CARBACHOL MODELS OF REM SLEEP: RECENT DEVELOPMENTS AND NEW DIRECTIONS

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INTRODUCTION

The decade spanning from 1953, when Aserinsky and Kleitman (1) reported the first systematic observations of a distinct phase of sleep characterized by rapid eye movements (REM), through the early '60s, when Jouvet (35), Hernández-Peón et al. (28) and George et al. (21) demonstrated that cholinergic mechanisms localized within the pons and midbrain underlie the generation of REM sleep, was a defining period for sleep research in the second half of this century. It provided the basis for analyzing sleep as a rhythmic process generated and regulated by neurochemically and spatially distinct neuronal populations (29, 66).

The discovery of REM sleep (1, 13), and of the powerful ability of cholinergic agonists such as carbachol (a broad-spectrum muscarinic cholinergic agonist) to produce a similar to REM sleep behavioral state following their injection into the medial pontine tegmentum (e.g., 28; see ref. 2 for a review) led to hundreds of studies in which carbachol and other cholinomimetics were applied locally or systemically in order to understand the neural mechanisms of the generation of REM sleep and its influence on autonomic, motor and sensory functions. Many aspects of this research have been reviewed recently, in particular in the context of the pharmacology of cholinergic systems (2), suppression of postural tone (10, 70), narcolepsy and related disorders (60, 74, 85), respiratory regulation during REM sleep (47, 58), the generation of hippocampal theta rhythm (106), and the anatomy and physiology of the pontomedullary neuronal network involved (24).

Over the last ten years, distinct “carbachol models” of REM sleep and/or its individual neural phenomena have been developed. In particular, “reduced” models were added to the repertoire of carbachol studies, and the rat became the primary experimental animal. The reduced models include acute in vivo studies in decerebrate, unanesthetized cats and rats, intact animals under anesthesia, and in vitro investigations of individual cells in slices of the rat pons containing the reticular formation regions known to be involved in generation of REM sleep. Since carbachol studies using varius models are expected to yield complementary results, it is timely to compare and integrate the observations that emerged from studies in rats and distinct reduced carbachol models with the earlier results from carbachol studies in chronically instrumented, behaving cats. The review consists of three parts that deal with the question of differences between rats and cats in their response to pontine cholinergic stimulation (particularly with respect to the
time course of the evoked response), the anatomical and neurochemical characterization of pontine regions producing REM sleep, and the neuroanatomy of the pontomedullary network generating REM sleep.

WHAT IS DIFFERENT BETWEEN THE EFFECTS OF PONTINE CARBAChOL IN RATS AND CATS?

The first two studies in chronically instrumented, behaving rats in which carbachol was injected into the pontine reticular formation revealed a statistically significant enhancement of REM sleep (23, 86). These initial observations were then confirmed and extensively analyzed in chronic rat studies using improved microinjection techniques (7, 14, 77, 104). Thus, they demonstrated that, in principle, cholinergic pontine stimulation in rats has effects comparable with those described earlier in chronic cats (see refs. 2, 29 for reviews). However, important differences were also noted. The magnitude of the REM sleep enhancement observed in cats often exceeds 300%, whereas it is less than 100% in rats. Also in contrast to cats, where REM sleep is often induced almost immediately following the microinjection (e.g., within 20-600 s), in rats the latencies of the first REM sleep episode following carbachol are usually longer than 30 min. In addition, whereas the carbachol-induced REM sleep occurs as frequent episodes whose individual durations are short and similar to those of natural REM sleep in rats, carbachol injections in cats produce REM sleep episodes lasting much longer (especially the first few following the injection) than natural bouts of REM sleep.

Even though pontine carbachol injections in chronic rats on the average result in a statistically significant enhancement of REM sleep, all studies in chronic rats report cases with an increased amount of wakefulness. The increase occurs at the expense of both slow-wave and REM sleep, and is particularly pronounced with larger carbachol doses (7, 14, 77). On the other hand, dissociated states, such as motor atonia (cataplexy) in otherwise awake animals or hippocampal theta rhythm occurring without other signs of REM sleep, that sometimes occur following carbachol in chronic cats (see 102 for earlier refs.) have not been reported in chronic rats.

The effective sites in rats overlap with analogous sites in cats (4, 102, 112), but are more widely distributed within the pontine reticular formation (see the next section). In addition, in rats, the effects produced from the same site are difficult to reproduce in repeated trials (14), whereas the effects in cats are relatively consistent. These differences, especially those concerning the timing of REM sleep following carbachol and the repertoire of carbachol-induced behavioral states other than REM sleep, suggest that important species differences between the rat and cat may exist in the pontine cholinergic mechanisms controlling behavioral state.

The decerebrate carbachol models were developed based on the observations that episodes of postural atonia analogous to those during REM sleep can occur spontaneously in chronic cats decerebrated at the precollicular level (35, 107), and
that the duration and frequency of such episodes can be increased by systemic administration of cholinesterase inhibitors (64; see 59 for earlier refs.). Morales et al. found that local pontine microinjections of carbachol very effectively produce a REM sleep-like postural atonia in acutely decerebrate cats (69). These atonias are accompanied by a stereotyped, REM sleep-like suppression of respiratory motor output (18, 38), silencing of medullary serotonin-containing neurons (109), and are often accompanied by intense and rapid eye movements (100). The latter are more regular and more frequent than those of natural REM sleep, and their occurrence in only some preparations is not obviously related to the injection site. Episodes of postural atonia and respiratory depression in many regards similar to those in decerebrate cats can be also produced by pontine carbachol injections in decerebrate rats (see below) (96).

As in chronic cats, carbachol effects in acute, decerebrate cats often develop within seconds following the injection, but in contrast to brain-intact, chronic cats, which have multiple REM sleep episodes, with the first few typically being much longer (30-60 min) than those of natural REM sleep, in decerebrate cats carbachol produces only one prolonged episode of atonia. A spontaneous recovery from the atonia occurs gradually over 30-90 min. Additional spontaneous episodes of atonia usually do not occur, and subsequent injections at the same site are ineffective for several hours, although a second atonia can be produced within this time frame by an injection in the contralateral pons. Such a second carbachol injection is effective even in the presence of atropine in the reticular formation on the opposite side (38). The effective region for producing postural atonia with the shortest latencies in decerebrate cats is relatively discrete, ca. 1.5 mm in diameter (38, 97, 100), and its location is consistent with the region producing REM sleep in chronic cats (102, 112).

In the only systematic study in unanesthetized, decerebrate rats, carbachol had effects similar to those observed in decerebrate cats. The injections produced only one prolonged (11-27 min) episode of atonia that occurred within 0.5-3 min following the injection (96). Unlike in the decerebrate cat, however, the spontaneous recovery was abrupt, not gradual, and additional episodes of atonia could be produced by a microinjection at the same site 20-30 min after the recovery from the preceding one. This lack of pronounced refractoriness could be related to slightly smaller (relative to brain size) injection volumes in decerebrate rats (10-40 nl) than those used in decerebrate cats (100-250 nl; 10 mM carbachol was used in both). In spite of the relatively small injection volumes used in decerebrate rats, the distribution of the injection sites was rather widespread, and similar to that observed in chronic rats (7, 14).

The use of decerebrate carbachol models allows one to study cellular behaviors and systemic changes under highly standardized experimental conditions. Other major assets are the ability to use invasive recording and stimulation techniques, and to place very small microinjections using calibrated glass capillaries, rather than metal cannulae. However, the state induced by carbachol in these preparations is but a pharmacological analogue of selected phenomena of REM sleep and lacks important components of natural REM sleep: cortical desynchronization, hippocampal theta rhythm, phasic motor events, and variability of the cardiorespiratory
parameters (see 47 for a review). Decerebration removes major descending influences (e.g., from the hypothalamus) that may play an important role in generating REM sleep and modulating the expression of its signs. To overcome these limitations without sacrificing other assets of acute preparations, the effects of pontine carbachol have been studied in brain-intact, anesthetized cats and rats. Initially, only selected REM sleep-like changes were observed; hippocampal theta rhythm in urethane-anesthetized rats (105), and inhibition of motoneuronal activity in α-chloralose-anesthetized cats (56). In rats, the episodes of hippocampal theta rhythm occur together with cortical desynchronization (39). By simultaneously recording cortical and hippocampal signals and hypoglossal nerve activity or genioglossal muscle EMG (as an index of changes in motor activity), we found that highly stereotyped, short (mean: 2.5 min) episodes comprising cortical desynchronization, hippocampal theta rhythm and motor suppression can be repeatedly produced in urethane-anesthetized rats with small injections (5-20 nL; 9-36 ng of carbachol) (19). To obtain these episodes, the level of anesthesia has to be adjusted so that the cortical EEG shows a steady delta-like frequency, while a moderately painful stimulus (e.g., tail pinch) produces a transient EEG desynchronization and hippocampal theta rhythm (cf. 25). Even when these conditions are met, in some animals carbachol produces a complex REM sleep-like episode, whereas in others only a period of motor suppression not accompanied by the electrocortical changes is produced. This may be related to the location of the injection site (next section). In both cases, however, the episodes are associated with a reduction in noradrenergic locus coeruleus (LC) cell activity (ref. 17 and Fenik, Ogawa, Davies and Kubin, unpublished observations). Thus, complex neuronal networks involved in the production of many hallmarks of natural REM sleep can be activated by pontine carbachol under general anesthesia.

The comparison of the observations made using different carbachol models shows that some of the species differences similar, or analogous, to those found in chronic/behaving rats and cats are also manifest in the decerebrate and/or anesthetized animals (e.g., those regarding the duration of REM sleep-like episodes or the distribution of effective sites in the pons (2, 14). A closer examination of the comparisons across different carbachol models suggests that most of these differences are quantitative rather than qualitative, and that they correspond to the patterns of natural REM sleep of the two species. The REM sleep latencies following carbachol injections in chronic rats are long (>30 min), but REM sleep-like responses occur within seconds in decerebrate or anesthetized rats. Therefore, it cannot be said that rats are unable to respond to pontine carbachol with REM sleep-like events with very short latencies. Rather, it is the excitability of brainstem neurons at the time of the injection that determines whether the animal will, or will not, respond almost immediately to carbachol with REM sleep-like pattern.

A similar argument can be made about the control of REM sleep durations. The carbachol-induced REM sleep-like episodes are short in both chronic/behaving and brain-intact/anesthetized rats, and their durations are similar to those of natural REM sleep bouts in rats (90 s), whereas in chronic/behaving cats the periods of carbachol-induced REM sleep are often much longer than the periods of natural
REM sleep. In decerebrate rats and cats, however, carbachol produces very long episodes of postural atonia. Thus, pontine carbachol can maintain prolonged episodes of atonia in both species even though it is unable to do so in chronic/behaving and brain-intact/anesthetized rats. These differences suggest that endogenous REM sleep-terminating influences can more easily oppose the effects exerted by carbachol in rats than in cats, and that such influences originate in suprapontine brain regions. In the absence of such descending, REM sleep-terminating effects (decerebrate preparations), the duration of carbachol-induced periods of atonia in rats and cats is close to the duration of their respective natural sleep cycles. Based on the latter observation, it was suggested that the species-specific period of the sleep cycle determines the duration of the response to carbachol in pontomedullary preparations (96). This period may be determined by functioning of the ultradian cycle referred to by Kleitman as the basic rest-activity cycle (BRAC) (40, 41). Indeed, the ultradian (or BRAC) cycle is functional in decerebrate cats, and predictably responds to alterations in brain metabolism and temperature (37), but less is known about this periodicity in rats. To date, only the circadian and homeostatic aspects of sleep received due attention and became firmly incorporated into current concepts of sleep regulation (6). The mechanisms and role of BRAC as a biological rhythm superimposed on the circadian cycle has been less investigated and remains poorly understood. Thus, an increased interest in the BRAC concept should ensure the continuation of Nathaniel Kleitman’s legacy into the 21st century.

While the differences between rats and cats may not be as sharp as they appear based on studies in chronic animals alone (14), what does appear to be more strongly expressed in rats than in cats, is the prominent wakefulness-enhancing effect of carbachol. This is consistent with the extensive evidence that the majority of pontine cholinergic neurons increase their activity during both active wakefulness and REM sleep (see 29, 65, 80 for refs). Since some of the neuronal events produced by pontine carbachol are characteristic of both behavioral states, the same pontine reticular formation regions and cells may contribute to both in cats and rats, but the wakefulness-promoting role of pontine cholinergic stimulation would be more pronounced in the latter. A stronger expression of the arousing effect of pontine carbachol in rats than in cats may be related to the differences in the environments in which the two species evolved, their different lifestyles (rodents vs. preying cats), and a stronger magnitude of circadian regulation of sleep in rats.

IDENTIFICATION OF THE EFFECTIVE PONTINE SITES AND THEIR PHARMACOLOGY

Two major goals of pontine microinjection experiments have been to delineate the sites of highest sensitivity and effectiveness in inducing REM sleep or its selected phenomena in response to cholinergic stimulation, and to determine which neurochemicals other than acetylcholine contribute to the triggering, maintenance
and/or modulation of REM sleep by their actions within the pontine tegmentum. Towards the first goal, carbachol and other cholinomimetics have been microinjected into the pons with increased precision and sophistication (e.g., 4, 7, 14, 20, 102, 105, 112). Different methods of carbachol delivery (e.g., microinjections or microinfusions using either carbachol solutions or carbachol attached to poorly mobile microspheres), volumes and concentration of carbachol (20-500 nl and 0.1 μM-100 mM, respectively), animal models (rats or cats; intact/behaving or decerebrate or anesthetized), and outcome measures (e.g., presence of a complex set of REM sleep-like phenomena – cortical and hippocampal rhythm changes + motor atonia + eye movements + ponto-geniculo-occipital waves – or only selected REM sleep-like events such as postural atonia or hippocampal theta rhythm) have been used. Consequently, there is little consensus as to the exact location of the most effective injection site(s). Selected REM sleep phenomena occurring in isolation can be produced from wider regions of the pontomesencephalic reticular formation than the fully developed REM sleep-like state (cf. refs. 102, 112 in the cat, and 7, 105 in the rat).

The sites producing a REM sleep-like state following carbachol injections into the medial pontine reticular formation (mPRF) of cats were localized to two distinct dorsoventral locations. The most effective site has been identified as being just ventral to LC, corresponding to the LCα and peri-LCα region (102), and perhaps extending ventrally towards the level of the trigeminal motor nucleus (4, 112). However, another group found the most effective region to be in a ventral part of the nucleus pontis oralis (20). In most of these studies, the onset latency of the REM sleep-like state was used as the principal measure of effectiveness. Unfortunately, this measure is not suitable for studies in chronic rats because the latencies of the first REM sleep episode following carbachol are usually longer than 30 min. Consequently, the REM sleep amount, rather than latency, is used in chronic rats as the principal measure of effectiveness. Diffusion during such a long latent period limits greatly the spatial resolution with which the most effective sites can be identified.

In chronic rats, REM sleep-enhancing sites were localized within the pontis oralis and caudalis nuclei, -7.8 to -10.3 from the bregma (7, 14), with the dorsoventral extent of this region covering both the location analogous to that described in chronic and decerebrate cats (4, 38, 79, 97, 102, 112) and the most ventral mPRF region of the cat (20). Our recent data from carbachol injections in urethane-anesthetized rats suggest that this species may also have distinct carbachol-sensitive sites within the mPRF that are relevant for the generation of REM sleep. In those studies, injections much smaller than those used in chronic rats (5-20 nl, rather than 50-100 nl), could repeatedly produce episodes of cortical desynchronization, hippocampal theta rhythm and suppression of hypoglossal nerve activity (or genioglossal muscle EMG). These effects occurred following injections into either the dorsal nucleus pontis oralis (19), or into the ventral mPRF region adjacent to the reticulo-tegmental nucleus (32) (Fig. 1). The injections placed in the dorsal mPRF of urethane-anesthetized, paralyzed and artificially ventilated rats produce, within seconds, stereotyped changes comprising acceleration of cortical EEG
Fig. 1. - Distribution of pontine carbachol injection sites from which REM sleep-like, or arousal-like, changes in cortical and hippocampal EEG, hypoglossal nerve and noradrenergic locus coeruleus cell activity are produced in urethane-anesthetized rats.

The injections (10 nl, 10 mM carbachol), each in a separate animal, were placed at A-P levels from -7.5 to -9.0 relative to bregma. From Ref. 19.

frequency, appearance of hippocampal theta rhythm, suppression of hypoglossal nerve activity, decreased respiratory rate, and silencing of LC cells (17). Figure 2 shows one example of a REM sleep-like episode produced by microinjection of carbachol into the dorsal portion of the mPRF.

Following ventral injections in paralyzed and artificially ventilated rats (Fig. 1), cortical desynchronization and hippocampal theta rhythm occur together with an increase of hypoglossal nerve activity and a moderate acceleration of respiratory rate (19). Importantly, however, these REM sleep-like electrocortical changes are accompanied by increases in noradrenergic LC cell activity (19), which is opposite to the change observed during natural REM sleep. Thus, the effects produced from the ventral sites may be related to the arousal-, rather than REM sleep-, promoting aspects of cholinergic stimulation within the mPRF (discussed in the previous section). It is also important to note, that ventral carbachol injections in spontaneously breathing, non-paralyzed, urethane-anesthetized rats (in which genioglossal EMG is used to monitor motor output), REM sleep-like cortical and hippocampal EEG changes are accompanied by decrements of hypoglossal nerve activity and increased respiratory rate (32). This apparent motor suppression is consistent with REM sleep, but its presence only in spontaneously breathing animals suggests that it is secondary to reflex hyperventilation resulting from the increased respiratory rate. Thus, the observations in anesthetized and non-paralyzed rats could lead to
the conclusion that both dorsal and ventral carbachol injections produce REM sleep-like changes, but studies in paralyzed rats in whom respiratory reflexes do not confound the response pattern show that, depending on the injection site, carbachol can produce two distinct responses; one that is similar to REM sleep and another that is more consistent with arousal. The sites producing REM sleep-like effects and arousal-like effects in urethane-anesthetized rats are clearly distinct (Fig. 1), whereas injections into the mPRF regions adjacent to, and interposed between, these two locations produce a moderate suppression of hypoglossal nerve and LC cell activity, but these are rarely accompanied by changes of cortical and hippocampal signals (not illustrated, but see refs. (32, 110). This intermediate area corresponds to the center of the region identified to be most effective in producing REM sleep in chronic rats (7, 14). Thus, it is probable that the REM sleep-like effects obtained in chronic rats reflect the net result of combined stimulation of the two regions identified in anesthetized rats, yielding responses having some properties similar, and other opposite, to natural REM sleep.

![Diagram](image)

**Fig. 2.** A typical REM sleep-like episode produced in urethane-anesthetized rat by pontine carbachol injection (10 nl, 10 mM) placed in the dorsal nucleus pontis oralis. From Refs. 17 and 19.

Both the use of small injection volumes and the presence of anesthesia might have helped enhance the contrast between the effects of pontine carbachol produced from the dorsal and ventral mPRF. The extent to which these regions are relevant to REM sleep alone, wakefulness (arousal) alone, or both requires further studies. At present, the studies in anesthetized rats with carefully monitored motor, electrocortical and noradrenergic cellular changes suggest that the sites producing a REM sleep-like state are localized and surrounded by sites from which isolated REM sleep-like phenomena can be evoked. A caution is, however, needed when interpreting those isolated phenomena as representing an incomplete activation of a REM sleep-like state because some of them also occur in entirely different
behavioral states, such as arousal (a state comprising theta rhythm, cortical desynchronization, respiratory activation and increase of LC cell activity), or the motor suppression characteristic of startle and other highly attentive states. An alternative possibility is that those widely distributed cholinceptive regions of the pontine reticular formation participate to a varying degree and with variable patterns of activation in all these apparently disparate behavioral states.

The contrast between the relatively small regions from which distinct REM sleep-like effects are produced in anesthetized rats and the relatively large regions found to be effective in earlier studies in chronic or anesthetized rats is likely to be due to the use of larger volumes and/or concentrations of carbachol in those earlier studies. Nicholson's assumptions (72, 73) can be used to assess the spatial resolution that can be achieved by microinjections into the mPRF. Following a fast microinjection into the brain, the injected fluid will initially produce a "cavity" having a volume equal to the injected volume. If the injection is sufficiently slow, the injected fluid will initially displace the extracellular fluid whose volume is about 25-50% of the tissue volume. The black dot and the surrounding dark-shaded circle in Fig. 3 show the size of the "cavity" and the area from which the extracellular fluid is initially displaced following a slow injection of 10 nl of a drug solution, respectively (assuming that the extracellular volume fraction is 25%). These areas are shown centered within mPRF region of the rat brainstem at level P8.3, where carbachol was repeatedly shown to be effective. The third, still larger and lightly shaded region in Fig. 3 shows the distribution of the same initial volume following a period of diffusion during which the injected solution becomes diluted 100 times (diffusion gradient neglected, i.e., the initial volume is evenly diluted in a 99 times larger volume of extracellular fluid that occupies 25% of the tissue volume). In spite of the small volume injected, the diameter of the area of diffusion is 1.97 mm, and covers almost the entire nucleus pontis oralis. This estimate is consistent with the dimensions of the area covered by horseradish peroxidase following a 10 nl microinjection into the dorsal pontine tegmentum in the cat (78). If the injected volume contained carbachol at concentration of 10 mM, a 10⁴-fold (rather than 10⁵) dilution would still result in a concentration of carbachol in the tissue that is more than 100 times the in vitro affinity of carbachol to muscarinic M₂ receptors (the ones most consistently implicated in eliciting REM sleep-like phenomena (3, 33, 104)). The area of the mPRF covered by 10 nl of 10 mM carbachol after it has been diluted 10⁴ times in the available extracellular space would have a diameter of 9.15 mm, i.e., would be larger than the width of a coronal cross-section of rat brain at P8.3!

While the latter estimate may be exaggerated because it neglects the long time required for such a widespread diffusion and the concomitant removal of the drug, it is clear that carbachol microinjection studies may be easily confounded by carbachol exerting its effect simultaneously (albeit with different concentrations) at several functionally distinct regions of the pontine reticular formation. Since, for technical reasons, microinjection volumes cannot be reduced below 1-5 nl in acute experiments, and below 10-20 nl in chronic studies, without sacrificing reliability and accuracy, these calculations show that the spatial resolution of microinjections
Fig. 3. - Estimation of the extent of diffusion following a microinjection into the dorsomedial pontine tegmentum of a rat.

The assumptions used are that the original injected volume is 10 nl (black circle), and that the available extracellular space is 25% (dark shaded area) (72, 73). The lightly shaded area represents the sphere occupied by the originally injected volume of fluid when it becomes uniformly diluted 100-fold in the extracellular fluid. In most carbachol injection studies, volumes and concentrations were 50 - 500 nl, and 1-100 mM, respectively. Thus, they were usually much larger than in the example.

is limited. Thus, the question as to whether the mPRF contains several spatially and functionally distinct cholinceptive regions relevant for REM sleep or, rather, a gradient of cells whose relative involvement in the generation of REM sleep, wakefulness, and/or their selected phenomena varies with behavioral state and experimental conditions may prove difficult to resolved in chronic animals using microinjections alone.

Another major goal of pontine microinjection experiments has been to identify cholinergic receptor subtypes and neurotransmitters other than acetylcholine, peptides and their receptors that may trigger, suppress, or otherwise modulate the expression of REM sleep by their actions within the mPRF (see 11, 65 for reviews). Figure 4 schematically shows those mediators and receptors effective in enhancing (right side), or suppressing (left side), REM sleep. Of the various neurotransmitters tested, some are co-localized with acetylcholine in pontine cholinergic neurons (e.g., NO - nitric oxide, ANP - atrial natriuretic peptide, NGF - nerve growth factor) (53, 68, 77, 114), others are released by neurons whose activity ceases during REM sleep (NE - norepinephrine, 5-HT - serotonin, H - histamine) (5, 12, 63, 101), some may mediate hypothalamic influences (H, CRF - corticotropin-releasing factor, VIP - vasoactive intestinal peptide) (8, 16, 51, 54), and still others
are likely to be released by local pontomesencephalic interneurons (GABA and glycine) (61, 92, 111). So far, studies addressing the nature of cellular interactions occurring within the mPRF and involving these mediators were performed only in vitro; i.e., under conditions when the role of the studied neurons in the generation of REM sleep an/or other behavioral states could not be ascertained (e.g., 22, 42, 75, 91-94). The studies primarily focused on the postsynaptic effects of agonists, as antagonist studies and those aiming to identify potentially important presynaptic effects on transmitter release require that the studied neurons be under a tonic influence of endogenously released transmitters. This requirement can be fulfilled only to a limited extent in in vitro conditions. The functional specificity of in vitro studies can be improved, however, by combining visualization of markers characteristic of different types of mPRF neurons using either immunohistochemistry or retrograde tracers with electrophysiological and pharmacological studies. This has been successfully applied to other groups of pontine neurons (e.g., 52, 57), but not to mPRF neurons.

The information from microinjection studies about the modulatory effects on REM sleep exerted by different neurotransmitters, peptides and their receptors can be used to designing in vivo experiments in which drug application by iontophoresis, rather than microinjections, is combined with identification of neuronal behaviors during either natural REM sleep (in chronic animals) or REM sleep-like episodes produced by carbachol under anesthesia. The acute studies allow one to determine, with relative ease, axonal projections, responses to selected sensory stimuli, and even the neurotransmitters of extracellularly studied neurons. Like with the in vitro studies, these approaches have been previously applied to other groups of pontomedullary neurons (e.g., 44-46), but not to the cells of the mPRF. There is a renewed interest in the use of anesthetized animals to study neurons involved in the control of sleep (e.g., 19, 62, 95), which is fully justified if we consider that many neuronal and network properties determined to be relevant for the generation and regulation of sleep in behaving animals were discovered and initially investigated in reduced animal models (see 88).

**Pontomedullary Neuronal Network Generating REM Sleep**

Particular emphasis has been placed on the role of mesopontine cholinergic neurons of the laterodorsal and pedunculopontine tegmental nuclei (LDT and PPT) as a critical source of the signal that triggers and/or maintains natural REM sleep (see 29, 65 for reviews). The ability to produce virtually all the electrophysiological signs of REM sleep by carbachol injections placed in the mPRF is consistent with anatomical data showing strong cholinergic afferent projections to this site (67, 79), and suggests that efferent projections of the mPRF neurons target many, if not all, sites and nuclei of the brain involved in generation of REM sleep (Fig. 4). Whether the mesopontine cholinergic neurons are important for the generation of a REM sleep-like state by pontine carbachol injections is not certain, for in many
Fig. 4. - A schematic summary of the list of neurotransmitters and peptides involved in generating and/or modulating REM sleep within the medial pontine reticular formation (mPRF), as demonstrated by microinjections.

Peptides and neurotransmitters having REM sleep-enhancing effects are shown to the left from the cholinergic input (ACh) originating principally from cells of the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei, and neurotransmitters having REM sleep-suppressing effects are shown on the right. See text for the explanation of abbreviations. NO is shown as having both positive and negative effects, as local inhibition of its production reduced the amount of REM sleep in the cat (53), but increased REM episode duration in the rat (77). The diagram also shows some of the potentially relevant receptor subtypes. All signs of REM sleep can be initiated by cholinergic stimulation within the mPRF, but the cellular interactions occurring at this site and involving the listed transmitters and peptides remain largely unknown.

studies using large volumes of carbachol, the activity of the dorsal mesopontine cholinergic neurons might have been suppressed while REM sleep was produced (15, 52, 57, 82; see also ref. 80). Thus, the neuronal networks downstream from, or not involving, LDT/PPT neurons may be capable of producing a REM sleep-like state in response to exogenous cholinergics. This highlights again that more information about the cellular properties and network connectivities of mPRF neurons is needed to further our understanding of the mechanisms generating REM sleep and its individual phenomena.

Studies of the afferent and efferent connections of the mPRF point out numerous
anatomical sites with which the mPRF may interact in generating and maintaining REM sleep. In particular, there is a very strong projection to the medial medullary reticular formation (mMRF), including the medial magnocellular, ventral gigantocellular and midline medullary raphe nuclei in cats (49, 84) and rats (26, 27, 34). In our recent attempts to evaluate the extent and magnitude of this projection in rats, we iontophoretically delivered an anterograde tracer, Phaseolus vulgaris agglutinin, into pontine sites identified as producing REM sleep-like phenomena in anesthetized rats (48, 76). Anterogradely labeled terminal boutons were found in many regions of the pontomedullary reticular formation, with the most dense projections targeting the ventromedial medullary reticular formation (Fig. 5). The highest labeled terminal density at this location was 500-1000 terminals per mm², when determined using a 0.18 mm² grid superimposed over the analyzed sections. There were, however, also less dense projections to other pontomedullary locations, including the sites that may be important for the generation of eye movements, such as vestibular nuclei and the nucleus prepositus hypoglossi (Fig. 5).

Electrical and chemical stimulation of the mMRF causes bilateral suppression of postural muscle tone (50), and lesions of this region abolish or attenuate the atonia of REM sleep (30). Consequently, the mMRF is seen as a relay site where activation first produced within the mPRF is transmitted to premotor neurons that function to inhibit motoneurons. Indeed, neurons having increases in activity closely correlated with REM sleep (and, in many cells, also during active wakefulness) are present in the mMRF (71, 83, 90, 99), and the same area has a large number of neurons expressing c-fos protein following the natural or cholinergically induced REM sleep (113). In a more direct support of the motor inhibitory role of the mMRF, inhibitory postsynaptic potentials correlated with the activity of individual mMRF neurons with REM sleep-specific increases in activity were recorded in motoneurons (9, 98).

There is, however, also evidence that mMRF is not just a relay site for motor atonia, but an important site that interacts with the mPRF in the generation and maintenance of REM sleep. Lesions of the mMRF produce a long-lasting suppression of REM sleep in addition to their attenuating effects on the atonia (30), and disconnecting the mMRF from the mPRF impairs the ability of carbachol to elicit REM sleep from the mPRF (103). In addition, muscular atonia cannot be produced by stimulation within the mMRF when the mPRF is inactivated or disconnected from the medulla (43, 87), and the generation of natural REM sleep is also impaired (108). Thus, the pathways descending from the mPRF and terminating in the mMRF must have functions other than just to activate the descending motor inhibitory system (cf. 36). Consistent with this, potentially important for REM sleep ascending pathways originate in the mMRF and terminate in the mPRF region (26, 55, 84, 89). The neurochemistry of the neurons in the mMRF that are targets of the axons descending from the mPRF remains largely unknown, but some of the ascending mMRF neurons are cholinergic (31, 81). Such neurons may provide an important cholinergic feedback to the mPRF neurons that supplements cholinergic inputs to mPRF originating from the LDT and PPT nuclei. Recent
Fig. 5. - Medial medullary reticular formation is a major target of axonal projections descending from the REM sleep-triggering region of the pons.

A: center of a iontophoresic microinjection of an anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L), into the medial pontine tegmentum in the rat. PHA-L was injected for 15 min using 5 µA current at 70% duty cycle. Following a 13 day survival, the tracer and serotonin-containing neurons were visualized in 35 µm coronal section of the brainstem. B: a high density of axon terminals in the ventromedial medullary reticular formation, with additional terminals less densely distributed throughout the entire medullary reticular formation resulting from the PHA-L injection shown in A. All labeled terminal boutons present in this medullary section ipsilaterally to the pontine PHA-L deposit were redrawn using camera lucida and superimposed on the outline of the section. Cell bodies of serotonin-containing neurons distributed along the midline and the dorsal edge of the inferior olive (IO) are also shown. Abbreviations: Amb - n. ambiguus; LPG - n. lateralis paragigantocellularis; PH - n. prepositus hypoglossi; RVL - rostral ventrolateral medullary nucleus. From Refs. 48 and 76.
improvements in the localization of the carbachol-sensitive mPRF region, improved antibodies for neuroanatomical studies, and availability of acute carbachol models of the REM sleep-like state should help dissect the neurochemical and electrophysiological features of the pontomedullary network underlying the generation of REM sleep.

CONCLUSIONS

Distinct carbachol models of REM sleep provide us with complementary information about the cellular processes responsible for this behavioral state. However, the information about the cellular processes involved cannot resolve the question of the function(s) of REM sleep; for this complementary behavioral and comparative studies are essential, as well as new conceptual frameworks. It is currently assumed that distinct states of sleep and wakefulness are controlled by state-specific, and spatially and neurochemically distinct, neuronal populations, and that REM sleep is a distinct state of sleep; i.e., a period characterized by lack of conscious perception of the external environment and relative unresponsiveness to external stimuli. Alternative assumptions, e.g., that discrete levels and patterns of activities generated simultaneously in distinct neuronal populations having an active role in more than one behavioral state define the various behavioral states of sleep and wakefulness, and that REM sleep derives from a particular state of wakefulness (or arousal), rather than sleep, may prove productive.

SUMMARY

Since the early ’60s, injections of a broad-spectrum muscarinic cholinergic agonist, carbachol, into the medial pontine reticular formation (mPRF) of cats have been extensively used as a tool with which to study the neural mechanisms of rapid eye movement (REM) sleep. During the last decade, new carbachol models of REM sleep were introduced, including chronically instrumented/behaving rats and “reduced” preparations such as decerebrate or anesthetized cats and rats. The combined results from these distinct models show interspecies similarities and differences. The dual nature, both REM sleep-promoting and wakefulness (or arousal)-promoting, of the cholinergic effects exerted within the mPRF is more strongly expressed in rats than in cats. This strengthens the possibility suggested by earlier central neuronal recordings that active wakefulness and REM sleep have extensive common neuronal substrates, and may have evolved from a common behavioral state.

Carbachol studies using different intact and reduced models also suggest that powerful REM sleep episode-terminating effects originate in suprapontine structures. In contrast, the timing of REM sleep-like episodes in decerebrate models is determined by a pontomedullary neuronal network responsible for the generation of an ultradian cycle similar to the basic rest-activity cycle of N. Kleitman. Other
presumed species differences, such as the more widespread distribution of carbachol-sensitive sites or the relative failure of carbachol to increase the duration of REM sleep episodes in rats when compared to cats, may be of a quantitative or technical nature.

While carbachol and many other neurotransmitters and peptides microinjected into the mPRF evoke, enhance or suppress REM sleep, the most sensitive site(s) of their actions have not been fully mapped, and the nature of the cellular and neurochemical interactions taking place at the sites where carbachol triggers the REM sleep-like state remain largely unknown. Similarly, little is known about the pathways between the mPRF and medial medullary reticular formation, but the existing evidence suggests that they are reciprocal and essential for the generation of both natural and carbachol-induced REM sleep. Studies of the mesopontine cholinergic neurons, which are hypothesized to be the main source of endogenous acetylcholine for the mPRF, need to be extended to neurons of the mPRF and cells located functionally downstream from this important site for REM sleep, or both REM sleep and active wakefulness.

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