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THE EFFECT OF IONIZING RADIATION ON AMINO ACID TRANSPORT SYSTEMS IN THYMOCYTES

L. KWOCK* and D. F. H. WALLACH

Division of Radiobiology, Tufts-New England Medical Center, 136 Harrison Avenue, Boston, Mass. 02111 (U.S.A.)

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SUMMARY

The radiation effect on K^+ transport has been implicated as a cause for the high radiosusceptibility of thymocytes. We have investigated the effects of radiation (0.05–10 krad) on the Na^+-dependent transport of 2-aminoisobutyric acid and the Na^+-independent transport of 1-aminocyclopentane-1-carboxylic acid and b-2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid to determine if these transport systems are similarly impaired. Irradiation with 0.05–10 krad lowers 2-aminoisobutyric acid uptake both in vitro and in vivo (15–70%) while not affecting 1-aminocyclopentane-1-carboxylic acid or b-2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid uptake. The 2-aminoisobutyric acid radiation response is time- and dose-dependent and parallels the effects observed for the K^+ system.

Lineweaver–Burk plots of $1/v$ vs $1/S$ for 2-aminoisobutyric influx at different radiation doses show alterations of V only (control 2.1 ± 0.05 nmoles 2-aminoisobutyric acid per 10^7 thymocytes per 10 min; 10 krad 0.71 ± 0.03 nmoles 2-aminoisobutyric acid per 10^7 thymocytes per 10 min). The K_m remains essentially unaltered (control $K_m = 0.48 \pm 0.03$ mM; at 5 krad $K_m = 0.57 \pm 0.05$ mM; and at 10 krad $K_m = 0.50 \pm 0.04$ mM). We obtain fractional 2-aminoisobutyric acid efflux coefficients of 0.021 ± 0.001 min^{-1} for unirradiated thymocytes vs 0.027 ± 0.003 min^{-1} and 0.025 ± 0.001 min^{-1} after 5 and 10 krad, respectively.

We found that insulin and concanavalin A which increase 2-aminoisobutyric acid influx by increasing V, protects the thymocyte 2-aminoisobutyric acid system against the effects of radiation. Trypsin which mimics the effect of insulin in fat cells and hepatocytes was also found to protect the transport system.

The results suggest Na^+–K^+ transport and 2-aminoisobutyric acid transport may be coupled in rat thymocytes and that the coupling factor is radiosensitive. This component affects either the rate of substrate turnover, by possible rearrangements of plasma membrane components, or the number of carrier sites.

* To whom correspondence should be addressed.

INTRODUCTION

Lymphoid cells succumb very readily to ionizing radiation in processes which take place during the interphase of the mitotic cycle. Thus, rat thymocytes die within a few hours after exposure to as little as 50 rad, without the intervention of cell division [1, 2]. The cause of this interphase death remains uncertain, but considerable evidence implicates an unusual radiosusceptibility of plasma membrane transport systems. For example, at 37 °C a 50% depletion of the normal intracellular K^+ occurs after as little as 30 rad of X-ray; moreover, the cells also become permeable to larger, normally more excluded substances, such as eosin [3, 4]. In addressing this matter, Chapman and Sturrock [5] compared the effects of X-ray with those of temperature, cardiac glycosides and substitution of choline for Na^+ and K^+ upon the cation transport of thymocytes in vitro. Their studies suggest two radiation-induced lesions: (a) after low irradiation levels (0.2–4 krad) the major factor affecting potassium loss is an impairment of the metabolic mechanisms involved in active transport and (b) higher levels of irradiation also change passive plasma membrane permeability. Exploring the possibility that other transport systems may also be generally impaired in cellular radiation damage, Archer [6] finds that irradiation of Ehrlich ascites carcinoma impairs active amino acid transport, as well as cation transport [7]. She suggests that radiation does not affect transport elements per se, but rather the macromolecules linking diverse transport systems. Unfortunately, these studies required high radiation doses.

In view of Archer's work, the known linkage between the transport of some amino acids and alkali cations as well as the established radiosensitivity of membrane cation transport in lymphoid cells, we have investigated the effect of radiation on the transport of three non-metabolizable amino acids; (a) 2-aminoisobutyric acid, whose transport requires a functioning Na^+ pump [8]; (b) 1-aminocyclopentane-1-carboxylic acid and (c) b-2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid, amino acids which do not require Na^+ for their transport [8, 9]. Our aims are 3-fold: (a) clarification of "linkage" mechanisms in thymocyte membrane transport; (b) definition of the molecular events involved in membrane radiation damage; and (c) development of techniques which will protect the immune response against ionizing radiation.

Our data show that low-radiation doses damage the transport of 2-aminoisobutyric acid (Na^+-dependent system) but not the transport of 1-aminocyclopentane-1-carboxylic acid and b-2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (Na^+ independent). We also demonstrate that insulin, concanavalin A and trypsin, which increase the maximal rate of uptake (V) of 2-aminoisobutyric acid prevent irradiation damage of this transport system.

MATERIALS AND METHODS

Wistar–Lewis female rats, 6–8 weeks old were purchased from Charles River Breeding Laboratory. 2-Aminoiso-[^{14}C]butyric acid (9.79–12.2 mCi/nmole), b-2-aminobicyclo-[2.2.1]-[^{14}C]heptane-2-carboxylic acid (4.54 mCi/mmole), 1-aminocyclo-[^{14}C]pentane-1-carboxylic acid (8.9 Ci/mole), [^{3}H]insulin (100 mCi/g) were purchased from New England Nuclear, porcine insulin (26.5 units/mg) from Schwarz-Mann, trypsin from Grand Island Biological Company, and concanavalin A from

Miles-Yeda Ltd. Other chemicals were of the highest grade of purity obtainable from commercial sources.

Isolation of thymocytes

6–8-week-old rats are anesthesized with chloroform and then exsanguinated by puncture of the inferior vena cava to reduce erythrocyte contamination of thymocyte preparations. We then transfer the thymocytes to iced RPMI 1629 medium* (Associated System, Inc.), mince gently with scissors, rinse with 0.017 M Tris–HCl, 0.14 M NH_4Cl, pH 7.2, to lyse remaining red blood cells, filter through four layers of cotton gauze and then centrifuge at $1500 \times g \cdot$ min (International Equipment Co. Clinical Centrifuge Model CL). After decanting the supernatant, we wash the cells with phosphate-buffered saline, pH 7.4 [10], containing 15 mM α-D-(d)-glucose (Grand Island Biological Company), pellet the cells at $1500 \times g \cdot$ min, remove the supernatant and resuspended in phosphate-buffered saline plus 15 mM glucose. (For the 8- and 24-h incubations the cells are resuspended in RPMI 1629 medium). At least 90% of the thymocytes prepared in this manner are viable as measured by the exclusion of 0.1% trypan blue.

Irradiation

For radiation levels between 50 and 250 rad, 3 ml of cells are suspended in RPMI 1629 in 4 cm×8.5 cm bags of 24-μm thick, internally etched FEP-Teflon (Instrimentation Laboratoreis, Inc.), and the cell suspension irradiated with a clinical ^{60}Co source (Theratron-80; Atomic Energy of Canada, Ltd.) with computer controlled dose levels and a dose rate of 102 rad/min.

For radiation levels between 0.5–10 krad we use a ^{137}Cs source (Radiation Machinery Corporation Model M38 Gammator) with a dose rate of 510.6±3.5% rad/min at the center of the radiation chamber. For those studies the cells are placed in 15-ml siliconized plastic culture tubes (Falcon) during irradiation. After irradiation the cells are incubated at 37 °C in a 95% air–5% CO_2 atmosphere for stated time periods before measuring amino acid uptake.

Amino acid uptake

The cells are incubated ($20 \cdot 10^6$–$40 \cdot 10^6$ per ml in phosphate-buffered saline plus 15 mM glucose) in 15-ml siliconized plastic centrifuge tubes (Falcon) at 37 °C. To each 0.9 ml of cell suspension (in phosphate-buffered saline plus 15 mM glucose), we add 0.1 ml amino acid containing phosphate-buffered saline plus 15 mM glucose (final amino acid concentration $1 \cdot 10^{-4}$ M) and allow uptake to proceed for 10 min before adding 3 ml of iced phosphate-buffered saline. After centrifuging at $5000 \times g \cdot$ min, at 4 °C (International Equipment Co. Model B20A centrifuge, Rotor Model 947) we aspirate the supernatant fluids and wash the cell pellets twice more, each time with 3 ml of iced phosphate-buffered saline with 15 mM glucose. To release the ^{14}C-labeled amino acid from the washed cell pellets, we add 1 ml of 10% $HClO_4$ to each, wait overnight, and centrifuge at $5000 \times g \cdot$ min. We then take 0.5-ml aliquots of the supernatants, add these to counting vials (Packard Instrument Co.) containing 10 ml of Bray's scintillation solution [11] and count the samples on a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3320). Quenching variations are negligible as determined by use of an automatic external standard. [^3H]Inulin is used as a

* For composition, see ref. 27.

marker for extracellular fluid and find that less than 0.1% of the ^{3}H radioactivity remains with the pellet after our washing procedure. Each assay is run in triplicate. Results are expressed in terms of viable (trypan-blue negative) cells, since independent studies show essentially no 2-aminoisobutyric acid uptake by trypan blue positive cells.

Efflux studies

We incubate $3 \cdot 10^{7}$ cells per ml under standard conditions with $1 \cdot 10^{-4}$ M ^{14}C-labelled 2-aminoisobutyric acid for 2 h. The cells are then transferred to 10 ml of iced incubation medium (phosphate-buffered saline plus 15 mM glucose), centrifuged at $5000 \times g \cdot$ min at 4 °C and the cell pellet washed twice with 12 ml iced phosphate-buffered saline plus 15 mM glucose. We then resuspend the cells in the buffer, allow the cells to thermally equilibrate to 37 °C and measure the rate of 2-aminoisobutyric acid efflux into the medium for 30 min at 5-min intervals. We centrifuge the replicate samples at $5000 \times g \cdot$ min and determine the amount of radioactivity remaining in the cell as previously described. The fractional efflux coefficient is the slope of the straight line obtained by plotting ln ([2-aminoisobutyric acid] at time $= t$)/([2-aminoisobutyric acid] at time $= 0$) vs time.

RESULTS

Uptake of 2-aminoisobutyric acid and effects of irradiation

Table I shows the initial uptake of 2-aminoisobutyric acid in rat thymocytes as a function of time after incubation. After 6–7 h incubation at 37 °C, 50–70% of the thymocytes remain viable, as judged by the exclusion of 0.1% trypan blue. For longer incubation periods, cell viability is usually 10–20% higher in both the control and irradiated samples when the cells are incubated in FEP-Teflon bags.

TABLE I

2-AMINOISOBUTYRIC ACID UPTAKE BY RAT THYMOCYTES

2-Aminoisobutyric acid uptake expressed in terms of viable cells.

Time of incubation (h)	2-Aminoisobutyric acid uptake (nmoles 2-aminoisobutyric acid/10^{7} thymocytes per 10 min)*	Viability** (%)
Control cells		
2	0.40±0.004	78
4	0.37±0.005	74
6	0.42±0.006	70
Irradiated cells (0.5 krad)***		
2	0.31±0.01	79
4	0.28±0.01	58
6	0.19±0.01	50

* Mean and ranges of a representative set of triplicate determinations.
** Viability determined by exclusion of trypan blue.
*** Irradiation ^{60}Co.

Table I also shows the increasing manifestations of radiation damage at increasing time after exposure to 0.5 krad γ-ray. The accumulation of 2-aminoisobutyric acid by the rat thymocytes decreases and the proportion of non-viable cells increases. 5 h after irradiation viability drops to 50% compared to 70% in the controls, and 2-aminoisobutyric acid accumulation declines by almost 60%.

Effects of irradiation on the 2-aminoisobutyric acid uptake of rat thymocytes

Table II shows the uptake of 2-aminoisobutyric acid at an initial concentration of $1 \cdot 10^{-4}$ M, 7–8 h after low level irradiation of thymocytes. After 50 rad the uptake drops by 15%; after 250 rad the decrement becomes 70%. Fig. 1 illustrates the radiation impairment of 2-aminoisobutyric acid uptake as a function of time. After thymocytes receive a radiation dose of 1–10 krads (^{137}Cs) we observe a sharp, dose-dependent drop in uptake within the first 20–40 min after exposure; a more gradual decrease in 2-aminoisobutyric acid accumulation follows. Irradiation with 0.5 krad (^{60}Co) produces maximum inhibition of uptake after about 7 h.

TABLE II

EFFECT OF LOW LEVEL RADIATION ON THE 2-AMINOISOBUTYRIC ACID UPTAKE OF RAT THYMOCYTES

Radiation dose (rad)*	Time after irradiation (h)	2-Aminoisobutyric acid uptake (nmoles 2-aminoisobutyric acid/10^7 thymocytes per 10 min)**	Decrease in 2-aminoisobutyric acid uptake (%)
0	8	0.72±0.01	0
50	8	0.61±0.01	15
150	8	0.34±0.004	43
250	8	0.22±0.01	70

* Irradiation ^{60}Co.

** Expressed in terms of viable cells (trypan blue negative).

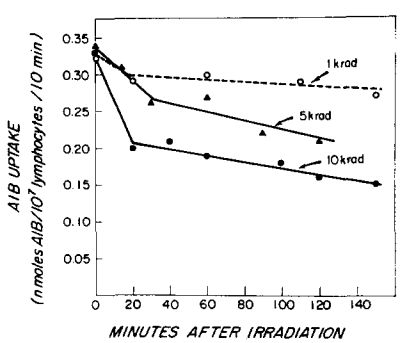

Fig. 1. 2-Aminoisobutyric acid (AIB) uptake as a function of time after irradiation. The final concentration of 2-aminoisobutyric added to each sample was $1.0 \cdot 10^{-4}$ M. Each point is the mean of triplicate determinations. The uptake of 2-aminoisobutyric is expressed in terms of viable cells.

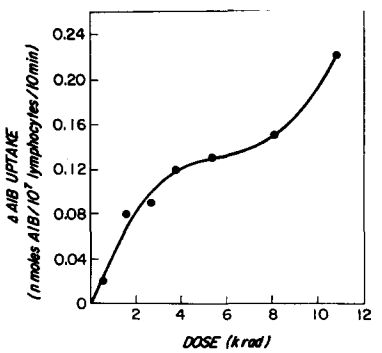

Fig. 2. Decrease in 2-aminoisobutyric acid (AIB) uptake as a function of radiation dose. The final concentration of 2-aminoisobutyric acid added to each sample was $1.0 \cdot 10^{-4}$ M and each is the mean of triplicate determinations. The uptake is expressed in terms of viable cells.

Fig. 2 depicts the decline of 2-aminoisobutyric acid uptake on irradiated thymocytes as a function of radiation dose. Here we incubate cells at 37 °C in phosphate buffered saline plus 15 mm glucose for 2–3 h after radiation before measurement of uptake. The dose response curve is sigmoidal in shape, with a plateau between 3 and 8 krad.

We find that the initial 2-aminoisobutyric acid uptake of unirradiated cells varies from one batch of animals to the next in the range of 0.3–0.7 nmole per 10^7 thymocytes per 10 min at an initial extracellular amino acid concentration of $1 \cdot 10^{-4}$ M. However, the percent radiation response under given conditions lies within $\pm 10\%$ of the mean in all our preparations. The irradiation effect can easily be demonstrated also in vivo. Thus, 24 h after treating rats with 0.5 krad, the thymuses exhibit dramatic loss of cellular mass and transport of 2-aminoisobutyric acid in the remaining viable cells decreases to 46% of normal.

Transport of 1-aminocyclopentane-1-carboxylic acid and b-2-aminobicyclo-[2.2.1]-heptane-2-carboxylic acid

Thymocyte 2-aminoisobutyric acid transport requires Na^+ [8]. To determine whether the radiation impairment observed for 2-aminoisobutyric acid represents a general effect on all amino acid systems or mainly damage of the amino acid transport machinery coupled to the Na^+–K^+ transport system, we examined the effect of radiation on the transport of 1-aminocyclopentane-1-carboxylic acid and b-2-bicyclo[2.2.1]

TABLE III

RELATIVE EFFECTS OF γ-IRRADIATION ON UPTAKE OF 1-AMINOCYCLOPENTANE-1-CARBOXYLIC ACID, b-2-AMINOBICYCLO-[2.2.1]-HEPTANE-2-CARBOXYLIC ACID AND 2-AMINOBUTYRIC ACID BY RAT THYMOCYTES

Cells ($20 \cdot 10^6$–$40 \cdot 10^6$ per ml) were incubated in phosphate-buffered saline, pH 7.4, containing 15 mM glucose at 37 °C for 15 min before addition of ^{14}C-labeled amino acid. The cells had previously been irradiated at 0.50–5 krad and incubated for 3–4 h before measurement of 1-aminocyclopentane-1-carboxylic acid, b-2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid and 2-aminobutyric acid uptake.

Amino acid	Initial concentration amino acid (M)	Dose (krad)*	Aminoacid uptake (nmoles amino acid per 10^7 thymocytes per 10 min)**
1-aminocyclopentane-1-carboxylic acid	$1 \cdot 10^{-4}$	0	0.38 ± 0.01
		0.5	0.38 ± 0.01
		5	0.37 ± 0.01
b-2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid	$2 \cdot 10^{-4}$	0	0.45 ± 0.02
		0.5	0.43 ± 0.01
		5	0.47 ± 0.02
α-aminobutyric acid	$1.0 \cdot 10^{-4}$	0	0.34 ± 0.01
		0.5	0.31 ± 0.01
		5	0.20 ± 0.01

* ^{137}Cs.

** Means and ranges of a typical set of triplicate determinations. Uptake in terms of viable (trypan blue-negative) cells.

heptane-2-carboxylic acid. Thymocyte 1-aminocyclopentane-1-carboxylic acid uptake is mediated by a distinct transport system, which does not require Na^+ [8] whereas the transport of the bicyclo-heptyl amino acid in several cell types also lacks a Na^+ requirement [9]. As shown in Table III, even high radiation levels do not impair the uptake of these two amino acids, although the same cell preparation show 2-aminoisobutyric acid uptake to be decreased by about 10 and 40% 2 h after irradiation with 0.5 krad and 5 krad, respectively.

Effect of radiation on the saturable transport parameters of 2-aminoisobutyric acid uptake in thymocytes

We have measured the rate of uptake of 2-aminoisobutyric acid at various substrate levels below 5 mM over 10 minute periods. A plot of the reciprocal uptake velocity, $1/v$, against the reciprocal substrate concentration, $1/S$, i.e. the reciprocal Lineweaver–Burk plot [12], yields a linear function for the amino acid over at least the first 10 min (Fig. 3).

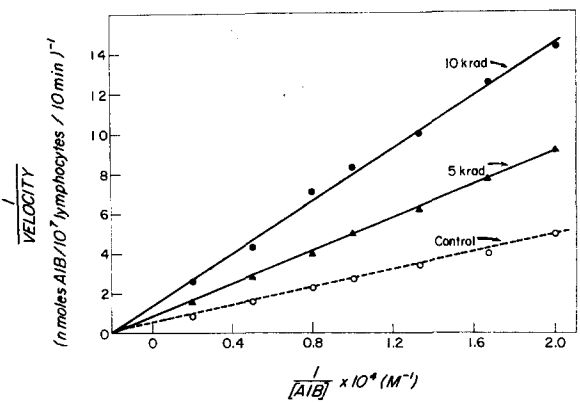

Fig. 3. The effect of radiation on the saturable component of 2-aminoisobutyric acid (AIB) influx. In this experiment $20 \cdot 10^6$–$40 \cdot 10^6$ cells per ml were incubated for 2–3 h after irradiation at 5 and 10 krad, 2-aminoiso-[^{14}C]butyric acid was then added to give concentrations of 0.05–0.5 mM, and 2-aminoisobutyric acid influx determined after 10 min. Each point is the mean of 5 determinations and lines were drawn by the least squares method.

Fig. 3 also shows the effect of X-ray irradiation at 5 and 10 krad on the kinetics of 2-aminoisobutyric acid influx. Irradiation alters mainly the uptake velocity of the amino acid as evidenced by the decrease in V from 2.1 ± 0.05 nmoles per 10^7 thymocytes per 10 min for the control to 0.71 ± 0.3 nmole per 10^7 thymocytes per 10 min for cells irradiated at 10 krad. The apparent affinity constant, K_m, remains essentially unaltered by irradiation (control $K_m = 0.48 \pm 0.03$ mM, at 5 krad K_m 0.57 ± 0.05 mM, and at 10 krad $K_m = 0.5 \pm 0.04$ mM).

Effect of irradiation on 2-aminoisobutyric acid efflux

In all cases we find that the natural logarithm of the intracellular 2-aminoisobutyric acid originally present as a function of time is linear for at least 30 minutes. Therefore, 2-aminoisobutyric acid efflux behaves as a first order process both in

control and irradiated cells. We obtain a fractional 2-aminoisobutyric acid efflux coefficient of 0.021 ± 0.001 min^{-1} for unirradiated thymocytes. This value lies close to the 0.0248 ± 0.002 min^{-1} reported for thymocytes from 11-day-old suckling rats [13]. Exposure of thymocytes to 5 and 10 krad slightly raises their fractional efflux coefficient to 0.027 ± 0.003 min^{-1} and 0.025 ± 0.001, respectively.

Radioprotective effects of insulin, trypsin, and concanavalin A

Insulin increases the V of 2-aminoisobutyric acid uptake in lymphocytes [13]. Since our experiments indicate that radiation primarily impairs V, we have explored the possibility that insulin might protect thymocyte in this amino acid transport system against radiation. For this, we have examined the effects of insulin added to thymocytes before irradiation, as well as at various time intervals after exposure.

TABLE IV

RADIOPROTECTION OF THYMOCYTE 2-AMINOBUTYRIC ACID UPTAKE BY INSULIN

Samples (irradiation with ^{137}Cs)	Time cells incubated before uptake determined (h)	2-Aminoisobutyric acid uptake* (nmoles per 10^7 thymocytes per 10 min)
Control	2	0.39 ± 0.01
Control + 5 krad	2	0.34 ± 0.02
Control + $1.7 \cdot 10^{-6}$ M insulin	2	0.43 ± 0.01
Insulin + 5 krad (cells pretreated for 2 h with $1.7 \cdot 10^{-6}$ M insulin before 5 krad irradiation)	4	0.44 ± 0.01
Insulin + 5 krad (cells pretreated with $1.7 \cdot 10^{-8}$ M insulin before 5 krad irradiation)	4	0.41 ± 0.01
5 krad + insulin (cells irradiated at 5 krad and allowed to incubate for 2 h before $1.7 \cdot 10^{-6}$ M insulin added)	4	0.32 ± 0.01

* Uptake in terms of viable (trypan blue-negative cells).

Table IV shows that insulin protects thymocyte 2-aminoisobutyric acid transport from the effects of even 5 krad γ-ray. Further experiments at insulin concentrations near physiological levels of $1.0 \cdot 10^{-8}$ M showed that the hormone, when added before irradiation and up to 1 h afterwards, protects this transport system. Insulin addition 2 h after irradiation does not protect. As reported in ref. 13, insulin augments 2-aminoisobutyric acid transport in lymphoid cells. Irradiated insulin-protected cells show the same 2-aminoisobutyric acid uptake as unirradiated, insulin-treated thymocytes, i.e. higher than controls. Moreover, the viability of insulin-treated thymocytes, irradiated with 5 krad, is identical to that of unirradiated insulin-treated cells.

TABLE V

RADIOPROTECTION OF THYMOCYTE 2-AMINOISOBUTYRIC ACID TRANSPORT BY TRYPSIN AND CONCANAVALIN A

In the trypsin experiments cells were exposed to 1 μg/ml of cell suspension for 30 min at 37 °C. After this time period, cells were spun down at $1500 \times g \cdot$ min and washed twice with phosphate-buffered saline. The cells were then resuspended in phosphate-buffered saline containing glucose and allowed to equilibrate at 37 °C for 15 min before irradiating at 5 krad. After 2 h, cells were examined for 2-aminobutyric acid uptake. Cells were also treated with trypsin for 30 min after cells had been irradiated at 5 krad and allowed to incubate for 2 h. In the concanavalin A experiments, cells were either pretreated with 5 μg/ml of concanavalin A for 2 h before irradiation at 5 krad then uptake measured 2 h later or irradiated first, allowed to incubate for 2 h then concanavalin A added and incubated for 2 h before uptake measure. Results in terms of viable (trypan blue-negative) cells. Data are means ± ranges of representative triplicate determinations.

Samples	Total time of incubation (h)	Uptake 2-aminoisobutyric acid (nmoles per 10^7 lymphocytes per 10 min)
Control + trypsin	2.5	0.50 ± 0.06
Trypsin + 5 krad	2.5	0.50 ± 0.01
5 krad + trypsin	2.5	0.43 ± 0.01
Control + concanavalin A	4.0	0.44 ± 0.01
Concanavalin A + 5 krad	4.0	0.42 ± 0.01
5 krad + concanavalin A	4.0	0.29 ± 0.01
Control	2	0.39 ± 0.01
Control + 5 krad	2	0.34 ± 0.02

Trypsin and concanavalin A which mimics the effect of insulin in several tissues [14, 15] also protect thymocyte 2-aminoisobutyric acid transport against radiation damage. The full protective effects appear only when trypsin or concanavalin A are present during irradiation. (Table V).

DISCUSSION

Our results show that in vitro exposure of rat thymocytes to 0.05–10 krad γ-irradiation impairs their capacity to accumulate the non-metabolizable amino acid, 2-aminoisobutyric acid in a time- and dose-dependent fashion. The dose–response curves obtained exhibit a plateau between 3 and 8 krad and parallel those observed for potassium transport [5], suggesting that a single radiation lesion impairs both translocation mechanisms. This notion is also consistent with the fact that two amino acids, whose uptake is not linked to alkali cation transport [8, 9], are accumulated normally even after high radiation doses.

Examination of the saturable component characterizing 2-aminoisobutyric acid influx in rat thymocytes shows that radiation alters only the maximum rate of amino acid uptake, i.e. V, without significantly affecting the apparent binding constant K_m; K_m, presumably reflects the nature of the 2-aminoisobutyric acid binding site, i.e. 2-aminoisobutyric acid affinity for a presumed "carrier protein". The V depends on the number of active 2-aminoisobutyric acid carrier sites as well as the rates at which these turn over, and its decrease following radiation may involve one or both

of these mechanisms. We cannot exclude the possibility that increasing irradiation doses preferentially eliminate increasing proportions of the amino acid carrier and that this process damages the cells beyond repair. However, this explanation does not deal with the parallel effects of irradiation on 2-aminoisobutyric acid and Na^+–K^+ transport.

Alternatively, radiation inactivation of the Na^+ pump might lead to Na^+–K^+ gradients unfavorable to inward transport of 2-aminoisobutyric acid. This effect could lower V without affecting K_m (since Na^+ is not involved in the binding of the amino acid to the cell membrane). This explanation also fits our observation that radiation does not impair uptake of amino acids known not to require Na^+ for trans port. However, while 2-aminoisobutyric acid transport does vary with experimentally induced Na^+ gradients, induced by replacing Na^+ with choline [8], we measure 2-aminoisobutyric acid transport at physiologic levels of extracellular Na^+ concentration and express our data in terms of viable (normally permeable) cells. Furthermore, ouabain, which is a specific inhibitor of the (Na^+–K^+)-ATPase, inhibits 2-aminoisobutyric acid uptake only slightly [15], indicating that 2-aminoisobutyric acid uptake is not directly related to (Na^+–K^+)-ATPase activity. The cation gradient hypothesis, thus, does not adequately explain our data and we therefore suspect existence of other lesions in membrane components essential to both cation and 2-aminoisobutyric acid transport.

Hadden, et al. [16] have recently demonstrated that insulin stimulates the plasma membrane (Na^+–K^+)-ATPase of human lymphocytes. Also, Goldfine et al. [13] show that insulin stimulates the uptake of 2-aminoisobutyric acid in thymocytes from 11-day-old suckling rats. This effect of insulin arises from both an increased V for 2-aminoisobutyric acid influx and a decreased K_m. Our own experiments are in accord but further show that insulin ($1.7 \cdot 10^{-6}$–$1 \cdot 10^{-8}$ M) fully protects 2-aminoisobutyric acid transport against radiation damage in rats.

Moreover, concanavalin A, which affects only the V of 2-aminoisobutyric acid transport [15] in rat lymphocytes, also fully protects 2-aminoisobutyric acid transport in rat thymocytes against radiation damage. Indeed, neither in the case of insulin nor concanavalin A does irradiation depress 2-aminoisobutyric acid transport from the stimulated level induced by these agents. A radioprotective phenomenon, possibly related to the concanavalin A effect, has been reported for kidney bean phytohemagglutinin [17]. This lectin, which increases 2-aminoisobutyric acid uptake in lymphocytes by affecting mainly V [18], protects human peripheral lymphocytes against the lethal effects of in vitro radiation [17].

Increasing evidence suggests a close relationship between insulin receptors and some lectin binding sites. For example, Cuatrecasas [19] shows that concanavalin A and wheat germ agglutinin perturb the insulin-receptor sites in fat and liver cells by a direct interaction between the plant lectins and the insulin-binding macromolecules. Moreover, Gavin et al. [20] find that the insulin receptors of human lymphocytes closely resemble those of hepatocytes and fat cells. These parallelisms suggests that the radioprotective and metabolic effects observed with the plant lectins may be due to the interaction of these substances with the insulin receptor site.

Trypsin has been shown to interact with the insulin receptor of fat cells and mimic the actions of insulin [21]. We show, herein, that mild trypsinization of thymocytes increases 2-aminoisobutyric acid influx and, when performed prior to irradiation,

radioprotects 2-aminoisobutyric acid transport. As in the case of insulin and concanavalin A, addition of trypsin 2 h after irradiation does not reverse the effects of irradiation. Thus, it appears that substances which can mimic the effects of insulin by binding onto or perturbing the insulin receptor site, can protect the Na^+-dependent 2-aminoisobutyric acid system in rat thymocyte against high levels of radiation.

We are in the process of characterizing the radiation induced thymocyte membrane lesion(s) at the molecular level.

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