PREFERENTIAL ENRICHMENT OF THE (+)-α-HEXACHLOROCYCLOHEXANE ENANTIOMER IN CEREBRAL MATTER OF HARBOUR SEALS

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Introduction

Organic substances that are transported by the blood towards the neurons of cerebral matter have to pass a special barrier, the so-called "blood-brain-barrier [BBB]". It is well-known that some hydrophobic substances are able to pass this barrier, while the passage of other compounds, e.g., carbohydrates requires additional energetic contributions of the endothel-cells and the gliacells. The function of the BBB depends on a membrane that wraps up the brain completely thus forming a barrier which regulates the transfer of substances into and from the cerebral matter[1]. Sometimes, it is assumed that this "barrier effect" is complemented by an "enzymatic barrier" in the endothel-cells[2].

In general, it is claimed that the BBB prevents many organic pollutants from penetrating this barrier. This hypothesis is in accordance with results which showed a decrease in concentration of relevant environmental pollutants in the brain in comparison with the concentration of these substances in the fat or other organs of the same animals. However, recently Portig et al.[3] and Rimkus[4] presented experimental evidence that this observation cannot be rigorously generalized and that exceptions have to be stated at least for α-hexachlorocyclohexane [α-HCH].

Portig et al. tried to explain the preferential distribution of α-HCH into cerebral white matter of rats relative to that in blood by stereospecific arguments. Herein, additional experimental evidence will be presented that supports this conjecture by demonstrating the highly enantioselective character of the transport mechanism.

Since α-HCH is the only chiral isomer of all eight conceivable HCH-isomers, the recently developed cyclodextrin-phases in chiral gas chromatography allow a new experimental access[5,6] to verifying the hypothesis of Portig et al.. Our experimental approach includes the gas chromatographic analysis of fat and brain tissues of harbour seals (Phoca vitulina) obtained from samples of highly polluted animals of the German Bight (North Sea) and of less polluted harbour seals from Iceland.

Experimental method

Tissue samples of dead harbour seals of the German Bight found in the year 1989 were obtained from the Institut für Haustierkunde, Kiel (F.R. Germany) , and samples from Iceland seals were kindly supplied by Dr. Skirnisson, University of Reykjavik (Iceland), in 1990.

The tissue samples of the seals were extracted and purified according to known procedures including Florisil adsorption chromatography and gel permeation chromatography [GPC]. After purification the extracts were dissolved in toluene and used for the subsequent chiral gas chromatographic analysis without further clean-up steps.

The concentrations of the HCH-isomers were determined gas chromatographically using conventional DB5, DB1701 and SE-54 columns , while the enantiomeric ratios [ER] of α-HCH were determined using a chiral fused-silica capillary column which had been specially prepared according to Faller et al.[5]: 25 m, i.d. 0.25 mm, coated with 50 % octakis(3-O-butyryl-2,6-di-O-pentyl)-γ cyclodextrin and 50 % OV-1701, carrier gas helium 60 kpa, on a Vega gas chromatograph Carlo Erba, 2 µl on-column injection, ^{63}Ni-ECD with nitrogen as make-up gas.

Results and Discussion

Typical gas chromatograms of α-HCH and γ-HCH obtained from water samples., and from blubber and brain tissues of harbour seals of the German Bight, respectively, are shown in Figure 1. In the upper part of Figure 1, conventional gas chromatograms using a SE-54 column are shown which illustrate the different enrichment factors of α-HCH and γ-HCH in blubber and brain tissues of harbour seals in the German Bight, respectively. For comparison, the chromatogram showing the γ-HCH/α-HCH relation in the first part of the ecological chain, in North Sea water, is also included in Figure 1. In the lower part of Figure 1, gas chromatograms obtained by means of the chiral stationary phase described above are shown. The enantiomeric ratios [ER] inferred from these chromatograms are given below the respective chromatograms. The α-HCH-concentrations and the enantiomeric ratios of α-HCH of all seal tissue samples investigated herein are summarized in Table 1.

The concentration values summarized in Table 1 clearly confirm the hypothesis that α-HCH is able to penetrate the BBB giving rise to enrichment factors of about 3 to 4 (German Bight) and up to 8 (Iceland) in seal brain tissue in relation to blubber of the same animal. Insofar earlier conclusions drawn by Portig et al.[3] for rats and by Rimkus[4] for harbour seals are confirmed.

A very unexpected result can, however, be inferred from the enantiomeric ratios determined by chiral gas chromatography. The ER of α-HCH in the water of the German Bight of about 0.87 reflect the enantioselective degradation of α-HCH by marine microorganisms which are known to decompose preferably (+)-α-HCH [7] thus giving rise to ER values < 1. The enzymatic decomposition of α-HCH indicated by ER values of 1.5 to 4.5 for seal blubber, however, leads to a preferable degradation of (-)-α-HCH. Similar observations were reported by Kallenborn et al.[8] for Common Eider ducks. The unexpected result is related to the ER values of the seal brain tissues which indicate that nearly exclusively (+)-α-HCH is able to penetrate the blood-brain-barrier, while (-)-α-HCH is largely held back by this barrier.

These results imply that a.) an active transport mechanism through the BBB can be assumed and b.) the BBB exhibits a strongly enantioselective character.

Further insight into the BBB can be gained by comparing the values obtained from harbour seals of the German Bight and from Iceland: basically, in both cases the BBB leads to an enantioselective enrichment of (+)-α-HCH in the brain tissue. However, in less polluted Iceland seals exclusively the (+)-enantiomer was found, while in more polluted seals from the German Bight also 5 to 11 percent of (-)-α-HCH was determined. Additional investigations are due, in order to verify our tentative hypothesis that higher concentrations of organic pollutants may lead to a partial "inactivation" of the BBB.

Figure 1: gas chromatograms of North Sea water, blubber and brain tissue of North Sea harbour seals. Upper part: α-HCH, γ-HCH, and internal standard ε-HCH measured with a SE-54 column. Lower part: Enantiomers of α-HCH, i.e., (+)-α-HCH and (-)-α-HCH, measured with the chiral cyclodextrin-phase (see experimental section).

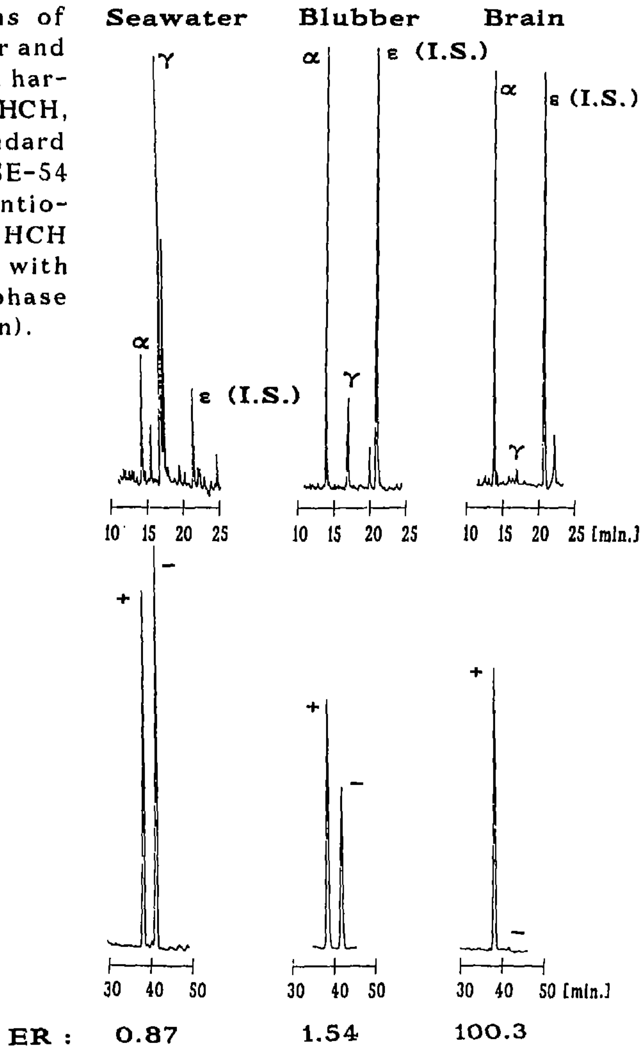

Table 2: Enantiomeric ratios, percentage and concentrations of the enantiomers of α-HCH determined in tissue samples of 3 harbour seals found in the German Bight, and of tissue samples of 8 Iceland harbour seals.

Sample	Tissue	Enantiomeric ratio [+/-]	Percent (%)		Concentration [µg/g lipid]	
			+	-	+	-
German Bight 1	Blubber	4.47	81	19	0.05	0.01
	Spinal marrow	19.89	95	5	0.21	0.01
German Bight 2	Blubber	1.54	60	40	0.03	0.02
	Brain	16.34	94	6	0.22	0.01
German Bight 3	Blubber	2.83	74	26	0.05	0.02
	Brain	7.9	89	11	0.23	0.03
Iceland 1	Blubber	1.36	58	42	0.006	0.004
	Brain	∞	100	--	0.08	--
	Spinal marrow	∞	100	--	0.05	--
Iceland 2	Blubber	1.26	56	44	0.011	0.009
	Brain	∞	100	--	0.11	--
	Spinal marrow	99.54	99	1	0.04	--
Iceland 3	Blubber	1.21	55	45	0.011	0.009
	Brain	∞	100	--	0.11	--
	Spinal marrow	∞	100	--	0.07	--
Iceland 4	Brain	∞	100	--	0.09	--
	Spinal marrow	∞	100	--	0.06	--
Iceland 5	Brain	66.24	98	2	0.08	--
	Spinal marrow	30.18	97	3	0.04	--
Iceland 6	Brain	55.64	98	2	0.10	--
	Spinal marrow	∞	100	--	0.06	--
Iceland 7	Brain	∞	100	--	0.06	--
	Spinal marrow	∞	100	--	0.03	--
Iceland 8	Brain	∞	100	--	0.06	--
	Spinal marrow	22.84	96	4	0.03	--

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