Paradoxical (REM) sleep genesis by the brainstem is under hypothalamic control.

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Abstract

The purpose of this review is to outline our latest hypothesis on the mechanisms responsible for the genesis ofparadoxical (REM) sleep (PS). Based on recent data, we propose that the onset and maintenance ofPSis due to the activation by intrinsic and extrinsic factors of MCH/GABAergic neurons located in the lateral hypothalamic area. These neurons would inhibit duringPS, GABAergic PS-off neurons located in the ventrolateral periaqueductal gray region. A number of results strongly suggest that these PS-off neurons gate the activation of thePS-on glutamatergic neurons located in the sublaterodorsal tegmental nucleus (SLD)and responsible for cortical activation and muscle atonia via descending projections to GABA/glycinergic neurons localized in the ventral medullary reticular nuclei.

Introduction

In most mammals, two sleep states, characterized by clear differences in electroencephalogram (EEG), electromyogram (EMG) and electro-oculogram (EOG) recordings have been identified. Slow-wave (SWS) sleep (or NREM sleep), is characterized by low-frequency, high-amplitude delta oscillations on the EEG, low muscular activity on the EMG and no ocular movement while rapid eye movement (REM) (or paradoxical, PS) sleep is characterized by an activated low-amplitude EEG close to the waking EEG, but with complete disappearance of muscle tone and rapid ocular movements.

Despite a wealth of neuropathological evidence dating back to the 19th century indicating that altered states of vigilance can be induced by focal brain lesions and that different neurochemical mechanisms are responsible for the succession of the three vigilance states across 24 hours [1], the mechanisms underlying the switch of cortical activity from an activated (desynchronized) state during waking to a synchronized state during deep SWSand then to the activated state of PS have not yet been precisely described.

This review examines possible neuronal networks and mechanisms responsible of the genesis ofPS.

The localization of the neurons generating PS in the pontine reticular formation

It was first shown that a state characterized by muscle atonia and REM persists following decortication, cerebellar ablation or brain stem transections rostral to the ponsand in the ”pontine cat”, a preparation in which all the structures rostral to the pons have been removed [2]. These results indicated that brainstem structures are necessary and sufficient to trigger and maintain the state of PS. By using electrolytic

and chemical lesions, it was then shown that the dorsal part of pontisoralis(PnO) and caudalis (PnC) nuclei also named peri-locus coeruleusα (peri-LCα), pontine inhibitory area (PIA,) andsubcoeruleus nucleus (SubC)contains the neurons responsible for PS onset [2]. More recently, a corresponding area has been identified in rats, and named the sublaterodorsal tegmental nucleus (SLD).It was also shown thata bilateral injection in cats of a cholinergic agonist, carbachol into the PnO and PnC dramatically increases PS quantities in cats [3,4].In addition, the PnO and PnCand the adjacent laterodorsal (Ldt) and pedunculopontine tegmental (PPT)cholinergic nuclei contain many neurons showing a tonic firing selective to PS (called "PS-on" neurons) [5,6]. From these results, it was thought for more than forty years that the PS-on neurons generating PS were cholinoceptive and cholinergic.

Paradoxical (REM) sleep generating neurons: the switch from acetylcholine to glutamate

However, in contrast to cats, carbacholiontophoresis into the rat sublaterodorsal tegmental nucleus (SLD) failed to induce a significant increase in PS quantities[7]. Further, only a few cholinergic neurons were stained for c-Fos in the LDT, PPT and SLD after PS hypersomnia [8,9]. Finally, neurochemical lesions in rats of both the LDT and PPT induced no effect on PS and cortical activation [10]. Then, Lu et al. [10] reported for the first time the presence of neurons expressing a specific marker of glutamatergic neurons, the vesicular glutamate transporter 2 (vGlut2) in the SLD. We recentlyfurther demonstrated that most of the Fos-labeled neurons localized in the SLD after PS recovery express vGlut2 [11]**. Altogether, these results indicate that the PS-on SLD neurons triggering PS are glutamatergic.

A number of recent results further suggest that PS-on glutamatergic neurons located in the SLD generate muscle atonia via descending projections to PS-on

GABA/glycinergic premotoneurons located at medullary level rather than directly in the spinal cord. First, by means of intracellular recordings during PS, it has been shown that trigeminal, hypoglossal and spinal motoneurons are tonically hyperpolarized by large inhibitory postsynaptic potentials (IPSPs) during PS. Further when these recordings were combined with local iontophoretic application of strychnine (a specific antagonist of the inhibitory neurotransmitter, glycine), motoneurons hyperpolarization was strongly decreased indicating that they are tonically inhibited by glycinergic neurons during PS [12-14]. It has then been shown that the levels of glycine but also that of GABA increase within hypoglossal and spinal motor pools during PS-like atonia suggesting that GABA in addition to glycine might contribute to motoneurons hyperpolarization during PS [15]. Further,it was recently shown that combined microdialysis of bicuculline, strychnine and phaclophen (a GABAb antagonist) in the trigeminal nucleus is necessary to restorejaw muscle tone during PS [16]**. Finally, mice with impaired glycinergic and GABAergic transmissions display PS without atonia [17]*.

In addition, it has been shown that the SLD sends direct efferent projections to GABA/glycinergic neurons located in the nucleus raphe magnus (RMg) and the ventral (GiV), alpha (Gia) gigantocellular and lateral paragigantocellular (LPGi) reticular nuclei[7,18]. Further, glutamate release increases specifically during PS in the Gia and GiV [19]. In addition, injection of non-NMDA glutamate agonists in these nuclei suppresses muscle tone while an increased tonus is seen during PS in cats with Gia and GiV cytotoxic lesion [20,21]. In addition, it has been previously shown in cats using antidromic activation that SLD PS-on neurons directly project to the ventral medulla but not to the spinal cord, whereas SLD neurons with a firing rate unrelated to PS display spinal cord projections [5]. Besides, GABA/glycinergic neurons of the

Gia, GiV, LPGi and RMg express c-Fos after induction of PS by bicuculline (Bic, a GABAa antagonist) injection in the SLD [7]. In addition, nearly all Fos-labeled neurons localized in these nuclei after 3h of PS recovery following 72h of PS deprivation express GAD67mRNA [22].At variance with these results, it has been shown combining retrograde tracing with vglut2 labeling that some of the glutamatergic neurons located in the SLD directly project to the spinal cord [10]. Further, it was shown that 10% of these neurons are Fos-labeled during PS enhanced by dark conditions. It was also recently shown that inactivation in mice of the glutamatergic but not of the GABA/glycinergic neurons of the GiV region induce an increased motor activity during REM sleep [23]. However, inactivation of GABA and glycinergic transmissions in the spinal cord induced only occurrence of small phasic movements during REM sleep in contrast to medullary lesions suggesting that spinal cord GABA/glycinergic interneurons might play a minor role compared to medullary ones [24].

In view of all these results, we propose that the SLD glutamatergic PS-on neurons induce muscle atonia during PS by means of direct projections to medullary RMg/GiA/GiV/LPGiand to minor extent spinal GABA/glycinergic PS-on neurons.These neurons hyperpolarize motoneurons mainly using glycine but also to a minor extent GABA acting on GABAa and GABAb receptors.

It has also been shown that a subpopulation of SLD PS-on neurons project to the intralaminar thalamic nuclei,the posterior hypothalamus and the basal forebrain. In addition to the SLD, it has also been shown that cholinergic neurons located in the pedunculopontine and laterodorsal tegmental nuclei and glutamatergic neurons located in the reticular formation active both during waking and REM sleep and projecting rostrally contribute to cortical activation during REM sleep [1,7].

Mechanisms of activation of SLD PS-on neurons during PS

In cats and rats, microdialysis administration in the SLDof kainic acid, a glutamate agonist induces a PS-like state [7,25]. A long-lasting PS-like hypersomnia can also be pharmacologically induced with a short latency in head-restrained unanesthetized rats by iontophoretic applicationinto the SLD of bicuculline or gabazine, two $GABA_A$ receptor antagonists[7]. Further, application of kynurenate, a glutamate antagonist, reverses the PS-like state induced by bicuculline[7]. In the head restrained rat, we also recorded neurons within the SLD specifically active during PS and excited following bicuculline or gabazineiontophoresis[26]. Taken together, these data indicate that the activation of SLD PS-on neurons is mainly due to the removal during PS of a tonic GABAergic tone present during W and SWScombined with a continuous presence of a glutamatergic input. Combining retrograde tracing with cholera toxin b subunit (CTb) injected in SLD and glutamate decarboxylase 67 (GAD67)immunohistochemistry or Fosimmunohistochemistrywith GAD67mRNA "in situ hybridization" after 72h of PS deprivation, we recently demonstrated that the ventrolateral part of the periaqueductal gray (vlPAG) and the adjacent dorsal part of the deep mesencephalic nucleus (dDPMe) are the only ponto-medullary structures containing a large number of GABAergic neurons activated during PS deprivation projecting to the SLD[22]. Further, injection of muscimol in the vlPAG and/or the dDpMe induces strong increases in PS quantities in cats [27] and rats [22]. Finally, neurochemical lesion of these two structures induces a profound increases in PS quantities [10]. These congruent experimental data leaded us to propose that PS-off GABAergic neurons within the vlPAG and the dDpMe are gating PS by tonically inhibiting PS-on neurons of the SLD during W and SWS. Our results indicate that these GABAergic neurons are crucial to gate PS although they do not rule out a

secondary role for monoaminergic neurons since increase in monoaminergic transmission either by reuptake blockers or agonists is well known to inhibit PS[28]*. The targets of monoaminergic neurons responsible for their inhibitory effects remain to be defined. They can either excite PS-off neurons or inhibit PS-on neurons. One possibility is that the monoaminergic neurons are exciting the GABAergic PS-off neurons during waking to preclude PS onset.

Mechanisms of inhibition of GABAergic and monoaminergic PS-off neurons at the onset and during PS

We previously reported that bicuculline application on serotonergic and noradrenergic neurons during SWS or PS restores a tonic firing in both types of neurons [29-31]. These results strongly suggest that an increased GABA release is responsible for the PS-selective inactivation of monoaminergic neurons. This hypothesis is well supported by microdialysis experiments in cats measuring a significant increase in GABA release in the DRN and LC during PS as compared to W and SWS but no detectable changes in glycine concentration [32,33].

By combining retrograde tracing with CTb and GAD immunohistochemistry in rats, we found that the vlPAG and the dorsal paragigantocellular nucleus (DPGi) [31,34] contained numerous GABAergic neurons projecting both to the DRN and LC. We then demonstrated by combining c-Fos and retrograde labeling that both nuclei contain numerous LC-projecting neurons selectively activated during PS rebound following PS deprivation [35,36]. Further, we found that the DPGi contains numerous PS-on neurons that are silent during W and SWS and fire tonically during PS[37].Taken together, these data highly suggest that the DPGi contains the neurons responsible for the inactivation of LC noradrenergic neurons during PS [37]. A

contribution from the vlPAG in the inhibition during PS of LC noradrenergic and dorsal raphe serotonergic neurons is also likely. Indeed, an increase in c-Fos/GAD immunoreactive neurons has been reported in the vlPAG after a PS rebound following deprivation in rats [9,22]. In summary, a large body of data indicates that GABAergic PS-on neurons localized in the vlPAG and the DPGi hyperpolarize the monoaminergic neurons during PS.

We first proposed that these neurons mightbe also responsible of the inhibition of the dDPMe/vlPAG PS-off GABAergic neurons during PS. To test this hypothesis, we recently localized the neurons active during PS hypersomnia projecting to the dDPMe/vlPAG PS-off GABAergic neurons[38]**.We found out that the vlPAG and the DPGi respectively contained a substantial and a small number of CTb/Fos double labeled neurons in PS hypersomniac rats. Although, the GABAergic nature of these neurons remains to be demonstrated, our results indicate that the vlPAG and the DPGi might contain PS-on GABAergic neurons inhibiting the vlPAG/dDPMe PS-off GABAergic neurons at the onset and during PS. However, we also demonstrated that the lateral hypothalamic area (LH) is the only brain structure containing a very large number of neurons activated during PS hypersomnia and projecting to the VLPAG/dDpMe. We further demonstrated that 44% of these neurons express the neuropeptide melanin concentrating hormone (MCH). These results indicate that LH hypothalamic neurons might play a crucial role in PS onset and maintenance by means of descending projections to the vlPAG/dDPMe PS-off GABAergic neurons. They confirmed previous datadiscussed below indicating that the posterior hypothalamus contains neurons implicated in PScontrol.

Role of the posterior hypothalamus, in particular MCH neurons in PS control

To localize all brain areas activated during PS, we extensively mapped the distribution of c-Fos+ neurons in control rats, rats selectively deprived of PS for 72h and rats allowed to recover from such deprivation [36,39]. Surprisingly, we observed a very large number of c-Fos+ cells in the posterior hypothalamus (PH), including zonaincerta (ZI), perifornical area (PeF) and the lateral hypothalamic area (LH). Only a few experimental results already supported the notion that the PH contributes to PS regulation. Bilateral injection of muscimol in the cat mammillary and tuberal hypothalamus induce a drastic inhibition of PS [40]. Further, neurons specifically active during PS were recorded in the PH of cats [41-43] or head-restrained rats [44]. By using double-immunostaining, we further showed that around 75% of PH cells labeled for c-Fos after PS rebound express GAD67 mRNA and are therefore GABAergic [45]. One third of these GABAergic neurons werealso immunoreactive for the neuropeptide Melanin Concentrating Hormone (MCH). Almost 60% of all the MCH-immunoreactive neurons counted in PeF, ZI and LHA were c-Fos+ [39,46]. We recently further demonstrated that these neurons co-contain nesfatin, another recently discovered peptide [47]. In support of our Fos data, it has recently been shown in head-restrained rats that MCH neurons fire exclusively during PS [48]. Importantly, MCH neurons start to fire simultaneously with the onset of PS and therefore can play a role in PS maintenance but not in PS induction. Nevertheless, rats receiving ICV administration of MCH showed a strong dose-dependent increase in PS and, to a minor extent, SWS quantities, due to an increased number of PS bouts [39]. Further, subcutaneous injection of an MCH antagonist decreases SWS and PS quantities [49] and mice with genetically inactivated MCH signaling exhibit altered vigilance state architecture and sleep homeostasis [50,51].In addition, disruption of Nesfatin-1

signaling by icv administration of Nesfatin-1 antiserum or antisense against the nucleobindin2 (NUCB2) prohormone suppressed PS. Further, the infusion of Nesfatin-1 antiserum after a selective PS deprivation precluded PS recovery[47].

In agreement with our results showing that MCH neurons constitute only one third of the GABAergic neurons activated during PS hypersomnia, it was recently shown that a large population of GABAergic neurons without MCH localized in the lateral hypothalamic area discharge maximally during PS [52]. These neurons are mostly silent during active W with high muscle tone, and progressively increase their discharge from quiet W through SWS to be maximally active during PS. Since these neurons anticipate PS onset, they can play a role in triggering the state.

To determine the function of the LH MCH+/GABA+ and MCH-/GABA+ neurons in PS control, we inactivated all LH neurons with muscimol(a GABAa agonist) or only those bearing alpha-2 adrenergic receptors using clonidine. We found that muscimol and to a lesser degree clonidine bilateral injections in the LH induce an inhibition of PS with or without an increase in SWS quantities, respectively.We further showed that after muscimol injection in the LH, the vlPAG/dDpMe region contains a large number of c-FOS/GAD67+ and of c-FOS/CTb+ neurons in animals with a CTb injection in the SLD. Our results indicate that the activation of PS-on MCH/GABAergic neurons localized in the LH is a necessary step for PS to occur. They further suggest that MCH/GABAergic PS-on neurons of the LH control PS onset and maintenance by means of a direct inhibitory projection to vlPAG/dDpMe PS-off GABAergic neurons. From our results, it can be proposed that MCH/GABAergic neurons of the LH constitute a master generator of PS which controls a slave generator located in the brainstem. At variance with this hypothesis, it is well accepted that the brainstem is necessary and sufficient to generate a state

characterized by muscle atonia and REM [2]. To reconcile these and our results, we therefore propose that after removal of the forebrain, the brainstem generator is sufficient to induce a state with muscle atonia and REM by means of a reorganization of the brainstem systems generating PS. However, the brainstem generatorwould beunder control of the LH generatorin intact animals.

In addition to thedescending pathway to the PS-off GABAergic neurons, the MCH/GABAergicPS-on neurons might also promote PS by means of other pathways to the histaminergic neurons, the monoaminergic PS-off neuronsand the hypocretinergic neurons [7,53,54].

The mechanisms at the origin of the activation of the MCH/GABAergic neurons of the LH at the entrance into PS remain to be identified. A large number of studies indicate that MCH neurons also play a key role in metabolism control [55]. Therefore, the activation of these neurons at the onset and during PS could beinfluenced by the metabolic state. In addition, we propose that yet undiscovered endogenous cellular or molecular clock-like mechanismsmayplay a role in their activation.

The cessation of activity of the MCH/GABAergic PS-on neurons and more largely of all the PS-on neurons at the end of PS episodes is certainly due to a completely different mechanism than the entrance into the state. Indeed, animals are entering PS slowly from SWS while in contrast they exit from it abruptly by a microarousal[56]. This indicates that the end of PS is induced by the activation of the W systems like the monoaminergic, hypocretin or the histaminergic neurons. The mechanisms responsible for their activation remain to be identified.

1. A network model for PS onset and maintenance (Figs. 1, 2)

The onset of PS would be due to the activation by intrinsic and extrinsic factors of PS-on MCH/GABAergic neurons localized in the lateral hypothalamic area (LH). These neurons would inhibit at the onset and during PSthe PS-off GABAergic neurons localized in the vlPAG and the dDpMetonically inhibiting during W and SWS the glutamatergic PS-on neurons from the SLD. The disinhibited ascending glutamatergic SLD PS-on neurons would in turn induce cortical activation via their projections to intralaminar thalamic relay neurons in collaboration with W/PS-on cholinergic and glutamatergic neurons from the LDT and PPT, mesencephalic and pontine reticular nuclei and the basal forebrain. Descending glutamatergic PS-on SLD neurons would induce muscle atonia via their excitatory projections to GABA/glycinergic premotoneurons localized in the raphe magnus, alpha and ventral gigantocellular reticular nuclei. PS-on GABAergic neurons localized in the LH, DPGi and vlPAG would also inactivate the PS-off orexin and aminergic neurons during PS. The exit from PS would be due to the activation of waking systems since PS episodes are almost always terminated by an arousal. The waking systems would reciprocally inhibit the GABAergic PS-on neurons localized in the LH, vlPAG and DPGi. Since the duration of PS is negatively coupled with the metabolic rate, we propose that the activity of the waking systems is triggered to end PS to restore crucial physiological parameters like thermoregulation.

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Figure 1. State of the neuronal network responsible for paradoxical (REM) sleep during waking. Abbreviations: 5HT, 5-hydroxytryptamine (serotonin), Ach, acetylcholine; BF, basal forebrain; DPGi, dorsal paragigantocellular reticular nucleus; dDPMe, deep mesencephalic reticular nucleus; DRN, dorsal raphe nucleus; GABA, gamma-aminobutyric acid; Gia, alpha gigantocellular reticular nucleus; GiV, ventral gigantocellular reticular nucleus; Gly, glycine; Hcrt, hypocretin (orexin)-containing neurons; His, histamine; LC, locus coeruleus; LdT, laterodorsal tegmental nucleus; LPGi, lateral paragigantocellular reticular nucleus; MCH, melanin concentrating hormone-containing neurons; NA, noradrenaline; PH, posterior hypothalamus; PPT, pedunculopontine tegmental nucleus; PS, paradoxical sleep; SCN, suprachiasmatic nucleus; SLD, sublaterodorsal nucleus; SWS, slow-wave sleep; TMN, tuberomamillary nucleus; vlPAG, ventrolateral periaqueductal gray; VLPO, ventrolateralpreoptic nucleus; W, waking.

Figure 2. State of the neuronal network responsible for paradoxical (REM) sleep at the onset and during PS.