THE EFFECT OF IONIZING RADIATION ON THE CONTENT OF FREE AND BOUND ACETYLCHOLINE IN THE LIVER AND BRAIN

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Analysis of the mechanisms of development of radiation affections indicates that in the pathogenesis of radiation sickness various changes in the neuro-humoral regulatory processes play a prominent part [1,10,12-15]. At the same time, considerable disturbances also originate in the metabolism of local origin. Therefore, of considerable interest is the effect of ionizing radiation on the metabolism of those natural chemical agents of high biological activity which directly participate in the neuro-humoral and local regulation of biochemical processes.

Acetylcholine [ACh] attracted our attention as one of these substances. On the one hand, its effect on the organism is directly connected with the activity of numerous neural elements. This fact determines the immense effect of ACh in the regulation of the tonus of blood vessels, cardiac rhythm, dynamics of the gastrointestinal tract, functions of the glands of internal and external secretion, muscular activity, and the activity of many central neural apparatuses. On the other hand, ACh in larger or smaller quantities is formed in the tissues of the liver, muscles, placenta which has no neural elements, etc. ACh as a "local hormone" apparently plays an important role in the regulation of the physicochemical state of various interphase borders within the cells by affecting their polarization and permeability. In this connection, the property of ACh of enhancing the metabolism of phospholipids, possibly as the result of disintegration of lipoproteid complexes, is of great importance [29,30]. It exerts considerable and diverse (depending on conditions) effect on the activity of a number of most important enzymic systems [2,4,6,7]. It should be pointed out also that from the specific radiobiological point of view the effect of ACh on sulfhydryl groups'activity is highly important [2,3,5,8,16,19], as well as on the oxidation processes [2,27,28,31] and, in particular, its property of enhancing the resistance of the organism to hypoxia [25,33,34].

Experiments of many authors elicited in radiation injuries various deviations of ACh metabolism from norm. In the majority of cases,during the remote period after irradiation a reduction of cholinesterase activity in the blood serum [11,17,18,26,32],as well as in some tissues [21-24,26],has been observed. Prior to this reduction, wave-like fluctuations of cholinesterase activity were observed, with rises during the early stages after irradiation [9,17,18,20]. A detailed investigation of ACh metabolism following external(x-ray) and internal (polonium and uranium fission products effects) irradiation was carried out on rats by E. N. Petrovnina [17]. She investigated, simultaneously with the determination of general cholinesterase activity in a number of tissues, also the changes in the total ACh content in them, as well as the cholinacetylase activity of the brain. She established, in particular, that the total ACh content in the tissues during the early stages of radiation sickness had been reduced and that, later, the tendency toward its increase was manifested.

The interpretation of the available data is hampered by the fact that in the overwhelming number of investigations only the total cholinesterase activity and the total ACh content (or of the free ACh only) were determined. We were intersted in the problem of the bound ACh. Apparently, bound ACh being impervious to the attack of cholinesterases is not biologically passive precisely on this account. By combining at various periods of time with biological objects, acetylcholine changes their properties, thus manifesting a "trigger" or "trophic" activity.

In the present series of investigations we carried out determinations of the free (conditionally ?) and bound ACh in the tissues of the liver and brain of white rats following total external radiation of Co^{60} gamma-rays in a dose of 800 r within 2,24, and 48 hours after irradiation

METHOD

Quite a number of methods of determination of bound ACh have been suggested consisting of denaturation of proteins and destruction of lipid formations. We decided on the heating method without introducing into the tests any other substances except cholinesterase inhibitors. For the determination of ACh we employed as a test object the dorsal muscle of a leech. Its reaction (contraction) was investigated by testing extracts where ACh had been specif-

Content of Free and Bound ACh in the Liver and Brain of White Rats following Total Irradiation (in microgram%)

Liver		Brain	
free	bound	free	bound
acetylcholine		acetylcholine	
Norm			
0,04	0,62	2,2	84
0,06	0,05	15,4	49
0,06	0,05	4,4	78
0,17	4,78	1,1	49
0,46	0,45	5,9	94
0,23	0,72	8,4	71
		1,1	72
0,44	4,29	28,6	70
0,06	0,05	15,4	66
0,11	0,99	29,7	39
		9,9	83
Average 0,18± ±0,06	1,33± ±0,61	11,1±3,1	69±5
Within 2 hr after irradiation			
traces	4,40	16,5	79
»	traces	11,0	76
0,04	0,06	5,5	76
0,99	1,76	10,7	97
traces	5,50	16,5	83
»	traces	5,5	60
»	0,65	2,2	34
»	0,10		
Average	—	9,7±2,1	72±8
Within 24 hr after irradiation			
0,01	0,10	7,9	25
0,03	2,39	9,9	100
0,03	2,17	6,6	67
0,04	0,05	5,5	79
none	0,06	28,1	51
»	0,29	4,9	108
0,04	0,05	32,5	41
none	0,08	3,3	97
0,04	4,03	1,1	34
		2,8	74
Average	—	10,3±3,5	68±9
Within 48 hr after irradiation			
traces	0,40		
0,10	2,40	8,8	68
0,02	0,08	0,1	44
0,30	11,10*	1,1	86
0,10	0,30	0,9	71
0,60	0,50	16,5	59
Average 0,22± ±0,027	0,74± ±0,35	5,5±3,1	66±7

* These data were not included in the average.

ically destroyed with a cholinesterase preparation. The tested tissue samples were homogenized and extracted with a Ringer solution (with pH 7.6) by heating (for the determination of bound ACh) or in cold (for the free ACh). The obtained extract was placed in a container with a preparation of the dorsal muscle of a leech, and the extent of its contraction was tested kymographically and compared with the extent of contraction induced by an ACh solution of known concentration. The quantity of bound ACh was determined according to the difference between the total ACh content and the free ACh content. Irradiation was carried out with gamma-rays an ÉGO-2 apparatus by an 800 r dose at a dose strength of 460 r/ min.

The obtained data indicate that in norm the content of free and bound ACh in the tissues of the liver and brain varies considerably. In regard to the total content of any given fraction, the ACh quantity in the brain exceeds by several ten-folds that of the liver. In view of the considerable divergence between the data of individual determinations we cited in the table the entire experimental material.

As seen in the table, the free ACh content of the liver varied from 0.04 to 0.46 microgram% and averaged 0.18 microgram%. Within two hours after irradiation, almost every test revealed only traces of free ACh; in one case, on the contrary, its quantity turned out to be approximately twice as high as the normal maximum. Subsequently, within 24 hours after irradiation the free ACh content in the liver tissue became accessible for quantitative determination in a number of cases, but it was still very low; within 48 hours after irradiation its quantity increased and reached almost normal levels.

The content of bound ACh of the liver in norm was several times higher than that of free ACh; with variations from 0.05 to 4.78 microgram%, it constituted 1.33 microgram% on the average. Within 2 hours after irradiation the quantity of bound ACh varied even more markedly than in norm—from traces to 5.50 microgram%. The data obtained within 24 and 48 hours after irradiation proved to be on the whole close to those in norm; we should mention only one very high figure-11.10 microgram%-elicited in one case 48 hours after irradiation.

In norm the free ACh content of the brain (see table) fluctuated between 1.1 and 29.7 microgram%, or 11 microgram% on the average. Within 2 and 24 hours after irradiation, no appreciable deviations from norm were observed. Within 48 hours after irradiation, a certain reduction of the free ACh content was also found in the cerebral tissue. The amount of bound ACh inthe cerebral tissue was also several times higher than free ACh. It constituted about 70 microgram% on the average. As shown by the above-cited data, no changes in the content of bound ACh in the cerebral tissue of rats were observed within 2,24, and 48 hours after irradiation.

Thus, the early stages of acute radiation sickness in rats are characterized by a considerable reduction (almost disappearance) of the free ACh content of the liver within 2 hours after irradiation, and its gradual normalization within 48 hours after irradiation. In the brain the free ACh quantity is also reduced, but this occurs only within 48 hours following irradiation and to a relatively small extent. The content of bound ACh, while there is a certain increase of the range of its fluctuations in the liver, remains on the whole fairly stable, especially in the brain.

SUMMARY

In testing on leech dorsal muscle the authors determined the contents of free (conditionally) and bound acetylcholine (ACh) in the liver and brain tissues of rats both in normal conditions and following γ-ray irradiation in a dose of 800 r. As demonstrated, the content of free and bound ACh was several times greater in the brain than in the liver. Considerable individual variations were noted. In 2 hours after the irradiation the content of free ACh in the liver decreased to only traces. Its initial level was gradually restored by the 2nd post-irradiation day. The content of free ACh in the brain dropped too, although to a much lesser extent. The concentration of bound ACh remained rather stable in the brain of irradiated animals, whereas the range of its variations in the liver had somewhat increased.

LITERATURE CITED

1. P. D. Gorizontov, Pathological Physiology of Acute Radiation Sickness [in Russian] (Moscow, 1958) p. 5.
2. N. N. Demin, Biochemical Activity of Acetylcholine. Author's abstract of doctoral dissertation [in Russian] (Moscow, 1953).
3. N. N. Demin, Biokhimiya 20, 3 (1955) p. 317.
4. N. N. Demin, Izv. AN SSSR. Seriya Biolog., 6 (1955) p. 67.
5. N. N. Demin, Theses of Reports at 8th All-Union Congress of Physiologists, Biochemistsand Pharmacologists [in Russian] (Moscow, 1955) p. 207.
6. N. N. Demin, Ukr. Biokhim. Zhurn. 27, 4 (1955) p. 460.
7. N. N. Demin and I. V. Kuznetsova, in the book: Modern Problems of Nervism in Physiology and Pathology, Dedicated to the 70th Anniversary of A. D. Speranskii [in Russian] (Moscow, 1958) p. 405.
8. N. N. Demin, S. N. Nistratova and L. S. Rozanova, Dokl. AN SSSR 100, 3 (1955) p. 597.
9. N. V. Korneeva, in the book: Collection of Reports on Radiation Medicine of the year 1957 [in Russian] 1 (Moscow, 1959) p. 38.
10. N. A. Kraevskii, Essays on Patholog. Anatomy of Rad. Sickness [in Russian] (Moscow, 1957).
11. N. E. Kuznetsova, Theses of Reports of the Sci. Conf. of the Central x-ray-Radiological Institute on the Problem: Pathogenesis, Clinic, Therapy and Prophylaxis of Radiation Sickness [in Russian] (Leningrad, 1957) p.6.
12. A. V. Lebedinskii, Reports of Soviet Deleg. at the International Conf. on Peaceful Uses of Atomic Energy [in Russian] (Geneva, 1955) (Moscow, 1955) p. 43.
13. M. N. Livanov, Med. Radiol. [in Russian] 1, 1 (1956) p. 19.
14. M. N. Livanov and D. A. Biryukov, Transact. of 2nd Internat. Conf. on Peaceful Uses of Atomic Energy [in Russian] 5 (Moscow, 1959) p. 74.
15. M. N. Livanov and I. N. Kondrat'eva, Med. Radiol. [in Russian] 4, 9 (1959) p.3.
16. S. N. Nistratova and T. M. Turpaev, Biokhimiya 24, 1 (1959) p. 171.
17. E. N. Petrovnina, Changes in the System Acetylcholine-Cholinesterase-Cholinacetylase in the Organs of Animals Affected by Ionizing Radiation. Candidate's diss. [in Russian] (Moscow, 1958).
18. N. E. Ponomarenko, Med. Radiologiya 1, 5 (1956) p. 13.
19. T. M. Turpaev, Dokl. AN SSSR 102, 2 (1955) p.323.
20. T. Baglioni and M. Piemonte, Boll Soc. ital. Biol. sper. 23 (1947) p. 372.
21. J. H. Burn, P. Kordik and R. Mole, Brit. J. Pharm. 7 (1952) p. 58.
22. R. A. Conard, Am. J. Physiol. 170 (1952) p. 418.
23. J. Doull, J. Pharm. exp. Ther. 110 (1954) p. 14.
24. W. Florsheim and M. E. Morton, Am. J. Physiol. 176 (1954) p. 15.
25. C. Franck, R. Grandpierre, P. Arnould, et al., C. R. Soc. Biol. 142 (Paris, 1948) p. 79.
26. A. B. French and P. E. Wall, Am. J. Physiol. 188 (1957) p. 76.
27. H. Gremels, Arch. exp. Path. Pharmak. 182 (1936) p. 1.
28. H. Gremels and F. Zinnitz, Ibid. 188 (1938) p. 79.
29. L. E. Hokin and M. R. Hokin, Biochim. Biophys. Acta 16 (1955) p. 229.
30. Idem, J. Biol. Chem. 209 (1954) p. 549.
31. M. Labbé and M. Rubinstein, C. R. Soc. Biol. 112 (Paris, 1933) p. 1040.
32. H. Lüthy, Radiol. Clin. 22 (1953) p. 491.
33. J. H. Welsh, Bull. Johns Hopk. Hosp. 83 (1948) p. 568.
34. J. H. Welsh and J. E. Hyde, Proc. Soc. exp. Biol. 55 (New York, 1944) p. 256.