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BIOGENIC AMINE RESPONSE TO WHOLE BODY IRRADIATION

Prevention by APTH

G. C. PRASAD, S. S. HASAN, S. N. PANDEYA, S. MAZUMDAR and P. M. SINGH

It is a well documented fact that irradiation brings about marked disturbances in the bioamine levels (NAIR 1965, BRINKMAN & VENINGA 1962, VENINGA & DE BOER 1963, VARAGIC et coll. 1967). The bioamine levels have furthermore been correlated with mortality by SIMMSON et coll. (1970), MATHEW (1973) and MODIGH (1974). They have also claimed that any drastic change in the bioamine levels proves to be lethal. However, the role of bioamines in connection with the use of a chemical radiation protector are still obscure and need to be probed further.

Unpublished data indicate marked changes in the bioamine levels followed by a 50 per cent mortality 15 days post-irradiation. This motivated a search for a drug which could diminish the post-irradiation changes and prevent the mortality. The influence of a radiation protective drug on the functional condition of certain biogenic amines of brain and blood was investigated. The experiments were also designed to throw light on the response of hypothalamic neurosecretory neurons to radiation.

Material and Methods

Ninety albino rats weighing 100 to 110 g were used. They were acclimatised for two weeks to laboratory conditions prior to commencement of the experiments. During the acclimatisation and experimental periods the animals were fed on balanced

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laboratory diet procured from Hindustan Livers Ltd. (Bombay, India), and water was permitted ad libitum. 1-acetyl-3-phenylamidine thiocarbamide hydrochloride (APTH), a newly synthesized organic compound (by S.N.P.), was used as a radiation protector. This compound is formed by interaction of phenylamidine chloride with N-acetyl thiocarbamide. It is a derivative of amidine thiocarbamide, which is a sulphur containing compound. The LD_{50} of the drug (intraperitoneal injection) exceeded 90.0 mg/kg body weight.

The experiments were carried out in 3 different groups, each containing 30 rats.

Group I received 1 ml physiologic saline and served as normal controls.

Group II received 1 ml of physiologic saline and was exposed to 154.8 mC/kg (600 R).

Group III received APTH 3 mg/100 g and was also exposed to 154.8 mC/kg.

The solution of APTH in physiologic saline was freshly prepared before testing to a pH of 9.0. The solution was injected intraperitoneally 30 min before irradiation with ^{60}Co. The rats in group II were irradiated simultaneously with the APTH treated rats and thereafter housed under identical conditions.

The mortality rate was recorded over a period of 30 days. The animals were killed at day 15 or day 30.

The brains were dissected out and were fixed in Bouin's fluid. The sections were 10 μ thick and were stained in Gomori's aldehyde fuchsin after Halmi's modification for the demonstration of neurosecretion.

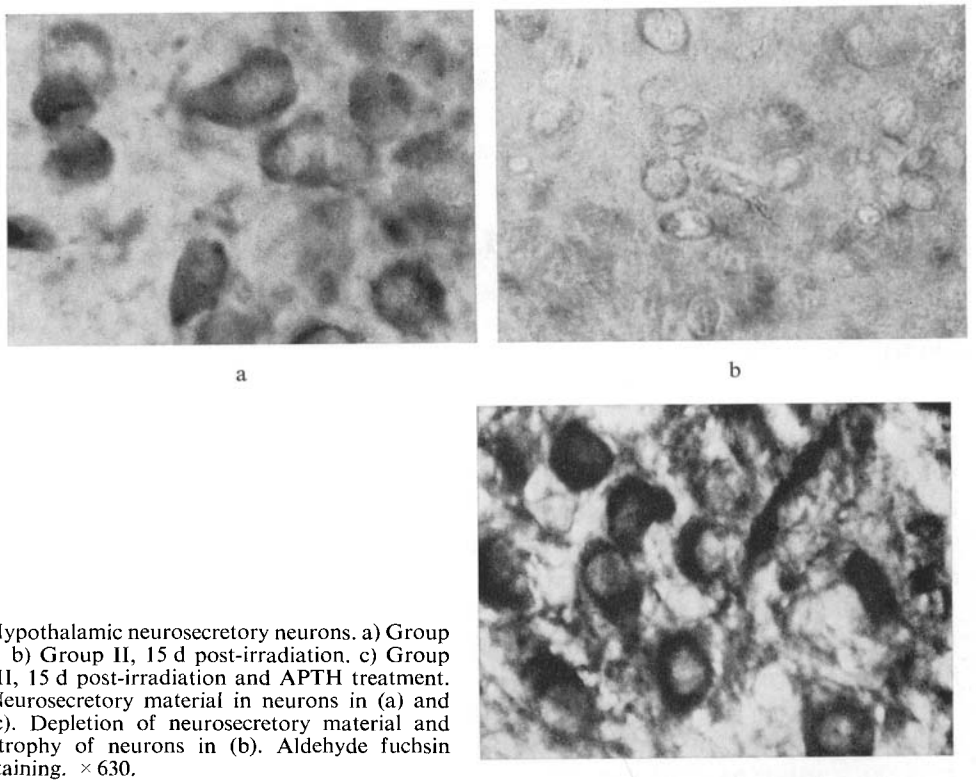
The blood from each animal was collected in a heparinised tube and subjected to biochemical analysis of acetylcholine (PANDEY et coll. 1975) and cholinesterase (CARAWAY 1956). The whole brain was removed and transferred to a solution of 0.4 N perchloric acid ($HClO_4$). After homogenisation 5-HT (SNYDER et coll. 1965) and catecholamine (CROUT 1961, with certain modifications) were extracted and estimated fluorometrically.

Results

Group II had an overall mortality of 60 per cent within a month; of those, 50 per cent occurred within the first two weeks. In groups I and III no mortality was recorded during this period.

Hypothalamic neurosecretory neurons. In group II a complete depletion of neurosecretory materials was found with a consequential reduction in the size of neurons (Fig. b). In groups I and III the neurons behaved identically and had a fair amount of neurosecretory material, the size of the neurons in groups I (Fig. a) and III (Fig. c) not being reduced.

Brain 5-hydroxytryptamine (5-HT) (Table 1). On day 15 the brain 5-HT level following irradiation had decreased significantly to 0.404 μg/g from the group I level of 0.591 μg/g ($p < 0.001$), whereas in group III the level rose to 0.799 μg/g. In com-



a b

Hypothalamic neurosecretory neurons. a) Group I. b) Group II, 15 d post-irradiation. c) Group III, 15 d post-irradiation and APTH treatment. Neurosecretory material in neurons in (a) and (c). Depletion of neurosecretory material and atrophy of neurons in (b). Aldehyde fuchsin staining. × 630.

parison with group I this was not highly significant ($p < 0.025$). At day 30 the 5-HT level had increased to 0.724 μg/g, which was statistically insignificant ($p < 0.05$) in comparison with the group I value of 0.539 μg/g. In group III the 5-HT level further increased to 0.899 μg/g, which was statistically significant ($p < 0.01$) in comparison with group I, but remained insignificant ($p < 0.05$) in comparison with group II (Table 1).

Brain catecholamine (Table 2). On day 15 after irradiation the brain catecholamine level had decreased to 0.811 μg/g, which was statistically significant ($p < 0.025$) in comparison with the group I value of 1.194 μg/g, whereas in group III the level increased significantly to 1.934 in comparison with groups I and II ($p < 0.05$ and $p < 0.01$, respectively). On day 30 following irradiation the catecholamine level had decreased to 0.615 μg/g, which was statistically significant ($p < 0.01$) as compared to group I (1.163 μg/g), while in group III the catecholamine level showed a little rise (1.810 μg/g), significance level $p < 0.05$ in relation to group I, $p < 0.001$ in relation to group II.

Table 1

5-hydroxytryptamine content (μg/g) in the brain tissue of control animals (group I), irradiated controls (group II) and drug-treated irradiated animals (group III)

Day 15			Day 30		
Group I	Group II	Group III	Group I	Group II	Group III
0.591 SD ± 0.148	0.404 SD ± 0.016	0.799 SD ± 0.048	0.539 SD ± 0.212	0.724 SD ± 0.301	0.899 SD ± 0.202

Table 2

Catecholamine content (μg/g) in the brain tissue in group I, II and III

Day 15			Day 30		
Group I	Group II	Group III	Group I	Group II	Group III
1.194 SD ± 0.432	0.811 SD ± 0.268	1.934 SD ± 1.029	1.163 SD ± 0.501	0.615 SD ± 0.144	1.810 SD ± 0.09

Table 3

RBC acetylcholine and cholinesterase of groups I, II and III

	Day 15			Day 30		
	Group I	Group II	Group III	Group I	Group II	Group III
RBC Acetylcholine (μg/ml)	0.990 SD ± 0.321	0.926 SD ± 0.169	2.105 SD ± 0.521	0.828 SD ± 0.415	0.972 SD ± 0.227	2.120 SD ± 0.426
RBC Cholinesterase (PU/ml)	79.00 SD ± 4.95	37.50 SD ± 3.62	56.00 SD ± 5.20	82.01 SD ± 1.39	51.00 SD ± 4.18	79.50 SD ± 8.10

RBC acetylcholine (Table 3). In group II the acetylcholine level on day 15 had decreased to 0.926 μg/ml as compared to group I (0.990 μg/ml), which was statistically insignificant ($p > 0.05$) whereas in group III the acetylcholine level rose to 2.105 μg/ml, which was highly significant ($p < 0.001$) in comparison with groups I and II. On day 30 after irradiation the acetylcholine level increased to 0.972 μg/ml, which was insignificant ($p > 0.05$) in comparison with group I (0.828 μg/ml). In group III the level increased significantly ($p > 0.001$) to 2.120 μg/ml in relation to groups I and II.

RBC cholinesterase (Table 3). On day 15 the cholinesterase level had decreased significantly (37.50 PU/ml, $p < 0.001$) in group II in comparison with group I (79.0

PU/ml). In group III the cholinesterase level increased and was highly significant ($p<0.001$) in comparison with groups I and II. On day 30 the cholinesterase level was found to be significantly lower in group II ($p<0.001$) in comparison with group I, whereas in group III the cholinesterase further increased in comparison to group II; it was statistically significant ($p<0.001$) but insignificant in comparison to group I ($p<0.05$).

Discussion

The results clearly demonstrate a mortality of 60 per cent within 30 days after 154.8 mC/kg (600 R) ^{60}Co irradiation but after treatment with APTH with a dose of 3 mg/100 g no deaths occurred. BONEFI & NUVOLONE (1958) recorded a 26 to 44 per cent survival after cysteamine acetic acid and N-glutanyl cysteamine before irradiation with 600 R. A similar observation was made by FOYE & MICKELS (1962) using 2-piperazinoethyl dithiocarbamic acid. Hence, APTH is more effective at this radiation dose.

The irradiation eliminates neurosecretory material from the perikaryons of the neurons and causes a marked atrophy of the nuclear volume of the supraoptic and paraventricular neurons. When the rats were irradiated 30 min after the injection of APTH, the neurons were still found loaded with neurosecretory material and no nuclear atrophy was observed. It seems that the drug counteracts the effect of irradiation. This agrees well with previous observations of DUCHESNE et coll. (1968).

It has been observed that irradiation causes a marked disturbance in the biogenic amine levels. In the present series irradiation decreased the 5-hydroxytryptamine (5-HT) and catecholamine (CA) contents of the brain during the first two weeks, the period with maximum mortality. After administration of APTH before irradiation the bioamine level was similar to that in the controls. After day 15, when the mortality is lower, the 5-HT was a little higher than in the controls. This may be attributed to a compensatory phenomenon in order to raise the decreased level of 5-HT. This partly substantiates the findings of BACQ et coll. (1954). They found normal content of cholesterol and ascorbic acid in the adrenals of protected rats 3 days after irradiation.

It was further observed that acetylcholine in the APTH treated group increased as well as did cholinesterase. It suggests that acetylcholine in the treated group is, like other biogenic amines, required in excess quantity. Probably the synthesis increases after APTH administration, although the degradation rate as indicated by the increased level of cholinesterase remains higher. Under such conditions it seems probable that the synthesis is much higher than the degradation and hence acetylcholine and cholinesterase levels remain increased.

On the basis of these results it is suggested that the irradiation protective property of APTH is caused by stimulating the synthesis of bioamines. It also supports the assumption that it counteracts the action of protein depletion owing to ionizing radiation, which in turn further corraborates the findings of DUCHESNE et coll.

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SUMMARY

^{60}Co whole body irradiation with 154.8 mC/kg (600 R) resulted in a mortality of 50 per cent within two weeks. Administration of 1-acetyl-3-phenylamidine thiocarbamide hydrochloride (APTH) 30 min before irradiation prevented this mortality. Irradiation eliminated neurosecretory material from the perikaryons of the supraoptic and paraventricular neurons, whereas APTH counteracted this action. APTH also increased the synthesis of bioamines (5-hydroxytryptamine, catecholamine and acetylcholine).

ZUSAMMENFASSUNG

^{60}Co Ganzkörperbestrahlung mit 154,8 mC/kg (600 R) führte zu einer Mortalität von 50 Prozent in 2 Wochen. Die Gabe von 1-Acetyl-3-Phenylamidin Thiocarbamid Hydrochlorid (APTH) 30 Minuten vor der Bestrahlung verhinderte diese Mortalität. Die Bestrahlung eliminierte neurosekretorisches Material von den Perikaryons der supraoptischen und paraventrikulären Neuronen, wobei APTH diesem Effekt entgegenwirkt. APTH steigerte auch die Synthese der Bioamine (5-Hydroxytryptamin, Catecholamin und Acetylcholin).

RÉSUMÉ

L'irradiation du corps entier par le ^{60}Co avec 154,8 mC/kg (600 R) a donné une mortalité de 50% en deux semaines. L'administration d'hydrochlorure de 1-acétyl-3-phénylamidine thiocarbamide (APTH) 30 minutes avant l'irradiation a empêché cette mortalité. L'irradiation a éliminé le matériel neurosécrétoire des périkaryons des neurones supra-optiques et para-ventriculaires, alors que l'APTH contrarie cette action. L'APTH augmente aussi la synthèse des bioamines (5-hydroxytryptamine, catécholamine et acétylcholine).

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