BRIEF REPORT

The Combination of VIP and Atropine Induces REM Sleep in Cats Rendered Insomniac by PCPA

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Twenty-four cats were implanted with electrodes for chronic sleep recordings. One week after the surgery, cats were treated with two intraperitoneal injections of parachlorophenylalanine (PCPA), an inhibitor of serotonin synthesis, to induce insomnia. Twenty-four hours after the second injection of PCPA, cats were at the peak of insomnia (strong reduction of both slow wave sleep 2 and rapid-eye movement [REM] sleep). During this period cats were divided into four groups (n = 6) and were injected with either atropine (0.5 mg/kg, IM [3.5 mmol/kg]), vasoactive intestinal peptide (VIP) (200 ng, ICV [60 pmol]) or atropine plus VIP (same doses and routes of administration). The control group received saline intramuscularly (IM) intracerebroventricularly and (ICV). Results showed that VIP and atropine injected alone and in combination increased mean total time of REM sleep in PCPA-treated animals. These findings are discussed in terms of a serotonin–acetylcholine interaction. ***[Neuropsychopharmacology 8:387–390, 1993]***

KEY WORDS: *REM sleep; Insomnia; Vasoactive intestinal polypeptide; Atropine; Serotonin; PCPA*

Acetylcholine (ACh) is one of the neurotransmitters associated with rapid-eye-movement (REM) sleep generation.
administration of cholinergic agonists into the pontine reticular formation (PRF) of cats and rats promptly induces REM sleep (for extensive review, see Shiromani et al. 1987; Hobson 1990). An increase in ACh release associated with REM sleep has also been observed in several brain structures, including the PRF (for review, see Gillin and Shiromani, 1990). Recently, Velazquez-Moctezuma et al. (1989) have reported evidence suggesting that the M2 cholinergic receptors are more critical in inducing this effect. However, M1 receptors may also play an important role in the generation of REM sleep (Salín-Pascual et al. 1992).

On the other hand, vasoactive intestinal polypeptide (VIP) is also a potent REM sleep inducer. For example, intracerebroventricular administration of VIP in normal rats, cats, and rabbits increases REM sleep (Riou et al. 1982; Drucker-Colín et al. 1984; Obal et al. 1989). In addition, VIP restores REM sleep in parachlorophenylalanine (PCPA)-treated insomniac rats (Riou et al. 1982) and cats (Prospero-García et al. 1986). Moreover, the inactivation of the endogenous VIP by its antagonists (Mirmiran et al. 1988) or antibodies anti-VIP (Riou et al. 1982; Drucker-Colín et al. 1988) reduce REM sleep.

From a functional viewpoint, ACh and VIP are closely related. Both substances coexist in the same terminals and one modulates the release of the other (Lapchak and Beaudet 1990; Wang et al. 1986; for extensive review, see Whittaker 1989; Magistretti 1990). Moreover, VIP may enhance the synthesis of ACh in structures like the hippocampus (Luini et al. 1984).

Due to this interaction, we have investigated

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whether VIP's REM sleep-inducing effect in PCPA-treated insomniac cats depends on cholinergic mechanisms. Earlier observations showed that carbachol, a cholinergic agonist, did not induce REM sleep in PCPA-treated cats when administered into the PRF (Drucker-Colín and Prospero-García 1988). However, it reduces the REM sleep-inducing effect of VIP by approximately 50% when both compounds are administered simultaneously (Drucker-Colín et al. 1988). These findings suggested that in PCPA-treated cats, the insomnia observed may be a result of the reduction in serotonin availability associated with an overactivation of the cholinergic system. To further our understanding of ACh–VIP-serotonin interaction in REM sleep induction, we decided to test the effect of atropine, VIP, and the combination of both in the sleep of PCPA-treated insomniac cats.

Twenty-four adult male and female cats weighing 2.5 to 3.5 kg were implanted, under pentobarbital anesthesia, with a set of electrodes for chronic recordings. Two screw electrodes were placed on the parietal bones for electroencephalographic (EEG) recording and two other electrodes were placed in the internal and external canthus of the orbit to record eye movements (EOG). Postural tone was recorded through two wire electrodes inserted in the muscles of the neck. An additional tripolar electrode placed into the lateral geniculate body recorded ponto-geniculo-occipital (PGO) spikes. A stainless-steel guide cannula was aimed to the fourth ventricle for the administration of VIP. During the surgery, the position of the cannula's tip into the fourth ventricle was corroborated withdrawing cerebrospinal fluid (CSF). In this position, the cannula was anchored to the skull with dental cement. A stylet was placed into the cannula to avoid CSF outflow. Male and female cats were housed separately in two rooms. Dimensions of both rooms were approximately 35 × 2 m. Light-dark cycle was controlled (12:12). Lights were turned on at 8:00 AM.

One week after the surgery cats were habituated to the recording conditions for 24 hours. Upon completion of the habituation period, cats were treated twice with 400 mg/kg (0.4 mol/kg) of PCPA. The two intraperitoneal injections were separated by a 24-hour period. Twenty-four hours after the second injection cats were divided into four groups ($n = 6$). and were challenged with either saline (ICV and IM), atropine (0.5 mg/kg IM [3.5 mmol/kg]), VIP (200 ng ICV [60 pmol]) or atropine plus VIP. Three cats in the control group received saline into the fourth ventricle, whereas the other three received saline intramuscularly. Since saline administered through either route did not modify the insomnia induced by PCPA, the six cats were analyzed together as a single group. Immediately after the injection, cats were recorded in a sound-attenuated, Faraday chamber (8:30 AM to 7:30 PM). Four cats (one from each group) were simultaneously recorded and constantly monitored through closed circuit television.

Sleep recordings were visually scored and four sleep states were determined according to standard criteria (Ursin and Sterman, 1981). Data were analyzed on the basis of total time in minutes of wakefulness, slow-wave sleep 1 (SWS1), slow-wave sleep 2 (SWS2), and REM sleep. Data were presented as percent of controls for each sleep stage. Frequency and duration of the individual periods of REM sleep were also analyzed. Statistical analysis of the results was carried out using an analysis of variance and then a Scheffé test (Scheffé, 1953).

The results summarized in Fig. 1 and Table 1 show that PCPA-treated cats receiving saline spent more than 80% of the total time of recording in wakefulness. Both VIP alone and atropine in combination with VIP reduced wakefulness induced by PCPA by approximately 40% ($p < .01$), whereas atropine alone reduced wakefulness by approximately 30% ($p < .01$). Vasoactive intestinal peptide alone and in combination with atropine increased SWS1 by approximately 150%, whereas atropine alone increased SWS1 by approximately 85% ($p < .01$). Rapid-eye movement sleep was increased by approximately 800% when VIP or atropine was injected alone, whereas the combination of these compounds increased REM sleep by about 1300% ($p < .001$). Slow-wave sleep 2 was not significantly increased by any of the drugs tested.

Vasoactive intestinal peptide and atropine alone and in combination augmented the total time of REM sleep, increasing the frequency of appearance and duration of this sleep stage ($p < .01$).

Another interesting result was that atropine and VIP alone and in combination reduced PGO activity during wakefulness and SWS1, but not during REM sleep. Such an effect was observed at the end of the first hour and lasted for the rest of the recording period. No further quantification of this signal was performed in this study.

These results show that both atropine and VIP are able to induce REM sleep in cats rendered insomniac by PCPA. It is also interesting that VIP potentiates atropine effects. We have previously observed that atropine (0.5 mg/kg) and VIP (200 ng) reduce REM sleep in normal cats (Prospero-García, 1989); thus, the induction of REM sleep in PCPA-treated cats may be dependent on the reduction of serotoninergic neurotransmission.

It has been suggested that serotonin and ACh interact to regulate SWS–REM sleep generation (Hobson, 1990). It is likely that a reduction in the activity of the serotoninergic system may disinhibit the cholinergic system within the dorsal tegmentum as has been suggested by in vitro studies (Luebke et al. 1992). Because ACh also facilities arousal, the resulting overactivation of the cholinergic system may account for the induc-

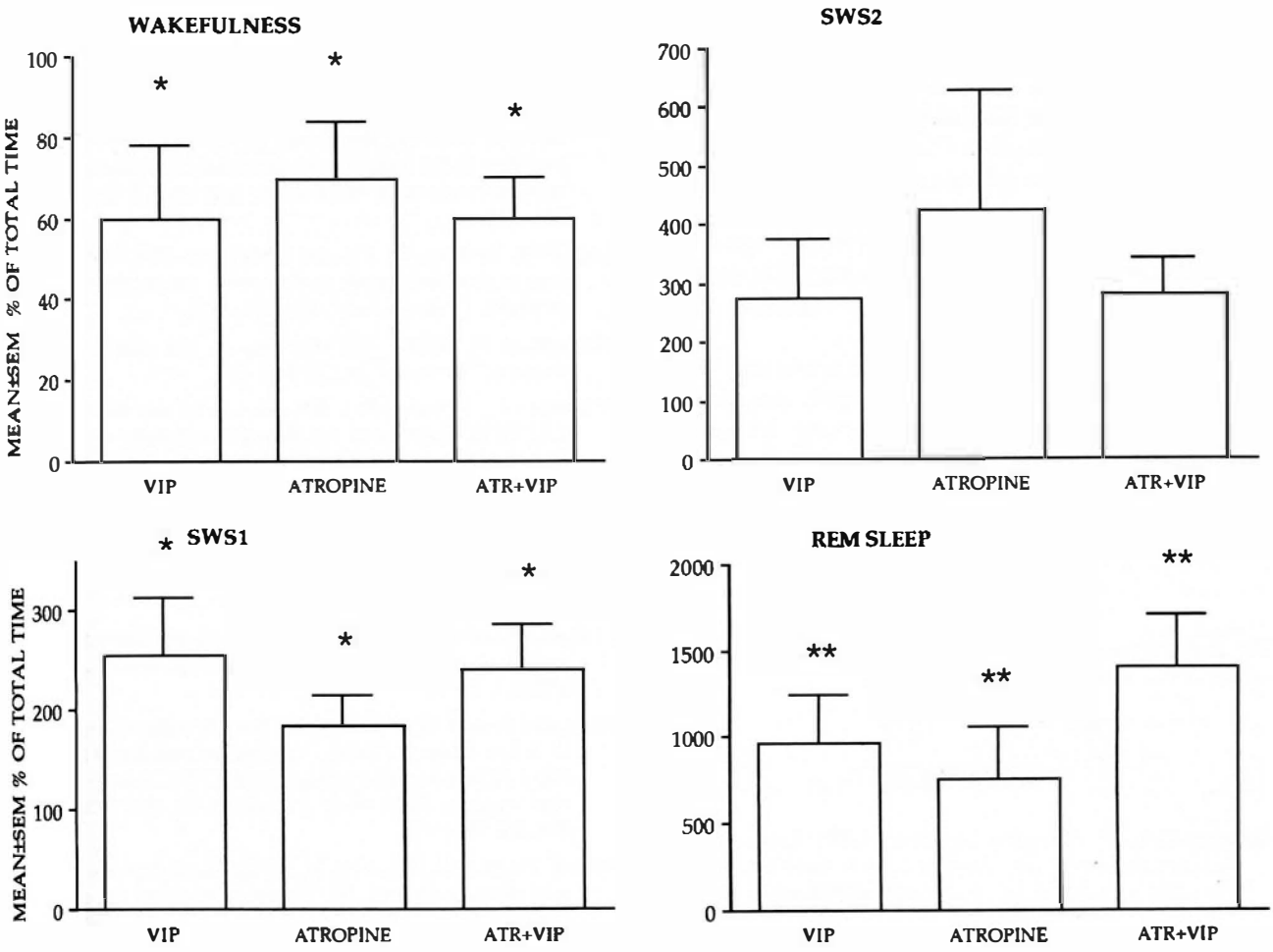

Figure 1. Atropine alone and in combination with VIP increases both SWS1 and REM sleep in PCPA-treated cats. Their main effect is exerted on REM sleep. Data are presented as percent of controls (100% is the effect of saline). ATRO + VIP: atropine plus VIP; Wakefulness: $F = 6.475$, $df = 3, 20$, $p < .01$. SWS1: $F = 3.563$, $df = 3, 20$, $p < .01$. SWS2: $F = 1.784$, $df = 3, 20$, $p = .18$; REM: $F = 5.185$, $df = 3, 20$, $p < .001$ as compared to saline.

tion of insomnia. For instance, it has been shown that physostigmine, an indirect cholinergic agonist, elicits either wakefulness or REM sleep, depending on the dose and when it is administered during SWS (Gillin et al. 1978). Similar results have been obtained with the iontophoretical administration of carbachol and physostigmine into the pons of unanesthetized head-restrained cats (López-Rodríguez et al. 1992). In this context, the administration of cholinergic agonists like carbachol in PCPA-treated insomniac cats, potentiates ACh activity, thereby facilitating insomnia instead of REM sleep (Drucker-Colín and Prospero-García 1988). In contrast, by reducing the activity of the cholinergic system atropine may be reestablishing a balance between serotonin and ACh, thereby eliciting REM sleep.

Table 1. Effect of Atropine or VIP and Their Combination on REM Sleep Parameters of PCPA-Treated Insomniac Cats

	Frequency	Duration	Latency
Saline	3.16 ± 3	0.5 ± 0.2	510 ± 103
VIP	14 ± 1.8**	2.5 ± 0.7*	97.2 ± 26.7**
Atropine	8.5 ± 4*	2.9 ± 0.8*	299.4 ± 109*
Atro + VIP	17.5 ± 5**	3.01 ± 0.6*	108.5 ± 91.5**

Values represent Mean ± SEM in minutes for duration and latency; for frequency the number of episodes during 11 hours of recording. Frequency: $F = 4.006$, $df = 3, 20$, $p < .01$; duration: $F = 4.074$, $df = 3, 20$, $p < .01$; latency: $F = 5.006$, $df = 3, 20$, $p < .001$ as compared to saline.

* $p < .01$.

** $p < .001$.

In addition, it is known that VIP exerts inhibitory or facilitatory effects on ACh release depending on its concentration (see Whittaker 1989). Such a dose-dependent effect is also observed on REM sleep. For example, 100 ng of VIP (ICV) enhance REM sleep, whereas 200 ng reduce it in normal cats (Drucker-Colín et al. 1984; Prospero-García 1989). In contrast, in PCPA-treated cats, 100 ng of VIP does not restore REM sleep, whereas 200 ng restores it. In addition, when VIP is injected in combination with atropine, REM sleep is potentiated.

We have previously shown that carbachol does not induce REM sleep in PCPA-treated cats (Drucker-Colín and Prospero-García 1988). The present results show that atropine induces REM sleep. Therefore, it is likely that insomnia observed in PCPA-treated cats may be due in part to an overactivation of the cholinergic system. In addition, since VIP (200 ng) reduces REM sleep in normal cats (Prospero-García, 1989) and has synergistic effects with atropine in inducing REM sleep in PCPA cats, it may be that at this concentration, VIP is antagonizing cholinergic activity.

In conclusion, the property of atropine and VIP to induce REM sleep in PCPA-treated cats, may depend on the reduction of serotoninergic activity. Ultimately, REM sleep generation may depend on a balance between serotonin and ACh in normal conditions.

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