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MINI-REVIEW

Role of Biological Membranes in Slow-Wave Sleep

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Abstract

Two involvements of cellular membranes in slow-wave sleep (SWS) are discussed. In the first the endoplasmic reticulum (ER) is focussed upon, and in the second, the plasmalemma, where specific binding sites (receptors?) for promoters of slow-wave sleep are believed to be located. The study concerning the ER focusses on an enzyme in the brain, glucose-6-phosphatase, which, although present at low levels, manifests greatly increased activity during SWS compared to the waking state. The work on the plasmalemma has to do with the specific binding of muramyl peptides, inducers of slow-wave sleep, to various cells, and membrane preparations of various sorts, including those from brain tissue. Such cells as macrophages from mice, B-lymphocytes from human blood, and cells from a cell line (C-6 glioma) have been examined in this context.

Key Words: Slow-wave sleep; specific binding; muramyl peptides; serotonin; glucose-6-phosphatase; endoplasmic reticulum; interleukin-1.

Introduction

Although the sleeping state occupies about one-third of the lifetime of humans, and slow-wave sleep is a large component of that state (Williams *et al.*, 1974) —perhaps the most available to examination at the molecular level— biochemical investigations of slow-wave sleep are few. Even fewer are considerations of the biochemical role(s) of cellular membranes in that phenomenon.

This mini-review is far from comprehensive, and addresses only two matters:

(I) Aspects of metabolic differences between the brains of waking and sleeping animals, insofar as these are membrane-related.

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(II) Considerations of naturally occurring slow-wave sleep-promoting substances with particular reference to specific binding sites for those agents on cellular membranes. This aspect is given additional point by the fact that the mode of action of many such slow-wave sleep promoting factors appears to have an immune basis or link (Krueger, 1990; Krueger and Karnovsky, 1987; Krueger and Majde, 1990).

Earlier reviews on biochemical aspects of sleep that are germane include a comprehensive coverage of the situation to 1977 (Karnovsky and Reich, 1977), and recent discussions of sleep-inducing substances such as that of Borbely and Tobler (1989), as well as more specialized reviews such as that by Hayaishi (1988).

Brain Metabolism in Slow-Wave Sleep

The major observation in this context that has links to cellular membranes is the finding that during slow-wave sleep in rats there apparently is increased activity of brain glucose-6-phosphatase, an enzyme that in liver and kidney is associated with the endoplasmic reticulum. This activity is often regarded in those organs as being an enzyme "system" rather than merely a hydrolase *per se* (reviewed by Sukalski and Nordlie, 1989), as discussed below.

The observation that brain glucose-6-phosphatase is involved in slow-wave sleep stems from the following experimental approach (Anchors and Karnovsky, 1975): Either $^{32}P_i$ or $^{33}P_i$ was administered intracerebrally to a sleeping rat. A closely matched waking rat received the other isotope. Twenty minutes after infusion of label the animals were killed by rapid freezing. The brains of matched sleeper and waker were extracted and purified together by polyacrylamide electrophoresis, or by chromatography in detergent solutions. A homogeneous fraction was obtained for which the ratio of "sleeper" isotope to "waker" isotope was maximal. The phosphate in this fraction was acid labile—as had been noted in earlier studies (Reich *et al.*, 1967, 1972, 1973), and it was shown that the entity isolated was a phosphoprotein and contained a histidine phosphate (Anchors and Karnovsky, 1975). The latter compound was isolated after aklaline hydrolysis of the purified phosphoprotein. When known histidine phosphoproteins were considered, the protein isolated was determined to be glucose-6-phosphatase (Fig. 1), and the phosphorylated entity that was isolated was presumably the enzyme intermediate previously reported (Feldman and Butler, 1972) in reactions *in vitro*, i.e.,

(a) Glc-6-P + Enz $\longrightarrow$ Glc + Enz-P

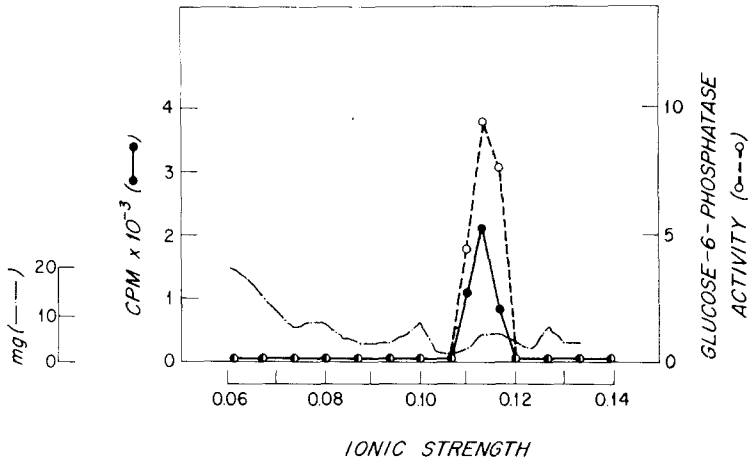

Fig. 1. Phosphoprotein radioactivity and glucose-6-phosphatase activity in a purification by a DEAE-chromatography step. A delipidated, radioactively labeled, detergent extract from the brain of a sleeping rat was loaded onto a column of DEAE-Sephadex. The column was eluted with a linear gradient of ionic strength, which contained 0.1% sodium deoxycholate. Fractions were assayed for protein (dot-dash line and scale in milligrams), trichloroacetic acid-insoluble (i.e., protein bound) radioactivity (filled circles), and glucose-6-phosphatase activity (open circles). The units of enzymatic activity used are μmol/min/mg of protein. (Details in Anchors and Karnovsky, 1975.)

(b)[2] Enz-P + H_2O ⟶ Enz + P_i

From these experiments it would appear that labeled inorganic P_i entered the brain ATP pool, that labeled glucose-6-P was then formed, and that there was increased traffic of that substance, via glucose-6-phosphatase to form glucose and the labeled enzyme intermediate (see above). Finally P_i was released. Other explanations are, of course possible, since the kinetic information is far from complete. Further experimentation, however, favored the one given above. For example, increased attack on 2-deoxyglucose-6-P during slow-wave sleep was demonstrated (Karnovsky, 1982).

In brain, though the enzyme is clearly associated with a membrane component, and is located in what is operationally a "microsomal" fraction (Fishman and Karnovsky, 1986), the situation is apparently less complex than what pertains in liver and kidney. In those two organs, the hydrolase of the endoplasmic reticulum has been considered to be lumenal in location and to require a transporter (T_1) to bring the substrate from the cytoplasmic side of the membrane to the lumenal hydrolase *per se* (Arion *et al.*, 1975; Gold and Widnell, 1976; reviewed by Sukalski and Nordlie, 1989). The transport

[2] Here a hydrolytic reaction is depicted; if ROH replaces H_2O, a transfer reaction results, with the formation of RP.

Table I. Kinetics of Hydrolysis of Glucose-6-phosphate and Mannose-6-phosphate in Intact and Disrupted Brain Microsomes[a]

Substrate concentration (mM)	Glucose-6-phosphate			Mannose-6-phosphate		
	I	D	L	I	D	L
2	0.20	0.38	47.4	0.20	0.37	45.9
5	0.42	0.78	46.2	0.40	0.75	46.7
10	0.56	1.04	46.2	0.52	0.98	46.9
20	0.68	1.20	43.3	0.70	1.20	41.7
80	1.14	2.14	46.7	1.16	2.20	47.3
K_m (mM)	9.2	8.4	—	8.7	8.5	—
V_{max}	1.13	1.98	43	1.07	1.94	45

[a]All assays were performed in triplicate on a single microsomal preparation, and the data listed are mean values. I = intact microsomes; D = disrupted microsomes (0.015% DOC); L = latency, as %; calculated from the formula (1 − activity in intact microsomes)/activity in disrupted microsomes. Data given as μmol P_i/mg protein/h. (See Fishman and Karnovsky, 1986 for details.)

protein confers specificity on the system, i.e., whereas the hydrolase in broken microsomal systems (e.g., in presence of detergents) will hydrolyze hexose-6-phosphates other than glucose-6-phosphate, the transport protein is specific for the latter sugar-phosphate. Thus, *intact* liver or kidney microsomes specifically hydrolyze glucose-6-phosphate (at low concentrations of hexose-6-phosphate). In the case of brain microsomes no transport protein could be detected, and the microsomes obtained from that tissue hydrolyze mannose-6-phosphate just as well as glucose-6-phosphate (Fishman and Karnovsky, 1986), as illustrated in Table I.

Such data are indeed most informative only at the lowest concentrations of substrate but the conclusion that a transport protein was not involved in the brain was reinforced by experiments in which covalently linking inhibitors which do not penetrate membranes were used (e.g., 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DIDS). This reagent, when applied to *liver* microsomes, was shown to be able to block the function of the transport protein mentioned above (Zoccoli *et al.*, 1982). It (DIDS) and other "nonpenetrating" reagents, however, failed to block accessibility of substrate to glucose-6-phosphohydrolase of brain microsomal preparations (Fishman and Karnovsky, 1986). One should note that the brain "microsomes" exhibit only a moderate degree of latency, ca. 50–60%. They might have been damaged, perhaps by the presence of amphipathic brain glycolipids during homogenization. However, when liver organelles were prepared *together* with those of brain, they were found to be intact by accepted criteria (Sukalski and Nordlie, 1989; Arion *et al.*, 1975; Gold and Widnell, 1976).

Irrespective of this particular matter, the relevant enzyme is membrane bound, and one might ask what the role of such a membrane-bound

hydrolase might be in slow-wave sleep. This is an especially vexing question since the brain is not a glucose-exporting organ, and the enzyme is present in brain at very low levels, i.e., one-fiftieth those of liver (Anchors *et al.*, 1977). (In fact, many textbooks deny the presence of glucose-6-phosphatase in brain!). One possibility for the experimental observation reported above for brain is that there is increased passage of glucose from one cell type to another during slow-wave sleep, i.e., from glucose-6-P of cell-type A → intracellular glucose → extracellular glucose → intracellular glucose of cell-type (B) → glucose-6-P of cell type B, presumably to be catabolized in the latter cells. Early enzymatic experiments from Hyden's laboratory (Hyden and Lange, 1965) on single neurons and glia might suggest a correlation of levels of metabolic activity between these two cell types and the sleep/wakefulness cycle, i.e., a "reciprocal" metabolic relationship relevant to sleep may pertain to these two cell types. In that work the enzyme followed was succinoxidase. It is interesting that, using the labeled 2-deoxyglucose method of Sokoloff (1982), experiments of Petitjean, Jouvet, Bobillier and their colleagues (Petitjean *et al.*, 1982), as well as unpublished experiments of Sokoloff's group with the present author, revealed only very minor insignificant differences in regional glucose utilization on comparing sleep and wakefulness in rats.

However, if the suggestion made above regarding the possible involvement of the cerebral glucose-6-phosphatase in intercellular traffic of glucose is indeed to be explored, attention would also have to be paid to the observation that rat brain glycogen almost doubles upon the onset of slow-wave sleep, compared with waking levels (Karnovsky *et al.*, 1983). It would be well to establish the cell type in which this occurs (astrocytes?) and a cytochemical approach to the problem is indicated.

Role of Cell Membranes in Functions of Slow-Wave Sleep-Promoting Substances

In 1982, the composition of a long sought naturally occurring sleep-promoting factor was determined (Krueger *et al.*, 1982). In 1984, more detailed chemistry and structure–function relationships of this substance were revealed (Martin *et al.*, 1984; Krueger *et al.*, 1984). The sleep-promoting factor that had been pursued for many years by the Harvard group was established as being a muramyl peptide—a bacterial cell wall monomer. Professor Edgar Lederer called it a 'sleep vitamin' since it was clearly not produced by mammals, but was a bacterial breakdown product, particularly of gram-positive bacteria. Lederer and his colleagues had established the role of this group of substances as immune adjuvants (reviewed by Adam and

Lederer, 1984), and had defined the minimal componment of the mycobacterial entity of Freund's complete adjuvant as muramyl dipeptide, i.e., *N*-acetylmuramyl-L-alanyl-γ-D-isoglutamine, which was synthesized. The sleep factor isolated from human urine at Harvard was *N*-acetyl-D-glucosaminyl-*N*-acetyl-D-1,6-anhydromuramyl-L-alanyl-D-isoglutaminyl-*meso*-diaminopimelyl-D-alanine. (The configurations of the components were assumed from previous work on bacterial cell walls.)

Soon it was demonstrated that a number of substances, related to muramyl peptide in one way or another (physiologically, if not in chemical structure), could also induce slow-wave sleep. This list of substances includes bacterial lipopolysaccharides (Cady *et al.*, 1989), interleukin-1 (Walter *et al.*, 1989), interferons (Krueger *et al.*, 1987), double-stranded RNA (poly I : C but not poly A : U) (Krueger *et al.*, 1988), growth hormone releasing factor (Obal *et al.*, 1988), and even insulin (Danguir and Nicolaidis, 1984). The full range of substances is reviewed in various articles cited above and others (Borbely and Tobler, 1989; Krueger and Johannsen, 1988; Krueger *et al.*, 1989). The substances mentioned and others form a virtual network that regulates sleep (Krueger, 1990). It may be recognized that an *order* of factors can be discerned from bacterial entities to cytokines, for example, and to more general modulators of metabolism. Krueger has indicated, in an elegant exposition, how such factors may interact in a hierarchical way (Krueger, 1990). The "leitmotif" throughout may well be interaction with cellular membranes.

In these considerations it becomes clear that IL-1 is a key substance. Its ability to raise the temperature of the subject could be separated from its sleep-inducing capacity by selection of appropriate administration site, or by countering the temperature-raising action of IL-1 *per se*, or of muramyl peptide which is known to induce IL-1 release from certain cell types (Walter *et al.*, 1989; Krueger *et al.*, 1978). As might be expected from the list of sleep-inducing substances, even the effect of microbial challenge on sleep was observed in rabbits (Toth and Krueger, 1989). It was found that "altered sleep patterns occur in response to infectious challenge in rabbits, and that these changes are related to the type of infectious organism involved" (Toth and Krueger, 1989). Further, in considering the effects of viral infections, one may point to the item mentioned above regarding double-stranded RNA (Krueger *et al.*, 1988) that this question inspired.

A key observation that emerged from both the earlier work on the immuno-adjuvant aspects of muramyl peptides reported by the French group, and the later sleep-related findings, was the pervasive sense that immune phenomena involving specific membrane-binding sites were crucial throughout. The bridge from the immune- to the neuro-phenomena was indicated early on by Masek and Kadlec (1983), and also by Root-Bernstein and Westall (1983). The former group had earlier pointed to the serotonergic

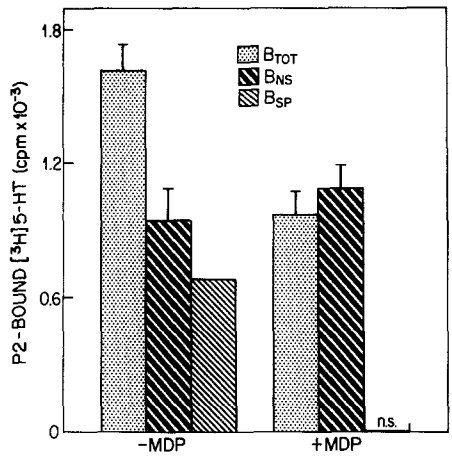

Fig. 2. Specific, nonspecific, and total binding of [^{3}H]5-HT to P2 fraction of C6 cells, in the presence and absence of MDP. All samples of membrane suspensions were incubated at 4°C with 20 nM [^{3}H]5-HT. Total (B_{TOT}) and nonspecific binding (B_{NS}) were determined in the absence and presence, respectively, of 100 μM unlabeled serotonin. Each of these determinations was in turn made in the absence and presence of 10 μM MDP. Binding is expressed as cpm per sample of P2 membranes originating from one million cells. Means and SEM are represented, $n = 3$. Specific binding component in the absence of MDP is significant ($p < 0.02$). No significant difference exists between B_{TOT} and B_{NS} in the presence of MDP. (See Silverman *et al.*, 1989.)

aspects of muramyl peptide action (Masek *et al.*, 1978). The latter group, basing its arguments on theoretical structural data, and later on experimental (NMR) binding measurements, provided strong indications that muramyl peptides (MPs) and serotonin (5-HT) might share specific cellular binding sites (Root-Bernstein and Westall, 1984; Westall and Root-Bernstein, 1985).

In the context of the present communication, a direct demonstration of specific binding by MPs to biological membranes would be crucial. This was done first with macrophages (Silverman *et al.*, 1986). That 5-HT shared the sites demonstrated was also shown directly with macrophages, and, in addition with brain tissue and brain-derived cell lines (Silverman *et al.*, 1985, 1989; Kaydalov *et al.*, 1987) (Fig. 2). Furthermore, it was shown that MPs and 5-HT could induce some of the same physiological cellular responses when they are bound to cell membranes. One example is the ability to release enhanced amounts of superoxide by macrophages upon binding of MPs and later challenge with phorbol myristate acetate (Silverman *et al.*, 1985). Another is the release of IL-1-like activity by C-6 (glioma) cells exposed to either agent, MP or 5-HT (Silverman *et al.*, 1989; Fontana *et al.*, 1983). Apart from the aspect of specific but shared binding sites for MPs and 5-HT is the fact that serotonin has for a long time been considered as a factor in the regulation of sleep (Jouvet, 1983). These matters, and some extensions

thereof concerning mutual MP/5-HT receptors and neuro-immune interactions, have been reviewed recently (Silverman and Karnovsky, 1989).

A series of observations from Hayaishi's laboratory gives further weight to thoughts of cell membrane involvement in slow-wave sleep. This group observed that prostaglandin D_2 is a slow-wave sleep promoter, while prostaglandin E_2 is associated with wakefulness (Hayaishi, 1988; Ueno *et al.*, 1983). Hayaishi's group has also shown that MPs induce release of prostaglandin D_2 from astroglia (Yamamoto *et al.*, 1988), as well as do lipopolysaccharide and IL-1—both known slow-wave sleep promoters (Cady *et al.*, 1989; Walter *et al.*, 1989). These particular observations do much to bridge the ideas of the various investigators, and indeed Hayaishi's group has offered direct comparison of several sleep-promoting substances (Inoue *et al.*, 1984). Further, the involvement of prostagalandin D_2 in the hierarchy of such substances (Krueger, 1990) tends to consolidate one's focus on cellular membranes as being of cardinal importance in the promotion of slow-wave sleep, since these prostaglandins are known to be formed from arachidonate released by phospholipase A_2 from membrane glycerophospholipids (reviewed by Samuelsson, 1978), and to function at membrane sites.

As a final matter that is intriguing, as well as being of help in tightening up our concepts, one might cite the correlation between muramyl peptides, sleep, and the histocompatibility antigens HLA-DR_2 and DQw1. Individuals who express this phenotype fail to bind MPs specifically (Silverman *et al.*, 1990). Furthermore, they show greatly impaired ability to bind 5-HT specifically (Johnson *et al.*, 1990). Almost all narcoleptics, who have severe sleep derangements, express this antigen (e.g., Terasaki, 1980; Juji *et al.*, 1984; Sachs and Moller, 1987; Pollack *et al.*, 1988). One is tempted in the first instance to relate their reported lack of nocturnal slow-wave sleep, Stage IV (Larkin, 1984), to this failure of binding of muramyl peptides and serotonin, but the situation is undoubtedly very complex and more data are needed. However, the involvement of this membrane-linked immunological feature with the failure to bind the slow-wave sleep promoting factor specifically on cell membranes is thought-provoking—and another pointer to the involvement of those membranes in a complex physiological function—slow-wave sleep. It is now of great importance to sort out the signal transduction mechanism(s) that link(s) together the various sleep-promoting ligands hierarchically (Krueger, 1990), the specific membrane-binding sites (receptors), and the physiological state *per se*. Are these inositide or cyclic AMP-connected? Are G proteins involved—or are there other, as yet undiscovered, mechanisms of signal transduction? These questions are only a few among the many that confront investigators working on sleep phenomena at the subcellular and molecular levels.

Acknowledgments

Table 1 is republished with the permission of the *Journal of Neurochemistry*; Fig. 1 with that of the *Journal of Biological Chemistry*, and Fig. 2 with that of *Peptide Research*. The author acknowledges support from the National Institutes of Health, Grant AI-03260, and from the Council for Tobacco Research U.S.A., Grant No. 2497.

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