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HIBERNATION-INDUCED CHANGES IN THE GANGLIOSIDE COMPOSITION OF DORMICE (*GLIS GLIS*)

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Abstract—1. The brain ganglioside pattern and the concentration of proteins, sialoglycoproteins and gangliosides in the whole brain and seven different brain parts of normothermic and hibernating dormice (*Glis glis*) were investigated and compared with corresponding data of laboratory rats.

2. The concentration of brain proteins (about 100 mg/g fresh wt), sialoglycoproteins (about 230 μg NeuAc/g fresh wt) of normothermic as well as hibernating dormice is very similar to that of laboratory rats. The concentration of gangliosides however is slightly higher in rats as compared to dormice and reaches values between 500 and 1000 μg NeuAc/g fresh wt, depending on the brain-structure.

3. The ganglioside pattern of normothermic dormice in comparison to that of laboratory rats is characterized by a higher content of the polar trisialoganglioside GT1b (+8.5% in the cerebellum) and the tetrasialoganglioside GQ1b (+4.5% in the cerebellum).

4. In hibernating dormice in contrast to their normothermic counterparts the brain gangliosides are even more polar resulting in an increase of the polar ganglioside fractions (GT1b, GQ1b, GP) from 30.5 to 35.8% of ganglioside-bound NeuAc.

5. There is not change in the ganglioside pattern with length of torpor. The polysialylation takes place during the first three weeks of hibernation.

6. The results are discussed with regard to the hypothesis, that neuronal membranes provided with more polar gangliosides at lower environmental temperatures might be more efficient with respect to the high complexation ability of gangliosides to Ca^{2+} ions.

INTRODUCTION

IN HOMEOTHERMIC vertebrates the ability to tolerate large changes in body temperature is only weakly developed. Nevertheless, in birds and mammals this thermal tolerance is evident to a much larger extent during the heterothermic phase of neonatal development and during torpor phase in hibernators, which in their non-hibernating state, of course, are competent homeotherms. According to Satinoff (1967), Mrosovsky (1968) and Williams & Heath (1970) the CNS is suggested to be the regulatory system for initiation and maintenance of hibernation; and within the CNS the functional contacts between the nerve cells, the synapses, were shown to be the most sensitive and adaptive parts of an organism. These synapses might be responsible for the regulation of the thermal tolerance (Katz & Miledy, 1970; Hazel & Prosser, 1974; Lagerspetz, 1974). So it was assumed that changes in the physico-chemical properties of the neuronal membrane are probably responsible for temperature dependent synaptic events, which were thought to be essential determinants of the thermotolerance of hibernators also.

Up to now, there are only few studies dealing with longterm effects of hibernation at the molecular level of the neuronal membrane itself. With regard to this Aloia & Pengelley (1979) showed, that in microsomal fractions of hibernating squirrel brains an increase in the amount of monoenoic fatty acids of several phospholipids occurred. But since these compounds are ubiquitous in biological membranes, the changes observed may not be specific so consequently, the interest is focussed on those compounds enriched in synaptic terminals.

In this regard gangliosides seem to be best qualified, because they are concentrated in synaptic terminals (Wiegandt, 1968; Dekirmenjan & Brunngraber, 1969; Morgan *et al.*, 1973; Eichberg *et al.*, 1974). These glycosphingolipids contain different numbers of sialic (neuraminic) acid (NeuAc), as a result they can carry a more or less strong negative charge and form chelate complexes with divalent cations, especially with Ca^{2+}. Therefore gangliosides are assumed to play a specific functional role in essential neuronal events (Rahmann, 1978, 1980; Rahmann *et al.*, 1976).

Recent evidence strongly suggests an important role for gangliosides in a variety of thermal adaptation processes (Breer & Rahmann, 1976; Rahmann *et al.*, 1976, 1978; Rahmann, 1980; Hilbig *et al.*, 1977; Hilbig & Rahmann, 1978, 1979; Rahmann & Hilbig, 1980; Rösner *et al.*, 1979; Rösner, 1977). The general conclusion from these investigations is: the lower the ambient temperature—the higher the degree of sialylation of neuronal gangliosides.

In an earlier study the brain ganglioside composition of a hibernator (golden hamster) during the torpor phase had also been investigated (Hilbig & Rahmann, 1979). The gangliosides showed slight polysialylation effects during torpor. But since golden hamsters do not belong to true hibernators with a longtime torpor (Pohl, 1961) normothermic and hibernating dormice (*Glis glis*) being true hibernators (Eisentraut, 1956) were taken for the examination. The present study investigates the brain ganglioside

concentration and especially the composition of the whole brain and seven different brain parts in normothermic and hibernating dormice (*Glis glis*).

MATERIALS AND METHODS

Source, maintenance and acclimation of animals

Twenty-four adult wild normothermic and hibernating dormice (*Glis glis*) and for comparison 10 adult laboratory rats of inbred colonies (Wistar, Han.) were investigated. Hibernation was induced by transferring the dormice from room temperature (ambient temperature $T_a = 22 \pm 2°C$, dark–light cycle; 14:10) to a dark cooling chamber at $T_a = 6 \pm 2°C$. Two animals were kept in one cage each and provided with nest materials, food (Altromin pellets, apples, acorns) and water *ad libitum*.

Dormice are true hibernators having a very strong critical sleeping temperature (18°C; Eisentraut, 1956), a low body temperature in torpor (lowest temperature about 4–0°C; König, 1960) and extremely long sleeping phases (about 7 months). After 4 weeks in the cold all animals fell into hibernation. In intervals of about 4 weeks they woke up again to eat apples because their air in the cooling chamber was relatively dry. But then they fell into torpor quickly again.

Brain preparation and analytical procedure

The dormice were killed by decapitation in successive 3-week periods after falling into torpor in order to find out chemical change and torpor length. The brain preparations of dormice and rats were carried out on ice. The different brain parts (cortex, cerebellum, pons, medulla oblongata, bulbus olfactorius, brain stem and regio quadrigemina from midbrain) were removed immediately and deep frozen to below −20°C until ganglioside-extraction according to Tettamanti *et al.* (1973) was carried out. A 10% aliquot of each brain part was pooled to provide a whole brain sample. The concentration of proteins was determined by the method of Lowry *et al.* (1951) and that of sialoglycoproteins by the method of Jourdian *et al.* (1971). The amount of the ganglioside-bound sialic acid (NeuAc) was assessed according to Svennerholm (1957) and Svennerholm & Fredman (1980). To determine the ganglioside pattern 5 μg NeuAc/g fresh wt were separated by TLC on precoated silicagel plates (HPTCL; Fa. Merck) and developed in chloroform–methanol–10^{-3} mol $MgCl_2$ solution–NH_3 = 60:36:8:0.4 by vol. The spots were visualized with resorcinol reagent (Svennerholm, 1957) and quantified by densitometric scanning (Zeiss KM3). The ganglioside fractions were numbered according to their chromatographic migration rates. They were identified by known standards (GM2, GM1, GD3, GD1b, GT1b, GQ1b) and in comparison with the well known pattern of chick brain gangliosides (Rösner, 1980). The gangliosides were named according to Svennerholm's nomenclature (Svennerholm, 1963).

RESULTS

For a first characterization of hibernation-induced changes in brain gangliosides of dormice, the concentrations of protein, sialoglycoproteins and gangliosides of the *whole brain* from normothermic dormice were determined and compared with corresponding values of laboratory rats. The results presented in Table 1 indicate that the content of proteins in rats, normothermic and hibernating dormice is in the same range. But the content of sialylated glycoproteins is about 12% less in normothermic and hibernating dormice. The average concentration of gangliosides in dormice (about 750 μg NeuAc/g fresh wt) is about 15% lower than that in rats (about 890 μg NeuAc/g fresh wt) and during hibernation it decreases to 650 μg/g fresh wt.

When analysing the content of proteins, sialoglycoproteins and gangliosides in *different brain parts* (cortex, cerebellum, brain stem, medulla, pons, olfactory bulb and regio quadrigemina) of normothermic and hibernating dormice (Table 2) the following results were obtained: there are no differences between active and hibernating dormice concerning the protein concentration in any of the brain parts. Only one statistically significant decrease in the amount of sialoglycoproteins (medulla) during hibernation appeared. On the other hand the ganglioside content was shown to be significantly reduced in the pons, olfactory bulb and midbrain of the hibernators.

On the basis of these results it was of special interest to analyze the *ganglioside pattern* of defined brain regions in normothermic and hibernating dormice in comparison to that of the homeothermic rats (Fig. 1). First the ganglioside pattern of cortex and cerebellum from normothermic dormice is more polar than that of rats. In the cortex of rats the relative portion of the G_{D1a}-fraction is about 32% of the total pattern, while

Table 1. Content of proteins, sialoglycoproteins and gangliosides from whole brains of laboratory rats, normothermic and hibernating fat dormice (± SEM)

	Rats	Dormice	
		Normothermic (T_a = 22°C)	Hibernating (T_a = 6°C)
Protein (mg/g fresh wt)	100.0 ± 3.5	110.8 ± 4.6	105.9 ± 3
Sialo glycoprotein (μg NeuAc/g fresh wt)	254.6 ± 8.1	212 ± 21.0	210.0 ± 10.0
Ganglioside (μg NeuAc/g fresh wt)	88.5 ± 42.0	749.0 ± 71.0	647.2 ± 34.0

Table 2. Content of (a) proteins (mg/g fresh wt); (b) sialoglycoproteins (μg NeuAc/g fresh wt); and (c) gangliosides (μg NeuAc/g fresh wt) in different brain structures of normothermic and hibernating dormice

(a) proteins (b) glycoproteins (c) gangliosides		Dormice			
		Fat dormice			
Structure		Normothermic $T_a = 22°C$	Hibernating $T_a = 6°C$	Diff. −%	Significance (*t*-test)
Cortex	(a)	107.3 ± 3.1	109.6 ± 3.9	1	NS
	(b)	262 ± 23.2	264 ± 13.4	1	NS
	(c)	845 ± 66	872 ± 45.9	1.3	NS
Cerebellum	(a)	107.5 ± 2.2	108.3 ± 2.8	0.7	NS
	(b)	248 ± 11	228 ± 9.5	7.9	NS
	(c)	776 ± 27	733.7 ± 26.4	5.5	NS
Brain stem	(a)	112.4 ± 3.7	107.8 ± 3.5	4.1	NS
	(b)	231 ± 12.4	218.5 ± 14.7	5.5	NS
	(c)	946.5 ± 49.6	852.5 ± 42.7	10	NS
Medulla	(a)	126 ± 7.8	124.3 ± 3.3	1.4	NS
oblongata	(b)	199 ± 11.4	181.3 ± 8.1	8.9	$P < 0.01$
	(c)	629 ± 22.4	626.7 ± 46.2	0.4	*NS*
Pons	(a)	121.3 ± 5.8	122.2 ± 3.8	0.7	NS
	(b)	196.8 ± 6.6	163.3 ± 7.2	7.1	$P < 0.05$
	(c)	702 ± 34	528 ± 14.8	24.8	$P < 0.01$
Bulbus	(a)	107.6 ± 7.2	106.5 ± 3.2	1	NS
olfactorius	(b)	250 ± 16.7	243.8 ± 20.8	8.5	NS
	(c)	822 ± 37.6	618 ± 33	24.8	$P < 0.01$
Regio	(a)	116.5 ± 5.2	117.9 ± 3.7	1	NS
quadrigemina	(b)	234.5 ± 12	230 ± 10.6	2	NS
	(c)	837 ± 47.3	676.9 ± 39.5	19.2	$P < 0.05$

in dormice it is only about 27%. On the other hand the G_{T1b} in rat cortex forms 24.5%, in that of dormice it is 28.4%. In dormice, however, an additional highly polar penta-sialo ganglioside fraction (G_{P1}) occurs. In the cerebellum with its more polar gangliosides a similar tendency can be seen: again there is a lower-concentration in mono- and disialogangliosides in dormice, whereas in the polar tetrasialoganglioside (G_{Q1b}) a difference from 8% (rat) to 14% (dormouse) is found as well as the additional polar G_{P1}-fraction. From these data it can be concluded that the basic composition of the different brain regions is to a certain extent similar in both species. Nevertheless the dormice have more polar ganglioside fractions in comparison with the rat.

Now it was of great interest to investigate whether there might be any variations in the ganglioside pattern of different brain regions from hibernating compared with normothermic dormice (Table 3 & Fig. 1). Generally, in all brain regions investigated, there is an increase of the higher sialylated tri- and tetrasialoganglioside fractions (G_{T1b} and G_{Q1b}) in hibernating dormice compared with that of the normothermic counterparts. These differences, which are partly due to concomitant decrease especially in the G_{D1b}- and G_{D2}-fractions were shown to be highly significant in the older brain structures of the pons, regio quadregemina (midbrain), oblongated medulla and brain stem, but not in the olfactory bulb. In the phylogenetically more modern structures (cortex, cerebellum)

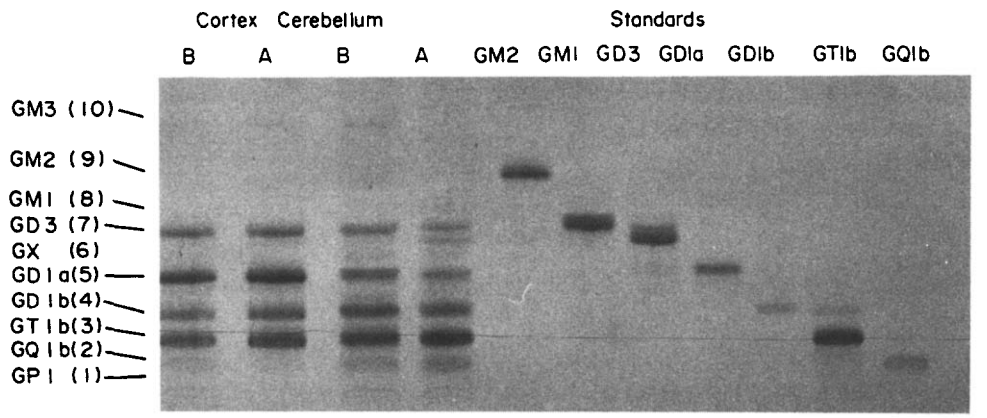

Fig. 1. Chromatograms of ganglioside mixtures of cortex and cerebellum from rat (B) and dormouse (A) in comparison with standards; development of the plate with chloroform–methanol–10^{-3} mol $MgCl_2$ solution–NH_3 = 60:36:8:0, by vol. Bands were visualized with resorcinol reagent.

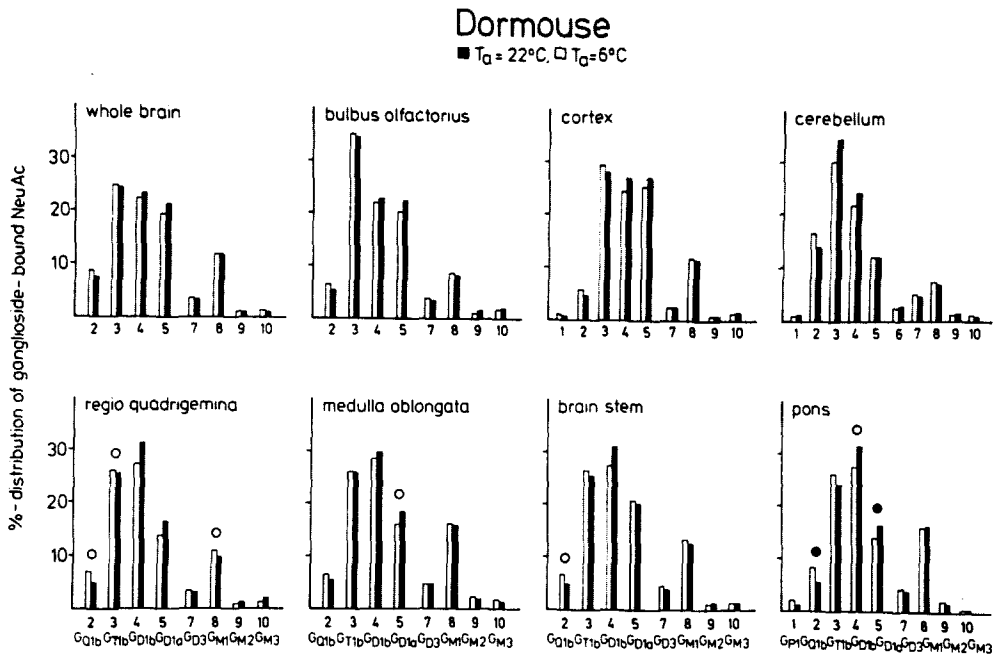

Fig. 2. Relative proportion of ganglioside-bound NeuAc to different ganglioside fractions (G_{P1} to G_{M3}) in various brain parts of normothermic (ambient temperature T_a = 22°C) and hibernating (T_a = 6°C) fat dormice. ○, $P < 0.01$; ●, $P < 0.001$. Migration rate of G_{D2} and G_{D1b} were identical in this solvent system (*cf* Fig. 1).

these differences were less distinct. Furthermore the brain ganglioside patterns of dormice did not depend on the length of torpor, thus indicating that in dormice the onset of a polysialylation of the midbrain gangliosides takes place early in the hibernation phase.

DISCUSSION

The present data reveal that the brain ganglioside pattern of the normothermic, active fat dormouse in comparison to that of the laboratory rat is more polar, due to a relatively larger amount (2–15%,

Table 3. Relative proportion of ganglioside-bound NeuAc to different ganglioside fractions in whole brain and various brain parts of normothermic and hibernating fat dormice

Structure	Ganglioside fraction	% Distribution of ganglioside-bound NeuAc in fat dormouse		
		Normothermic (T_a = 22°C)	Hibernating (T_a = T_b = 6°C)	Significance (*t*-test)
Whole brain	G_{M3}	0.85 ± 0.14	1.26 ± 0.07	NS
	G_{M2}	1.12 ± 0.18	1.06 ± 0.06	NS
	G_{M1}	12.1 ± 0.4	11.98 ± 0.45	NS
	G_{D3}	3.5 ± 0.18	3.8 ± 0.13	NS
	G_X			—
	G_{D1a}	21.7 ± 0.8	19.83 ± 0.37	NS
	G_{D1b}	23.6 ± 0.4	22.8 ± 0.48	NS
	G_{T1b}	29.4 ± 0.33	29.9 ± 0.46	NS
	G_{Q1b}	7.46 ± 0.6	9.2 ± 0.7	NS
Bulbus olfactorius	G_{M3}	1.77 ± 0.24	1.8 ± 0.19	NS
	G_{M2}	1.27 ± 0.37	1.17 ± 0.15	NS
	G_{M1}	8.14 ± 0.25	8.75 ± 0.2	NS
	G_{D3}	3.54 ± 0.34	3.8 ± 0.15	NS
	G_X			—
	G_{D1a}	22.44 ± 0.46	20.42 ± 0.5	NS
	G_{D1b}	23.02 ± 0.38	22.06 ± 0.48	NS
	G_{T1b}	34.73 ± 0.69	35.27 ± 0.85	NS
	G_{Q1b}	5.47 ± 0.4	6.56 ± 0.27	NS

Table 3—*continued.*

Structure	Ganglioside fraction	% Distribution of ganglioside-bound NeuAc in fat dormouse: Normothermic (T_a = 22°C)	Hibernating (T_a = T_h = 6°C)	Significance (*t*-test)
Cortex				
	G_{M3}	1.43 ± 0.1	1.4 ± 0.16	NS
	G_{M2}	0.55 ± 0.07	0.69 ± 0.07	NS
	G_{M1}	11.44 ± 0.47	11.52 ± 0.32	NS
	G_{D3}	2.38 ± 0.09	2.49 ± 0.09	NS
	G_X			—
	G_{D1a}	26.82 ± 0.46	25.49 ± 0.4	NS
	G_{D1b}	27.05 ± 1.13	24.39 ± 0.83	NS
	G_{T1b}	28.24 ± 0.6	29.4 ± 0.43	NS
	G_{Q1b}	4.07 ± 0.22	5.73 ± 0.57	NS
	G_{P1}	0.5 ± 0.1	0.84 ± 0.17	NS
Cerebellum				
	G_{M3}	0.73 ± 0.02	0.77 ± 0.03	NS
	G_{M2}	1.34 ± 0.22	1.16 ± 0.09	NS
	G_{M1}	6.98 ± 0.35	7.28 ± 0.39	NS
	G_{D3}	4.57 ± 0.29	4.8 ± 0.17	NS
	G_X	2.87 ± 0.26	2.47 ± 0.1	—
	G_{D1a}	11.69 ± 0.26	11.6 ± 0.32	NS
	G_{D1b}	24.4 ± 0.95	22.09 ± 0.77	NS
	G_{T1b}	34.97 ± 1	33.3 ± 0.78	NS
	G_{Q1b}	14.1 ± 0.4	16.4 ± 0.63	NS
	G_{P1}	1.17 ± 0.16	0.97 ± 0.08	NS
Regio quadrigemina	G_{M3}	1.76 ± 0.24	1.03 ± 0.09	NS
	G_{M2}	1.05 ± 0.2	0.68 ± 0.06	NS
	G_{M1}	9.65 ± 0.43	11.2 ± 0.33	NS
	G_{D3}	3.17 ± 0.06	3.38 ± 0.17	$P < 0.01$
	G_X			—
	G_{D1a}	16.21 ± 0.7	13.97 ± 0.69	NS
	G_{D1b}	32.28 ± 0.8	28.25 ± 1.1	NS
	G_{T1b}	28.85 ± 0.66	31.22 ± 0.72	$P < 0.01$
	G_{Q1b}	5.4 ± 0.53	8.7 ± 0.89	$P < 0.001$
Medulla oblongata	G_{M3}	1.08 ± 0.22	1.36 ± 0.12	NS
	G_{M2}	1.57 ± 0.01	2.07 ± 0.17	NS
	G_{M1}	15.7 ± 0.45	16.19 ± 0.33	NS
	G_{D3}	4.5 ± 0.17	4.6 ± 0.15	NS
	G_X			—
	G_{D1a}	18.06 ± 0.66	15.08 ± 0.5	$P < 0.01$
	G_{D1b}	30.06 ± 0.75	28.7 ± 0.62	NS
	G_{T1b}	25.9 ± 0.37	26.02 ± 0.34	NS
	G_{Q1b}	5.23 ± 0.44	6.17 ± 0.6	NS
Brain stem	G_{M3}	0.96 ± 0.21	1.02 ± 0.1	$P < 0.01$
	G_{M2}	1.07 ± 0.1	0.88 ± 0.1	NS
	G_{M1}	12.5 ± 0.48	13.2 ± 0.41	NS
	G_{D3}	3.8 ± 0.1	4.5 ± 0.68	NS
	G_X			—
	G_{D1a}	20.19 ± 0.66	20.58 ± 0.42	NS
	G_{D1b}	31.44 ± 1.7	27.5 ± 1.33	NS
	G_{T1b}	25.26 ± 0.5	26.37 ± 0.74	NS
	G_{Q1b}	4.6 ± 0.23	6.4 ± 0.53	NS
Pons	G_{M3}	0.92 ± 0.14	1.06 ± 0.13	NS
	G_{M2}	1.67 ± 0.17	1.93 ± 0.17	NS
	G_{M1}	16.43 ± 0.53	16.44 ± 0.36	NS
	G_{D3}	3.94 ± 0.16	4.34 ± 0.12	NS
	G_X			—
	G_{D1a}	16.32 ± 0.31	14.2 ± 0.49	$P < 0.001$
	G_{D1b}	31.59 ± 0.84	27.77 ± 0.75	$P < 0.01$
	G_{T1b}	24.16 ± 0.95	25.7 ± 0.82	NS
	G_{Q1b}	5.3 ± 0.3	8.23 ± 0.58	$P < 0.001$
	G_{P1}	1.05 ± 0.18	1.83 ± 0.67	NS

dependent on brain region) of the higher sialylated tri- and tetrasialoganglioside fractions. Moreover these investigations show that in hibernating dormice in comparison with their normothermic counterparts significant changes in the ganglioside pattern (polysialylation effects) occurred. These are most significant in those brain parts which are according to Reaves & Hayward (1979) the thermosensitive regions of the CNS (pons, brainstem, oblongated medulla, regio quadrigemina of midbrain), with the exception of the cortex which is inactive during hibernation (Kayser *et al.*, 1951). Structures which are not involved in the thermoregulation (cerebellum, olfactory bulb) show no profound differences. Similar but not so distinct differences already had been shown for the whole brain of golden hamsters (Hilbig & Rahmann, 1979) but did not occur in the dwarf hamster (*Phodopus dsungorus*; Hilbig *et al.*, in preparation). Now it could be supposed that the differences in the sialylation- (= polarity) degree of brain gangliosides shown above, directly corresponds with the ability of mammals to maintain their body temperature at a constant level, when the ambient temperature decreases. So in the fat dormouse as a true hibernator with extreme long torpor phases the degree of polysialylation is significant, followed by that of the golden hamster, which has only very short torpor phases. On the other hand, the dwarf hamster, which can tolerate extreme low temperatures (−30°C) in its natural habitat (Mongolian deserts) by changing the body temperature only for short circadian periods but never below 20°C (Heldmaier, 1979), doesn't show typical variations of a polysialylation in the ganglioside metabolism.

On the basis of these results only speculative assumptions can be made concerning the possible causal interactions of neuronal gangliosides in adaptation to lowered ambient temperatures. Goldman (1975) postulated that an increase in the unsaturation of fatty acids in phospholipids induces changes in the fluidity of the membrane in order to maintain membrane function under lowered temperature conditions. Cossins *et al.* (1977) reported that these changes in phospholipids happen within a few days after a transfer to lowered temperatures. On the other hand, according to our results (Hilbig & Rahmann, 1979; Rahmann, 1980) a compensatory reconstitution (de- or polysialylation) of neuronal gangliosides took place over much longer periods of about 5–7 weeks. Considering the data concerning the complexation ability of gangliosides together with Ca^{2+}-ions (Probst *et al.*, 1979; Rahmann *et al.*, 1978) and the thermosensitivity of these complexes (Probst & Rahmann, 1980) it is more likely that during hibernation torpor the temperature changes influence neuronal gangliosides in a way that with decreasing temperatures more polar gangliosides of fractions are synthetized to form more stable Ca^{2+} complexes in the cold. These polar gangliosides probably maintain the neuronal membrane function, especially in its synaptic terminals comparable with the less sialylated gangliosides under homeothermic temperature conditions.

SUMMARY

The composition of the brain ganglioside pattern of normothermic dormice (*Glis glis*) is more polar (higher sialylated) than that of laboratory rats. When comparing normothermic with hibernating dormice a polysialylation of brain gangliosides can be shown which is most distinct in those brain regions, which regulate the temperature during hibernation torpor (pons, brainstem, oblonged medulla, parts of midbrain). The present results support the hypothesis according to which a lowering of the environmental temperature induces polysialylation effects in neuronal membranes.

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