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REVUE ROUMAINE DE BIOLOGIE

SÉRIE DE ZOOLOGIE

TOME 14 1969 Nº 3

TIRAGE À PART

EDITURA ACADEMIEI REPUBLICII SOCIALISTE ROMANIA

CORRELATION BETWEEN THE RADIOPROTECTIVE EFFECT OF SOME SUBSTANCES WITH NEUROTROPIC ACTION AND THE INHIBITION OF RESPIRATORY ENZYMES

BY

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AET and some neurotropic substances (imipramine, desmethylimipramine, chlorpromasine, dibenzocycloheptatriene derivatives) reduces cytochrome oxidase and succinoxydase activity of liver mitochondria.

The presence of these substances in cells induced a reversible alteration of the mitochondrial respiration. Biochemical modifications in the first short time period caused by radioprotectors may be responsible for protection against irradiation.

Zins and co-workers [19] have found a correlation between the radioprotective effects and mitochondrial respiratory activity. Laser [9], Cohen [6] and Liebecq [11] have demonstrated an increase of radiosensitivity in the case when respiratory enzymes occur in oxidative state.

AET, cisteamine and other well-known radioprotectors were studied from this point of view [1—4]. In this paper, we have studied the action of some neurotropic substances, with radioprotective effect, on the activity of succinoxidase and cytochrome oxidase from mouse liver.

MATERIAL AND METHODS

AET * (S-2-/Aminoethyl/-isothiouree Br. HBr) — as a reference substance — and some neurotropes: iminodibenzene derivatives (imipramine = N-γ-dimethylaminopropyl-iminodibenzene, desmethylimipramine = N-γ-methyl-aminopropyl-iminodibenzene **); dibenzocyclc-

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heptatriene derivatives (Ro-4-1577, Ro-4-6011, Ro-4-8093, Ro-4-7960, Ro-4-8624**) and imipramine associated with chlorpromazine were used in our experiments.

White male mice were kept in normal conditions. The body weight of the animals was of 20 ± 2g. A group of animals were treated intraperitoneally with the above mentioned substances, 20 min. before killing. Succinoxidase (SuOx) and cytochrome oxidase (CyOx) activity was determined in liver homogenate [15]. Lactic acid (LA) concentration was determined in liver and blood. Pyruvic acid concentration (PA) was determined in the blood [5], [16].

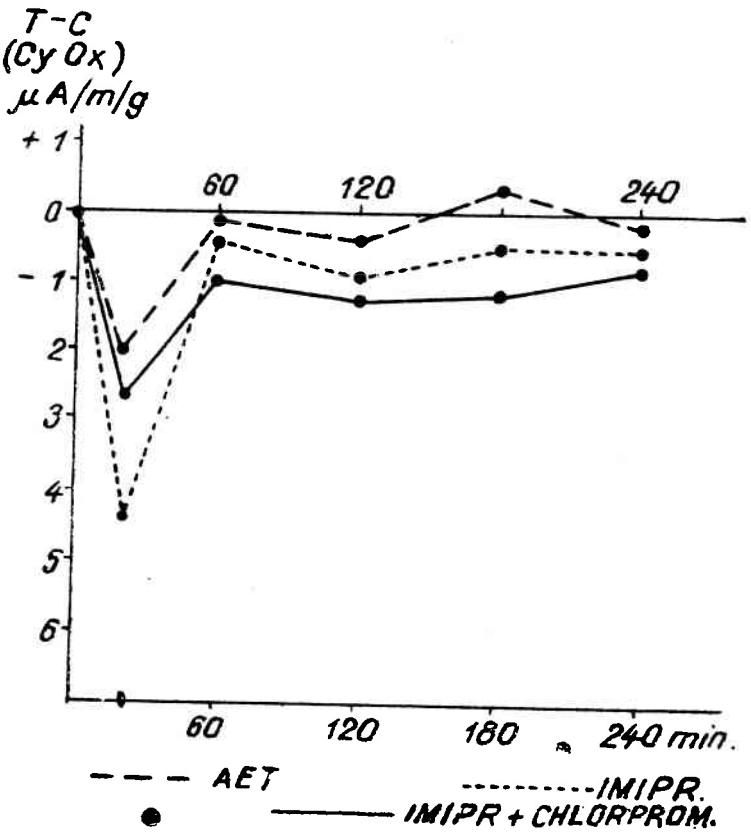


Fig.1. — Inhibitory action of AET, imipramine and imipramine + chlorpromazine on CyOx activity in the liver depending on the time after administration. (T—C = difference between treated and control).

In vitro effect of these substances was studied by using isolated liver mitochondrial fractions. We have used mitochondrial fractions obtained by a density gradient method [7]. The 0.340 M and 0.636 M saccharose suspensions of mitochondria have been obtained after 10 min. centrifugation of homogenate at 5000 g and 4°C. Mitochondrial sediments were obtained after 15 min. centrifugation at 18000 g. The oxygen consumption of mito hondrial suspensions in 0.36 M saccharose containing 30 mM succinate [8] and tris-HCl pH = 7.4, 10 mM + EDTA 1 mM + ADP and ATP 0.5 mM + KCl 30 mM and phosphate buffer pH = 7.4, 10 mM. Every vessel contained mitochondria corresponding to 7.6 mg proteins. The results obtained were calculated and evaluated by modern statistic methods.

RESULTS

A. In vivo action of AET, imipramine and imipramine + chlorpromazine on CyOx activity, depending on time

AET, imipramine and imipramine + chlorpromazine have considerable radioprotective effect when they are administered before 15—20 min. of exposure of animals to lethal dosis of X-rays. The survival after irradiation represented 85 per cent, 30 per cent, respectively 40 per cent of the total number of irradiated mice.

In our experiments 300 mg/kg b. w. AET, 62.5 mg/kg imipramine and 25 mg + 25 mg imipramine + chlorpromazine were used. The treated animals were sacrificed at different periods of time after administration

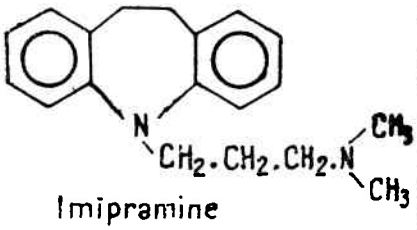
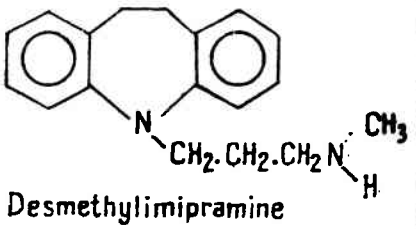
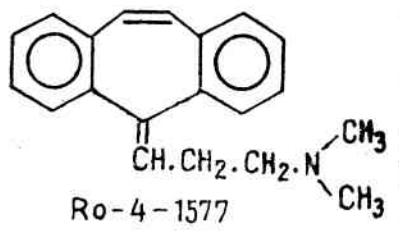
for determining the action of these substances on the CyOx activity in the liver of mice. The results obtained showed a maximum decrease of CyOx activity 20 min. after the administration (Fig. 1).

B. In vivo action of AET and neurotropes on CyOx and SuOx activity in the mouse liver

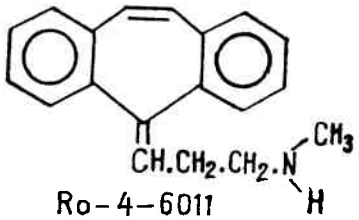
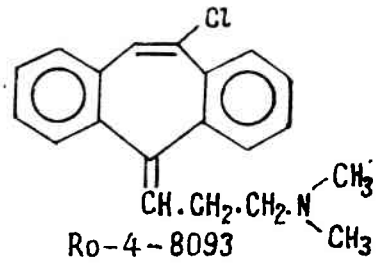
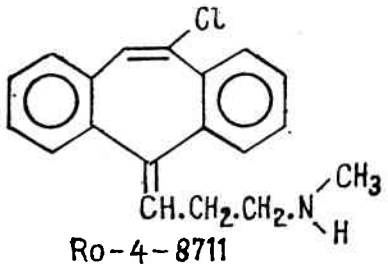
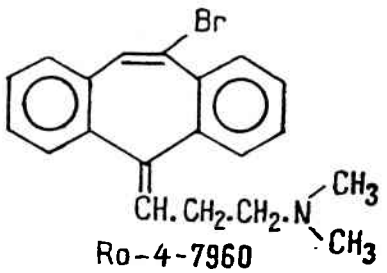
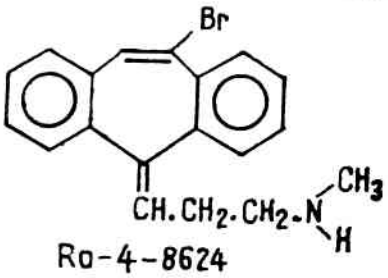
The results obtained are presented in table 1. It can be seen that AET significantly reduced CyOx and SuOx activity (19.2 per cent, respectively 26.7 per cent). Imipramine had a stronger effect than AET on CyOx activity (—38.5 per cent). Imipramine associated with chlorpromazine

Table 1

In vivo effect of AET and some neurotropes on CyOx and SuOx activity of liver 20 min. after the administration (Results are given in microatoms oxygen per min. and 1 g fresh tissue)

Substances	Dosis mg/kg		CyOx		SuOx	
			Control	Treated	Control	Treated
AET	300	X ±SE n p	9.48 0.20 (6) —	7.61 0.06 (6) < 0.01	7.72 0.13 (6) —	5.66 0.24 (6) <0.01
 Imipramine	62.5	X ±SE n p	12.94 0.78 (8) —	8.04 0.85 (8) <0.01	8.23 0.47 (8) —	6.28 0.42 (8) 0.02
 Desmethylimipramine	50	X ±SE n p	5.49 0.68 (5) —	3.66 0.40 (5) <0.01	13.08 0.66 (5) —	9.44 0.56 (5) <0.01
 Ro-4-1577	50	X ±SE n p	11.02 0.96 (6) —	8.40 0.36 (6) <0.05	18.42 2.20 (6) —	11.84 1.90 (6) <0.05

(Table 1 continued)

Substances	Dosis mg/kg		CyOx		Su Ox	
			Control	Treated	Control	Treated
 Ro-4-6011	50	X ±SE n p	9.68 0.78 (6) —	7.42 0.77 (6) <0.05	7.51 0.96 (6) —	3.52 0.45 (6) <0.01
 Ro-4-8093	50	X ±SE n p	9.76 0.77 (6) —	9.52 0.63 (6) >0.05	11.26 1.12 (6) —	11.20 1.96 (6) >0.05
 Ro-4-8711	50	X ±SE n p	8.41 0.96 (6) —	9.20 0.74 (6) 0.05	10.64 1.70 (6) —	8.26 0.53 (6) >0.05
 Ro-4-7960	50	X ±SE n p	9.71 1.81 (6) —	8.35 0.56 (6) 0.05	13.30 1.30 (6) —	12.55 1.96 (6) 0.05
 Ro-4-8624	50	X ±SE n p	3.74 0.64 (5) —	3.56 0.64 (5) >0.05	12.14 1.40 (5) —	10.26 0.76 (5) >0.05
Imipramine+ Chlorpromazine	25+ 25	X ±SE n p	11.00 0.52 (6) —	8.41 0.60 (6) <0.01	8.85 0.46 (6) —	7.90 0.51 (6) >0.05

inhibited only the CyOx activity of liver. Desmethylimipramine had a weaker effect than imipramine on CyOx activity, but in the same manner it inhibited SuOx activity.

Dibenzocycloheptatriene derivatives, especially the derivatives without halogen atoms in molecule (Ro—4—1577 and Ro—4—6011) had a significant inhibitory effect, but a weaker one than imipramine. Halogen derivatives containing Cl or Br did not show any action on hepatic CyOx or SuOx enzymes.

C. In vitro action of AET, imipramine and imipramine + chlorpromazine on the respiratory activity of isolated hepatic mitochondrial fractions

These experiments were made in order to elucidate the direct action of substances used on the respiratory activity of mitochondria. The substances were added to the incubation media. The results obtained are presented in table 2.

Table 2

Effect of AET, imipramine and imipramine + chlorpromazine on hepatic mitochondrial succinate oxidation (Results are given in microatoms oxygen per min. and mitochondrial proteins in mg)

Dosis per 7.2 mg mitochondrial proteins		AET 300 μg			Imipramine 300 μg			Imipramine + Chlorpromazine 150+150 μg		
		C	T	T—C	C	T	T—C	C	T	T—C
	X	0.88	0.52	—0.36	1.12	0.57	—0.59	1.18	0.69	—0.49
	±ES	0.02	0.02	0.02	0.04	0.03	0.07	0.13	0.15	0.14
	n	(8)	(8)	(8)	(6)	(6)	(6)	(5)	(5)	(5)
	p	—	<0.01	<0.01	—	<0.01	<0.01	—	<0.05	<0.02

C = control, T = treated

These date showed that AET and neurotropes induced a moderate decrease of oxygen consumption. This phenomenon determined a relative hypoxia of mitochondria, which is in full agreement with the so-called "biochemical shock" discovered by Prof. Bacq and co-workers [1], [2]. The reversible alteration of subcellular units may have an important significance in chemical protection. This hypoxia determines some modifications in cell metabolism, for instance : accumulation of reduced coenzymes (NADH, NADPH). This phenomenon is strongly supported by the increase of LA in liver and blood (Table 3) and the modification of LA/PA ratio in the blood after administration of AET, imipramine and imipramine + chlorpromazine.

Table 3

Effect of AET, imipramine and imipramine + chlorpromazine on LA concentration and LA/PA ratio after in vivo administration of substances 20 min. before sacrification

Substance and dosis	Liver	Blood	
	LA μg/0.1 g	LA μg/ml	LA/PA
Control	3.59 ± 0.09 n = 6	0.12 ± 0.027 n = 8	9.7
AET 300 mg/kg	4.19 ± 0.17 n = 5 p ~ 0.05	0.28 ± 0.027 n = 8 p < 0.01	12.2
Imipramine 62.5 mg/kg	4.76 ± 0.47 n = 6 p ~ 0.05	0.30 ± 0.036 n = 8 p < 0.01	16.9
Imipramine + Chlorpromazine 25 + 25 mg/kg	4.33 ± 0.36 n = 5 p ~ 0.05	0.29 ± 0.043 n = 6 p < 0.01	16.3

DISCUSSION

The obtained data revealed the effect of AET and radioprotective neurotropes (imipramine, desmethylimipramine, Ro—4—1577, Ro—4—6011, imipramine+chlorpromazine) in moderating liver respiratory mitochondrial enzymes activity. Radiosensitivity is always associated with the oxidative state of intracellular catalysts. A reduced state of these enzymes may ensure radioprotection, a phenomenon that could be in relationship with the action of CyOx inhibitors to produce the hypoxia of all enzymes involved in mitochondrial respiration. CyOx is a key enzyme, which reacts with molecular oxygen. The central position of CyOx is represented by Lehninger [10] in scheme 1 :

$$\text{succinate} \rightarrow \text{Fs} \qquad \text{NADH} \rightarrow \text{Fp} \qquad \Big\rangle \; Q \; \Big\langle \begin{matrix} \text{cit b} \\ \text{cit } c_1 \end{matrix} \rightarrow \text{cit c} \rightarrow \underline{\text{CyOx}} \rightarrow O_2$$

It appears likely that the protective substances studied in these experiments should act in such a way when the reduced coenzymes accumulated (as a consequence of the action of CyOx inhibitors) and they can react with free radicals formed by irradiation. Pihl and Sanner [14] suggested the interesting possibility that reduced coenzymes NADH and NADPH may act as hydrogen donors in transfer reactions, repairing target radicals by the following reaction :

$$R^{\cdot} + NADH \dashrightarrow RH + NAD^{\cdot}$$

The NAD· radicals formed would be expected to disappear in a dismutation reaction forming reduced and oxidized NAD :

$$2\ NAD^{\cdot} + H^{+} \longrightarrow NADH + NAD^{+}$$

Repair of target radicals by reduced pyridine coenzymes might partially explain the oxygen effect in biological systems.

LA concentration increased because extramitochondrial NADH could not be reoxidated by the respiratory chain, only to a smaller extent. NADH reoxidation is carried out by pyruvate and by other coenzymes (NADP), which likewise participate in free radical uptake.

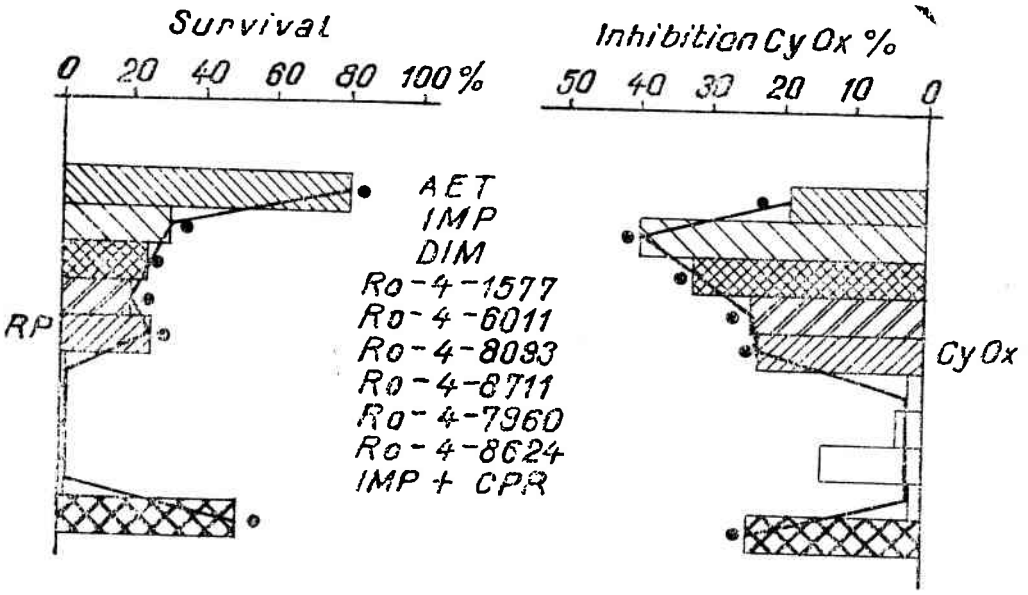


Fig. 2. — Comparison between the inhibitory effect of AET and neurotropes on the CyOx activity from liver and the survival data (in per cent) of white mice irradiated with lethal doses of X-rays.
● significant effects
IMP = imipramine
DIM = desmethylimipramine
CPR = chlorpromazine

In our experiments only the substances with radioprotective effect diminished the activity of liver respiratory enzymes but we did not find a good correlation between these two actions. Comparing the inhibitory effect of AET and neurotropes on the respiratory enzymes studied with the radioprotective one (after 30 days of irradiation with 800 R in the case of mice) (Fig. 2), we can conclude :

AET had 80—100 per cent radioprotective effect and an inhibitory action on CyOx activity equal to 19 per cent. Imipramine + chlorpromazine a 30—50 per cent radioprotective effect and an inhibitory action on CyOx activity equal to 24 per cent. Imipramine, desmethylimipramine, Ro-4-1577, Ro-4-6011 determined 10—30 per cent radioprotection and inhibited CyOx activity of liver by 25—40 per cent. The halogenated dibenzocycloheptatriene derivatives had no radioprotective action and no inhibitory effect on CyOx activity. We suppose that a moderate inhibition of respiratory chain might be significant in the radioprotection of the cells. A strong inhibition of CyOx and the other respiratory enzymes, as well as a weak effect, increases the sensitivity of the cells. Hypoxia produced by a moderate respiratory enzyme activity determines an increase in the rate of glycolysis and an accumulation of reduced coenzymes and LA concentration in liver cells and probably in the other radiosensitive organs.

These coenzymes may function as scavengers and modify the redox potential of cells, meeting the requirements of a relative radioresistant state of the animal organism.

REFERENCES

1. Bacq Z. M., *Chemical protection against ionizing radiation.* Ch. Thomas Publ., Springfield, 1965.
2. Bacq Z. M., Liebecq C., *Effets métaboliques de quelques radioprotecteurs,* in *Proc. II. Inter. Pharmacol. Meeting. Aug. 20—23, 1963,* Pergamon Press, New York, 1965.
3. Bacq Z. M., Beaumariage M. L., Van Caneghem P., Annal. Inst. Super. Sanita., 1965, *1,* 639—645.
4. Bacq Z. M., Bull. Acad. Royal. Méd., 1966, *4, 2.*
5. Barker I. B., Summerson W. H., J. Biol. Chem., 1941, **138**, *2,* 535—554.
6. Cohen J. A., Vos O., Van Bekkum D. W., *Advances in Radiobiology.* Oliver and Boyd, Edinburgh, 1957, p. 134.
7. Keil B., Šormova Z., *Laboratoriumstechnik für Biochemiker,* Akad. Verlagsgeselschaft. Geest et Portig K.-G., Leipzig, 1965, p. 759—776.
8. Kovách A., *A kisérletes orvostudomány vizsgáló módszerei.* Vol. IV, Akadémiai Kiadó, Budapest, 1961.
9. Laser H., Nature, 1954, **174**, 753.
10. Lehninger A., *The mitochondrion,* Benjamin W. A., New York-Amsterdam, 1965.
11. Liebecq C., *Radiosensitivity and cellular redox-potential* in "*Oxygen in the animal organism*", *Proc. Symp., London, 1963,* Pergamon Press, Oxford, 1964, p. 535—536.
12. Locker A., *Strahlenschutzwirkung von hypothermiesierenden und stoffwechselsenkenden Psychopharmaca.* Pharmacol. Geselschaft der D.D. R., 9 Jahrstagung, Dresden, 29. Nov. —2. Dez. 1967.
13. Маноилов С. Е., Радиобиол. радиотерапия, 1966, **7**, *5,* 611—626.
14. Pihl A., Sanner T., Progr. Biochim. Pharm., 1965, **1**, 85.
15. Potter V. R., Schneider W. C., J. Biol. Chem., 1943, **149**, 217.
16. Rindi G., Ferrari G., Experientia, 1956, **12**, *10.*
17. Uray Z., Holan T., Naturwissenschaften, 1966, **21**, 53.
18. Uray, Z., Holan T., Derevenco V., II Nat. Conf. Radiobiol., Varna. Bulgaria, 25—27 May 1967.
19. Zins G., Raymund A. B., Du Bois K. P., Rad. Labor. Quat. Report of Univ. Chicago, U.S.A., 1958, **29**, *1.*

Received December 2, 1968

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