Archives Italiennes de Biologie, 142: 487-500, 2004.

POSTERIOR HYPOTHALAMUS AND REGULATION OF VIGILANCE STATES

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INTRODUCTION

The hypothalamus serves as the main centre integrating endocrine functions and autonomic responses such as regulation of body temperature, blood pressure, body weight, vigilance states and pain perception, and may influence behaviour as the result of alterations in endocrine and autonomic functioning.

The posterior hypothalamus (PH) is a highly heterogeneous brain region limited dorsally by the thalamus, medially by the third ventricle and laterally by the subthalamic nucleus. Its rostrocaudal extension is bordered caudally by the ventral tegmental area (VTA) and rostrally by the pre-optic area.

A large number of data have shown that the PH plays a crucial role in vigilance states regulation, especially for promoting wake. It was first recognized by von Economo during World War 1, based on observations of patients from the encephalitis lethargica epidemic (70). He reported that posterior hypothalamic and midbrain junction lesions resulted in sleepiness, and further predicted that the posterior hypothalamus (PH) contains neurons that promote wakefulness (70).

In subsequent years, experimental studies aimed to reproduce these effects by making electrolytic lesions of the posterior hypothalamus in monkeys (52), rats (47) and cats (61). They all induced sleepiness suggesting strongly that the PH contains neuronal population promoting waking.

Although, these results were quite strong, little attention was given to the PH in the subsequent years, probably because Moruzzi and Magoun (45) showed that the reticular formation is a wake promoting centre sending, through the PH, ascending projections responsible for thalamocortical activation The sleepiness observed after of PH electrolytic lesions was then attributed to the damage of fibers of passage originating from the reticular formation and not to neurons located in the PH.

It is only end of the 80s, early nineties that Jouvet's group brought additional data showing that the PH is a wake-promoting centre. Indeed, these authors induced sleepiness for more that 2 days following ibotenic acid lesion, a chemical agent that induce death of neurons only (56, 61), or by inactivation of the PH neurons with muscimol applications, a GABAa agonist (32, 57).

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In the same lines, Lin and coworkers showed that the effect of modafinil and amphetamine, two wake-promoting agents, is suppressed by injection of muscimol in the cat PH (34, 36). In agreement with the above observations, Vanni-Mercier *et al.* (67) recorded neurons active only or mainly during waking in the PH of cat by extracellular electrophysiology. Unfortunately, these authors did not identified the chemical nature of these neurons. It's only during the 90s, that two neuronal populations responsible for the wake promoting effect of the PH were discovered, the histaminergic neurons and hypocretin/orexin neurons.

Looking carefully at the literature, additional observations from Jouvet's group, indicated that the PH could also play a role in Paradoxical Sleep (PS, also called rapid eye movement, REM sleep) regulation. Jouvet and his colleagues were studying "pontine cats", animals in which all brain structures rostral to the brainstem including the PH were supressed. These cats could be kept alive only for 7 days. They described that in pontine cats, a PS-like state occurs indicating that the brainstem is sufficient to induce PS. However, PS gradually disappeared after 5 days in favour of waking and PS rebound usually observed after PS sleep deprivation did not occur in pontine cats (26). Therefore, the brainstem contains the structures responsible for the PS to occur but not those responsible for its homeostatic regulation. If an isolated hypothalamus was left intact in the skull but separate from the pons, these pontine-like cats showed normal PS-like quantities and could survive several months. In support of these observations, several studies mentioned that 9 to 15% of neurons recorded in the PH were specifically active during PS (1, 28, 60). These observations are of great interest and it is surprising that they brought so little attention until now. Last year, we reported crucial data indicating that PH is involved in the homeostatic regulation of PS. We also identified one of the neuronal population involved, the neurons containing melanin-concentrating hormone (MCH) (69).

To better present the dual function of the PH, we will first review how histaminergic and hypocretinergic neurons, exclusively located in the PH, are involved in the promotion of wakefulness, and in a second part, we will present our recent data indicating that posterior hypothalamus is also implicated in PS regulation. At last, we will comment on possible interactions between the three neuronal populations identified in the PH, the histaminergic, hypocretin and MCH neurons.

THE POSTERIOR HYPOTHALAMUS AND WAKING

Several lines of evidence indicate that the role of the posterior hypothalamus in waking is mediated, at least in part, by histaminergic (HA) neurons. The main reason leading to this assumption is based on observations that anti-histaminics cause drowsiness in men (58).

Histaminergic neurons are found exclusively in the tuberomammillary nucleus (TMN) of the PH and send widespread projections in the brain, in particular to structures known to be important in sleep-wake control, such as the cortex, thalamus, pre-

optic area, periaqueducal gray and to the brainstem and, in particular, they contact cholinergic and monoaminergic neurons (37).

In vitro electrophysiological studies have shown that histaminergic neurons display characteristics very similar to the other aminergic neurons involved in the promotion of waking such as the noradrenergic neurons of the locus coeruleus and the serotonergic neurons of the dorsal raphe nucleus. They fire spontaneously in a slow regular, pacemaker fashion, have broad action potential and deep long-lasting after-hyperpolarisation (18).

In freely moving animals, neurons in the TMN discharge tonically, with similar characteristics as those observed on histaminergic neurons *in vitro*, but only during waking (54, 60), as the other monoaminergic neurons do. Based on their location and their pattern of discharge, these neurons are probably histaminergic in nature. In support of this statement, an additional study has provided some evidence for the histaminergic nature of these "Wake-on" neurons. Indeed, after electrophysiological recordings in the Tmn of freely moving cats, combined with injection of H3-receptor agonist, (imetit and α-MHA) decrease in the firing rate of wake related neurons was observed while when an HA antagonist, the ciproxifan, was applied, their firing rate was enhanced (68).

Finally, drugs which enhance HA neurotransmission produce arousal (31-33, 35, 36, 42-44), while those that interfere with HA neurotransmission, such as antagonists at central HA receptors (48), drugs that inhibit HA synthesis (27), or doses of H3 agonists that decrease synaptic release of HA via action at the HA autoreceptor (33) enhance sleep. Consistent with the high discharge rate of presumed histaminergic neurons during waking, it has been demonstrated, in rodents, that the release of histamine is high during darkness, the period in which animals are active and spend a large part of their time awake (41, 49).

To determine the level of implication of HA neurons in waking regulation and to see the effect of selective long-term abolition of HA neurons on the sleep-wake cycle, Parmentier *et al.* used mice lacking histidine decarboxylase (HDC-/-), the unique enzyme responsible for HA synthesis. On polysomnographic baseline recordings, HDC-/-mice showed a more than 23% increase in PS over 24 hours, a deficit in waking just before and after lights-off (more than 40% during the period of 6:00 to 10:00 PM, with lights-off at 7:00 PM) and were unable to remain awake when facing behavioral and cognitive challenges such as simulation of intra-peritoneal injection or new environment (50). Such results suggest that the lack of histamine (HA) affect not only vigilance states regulation, but also cognitive processes. It is of interest to note that the implication of HA in such processes is also supported by the fact that HA is implicated in the regulation of emotional behavior (59). Indeed, it has been shown that, in humans, HA is involved in the maintenance of arousal and cognition (63) and that, in mice lacking H1 receptors, exploratory behavior is decreased in new environments (23).

If the involvement of the posterior hypothalamus in the regulation of wakefulness was fulfilled uniquely by the HA neurons until 1998, the identification of novel neuropeptides named hypocretins (Hcrt or orexins) brought a new vision for the mechanisms of regulation of wakefulness.

The hypocretins are two peptides, Hcrt-1 (orexin-A) and Hcrt-2 (orexin-B), generated from a single preprohypocretin and synthesized by a small number of neurons restricted to the perifornical area of the PH (10, 51, 55). In contrast, Hcrt axons are found throughout the CNS, with innervation of the hypothalamus, locus coeruleus (LC), raphe nuclei, Tmn, midline thalamus, all levels of spinal cord, sympathetic and parasympathetic centers, and many other brain regions (51, 66). Two G protein–coupled receptors that respond to the Hcrt have been identified (55). In parallel to the wide distribution of axons, the two Hcrt receptors (HcrtR-1 and HcrtR-2) show a widespread and heterogeneous pattern of expression throughout the CNS (20, 39, 65). Interestingly, HcrtR-1 and HcrtR-2 are densely packed in monoaminergic and cholinergic nuclei which are well known to be involved in the regulation of sleep and wakefulness (24). Thus, Hcrt here may control vigilance by modulating the activity of monoaminergic and cholinergic neurons. Pharmacological studies indicate potent wake-promoting (+ 160%) and PS (- 68%) reduction effects following i.c.v. administration and local injections in the LC of Hcrtr- (7, 19). Furthermore, the arousal effect of Hcrt-1 when injected in the lateral ventricle is blocked by systemic injections of antagonist of the H1 HA receptors (71). This arousing effect is also absent in H1 knockout mice (22). These data, together with the observation that *in vitro* Hcrt applications on rat brain slices strongly stimulate firing rate of the noradrenergic LC (19, 21), dopaminergic VTA (46), serotonergic raphe dorsalis (8, 38) and histaminergic TMN cells (3, 12), is thus consistent with a global stimulatory effect of Hcrt on monoaminergic tone to maintain wakefulness.

Using functional neuroanatomy, it has been also showed that Hcrt cells are Fos positive after active wake or treatment with stimulants such as amphetamine or modafinil but are not Fos positive after PS rebound suggesting that Hcrt neurons are active during waking and silent during PS (64, 69) and therefore that Hcrt stimulates waking but has no direct inhibitory effect on PS. Consistent with these observations, it has been showed by continuous microdialysis or CSF puncture through 24 hours that the level of Hcrt released *in situ* or in the CSF is higher during the active period in rat (72) and in monkey (73).

Although the role of Hcrt as a wake promoting compound is now well accepted, its mode of action on regulation of wakefulness remains to be identified. To have a better understanding, it is important to look at pathologies. Deficiencies in Hcrt neurotransmission due to a lack of preprohcrt in human or knock-out mice, or mutations of the HcrtR-2 in dogs is the fundamental cause of narcolepsy.

Narcolepsy is a unique model for dysfunction in mechanisms that regulate wakefulness and transitions between vigilance states. The narcolepsy syndrome is characterized by the narcolepsy tetrad: excessive daytime sleepiness, fragmented sleep at night, cataplexy (sudden loss of muscle tone triggered by emotions such as laughter, anger), hypnagogic hallucinations (dream-like episodes occurring at sleep onset) and sleep paralysis (2). Narcolepsy appears to consist of two major problems: an inability to maintain wakefulness and intrusion of PS into wakefulness. The total daily amount of total sleep and PS however, is similar of those of healthy subjects. In regard to these results, we can propose that the role of Hcrt for the regulation of wakefulness would be to maintain wake and resist sleep.

THE POSTERIOR HYPOTHALAMUS AND PARADOXICAL SLEEP

Little is known about a possible implication of the PH in PS regulation. Only a few observations have been reported prior to our publication released in 2003. In this recent study (69), we showed that the PH and in particular the MCH neurons of the PH play an important role in PS homeostatic regulation.

Using an indirect method of Fos immunolabelling, a marker of neuronal activity, combined with a significant PS rebound consecutive to a specific PS deprivation, we found a very large number of activated neurons in the entire PH after PS rebound (PSR) (Fig. 1). Although their location overlap on the Hcrt and HA areas, the distribution of Fos imunoreactive neurons was widely spread in the PH. The largest number of Fos+ neurons in PSR condition was localized in the lateral hypothalamic area.

A significant number of Fos immunoreactive neurons was also observed in the PH after PS deprivation. Their distribution was quite different from the PSR condition (Fig. 1).

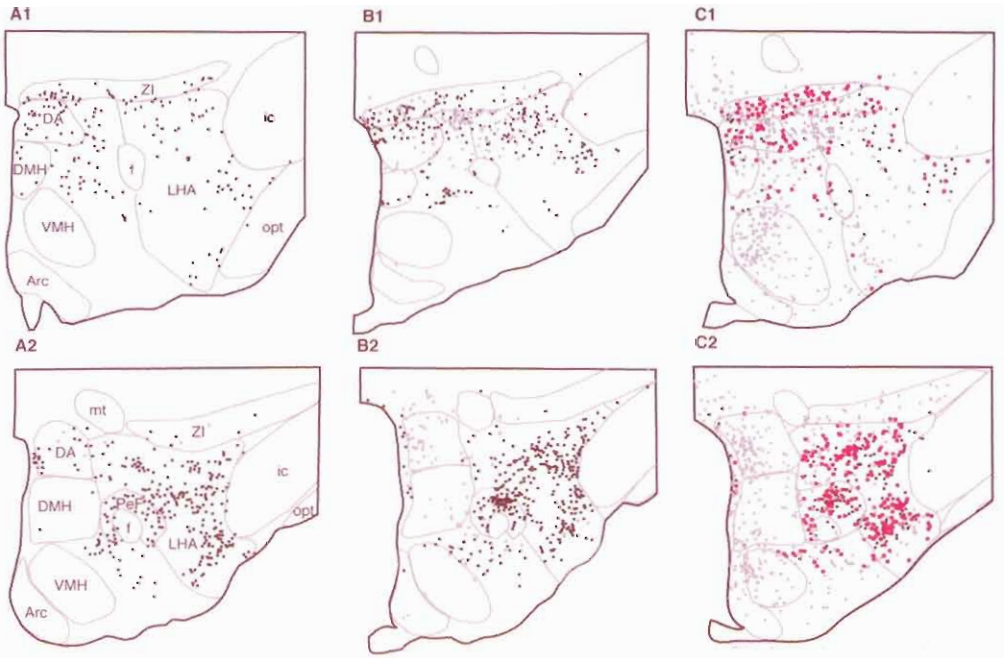

Fig. 1. - *Schematic distribution of Fos+ (grey dots), MCH+ (black dots) and MCH+/Fos+ (red dots) neurons on two coronal hemi-sections of the hypothalamus in a representative animal for PS-Control (A1, A2), PS-Deprivation (B1, B2) and PS-Recovery (C1, C2) conditions.*

Note the very large number of MCH+/Fos+ cells specifically in the PSR condition. Abbreviations: Arc, arcuate nucleus; DA, dorsal hypothalamic area; DMH, dorso-medial hypothalamic area; f, fornix; ic, internal capsule; LHA, lateral hypothalamic area; mt, mammillothalamic tract; opt, optic tract; PeF, perifornical area; VMH, ventro-medial hypothalamic area; ZI, zona incerta.

We reported that only 2% of the Hcrt neurons expressed Fos after PSR and was not correlated with the percent of PS. In contrast, a majority of MCH+ cells were immunoreactive to Fos after PSR (58% of all the MCH neurons counted) (Fig. 2). The number of MCH+/Fos+ cells was significantly and positively correlated with the percent time spent in PS and negatively correlated with the percent waking. In the PSR condition, the MCH+/Fos+ neurons made up for 76% of the Fos+ neurons localized in the perifornical area, 60% of the Fos+ neurons in the lateral hypothalamic area and 34% of the Fos+ neurons in the rostral zona incerta.

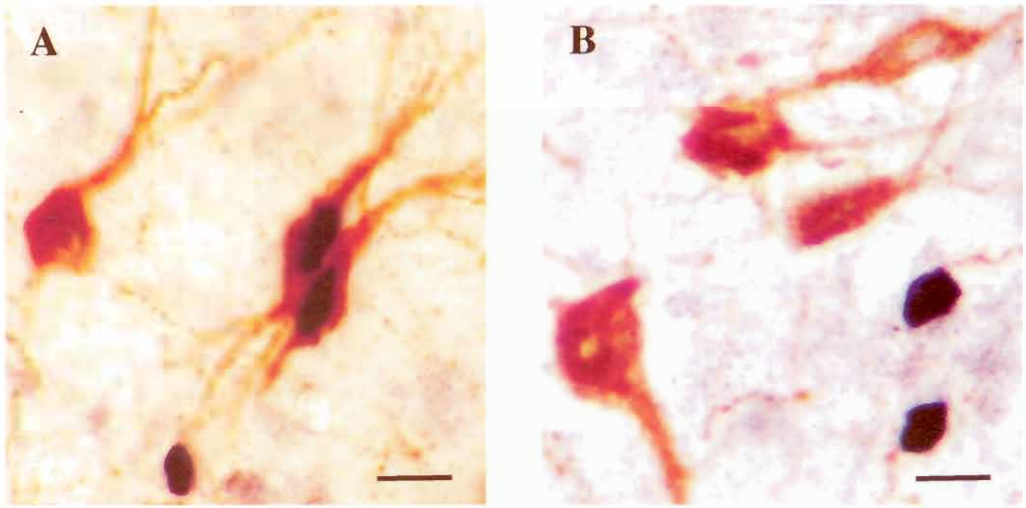

Fig. 2. - *Photomicrographs of double-immunostained sections from a rat belonging to the PS-Recovery condition.*

A, Two double-labeled MCH+/Fos+ cells with their cytoplasm colored in brown and their nucleus labeled in black, one single labeled MCH+ cell and one single labelled Fos+ cell. B, Fos+ (with a black nucleus) and hypocretin (with a brown cytoplasm) single labeled cells. Scale bars: 20 µm.

Our results suggest that MCH neurons, in addition to other non-identified neuronal populations present in the PH, are specifically and strongly active during PS. They are in agreement with electrophysiological studies showing the presence of neurons strongly active during PS in the PH (1, 28, 60).

These results, although very interesting, are not sufficient to determine whether MCH neurons play an active role in PS regulation. It is unlikely that they are responsible for PS onset and maintenance since the pontine cats experiments have established that PS generating structures are restricted to the brainstem (25). However, MCH neurons could play a role in PS homeostatic regulation.

To test this hypothesis, we conducted ICV administrations of MCH and found that injections of 0.2, 1 and 5 µg induce a dose-dependant increase in PS (Figs. 3 and 4),

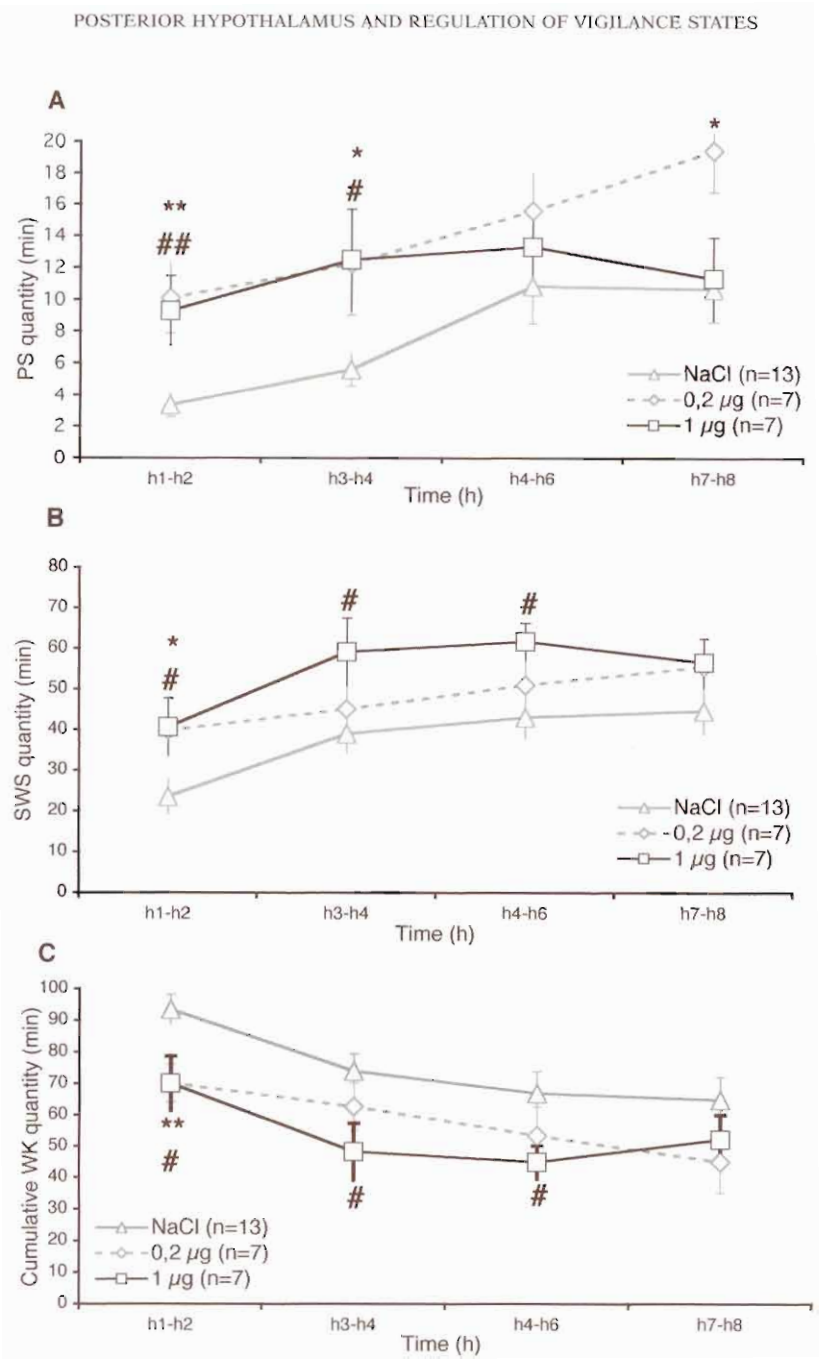

Fig. 3. - *Increase of paradoxical sleep and slow wave sleep quantities by ICV administration of MCH.*

Administration of 1 µg (black line) and 0.2 µg (dashed line) of MCH induced a strong increase in the quantities of PS (A) and SWS (B) and a decrease in W (C) in male rats compared to NaCl (Gray line). The values are expressed in minutes per two hours. Significance values indicated for individual points are: 0.2 µg MCH versus NaCl (*$p < 0.05$, **$p < 0.01$), 1 µg MCH versus NaCl (#$p < 0.05$, ##$p < 0.01$).

due to an increase in the number of PS bouts and not in their duration. At all doses, the latency of the first PS episode was unchanged compared to NaCl administrations. To a minor extent, a higher quantity of SWS was also observed after MCH administration.

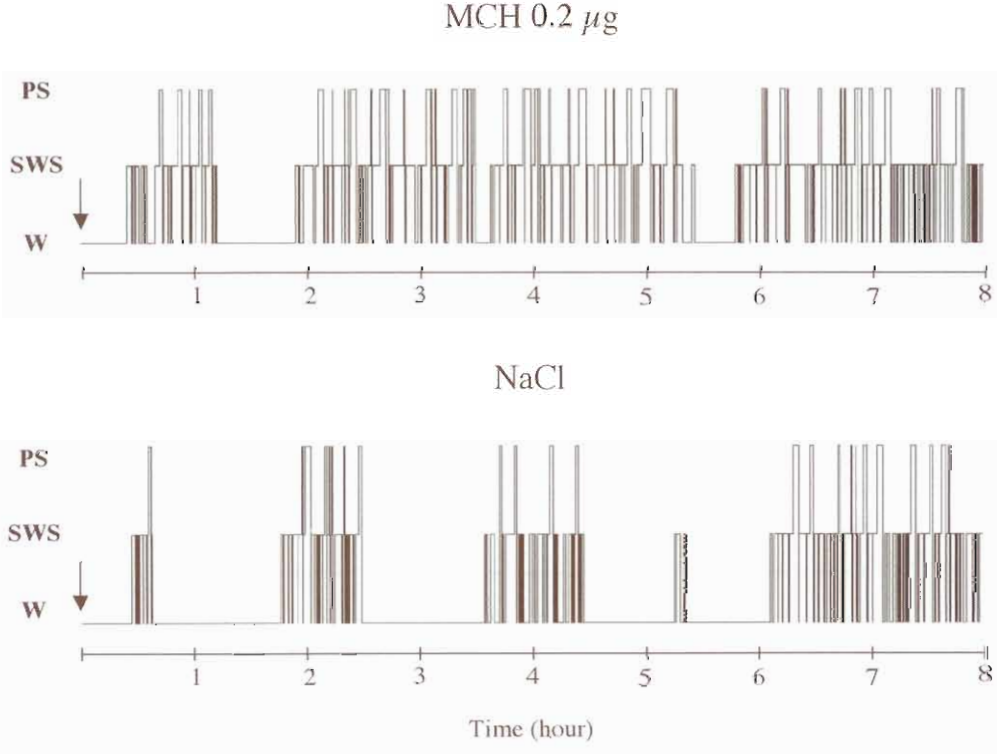

Fig. 4. - *Increase in the PS quantities after ICV administration of 0.2 µg of MCH.*

Representative Hypnograms obtained from one rat during 8 hours after administration in the lateral ventricle (arrow) of 0.2 µg of MCH (top) and NaCl (bottom).
Note the strong increase in the number of PS episodes between two and five hours post-injection.

These results suggest therefore that MCH neurons regulate PS and SWS quantities. They are supported by the observation that MCH-receptor 1 deficient mice are hyperactive (40).

Based on these results, we proposed that the MCH neuropeptide is implicated in PS homeostatic regulation. However, its mechanism of action remains to be determined.

CONCLUSION: INTERACTION BETWEEN HISTAMINE, HYPOCRETIN AND MCH NEURONS

Three neuronal populations, which promote either sleep or wakefulness, have now been identified in the posterior hypothalamus. Many additional experiments are needed to understand how they interact with each other and how they participate to sleep-wake regulation.

HA neurons are GABAergic neurons (but histamine and GABA are not costored in the same structures in TMN neurons (29)) and have been shown to have either excitatory or inhibitory effects depending on the targeted neuronal population (review in (9)). Hrct neurons are glutamatergic (53) and have always been found to be excitatory on targeted neurons (see (62) for review). MCH has been shown to be an inhibitory peptide (13, 14) and GABA has been shown to be co-localized with MCH (11).

It is interesting to note that HA neurons project to Hcrt neurons, although it has been shown that HA has no effect on Hcrt neurons as tested by patch clamp on in vitro slices (30). Hcrt neurons send excitatory projections to all monoaminergic cell groups, including the HA cells, known to be implicated in the regulation of wake. It is therefore likely that monoaminergic and Hcrt systems work in concert during wakefulness, with partial specialization. As mentioned above, HA would maintain a high level of arousal when the animals experienced behavioural challenges while Hcrt would have the function of resisting sleep.

Based on electron and photonic microscopy observations, it has been showed that MCH and Hcrt neurons are interconnected (4, 17). It is unknown whether MCH and HA neurons are interconnected although it is likely.

We can propose that MCH neurons modulate SWS and PS quantities via an inhibitory action on the intermingled hypocretin neurons and on the HA neurons. We showed that MCH neurons are active during PS rebound but not during wake. Therefore, it is likely that MCH neurons promote PS indirectly by inhibiting neurons, themselves inhibiting the PS executive neurons during W and SWS (PS-off neurons). The monoaminergic neurons in the brainstem and the HA neurons in the caudal hypothalamus belong to this category. They are active during W, decrease or nearly cease their activity during SWS and are silent during PS (15, 16, 60). Based on our and others results, it is likely that the Hcrt neurons also have this pattern of activity across the sleep-wake cycle. In previous studies, we have shown that GABA tonically inhibits the pontine PS executive neurons localized in the sublaterodorsal nucleus (5). Further, we localized in the ponto-mesencephalic reticular formation the GABAergic neurons potentially responsible for this inhibition (6). We can then propose that the increase of PS induced by MCH could be due to a direct inhibition of the GABAergic neurons of the ponto-mesencephalic reticular formation and that Hcrt neurons, that also project to this area, would be excitatory on these GABAergic neurons and by this mean, would prevent PS.

Acknowledgments. - This work was supported by CNRS (UMR5167), and Université Claude Bernard Lyon 1.

REFERENCES

1. ALAM, M.N., GONG, H., ALAM, T., JAGANATH, R., MCGINTY, D. AND SZYMUSIAK, R. Sleep-waking discharge patterns of neurons recorded in the rat perifornical lateral hypothalamic area. *J. Physiol., Lond.*, **538**: 619-631, 2002.
2. BASSETTI, C. AND ALDRICH, M.S., Narcolepsy. *Neurol. Clin.*, **14**: 545-571, 1996.
3. BAYER, L., EGGERMANN, E., SERAFIN, M., SAINT-MLEUX, B., MACHARD, D., JONES, B. AND MUHLETHALER, M. Orexins (hypocretins) directly excite tuberomammillary neurons. *Eur. J. Neurosci.*, **14**: 1571-1575, 2001.
4. BAYER, L., MAIRET-COELLO, G., RISOLD, P.Y. AND GRIFFOND, B. Orexin/hypocretin neurons: chemical phenotype and possible interactions with melanin-concentrating hormone neurons. *Regul. Pept.*, **104**: 33-39, 2002.
5. BOISSARD, R., GERVASONI, D., SCHMIDT, M.H., BARBAGLI, B., FORT, P. AND LUPPI, P.H. The rat ponto-medullary network responsible for paradoxical sleep onset and maintenance: a combined microinjection and functional neuroanatomical study. *Eur. J. Neurosci.*, **16**: 1959-1973, 2002.
6. BOISSARD, R., FORT, P., GERVASONI, D., BARBAGLI, B. AND LUPPI, P.H. Localization of the GABAergic and non-GABAergic neurons projecting to the sublaterodorsal nucleus and potentially gating paradoxical sleep onset. *Eur. J. Neurosci.*, **18**: 1627-1639, 2003.
7. BOURGIN, P., HUITRON-RESENDIZ, S., SPIER, A.D., FABRE, V., MORTE, B., CRIADO, J.R., SUTCLIFFE, J.G., HENRIKSEN, S.J. AND DE LECEA, L., Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. *J. Neurosci.*, **20**: 7760-7765, 2000.
8. BROWN, R.E., SERGEEVA, O., ERIKSSON, K.S. AND HAAS, H.L., Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. *Neuropharmacology*, **40**: 457-459, 2001.
9. BROWN, R.E., STEVENS, D.R. AND HAAS, H.L., The physiology of brain histamine. *Progr. Neurobiol.*, **63**: 637-672, 2001.
10. DE LECEA, L., KILDUFF, T.S., PEYRON, C., GAO, X., FOYE, P.E., DANIELSON, P.E., FUKUHARA, C., BATTENBERG, E.L., GAUTVIK, V.T., BARTLETT, F.S., 2ND, FRANKEL, W.N., VAN DEN POL, A.N., BLOOM, F.E., GAUTVIK, K.M. AND SUTCLIFFE, J.G. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. USA*, **95**: 322-327, 1998.
11. ELIAS, C.F., LEE, C.E., KELLY, J.F., AHIMA, R.S., KUHAR, M., SAPER, C.B. AND ELMQUIST, J.K., Characterization of CART neurons in the rat and human hypothalamus. *J. Comp. Neurol.*, **432**: 1-19, 2001.
12. ERIKSSON, K.S., SERGEEVA, O., BROWN, R.E. AND HAAS, H.L. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus, *J Neurosci*, **21**: 9273-9279, 2001.
13. GAO, X.B. AND VAN DEN POL, A.N. Melanin concentrating hormone depresses synaptic activity of glutamate and GABA neurons from rat lateral hypothalamus. *J. Physiol. Lond.*, **533**: 237-252, 2001.
14. GAO, X.B. AND VAN DEN POL, A.N. Melanin-concentrating hormone depresses L-, N- AND P/Q-type voltage-dependent calcium channels in rat lateral hypothalamic neurons. *J. Physiol., Lond.*, **542**: 273-286, 2002.
15. GERVASONI, D., DARRACQ, L., FORT, P., SOULIERE, F., CHOUVET, G. AND LUPPI, P.H. Electrophysiological evidence that noradrenergic neurons of the rat locus coeruleus are tonically inhibited by GABA during sleep. *Eur. J. Neurosci.*, **10**: 964-970, 1998.
16. GERVASONI, D., PEYRON, C., RAMPON, C., BARBAGLI, B., CHOUVET, G., URBAIN, N., FORT, P. AND LUPPI, P.H. Role and origin of the GABAergic innervation of dorsal raphe serotonergic neurons. *J. Neurosci.*, **20**: 4217-4225, 2000.

17. GUAN, J.L., UEHARA, K., LU, S., WANG, Q.P., FUNAHASHI, H., SAKURAI, T., YANAGIZAWA, M. AND SHIODA, S., Reciprocal synaptic relationships between orexin- and melanin-concentrating hormone-containing neurons in the rat lateral hypothalamus: a novel circuit implicated in feeding regulation. *Int. J. Obes. Relat. Metab Disord.*, **26**: 1523-1532, 2002.
18. HAAS, H.L. AND REINER, P.B. Membrane properties of histaminergic tuberomammillary neurones of the rat hypothalamus in vitro. *J. Physiol., Lond.*, **399**: 633-646, 1988.
19. HAGAN, J.J., LESLIE, R.A., PATEL, S., EVANS, M.L., WATTAM, T.A., HOLMES, S., BENHAM, C.D., TAYLOR, S.G., ROUTLEDGE, C., HEMMATI, P., MUNTON, R.P., ASHMEADE, T.E., SHAH, A.S., HATCHER, J.P., HATCHER, P.D., JONES, D.N., SMITH, M.I., PIPER, D.C., HUNTER, A.J., PORTER, R.A. AND UPTON, N., Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc. Natl. Acad. Sci. USA*, **96**: 10911-10916, 1999.
20. HERVIEU, G.J., CLUDERAY, J.E., HARRISON, D.C., ROBERTS, J.C. AND LESLIE, R.A. Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. *Neuroscience*, **103**: 777-797, 2001.
21. HORVATH, T.L., PEYRON, C., DIANO, S., IVANOV, A., ASTON-JONES, G., KILDUFF, T.S. AND VAN DEN POL, A.N. Hypocretin (orexin) activation and synaptic innervation of the locus coeruleus noradrenergic system. *J. Comp. Neurol.*, **415**: 145-159, 1999.
22. HUANG, Z.L., QU, W.M., LI, W.D., MOCHIZUKI, T., EGUCHI, N., WATANABE, T., URADE, Y. AND HAYAISHI, O. Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl. Acad. Sci. USA*, **98**: 9965-9970, 2001.
23. INOUE, I., YANAI, K., KITAMURA, D., TANIUCHI, I., KOBAYASHI, T., NIIMURA, K. AND WATANABE, T. Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. *Proc. Natl. Acad. Sci. USA*, **93**: 13316-13320, 1996.
24. JONES, B.E. The organization of central cholinergic systems and their functional importance in sleep-waking states. *Progr. Brain Res.*, **98**: 61-71, 1993.
25. JOUVET, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.*, **64**: 166-307, 1972.
26. JOUVET, M. The regulation of paradoxical sleep by the hypothalamo-hypophysis. *Arch. Ital. Biol.*, **126**: 259-274, 1988.
27. KIYONO, S., SEO, M.L., SHIBAGAKI, M., WATANABE, T., MAEYAMA, K. AND WADA, H. Effects of alpha-fluoromethylhistidine on sleep-waking parameters in rats. *Physiol. Behav.*, **34**: 615-617, 1985.
28. KOYAMA, Y., TAKAHASHI, K., KODAMA, T. AND KAYAMA, Y. State-dependent activity of neurons in the perifornical hypothalamic area during sleep and waking. *Neuroscience*, **119**: 1209-1219, 2003.
29. KUKKO-LUKJANOV, T.K. AND PANULA, P. Subcellular distribution of histamine, GABA and galanin in tuberomamillary neurons in vitro. *J. Chem. Neuroanat.*, **25**: 279-292, 2003.
30. LI, Y., GAO, X.B., SAKURAI, T. AND VAN DEN POL, A.N. Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. *Neuron*, **36**: 1169-1181, 2002.
31. LIN, J.S., SAKAI, K. AND JOUVET, M. Evidence for histaminergic arousal mechanisms in the hypothalamus of cat. *Neuropharmacology*, **27**: 111-122, 1988.
32. LIN, J.S., SAKAI, K., VANNI-MERCIER, G. AND JOUVET, M. A critical role of the posterior hypothalamus in the mechanisms of wakefulness determined by microinjection of muscimol in freely moving cats. *Brain Res.*, **479**: 225-240, 1989.
33. LIN, J.S., SAKAI, K., VANNI-MERCIER, G., ARRANG, J.M., GARBARG, M., SCHWARTZ, J.C. AND JOUVET, M. Involvement of histaminergic neurons in arousal mechanisms demonstrated with H3-receptor ligands in the cat. *Brain Res.*, **523**: 325-330, 1990.
34. LIN, J.S., ROUSSEL, B., AKAOKA, H., FORT, P., DEBILLY, G. AND JOUVET, M. Role of catecholamines in the modafinil and amphetamine induced wakefulness, a comparative pharmacological study in the cat. *Brain Res.*, **591**: 319-326, 1992.

35. LIN, J.S., SAKAI, K. AND JOUVET, M. Hypothalamo-preoptic histaminergic projections in sleep-wake control in the cat. *Eur. J. Neurosci.*, **6**: 618-625, 1994.
36. LIN, J.S., HOU, Y. AND JOUVET, M., Potential brain neuronal targets for amphetamine-, methylphenidate- AND modafinil-induced wakefulness, evidenced by c-fos immunocytochemistry in the cat. *Proc. Natl. Acad. Sci. USA*, **93**: 14128-14133, 1996.
37. LIN, J.S. Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med. Rev.*, **4**: 471-503, 2000.
38. LIU, R.J., VAN DEN POL, A.N. AND AGHAJANIAN, G.K. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J. Neurosci.*, **22**: 9453-9464, 2002.
39. MARCUS, J.N., ASCHKENASI, C.J., LEE, C.E., CHEMELLI, R.M., SAPER, C.B., YANAGISAWA, M. AND ELMQUIST, J.K. Differential expression of orexin receptors 1 and 2 in the rat brain. *J. Comp. Neurol.*, **435**: 6-25, 2001.
40. MARSH, D.J., WEINGARTH, D.T., NOVI, D.E., CHEN, H.Y., TRUMBAUER, M.E., CHEN, A.S., GUAN, X.M., JIANG, M.M., FENG, Y., CAMACHO, R.E., SHEN, Z., FRAZIER, E.G., YU, H., METZGER, J.M., KUCA, S.J., SHEARMAN, L.P., GOPAL-TRUTER, S., MACNEIL, D.J., STRACK, A.M., MACINTYRE, D.E., VAN DER PLOEG, L.H. AND QIAN, S. Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive AND hyperphagic and have altered metabolism. *Proc. Natl. Acad. Sci., USA*, **99**: 3240-3245, 2002.
41. MOCHIZUKI, T., YAMATODANI, A., OKAKURA, K., HORII, A., INAGAKI, N. AND WADA, H. Circadian rhythm of histamine release from the hypothalamus of freely moving rats. *Physiol. Behav.*, **51**: 391-4, 1992.
42. MONNIER, M., SAUER, R. AND HATT, A.M. The activating effect of histamine on the central nervous system. *Int. Rev. Neurobiol.*, **12**: 265-305, 1970.
43. MONTI, J.M., PELLEJERO, T. AND JANTOS, H. Effects of H1- and H2-histamine receptor agonists and antagonists on sleep and wakefulness in the rat. *J. Neural. Transm.*, **66**: 1-11, 1986.
44. MONTI, J.M., JANTOS, H., BOUSSARD, M. ALTIER, H., ORELLANA, C. AND OLIVERA, S., Effects of selective activation or blockade of the histamine H3 receptor on sleep and wakefulness. *Eur. J. Pharmacol.*, **205**: 283-287, 1991.
45. MORUZZI, G. AND MAGOUN, H.W. Brain stem reticular formation and activation of the EEG. 1949. *J. Neuropsychiatry Clin. Neurosci.*, **7**: 251-267, 1995.
46. NAKAMURA, T., URAMURA, K., NAMBU, T., YADA, T., GOTO, K., YANAGISAWA, M. AND SAKURAI, T. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res.*, **873**: 181-187, 2000.
47. NAUTA, W.J.H. Hypothalamic regulation of sleep in rats: an experimental study. *J. Neurophysiol.*, **9**: 285-316, 1946.
48. NICHOLSON, A.N., PASCOE, P.A. AND STONE, B.M. Histaminergic systems and sleep. Studies in man with H1 and H2 antagonists. *Neuropharmacology*, **24**: 245-250, 1985.
49. ORR, E. AND QUAY, W.B. Hypothalamic 24-hour rhythms in histamine, histidine, decarboxylase and histamine-N-methyltransferase. *Endocrinology*, **96**: 941-945, 1975.
50. PARMENTIER, R., OHTSU, H., DJEBBARA-HANNAS, Z., VALATX, J.L., WATANABE, T. AND LIN, J.S. Anatomical, physiological AND pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. *J. Neurosci.*, **22**: 7695-7711, 2002.
51. PEYRON, C., TIGHE, D.K., VAN DEN POL, A.N., DE LECEA, L., HELLER, H.C., SUTCLIFFE, J.G. AND KILDUFF, T.S. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.*, **18**: 9996-10015, 1998.
52. RANSON, S. Somnolence caused by hypothalamic lesions in the monkey. *Arch. Neurol. Psychiatry*, **41**: 1-23, 1939.

53. ROSIN, D.L., WESTON, M.C., SEVIGNY, C.P., STORNETTA, R.L. AND GUYENET, P.G. Hypothalamic orexin (hypocretin) neurons express vesicular glutamate transporters VGLUT1 or VGLUT2. *J. Comp. Neurol.*, **465**: 593-603, 2003.
54. SAKAI, K., EL MANSARI, J.S., LIN, J.S., ZHANG, G. AND VANNI-MERCIER, G. The posterior hypothalamus in the regulation of wakefulness and paradoxical sleep. Pp. 171-198, *The Diencephalon and Sleep.* Raven Press; New York, 1990.
55. SAKURAI, T., AMEMIYA, A., ISHII, M., MATSUZAKI, I., CHEMELLI, R.M., TANAKA, H., WILLIAMS, S.C., RICHARDSON, J.A., KOZLOWSKI, G.P., WILSON, S., ARCH, J.R., BUCKINGHAM, R. E., HAYNES, A.C., CARR, S.A., ANNAN, R.S., MCNULTY, D.E., LIU, W.S., TERRETT, J.A., ELSHOURBAGY, N.A., BERGSMA, D.J. AND YANAGISAWA, M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, **92**: 573-585, 1998.
56. SALLANON, M., SAKAI, K., BUDA, C., PUYMARTIN, M. AND JOUVET, M., Increase of paradoxical sleep induced by microinjections of ibotenic acid into the ventrolateral part of the posterior hypothalamus in the cat. *Arch. Ital. Biol.*, **126**: 87-97, 1988.
57. SALLANON, M., DENOYER, M., KITAHAMA, K., AUBERT, C., GAY, N. AND JOUVET, M., Long-lasting insomnia induced by preoptic neuron lesions and its transient reversal by muscimol injection into the posterior hypothalamus in the cat. *Neuroscience*, **32**: 669-683, 1989.
58. SCHWARTZ, J.C., Histaminergic mechanisms in brain. *Ann. Rev. Pharmacol. Toxicol.*, **17**: 325-339, 1977.
59. SCHWARTZ, J.C., ARRANG, J.M., GARBARG, M., POLLARD, H. AND RUAT, M. Histaminergic transmission in the mammalian brain. *Physiol. Rev.*, **71**: 1-51, 1991.
60. STEININGER, T.L., ALAM, M.N., GONG, H., SZYMUSIAK, R. AND MCGINTY, D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain Res.*, **840**: 138-147, 1999.
61. SWETT, C.P. AND HOBSON, J.A. The effects of posterior hypothalamic lesions on behavioral and electrographic manifestations of sleep and waking in cats. *Arch. Ital. Biol.*, **106**: 283-293, 1968.
62. TAHERI, S., ZEITZER, J.M. AND MIGNOT, E. The role of hypocretins (orexins) in sleep regulation and narcolepsy. *Ann. Rev. Neurosci.*, **25**: 283-313, 2002.
63. TASHIRO, M., MOCHIZUKI, H., IWABUCHI, K., SAKURADA, Y., ITOH, M., WATANABE, T. AND YANAI, K. Roles of histamine in regulation of arousal and cognition: functional neuroimaging of histamine H1 receptors in human brain. *Life Sci.*, **72**: 409-414, 2002.
64. TORTEROLO, P., YAMUY, J., SAMPOGNA, S., MORALES, F.R. AND CHASE, M.H. Hypocretinergic neurons are primarily involved in activation of the somatomotor system. *Sleep*, **26**: 25-28, 2003.
65. TRIVEDI, P., YU, H., MACNEIL, D.J., VAN DER PLOEG, L.H. AND GUAN, X.M. Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett.*, **438**: 71-75, 1998.
66. VAN DEN POL, A.N. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. *J. Neurosci.*, **19**: 3171-3182, 1999.
67. VANNI-MERCIER, G., SAKAI, K. AND JOUVET, M., Specific neurons for wakefulness in the posterior hypothalamus in the cat. *C. R. Acad. Sci. III*, **298**: 195-200, 1984.
68. VANNI-MERCIER, G., GIGOUT, S., DEBILLY, G. AND LIN, J.S. Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. *Behav. Brain Res.*, **144**: 227-241, 2003.
69. VERRET, L., GOUTAGNY, R., FORT, P., CAGNON, L., SALVERT, D., LEGER, L., BOISSARD, R., SALIN, P., PEYRON, C. AND LUPPI, P.H. A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. *BMC Neurosci.*, **4**: 19, 2003.
70. VON ECONOMO, C. Sleep as a problem of localization. *J. Nerv. Mental. Dis.*, **71**: 249-269, 1931.

71. YAMANAKA, A., TSUJINO, N., FUNAHASHI, H., HONDA, K., GUAN, J.L., WANG, Q.P., TOMINAGA, M., GOTO, K., SHIODA, S. AND SAKURAI, T. Orexins activate histaminergic neurons via the orexin 2 receptor. *Biochem. Biophys. Res. Commun.*, **290**: 1237-1245, 2002.
72. YOSHIDA, Y., FUJIKI, N., NAKAJIMA, T., RIPLEY, B., MATSUMURA, H., YONEDA, H., MIGNOT, E. AND NISHINO, S. Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. *Eur. J. Neurosci.*, **14**: 1075-1081, 2001.
73. ZEITZER, J.M., BUCKMASTER, C.L., PARKER, K.J., HAUCK, C.M., LYONS, D.M. AND MIGNOT, E. Circadian and homeostatic regulation of hypocretin in a primate model: implications for the consolidation of wakefulness. *J. Neurosci.*, **23**: 3555-3560, 2003.