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Scopolamine-Induced Suppression of Paradoxical Sleep is Reversed by the Somatostatin Analogue SMS 201–995 in Rats

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DANGUIR, J. AND S. DE SAINT-HILAIRE-KAFI. *Scopolamine-induced suppression of paradoxical sleep is reversed by the somatostatin analogue SMS 201–995 in rats.* PHARMACOL BIOCHEM BEHAV 30(2) 295–297, 1988.—The intraperitoneal administration of the octapeptide somatostatin analogue SMS 201–995 produced a significant increase in paradoxical sleep (PS) in rats. The suppression of PS by the muscarinic receptor blocker scopolamine was reversed by SMS 201–995. These findings confirm previous results demonstrating a role of somatostatin in the generation of PS. In addition they suggest that the suppression of PS by scopolamine may be due to an inhibitory effect on somatostatin release, rather than to an alteration of cholinergic function alone.

Paradoxical sleep Somatostatin Somatostatin analogue Scopolamine

WE recently reported that somatostatin (SRIF) selectively increased paradoxical sleep when infused into the cerebral ventricles of rats [5]. Conversely, a specific suppression of paradoxical sleep (PS) was observed following the intracerebroventricular (ICV) infusion of cysteamine, a particularly potent SRIF depletor [5], and also when central endogenous SRIF was neutralized by ICV infusion of somatostatin antiserum (Danguir, unpublished data).

The case for a role of SRIF in the generation of PS was strengthened by our recent demonstration that the increase of PS which follows the injection of cholinergic agonists such as carbachol could be blocked by the administration of SRIF antiserum [6]. In that report, we suggested that contrary to the prevailing concept that the relationship between cholinergic mechanisms and PS is direct [1–3, 7, 8], acetylcholine may influence the generation of PS via some intervening action on SRIF. If this suggestion were correct, SRIF should reverse the suppression of PS seen after the administration of muscarinic receptor blockers, such as scopolamine [2, 9, 10].

The present experiments were designed to test this prediction. We tried to reverse the well known insomnia observed after treatment with scopolamine by using the octapeptide somatostatin analogue, SMS 201–995. We used this analogue, rather than native SRIF, for two reasons. First, it is much more potent than native SRIF [11], and second, its biological activity is not altered when it is administered through intraperitoneal routes [4].

METHOD

Animals and Housing

Six adult, male Wistar rats (Iffa Credo) weighing 280–320 g were used. After surgery, the rats were housed individually in an environmentally controlled room on a 12-hr light/dark schedule (lights on at 08:00). The cages were Plexiglas cylinders open at the top to allow chronic electrocorticographic (ECoG) sleep recordings. The rats were maintained with ad lib water and standard laboratory chow.

Surgery

All surgery was performed under pentobarbital anesthesia (Nembutal, 40 mg/kg). The rats were implanted with two cortical electrodes made with chloridized silver wire terminating in a 1 mm diameter sphere which was pushed through a hole made in the skull to come into contact with the dura mater. These electrodes were positioned on each side of the saggital suture, one just anterior to the bregma and the other in front of the lambda. The ground electrode, also made of silver wire, was positioned subcutaneously. All electrodes were attached to a micro-connector (Ouest Electronic) mounted on the skull with dental cement and with the aid of anchoring screws.

Experimental Procedure

One week after recovery from surgery, the rats were con-

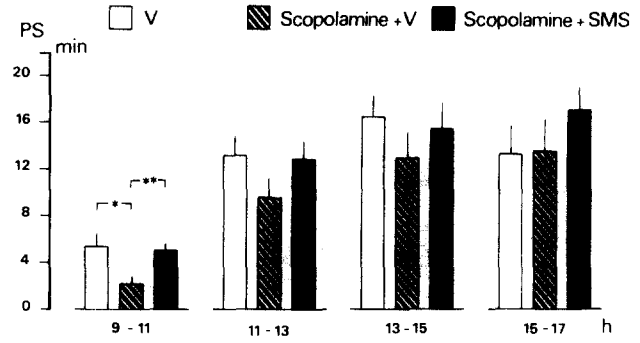

FIG. 1. Duration of paradoxical sleep, expressed in minutes (mean±SEM) every 2 hours following IP injection of the vehicle of SMS (V), scopolamine plus V, on scopolamine plus the somatostatin analogue SMS 201–995. *$p<0.02$, **$p<0.01$ (paired t-test).

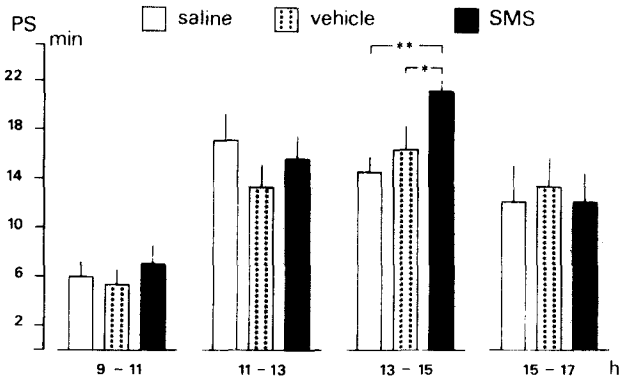

FIG. 2. Duration of paradoxical sleep, expressed in minutes (mean±SEM) every 2 hours following the IP injection of saline, the vehicle of somatostatin analogue SMS 201–995, or SMS alone. *$p<0.02$, **$p<0.01$ (paired t-test).

nected to the recording cables and allowed two days for adaptation. In all rats, sleep recordings were made from 09:00 to 17:00 hr on five days, each separated from the next by an intervening day on which no treatment was given and no recordings were made. All rats received the following types of injection intraperitoneally in a random fashion: saline, the SMS 201–995 vehicle, scopolamine dissolved in saline (10 mg/kg, Sigma Chemicals) immediately followed by the SMS 201–995 vehicle (acetic acid=3.2 mg, sodium chloride=7 mg, sodium acetate=1.8 mg, in 1 ml of distilled water (pH=4); Lab. Sandoz, France). The somatostatin analogue alone (0.15 mg/kg, the generous gift of Dr. Chaumet-Riffaud, Lab. Sandoz, France) and scopolamine plus SMS 201–995 (0.15 mg/kg).

Statistical Analysis

One-way analysis of variance and post hoc paired Student's t-test (2-tailed) were used for statistical analysis.

RESULTS

As expected, the administration of scopolamine resulted in a suppression of PS. This suppression was specific for PS, since scopolamine had no effect on slow-wave sleep. The suppression of PS was largest during the two hours (9:00 to 11:00) which was followed by the administration of the drug (Fig. 1). No statistically significant differences in PS were observed during the subsequent 6 hours of recording (from 11:00 to 17:00).

When the somatostatin analogue SMS 201–995 was injected immediately after scopolamine, the result was a complete reversal of the PS suppression induced by scopolamine alone during the two first hours which followed the injections (Fig. 1).

The IP administration of SMS alone brought about a significant increase in PS four hours following its administration, compared to the saline and the vehicle injection days (Fig. 2). Once again, the duration of slow-wave sleep was unaffected.

DISCUSSION

The major findings of this report were: (1) the peripheral administration of the octapeptide somatostatin analogue SMS 201–995 resulted in a significant and selective increase of PS, and (2) the somatostatin analogue reversed the suppression of PS induced by the muscarinic receptor blocker scopolamine.

The results confirm those we have previously observed with intracerebroventricular administration of native somatostatin [5] and provide further evidence for a role of this hormone in the induction of paradoxical sleep. In addition, the results show that not all of the native hormone's molecular structure is necessary for the full action on PS.

The fact that the somatostatin analogue promotes PS even when administered *peripherally* is interesting for two reasons. First, it confirms the ability of this octapeptide analogue to maintain its functional properties when given intraperitoneally. Second, it supports the idea that peripheral factors, which have been largely neglected in sleep studies, may be of primary importance in triggering sleep.

Recently, we have shown that microinjection of a cholinergic agonist such as carbachol into the nucleus of tractus solitarius results in an increase of PS [6]. We have also shown that such carbachol-induced increase of PS can be blocked by the administration into the same site of somatostatin antiserum [6]. This result suggests that the carbachol-induced increase of PS is not due to cholinergic stimulation alone, but could rather be mediated by somatostatin. The restoration of PS by the somatostatin analogue in scopolamine-pretreated rats, as described in the present report, would support such a hypothesis. However, arguing against the somatostatin mediation hypothesis are the different time courses, for direct effects of SMS 201–955 upon brain and effects of scopolamine (see Figs. 1 and 2). This could mean that the opposing effects of the somatostatin peptide and the cholinergic agonists are not sharing common postsynaptic mechanisms. Should this be the case, the reversal of scopolamine-induced suppression of PS by somatostatin could be accounted for by an additive effect, whereby scopolamine's effect on PS is negative, somatostatin's effect is more strongly positive.

Although no data is available concerning the effects of scopolamine administration on peripheral and/or central somatostatin levels, the present results favor an inhibitory action of this muscarinic receptor blocker on somatostatin secretion. Such an action would explain the suppression of PS observed after scopolamine treatment and its reversal by

the somatostatin peptides. It would also strengthen the suggestion made by Gnadt and Pegram [8] that the reduction of PS by peripheral administration of scopolamine may not be acting by blocking the brainstem receptors which stimulate PS [1–3].

In summary, it seems clear that somatostatin plays a key role in inducing PS. In addition, our previous and present results suggest that the cholinergic effects on PS may be mediated by somatostatin systems. However, evidence concerning the biochemical basis as well as the exact locus of action of the somatostatin-acetylcholine interaction is still needed to support such a theory.

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