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PONTINE STRUCTURES AND MECHANISMS INVOLVED IN THE GENERATION OF PARADOXICAL (REM) SLEEP

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INTRODUCTION

Following the discovery in 1953 of rapid eye movement (REM) sleep in humans by Aserinsky and Kleitman (1), Kleitman, Aserinsky, and Dement (2, 7) established that REM sleep recurs approximately every 90 min throughout the night (a basic rest-activity cycle) and is closely associated with dream activity. Shortly after this landmark discovery of REM sleep in humans, Dement (6) and, subsequently, Jouvet and his colleagues (19), identified a similar sleep state in the cat, called desynchronized sleep, active sleep, deep sleep, or paradoxical sleep (PS).

In early brain lesion and transection studies, Jouvet (15) showed that a state nearly identical to PS could be observed even after removal of all neural tissues above the level of the rostral pons, and that the pons is the most critical region in the brain for PS generation. Efforts were then directed at identifying brain structures within the pons critical for the generation of PS and its major tonic and phasic events. Early lesion experiments first pointed out the important role played by the caudal part of the nucleus pontis oralis (Poo) and the rostral part of the nucleus pontis caudalis (Poc) (3, 16, 17, 62); bilateral electrolytic destruction of these regions led to the complete disappearance of PS, which is defined by the simultaneous appearance of neocortical EEG activation, rapid eye movements, and muscle atonia. Later, Jouvet (18) proposed that noradrenergic neurons in the nucleus locus coeruleus (LC) of the dorsolateral pontine tegmentum are critical for the generation of PS and its signs. Hobson, McCarley, and co-workers (10, 25) refocused attention on the pontine reticular formation (RF) and proposed that a population of "cholinergic" pontine RF gigantocellular neurons have executive functions in the generation of both PS and the tonic and phasic events which characterize this phase of sleep (53). Subsequent experiments, however, have not supported these early proposals that were based on the concept of a single primarily localized "center" of PS control (11, 32, 59). Several experimental studies have suggested that various neuronal groups, located in both the pons and medulla, are involved in PS generation, and that these pontine and medullary mechanisms must interact to produce PS (13, 34, 35, 52, 58).

The recent search for the neurophysiological and neurochemical mechanisms involved in PS generation has focused on rostral pontine structures. In this paper, we will summarize our recent experimental evidence showing that the peri-LCα of the mediodorsal pontine tegmentum plays a central role in PS generation within the

pons, and that PS is generated as a result of a combination of the tonic excitation of cholinergic and non-cholinergic (presumably glutamatergic) PS-on neurons, which serve an executive function, and the cessation of activity of monoaminergic and non-monoaminergic (possibly GABAergic) PS-off neurons, which play an inhibitory or permissive role in PS generation by inhibiting PS-on neurons during wakefulness (W) and slow-wave sleep (SWS).

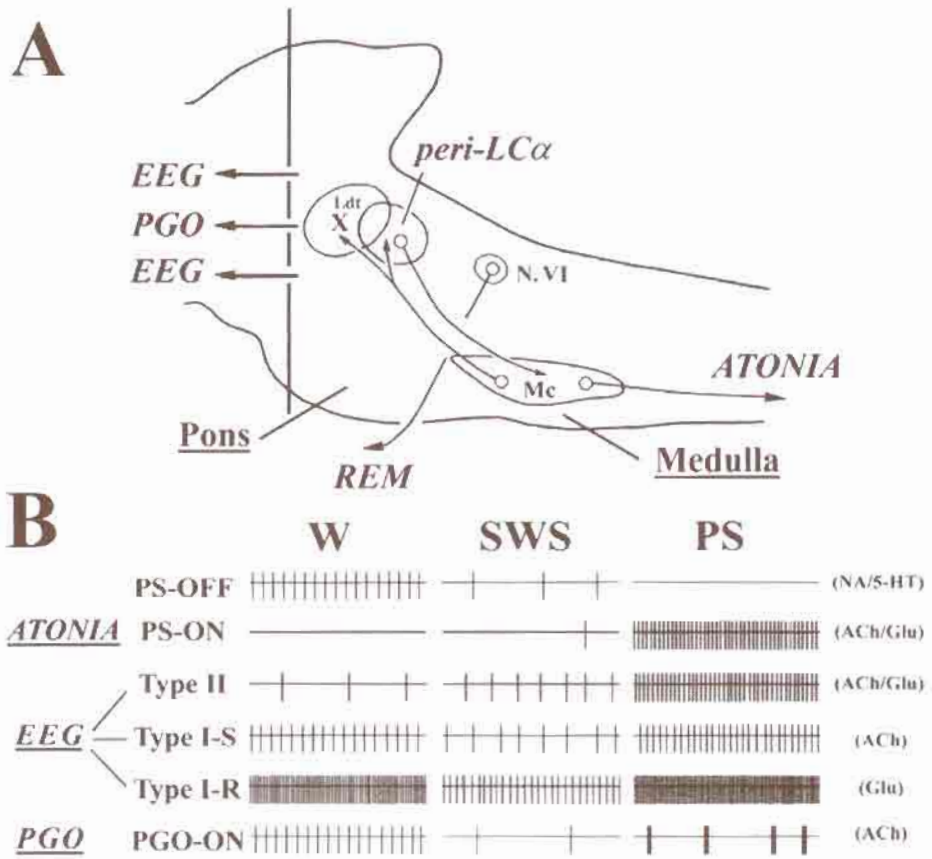

Fig. 1. - *Diagrams illustrating the lower brain stem structures implicated in the executive mechanisms of PS generation (A) and the discharge patterns during the sleep-wake cycle of neurons found in the dorsal mesopontine tegmentum (B).*

PS-on neurons located in the peri-LCα of the mediodorsal pontine tegmentum and in the ventromedial (the nucleus reticularis magnocellularis; Mc) and lateral medulla play a central role in the executive mechanisms of PS generation in general, and in the generation of tonic and phasic events of PS in particular. Ascending tonic neurons (types II, I-S, and I-R) and PGO-on neurons located in the X area and the rostral part of the peri-LCα play an important role in cortical electroencephalogram (EEG) desynchronization and the occurrence of thalamo-cortical PGO waves, respectively. Descending PS-on neurons located in the caudal peri-LCα play a crucial role in the generation of atonia via descending projections to the Mc. Other abbreviations: Ldt, laterodorsal tegmental nucleus; N. VI, abducens nucleus; REM, rapid eye movement; X, X area or cholinergic component of the nucleus tegmenti pedunculopontinus, pars compacta.

EXECUTIVE MECHANISMS OF PS GENERATION

PS-on neurons.

Single unit recording studies in freely moving cats have revealed the existence of neurons which satisfy basic criteria for PS-generator or PS-executive neurons responsible for the initiation and maintenance of this state of sleep, referred to as PS-on neurons (35, 36, 45). These criteria include: 1) selectivity (the neurons should exhibit their highest rate of discharge during PS); 2) anticipation (the activity of PS-executive neurons should anticipate the onset of PS); and 3) tonicity (the activity of the neurons should be tonic throughout the PS episode). PS-on neurons remain completely silent during W, but exhibit a significant increase in discharge rate prior to the onset of PS, maintain a sustained tonic discharge throughout the PS episode, and show complete cessation of discharge during the transition from PS to SWS or from PS to W (Figs. 1B, 2B, C). PS-on neurons are located in both the pons and medulla (35, 36, 45) and their excitatory interactions appear to be necessary for PS generation, since PS is no longer seen after lesion or section of the fibers connecting these two structures (13, 52, 58). In the pons, PS-on neurons are found almost exclusively in the peri-LCα, located ventromedial to the LCα which contains tightly packed noradrenergic neurons (Fig. 2A). The rostral part of the peri-LCα contains a dense population of cholinergic neurons that send axons to the thalamus and/or hypothalamus, while the caudal peri-LCα contains mainly non-cholinergic and non-monoaminergic descending neurons (37).

Two different types of PS-on neurons can be distinguished in the dorsal pontine tegmentum, one of which is characterized by a broad action potential, slow conduction velocity, and an inhibitory response to iontophoretically-applied carbachol, a potent cholinergic agonist (therefore termed Carb-I PS-on neurons), while the other is characterized by a short action potential, fast conduction velocity, and an excitatory response to applied carbachol (Carb-E PS-on neurons). Carb-I PS-on neurons are located exclusively in the mediodorsal pontine tegmentum which contains cholinergic neurons, while Carb-E PS-on neurons are found in both the cholinergic (rostral) and non-cholinergic (caudal) parts of the peri-LCα, as well as in the medial X area (the cholinergic part of the nucleus tegmenti pedunculopontinus, pars compacta), located adjacent to the rostral peri-LCα (Fig. 2A). Carb-I neurons are considered as cholinergic, while Carb-E PS-on neurons are regarded as non-cholinergic (39). Both types of PS-on neurons are excited by iontophoretically applied glutamate (Glu), a well-known excitatory amino acid, or bicuculline, a $GABA_A$ receptor antagonist. Although serotonin (5-HT) has no effect, microiontophoretic application of norepinephrine (NE) to presumed non-cholinergic Carb-E PS-on neurons results in inhibition of either their spontaneous tonic discharge during PS or their tonic discharge induced by carbachol application during W or SWS, but has no effect on presumed cholinergic Carb-I PS-on neurons (39). These data suggest that both cholinergic and glutamatergic mechanisms are involved in the executive mechanisms of PS and that both adrenergic and GABAergic mechanisms are implicated in the permissive mechanisms of PS generation.

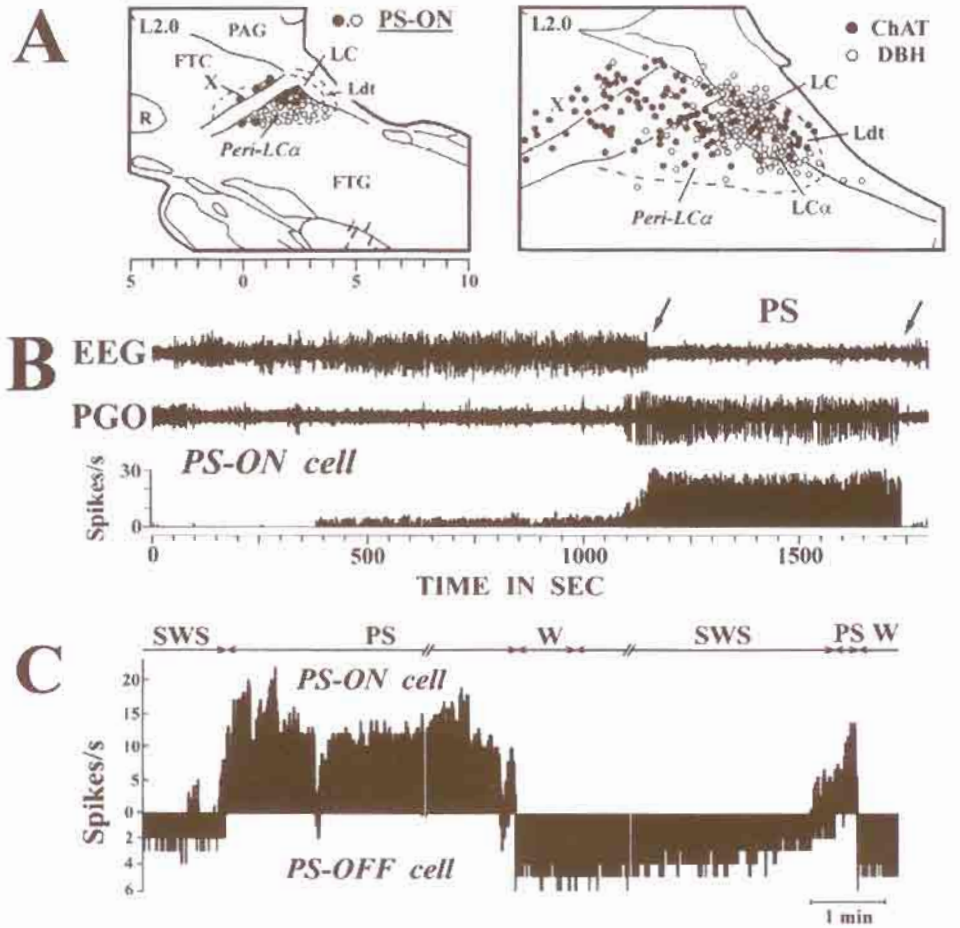

Fig. 2. - *A. Drawings of sagittal sections of the cat brainstem at L2.0 illustrating the localization of (left) presumed cholinergic (closed circles) and non-cholinergic (open circles).*

PS-on neurons and (right) cholinergic (closed circles) and noradrenergic (open circles) neurons revealed by choline acetyltransferase (ChAT) and dopamine-β-hydroxylase (DBH) immunohistochemistry.

B. Polygraphic recording showing activity of a pontine PS-on neuron during the wake-sleep cycle including one PS episode (indicated by arrows).

C. Single unit discharge rates of PS-on (upper) and PS-off (lower) cells recorded in the medulla during sleep-waking cycles with two PS episodes.

Note that the changes in unitary activity are inversely correlated.

Abbreviations: FTC, FTG, central and gigantocellular tegmental field, respectively; LC, nucleus locus coeruleus; PAG, periaqueductal gray; R, red nucleus; X, X area.

Most Carb-E, but not Carb-I, PS-on neurons are invaded antidromically by stimulation of the nucleus reticularis magnocellularis (Mc) of the ventromedial medulla, which contains PS-on neurons that send axons to the spinal cord (45). Stimulation of the peri-LCα results in synaptic excitation of Mc PS-on neurons. In

addition, bilateral lesioning of the peri-LCα causes complete suppression of atonia during PS (45) and thereby induces several stereotyped behaviors during the PS episode without atonia (47). The descending PS-on neurons in the peri-LCα are therefore regarded as atonia-executive neurons. As described below, these are under the control of excitatory cholinergic and inhibitory catecholaminergic systems.

Ascending tonic and PGO-on neurons.

In line with the idea that the caudal mesencephalic and rostral pontine tegmentum play an important role in the generation of EEG desynchronization (27), single unit recording studies in cats have revealed the existence of several types of ascending tonically active neurons in this brain area (8, 55); these can be divided into two groups: 1) those showing a higher rate of tonic discharge during both W and PS compared with during SWS, referred to as tonic type I and subdivided into two groups on the basis of their slow (type I-S) or rapid (type I-R) spontaneous discharge; and 2) those exhibiting a tonic discharge selective (PS-on neurons), or highly selective (tonic type II neurons), for periods of PS (cf. Fig. 1B) (37). Type I-S and type II neurons show a significant increase in tonic discharge rate 20-25 s or more before the onset of EEG desynchronization occurring either during W and PS or only during PS (8, 9, 55).

In line with the view that the mesopontine tegmentum is critical for the generation of PGO waves, single unit recording experiments have demonstrated neurons showing tonic discharge during W, but exhibiting phasic burst discharge during sleep just prior to, and during, PGO waves, referred to as PGO-on or PGO-burst neurons (26, 33, 38, 56). PGO-on neurons are mainly found in the X area and rostral peri-LCα (21, 37, 43). Antidromic invasion experiments demonstrated that PGO-on neurons send axons to the LGNd, as well as to the pulvinar and/or intralaminar thalamic nuclei (38, 43, 56). Electrical stimulation of brain structures containing PGO-on neurons elicits PGO waves, while bilateral lesioning of these structures results in the total disappearance of PGO waves in the thalamo-cortical structures (42).

Both type I-S and PGO-on neurons are located exclusively in the cholinergic part of the mesopontine tegmentum, and, consistent with previous evidence from anatomical studies (22) and *in vitro* and *in vivo* experiments on identified cholinergic mesopontine neurons (20, 23, 24, 28), their activity is characterized by a long action potential, slow conduction velocity, and an inhibitory response to carbachol via muscarinic inhibitory autoreceptors. Type I-R neurons are found in both cholinergic and non-cholinergic regions of the mesopontine tegmentum and are characterized by a short action potential, fast conduction velocity, and either an excitatory, or no, response to carbachol. Type I-S and PGO-on neurons are therefore considered as cholinergic, while type I-R neurons are regarded as non-cholinergic, presumably glutamatergic. A cholinergic or a non-cholinergic nature has been suggested for PS-on and tonic type II neurons (9, 39).

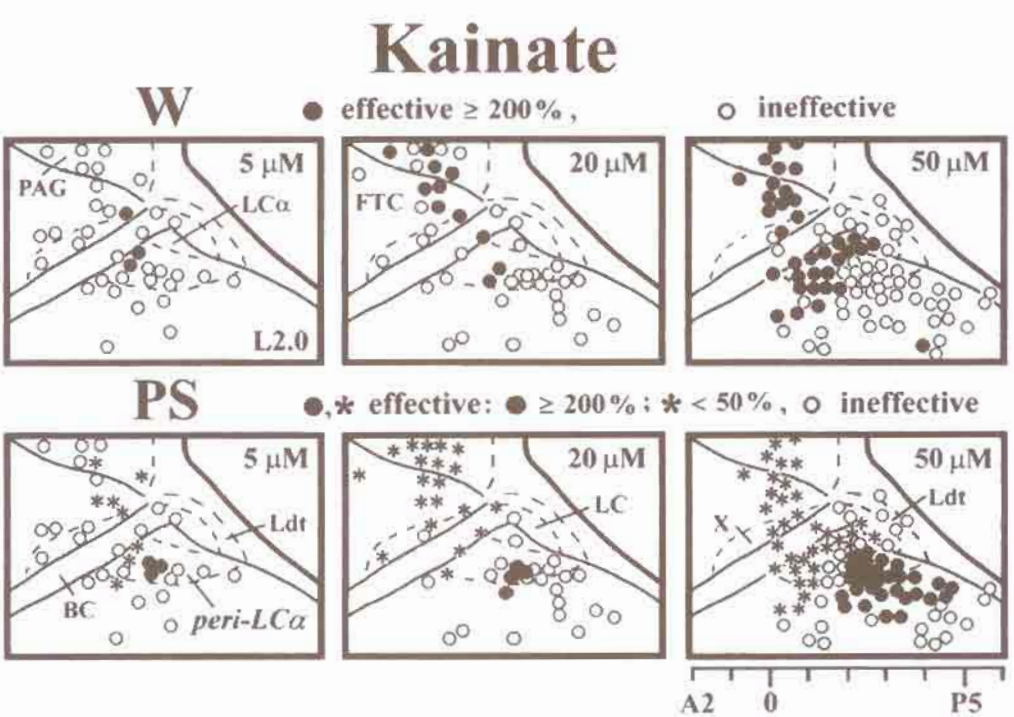

Fig. 3. - *Effects of microdialysis application of kainate (KA) on W and PS.*

Drawings of a sagittal section at L2.0 with the KA infusion sites (tip of the microdialysis probe) indicated by different symbols according to their effect on W or PS as compared with the paired-control. In this and the following figures, all drugs were applied unilaterally for 2 consecutive hours using a microdialysis probe (membrane 1 mm in length and external diameter 0.24 mm). Abbreviation: BC, brachium conjunctivum.

GLUTAMATERGIC MECHANISMS OF PS GENERATION

As shown in Fig. 3, a marked increase in PS is seen when kainate (KA), an excitatory amino acid agonist, is applied to the peri-LCα. The effect is dose- and site-dependent (29). Application of 50 μM KA to the caudal peri-LCα results in a greater than 300% increase in PS, while application to the rostral peri-LCα produces EEG desynchronization and behavioral arousal. In the middle to rostral part of the peri-LCα, application of 5 or 20 μM KA has a PS-inducing effect, while, in the most rostral part of the peri-LCα, these low concentrations of KA induce EEG and behavioral arousal (30). However, no PS-inducing effect is seen when KA is applied to other brain structures, such as the cholinergic X area or laterodorsal tegmental nucleus (Ldt), the noradrenergic LC or LCα, the non-cholinergic and non-monoaminergic Poo, the periaqueductal gray (PAG), or the central tegmental field (FTC) (Fig. 3). These results are in general agreement with those obtained in previous lesion experiments (14, 48, 49, 51, 60). Our data indicate the special importance of the peri-LCα in the executive mechanisms of PS generation and

have also revealed several PS-permissive structures, such as the PAG, X area, rostral peri-LCα, and a region between the PAG and medial X area (see below). The PS-inducing effect appears to be mainly due to activation of KA receptors, since application of AMPA (5-100 μM) or NMDA (50-500 μM) has little or no effect on PS induction, and the PS-inducing effect of KA is completely blocked by GAMS, a preferential KA receptor antagonist, but not by AP-5 or MK-801, selective NMDA receptor antagonists. Atropine, a selective muscarinic receptor antagonist, also does not block the KA effect (29).

CHOLINERGIC MECHANISMS OF PS GENERATION

In agreement with previous microinjection studies (57, 61), microdialysis application of carbachol to the peri-LCα, especially its caudal part, results in a dose-dependent increase in PS (40). Although 20 μM carbachol has no effect, concentrations of 50, 100, or 200 μM induce, respectively, a 2-fold, 3-fold, or greater than 3-fold increase in PS. As illustrated in Fig. 4A, little or no PS-inducing effect is seen when carbachol is applied to other regions of the mesopontine structures. In contrast, carbachol significantly inhibits PS when applied to the ventral part of the PAG (44). The PS-inducing effect of 200 μM carbachol is antagonized by 200 μM atropine or low concentrations (5-10 μM) of 4-DAMP, an M_1/M_3 muscarinic receptor antagonist, but not by high concentrations (200-500 μM) of pirenzepine, an M_1 muscarinic receptor antagonist, or methoctramine, an M_2 muscarinic receptor antagonist. Single application of 5 μM 4-DAMP to the caudal peri-LCα produces a great reduction in PS and PS without atonia (40). These findings indicate that M_3 muscarinic receptors, located in the caudal part of the peri-LCα, play a crucial role in the generation of PS in general, and in the generation of postural atonia in particular. The PS-inducing effect of carbachol is not antagonized by co-application of GAMS, even at high concentrations. This, and the finding that atropine does not block the PS-inducing effect of KA, indicate that two distinct (cholinergic and glutamatergic) mechanisms are involved in the executive mechanisms of PS generation.

The peri-LCα receives cholinergic afferent projections from regions of the pons and medulla, such as the X area in the pons, and the Mc and nuclei reticularis parvocellularis (Pc) and paragigantocellularis lateralis (PGCL) in the medulla, all of which contains PS-on neurons (36). The non-cholinergic caudal peri-LCα is also innervated by cholinergic neurons of the rostral peri-LCα (our unpublished data).

PS-EXECUTIVE STRUCTURES AS DEFINED BY LOCAL APPLICATION OF MUSCIMOL

PS-executive structures can be defined as structures stimulation or inhibition of which leads, respectively, to induction or suppression of PS. Fig. 4B summarizes the functional anatomy of PS-executive structures as determined by microdialysis

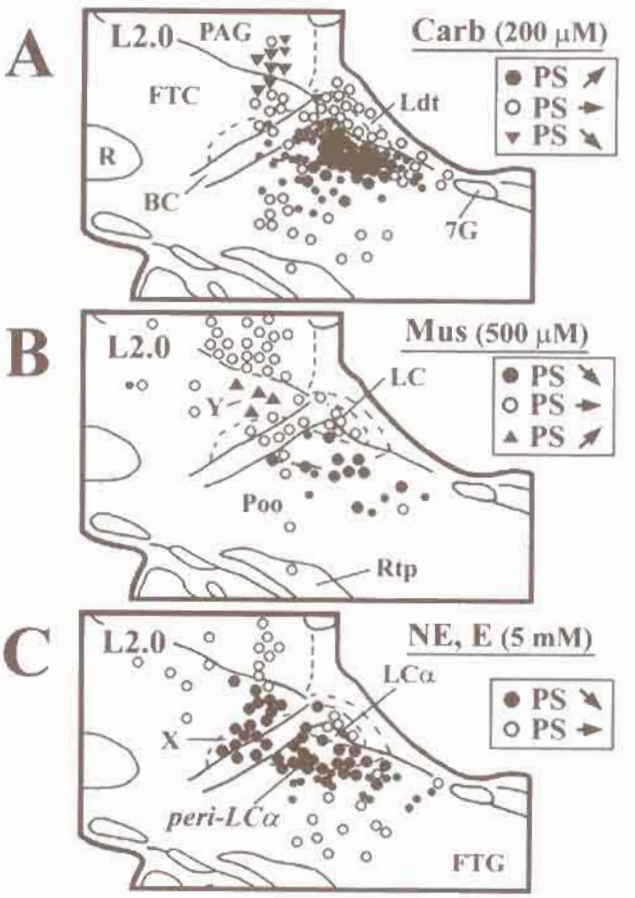

Fig. 4. - *Effects of microdialysis application of (A) carbachol (Carb), (B) muscimol (Mus), and (C) norepinephrine (NE) or epinephrine (E) on PS.*

Drawings of a sagittal section at L2.0 with drug infusion sites (tip of the microdialysis probe) indicated by different symbols according to their effects on PS as compared with the paired-control. The large empty or filled circles and large filled downward-pointing triangles designate sites at which a major effect (greater than 3-fold increase or decrease in the amount of PS) was seen during the 2 h drug application period, while the small filled circles and triangles indicate sites at which the increase or decrease in the amount of PS was greater than 2-fold, but less than 3-fold. The large filled triangles in B indicate sites at which 500 mM muscimol induced a greater than 2-fold increase in the amount of PS. Abbreviations: 7G, genu of the facial nerve; LCα, nucleus locus coeruleus alpha; Poo, nucleus reticularis pontis oralis; Rtp, nucleus reticularis tegmenti pontis; Y, Y area.

application of muscimol, a potent $GABA_A$ receptor agonist. As shown in the Figure, 500 μM muscimol significantly inhibits PS when applied to the peri-LCα, but has no effect on other behavioral states. Muscimol has little or no effect when applied to the Ldt, X area, PAG, FTC, or Poo, but significantly increases the amount of PS when applied to an area located at the pontomesencephalic junction between the PAG and medial X area, recently referred to as the "Y area" (4).

BRAIN STRUCTURES AND MECHANISMS INVOLVED IN PERMISSIVE MECHANISMS OF PS GENERATION

Muscimol microapplication studies confirm the important executive role of the peri-LCα in PS generation and further point to an important permissive role played by the Y area. In agreement with this view, application of KA to the Y area results in dose-dependent inhibition of PS (Fig. 3). In addition, early lesion experiments demonstrated that bilateral lesioning of the Y area leads to a marked and long-lasting increase in both SWS and PS (31). A dramatic increase in PS following bilateral microinjection of muscimol into this area has also recently been reported (50). As mentioned above, KA application has also revealed several other PS-inhibitory brain structures, such as the PAG, X area, and rostral peri-LCα (Fig. 3). The PS-suppressing effect seen at these sites is paralleled by a significant increase in W, suggesting that these brain structures are critically involved in the mechanisms of W and that the PS-suppressing effect may be secondary to the waking effect.

MONOAMINERGIC MECHANISMS OF PS INHIBITION

The entire peri-LCα receives dense monoaminergic afferent projections, which consist of: 1) serotonergic afferent projections from the dorsal raphe nucleus (RD); 2) noradrenergic afferent projections from pontine (A4, A5, A6 and A7) and medullary (A1 and A2) cell groups; 3) adrenergic afferent projections from the medulla (C1 cell group); 4) dopaminergic projections from the posterior hypothalamus (A11); and 5) histaminergic afferent projections from the tuberomammillary nucleus (TM) of the posterior hypothalamus (37). Single unit recording experiments have demonstrated that serotonergic, noradrenergic, histaminergic, and possibly adrenergic, but not dopaminergic, brainstem neurons selectively cease firing during PS (PS-off neurons) (12, 43, 54). In addition to these monoaminergic PS-off neurons, a population of non-monoaminergic (possibly GABAergic) PS-off neurons has recently been discovered in the medulla (41). As shown in Fig. 2C, when simultaneous recordings are made from PS-on and putative monoaminergic PS-off neurons, a mirror image, or exact inverse, relationship in terms of cellular discharge is seen, suggesting a mutual inhibitory interaction between these two distinct neuronal populations (34, 36).

Although application of 5-HT or histamine (HA) has no PS-inhibiting effect, application of 5 μM NE, epinephrine (E), or dopamine (DA) to the caudal peri-LCα causes a marked decrease in PS without affecting other behavioral states and induces PS without atonia (4). PS inhibition by NE and E in the caudal peri-LCα is mediated by α_2-adrenoceptors, since the PS-inhibiting effect is completely blocked by co-application of rauwolscine or RX 821002, specific α_2-adrenoceptor antagonists, but not by co-application of α_1- (benoxathian) or β_1-(atenolol) adrenoceptor antagonists. Furthermore, microdialysis application of clonidine, a specific α_2-

adrenoceptor agonist, mimics the effect of NE and E, whereas application of methoxamine, an α_1-adrenoceptor agonist, or isoproterenol, a β-adrenoceptor agonist, does not (5). The PS-inhibiting effect of NE and E seen in the caudal peri-LCα appears to result from direct inhibition of non-cholinergic PS-on neurons. The exact receptor subtypes that mediate PS inhibition by DA remain to be elucidated.

In both the rostral peri-LCα and the X area, NE and E induce marked inhibition of PS with a significant increase in W and a decrease in deep SWS (Fig. 4C). As described above, these structures contain PS-on neurons, as well as ascending neurons critically implicated in arousal mechanisms. The PS-inhibiting effect therefore appears to result from both inhibition of PS-on neurons and excitation of the ascending neurons. As in the caudal peri-LCα, 5-HT and HA have no PS-suppressing effect, although HA has strong waking and EEG desynchronizing effects and PS may occur, as in narcolepsy, during HA application. DA also has no effect at these sites. Although NE and E have no effect when applied to the PAG, FTC, or Poo, they have a significant PS-inhibiting effect when applied to the non-cholinergic and non-monoaminergic Y area (4), suggesting that they also have an important PS-inhibitory function via activation of non-monoaminergic PS-permissive structures.

Taken together, these findings demonstrate a particular importance of the medullary adrenergic, ponto-medullary noradrenergic and, to a lesser extent, hypothalamic dopaminergic systems in the inhibitory or permissive mechanisms of PS generation, and of the adrenergic, noradrenergic, and hypothalamic histaminergic systems in the control of arousal. The data also point to an important role of the PAG, Y area, X area, and rostral peri-LCα in the permissive mechanisms of PS generation. It appears that NE and E inhibit PS either by a direct inhibitory action on PS-on neurons via α_2-adrenoceptors or by an excitatory action on non-monoaminergic PS-permissive structures through as yet unknown mechanisms.

Although non-monoaminergic PS-off neurons have been discovered in the medulla (41), there is, as yet, no report of the existence of such neurons in the mesencephalon and pons. Our recent data show that both the PAG and Y area contain populations of type I-R and type II neurons exhibiting marked phasic increases in firing rate during PS as compared with during SWS. It is tentatively hypothesized that these neurons inhibit PS via activation of both monoaminergic and non-monoaminergic PS-off neurons during W, and that their marked increase in activity during PS promotes the end of this state of sleep

CONCLUSIONS

Our recent experimental results support the view that the peri-LCα, which contains PS-on neurons, plays a crucial role in the executive mechanisms of PS generation within the pons. It appears that excitatory interactions between ponto-medullary cholinergic and glutamatergic PS-on neurons, via activation of M_1 muscarinic and KA receptors, are important for the initiation and maintenance of

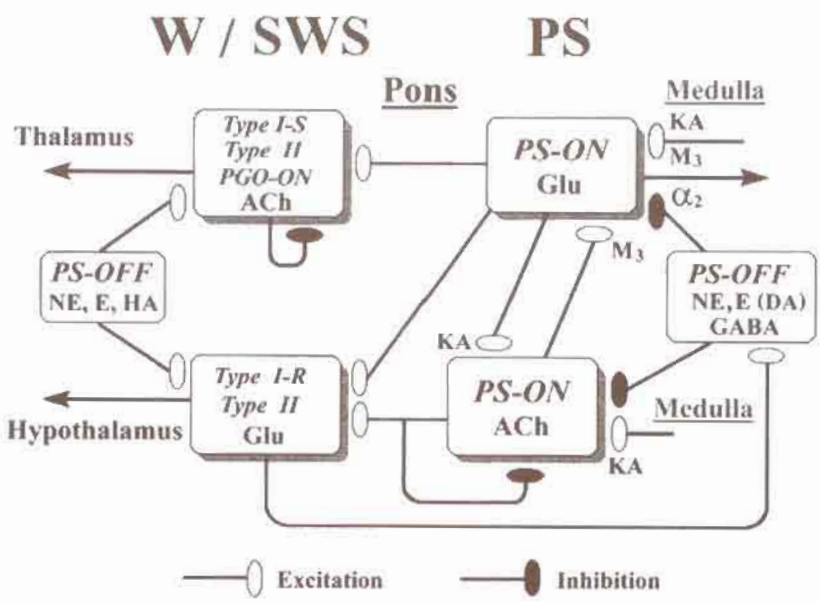

Fig. 5. - *Diagram illustrating the current state of knowledge and hypotheses concerning the pontine mechanisms of PS generation.*

Both glutamatergic (Glu) and cholinergic (ACh) PS-on neurons located in the peri-LCα play a central role in the executive mechanisms of PS generation. Pontine glutamatergic PS-on neurons receive excitatory inputs from ponto-medullary cholinergic and medullary glutamatergic PS-on neurons, while pontine cholinergic PS-on neurons receive excitatory inputs from ponto-medullary glutamatergic PS-on neurons. Excitatory interactions between glutamatergic and cholinergic PS-on neurons mediated by M_3 muscarinic and kainate (KA) receptors are important in the initiation and maintenance of PS. Pontine PS-on neurons are under the inhibitory control of medullary adrenergic and ponto-medullary noradrenergic neurons, acting via α_2 adrenoceptors, and of possibly GABAergic PS-off neurons in the medulla. NE, E, and HA also play a PS-inhibitory role either via activation of ascending tonic neurons, which are critically implicated in the mechanisms of arousal and thus in the inhibitory mechanisms of PS, or via activation of a population of non-monoaminergic and non-cholinergic descending PS-permissive neurons, which excite PS-off neurons and thus inhibit PS.

PS. The pontine PS-on neurons appear to be under the powerful inhibitory control of medullary adrenergic, ponto-medullary noradrenergic, hypothalamic dopaminergic, and possibly GABAergic medullary neurons. Both adrenergic and noradrenergic neurons, as well as hypothalamic histaminergic neurons, are critically involved in the control of arousal and thereby implicated in the permissive mechanisms of PS generation. Adrenergic and noradrenergic neurons may inhibit PS either by direct inhibitory actions on PS-on neurons via α_2-adrenergic receptors or by activation of non-monoaminergic PS-permissive structures, such as the Y area, which excite monoaminergic and non-monoaminergic PS-off neurons and thus inhibit PS. Figure 5 summarizes the current state of knowledge about the central mechanisms of W, SWS and PS.

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