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Adrenergic and Cholinergic Inputs in Preoptic Area of Rats Interact for Sleep–Wake Thermoregulation

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MALLICK, B. N. AND M. M. J. JOSEPH. *Adrenergic and cholinergic inputs in rat preoptic area interact for sleep–wake thermoregulation.* PHARMACOL BIOCHEM BEHAV **61**(2) 193–199, 1998.—Isolated studies have shown that both norepinephrine and acetylcholine into the medial preoptico-anterior hypothalamic area tonically regulate sleep–wake and body temperature. A possible interaction between these neurotransmitters for the regulation of such functions has been investigated in this study. To study this interaction a combination of either prazosin and carbachol or, scopolamine and methoxamine was injected into the medial preoptico-anterior hypothalamic area and the effect on sleep, wake, and rectal temperature recorded simultaneously. The combination of chemicals were selected based on our previous studies where it was observed that each of the chemicals in a combination had opposite effects. It was observed that injection of the combination expressed a resultant summated effects of individual component chemicals when injected in isolation (observed in previous studies). Because effect of neither of the chemicals in the combination was dominant, the results suggest an interaction and integration of the adrenergic and cholinergic inputs in the medial preoptico-anterior hypothalamic area for the regulation of sleep–wakefulness and body temperature. © 1998 Elsevier Science Inc.

Adrenergic Agonist-antagonist Cholinergic Interaction Medial preoptico-anterior hypothalamic area Sleep–wakefulness Thermoregulation

SLEEP–WAKEFULNESS and body temperature (Tb) go hand in hand and influence each other (7,27,38). It has been proposed that one of the functions of sleep–wakefulness is to maintain the Tb within physiological limit (17,22). The medial preoptico-anterior hypothalamic area (mPOAH) regulates both these functions (11,22,24,33). Because neurons related to either or both the functions (3,12,13,19,28,34) have been identified in this area, it is reasonable that the mPOAH influences these functions simultaneously as well as independently (17,18,21). Presence of norepinephrine (NE) as well as acetylcholine (ACh) sensitive neurons in the mPOAH has been identified iontophoretically (23,26). Independent studies have shown that both NE and ACh affect sleep–wakefulness states (18,20,43) as well as Tb (5,10,14,18,20,26,30). However, in a normal living system ACh and NE are present together. Although an interaction between the cholinergic and the adrenergic systems in mPOAH for the regulation of sleep–wakefulness and Tb has been suggested (11,16), it was yet to be experimentally verified. This is significant because NE and ACh are effective mediators of sympathetic and parasympathetic nervous system, respectively, which functionally oppose and tend to balance each other.

Although AChergic (9,32,35,41,42) as well as NEergic (6,9, 35,36,41) inputs and respective receptors have been identified, AChergic neurons have not been reported in the mPOAH. Therefore, the AChergic and the NEergic inputs into the mPOAH must act on noncholinergic neurons and interact for the regulation of sleep–wakefulness and Tb. To investigate this interaction in maintenance of such functions, suitable combination of AChergic and NEergic agonist and antagonist were microinjected into the mPOAH, and the effects on sleep–waking and Tb were recorded simultaneously. The combination of the chemicals were selected based on previous reports, where components of chemicals in a combination were independently injected (18,20) and opposite effects were observed. This was preferred with the assumption that a neutralizing effect would be observed.

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METHOD

Male Wistar inbred rats (250–300 g), maintained under a 12 L:12 D cycle with food and water ad lib, were used in this study. The experimental rats, divided into eight groups (Table 1), were acclimatized to the recording environment. Details of surgical and recording procedures have been mentioned earlier (1,2). In brief, under Nembutal (35 mg/kg IP) anesthesia two stainless steel screw electrodes were fixed on the skull to record bipolar EEG; while stainless steel flexible insulated (except at the tip) wires were used to record bipolar EMG and EOG from the dorsal neck muscles and external canthus of the eyes, respectively. Another screw electrode was implanted in the midline over the frontal sinus to serve as animal ground. The other ends of these electrodes were connected to a nine pin female plug that was fixed on the skull with dental cement. A pair of stainless steel guide cannulae (chemitrode) along with their blockers were introduced bilaterally at stereotaxic coordinate A −0.5 to −1.0 mm, L 0.8 to 1.2 mm (29) until the tips reached H7.5 ± 0.5 mm (i.e., approximately 1 mm above the target, the mPOAH) and anchored to the skull with dental cement. The recording started after recovery from surgical trauma. From the third recovery day onwards the rats were acclimatized by maintaining them in the recording cage with the recording plug and the rectal probe connected.

On the day of experiment the rat connected to the recording plug and rectal probe was left in the recording chamber for at least 1 h before the actual recording was started. Because rats are polycyclic animals and normally sleep during the light period, recording after injection of the chemicals were done both during the day (09.30–15.30 h) and the night (21.30–02.30 h). The effects of injection of individual agonist and antagonist have been reported earlier (18,20). The baseline bipolar EEG, EOG, and EMG were continuously recorded with a Grass polygraph for at least 1 h. The rectal temperature (Trec) was recorded because comparisons were made with previous findings under similar conditions. The temperature was recorded every 10 min. Following baseline recording 0.2 μl of either saline, *N,N*-Dimethyl Acetamide (*N,N*-DA vehicle used for dissolving prazosin), or a combination of agonist and antagonist of cholinoceptor and adrenoceptor was injected bilaterally into the mPOAH at a rate of 0.1 μl per min (1). When a combination of an antagonist and an agonist was used, injection of the former was followed by the latter. The combination of prazosin and carbachol or scopolamine and methoxamine were selected on the basis of results obtained under identical conditions from our previous studies (18,20) when the chemicals were injected separately and an opposite effect was obtained. It was done with the assumption that if an interaction was taking place, the effects of injection of the chemicals in combination would cancel out. The injector, projected by about 1 mm beyond the guide cannulae, was retained in the same position for at least 1 min at the end of injection and then replaced by the blocker. Thus, the entire injection procedure lasted about 6–12 min, depending on injection of a single chemical or a combination of chemicals. The injection was made once in each animal and the recording continued at least for 1 h or until the effects lasted on sleep–wakefulness. The behavior of the rats was monitored throughout the period of recording.

At the end of experiment, under deep anaesthesia, 0.4 μl of ferric chloride solution was injected into the same site where the chemicals were injected. After about 30 min the rat was intracardially perfused first with saline followed by with 10% formol-saline containing 2% potassium ferrocyanide. The site and spread of injected chemicals were histologically confirmed by the presence and extension of prussian blue coloration (Fig. 1). Data were taken only from those animals where the prussian blue color extended within the mPOAH. For statistical analysis the pre- and postinjection records were divided into bins of 10 s each. On the basis of EEG, EOG, and EMG each bin of the records was classified into sleep and waking states (40). Because the recording was done for relatively shorter period (as long as the effects lasted), rapid eye movement sleep was encountered infrequently, and if present, was considered within the sleep stage. Although the records were divided into two states (sleep and waking) only, for convenience and to maintain uniformity in statistical analysis and representation, only one state, the waking values, were considered, as reported earlier (1). The Wilcoxon matched-pairs signed-rank test was applied to compare the values obtained from the same group of rats, while the Mann–Whitney test was applied to compare the values obtained from different groups of rats (1).

RESULTS

The rats normally spent more time in sleep during the day while in waking during the night recording period. The mean

TABLE 1
EXPERIMENTAL PROTOCOL

Group	Number of Rats	Chemical Injected	Amount Injected	Time of Experiment	Effects on	
					W	Tb
1	4	normal saline	0.2μl	9.30–15.30	NC	NC
2	4	same	same	21.30–2.30	NC	NC
3	4	20% N,N DA	same	9.30–15.30	NC	NC
4	4	same	same	21.30–2.30	NC	NC
5	5	1% prazosin +	2 ng/0.2 μl (0.02 M)			
		1% carbachol	2 ng/0.2 μl (0.05 M)	9.30–15.30	I	NC
6	5	same	same	21.30–2.30	NC	NC
7	5	1% scopolamine +	2 ng/0.2 μl (0.02 M)			
		1% methoxamine	2 ng/0.2 μl (0.04 M)	9.30–15.30	NC	NC
8	5	same	same	21.30–2.30	NC	NC

I—increase; D—decrease; NC—no change.

Trec of all the rats during day was lower [37.83 ± 0.030°C (SEM)] than that during the night [38.33 ± 0.030°C (SEM)]. Both sleep–wakefulness and Trec were recorded simultaneously before and after injection of either saline, *N,N*-DA, or a combination of agonist and antagonist of adrenoceptor and cholinoceptor.

Control Studies

Effects of saline and N,N-DA on sleep, wakefulness, and Trec. Both during the day and the night recording an injection of either saline (groups 1 and 2) or *N,N*-DA, used as a vehicle for prazosin (groups 3 and 4) did not influence either sleep–wakefulness or Trec, except for the initial 10 min of the postinjection. This effect for the 10 min immediately after injection was due to nonspecific factors, because just handling the rats for an equivalent period of time taken for microinjec-

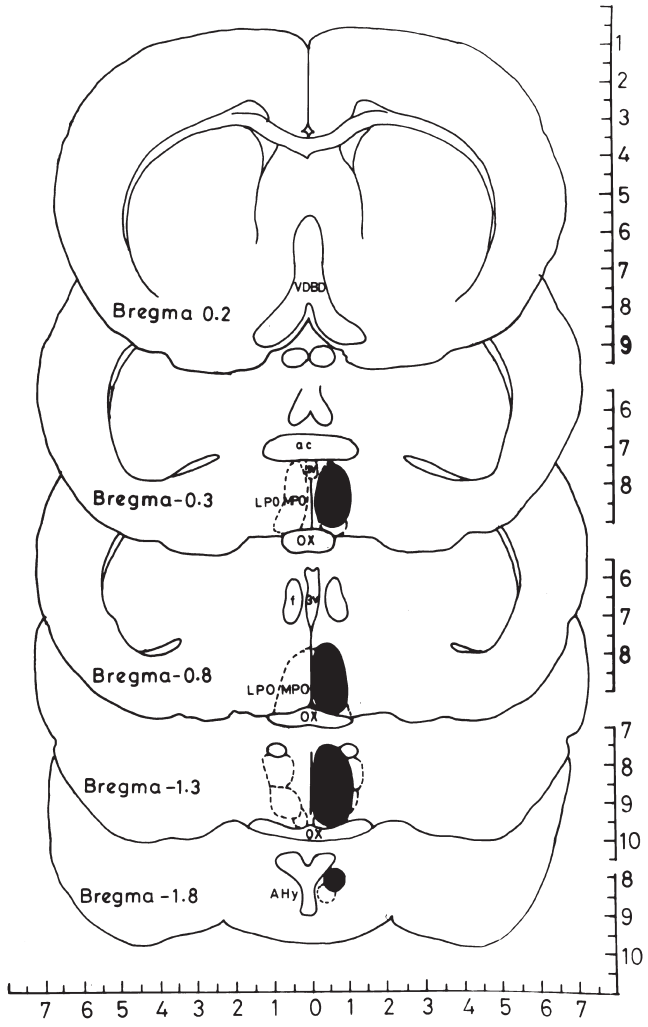

FIG. 1. Reconstruction diagram through medial preoptico-anterior hypothalamic area (29) is shown in this figure. The filled areas represent the maximum diffusion of prussian blue coloration, as observed in the histological sections from experimental and control rats. Although the injections were made bilaterally, is represented here on one side only.

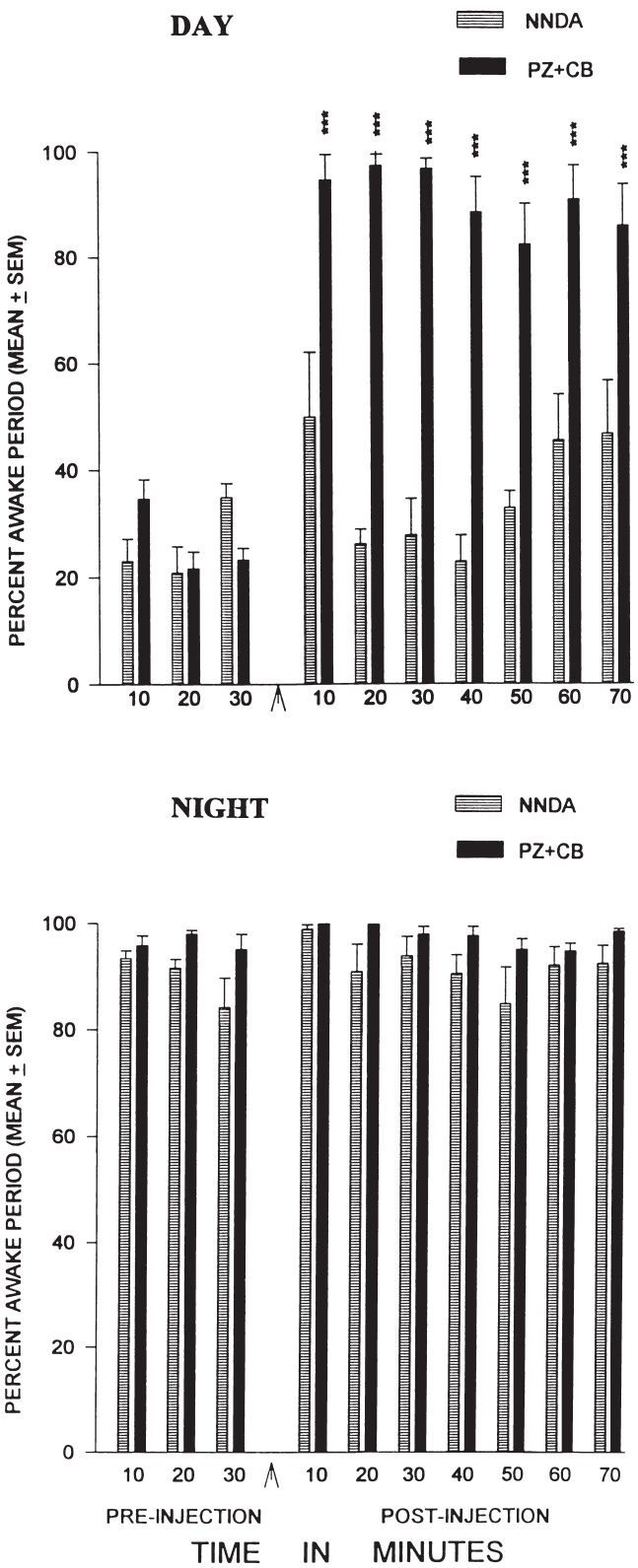

FIG. 2. The percent changes in mean (±SEM) wakefulness after microinjection of prazosin followed by carbachol (PZ + CB) and *N,N*-DA into the mPOAH during the day and the night recordings are shown in this figure. ***$p < 0.001$. Significant compared to *N,N*-DA injection at corresponding period.

tion (10–12 min) and sham injection also induced a comparable effect.

Effects of Combination Injection

Effects of combination of alpha-1 antagonist (prazosin) and cholinergic agonist (carbachol) on sleep–wakefulness. During the day recording (group 5) injection of this combination induced waking for at least 70 min. This increased waking was statistically significant compared to both the baseline ($p < 0.025$) and postsaline injection ($p < 0.01$) (Fig. 2). However, during the night recording (group 6) this combination did not affect either sleep or wakefulness significantly.

Effects of combination of alpha-1 antagonist (prazosin) and cholinergic agonist (carbachol) on Trec. Injection of prazosin followed by carbachol did not significantly affect the Trec either during the day (group 5) or the night (group 6) recording period except for the initial 10 min, which was likely to be handling effects (Fig. 3).

Effects of combination of cholinergic antagonist (scopolamine) and alpha-1 agonist (methoxamine) on sleep and wakefulness. Neither sleep nor wakefulness was significantly affected by the injection of this combination into the mPOAH either during the day (group 7) or the night (group 8) recording periods (Fig. 4).

Effects of combination of cholinergic antagonist (scopolamine) and alpha-1 agonist (methoxamine) on Trec. The Trec was also not significantly affected (except the initial handling effects) by the injection of this combination into the mPOAH both during the day (group 7) and the night (group 8) recording periods (Fig. 5).

DISCUSSION

Isolated studies have shown that NE (5,10,18,30) and ACh (5,14,20,25,30) in the mPOAH influence both sleep, waking, and Tb. Because both these inputs are simultaneously and tonically present, their possible interaction for the regulation of sleep–wakefulness and Tb (as reflected in Trec) have been investigated in this study. The experiments were conducted under similar conditions as that of the isolated studies (1,18,20) reported earlier and, therefore, the arguments put forward in those studies to rule out the effects due to nonspecific factors hold true for this study also. It was reported that effects of individual microinjection of scopolamine were opposite to that of carbachol on both sleep–waking and Tb (20). However, α_1 adrenoceptor antagonist and agonist, prazosin, and methoxamine, respectively, although had opposite effect on the Tb (the former induced hyperthermia while the latter hypothermia), the effects on sleep and waking were similar (both induced wakefulness) (18). Therefore, to study a possible interaction between them, effects of such combinations of cholinergic and adrenergic agonist and antagonist were selected in this study that if the effects of the two inputs were opposite, a nullifying result should be observed. Hence, in this study combinations of prazosin and carbachol or scopolamine and methoxamine were injected into the mPOAH. The effects were studied simultaneously on both sleep–wakefulness and Tb because they influence each other; also, mPOAH influences those functions.

Because rats spend more time in sleep during the day and in waking during the night, the experiments were conducted both during the day and the night. It has been reported by individual microinjection studies that hyperthermia was induced by prazosin (18), while hypothermia (20) was induced by carbachol. In this study, when those chemicals were injected in combination, there was no significant change in Trec (Fig. 3). This is likely to be a resultant neutralizing effect of the two chemicals having opposite responses on Tb when injected separately. Similarly, it is reported that isolated injection of methoxamine (18) and scopolamine (20) induced

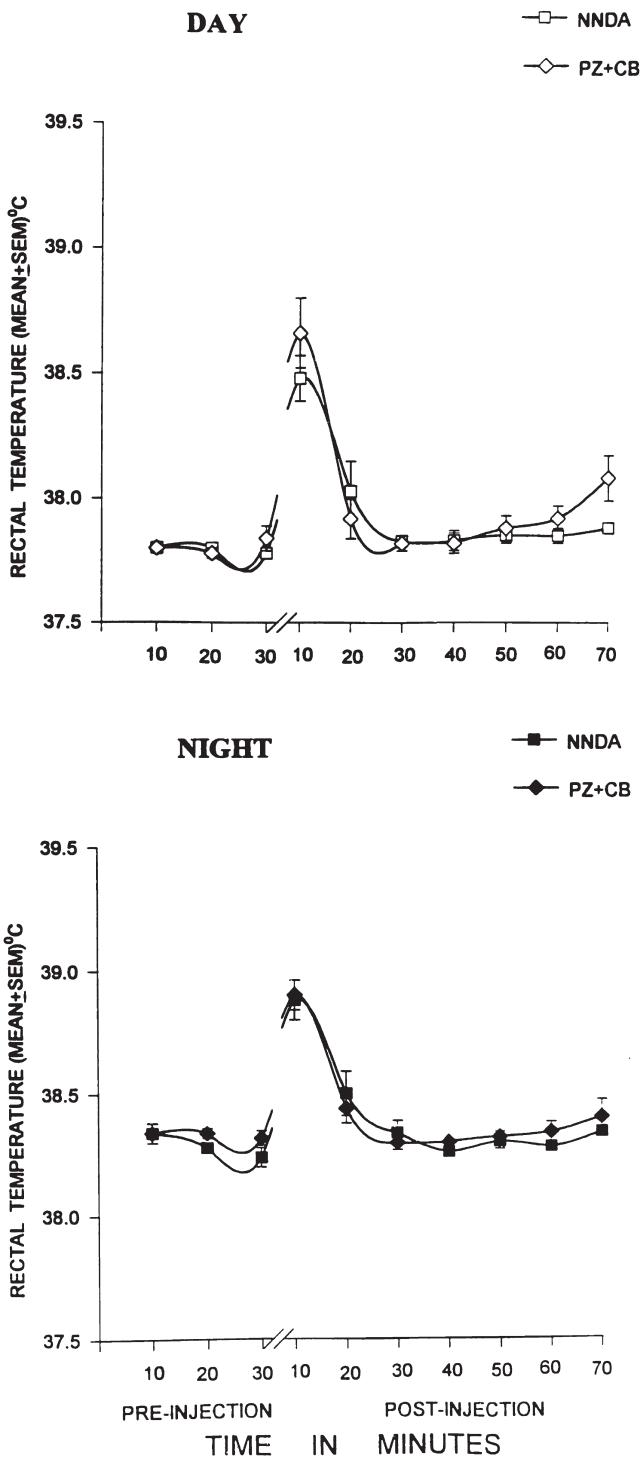

FIG. 3. The effect of prazosin followed by carbachol (PZ + CB) microinjection into the mPOAH compared to corresponding period of *N,N*-DA injection on rectal temperature during the day and the night recording periods are shown in this figure.

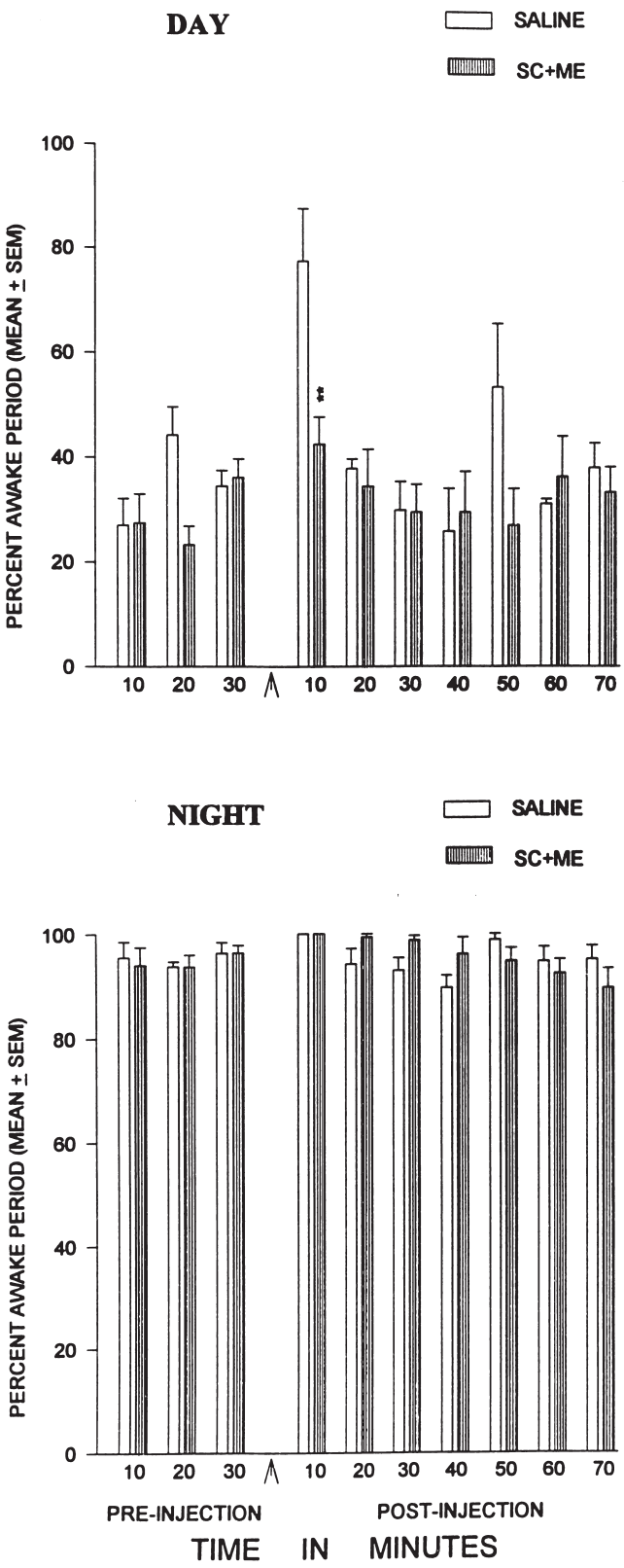

FIG. 4. The effects of saline and scopolamine followed by methoxamine (SC + ME) microinjection into the mPOAH on wakefulness (mean ± SEM) during the day (upper) and the night (lower) recording periods are shown in this figure. The arrows indicate the time of injections. Significance is as compared to saline injection during the corresponding postcombination injection period. $^{**}p < 0.01$.

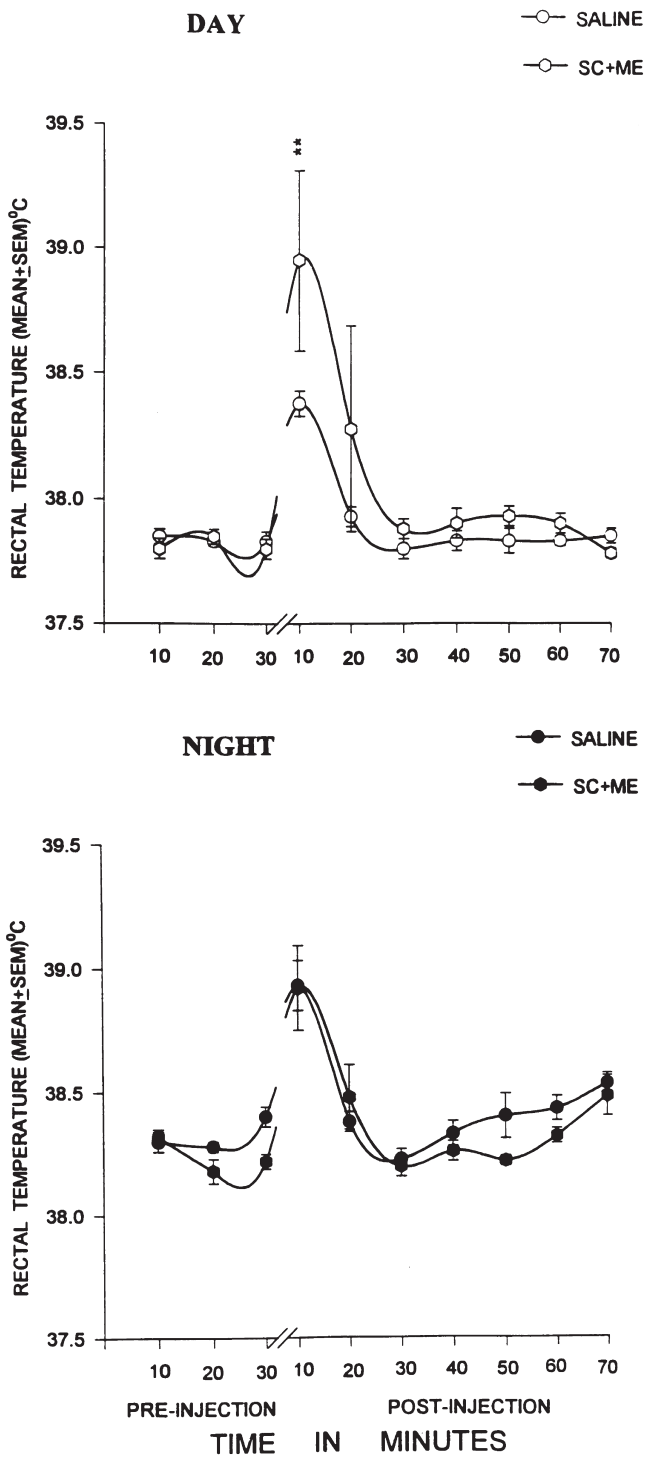

FIG. 5. The mean (±SEM) rectal temperature before and after saline and scopolamine followed by methoxamine (SC + ME) microinjection into the mPOAH during the day (upper traces) and the night (lower traces) are shown in this figure. The breaks indicate the time of injection. $^{**}p < 0.01$ significant compared to saline injection.

hypo- and hyperthermia, respectively; however, when they were injected in combination, the Trec remained unaffected (Fig. 5). The combination injection had a resultant effect on sleep–waking also, although in certain conditions it was not as apparant as that of the effect on Tb, which can, however, be explained as follows. Isolated injection showed that prazosin and carbachol both induced wakefulness, although the effect of the former was stronger. In this study, when both were injected in a combination during the day, wakefulness was precipitated, and the effect (i.e., wakefulness) was stronger than individual injection [compare isolated injection Fig. 4 of (18) and Fig. 2 of (20)]. Similarly, a combination of scopolamine and methoxamine did not significantly affect sleep and waking because in isolated injections the former induced sleep [Fig. 4 of (20)], while the latter, waking [Fig. 4 of (18)]. One could possibly argue that scopolamine might be ineffective, and methoxamine induced wakefulness during the day; hence, a combination of these chemicals induced wakefulness during the day. This is unlikely, because in previous studies independent injections of scopolamine induced sleep during the night in a background of wakefulness [Fig. 4 of (20)]; however, it was not statistically significant during the day because the rats were normally asleep. This was more apparent in this study when this combination was injected where the wakefulness-inducing effect of methoxamine was neutralized by a simultaneous sleep-inducing effect of scopolamine, and vice versa. The effects of these chemicals during the night can also be explained in the same way.

Because NE and ACh influence sleep, waking, and Tb, it is likely that these two inputs would interact for their regulation. Both adrenoceptive as well as adrenergic neurons are reported in the mPOAH (6,11,36,37). However, although cholinergic inputs and cholinoceptive neurons are present, cholinergic neurons have not been reported in the mPOAH (8,39). Therefore, the AChergic inputs to the mPOAH may be acting on cholinoceptive but noncholinergic neurons. The cholinergic inputs in the mPOAH may modulate release of other neurotransmitters by acting on heteroreceptors (31). It is unlikely that the cholinergic inputs act on the adrenergic neurons because in that situation the effect of adrenergic influence should have been expressed when a combination injection was made. On the contrary, the results of this study showed that a combination of prazosin and carbachol induced waking without affecting the Trec, while a combination of scopolamine and methoxamine neither affected the sleep–waking nor the Trec. One would expect such responses if the injected chemicals (in a combination) would induce a summated resultant effect by acting on non-NEergic neurons. At least GABAergic (4) and histaminergic (15) neurons and inputs are present in the mPOAH, and are known to influence sleep and wakefulness. These may play a significant role in modulating and integrating the responses that need to be studied.

Thus, ACh and NE interact in the mPOAH to regulate sleep–waking as well as Tb, and a resultant effect is expressed. However, it is difficult to comment if they act on the same or separate neuron(s) or group of neurons. This interaction provides the system (mPOAH in this case) a better flexibility of regulating sleep–wakefulness and Tb within a physiological limit that may possibly be explained as follows. In the case of derangement in one of the neurotransmitters or its receptor concentration, an appropriate change may be made in the other neurotransmitter systems, and homeostasis may be maintained. Needless to say, this is in addition to the capability of modulating the deranged system per se. Because NE and ACh are parts of sympathetic and parasympathetic regulatory mechanisms, which functionally are antagonistic to each other, this mechanism also allows for a better and finer tuning of the system.

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