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SHORT COMMUNICATION

Effects of Gamma Radiation on the Concentration of 5-Hydroxy-*L*-Tryptophan & 5-Hydroxytryptamine in Presence of Radioprotector in Sprague Dawley Rats

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ABSTRACT

The result of variation of 5-hydroxy-*L*-tryptophan (HT) and 5-hydroxytryptamine (5-HT) in different tissues of control and gamma-irradiated Sprague Dawley rats with and without a radioprotector β-amino-ethylisothiuronium bromide hydrobromide (AET) combination e.g. (HT + AET) have been studied. The retention of HT, in the tissues studied, decreased after lethal dose (10.5 Gy) but for 5-HT, no such trend was observed after incorporation of HT + AET. A slight tendency of both metabolites to come back to control level was also observed for Sprague Dawley rats. In urine, concentration of HT was less compared to 5-HT with a lethal dose (10.5 Gy). After incorporation of HT + AET the turnover rate of HT and 5-HT were found to be maximum when it was injected through intraperitoneal route.

NOMENCLATURE

HT	5-hydroxy-*L*-tryptophan
5-HT	5-hydroxytryptamine
	β-amino-ethylisothiuronium bromide hydrobromide
g	Gram
Gy	Gray
s	Second
Ex	Excitation
	Emission
nm	Nanometer
ml	Millilitre
	Micromolar
µg	Microgram
ng	Nanogram

1. INTRODUCTION

Two important metabolites 5-hydroxy-*L*-tryptophan (HT) and 5-hydroxytryptamine (5-HT) are considered in the functioning of different organs under normal and stressed conditions[1-3]. The activation or suppression of some of their receptors are linked with many physiological functions[4-8]. The combination of HT with β-amino-ethylisothiuronium bromide hydrobromide (AET) renders radioprotection to different biological systems[9-12]. Survival studies have also indicated the effectiveness of this particular radioprotective combination. Since different receptors are operative from different tissues for the release of HT and 5-HT[13,14], it is worthwhile to find out the effect of gamma radiation and its modification by HT+AET on the concentration of these two compounds in different tissues of Sprague Dawley rats. The mechanism of action of this particular

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radioprotector combination has been reported to be through receptors[10]. A synergistic action takes place when these two compounds are mixed. The enhanced radioprotective efficacy is through a complex which is formed between these two compounds[15,16].

Nuclear catastrophy and accidental exposure to radiation of workers can cause different types of radiolytic changes in all the tissues. Studies with experimental animals like mice[12] and monkeys[17] have proved beyond doubt, the efficacy of the above radioprotector combination against ionising radiation. This will help in extrapolating these data to human beings.

2. MATERIALS & METHODS

5-hydroxy-*L*- tryptophan (lot No. 33F 0402, H 3753, mol wt 220.2); 5-HT (lot No. 7752 H, mol wt 221.69) and AET (lot No. 122 F, 0369 mol wt 281.02) were obtained from Sigma Chemical Company, USA. The activated charcoal of Loba Chemie grade was used whereas the other reagents were of analytical grade. The male Sprague Dawley rats (150-200 g) were used during the present investigation. These were taken in liquid nitrogen for 15 min for sacrifice and subsequently brought to room temperature. The tissues were then dissected, separated and weighed. These were then homogenised at 800 g for 15 min at 4 °C. The estimation of HT and 5-HT from different tissues was done by solvent extraction method[18]. Six animals were taken for each group viz., control, gamma-irradiated and radioprotected. The fine suspension of homogenised tissues were gamma-irradiated to a dose of 10.5 Gy, at a dose rate of 0.077 Gy/s[19]. The concentrations of tryptophan and tryptamine in different tissues were estimated by measuring the fluorescence intensities of HT and 5-HT at Ex 285 nm, Em 360 nm for HT and Ex 365 nm and Em 440 nm for 5-HT, respectively[20,21]

For urine analysis, in a group of three animals, to each rat 1.5 ml of HT+AET of concentration (90.82 + 14.23) µM i.e (20 + 4) µg, was injected intraperitoneally, intramuscularly and subcutaneously. The urine collected after a fixed

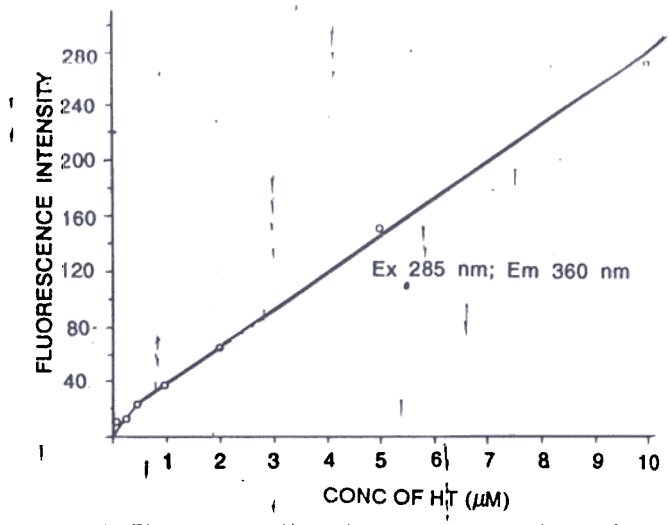

Figure 1. Florescence intensity as a function of HT concentration (Ex 285 nm; Em 360 nm). p*H* range 4.73-5.53.

interval was passed through activated charcoal and then HT and 5-HT was measured by fluorimetric method. Stock solutions of concentrations 50 µg/ml of HT and 5-HT each were prepared in water for calibration purposes. The p*H* measurements were carried out up to an accuracy of ±0.01 p*H* unit (Toshniwal p*H* meter). The pH values were adjusted by using appropiate quantity of 0.01 (N) *HCl*. Fluorescence spectra were taken by Aminco-Bowmann spectrophoto-fluorimeter (accuracy 0.001 unit). Concentration of HT and 5-HT were expressed in µM in the calibration curves whereas the same compounds when measured in different tissues were expressed in ng/g of wet tissue. The conversion of µM to ng was done by taking into account, the molecular weights of HT and 5-HT.

3. RESULTS

Calibration curves were drawn for HT and 5-HT (Figs 1 and 2 respectively). Tables 1 and 2 show the concentration of HT and 5-HT in ng/g of wet tissue homogenate in the control and gamma-irradiated animal with and without HT + AET. A marginal increment in 5-HT levels after irradiation in some tissues was observed (Table 1), but after incorporation of HT + AET there was a tendency to come back to the normal value.

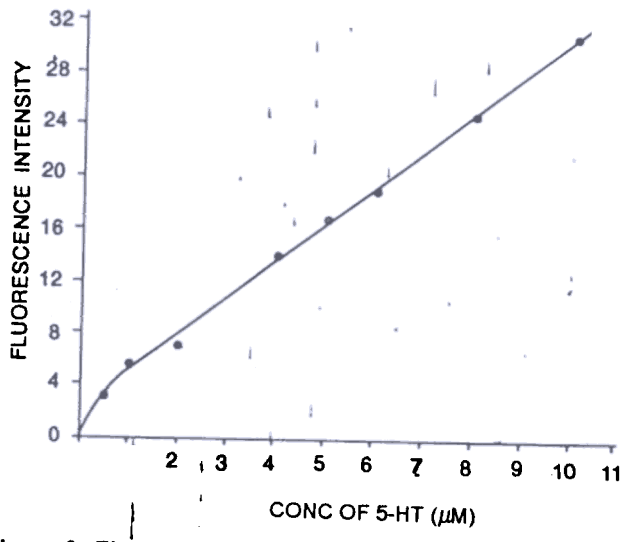

Figure 2. Florescence intensity as a function of 5-HT concentration (Ex 365 nm; Em 440 nm). *pH* range 4.47-5.53.

However, there is a decrease in the concentration of HT after irradiation (Table 2), and after incorporation of HT + AET, there was a tendency to come back to the normal value. Table 3 shows the concentration of HT and 5-HT in urine samples of Sprague Dawley rats collected after 24 hr of injection of 1.5 ml of HT + AET of concentration (90.82 + 14.23) μM i.e. (20+4) μg, administered through different routes. The turnover rate of HT is found to be in the following order:

- Intraperitoneal > subcutaneous > intramuscular

and the turnover rate of 5-HT is in the order

- Intraperitoneal > intramuscular > subcutaneous.

In the analysis of urine samples (Table 4), amount of HT is much less in comparison to the amount of 5-HT after 120 hr of gamma-irradiation with a dose of 10.5 Gy.

4. DISCUSSIONS

A linear plot of concentration *vs* fluorescence intensities of HT & 5-HT was used for the determination of various levels of these metabolites in tissues. (Figs 1 and 2 respectively).

Table 1. 5-HT concentration (ng/g wet tissue) in control & gamma-irradiated Sprague Dawley rats with and without HT + AET (No. of animals: six)

Tissue	Control	Dose 10.5 Gy	Dose 10.5 Gy + HT + AET
Lungs	200 ± 17		150 ± 10
Small intestine	150 ± 8	150 ± 8	100 ± 4
Large intestine	125 ± 10	100 ± 15	100 ± 5
Heart	100 ± 7	150 ± 12	75 ± 3

Table 2. HT concentration (ng/g wet tissue) in control & gamma-irradiated Sprague Dawley rats with and without HT + AET (No. of animals: six)

Tissue	Control	Dose 10.5 Gy	Dose 10.5 Gy + HT + AET
Lungs	1300 ± 23	350 ± 13	475 ± 10
Small intestine	700 ± 15	500 ± 23	550 ± 13
Large intestine	900 ± 75	475 ± 25	500 ± 15
Heart	700 ± 25	300 ± 18	500 ± 18

The control value of 5-HT for all these tissues is less compared to that of HT! This could be explained due to the fact that HT is a metabolic precursor of 5-HT[21]. Since the formation of 5-HT from HT is controlled by two pathways (metabolic and radiolytic), the concentration of HT is considerably less. The radiolytic decomposition of different tissues producing HT and other compounds has been reported[22].

For an absorbed dose of 10.5 Gy, 5-HT concentration is more and HT concentration is less in general for gamma-irradiated tissues compared to control. The reasons are as follows[23,24] :

(a) Receptors for the release of 5-HT and HT from tissues are different and these are susceptible to gamma-irradiation to different extents. After gamma-irradiation, the receptors may release or obstruct HT or 5-HT by the formation of different metabolic products.

(b) Moreover, on gamma-irradiation, HT gets converted to 5-HT along with other radiolytic

Table 3. Concentration of HT and 5-HT in urine samples of Sprague Dawley rats collected after 24 hr of injection of HT + AET in the unirradiated state (No. of animals: six)

Samples	HT (ng/g of urine)	5-HT (ng/g of urine)
Control		300 ± 17
Subcutaneous	275 ± 12	350 ± 18
Intramuscular	125 ± 10	370 ± 18
Intraperitoneal	300 ± 13	400 ± 20

Table 4. Concentration of HT and 5-HT in urine samples of Sprague Dawley rats collected after 120 hr of irradiation. (dose 10.5 Gy) (No. of animals: three)

Samples	5-HT (ng/g of urine)	HT (ng/g of urine)
4	88.8	26.4
5	77.7	24.2
6	88.8	28.6
7	77.7	27.5
8	88.8	26.4
	Av = 77.7 ± 3.93 (S.D.)	Av = 24.9 ± 1.18 (S.D.

products (e.g., 5-hydroxyindole-3-carboxylic acid, 5-hydroxyindole, 2-carboxylic acid, N-formylkyneurenine and kyneurenine).

Hence, the concentration of HT falls and that of 5-HT increases on gamma-irradiation (Tables 1, 2 and 4). Since the receptor sites for release of HT and 5-HT are different and the control mechanism offered by HT + AET is also different, the absolute values of HT and 5-HT after radioprotection of the tissues are different.

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