8. Biochemical regulation of sleep

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In order to understand the biochemical regulation of sleep, it is important to emphasize the importance of the conclusions of G.F. Rossi's report on the neural regulation of wakefulness and sleep. Two groups of neuronal systems, with antagonistic effects, are involved. The 1st group consists of activating systems inducing arousal and waking: the reticular ascending system from the upper brainstem (fig. 1, F. retic.) and the posterior ventral hypothalamus (Hypoth. p.v.). The 2nd group consists of systems inducing sleep. They operate by deactivating the waking systems or by inhibiting the effectory mechanisms of wakefulness in the cerebral cortex or subcortex. The sleep inducing systems are: a) the bulbar area, close to the nucleus of the solitary bundle (Medulla: Nc. f. solit.), which receives depressory visceral afferent impulses; b) the midline raphe nucleus of the midbrain (Nc. raphe); c) the intralaminar mediocentral thalamus (Thalam. med. centr.); d) the oromedio-ventral hypothalamus (Hypoth. o.m.v.) or preoptic area reconsidered by Bremer[1,2].

The neurophysiological investigations of the last 3 decades revealed that definite waking- or sleep-inducing neurons liberate, at the end of their nervous fibre (axon) and juction with another neuron (synapse) a 'chemical transmitter'. This substance acts on a specific receptor of the corresponding post-synaptic neuron. The excitability of this post-synaptic neuron is influenced chemically by the transmitter released from the presynaptic neuron at the synapse. We distinguish today between excitatory transmitters, which stimulate the post-synaptic neuron by 'depolarizing' its membrane at the synapse, and inhibitory transmitters which increase the resistance of the post-synaptic neuron by hyperpolarizing its membrane. In the first case, the post-synaptic receptor-neuron becomes more excitable and in the second case less excitable.

There are transmitters which induce arousal by stimulating (depolarizing) neurons of the reticular activating system, whereas other transmitters induce sleep by inhibiting (hyperpolarizing) the reticular system or by stimulating directly the inhibitory sleep-inducing systems.

Other chemical substances act as modulators, i.e. they do not operate directly by means of a postsynaptic transmitter mechanism at the end of the axon, but indirectly by altering the metabolic activity of the neuronal cell body. The so-called presynaptic inhibition is another mechanism modulating the excitability of the cell body. Transmitters like amino-acids or biogenic amines, are substances acting at an effectory operational level; by contrast, the modulators, like polypeptides or proteins, are large compounds acting at supra-transmitter level.

We shall deal with the following transmitters and modulators involved in wakefulness and sleep: biogenic amines, acetylcholine, amino-acids, polypeptides, proteins and hormones. A distinction will be made between transmitters of orthodox sleep (slow-wave delta sleep), with no rapid eye movements (NREM), and paradoxical sleep (PS) with rapid eye movements (REM) and dreaming (cf. J.M. Gaillard's EEG report). Pharmacological and biochemical agents often produce effects comparable to those of experimental stimulation or lesions (cf. G.F. Rossi's and F. Bremer's reviews).

1. *Biogenic amines*

A. Serotonin (5-HT). The involvement of brain serotonergic systems in the regulation of sleep stems from 2 lines of experiments: lesions of the nuclei containing 5-HT cell bodies in the brain and impairment of 5-HT metabolism by pharmacological means. The nerve cells which synthesize 5-HT are mainly localized in the median raphe nuclei of the brain stem, extending from the medulla to the midbrain. After coagulation of this raphe system, a state of more or less continuous EEG and behavioral wakefulness is observed[3]. The amount of remaining sleep is negatively correlated with the extent of the lesions and with the level of 5-HT in the brain orally to the lesion[4]. However, after longer observation time, a partial recovery may occur[5]. In the cat, selective lesions of different parts of the raphe yield different results. The destruction of the anterior part (raphe centralis and dorsalis) induces

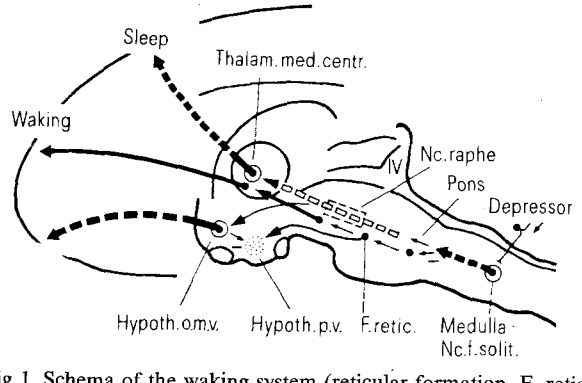

Fig. 1. Schema of the waking system (reticular formation, F. retic., and posterior ventral hypothalamus, Hypoth. p.v.) antagonized by the sleep-inducing systems (medulla close to the nucleus of the solitary bundle, Nc. f. solit.; the serotonergic midline raphe nucleus, Nc. raphe; the oromedial-ventral hypothalamus, Hypoth. o.m.v.). The intralaminar mediocentral thalamus (Thalam. med. centr.) modulates waking or sleep.

a permanent wakefulness, but paradoxical sleep (PS = REM sleep) still appears during 5–10% of the time, without being preceded by NREM sleep[3]. By contrast, destruction of the posterior part (raphe pontis and nucleus magnus) results in less of a decrease in NREM sleep, to about 40% of controls, whereas PS is completely abolished. Thus, there seems to be some specialization of the raphe system, the anterior part being mainly responsible for NREM sleep, whereas the posterior part may be involved in the priming of PS[6].

Parachlorophenylalanine (PCPA) inhibits 5-HT synthesis by blocking tryptophan hydroxylase, the rate-limiting step in this synthesis. An acute treatment with PCPA produces an almost complete insomnia in the cat and rat[7,8], but not in man[9]. This insomnia can transiently be suppressed by administration of 5-HTP, the direct precursor of 5-HT. Similarly, PCPA abolishes the inhibitory action of 5-HT on ponto-geniculo-occipital spikes ('pointes géniculo-occipitales') one of the main phasic components of REM sleep. Whereas 5-HT normally restricts these PGO to the PS stage, PCPA suppresses the control of 5-HT on the PGO, which are no longer restricted to PS, so that they now occur in NREM sleep and waking. These experimental results indicate a role of 5-HT in the maintenance of NREM sleep, and possibly in the priming of PS. The action of tryptophan as precursor of 5-HT[10] will be discussed below (amino-acids).

B. Catecholamines (noradrenalin, dopamine). Lesions in animals show that the anterior part of the locus ceruleus is responsible for the tonic cortical activation which accompanies waking: interruption of the dorsal noradrenergic (NA) bundle resulted in hypersomnia, with increased NREM sleep and PS[11]. Coagulation of dopamine (DA)-containing cells produced a motionless state (akinesia), however, with normal alternating slow-wave and arousal EEG[12]. This suggests that DA systems are implicated in the control of behavioral wakefulness, whereas NA systems are necessary for EEG activation. Pharmacological blockade or stimulation of DA receptors also affects the central activation[13]. Lesions of the lower part of the locus ceruleus alter PS by suppressing the inhibition of muscle tone normally present in this stage. The animal exhibits a variety of behavior while being actually asleep, as assessed by the EEG, the pupil constriction and the total relaxation of the nictitating membrane[6]. Lesions of the intermediate part of the locus ceruleus decrease PS[14]; the latter result was also obtained after administration of 6-OH–DA, a compound which destroys catecholaminergic (CA) terminals. Whatever the pharmacological means by which the activity of CA systems is impaired (inhibition of synthesis of the transmitter, inhibition of its presynaptic release, postsynaptic blockade or cellular leakage by reserpine), the production of PS is dose-dependently decreased[15]. These findings support the concept of a positive correlation between brain CA activity and PS. The occurrence of PS apparently requires an intact transmission in brain CA synapses; it is even possible to distinguish between a presynaptic and a postsynaptic participation in these regulations[16]. When the synthesis of NA is pharmacologically blocked, waking disappears almost completely in the cat and an abnormal EEG activity (continuous spindling) is detectable. Thus brain CA systems appear to play a crucial role in the control of waking and PS. NA is involved in cortical activation, with a possible participation of DA synapses, whereas midbrain DA structures are more specifically linked to behavioral activation.

2. *Acetylcholine (ACh)*

Acetylcholine is the specific excitatory transmitter of cholinergic somatic central and peripheral neurons, activating other neurons or striate muscles. It is also liberated in central parasympathetic preganglionic and postganglionic neurons of the visceral autonomic nervous system. Its effect is antagonized by the cholinesterase enzyme, which in turn may be blocked by anticholinesterase agents like physostigmine (eserine). Acetylcholine and agonistic drugs (physostigmine, pilocarpine) injected into the carotid artery of the rabbit produce arousal with desynchronized EEG in the neocortex, synchronized EEG in the hippocampus, caudate nucleus, thalamus and midbrain reticular system. The arousal reaction to sensory and reticular stimuli also increases. These effects are abolished by atropine[17,18]. Arousal, produced either by external stimuli or by direct stimulation of the reticular formation, is accompanied by a release of ACh at the cortical level, as well as in the striatum[19]. The release of ACh is lower in slow-wave sleep than in PS, where it may be even higher than in waking. A waking system corresponding to the ascending cholinergic system of Shute and Lewis[20] has been mapped by local injection of ACh crystals.

Atropine produces in animals a dissociation between cortical slow-wave EEG and waking behavior, tentatively attributed to a stronger impairment of cortical than subcortical structures. PS is facilitated by cholinmimetic agents, whereas anticholinergic drugs tend to suppress this stage[21]. Some data speak for a muscarinic effect as far as PS is concerned, whereas the nicotinic arousing effect is well-known.

3. *Amino-acids*

Some of these elementary components of proteins act as specific transmitters. This is the case of glycine, a true physiological inhibitory transmitter released at the axon terminal of neurons in the spinal cord and medulla[22,23]. Other inhibitory aminoacids, such as GABA (gamma-amino-butyric acid) have a depressing action on cortical neurons complementary to that of

the activating L-glutamic acid[24]. However, only tryptophan, an amino-acid precursor of serotonin (5-HT), has an effect on wakefulness. In man, nocturnal administration of L-tryptophan at doses of 0.25–1 g increases the duration of SWS (stage 4) and reduces the sleep latency[25].

4. *Peptides and humoral 'sleep factors'*

Legendre and Piéron[26] reported in 1910 that cerebrospinal fluid from deep-sleeping dogs (after sleep deprivation) induces behavioral sleep after infusion to normal dogs. Monnier and co-workers in 1963 demonstrated in rabbits with crossed cerebral circulation that sleep induced by electric thalamic stimulation in the donor evokes, by humoral transmission, a similar sleep in the recipient partner[27]. This humoral transmission of sleep was confirmed by dialyzing in vivo, with an artificial kidney, the cerebral venous blood from rabbits kept asleep by thalamic stimulation. The intraventricular or intravenous infusion of this cerebral blood dialysate to normal rabbits induced EEG and behavioral sleep. In the following years (1971/75), the team of Monnier and Schoenenberger achieved the physico-chemical characterization of the delta sleep factor isolated from the cerebral blood dialysate; it was a peptide consisting of 9 amino acids with tryptophan as the amino-terminal and mol. wt of 849[28,29]. This nonapeptide was synthesized in 1976/77 and called delta-sleep-inducing-peptide (DSIP); its delta EEG enhancing activity in the rabbit's cortex was significantly higher (54%) than that of a peptide analog used as control[30–32]. Biotests in rabbits, cats and rats revealed that DSIP produces a prolonged EEG and behavioral sleep at a dose of 6 nmoles/kg after intraventricular, and 30 nmoles/kg after i.v. injection[33–35]. Biotests on the isolated rat head exclude a peripheral somato-visceral origin of the DSIP effect[36]. From these data, it was concluded that the action of DSIP is not limited to one species. Furthermore, the parabolic, nonlinear dose-response curve suggests that DSIP represents a new group of physiologic hypnogenic compounds, inasmuch as it seems to act as a 'programming co-ordinator' at supra-transmitter level.

Other hypnogenic factors were obtained from cerebrospinal fluid or brain of various animals, but their final physical-chemical characterization and synthesis have not yet been reported. The 'sleep promoting factor S' of Pappenheimer was extracted from the cerebrospinal fluid, then from the brain of goats[37,38]. Another sleep promoting material was extracted by the Uchizono group from the brain of sleep-deprived rats[39]. Finally a factor reducing motor activities was obtained by Borbély's team[40] from cerebrospinal fluid of rat donors, withdrawn during their light period, then infused to rat recipients during their dark period. Other neuropeptides recently identified will not be discussed as they are not directly involved in sleep.

5. *Proteins and hormones (anabolic functions of sleep)*

The concept of the 'trophotropic function' of sleep (W.R. Hess 1925)[41] contributing to restoration of the cells and tissues in the 'milieu interne' for a better performance of the individual in his external surroundings, has received increased support during the past decades.

a) Growth hormone. The interest for the restoring action of sleep recurred when a link between slow-wave sleep (SWS) and nocturnal secretion of growth hormone (GH) was discovered in man[42,43]. The secretion of GH depends on SWS, which facilitates anabolic processes. GH stimulates amino acid uptake by the tissues and promotes protein or RNA synthesis[44]. Furthermore, it stimulates erythropoiesis through erythropoietin[45] and increases the free fatty acids in the blood; their degradation acts as a source of cellular energy (ATP), thus reducing amino acid catabolism and favouring protein synthesis during sleep. The nocturnal peak of GH secretion coincides with the lowest cortico-steroid level within the 24-h cycle, a condition which enhances protein synthesis[46]. In human subjects, the first increase of GH in plasma coincides with a rapid power increase in the EEG delta band[47,48]. Besides GH, prolactin, luteinizing hormone and testosterone are also sleep dependent and promote anabolic processes[49].

b) Protein synthesis and other anabolic processes. Adam and Oswald[50] demonstrated that 'Sleep is for tissue restoration' since the sleeping/waking cycle induces in mammals concomitant fluctuations in cellular work and energy charge. Degradation processes are stimulated during activity or waking, whereas restoring and synthetic processes are favoured by inactivity and sleep. In rats, brain protein synthesis, and in golden hamsters ATP concentrations, reach their highest levels during sleep. Similarly, the protein and RNA contents of the supra-optic nuclei increase in sleeping rats; the ATP, ADP and AMP concentrations in brain and the cellular energy charge are higher during sleep or during the first hour following sleep deprivation. All these restoring processes seem to increase primarily during the successive stages of NREM sleep; the higher protein levels reached during EEG SWS might even overlap on the following short stage of paradoxical REM sleep.

Conclusions. The organization of sleep and of the different wakingsleeping states involve highly elaborated neural and humoral regulations. Among the latter, the chemical transmitters, acting at effectory operational levels (amino-acids, biogenic amines, acetylcholine) are involved. At a higher supra-transmitter level, modulators and 'programming coordinators', such as polypeptides, proteins, hormones, operate as agonistic and antagonistic factors.

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9. Pathology of sleep

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The practice of all night or 24-h polygraphic recordings as well as a better understanding of sleep mechanisms have yielded extensive information on sleep pathology. Present data refer to hypersomnias and insomnias. They also concern other anomalies such as sleep incidents or parasomnias and sleep epilepsy.

Hypersomnias

Several types have been recognized and specified owing to certain findings such as: 1. the inversion of the 2 kinds of sleep at the onset of sleep, 2. the appearance of periodic sleep apneas, 3. the prolonged duration of a normal sleep.

1. Inversion of the 2 kinds of sleep: Sleep onset REM periods characterize narcolepsy[1,2]. This disease which was first described in 1880 by Gelineau is well defined by its clinical features: sudden daytime sleep 'attacks', attacks of cataplexy induced by emotions, hypnagogic hallucinations and sleep paralysis.

The inversion of the 2 types of sleep enables an understanding of the frequent occurrence of dreams in narcoleptics and the richness of hypnagogic hallucinations. The loss of muscle tone, attacks of cataplexy and sleep paralysis, have been equated to the muscle atony characteristic of REM sleep and interpreted as a dissociation of this sleep.

Furthermore polygraphic recordings have shown a poor quality sleep; insomnia is a frequent feature of this disease. Thus narcolepsy looks more like a dysomnia than an hypersomnia[2].

The circadian rhythm of wakefulness and sleep, that comes with age is deeply disturbed in narcoleptics. During the evolutive periods of this disease, the total