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UPTAKE AND SUBCELLULAR DISTRIBUTION OF INTRAVENTRICULARLY INJECTED [1-^{3}H]DOLICHOL IN RAT BRAIN**

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The uptake of C_{95}[1-^{3}H]dolichol in the form of liposomes into rat brain after the intracerebral and intraperitoneal injection was investigated. Efficient, time-dependent uptake of dolichol into the brain was observed exclusively after the intraventricular injection. Within 24 h after the injection about 10% of the applied dolichol was found in the brain and 1.5% in liver. The distribution of dolichol in various parts of rat brain decreased in the order: cerebellum > midbrain > grey matter and brain stem > white matter. Seven days after the injection total radioactivity in the brain decreased and concomitantly a significant increase was observed in blood circulating and liver of the rat. The highest activity was found in grey matter and it remained a few times higher in comparison with that in white matter. About 80% of the dolichol taken up by the brain membrane was recovered in the following subcellular fractions: crude nuclear fraction > microsomes > mitochondria > synaptosomes > myelin.

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These results demonstrate for the first time that dolichol is actively taken up by the brain membrane exclusively after intraventricular injection of dolichol-phosphatidylcholine in the form of liposomes; this method may be useful in studies on the role of dolichol in brain function.

Dolichols are very long-chain polyprenols, with a saturated α-isoprene unit, typical of animal tissues. All so far examined animal organs contain a mixture of these lipids with chain length ranging from C_{85} to C_{120} [3]. Dolichols occur in the form of phosphate esters acting as lipid carriers of glycosyl residues in biosynthesis of asparagine-linked glycoproteins (90% of brain glycoproteins) and as neutral lipids of unknown function: free alcohols, esters with fatty acids [1, 2].

The content of nonpolar forms of dolichols in different parts of the brain varied greatly, and accumulation reaching several times the neonatal level during the life span in the cortical and the subcortical grey matter was observed in mammalian and human brain [3, 4]. Moreover, dramatic accumulation of these lipids occurred in the cerebral cortex of patients with ceroid-lipofuscinosis and Alzheimer's disease [5, 6]. In contrast, dolichol phosphate is more evenly distributed in different parts of the brain [7]. Its concentration increases moderately and reaches a plateau in the maturation period characterized by neuronal and glial differentiation and myelination, when rapid synthesis of glycoproteins, critical in mediation of these major developmental events, is achieved.

Although the role of the neutral form of dolichol in cellular structure and function has not been established, the considerable influence exerted by dolichol and its derivatives on organization and packing of different lipids in model membranes is well documented [8, 9]. As a result of increasing concentration of dolichols, enhanced formation of inverted micelles in the phosphatidylethanolamine-containing membranes and liposome fusion were observed [10]. In this way dolichols and their derivatives could be involved in important cellular processes such as vesicular organelles traffic and fusion, could facilitated transmembrane transport, and influence the activity of membrane-bound enzymes. Indeed, exogenous dolichol incorporated into the synaptosomal plasma membrane influences significantly agonists binding to the $GABA_A$ receptor [11], the activity of synaptosomal Na^+, K^+-ATPase and, only moderately, the activity of acyltransferase [12]. An increase in dolichol content in membranes ob-

served with age may be an important factor in the decline of the neuronal membrane function.

The aim of the present studies was to investigate the uptake of dolichol into different parts of brain and into particular subcellular fractions.

MATERIALS AND METHODS

Chemicals. [1-^{3}H]Dolichol C_{95} (spec. act. 16 mCi/mol) was from the Collection of Polyprenols, Institute of Biochemistry and Biophysics, Warsaw, Poland.

Tris[(2-chloroethyl)-amine hydrochloride] and sucrose, were purchased from Sigma, St. Louis, U.S.A. T.l.c. plates were from Merck.

Dolichol was dispersed in 0.9% saline by injection of ethanolic solution into vigorously mixed saline or together with phosphatidylcholine in the form of liposomes.

Liposomes were prepared by sonication of a suspension of egg yolk phosphatidylcholine with radioactive [1-^{3}H]dolichol C_{95} in 0.9% NaCl. Liposomes were injected intraventricularly or intracerebrally (10 µl) or intraperitoneally (500 µl) into 50-day-old Wistar rats. At the indicated time the rats were decapitated, the brains removed and transferred into 20 ml of ice cold 0.9% saline, then the brain was washed four more times with the same solution and dissected. The separated parts of the brains were homogenized with the same volume of saline and aliquots were taken for measurement of radioactivity. For the subcellular fractionations brains were homogenized in 0.25 M sucrose with 50 mM Tris/HCl, pH 7.4, 1 mM EDTA using a Dounce type glass homogenizer. The 10% (w/v) homogenate was centrifuged for 3 min at 1 100 × g. The resulting supernatant was centrifuged for 10 min at 17 000 × g to yield a crude mitochondrial fraction. This fraction was resuspended in 0.32 M sucrose, 10 mM Tris/HCl, pH 7.4 and further fractionated on a Ficoll-sucrose discontinuous gradient as described by Booth & Clark [13].

The interphase containing synaptosomes and myelin was collected, resuspended in 0.32 M sucrose, 10 mM Tris/HCl, pH 7.4 and centrifuged at 17 000 × g for 10 min for synaptosomes and at 73 000 × g for 60 min for myelin. The mitochondria containing pellet was resuspended in the isolation medium. The microsomal fraction was obtained after centrifugation of the postmitochondrial supernatant at 100 000 × g for 60 min.

Lipids were extracted according to Folch *et al.* [14]. Dolichol and dolichol phosphate were separated on t.l.c. using the chloroform : methanol : H_2O (1:1: 0.2, by vol.) solvent system.

Radioactivity of lipid samples after evaporation was determined in a Beckman LS-9000 scintillation counter.

RESULTS AND DISCUSSION

In our preliminary studies on application of dolichol into rat brain *in vivo*, an aqueous suspension and phosphatidylcholine liposomes containing dolichol were injected.

The results indicated that dolichol was 10 times more efficiently taken up by the brain membrane from phosphatidylcholine liposomes containing [^{3}H]dolichol than from the ethanol suspension (not shown). In further experiments the liposome suspension was used.

When dolichol was injected into the parieto-occipital part of the left hemisphere, a four times higher activity was found at the place of injection as compared with the right hemispheres.

Intraventricular injection resulted in uniform uptake of radioactive dolichol by the brain. After intracerebral injection about 10% of the injected radioactivity was found in the brain and about 1.5% in liver.

After intraperitoneal application of radioactive dolichol the distribution was reversed. About 10% of radioactivity was found in the liver and about 1% in the brain (Table 1).

Thus the intraventricular injection was found to be the most convenient for the studies on dolichol uptake into the brain membrane.

Figure 1 presents the distribution of [^{3}H]dolichol in the brain cortex (in grey and white matter), in midbrain, stem and cerebellum 1 h, 24 h and 7 days after the intraventricular injection. At 24 h after the intraventricular injection the highest radioactivity was found in cerebellum, a lower one in midbrain > grey matter and stem, and very low in white matter.

The pattern of radioactivity distribution changed with time and on the 7th day after the injection the highest accumulation of dolichol radioactivity was observed in the grey matter of the brain cortex, but it remained very low in the white matter. The uptake of dolichol by cortical grey matter was 5 - 7 times more intensive as compared with that in the white matter of the cerebral cortex.

Table 1

Uptake and distribution of labeled dolichol after intracerebral, intraventricular and intraperitoneal injection

Dolichol (4×10^7 c.p.m. of [1-^{3}H]C$_{95}$) was injected as a liposomal suspension with egg yolk lecithin in 10 µl of saline intraventricularly and in 500 µl intraperitoneally. The animals were decapitated 24 h after the injection, the brains and liver quickly removed and homogenized, and the lipids extracted. The radioactivity of lipid extracts from the brain and liver was measured. The values represent means from three experiments

Place of injection	Radioactivity (c.p.m. $\times 10^5$)		
	Cerebral hemispheres		Liver
	Right	Left	
Left cerebral hemisphere	2%	8%	1%
Intraventricular	5%	5%	1.5%
Intraperitoneal	0.5%	0.5%	10%

This pattern of dolichol accumulation in different parts of the brain follows the highly differentiated distribution of internal dolichols in the grey- and white matter-rich parts of the brain [15].

Table 2 presents the distribution of dolichol in the brain subcellular fractions after the intraventricular injection. About 50% of the whole homogenate radioactivity was found in the crude nuclear fraction, 17% in microsomes, 9% in mitochondria and 3.0% and 1.5%, respectively, in synaptosomes and the myelin fraction.

A similar endogenous dolichol distribution among the brain mitochondrial and synaptosomal fractions was described by Sun *et al.* [12].

In the studies of Rip & Carroll [2], when dolichol was injected intravenously to rats, most of the radioactivity was recovered from the liver.

In this and in the other types of experiments the brain did not seem to absorb any significant amount of the injected dolichol. Our experiments showed for the first time that 24 h after intraventricular injection of labeled dolichol, about 10% of the radioactivity was found in the brain and 1.5 % in the liver.

After 7 days, the radioactivity in the midbrain decreased significantly and that in liver increased. The intraventricular injection of dolichol and active uptake of dolichol into the subcellular brain membrane may be useful in the studies on the role of dolichol in the brain membrane function.

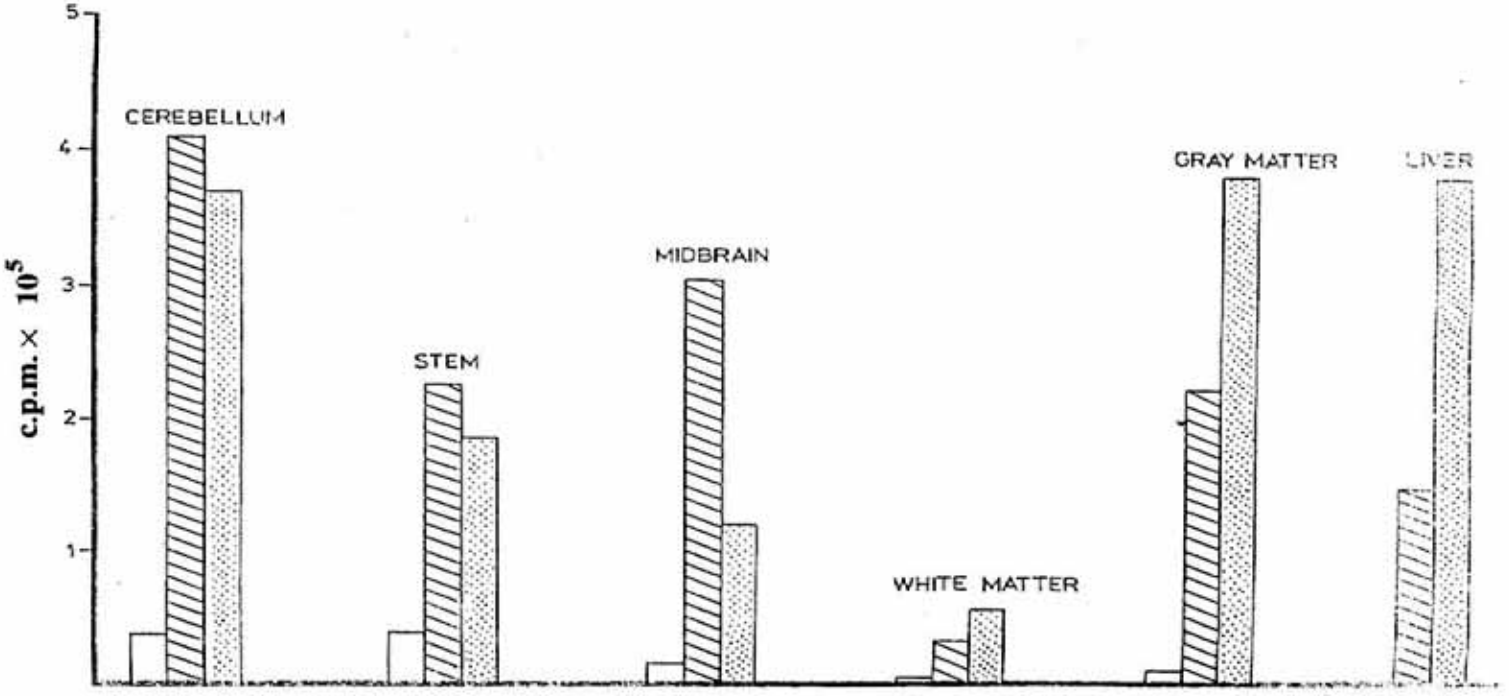

Fig. 1. Time-dependent uptake into and distribution of [1-^{3}H]dolichol in different parts of rat brain and in liver after intraventricular injection of dolichol.The results are the mean from two determinations. ▭, 1 h; ▨, 24 h; ▩, 7 days

Table 2

Distribution of intraventricularly injected [^{3}H]dolichol in brain subcellular fractions

Dolichol (4×10^7 c.p.m. of [1-^{3}H]C_{95}) was injected intraventricularly as a liposomal suspension with egg yolk lecithin in 10 µl of saline. After 7 days the whole brain homogenate containing 10% of the injected radioactivity was fractionated as described in Methods. Aliquots of homogenate and of each subcellular fraction were taken for radioactivity determination. The values represent the mean ±S.D. from three experiments

	Radioactivity (c.p.m. $\times 10^5$)	Homogenate radioactivity (%)
Homogenate	40.40 ± 0.11	
Crude nuclear fraction – whole cells	19.83 ± 0.31	49.0
Microsomal fraction	6.90 ± 1.87	17.1
Mitochondrial fraction	3.70 ± 0.17	9.2
Synaptosomal fraction	1.22 ± 0.14	3.0

It seems that the observed significant increase in the content of dolichols in brain membranes with age may be an important factor in the decline of neuronal membrane function.

Moreover, a characteristic feature of aged neurons as compared with young ones, is also the accumulation of lipofuscin pigments which contain dolichol and which may correlate with the loss of learning capacity, short-term memory and arousal [16].

At present the role of dolichol in the signal transduction pathway connected with inositol phospholipid metabolism is under study.

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