EFFECT OF CARBACHOL INJECTION IN THE MEDIAL PREOPTIC AREA ON SLEEP-WAKEFULNESS AND BODY TEMPERATURE IN FREE MOVING RATS

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Abstract: The aim of the present study was to find out the changes in sleep-wakefulness and body temperature brought about by application of cholinergic agonist, carbachol, in the medial preoptic area (mPOA). Carbachol, when injected bilaterally into the mPOA of male rats, through chronically implanted cannulae, produced a fall in rectal temperature and long lasting arousal. There was temporal dissociation in the duration of changes produced in the two parameters. It is suggested that the cholinergic system at the medial preoptic area brings about arousal response and fall in body temperature through different circuits.

Key words: cholinergic system   medial preoptic area   sleep

wakefulness   thermoregulation   carbachol

INTRODUCTION

The medial preoptic area (mPOA) has a definite role to play in sleep-wakefulness (24, 26) and thermoregulation (6, 25). This area has acetylcholine containing nerve terminals (17, 21, 32, 37, 41). The probable functions of these terminals have been studied by local application of cholinergic agonist in the mPOA. Both hypothermic (5, 18, 20, 30) and hyperthermic (1) changes have been reported after the application of these drugs in the mPOA. Reports of changes in sleep-wakefulness produced by application of cholinergic agonists in the mPOA are scanty. According to some it produces sleep (16, 40), whereas some other reports have shown that it produces increased locomotor activity (29, 30). It has been shown to produce wakefulness when applied at the basal forebrain area (4).

This study was undertaken to clarify the changes in sleep-wakefulness and body temperature produced by the local application of carbachol into the mPOA, and to find out the possible correlation between changes in these two parameters.

METHODS

Experiments were conducted at an ambient temperature of 25±2°C on adult male wistar rats weighting between 200 and 300 gms. The animals were housed in separate cages in a room having a temperature of 25±2°C and constant light on from 5.00 hr to 19.00 hr. Food and water were given *ad lib*. Rats which showed behavioural sleep for 75% of the time during the day, were only taken for this study.

A study was conducted on two groups of rats (six each) under identical conditions. In group I rats, sleep-wakefulness was assessed on the basis of electrophysiological recordings (i.e. EEG, EMG, EOG) and behavioural observations. EEG, EMG and EOG were recorded as described earlier (11, 21). In group II rats, rectal temperature was recorded with the help of a rectal probe, taped at the base of the tail and connected to a digital telethermometer (11). These rats were trained to retain the rectal probe for the recording of the rectal temperature. Only those rats which tolerated the rectal probe for about two and a half hours were selected for thermal recording studies.

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Rest of the experimental procedures were identical in both the groups.

The basal rectal temperature of the animals was recorded for 4-5 days, before operation in all the animals. Guide cannulae (made from 22 gauge needle for intracerebral injection), along with its stylette, were implanted stereotaxically, 1 mm above the mPOA, under nembutal (35 mg/kg) anaesthesia. In both the groups, recordings were done on free moving animals, between 12.00 and 15.00 hrs, 4-5 days after the operation. By this time the animals had recovered from operative trauma, was evident from their rectal temperature and behaviour. Before recording, the animals were habituated to the recording room and cage.

In all the animals, the recording was done for 1/2 hr before and 2 hrs after the injection. After removing the stylette from the guide cannula, 1.0 µg of carbachol in 0.2 µl was injected bilaterally into the mPOA with the help of the injector cannula, connected to a microlitre syringe with a polyethelene tubing. The selection of the drug dose was based on the earlier report (3), and not more than one injection was given to any animal to avoid the effect of injury (2, 3). The injector cannula was left in place for 1 min after completion of injection to allow drug diffusion. During the process of injection, rats were restrained by hand. The site and spread of injection was confirmed by the histology of the brain after infusing ferric chloride (2%) into the same site of injection (2, 3). The injection sites are shown in the reconstructed (Fig. 1).

Readings obtained during the two and half hr recording time, were analysed. In the group I, five second epochs of electrophysiological recordings were considered together to quantify the periods of wakefulness, slow wave sleep and REM sleep on the basis of the criteria adopted in the earlier studies (22, 35, 38). An epoch was included in awake/sleep period if it was present for more than 2.5 sec. Rectal temperature was noted at five min interval in group II.

In both the groups, as the first step of analysis, Friedman test was used to find out the significance of the difference in the preinjection data (of different animals) over a period of 30 min. Readings were then
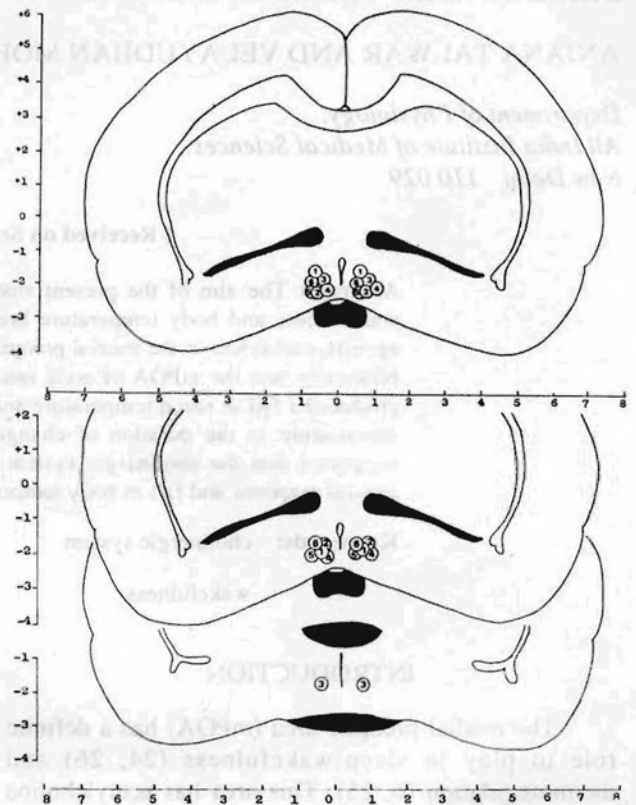

Fig. 1 :   Shows diagramatically the sites of injection of carbachol in different animals from where changes in sleep-wakefulness (A) and rectal temperature (B) were elicited. Brain section was reconstructed from the histological sections and drawn with the help of Degroot atlas.

analysed by two way ANOVA to see whether there was a significant difference after the injection. In the last step of analysis a comparison was made between preinjection mean readings with postinjection readings at every five mins, by multiple range test, to identify the readings which were significantly changed from the preinjection values.

RESULTS

Group I : There was no significant variation in readings from different animals and those obtained over a period of 30 min, before injection. Animals slept for 60-85% of this time, 8-12% of which was occupied by REM sleep. Microinjection of carbachol into the mPOA produced long lasting arousal as assessed by EEG, EMG
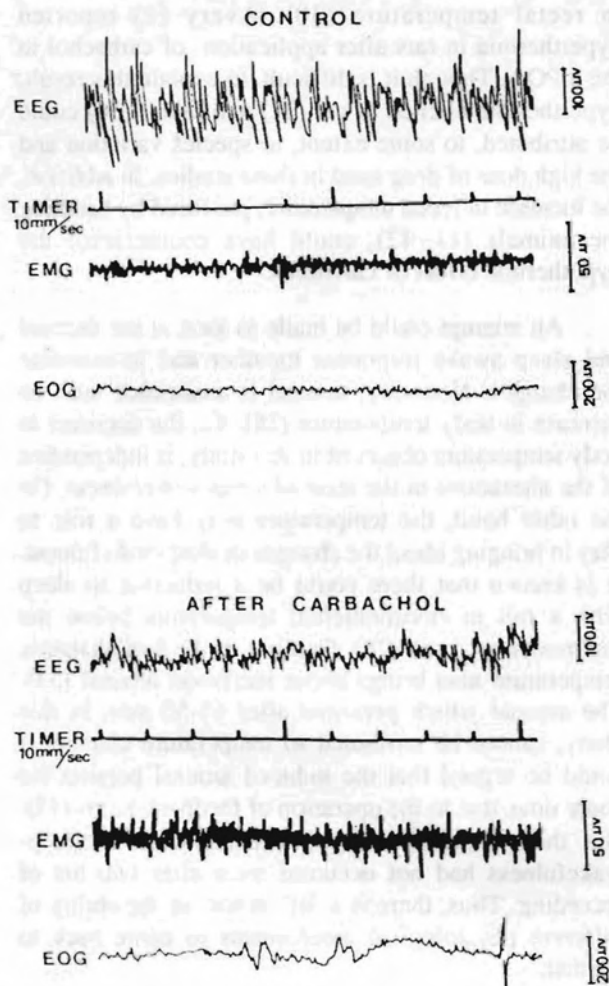
CONTROL

Fig. 2 : Shows representative record of EEG, EMG and EOG of a rat before and after injection of carbachol in the mPOA.
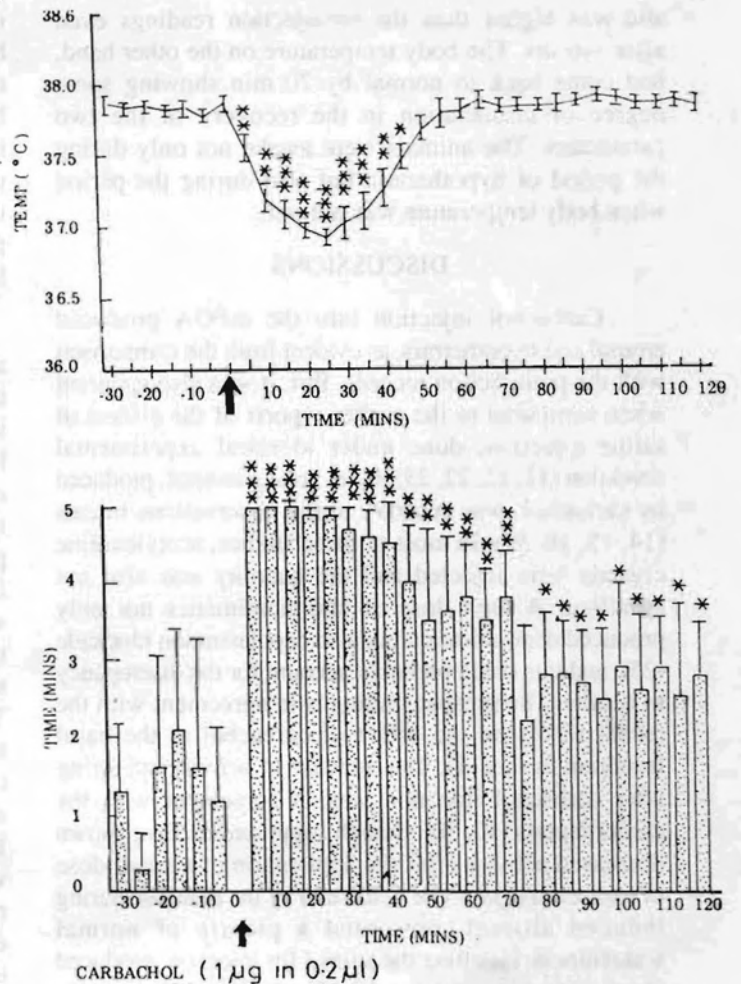

Fig. 3 : Shows the effect of injection of carbachol in the mPOA on the rectal temperature and awake period of two sets of rats during the day. Changes in rectal temperature shown above. The bar diagram below shows changes in awake period. *P<0.05; **P<0.01, ***P<0.001 level of significance of change compared to its own preinjection values.

EOG recordings (Fig. 2) and behavioural observation. The increased wakefulness was significant (P<0.01) upto 70 min (Fig. 3). During this period, rats were hyperactive and showed exploratory activity along with licking, grooming and scratching. The maximum arousal, observed upto 20-25 min, subsequently decreased gradually towards normal. But even after two hours, the basal level of wakefulness was not attained.

Group II : There was no significant intra group variation in the basal temperature readings of different animals. Basal rectal temperature during the day was 37.82±0.07°C. Microinjection of carbachol lowered the rectal temperature, which came back to normal by 45-

50 min (Fig. 3). The readings, for 45 min after the injection were significantly decreased (P<0.01). The decline in rectal temperature started immediately after the injection and the maximum fall occurred at 25 min after the injection.

The temporal sequence of decline in rectal temperature, observed in Group II animals, coincided with the arousal in Group I. The peak changes in the two parameters occurred around 20-25 min, but the increased wakefulness was significant up to 70 min

and was higher than the preinjection readings even after two hrs. The body temperature on the other hand, had come back to normal by 70 min showing some degree of dissociation in the recovery of the two parameters. The animals were awake not only during the period of hypothermia but also during the period when body temperature was normal.

DISCUSSIONS

Carbachol injection into the mPOA produced arousal and hypothermia, as evident from the comparison with the preinjection records. But, it was also apparent when compared to the earlier reports of the effects of saline injection, done under identical experimental condition (11, 12, 22, 23). Long lasting arousal, produced by carbachol, was contrary to the observations in cats (14, 15, 16, 39). In most of these studies, acetylcholine crystals were injected and the quantity was also not specified. A high dose of cholinomimetics not only produced depolarisation but also depolarisation blockade (25), and this could probably account for the discrepancy in results. The present finding is in agreement with the recent report on the effect of carbachol at the basal forebrain in rats (4). The increase in activity occurring after carbachol injection was in agreement with the earlier reports (10, 30), though some studies have shown decreased activity after the application of a small dose of carbachol (7, 9). The behaviour of the animals, during induced arousal, presented a picture of normal wakefulness. Handling the animal for injection produced short lasting arousal for 10-15 min (12, 23), but this cannot account for the prolonged wakefulness observed after the carbachol injection.

The rats were generally asleep during the afternoon (22, 35, 38) during which recordings were done. The duration of slow wave sleep, during pretreatment is comparable to that already reported but REM sleep was slightly less (22, 35, 38). Insufficient preinjection recording time may contribute towards this shorter duration of REM, though one hr period of habituation was considered sufficient for sleep studies (35).

Hypothermia, produced by injection of carbachol, is in agreement with the earlier reports (5, 18, 19, 20, 27, 29, 30) but the rebound increase in rectal temperature, as described earlier (10, 29), was not seen in our study. The cutaneous vasodilation may contribute to this fall

in rectal temperature (30). Avery (1) reported hyperthermia in rats after application of carbachol in the mPOA. Though it is difficult to explain this result, hyperthermia elicited in cat (31) and rabbit (36) could be attributed, to some extent, to species variation and the high dose of drug used in these studies. In addition, the increase in rectal temperature, produced by handling the animals (11, 12), could have counteracted the hypothermic effect of carbachol.

An attempt could be made to look at the thermal and sleep awake responses together and to correlate the changes. Normally, arousal is associated with an increase in body temperature (28). So, the decrease in body temperature observed in this study, is independent of the alterations in the state of sleep-wakefulness. On the other hand, the temperature may have a role to play in bringing about the changes in sleep-wakefulness. It is known that there could be a reduction in sleep with a fall in environmental temperature below the thermoneutral zone (28). Cooling of the hypothalamic temperature also brings about increased arousal (33). The arousal which persisted after 45-50 min, in this study, cannot be attributed to temperature change. It could be argued that the induced arousal persists for some time, due to the operation of feedback loops (13). But the restoration of the normal state of sleep-wakefulness had not occurred even after two hrs of recording. Thus, there is a difference in the ability of different physiological mechanisms to come back to normal.

Injection of norepinephrine in the mPOA also produced changes in sleep-wakefulness and body temperature which are similar in nature to that produced by carbachol (11, 23). Norepinephrine induced responses also had a different time course and were independent of each other. So, an involvement of identical efferent pathways for the changes produced by carbachol and norepinephrine cannot be ruled out. But, a common pathway for thermoregulatory response is unlikely as cold acclimatised animals responded differently to these drugs (30). A criticism which can be raised against norepinephrine injection study (11, 23), where temperature and sleep-wakefulness were simultaneously recorded in the same animal, is that the rectal probe would be a source of irritation which would affect the sleep-wakeful state of the animal. This

criticism was avoided by recording the two parameters in different sets of rats, under identical conditions, in this study.

On the basis of local injection studies, some functional role for the cholinergic system can be proposed. The different cholinergic fibres, arising from mesopontine regions (21, 32), might be having independent sleep regulatory and thermoregulatory functions at the level of the mPOA. Recent iontophoretic application studies have shown that some of the POA neurons, especially the cold sensitive ones, are stimulated

by acetylcholine (8, 34). The arousal response elicited by carbachol in the basal forebrain has been shown to be so powerful as to override the influence of cholinergic REM sleep inducing pontine mechanism (4). As a substantial number of brainstem cholonergic cells project both to the thalamus and the basal forebrain areas (21), the involvement of this system in the generalised arousal is indicated. The kind of receptors involved in these responses could not be assessed from the present study, but the involvement of M1 and M2 muscaranic receptors have been shown for locomotor activity in another study (9).

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