TIME COURSE OF DISTRIBUTION OF LABELED ETHIMIZOLE IN BRAIN CELL STRUCTURES

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A study of the effect of the neurotropic drug ethimizole (ethylnorantiphein*) on memory and learning processes has shown that it acts mainly on the stage of consolidation of the engram of the acquired skill [3]. A single dose of ethimizole lengthens the retention time of a learned skill. This effect is exhibited in rats for 2 months [2], which shows that the drug has long-term activity. Meanwhile changes in very slow waves of membrane potential in rabbit brain tissue and the modulating effect of membrane potential on the shape of the action potential in neurons of the visceral ganglia of mollusks can be recorded as early as 5-8 min after administration of the drug [4, 5, 10]. A study of the action of ethimizole on metabolism of various brain cell structures showed that its targets may be both cell nuclei [8, 9] and membrane structures [3, 9, 12]. The velocity of conduction of the metabolic effects of ethimizole varies from a few minutes to 1 h, evidence of the wide time range of its action. It has been shown that ethimizole affects adenylate cyclase activity [9], but the precise molecular mechanisms of its effects are still unknown. For further studies of the ethimizole accepting system the most useful method was to study the time course of its distribution in intracellular structures.

The object of this investigation was to study the time course of distribution of [^{14}C]-ethimizole in intracellular structures of the rat cerebral cortex.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar rats weighing 150-200 g. Ethimizole, labeled at the second carbon atom with ^{14}C, was injected intraperitoneally in a dose of 3 mg/kg (1 mCi/kg). After various time intervals (from 2 min to 7 days) the animals were killed and nuclei [16], mitochondria, synaptic membranes, myelin [13, 17], microsomes, and cytosol (by centrifugation of the postmitochondrial supernatant at 105,000g for 1 h) were isolated from their cerebral cortex. The protein fraction extractable with 0.14 M NaCl also was isolated from the microsomes [6]. Fractions obtained as residues were suspended in physiological saline. An aliquot sample was taken from each fraction and its radioactivity counted, using Bray's scintillation fluid, on an Isocap-300 counter (Nuclear Chicago, USA). Protein in the fractions was determined by Lowry's method [15].

Five animals were used in each experiment; the result of each experiment, consequently, is given as an averaged value. Thus in cases where n = 3, 15 animals were used; when n = 4, 20 animals. However, the mean error m was calculated for n = 3 and n = 4.

EXPERIMENTAL RESULTS

The results are given in Table 1 and Fig. 1. Ethimizole penetrated into the brain cells only 2 min after its injection. A small fraction of it (1.6%) remained in the synaptic membranes, but most (46.3%) was found in the cytosol. A considerable proportion (27.2%) after this short period was incorporated into the fraction of proteins extractable from microsomes with 0.14 M NaCl. These proteins are located on the surface of the microsomal membranes, as

*1-Ethylimidazole-4,5-dicarboxylic acid-bis-methylamide.

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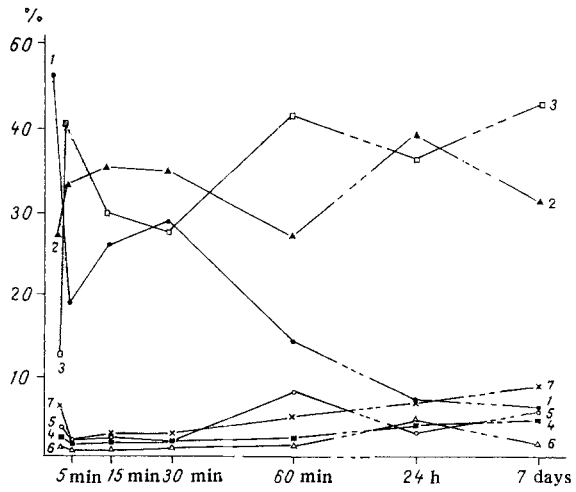

Fig. 1. Relative percentages of radioactivity of cell fractions. Total radioactivity taken as 100. 1) Cytosol, 2) surface proteins of microsomes, 3) nuclei, 4) microsomes, 5) mitochondria, 6) synaptic membranes, 7) myelin. Abscissa, time after injection of ethimizole; ordinate, % incorporation of label.

TABLE 1. Time Course of Distribution of [^{14}C]Ethimizole (in cpm/mg protein × 1000) in Fractions of Rat Cerebral Cortical Cells (M ± m)

Cell fraction	Time after injection of ethimizole						
	2 min (n=3)	5 min (n=3)	15 min (n=3)	30 min (n=3)	60 min (n=3)	24 h (n=3)	7 days (n=3)
Homogenate	12 208 ± 1 333	23 335 ± 2 898	16 840 ± 2 730	23 010 ± 2 859	13 344 ± 2 720	16 882 ± 1 456	5 775 ± 683
Cytosol	5 119 ± 143	4 464 ± 1 635	4 518 ± 1 535	8 631 ± 2 978	1 940 ± 192	1 180 ± 166	383 ± 106
Nuclei	1 371 ± 418	9 462 ± 722	4 866 ± 299	6 390 ± 1 958	5 542 ± 2 249	6 124 ± 1 318	2 459 ± 236
Microsomes (without soluble proteins)	274 ± 44	410 ± 98	377 ± 81	439 ± 102	352 ± 78	641 ± 112	269 ± 38
Microsomal proteins extracted with 0.14 M NaCl	2 998 ± 536	7 813 ± 1 243	5 999 ± 2 539	8 041 ± 1 986	3 584 ± 696	6 612 ± 904	1 783 ± 610
Mitochondria	407 ± 100	418 ± 137	458 ± 138	490 ± 177	1 069 ± 648	570 ± 81	303 ± 67
Total synaptic membranes	183 ± 89	272 ± 104	149 ± 29	243 ± 114	183 ± 48	620 ± 119	97 ± 18
Myelin	707 ± 127	495 ± 167	536 ± 144	776 ± 324	674 ± 226	1 135 ± 419	481 ± 71

was shown previously by an iodination method [6]. Of the other cell structures the greatest fraction (12.4%) was bound with cell nuclei. Most label 5 min after injection had moved from the cytosol into the cell structures. The highest level of radioactivity was found in the nuclei and surface proteins of the microsomes (40.5 and 33.5%, respectively). Measurement of radioactivity of the TCA residue of the cytosol showed that it was much lower (an order of magnitude) than the radioactivity of the nuclei and surface proteins of the microsomes; hence it could be concluded that the high level of incorporation of radioactivity into these fractions was not due to contamination with cytosol proteins. Most of the ethimizole dissolved in the cytosol was evidently not bound with proteins.

The radioactivity of the cytosol 1 h after injection of ethimizole was considerably reduced on account of increased binding of label by the nuclei and, in particular, by the mitochondria (From 2 to 8%). After 24 h only 7% of the label remained in the dissolved state and most of it was uniformly distributed among the nuclei (36.3%) and surface proteins of the microsomes (39.2%). Toward the end of 1 week not more than one-third of injected label remained in the brain cells, but most radioactivity at this time also remained bound with the nuclei and fraction of soluble microsomal proteins.

The most powerful acceptors of labeled ethimizole were thus the cell nuclei and surface proteins of the microsomal membranes. This by no means implies that ethimizole does not affect other cell strucures. For example, preliminary data obtained in the writers' laboratory show that in the concentration in which ethimizole is bound by synaptic membranes, it can activate phosphorylation of synaptosomal proteins. However, the fact that binding of ethimizole by fractions of nuclei and microsomal surface proteins took place more intensively is

undoubtedly a matter that deserves close attention. The ability of these fractions to bind calcium has been demonstrated recently [1, 7]. Meanwhile it has been shown that high doses of ethimizole increase the total calcium concentration in brain tissue [9]. Since ethimizole is largely antagonistic in its effects to caffeine, which inhibits Ca^{++} uptake by intracellular membranes, the possibility cannot be ruled out that it may have a specific influence on the calcium metabolism of particular cell structures, which is closely bound with the cyclic nucleotide system and modified when there is a change in state of the excitable cell membrane [1, 11].

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EFFECT OF ANTIOXIDANTS ON [^{3}H]SEROTONIN RELEASE BY RAT BRAIN SYNAPTOSOMES

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Release of neurotransmitters mediating Ca^{++}-dependent mechanisms is a key stage in the chain of events lying at the basis of synaptic transmission [1]. It has recently been established that certain lipids and, in particular, peroxidation products of free fatty acids and glycerophosphatides are highly efficient Ca^{++} ionophores [6]. Hence it follows that compounds which can affect free-radical lipid peroxidation (LPO) and, consequently, which can change the content of Ca^{++} ionophores in the synaptic membrane, may be regulators of neurotransmitter secretion.

The object of this investigation was to study the effect of LPO inhibitors of different chemical nature on release and reuptake of [^{3}H]serotonin by the synaptosomal fraction of rat cerebral cortex.

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