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DISTRIBUTION PATTERN OF ALKANES IN WHOLE BRAIN MITOCHONDRIA, MICROSOMES, SYNAPTOSOMES AND MYELIN ISOLATED FROM NORMAL MOUSE

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SUMMARY

An homologous series of *n*-alkanes, which are the major aliphatic hydrocarbons, has been identified in normal mouse brain and in brain subfractions: myelin, mitochondria, microsomes, synaptosomes.

The distribution patterns are qualitatively similar within all the studied fractions and show approximately equal amounts of odd and even chains. These alkanes, present in minor amounts in all the brain subfractions, accumulate in the myelin.

Alkanes are ubiquitous in nature; their occurrence in microorganisms, higher plants, insects, birds, mammals, marine animals and plants have been recently summarized [10].

Only fragmentary data about mammalian alkanes are as yet available; alkanes are part of the surface lipids [10] and they are also widely distributed in different internal organs: bovine [12] and human liver [15], beef heart [1] and brain [13]. The lack of systematic analysis concerning mammal distribution of *n*-alkanes may be attributed both to the related serious problems of exogenous contamination and the very low amounts usually found in mammals, in marked contrast to higher plants or microorganisms [7].

Nevertheless the composition of the hydrocarbons recovered from different tissues showed striking differences: in human liver [15], beef liver [12] and beef brain [13], alkanes were mostly of the cyclic and branched-chain type. In human meninges [10], serum lipoproteins [16], sweat [15] *n*-alkanes are ranging from C18 to C34 with an equal amount of odd and even chains, whereas in bovine heart [1], in human urine [15], in human [15] and bovine liver [12], odd chains are predominant. These results suggest preferences in the

accumulation of certain classes of hydrocarbons in different organs of the same species.

We previously studied the brain myelin from normal and Quaking mice (Quaking mice are neurological mutants with defective myelination). We showed that the alkane level in the normal brain myelin is higher than that established for any other organ. Moreover the Quaking myelin contained 3 times less alkanes than the normal one [4]. On the other hand, there is a diminution in the Quaking myelin of the level of the very long chain fatty acids, (VLCFA, ranging from C20 to C30), [2,5]. This observation is in good agreement with a relationship between VLCFA and alkanes in mammals as it has been demonstrated in other systems [8,11] and led us to made a systematic work on the level and the distribution pattern of alkanes in different fractions isolated from normal mouse brain (myelin, mitochondria, microsomes, synaptosomes) in order to determine if there is a preferential accumulation in myelin, if one pattern could correspond to one subfraction and to investigate further the biological role of alkanes in the membrane structure.

MATERIALS AND METHODS

Isolation of different fractions

Myelin [4], microsomes [3], mitochondria [14] and synaptosomes [6] were prepared as previously described, the purity of each subcellular fraction was performed by electron microscopy and marker enzymes.

Alkane analysis

Great caution was exercised throughout the experiments to avoid contaminations. During this work a strict absence of any paraffin in our laboratory was insured.

All solvents ($CHCl_3$, MeOH, Spectrograde Hexane) were freshly redistilled. All glassware was carefully cleaned, boiled with redistilled methanol and washed with spectrograde hexane. 50 ml of each purified solvent was evaporated to about 100 μl and they were tested by gas chromatography for the presence of hydrocarbons. As negative results were obtained, corrections for contaminations were not necessary.

For each fraction analysed (brain, myelin, microsomes, mitochondria, synaptosomes) the following procedure was done. The sample (10—30 mg) plus hexadecane (60 μg) as internal standard was saponified with KOH 5 M (0.5 ml) at 70°C for 1 H. The non-saponifiable material (including alkanes) was extracted thrice with 2 ml spectrograde hexane. The hexane solution was washed twice with water, dried with Na_2SO_4 and concentrated under N_2 to 1 ml. The residue was chromatographied with spectrograde hexane on a glass column (30 cm × 3 mm) containing prewashed alumina (activity degree III). The eluate was evaporated to 100 μl under N_2 and kept frozen for analysis.

For infrared spectra, determined with a Perkin Elmer 457 spectrophotometer, this eluate was evaporated to dryness to eliminate the hexane and then

dissolved in spectrograde CCl_4. In these conditions, IR spectra showed the presence of CH_2 and CH_3 groups only. The blank run had only the absorption bands due to CCl_4.

Mass spectrometry was also performed and all the fragmentation peaks were characteristic of saturated aliphatic chains.

Analysis were done by GLC (Intersmat 14 C 120 DFL) on a 3% OV 17 column programmed from 150 to 310°C at 4°C/min. The identification of the different peaks was done by comparison of their retention times with those of known standards. The relative abundances were estimated by calculation of their peak areas as compared to that of the internal standard, assuming a similar response of the detector for all the alkanes. Each experiment was performed at least 3 times.

RESULTS AND DISCUSSION

Each subcellular fraction contained alkanes ranging from C19 to C33 in myelin and from C23 to C35 in the others. The concentrations were 0.66 ± 0.07 μg/mg in the whole brain, 7.1 ± 0.6 μg/mg in myelin, 0.53 ± 0.02 μg/mg in microsomes, 0.48 ± 0.15 μg/mg in mitochondria and 0.13 ± 0.05 μg/mg in synaptosomes. All these results are an average of 3—5 assays (Table I).

These results show that alkanes occur, in each fraction studied under similar conditions. They are at rather comparable yields in each fraction excepting myelin where they appear at least 10 times more abundant.

The levels we have observed in mitochondria, microsomes, synaptosomes seem to be higher than those previously reported by other authors in different organs (4 μg/g in bovine liver [12]; 10 μg/g in human liver [15]; 32 μg/g in beef heart [1] for example) and even in the beef brain [13] where the small isolated amount is consistent with inevitable losses occurring during isolation.

Table I shows the distribution pattern of the identified alkanes in each fraction. These values represent at the same time, an average of the results established for each trial. It can be observed also an almost equal distribution among odd and even straight chains.

When we compare the distribution profile of alkanes in the analyzed materials, each pattern resembles to a Gaussian curve, but with a variable maximum; around nonacosane (C29) for mitochondria, microsomes and synaptosomes; heptacosane (C27) for whole brain and pentacosane (C25) for myelin. C29 accounts for respectively 15% in microsomes and 18% in synaptosomes; in mitochondria, C27, C28, C29, C30, which are almost at the same level, represent 51%; in whole brain C26, C27, C28 represent near 40% and in myelin, C24, C25, C26 : 46%.

As seen from the table, these distribution patterns with an equal amount of only straight odd and even chains are very similar in each fraction, and agree very well with those already described in sweat by Schlunegger [15] and human serum by Skipski et al. [16] but contrast with several other organs [1,13,15] where either cyclic and branched-chain occur or odd chain are prominent.

TABLE I

DISTRIBUTION PATTERN OF ALKANES IN NORMAL MOUSE BRAIN AND ISOLATED SUBFRACTIONS

Each value is expressed as % of the total and represent an average of 3 to 5 assays. For experimental details see under methods.

	Whole brain	Brain fractions			
		Microsomes	Mitochondria	Synaptosomes	Myelin
Total amount (μg alkane/mg dry tissue).	0.66 ± 0.07	0.53 ± 0.02	0.48 ± 0.15	0.13 ± 0.05	7.1 ± 0.6
Carbon Number					
C19	—	—	—	—	3.1
C20	t	—	—	—	3.3
C21	t	—	—	—	4.5
C22	t	t	t	t	9.7
C23	9.2	8.1	4.2	4.2	8.2
C24	6	7.6	4.2	3.5	12.5
C25	7	10.6	6.1	8.1	13.3
C26	11.9	9.9	8.6	10.3	10.6
C27	13.9	11.6	11.8	13.6	7.7
C28	13.9	12.2	12.3	12.8	7.4
C29	9	15.2	13.4	18	5.1
C30	7.5	9	13.5	11.2	2.9
C31	5	7.1	9.4	6.8	6.7
C32	6.5	7	9.1	8.1	2.9
C33	2.4	1.6	4.1	1.3	6
C34	1	t	2.3	2.1	—
C35	3.9	t	0.9	t	—
C36	2.9	—	—	—	—
Even chains (% of total)	50.4	54.2	49.9	52	48
Odd chains (% of total)	49.7	45.8	49.9	48	52

Our results indicate that alkanes accumulated preferentially in brain myelin. When related to the myelin yield per brain alkane amount in myelin represents more than 80% of the alkane level found in one whole brain so that the other brain subfractions including plasma membrane not studied here, may account for less than 20%.

The alkane which accumulated in myelin could have an exogenous origin or may reflect a local synthesis in the brain. Although the second hypothesis is considered as unlikely in the mammalians [10], it cannot be ruled out, since we observed recently an alkane synthesis by a microsomal pellet from a rabbit sciatic nerve [9]. This point will be further investigated in our laboratory.

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