exchangeability of irradiated cells was further investigated with time by the $^{42}$K pulse-labelling method. As described in the preceding section, the K+ exchange means the simultaneous uptake and loss of K+ in cells. In this experiment, however, K+ uptake was used as an index of K+ exchange because of difficulty in pulse-labelling intracellular K+. Cell suspensions were incubated with $^{42}$KCl for 10 min at appropriate times after irradiation, and $^{42}$K in cells was determined. The results are given in Fig. 3. The $^{42}$K uptake increased over the first 3 hr of incubation after irradiation with 21 krad, and hereafter decreased with time, resulting in no difference at 8 hr.

![Fig. 3. Time course of K+ exchangeability of cells (2 ml, Ht = 0.2) after irradiation with 21 krad of $^{60}$Co $\gamma$-rays using $^{42}$K pulse-labelling method.](image)
3.5. K$^+$ and ATP contents in cells

The K$^+$ and ATP contents in cells during the time that radiation-induced K$^+$ exchange occurred were examined to know whether any correlation exists between these contents. As shown in Fig. 4, the K$^+$ and ATP contents were not altered significantly at 5 hr after irradiation with the doses of up to 25 krad, which implies no meaningful correlation.

As shown in Fig. 5, 20 mM NaF that inhibited the radiation-induced K$^+$ exchange decreased total ATP content of cells due to inhibition of glycolysis, although it did not affect the radiation effect on ATP content.

![Graph showing ATP and K+ contents](image)

**Fig. 4.** ATP (○) and relative K$^+$ (■) contents in cells after 5 hrs post-irradiation incubation.
4. DISCUSSION

Actions of ionizing radiation on living cells or their functions are virtually inhibitory, and little is known of its stimulatory action (7). The present experiments suggest this type of radiation action. The ouabain-insensitive K⁺ exchange in erythrocytes was induced by radiation, but this is not simply considered attributable to increased passive transport of K⁺, because intracellular K⁺ concentration is much higher than extracellular K⁺ concentration and was not changed by radiation (Fig. 4).

The radiation-induced ouabain-insensitive K⁺ exchange was depressed by inhibition of glycolysis, implying a correlation between the radiation-induced K⁺ exchange and glycolysis. Therefore, it seems probable that the radiation-induced K⁺ exchange in cells is correlated with hydrolysis of ATP supplied from glycolysis. However, intracellular K⁺ and ATP contents did not vary during the
radiation-induced K⁺ exchange, which seemingly denies that ATP participates in the K⁺ exchange. But a constant intracellular ATP content does not necessarily mean the absence of ATP hydrolysis, because a rapid phosphorylation of ADP may occur. Taking this into consideration, the observation that ouabain did not alter the radiation-induced K⁺ exchange suggests the involvement of ouabain-insensitive ATPase in the radiation-induced K⁺ exchange, although hydrolysis of ATP must be proved.

The present results also indicate that there are two types of radiation-induced change in K⁺ transport at 37°C. One is, as described in detail, the radiation-induced K⁺ exchange without net transport of K⁺ occurring mainly during and up to 10 hr after irradiation. The other is the well-known K⁺ loss in cells occurring long after irradiation. Of course, the magnitude and time course of these changes are dependent on the dose of radiation. However, these two types of changes in intracellular K⁺ should be repaired by incubation at higher temperatures (5) (Fig. 5). The relation between the two, if any, is of interest, and requires further investigation.

It is uncertain whether these changes and repair were affected by membrane lipids, but the interaction is likely because the activity of membrane-bound, especially intrinsic, enzyme is regulated by the micro-(lipid)-environment in membranes (7) and because radiation damages to the modified membrane are varied by its substituted unsaturated-fatty acids (8-10). The radiation-induced change in the environment of enzymes might alter their activity under the experimental conditions in vitro different from the conditions in situ.

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