Neuropharmacology and Neurochemistry of Canine Narcolepsy

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Summary: It is believed that narcolepsy involves abnormalities of rapid eye movement (REM) sleep, especially of REM sleep atonia. Compelling evidence suggests that the regulation of REM sleep and REM sleep atonia involves a reciprocal interaction of cholinergic and monoaminergic systems. Using our canine model of narcolepsy and a pharmacological approach, we have previously demonstrated a similar interaction in the regulation of cataplexy. Global activation of cholinergic or monoaminergic transmission aggravates or suppresses canine cataplexy, respectively. We have also identified the subtypes of monoaminergic and cholinergic receptors specifically involved in this interaction. Cataplexy is aggravated by activation of the cholinergic system via M2 stimulation, as well as deactivation of the catecholaminergic systems by either blockade of postsynaptic α-1b receptors or stimulation of α-2 or D2 inhibitory autoreceptors. These pharmacological results correspond to previously identified neurochemical abnormalities in canine narcolepsy, such as significant increases in M2 receptors in the pons, α-1 receptors in the amygdala, α-2 receptors in the locus coeruleus and D2 receptors in the amygdala and nucleus accumbens, when compared to control animals. Using local perfusion of active compounds, we have further demonstrated that cholinceptive sites in the pontine reticular formation, as well as in the basal forebrain, are involved in the regulation of cataplexy. Although the specific sites of action of the monoaminergic compounds remain unknown, the results of our pharmacological and neurochemical studies to date suggest that a widespread hyperactivity of cholinergic systems within the central nervous system together with a hypoactivity of catecholaminergic systems underlie the pathophysiology of narcolepsy. Key Words: Canine narcolepsy—Cataplexy—REM sleep—Cholinergic systems—Monoaminergic systems—Receptor subtypes.

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, sleep onset rapid eye movement (REM) periods and dissociated REM sleep processes such as cataplexy, sleep paralysis and hypnogogic hallucinations (1,2). Canine narcolepsy is a naturally occurring animal model of this condition, which presents behavioral, pharmacological and electrophysiological similarities to the human disorder (3–5). Although several histopathological studies have been performed in idiopathic human (6,7) and canine narcolepsy (3,8,9), no clear morphological abnormalities have been consistently reported. This suggests that the pathophysiology of narcolepsy involves a minute imbalance in the neurochemical mechanisms regulating sleep rather than a localized lesion or a large developmental abnormality. Thus, neurochemical and neuropharmacological experiments in narcolepsy, such as those using the canine model, are especially useful in elucidating the pathophysiology of narcolepsy. These studies will also increase our understanding of how REM sleep is generated and will lead to the development of better treatments for human narcolepsy.

NEUROPHARMACOLOGICAL STUDIES IN CANINE NARCOLEPSY

Knecht et al. in 1973 (8) and Mitler et al. in 1974 (3) independently reported the existence of idiopathic cases of canine narcolepsy similar to the human disorder. Subsequently, the latter group discovered several familial cases of narcolepsy in Doberman pinschers and Labrador retrievers, and a colony of genetically narcoleptic (autosomal recessive with full penetrance) Dobermans and Labradors was established at the Stanford Sleep Research Center (10).
Canine narcolepsy is characterized mainly by the existence of cataplexy (abrupt flaccid paralysis of the postural muscles induced by emotions), an easily observed symptom. Sleepiness, a more subjective symptom, has also been documented in these animals using a canine version of the multiple sleep latency test (MSLT) (11), and polysomnographic recordings of narcoleptic and control canines (12,13). Polysomnographic recordings of narcoleptic Dobermans are now routinely carried out at the Stanford Sleep Laboratory to assess the effects of pharmacological compounds on alertness (14).

Canine cataplexy, like human narcolepsy, is elicited by emotional stimulation, such as feeding or playing. Using this characteristic, the Stanford Sleep Laboratory has developed the food elicited cataplexy test (FECT), a biological assay for quantifying cataplexy in narcoleptic canines. This test has been successfully used to assess the effects of pharmacological compounds on cataplexy for many years (15-28). In the FECT, 12 pieces of food (1 cm³) are placed 30 cm apart in a circle on the floor. Dogs, which have been previously trained to eat all the pieces of food in serial order, are introduced into the testing room. The experimenter records both the number and duration of cataplectic attacks, as well as the time required for the dog to complete the test. This simple behavioral bioassay method can be used to assess activity, potency and duration of action for a large number of compounds using dose-response and time-course experiments. Furthermore,
the effects of the compounds on the general behavior of the animal (locomotor activity, agitation, appetite or salivation) as well as changes in cardiovascular parameters (heart rate and blood pressure) or rectal temperature can be monitored simultaneously with the behavioral assay (23,24). Thus, the influence of any possible nonspecific effects can be considered in the interpretation of the effects of the compounds on cataplexy (19,23,24). The results of typical time-course and dose–response experiments are shown in Fig. 1. When FECTs are repeated several times after a vehicle injection in six narcoleptic canines, the mean number of attacks is very stable, with some compounds significantly suppressing, and others aggravating it. This assay method can also be used to quantify the in vivo potency of compounds, which significantly suppress or aggravate cataplexy by dose–response experiments. The ED50 (the dose producing 50% of the maximal effect) is then calculated from the dose–response curve and can be correlated to the in vitro potency (inhibitory constant; K, for receptor ligands or IC50 for uptake inhibitors) of the compounds to confirm that the proposed pharmacological property is indeed involved in the effect on cataplexy (25–28).

RECEPTOR MECHANISMS INVOLVED IN THE REGULATION OF CATAPLEXY

There is compelling evidence that cholinergic mechanisms are important in controlling REM sleep and REM sleep atonia (29–33). Pharmacological stimulation of the cholinergic systems using acetylcholine esterase inhibitors or muscarinic agonists enhances REM sleep (34), and a local injection of cholinergic agonists into the pontine reticular formation (PRF) can trigger REM sleep and/or episodes of muscle atonia that resemble cataplectic attacks (35–39).

It is widely believed that narcolepsy involves abnormalities of REM sleep, especially pathological manifestation of REM sleep atonia. Thus, acetylcholine should be one of the major neurotransmitters involved in the regulation of cataplexy. In agreement with this hypothesis, global activation of the cholinergic system using cholinergic agonists or acetylcholinesterase inhibitors can trigger REM sleep and/or episodes of muscle atonia that resemble cataplectic attacks (35–39).

Monoamines are the second set of neurotransmitters implicated in the regulation of REM sleep and thus, in the pathophysiology of narcolepsy. The firing rate of monoaminergic neurons in the brainstem (adrenergic and serotonergic neurons of the locus coeruleus and dorsal raphe, respectively) dramatically decreases during REM sleep (43,44), whereas the activity of cholinergic neurons increases during REM sleep (45). The involvement of the monoaminergic systems in narcolepsy can also be inferred from the fact that all therapeutic agents currently used to treat narcoleptic patients (monoaminergic stimulants, antidepressants and monoamine oxidase inhibitors) act via these neurotransmitters.

However, these compounds are not selective enough to activate a single monoaminergic system; rather, they globally enhance transmission of all monoaminergic systems. Using newer antidepressants (uptake inhibitors) and stimulants (releasing agents) with selective effects on single classes of monoamines, we have demonstrated that presynaptic activation of the adrenergic system, but not of the dopaminergic or serotonergic system, is the main regulator of canine cataplexy (21,27). These results suggest that cataplexy and possibly also REM sleep atonia are more selectively modulated by the adrenergic systems, whereas other features of REM sleep (for instance the control of phasic events such as pontogeniculate-occipital [PGO] spikes) may be more selectively controlled by the serotonergic system (46,47). Presynaptic activation of dopaminergic receptors with dopaminergic uptake inhibitors or releasing agents had a potent alerting effect (experiments in progress) but had absolutely no effect on cataplexy (21). This somewhat surprising result is, however, in agreement with the clinical impression that stimulants have little effect on cataplexy when administered at low doses. This result is also consistent with the fact that the activity of dopaminergic neurons of the ventral tegmental area and substantia nigra does not seem to change significantly during the sleep cycle (48,49) and with results showing that dopaminergic stimulants have much greater effects on alertness than on REM sleep (50).

Recently, many receptor subtypes selective for individual neurotransmitters (acetylcholine [Ach], dopamine [DA], norepinephrine [NE], serotonin [5-HT], etc.) encoded by distinct genes have been identified. These receptor subtypes may be involved in specific neurological functions. In order to determine the receptor subtypes that are most likely to be involved in the pathophysiology of narcolepsy, we have used the canine model to carry out pharmacological studies of...
subtype-selective receptor ligands. More than 150 compounds with various pharmacological profiles (cholinergics, adrenergics, dopaminergics, serotoninics, prostaglandins, opioids, benzodiazepines, γ-amino­butyric acid [GABA]-ergics and adenosinergics) have been studied in this model, and a summary of the results is shown in Table 1. Although many groups of compounds significantly reduced cataplexy, we believe that these anticatatonic effects are often nonspecific, since in many cases side effects that could interfere with the measurement of cataplexy were observed especially at high doses. On the other hand, aggravation of cataplexy is usually highly specific, and very few groups of compounds were found to aggravate cataplexy significantly (Table 1).

Among receptors for the monoaminergic systems, the postsynaptic adrenergic α-1b receptors (20,26) and presynaptic α-2 receptors (22) were specifically involved in the regulation of cataplexy, a result consistent with our adrenergic model of control of cataplexy. One of the compounds that most potently stimulates cataplexy is the α-2 agonist BHT-920 (22). This compound is also known to modulate dopaminergic activity via D2 autoreceptor mechanisms (51). This result led us to reexamine the role of this receptor subtype (25), and we were surprised to find that stimulation of this receptor subtype (with very low dose of agonists) can aggravate cataplexy. This result was consistent with the report that small doses of D2 agonists increase REM sleep (52,53), but is difficult to reconcile with the fact that dopaminergic uptake blockers and releasing agents have absolutely no effect on cataplexy (21). A possible explanation might be that dopaminergic uptake inhibitors do not act at the level of the dopaminergic terminals involved in the regulation of cataplexy, or alternatively, that “presynaptic (D2-type) heteroreceptors”, possibly located on the terminals noradrenergic neurons (54) and involved in the synthesis and release of NE, are involved in the regulation of cataplexy.

Several pharmacological experiments also suggest that narcoleptic dogs are more sensitive than control dogs to the various receptor ligands, which significantly modify cataplexy. We have for instance recently demonstrated that yohimbine, an α-2 antagonist, reduces cataplexy and increases locomotor activity in narco­lep tic canines. The dose of yohimbine, which significantly increases locomotor activity in narcoleptic animals, however, had no effect on the locomotor activity of control animals (22).

These pharmacological findings correspond well to the neurochemical abnormalities reported in canine narcolepsy, such as significant increases in α-2 receptors in the locus coeruleus (55), D2 receptors in the amygdala and nucleus accumbens (56), α-1 receptors in the amygdala (18) and M2 receptors in the pons (57,58). Furthermore, studies on the measurement of monoamines and their metabolites in specific brain regions of narcoleptic canines have demonstrated region-specific alterations in DA and NE metabolism (59,60). Conversely, no alteration of other neurotransmitter mechanisms such as benzodiazepine receptors (61) or 5-HT metabolism (59,60) in the narcoleptic brain has been identified, and these results are consistent with the negative results of the effects on cataplexy of the compounds acting on these neurotransmitter mechanisms (21,28).

These results could be explained by primary abnormalities in the activity of key enzymes or reuptake systems of monoaminergic or cholinergic neurons. Such abnormalities could induce the changes in monoamine contents or upregulation of various receptor systems observed in narcolepsy. This hypothesis seems unlikely, however, since there was no significant difference in the activity of tyrosine hydroxylase in the amygdala or choline acetyltransferase or acetylcholin-
esterase in the brainstem between narcoleptic and control animals (unpublished data). There were also no differences in synaptosomal uptake of \([^{3}H]\)NE and \([^{3}H]DA in the cortex or amygdala between narcoleptic and control canines (62).

**MONOAMINERGIC AND CHOLINERGIC INTERACTIONS IN NARCOLEPSY**

Narcolepsy in Dobermans is transmitted as an autosomal recessive trait. Thus, heterozygous dogs never show spontaneous cataplexy. However, we recently discovered that combining drugs which enhance cholinergic transmission with drugs that inhibit monoaminergic transmission (for example, physostigmine plus prazosin \([\alpha-1\) antagonist], BHT-920 \([\alpha-2/D2\) agonist\) or quinpirole \([D2/D3\) agonist\)) induces cataplexy in about \(\frac{1}{2}\) of asymptomatic heterozygous animals, while no control dogs responded to these drug challenges by exhibiting cataplexy (63). Conversely, single drug administration (physostigmine, prazosin, BHT-920 or quinpirole) or coadministration of two monoaminergic compounds (for example, prazosin and BHT-920) failed to induce cataplexy in heterozygous canines (63).

Based on our most recent pharmacological and neurochemical results, we have developed a model for the control of cataplexy (Fig. 2), similar to the model for REM sleep regulation, in which cholinergic and monoaminergic systems interact reciprocally (43,62). In our model, activation of cholinergic or deactivation of monoaminergic systems produces an aggravation of cataplexy. Among the cholinergic receptor mechanisms, muscarinic M2 receptors are specifically involved in this interaction, whereas the monoaminergic receptors involved are the presynaptic \(\alpha-2\) or D2 type \((D2/D3)\) and postsynaptic \(\alpha-1\) receptors. The result that combinations of an \(\alpha-1\) antagonist and an \(\alpha-2\) agonist failed to induce cataplexy in heterozygous animals (see above) further suggests that manipulating postsynaptic \(\alpha-1b\) and presynaptic \(\alpha-2\) or D2/D3 receptors affects the same pathway additively, while manipulating these monoaminergic receptors and M2 muscarinic receptors simultaneously affects a distinct and synergistic pathway (Fig. 2).

**INVOLVEMENT OF CHOLINOCEPTIVE SITES IN THE PRF AND BASAL FOREBRAIN IN THE REGULATION OF CATAPLEXY**

The neurochemical substitution underlying the effects of cholinergic and monoaminergic compounds on cataplexy was tested also using in vivo microdialysis and local injection experiments in specific brain areas.

![Schematic representation of the neuroreceptor mechanisms involved in the regulation of cataplexy. As detailed in the text, cataplexy is regulated by the balance of activity of cholinergic and monoaminergic systems. The neuroreceptors involved include the M2 muscarinic receptors, \(\alpha-1b\) adrenergic receptors and \(\alpha-2/D2\) inhibitory autoreceptors.](image)
to induce cataplexy-like behavior in control animals (Fig. 3).

We also measured Ach release in the PRF during spontaneous cataplexy and found that it significantly increases during cataplexy but not during feeding or movement alone (Fig. 4) (66). This suggests that the cholinergic systems projecting to the PRF are activated during cataplexy. Taken together with the fact that Ach release is also known to be enhanced in the same area during REM sleep (67), this result further demonstrates the relationship between the mechanisms of cataplexy and REM sleep regulation. The fact that narcoleptic canines are also hypersensitive to cholinergic stimulation in the PRF, as demonstrated using local injection experiments and biochemical techniques (increased M2 receptor density has been shown in this area), suggests that the cholinergic systems are abnormally hyperactive at several levels (release and receptors) in narcoleptic canines.

In addition to the involvement of this cholinceptive site in the PRF, we recently discovered that carbachol injected unilaterally or bilaterally (2-10 nmol per site) into the basal forebrain (magnocellular basal forebrain) dose-dependently aggravates cataplexy and induces a long-lasting muscle atonia with desynchronized EEG, while carbachol injections into the lateral preoptic area and amygdala had little or no effect (68). Furthermore, bilateral injection of atropine, a muscarinic antagonist, in the same site (50 nmol for each site) significantly suppresses cataplexy (68). These results suggest that cholinceptive sites located in the forebrain and distinct from PRF are also involved in the regulation of cataplexy, and thus, in the pathophysiology of narcolepsy.

Several studies on human subjects have demonstrated that symptomatic narcolepsy occurs more frequently in association with lesions of the diencephalon (hypothalamus and 3rd ventricle) than those in the midbrain or pons (69,70). The discovery of a cholinceptive area in this region suggests that the primary abnormality in narcolepsy may lie within the basal forebrain. It also demonstrated that the cholinergic hypersensitivity observed in the PRF extends to brain regions other than the brainstem. Since the basal forebrain is a region known to be connected tightly with the limbic system, cholinergic stimulation in this area could be triggered by limbic input during emotions, thus resulting in cataplexy and a global REM-sleep-like activation of cholinergic systems.

Although much progress has been made in identifying the sites and mechanisms of action of cholinergic compounds, the site of action of α-1 antagonist and α-2 or D2/D3 agonists remains to be determined, since administration of these compounds into the cerebroventricle and several brain structures including the PRF and the locus coeruleus, induced only a slight modification of cataplexy (66). Further studies are now in progress to address this question.

CONCLUSION

Since no consistent major morphological brain changes have been reported in canine narcolepsy and idiopathic human narcolepsy, it is likely that minute abnormalities of neurotransmitter systems are involved in the pathophysiology of narcolepsy. Using our canine model of narcolepsy, we have previously demonstrated that the cholinergic and monoaminergic

FIG. 3. Modulation of cataplexy by unilateral and bilateral perfusion of a cholinergic agonist, carbachol, in the PRF of narcoleptic and control animals. Carbachol perfusion with a microdialysis probe into the PRF dose-dependently aggravated canine cataplexy in narcoleptic canines. Note that bilateral perfusion and higher doses are required to induce cataplexy-like behavior in control animals.
Acetylcholine levels in the PRF increased 60% during FECT-induced cataplexy (after four consecutive FECT cataplexy trials) in narcoleptic animals (n = 5). In behavioral control studies such as feeding or motor activity without cataplexy, acetylcholine levels in the PRF did not change. Additionally, no change in acetylcholine release was observed in control animals with FECT [see detail in Reid et al. (66)].

systems interact reciprocally in the regulation of cataplexy, with global activation of the cholinergic system aggravating, and global activation of monoaminergic systems suppressing cataplexy. We have also identified the subtypes of monoaminergic and cholinergic receptors specifically involved in this interaction and have found that activation of the cholinergic system by M2 stimulation or deactivation of catecholaminergic systems by either blockade of postsynaptic α-1b receptors or stimulation of α-2 or D2 type inhibitory autoreceptors aggravates cataplexy.

The role of the cholinergic system in this process is now starting to be defined anatomically. Cholinceptive sites in the PRF, as well as in the basal forebrain, have been characterized and results suggest widespread cholinergic abnormality in narcolepsy. In contrast, sites of action of monoaminergic compounds remain to be found. Based on available neuropharmacological and neurochemical results on canine narcolepsy, we believe that a hyperactivity of the cholinergic system (increase in acetylcholine release during cataplexy plus hypersensitivity of M2 receptors) and a hypoactivity of catecholaminergic systems (hypersensitivity of α-2 and D2-type inhibitory autoreceptors) most likely underlie the pathophysiology of narcolepsy.

Canine narcolepsy is transmitted by a single autosomal recessive gene with full penetrance. It is thus difficult to explain how several neurochemical abnormalities could be involved in the pathophysiology of the disease. It is however possible that a common abnormality among different receptor groups and neurotransmitters is involved, or that a deficit, which is not directly related to receptor mechanisms, secondarily induces changes in sensitivity of several receptors. We hope that a further accumulation of neuropharmacological and neurochemical data, in conjunction with the results gained from other approaches, especially molecular genetics, will illuminate the etiology and pathophysiology of narcolepsy in the near future.

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REFERENCES
17. Foutz AS, Delashaw JB, Guilleminault C, Dement WC. Mono-


62. Valtier D, Dement WC, Mignot E. Monoaminergic uptake in


