Vasoactive Intestinal Polypeptide Induces REM Recovery in Insomniac Forebrain Lesioned Cats


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Summary: Basal forebrain (BF) lesions in cats produces insomnia by reducing both slow wave sleep (SWS) and rapid-eye-movement (REM) sleep time. Recently it has been shown that vasoactive intestinal polypeptide (VIP) may be a specific REM inductor in the parachlorophenylalanine (PCPA) insomniac model. The purpose of this study was to test the hypnogenic properties of VIP in a nonpharmacological model of insomnia. Cats were rendered insomniac by delivering a DC current through stainless steel tripolar electrodes implanted in the basal forebrain area (BFA). Sleep-waking cycle recordings were done prior to lesions and on days 7, 9, 10, 11, 14, and 21 days after BF lesion. On day 10 after the lesion, 200 ng of VIP was injected into the 4th ventricle. Results showed that on postlesion days 7 and 9, SWS and REM sleep total times decreased, while waking time increased significantly. VIP restored REM sleep total time and frequency for almost 48 h, and SWS sleep total time for 24 h. On days 14 and 21 postlesion, insomnia was reestablished. Results are discussed in terms of the possible anatomical and neurochemical substrates whereby VIP can induce the recovery of sleep-waking control values. Key Words: Forebrain—Vasoactive intestinal polypeptide—Rapid-eye-movement sleep—Insomnia—Sleep.

It has long been shown that basal forebrain areas (BFAs) are involved in electroencephalogram (EEG) and behavioral sleep mechanisms. In 1918 von Economo (1) when studying patients who suffered encephalitis lethargica postulated the existence of a Sleep Center located in the forebrain. Later, in 1946, Nauta (2) gave additional support for the concept of an active forebrain sleep mechanism. Lesions of these regions resulted in a significant total time reduction in both slow wave sleep (SWS) and rapid-eye-movement (REM) sleep (3). Moreover, recent unit activity studies have shown a
significantly higher discharge rate of basal forebrain (BF) cells during SWS sleep when compared to REM sleep (4,5).

Recently, the classical concept of humoral control of sleep, postulated by Legendre and Pieron (6,7), has been revived. Many substances obtained from blood (8), cerebrospinal fluid (CSF) (9,10), urine (11), and brain (9,11,12) have shown to have sleep-inducing properties, thus possibly acting as endogenous sleep factors. Since the 1970s peptides have emerged as the best candidates. Among them, gastropancreatic peptides, recently localized in the CNS, are particularly evident in those brainstem regions involved in sleep regulation (13). It is thus possible that these peptides participate in the regulation of sleep parameters. In fact, this has been demonstrated in rats (14) as well as intact (15) and insomniac cats (14). Moreover, we have shown that cholecystokinin (16) and vasoactive intestinal polypeptide (VIP) (17) induced significant REM sleep recovery in parachlorophenylalanine (PCPA) insomniac cats. It has also been shown that the intraventricular application of a VIP-antagonist decreased REM sleep in rats (18).

This study was therefore designed to determine whether the hipnogenic properties of VIP can occur in cats rendered insomniac by (BF) lesions.

**METHODS**

Experiments were carried out on five cats of either sex weighing between 2.5 and 3.5 kg. Surgery was made under pentobarbital anesthesia (35 mg/kg) under aseptic conditions. All animals were implanted according to the stereotaxic coordinates of Snider and Niemer (19). Screw electrodes were placed over the sensorimotor cortex for EEG recording, and on the external canthus of the eye for electro-oculographic (EOG) recording. Electromyography (EMG) was recorded through stainless steel wires inserted in the neck muscles, and PGO activity was recorded through a tripolar electrode placed in the lateral geniculate body. Additionally, two bilateral tripolar electrodes were directed to the BF area (A = 14.0 to 16.0, L = 1.0 to 5.0, and H = −4.0 to −1.0), and a stainless steel guide cannula (21 gauge) was directed into the fourth ventricle. The final position of the cannula was verified by extracting CSF with the aid of a Hamilton syringe. At this point the cannula was fixed with dental acrylic. Animals were allowed to recover for 1 week after surgery before recording. Two days before the first recording session animals were kept inside a cage within a sound-attenuated recording chamber for habituation.

Sleep-waking cycle recordings were taken during 8-h periods (1000 to 1800) with a Grass 79D polygraph. All animals were recorded twice before lesioning and used as their own controls. Electrolytic lesions of the BF were made by applying a DC current through each forebrain tripolar electrode tip. The current was delivered by a Grass S88 stimulator through a constant unit. Lesion parameters used were 3.5 ma/min/tip. Recordings were made on days 7, 9, 10, 11, 14, and 21 after the lesion.

Based on a dose-response curve established previously in our laboratory, we decided to use the dose with maximum potency (200 ng) over total REM sleep time in normal and PCPA insomniac cats. Thus, on day 10 postlesion, a 200 ng/100 µl solution of VIP was injected into the 4th ventricle through a guide cannula using a Hamilton syringe.

At the end of the experiment all cats were sacrificed intracardially. They were perfused with 0.9% saline solution and 10% buffered formol. The brains were removed sectioned and stained with the Kluver–Barrera method for histological analysis.

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Sleep recordings were scored visually according to standard criteria. All data were analyzed on the basis of total time in min of waking and SWS and REM sleep. An analysis of correlated student's t test was performed to determine significance, comparing postlesion data with the prelesion values. The criteria used to consider a cat as insomniac was to have had less than the 50% of the total sleep time. For comparative effects, two cats received vehicle injections in the fourth ventricle in the same way as VIP was injected.

RESULTS

Basal forebrain lesions (BFLs) yielded important changes in the sleep-waking cycle in the five animals. From day 7 after BFL, cats were rendered partially insomniac; SWS and REM sleep times showed a significant decrease (SWS: t = 6.89, p < 0.05; REM: t = 5.86, p < 0.05), while waking significantly increased (t = 7.62, p < 0.05). Nine days postlesion, REM sleep occupied less than 50% with respect to the normal control, as seen in Fig. 1. Some individual differences were observed as a result of the lesion. For

![Graphs showing changes in wakefulness, SWS, and REM sleep after BFL](image-url)

**FIG. 1.** Histograms of the mean time ± SEM spent in wakefulness, slow wave sleep (SWS), and rapid-eye-movement (REM) sleep: C = control; 7, 9, 10 [vasoactive intestinal polypeptide (VIP)], 11, 14, and 21 days after basal forebrain lesion (BFL). *p < 0.05, **p < 0.01 compared to control values.
example, recordings of cats showed abortive REMs and not a single complete REM during the 8 h of recordings. This latter state was completely eliminated during at least 1 recording session, 1 on day 7, 2 on day 14, and 1 on day 21.

On this basis VIP or its vehicle were injected on day 10. Our results showed that VIP was able to normalize REM sleep time as well as waking and SWS on this day. However, on day 11 only REM sleep remained similar to control values. No statistical differences were seen since waking and SWS returned to values similar to postlesion days 7 and 9. This means that VIP effects on REM sleep lasted 48 h, while its effects on waking and SWS lasted only 24 h (Fig. 1). Saline solutions did not modify the insomnia produced by forebrain lesions (day 10: Waking [W] = 294.5 min; SWS = 181.4 min; REM = 5.3 min. Day 11: W = 347.3 min; SWS = 126.9 min; REM = 5.8 min). On days 14 and 21 insomnia reappeared. Sleep–waking time values and normal control values were again significantly different (p < 0.01). Behaviorally the cats were restless, alternating with long periods of quiet sitting and standing. Only 1 cat presented drinking and feeding behavior deficits, with consequent weight loss requiring special care.

Neither REM sleep duration nor REM latency were significantly modified with the lesion or with the VIP injection. However, BFL and VIP significantly affected REM epoch frequency. BFL significantly reduced REM frequency (t = 4.35, p < 0.05) from day 7 and more than 50% on days 14 (t = 4.44, p < 0.05) and 21 (t = 12.8, p < 0.001). VIP was able to restore REM frequency almost to control amounts in the first 24 h. This effect was observed 48 h later. One day after its application, REM frequency began to show a reducing tendency. The percentage decrease of REM sleep was always greater than the decrease of SWS sleep.

Histological analysis showed that the lesioned areas included part of the Diagonal Band of Brocca, the Anterior Commisure, the Preoptic Area, and the Substantia Innominata (Fig. 2).

DISCUSSION

Our results support the idea that the BFA contains neural structures whose integrity is essential for the normal pattern of the sleep–waking cycle, as postulated by McGinty and Sterman (3). The insomnia produced by BFA lesions provides a useful model of studying the effects of postulated sleep factors, in spite of the fact that the underlying mechanisms are not well known.

This study is an attempt to use the BFA lesioned cats as an insomniac model for the study of VIP effects upon the sleep–waking cycle. Our results show that VIP was capable of reverting insomnia by normalizing REM sleep time and REM frequency. These findings are in agreement with the effects produced by VIP in the well known pharmacological model of insomnia using PCPA in rats (13) and cats (13,14). However, in this model VIP appeared to restore waking and SWS sleep time to control on the injection day, but not subsequently. REM sleep, on the other hand, returned to control levels on injection day as well as on the following day. This effect could be expected since VIP was applied to the 4th ventricle close to the brainstem areas involved not only in REM sleep, but also in SWS and waking states. These areas coincide with the sites where VIP has been localized (13).

The increase in waking time in our results could suggest that BFLs may release ascending influences from brainstem areas involved in waking expression. This supports the idea that the forebrain-brainstem integrity is essential for the excitability

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balance of both areas, and that when this balance is disrupted, the function of the nondamaged area predominates (3,20).

This excitability relationship can be modulated by different sensory stimuli (21) and VIP as well, perhaps modifying the excitability of certain brainstem areas to the necessary levels needed to produce REM sleep (22). Since acetylcholine and cholinergic agents can induce REM sleep (23,24) and VIP is able to modulate the muscarinic activity in sympathetic ganglia (25–27), it has been suggested that the cholinergic system could be playing an important role in the VIP hypnogenic effect of the PCPA insomniac rats (14). However, recent studies have shown that simultaneous administration of carbachol into the pontine cells and intracerebroventricular injection of VIP failed to induce REM sleep (28). These results suggest that the REM-enhancing properties of VIP may not involve cholinergic actions (29).

The way in which VIP is capable of eliminating the insomniac effects of BFL in our model could include stimulation of brainstem regions involved in both REM and waking (30–33). Since VIP has a synergistic effect with noradrenaline in the cerebral cortex (34), a noradrenergic-VIP interaction via the locus coeruleus could possibly modify waking and REM time values in our model.

On the other hand, the forebrain and especially the hypothalamus contain high concentrations of VIP (35) and are densely innervated by serotonin positive nerve terminals originating from the raphe nuclei (35). In normal conditions, the relationship of the hypothalamus-raphe nuclei must be important to regulate SWS sleep. It may be reasonable to suggest that in this case VIP may play an important modulatory effect over neurotransmitter receptor sensitivity. After BFA lesions this relationship is broken and VIP modulatory action is lost, causing SWS sleep to decline. VIP administration may
restore its modulatory action including the raphe nuclei to act upon other synchronizing structures, like the thalamus. As a result, we observe a clear transient increase of SWS sleep lasting for the first 24 h after VIP administration. However, the possibility that SWS sleep time recovery is a consequence of the recovery of waking and REM sleep time cannot be ruled out. In sum, it can be suggested that VIP is a powerful modulating influence on the mechanisms serving REM sleep.

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