diversity of these previous results, as compared to the present study, may be due to the differences in animal species and in the exact loci of implantation of the carcinogen. It is of interest to note that the duration of the experiment, i.e. 9 months, was identical in the different studies. The convenience of production of neoplasms such as osteosarcoma by the administration of carcinogens intrigued us to study this experimental model. However, further evaluation of this method aiming at the induction of osteosarcoma is required.

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The effect of X-irradiation on the amount of dopamine in corpus striatum of the rat

S.R. Stepanović and Jelena V. Nikolić

Boris Kidrič Institute, P.O. Box 522, YU-11000 Beograd (Yougoslavia), 3 May 1978

Summary. The effect of ionizing radiation on the amount of dopamine in corpus striatum was investigated in rats exposed to 650 or 850 R of X-rays. The amount of dopamine in the corpus striatum was measured fluorimetrically in various periods of time after irradiation. It was found that, irrespective of the dose applied, the ionizing radiation caused a significant depletion of dopamine in the striatum.

The dopamine was first detected by Goodall[1,2] in the heart and in the adrenal medulla. The presence of this catecholamine was later demonstrated in a variety of organs and in the central nervous system[3-6], the highest concentration of dopamine being found in the corpus striatum[6-8]. Ionizing radiation has been known to affect catecholamine (noradrenaline, adrenaline) stores in the adrenal medulla, heart and brain[9-14]. However, until now no data have been available concerning the changes of dopamine content in the brain of irradiation animals. It was therefore decided to study the effect of irradiation on the amount of dopamine in its typical store, the corpus striatum.

Materials and methods. Male rats weighing 190–210 g were used. The animals were whole-body X-irradiated with 650 or 850 R. Irradiation parameters were: 200 kV; 0.5 mm Cu; D-42 cm. The dose rate was 112 R/min. The irradiated animals were sacrificed 24 and 48 h, 5, 7 and 14 days after irradiation. Each experimental group had its day-to-day controls. The brain was rapidly removed and the striatum of both sides was dissected on an ice-cold beaker. The mean weight of the striatum was about 90 mg. The striata from 2 rats were pooled. Method of Manuhin et al.[15], based on the methods of Carlsson and Waldeck[16] and Laverty and Taylor[17], was used for extraction and quantitative estimation of dopamine. Recoveries of dopamine were 80–90% throughout the experiment. Fluorimetric estimation was done on an Aminco-Bowman spectrophoto-fluorimeter.

Results and discussion. The present experiments show that the amount of dopamine in the corpus striatum of rats irradiated with 650 R was significantly decreased 24 h to 7 days following irradiation, as compared with the control values. However, dopamine stores were completely restored 14 days after irradiation. The amount of dopamine in corpus striatum of irradiated animals was the same as non-irradiated controls. The results are presented in table 1. In rats irradiated with the dose of 850 R, the amount of dopamine in the striatum was also significantly decreased at all time intervals from 24 h to 7 days after irradiation (table 2). In these animals, there was no replenishment of normal dopamin stores during the observed period of time. The normal noradrenaline content in the corpus striatum of rats is small and amounts only to few percent of the total catecholamine content. Therefore, the presence of noradrenaline in corpus striatum could not be detected, using

Table 1. The amount of dopamine in the corpus striatum of the rat irradiated with 650 R at different time intervals after irradiation (mean ± SE µg/g fresh tissue). The number of experiments is indicated in parenthesis

Controls	Period after irradiation				
	24 h	48 h	5 days	7 days	14 days
1	*2*	*3*	*4*	*5*	*6*
8.58±0.23 (25)	5.21±0.28 (14)	4.80±0.37 (14)	5.57±0.26 (18)	5.86±0.19 (18)	8.39±0.46 (15)

p *(1:2)* < 0.001; p *(1:3)* < 0.001, p *(1:4)* < 0.001, p *(1:5)* < 0.001.

Table 2. The amount of dopamine in the corpus striatum of the rat irradiated with 850 R at different time intervals after irradiation (mean ± SE µg/g fresh tissue). The number of experiments is indicated in parenthesis

Controls	Period after irradiation			
	24 h	48 h	5 days	7 days
1	*2*	*3*	*4*	*5*
8.50±0.31 (20)	5.18±0.35 (15)	5.27±0.26 (15)	5.00±0.21 (19)	4.12±0.33 (11)

p *(1:2)* < 0.001; p *(1:3)* < 0.001; p *(1:4)* < 0.001; p *(1:5)* < 0.001.

the method employed. The results obtained are in agreement with our previous findings that, in animals exposed to 650 R, there was a clear tendency towards replenishment of noradrenaline and adrenaline stores in the heart and the brain 7 days following irradiation; but no such tendency was observed during the same period in animals exposed to 850 R[11-14]. It was also found earlier that irradiation with 900 R caused a significant depletion of noradrenaline content in whole brain 23 h after irradiation[18]. Changes in the catecholamine stores following irradiation have been found in other species as well. For example, a lethal dose of gamma-irradiation reduced catecholamine content in adrenal medulla and hypothalamus of monkey and these changes were observed during the first 24 h after irradiation[10]. In contrast, Johnsson et al.[19] found no significant changes in the amount of noradrenaline in the heart, vas deferens and brain 24 and 48 h after whole-body X-irradiation of rats with 850 R.

The results presented indicate that, during the time interval from 24 h to 5 days irradiation, there is no significant difference between the animals exposed to 650 R and those exposed to 850 R of X-rays in respect to the absolute decrease of dopamine content in the corpus striatum. However, the 2 groups of animals differ substantially in respect to the duration of the decrease of dopamine stores. Thus, in animals which received 650 R, reestablishment of physiological values of dopamine stores in the corpus striatum occured, indicating that the said dose of X-rays does not impair permanently the biosynthetic processes. On the other hand, replenishment of dopamine stores in the investigated organ was not observed in animals with 850 R, suggesting that high doses of X-rays produce irreversible defects in synthesis of catecholamines. In order the better to understand the extent of damage produced by ionizing radiation on the mechanisms controlling the biosynthesis and metabolism of catecholamines, it would be necessary to study the catecholamine turnover in the central nervous system using labelled DOPA, dopamine or noradrenaline.

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Immunochemical studies on rabbit calcitonin

A. Jullienne, J.M. Garel, C. Calmettes, D. Raulais, M.S. Moukhtar and G. Milhaud

U. 113 INSERM, Hôpital Saint-Antoine, 184, rue du Faubourg St-Antoine, F-75012 Paris (France), 23 January 1978

Summary. Cross reaction studies using radioimmunoassays specific for human and porcine calcitonin showed that rabbit calcitonin is structurally more closely related to human than to porcine calcitonin.

Calcitonin in mammals has been considered as an example of unusual biochemical evolution[1] due to the large variations encountered in the 10-31 sequence of the molecule. For example, human (HCT) and porcine (PCT) calcitonin share only 6 residues in common in this region. This indicates a high degree of mutation in the CT molecule during evolution.

We have shown that murine calcitonin (RCT) showed total cross reaction with human calcitonin using antibodies directed against HCT[2]. We concluded from these studies that RCT should have a high degree of similarity in its tertiary structure and probably in its primary amino acid sequence with HCT. Recently elucidation of the amino acid sequence of RCT[3] has strikingly confirmed these predictions based on immunological studies, as the 2 molecules differ by only 2 amino acids. Thus CT in primates and rodentia are structurally quite similar but very different from CT in artiodactyls.

We have undertaken an immunochemical study of rabbit calcitonin (lagomorphs) to establish whether in this species CT was related to the artiodactyl group or to the primate rodentia group. Such a study within the limits of immunological methods would be helpful in establishing whether CT in this order evolved separately or shared a common ancestor with either one of these 2 groups.

Material and methods. Thyroids from young (1 kg) male rabbits were extracted with cold 0.1 N HCl and freeze dried. CT content of the extract was estimated by a 4 point bioassay using synthetic HCT[4] (specific activity 100 MRC units/mg as a standard).

Aliquots were dissolved in the appropriate radioimmunoassay (RIA) buffer. Several RIA specific for either HCT or PCT were used in the study. The RIA were specific for each species as no cross reaction was present. 4 antisera specific for PCT and 3 antisera specific for HCT were used.

The antibodies specific for HCT were directed either towards mid (As 15-As 16) or carboxy terminal (As 36) portions of the human molecule. The detailed procedures for the RIA's used have already been published[2,5]. Histological localization of rabbit C cells was performed by an indirect immunofluorescent technique using antibodies specific for HCT according to the procedure used for RCT[2].

Results. The extract used in the study contained the equivalent of 230 ng of HCT/mg, as estimated by bioassay; this corresponded to 23 munits MRC of calcitonin. Rabbit thyroidal extracts displaced labelled HCT in the 3 RIA used (figure 1). In each system, rabbit extract and HCT displaced the human tracer in an identical fashion. No displacement of labelled PCT was observed when increas-