DISCUSSION
The observations made in this study suggest that:

i) both mPOA and lPOA are involved in S-W as well as thermoregulation;
ii) mPOA predominantly regulates sleep whereas lPOA is more involved in wakefulness;
iii) both mPOA and lPOA are involved in thermoregulation;
iv) mPOA is relatively more potent in Tb regulation as compared to that of lPOA;
v) mPOA also has the mechanism for independent regulation of S-wakefulness and Tb;
vii) the normal alterations in S-W cycle probably maintains the Tb within physiological limit;
viii) the m- and lPOA ARs are involved in S-W and Tb regulation;
ix) alpha-2 ARs are more potent for hypnogenesis;
ix) beta ARs are more potent for induction of wakefulness.

All these findings are discussed under following headings, for convenience:

[A] Observations are not artifacts or nonspecific effects
[B] Differential influence of mPOA and lPOA on S-W regulation
[C] Differential influence of mPOA and lPOA on Tb regulation
[D] mPOA also has mechanism for the independent regulation of S-W and Tb
[E] Influence of mPOA and lPOA ARs on S-W and Tb

[F] Influence of mPOA beta ARs on S-W and Tb

[G] Specificity of mPOA ARs in mediating S-W and Tb

[A] OBSERVATIONS ARE NOT ARTIFACTS OR NON-SPECIFIC EFFECTS:

Since the experiments were conducted on free moving animals, the handling of the animals could be a factor which may affect the observations. Therefore, before going into the details of the justifications and significance of the observations made, it will be pertinent to discuss, at the very outset, the validity of the observations made. The contention that the observed effects were specific due to the chemicals injected and not nonspecific ones or artifacts may be supported with the following observations and control studies:

i) The effects were chemical specific and even the effects during the day and the night were opposite on inactivation of the area;

ii) The effects were consistent and reproducible

iii) The effects were dose dependent;

iv) Different doses showed different responsiveness on the two physiological parameters e.g. 0.2 μl marcain was ineffective in influencing S-W whereas effective in influencing Tb;

v) The effects were reversible with time i.e. the effects were over after the duration of pharmacological action of the chemical was over;
vi) The effects were site specific;

vii) The degree of response was significantly different when injected into m- and lPOA;

viii) The effects were significantly different when compared with that of the control studies.

As control studies:

1) equal quantity of saline (used to dissolve all the chemicals except prazosin) and N,N-DA (used as a vehicle for dissolving prazosin) were injected into the same site, and

ii) same chemicals were injected into adjacent sites and the effects were observed.

It was found that control injections of saline and N,N-DA injection into the mPOA/lPOA induced wakefulness only for about 20 min during the day and was ineffective at night. This length of wakefulness after saline/N,N-DA injection was most likely due to handling of the rats which may be supported by the following observations:

1) The results were similar:

i) during both day and night (during day wakefulness was induced whereas at night when the rats were normally awake, it was maintained);

ii) after injection of either saline or chemicals which was ineffective in influencing S-W (e.g. propranolol);

iii) after injection of saline and other chemicals into surrounding areas;
iv) after injection of chemicals into surrounding areas not effective in influencing S-W;
v) on handling the rats for such a length of time (as for injection);
vii) after introduction of the injector cannula but without actually injecting any chemical (sham injection).  
2) Such type of handling effect has also been reported in other studies (Mohan kumar et al., 1984)

Similarly, the increase in Tb induced for 10-15 min after injection of saline/N,N-DA during day was likely due to because of handling and other nonspecific effects. This inference not only gets its support from the similar observations made above for S-W, but also from the following observations:

1) Injection of even those chemicals which induced hypothermia (clonidine, methoxamine, isoproterenol (Figs. IV. 3A&3C; VI. 1D) induced increase in Tb for this length of time during day

ii) Increase in the Tb due to saline/N,N-DA was of the order of the normal Tb at night. Normally the Tb during the day, when the rats usually sleep, was low (37.84 ± 0.03°C) as compared to that of night (38.34 ± 0.04°C) when the rats were more active;

iii) At night when the Tb was already high, the same dose of saline did not further affect the Tb.

Thus the above observations provide sufficient evidence to conclude that saline and N,N-DA did not affect S-W and Tb in the experiments mentioned here and the observations were not
not nonspecific effects or artifacts.

[B] DIFFERENTIAL INFLUENCE OF mPOA AND lPOA ON S-W REGULATION:

The results obtained in this study suggest that short term reversible anaesthetization of the m- and the lPOA by micro-injection of local anaesthetic, marcain, precipitated wakefulness during the day and sleep during the night. The rats normally spent more time in sleep during the day and in wakefulness during the night (Timo-Iaria et al., 1970; Mohan kumar et al., 1984). The wakefulness induced after inactivation of the mPOA/lPOA during day and increase in sleep at night was significantly higher as compared to respective control studies. Further, the wakefulness induced after inactivation of the mPOA during day was significantly higher, as compared to anaesthetization of the lPOA, where as inactivation of the lPOA was more effective than inactivation of the mPOA in inducing sleep during the night. This suggests that the m- and the lPOA have significantly different degree of tonic influence on sleep and wakefulness in freely moving rats.

Inactivation of the m- or the lPOA induced wakefulness during the day and sleep during the night. This suggests that both the areas of the POA have a tonic influence on sleep and wakefulness. It is consistent with earlier reports where mostly the role of POA as a whole has been studied in relation to sleep-wakefulness (Nauta, 1946; Velluti and Hernandez-Peon, 1963; Moruzzi, 1972; Lucas and Sterman, 1975; Mohan kumar et al., 1984). In earlier reports the tonic influence of POA was suggested mostly by lesion experiments where the effect could
not be studied after the recovery of the lesioned area. However, in this study normal sleep-wakefulness could be observed after the recovery of POA from the anaesthetizing effect of marcain. Though pharmacologically the anaesthetizing effect of marcain lasts for about 3 hr in the peripheral nervous system (Korolkovas, 1988), it was effective for 75-90 min in this study. The reasons may be: i) lower concentration or micro-quantity of marcain being injected; and ii) the period of marcain action in the peripheral and central nervous system may differ.

It is interesting to know that mPOA and lPOA have opposite influence on sleep and wakefulness depending on the time of recording viz. day or night. This observation supports the earlier findings that the POA is involved in both sleep and wakefulness (Nauta, 1946; Velluti and Hernandez-Peon, 1963; Moruzzi, 1972; Lucas and Sterman, 1975; Mohan kumar et al., 1984). This suggests that the POA probably is a modulator of the S-W. The effects induced after inactivation of mPOA might be because of withdrawal of POA influence on the caudal and the rostral brain stem reticular structures related to S-W (Bremer, 1970; 1975; Mancia et al., 1976; Edinger et al., 1977) and/or, vice versa (Mallick et al., 1986; Mohan Kumar et al., 1988). On the other hand, it may be said that the sleep and the awake active neurons or the concomitant EEG related neurons of the POA (Findlay and Hayward, 1969; Lincoln, 1969; Mallick et al., 1983; Kaitin, 1984; Szymusiak and McGinty, 1986; McGinty and Szymusiak, 1989) are preferably more active during the day and the night, respectively, causing the animals to exhibit
predominantly sleep during the day and wakefulness during the 
night in normal situation. Since during day more number of 
sleep related neurons of the POA were active, the inactivation 
of POA led to wakefulness. Conversely, at night more number of 
washing active neurons were active, the inactivation of POA by 
margin precipitated the opposite response i.e. sleep. 
Therefore, inactivation of POA precipitated sleep or 
wakefulness, opposite to that of the existing behavior, 
depending on the time of the day of inactivation (Alam and 
Mallick, 1990). This study, however, does not allow us to 
comment if the observed changes were due to inactivation of the 
cell bodies or the fibers passing through the POA. However, it 
has been reported (Szymusiak and McGinty, 1986b) that 
destruction of the cell bodies of the basal forebrain area 
including the lPOA leads to a disturbance in sleep and 
wakefulness.

The most important finding of this study is that though 
the m- and the lPOA could influence both sleep and wakefulness 
the magnitude of the effect differed (Fig I. 1&2). During day 
the wakefulness induced after inactivation of the mPOA was 
significantly higher than that of inactivation of the lPOA, 
while sleep induced after inactivation of the latter during 
night was significantly higher than that after inactivation of 
the former. These findings suggest that though both the 
divisions of the POA may influence sleep-wakefulness, the mPOA 
is likely to have a stronger sleep inducing effect while the 
lPOA is more effective in influencing tonic wakefulness. This
line of observation gets its support from earlier studies where it has been shown that though neurons related to sleep and wakefulness (Findlay and Hayward, 1969) and concomitant EEG changes (Lincoln, 1969) are scattered throughout the POA, the mPOA is rich in neurons whose activity increases with sleep (Kaitin, 1984) and cortical EEG synchronization (Mallick et al., 1983) while the lPOA is abundant in awake active neurons (Szymusiak and McGinty, 1986). During night 0.6 μl of marcain was required to affect sleep-wakefulness significantly when injected into the lPOA, as compared to only 0.4 μl into the mPOA. This is possibly because of the fact that histologically the neurons in the lPOA are more loosely arranged, as compared to that of the mPOA (Bleir, 1979, Morgane, 1979), which might necessitate larger quantity of marcain for inactivation of sufficient number of widely distributed awake active neurons present in the former. However, 0.4 μl marcain could significantly affect when injected into the mPOA during night and into the lPOA during the day. It may suggest that the sleep active neurons are probably closely packed as compared to that of the awake active neurons.

Present results show the differential role of the m- and the lPOA in sleep-wakefulness. Heuser et al. (1967) in their report found that local injection of progesterone only into mPOA could influence the sleep, while the same injected into the lPOA could not. Those results may be explained on the basis of differential influence of the m- and the lPOA on sleep-wakefulness as observed in this study. It is also possible that some of the variations in earlier studies (Sterman and
Clemente, 1968; Moruzzi, 1972; Sterman and Shouse, 1985) might have been because of such anatomical difference which was not taken into account so critically while placement of the electrodes.

[C] **DIFFERENTIAL INFLUENCE OF mPOA AND lPOA ON Tb REGULATION:**

The results of this part of study can be summarized as follows:

i) that reversible inactivation of the mPOA and the lPOA induced an increase in the Tb (Trec was taken as the representative of Tb) both during the day and the night;

ii) that inactivation of mPOA by even a low dose (0.2 µl) of marcain induced hyperthermia;

iii) that the inactivation of mPOA was more effective in influencing Tb than that of lPOA; and

iv) the POA mediated influence on absolute degree of change in the Tb was lesser in magnitude if there was simultaneous alteration in S-W/cortical EEG.

Normally the Tb was higher at night than during day. Further, the baseline mean Tb in the experimental before injections and the normal rats (without surgery) was comparable which suggests that recovery from surgery was complete and it is unlikely that surgical trauma and recording set up influenced the results. The higher Tb at night and relatively lower Tb during day is consistent with the earlier observations (Heller et al., 1983; Szymusiak and Satinoff, 1984; Szymusiak et al., 1985).

Inactivation of both mPOA and lPOA induced an increase in
Tb both during the day and the night, probably because POA is primarily a heat dissipating center (Boulant, 1980, 1991) which when knocked off, due to inactivation by marcain, precipitated an opposite response. Similar responses have been reported earlier. In earlier lesion studies (Gamble and Patton, 1953; Lipton, 1968; Nagel and Satinoff, 1980; Szymusiak et al., 1985) in rat and other mammalian species (Boulant, 1980, 1990) it has been reported that lesion of the POA as a whole induced hyperthermia. Thermoregulatory role of POA is further confirmed by the experiments where local warming and cooling of the POA is reported to induce heat production and heat dissipation, respectively, in different mammalian species (Hellstrom and Hammel, 1967; Jacobson and Squires, 1970; Boulant, 1980). The involvement of lPOA in Tb regulation also gets its support from unit activity studies where temperature sensitive neurons has also been reported in lPOA (Wit and Wang, 1967; Dean and Boulant, 1989). The anaesthetization of mPOA/lPOA by marcain probably inactivates the thermosensitive neurons assembly in the POA, and since the POA is a heat dissipating area, the heat dissipating function gets inactivated which results into hyperthermia.

Local application of marcain caused a steady increase in Tb which attained its peak within 2.5 h, in case of both m- and lPOA, which corresponds to its duration of pharmacological action (Korolkovas, 1988). The advantage of this study is that the specific tonic influence of the m- and lPOA could be studied by reversible inactivation where the effect of recovery
of the inactivated areas could also be observed.

Second important finding of this study is that the hyperthermia induced on inactivation of the mPOA was significantly higher than that of the lPOA. It is probably because comparatively more number of warm sensitive neurons are present in the mPOA (Wit and Wang, 1967; Dean and Boulant, 1989) than that of lPOA. No such study is available where the Tb has been studied after bilateral lesion/stimulation/inactivation of m- and lPOA. However, there is one study where the effects of unilateral anodal and cathodal lesion of m- and lPOA has been reported (Szymusiak and Satinoff, 1982). In that study, notwithstanding this finding, unilateral anodal lesion of mPOA and lPOA resulted in opposite influences on Tb viz hyper- and hypothermia respectively, though the cathodal lesion of both the areas produced only hyperthermia. The effect of anodal lesion which apparently seems contradictory to our report, may be explained as follows. Anodal lesion of the mPOA though damaged the area, the accumulated metal ions possibly stimulated the adjacent lPOA, and similarly, lesion of the lPOA probably stimulated the mPOA. Since mPOA is tonically more effective in affecting Tb regulation (as found in this study), its damage possibly caused withdrawal of its function but as lPOA was less effective, its stimulation (due to deposition of metal ions in mPOA) did not produce any effect and thus, hyperthermia was precipitated. On the other hand, damage to the lPOA resulted in stimulation of the mPOA (due to the deposition of metallic ions in lPOA), which is heat dissipation area, and there by hypothermia was precipitated.
(Alam and Mallick, 1991). Thus, though the mPOA is more potent in Tb regulation, it is unlikely that the m- and the lPOA have opposite influences. Since both types of lesions per se caused tissue damage, the investigators suggested that hypothermia was caused probably due to deposition of metallic ions causing irritation of the surrounding tissues away from the site of lesion. Further in unilateral lesion studies, the compensatory effect of the other counter part cannot be ruled out.

To rule out the effect of alteration in S-W and physical movement on Tb, the effect of inactivation of the mPOA/lPOA was studied in the background of PB treated rats. The Tb of PB treated rats decreased. The hypothermia and EEG synchronization and sleep induced after PB (i.p.) injection is consistent with the earlier reports (Killam, 1962; Olds and Olds, 1969; Nimmo, 1976; Kaitin, 1985). In such preparations (PB treated), though the EEG as well as physical movement was not influenced, injection of marcain into mPOA/lPOA induced a change in the Tb which reached a comparable peak temperature to that when marcain was injected into freely moving rats. This suggests that the POA mediated change in Tb is independent of simultaneous change in the S-W/cortical EEG. However, the absolute change in the Tb under PB treated condition was higher than when marcain was injected into the mPOA/lPOA in freely moving rats. It probably reflects that though inactivation of mPOA/lPOA per se is capable of inducing a higher change in the Tb independently, in normal situation the simultaneous
alterations in the S-W/EEG probably did not allow the Tb to change further and restricted it within limit (Alam and Mallick, 1991). The Tb did not increase further probably because the peak pharmacological effect of marcain was reached by then (Korolkovas, 1988). Possible involvement of different neuro-transmitters and/or receptor subtypes in influencing S-W and Tb independently cannot be ruled out. It was taken up for further study and are discussed below.

This study, however, does not claim to establish that the POA mediated influence on Tb and S-W can not be modulated by each other. It provides an experimental evidence that the POA mediated influence on the Tb can take place independent of simultaneous change in S-W state or vice versa. However, the simultaneous change in S-W/EEG probably maintains the Tb within the physiological limit.

[D1 mPOA ALSO HAS MECHANISM FOR THE INDEPENDENT REGULATION OF S-W AND Tb]

Normal recording as well as some of the earlier studies (Roberts and Robinson, 1969; McGinty and Szymusiak, 1990; Szymusiak et al., 1991) suggest a strong interdependent modulation of S-W and Tb. However, the results of this study suggest that POA mediated independent modulation of S-W and Tb is also possible. This is because of the fact that the S-W and the Tb (as the representative of Tb) responses could not be temporally correlated. If the changes in S-W and Tb were mediated through the same mechanism and/or they were dependent on each other, one would probably expect that:
i) the two effects would go hand-in-hand in magnitude and duration;

ii) the effect on Tb would have been opposite - hyper- and hypothermia along with precipitation of wakefulness and sleep, respectively; and

iii) 0.2 µl marclain would have also affected the S-W.

On the contrary,

i) 0.2 µl of marclain induced hyperthermia whereas this dose was ineffective in influencing S-W;

ii) Though 0.4 µl of marclain influenced both S-W and Tb
   a) only hyperthermia was induced both during day and night, whereas
   b) wakefulness was induced during day and sleep at night irrespective of hyperthermia; and
   c) the effect on S-W lasted for a very short period only for 30-90 min whereas the effect on Tb lasted for 6.30 to 7.00 hr.

Therefore, it is unlikely that alteration in one of the parameters was associated with simultaneous modulation in the other. One may inconclusively argue that during the day the wakefulness might have induced/triggered the hyperthermia but it cannot be supported from the observations in the night where hyperthermia was induced irrespective of S-W.

Neurons related to S-W (Mallick et al., 1983; Kaitin, 1984; Mallick et al., 1986) as well as sensitive to thermoregulation (Fox, 1974; Boulant, 1980, 1990) are present in the mPOA. It is unlikely that the marclain have inactivated neurons selectively. In an attempt to explain the mechanism of such dissociated effects on S-W and Tb, it may be suggested
that since the mPOA is the primary thermoregulatory center in the brain (central nervous system), even lower dose inactivation (with 0.2 μl) induced prolonged hyperthermia but could not influence S-W. On the other hand, on inactivation of the mPOA, one among several hypnogenic and S-W modulating areas in the brain (Moruzzi, 1972), other hypnogenic areas could effectively compensate for the function and hence the effect did not last longer (even with 0.4 μl). Duration of pharmacological action of marcain (Korolkovas, 1986) corresponded to its action on the Tb. Temporally dissociated responses of locomotor activity and body temperature after lesion of POA (Szymusiak and Satinoff, 1982) may also be explained in the same way. Further, the dissociated regulation of Tb and S-W has also been referred in earlier studies (Davenne and Krueger, 1987; Cady et al., 1987; Opp et al., 1991; Kapas et al., 1991). However, in all those studies, either i.c.v. or i.p. injections were made.

Though this study does not contradict that Tb and S-W may be correlated (Parmeggaini et al., 1987; McGinty and Szymusiak, 1990; Szymusiak et al., 1991), it does not favor that in normal situation the mPOA tonically influences S-W only by modulating Tb or vice versa. This study provides experimental evidence that mPOA may affect tonic S-W independent of its simultaneous influence on body temperature. Though the two functions may modulate each other and the mPOA may influence both of them, in normal situation mPOA probably exerts independent/dissociated influence on those functions. One of the advantages of this
study is that the tonic influence of the mPOA on S-W and Tb along with recovery could be studied, which were not possible in earlier lesion experiments. However, it is difficult to comment if the observed effects were due to inactivation of cell bodies or fibers passing through the mPOA.

**[I] INFLUENCE OF mPOA AND lPOA ALPHA ADRENERGIC RECEPTORS ON S-W AND Tb REGULATION:**

(1) **Role of Alpha-2 Adrenoceptors:**

The results of this study showed:

(i) that activation of mPOA alpha-2 AR by its agonist, clonidine induced sleep and hypothermia whereas the inactivation of mPOA alpha-2 AR by its antagonist, yohimbine induced wakefulness and hyperthermia. The responses were dose dependent.

(ii) that activation of lPOA alpha-2 AR by clonidine also induced sleep and hypothermia whereas their inactivation by yohimbine induced wakefulness and hyperthermia.

The onset of sleep on activation of alpha-2 and its inactivation leading to wakefulness suggests that alpha-2 AR is involved in sleep. This finding is confirmatory to the earlier suggestion made for alpha-2 AR after intrahypothalamic (Tsoucharis-Kupfer and Schmitt, 1972), intraperitoneal (Makela and Hilakivi, 1986) and oral administration of clonidine (Nicholson and Pascoe, 1991). In all those studies sleep was induced after clonidine injection. Stimulation of alpha-2 ARs probably leads to presynaptic inhibition to the release of NA which in turn induced sleep (Nicholson and Pascoe, 1991).
Specificity of alpha-2 AR in mediating clonidine induced sleep gets confirmed by induction of wakefulness after alpha-2 AR antagonism by yohimbine. Yohimbine possibly facilitates the release of NA by presynaptic alpha-2 AR inhibition leading to wakefulness. Intraperitoneal injection of yohimbine is reported to induce wakefulness in cats (Leppavuori and Putkonen, 1980). Idazoxan (oral route), a specific and potent antagonist of alpha-2 AR is also reported to increase the wakefulness (Nicholson and Pascoe, 1991). So far the mechanism is concerned, probably clonidine leads to hyperpolarization leading to inhibition of NA release whereas yohimbine blocks that hyperpolarization (Aghajanian and VanderMaelen, 1982). Further, the effect of clonidine on sleep is unlikely to be secondary to a change in Tb, because phenylephrine (a relatively more potent agonist for alpha-1) causing hypothermia did not cause sedation (Drew et al., 1979).

Moreover, yohimbine and clonidine injection during night and day did not produce any significant effect on S-W which suggests that probably POA neurons have variable responses to exogenous yohimbine/clonidine depending on the time of the day and night condition.

Hypothermia induced by activation of m- & lPOA alpha-2 AR by clonidine also gets its support from the earlier studies. Tsoucharis-Kupfer and Schmitt (1972) reported a dose dependent fall in body temperature after both systemic and intrahypothalamic (including POA) injection of clonidine. Some of the i.p., i.c.v. or intrahypothalamic injection
including POA are available (Review, Clark and Lipton, 1986). The findings of this study supports some of them (Tsoucaris-Kupfer and Schmitt, 1972; Lin et al., 1983; Lin et al., 1984). The primary reason for this discrepancy with other reports (Chu and Lin, 1983; Van Oene et al., 1982; Review, Clark and Lipton, 1986) seems to be the route and site of injection. Particularly in case of i.p. injection the injected agonists and antagonists acts primarily on the peripheral system and the effect on the central system becomes very little or negligible. However, in those earlier studies:

1) in most of the cases i.c.v. or i.p. injections were made and in a very limited number of studies POA along with some adjoining areas were studied;
2) both S-W and Tb were not studied simultaneously;
3) studies were not made exclusively in terms of m- and lPOA.

The advantages of the present study is that:

1) injections were made into POA, a very important central structure involved in both S-W and Tb regulation;
2) both S-W and Tb were studied simultaneously; and
3) studies were made exclusively in terms of m- and lPOA.

However, on the basis of this study, it is difficult to comment about the exact mechanism of clonidine and yohimbine action on alpha-2 ARs.

[2] Differential Involvement of m- and lPOA Alpha-2 System on S-W and Tb:

The results obtained after activation and inactivation of
alpha-2 AR of m- and lPOA by clonidine and yohimbine, respectively, suggest that:

i) the sleep and hypothermia induced due to activation of mPOA alpha-2 AR was of higher magnitude than that of lPOA

ii) wakefulness and hyperthermia induced due to inactivation of mPOA alpha-2 AR was more than that of lPOA.

These findings suggest that mPOA, through the mediation of alpha-2 AR, has a stronger influence on S-W as well as Tb regulation as compared to that of lPOA. Such type of systematic study is not available and this is the first study showing the differential role of m- and lPOA alpha-2 AR on S-W and Tb regulation. However, the differential responsiveness of m- and lPOA alpha-2 AR on S-W and Tb regulation can be supported by the distribution of alpha-2 AR as reported in earlier autoradiographic studies (Young and Kuhar, 1960; U'Prichard et al., 1983; Boyajian et al., 1987; Holets, 1990). These studies suggest that though alpha-2 AR are distributed both into the mPOA as well as the lPOA, a relatively higher concentration of alpha-2 AR is present in some periventricular area including mPOA. Probably this higher concentration of alpha-2 AR in mPOA results into higher degree of alpha-2 AR stimulation, thereby inducing more sleep as compared to that of lPOA where the concentration of alpha-2 AR is relatively less. In the same way, the inactivation of mPOA alpha-2 AR (by the same dose given into lPOA) causes inactivation of relatively more number of alpha-2 AR leading to comparatively more wakefulness after mPOA inactivation. The higher degree of hypo- and hyperthermia induced after mPOA alpha-2 AR activation and inactivation,
respectively, can also be justified on those criteria.

[3] Role of Alpha-1 Adrenoceptor:

The results of this study showed:

(i) that activation (by methoxamine) and inactivation (by prazosin) of mPOA alpha-1 AR induced wakefulness in a dose dependent manner;

(ii) that activation of nPOA alpha-1 AR induced hypothermia whereas the inactivation induced hyperthermia in a dose dependent manner; and

(iii) Similar responses (though of lesser degree) were recorded in case of lPOA.

Methoxamine induced wakefulness, probably because NA also acts to some extent on alpha-1 AR to mediate its wakefulness related response. However, prazosin even after being antagonist of alpha-1 AR induced wakefulness. Apparently this appears to be a paradox, however, such type of finding has also been reported in earlier studies (Vizzi et al., 1983; Pellejero et al., 1984; Makela and Hilakivi, 1986; Kleinlogel, 1989). In those studies, prazosin has been reported to induce wakefulness, at least for the initial 1-2 hr (Makela and Hilakivi, 1986), for the first half of the light period (Pellejero et al., 1984) and during eight hr of recording (Kleinlogel, 1989). Probably this happens because of the reason that prazosin exhibit a modest degree of prejunctional alpha-2 AR blocking activity as reported in peripheral nerves (Vizzi et al., 1983) and cardiac AR (Pieter et al., 1979). Probably, due to the blocking of alpha-1 AR, the NA which was already
available in the system together with the NA released due to blocking of presynaptic AR (by prazosin) became available for and acted on beta AR resulting into wakefulness.

Hypothermia induced by activation of mPOA alpha-1 AR (by methoxamine) and hyperthermia after blocking alpha-1 AR (by prazosin) suggest that NA mediated influence on Tb is probably mediated through alpha-1 AR. The discrepancy in the effect of alpha-1 agonist and antagonist on S-W and Tb i.e. similar effect of agonist and antagonist on S-W but different effect on Tb is probably because alpha-1 AR is predominantly involved in Tb regulation hence, its activation and inactivation induced opposite response on Tb. On the other hand wakefulness after both activation and inactivation of alpha-1 AR is caused probably due to the influence of the NA on other receptors where beta may a probable candidate. The combination studies provides probable answer of such discrepancies which is discussed later.

[4] Differential Involvement of m- and lPOA Alpha-1 System on S-W and Tb:

The results suggest that:

1) The wakefulness and hypothermia induced due to activation of mPOA alpha-1 AR were much intense than that of the lPOA

2) wakefulness and hyperthermia induced due to inactivation of mPOA alpha-1 AR were significantly higher than that of lPOA.

These findings suggest that the mPOA alpha-1 AR is more effective in influencing both S-W and Tb as compared to that of lPOA. Though such type of systematic study is not available,
yet these findings can be supported by the distribution of alpha-1 AR as reported by earlier autoradiographic studies (Jones et al., 1985; Palacios, 1987; Holets, 1990). These studies suggest that though alpha-1 AR are distributed both into mPOA as well as lPOA, the concentration of alpha-1 AR is relatively more in mPOA. The higher concentration of alpha-1 AR in mPOA (as compared to lPOA) possibly leads to activation of more number of alpha-1 AR and consequently induce more wakefulness and thermal changes as compared to that of lPOA where the concentration of alpha-1 AR is relatively less.

[F] INFLUENCE OF mPOA BKTA ADRENOCEPTORS ON S-W AND Tb:

In individual injection studies, it is difficult to rule out the possibility of endogenous NA acting on receptors in addition to the activation or inactivation of any one particular type of receptor. Since beta AR can be a potential target, the role of beta AR on S-W and Tb was also studied. The results suggests that:

i) beta AR activation induced wakefulness whereas its inactivation was ineffective;

ii) beta activation and inactivation induced hypo- and hyperthermia respectively.

Activation of beta AR leading to wakefulness suggests that probably beta is a predominantly involved post-synaptic AR to be involved in NA mediated wakefulness response. This can be supported from NA like responsiveness of isoproterenol, though in other system. Atkinson and Minneman (1991) reported that ISO increased cAMP accumulation by 17 fold in the neuronal clusters.
which was exactly similar to that induced by NA.

However, PRO even after being an antagonist of beta AR failed to induce sleep. Though this appears to be contradictory, however, ineffectiveness of PRO in influencing S-W has also been reported after injection into mPOA (Mohan Kumar et al., 1986). The hypo- and hyperthermia induced after activation and inactivation of beta receptors suggests that beta AR too is involved to some extent in hypothermic responsiveness of NA. This type of finding is also supported by earlier studies where injections were made into AHA/POA (Taucarisa-Kupfer and Schmitt, 1972; Clark and Lipton, 1986).

SUMMARY of INDIVIDUAL INJECTION STUDIES:

All the individual agonist/antagonist studies when taken together suggest that:

1) the local application of all the agonists and antagonists (for alpha-1, alpha-2 or beta AR) into the mPOA induced hypothermia whereas all the antagonists induced hyperthermia.

2) the effect of different agonists and antagonists on S-W responses were different:

a) the beta agonist (Isoproterenol) was effective in inducing wakefulness, while antagonist (Propranolol) was ineffective;

b) Alpha-2 agonist (Clonidine) and antagonist (Yohimbine) had opposite responses - the former induced S while the latter wakefulness; and

c) the alpha-1 agonist (Methoxamine) and antagonist (Prazosin) had identical (waking) responses.
SPECIFICITY OF mPOA ADRENOCEPTORS IN MEDIATING S-W and Tb:

From the results of single injection studies of either agonist or antagonist of ARs, one may be tempted to suggest that probably all the receptors (alpha-1, alpha-2 and beta) are involved in the regulation of S-W as well as Tb. But such an inference would be too much simplification of a complex system. This can be said because of the reason that in such an experimental design where an agonist or an antagonist (one out of at least the three receptors present) is locally injected, the effect of endogenous NA on other receptors present in the system cannot be ruled out (e.g. when alpha-1 is blocked by prazosin the role of endogenous NA on alpha-2 and beta can not be ruled out). Therefore, the results should be interpreted after keeping in mind the inactivation/activation of one type of receptor along with the availability and effect of endogenous NA on other receptors. Therefore, in order to understand the exact role of specific AR, at one point of time, the combination studies were conducted.

Before going into the details of the results obtained after combination studies, it is pertinent here to mention that in combination studies, notwithstanding with our earlier experimentations (where only one injection was made), a second penetration of the injector cannula was carried out which needs discussion. Second penetration of the cannula was unlikely to be nonphysiological and its use may be justified on following grounds:

1) That the second penetration was done within 10 min of the first injection and, therefore, for all practical purposes it
may possibly be considered to be one injection only; 

ii) that in separate pilot studies it was found that the chemical injection after second penetration reproduced a response similar to that induced by single injection; 

iii) that the responses induced by the second penetration also returned to normal after the effect of the injected chemical was over; and 

iv) that multiple injections in the same site has been reported (Lomax, 1969; Tsoucaris-Kupfer and Schmitt, 1972; Schenkel and Siegel, 1989; Clark et al., 1991).

Therefore, the results obtained after injection of combinations of chemicals were likely to be nonspecific effects or artifacts.

Two major conclusions have been derived on the basis of individual along with combination studies which are as follows:

[1] Sleep is Mediated by Alpha-2 and Wakefulness by Beta Adrenoceptors: 

The results from single and combination of chemical studies suggest that: 

i) activation of mPOA alpha-2 AR (by clonidine) always induced sleep (Fig. IV. 2A); 

ii) both activation and inactivation of alpha-1 AR failed to antagonize/nullify the sleep inducing effect of alpha-2 activation (Fig VI. 2E); 

iii) alpha-2 inactivation which normally induced wakefulness failed to do so when beta AR was blocked (Fig. IV.3D); 

iv) activation of mPOA beta AR (by isoproterenol) always
induced wakefulness (Fig. VI. 1A);
v) Beta activation not only nullified alpha-2 activation induced sleep rather also induced wakefulness (Fig. VI. 3E);
vi) beta inactivation failed to induce sleep (Fig. VI. B);
vii) even the inactivation of both alpha-1 and beta AR failed to induce sleep (Fig. VI. 2A);
viii) Inactivation of all the three receptors did not induce any effect on S-W (Fig. VI. 3C);

All these findings suggest that:
[1] alpha-2 AR is involved in sleep;
[2] beta AR is involved in wakefulness; and
[3] both sleep and wakefulness are active processes and are caused by activation of receptors.

The validity of these inferences can be justified on the following grounds:

[1] **Alpha-2 AR is Involved in Sleep:**

The justification for arriving at this conclusion is based on the following experimental observations:

i) alpha-2 activation always induced sleep - a response quite expected if the receptor is involved in sleep (Fig. IV. 2A);

ii) the sleep inducing effect of alpha-2 activation could not be nullified by both alpha-1 activation or inactivation which normally induced wakefulness (Fig. VI. 2E). This suggest that activation of alpha-2 is involved in sleep regulation.

However, at this point one may reasonably argue that if it is so then why the independent application of alpha-2 blocker (yohimbine) induced wakefulness. The induction of wakefulness
after yohimbine can be explained on the ground that in such situation more NA (out of pool in the system) probably became available for and activated beta receptor leading to the precipitation of wakefulness. The possibility of alpha-2 inactivation to be responsible for wakefulness further gets ruled out on the ground that inactivation of alpha-2 even with the inactivation of alpha-1 (normally inducing wakefulness) failed to induce wakefulness in a condition when beta receptor was blocked (Fig. VI. 3E).

[2] Beta AR is Involved in Wakefulness:

This conclusion can be supported on the grounds:

1) that the activation of beta receptors always induced wakefulness - a response quite expected if the receptor is involved in wakefulness (Fig. VI. 1A);

ii) beta activation not only nullified alpha-2 activation induced sleep, rather also induced wakefulness or in other words the wakefulness induced due to activation of beta receptors could not be nullified by even alpha-2 AR activation (Fig. VI. 3A). This suggests that activation of beta AR alone is sufficient and must to induce wakefulness;

iii) both alpha-2 and alpha-1 inactivation which induced wakefulness failed to do so when beta AR was blocked (Fig. VI. 3C). This suggests, that the presence of even more NA in the system can not induce wakefulness without the activation of beta AR.

Taken together, all these observation support the idea that activation of beta receptors is responsible for
[3] Both Sleep and Wakefulness are Active Processes:

The above results also support the idea that both sleep and wakefulness are active processes and are induced by the activation of alpha-2 and beta receptors, respectively. On the other hand, had sleep and wakefulness been passive processes they would have been induced by the inactivation of the beta and alpha-2 ARs, respectively. On contrary:

i) inactivation of beta AR did not induce sleep, rather was ineffective in inducing any effect on S-W (Fig. VI. 1);

ii) even the inactivation of both beta and alpha-1 AR failed to induce sleep (Fig. VI. 2A);

iii) the involvement of alpha-2 and alpha-1 inactivation on wakefulness gets ruled out on the ground that if alpha-1 and alpha-2 blocking could primarily induce wakefulness, they could have able to induce the same when injected in a combination along with beta blocker, propranolol. On the other hand, combination of alpha-1 and alpha-2 AR failed to induce wakefulness when beta AR was blocked (Fig. VI. 3C).

These observations also gets its support from the earlier concept that both sleep and wakefulness are induced by active mechanism (Moruzzi, 1972; Mancia and Marini, 1980).

The above interpretations may be supported with the general findings that alpha-2 AR is concentrated more in the medial POA (Bockaert et al., 1990; Young and Kuher, 1980) and probably therefore, the medial POA is a sleep inducing center. Further, probably an increase in beta receptor concentration in
the POA with age (Weiland and Wise, 1986) may be responsible for reduction in sleep with age.

[II] Body Temperature is Regulated by Alpha-1 Adrenoceptor:

A look at the results obtained after injection of individual chemicals suggest that the independent application of an agonist or an antagonist of alpha-1, alpha-2 and beta ARs induced hypo- and hyperthermia, respectively. Though from the results obtained after individual injection studies, one can infer that all the ARs are involved in POA mediated influence of NA on thermoregulation. However, the predominant involvement of alpha-1 AR in thermoregulation can be justified on the basis of the following observations, made after combination studies:

i) alpha-1 inactivation not only nullified the hypothermia induced by the alpha-2 AR activation rather induced hyperthermia afterwards (Fig. VI. 2F). On the other hand, alpha-1 activation potentiated the hypothermia induced by the alpha-2 AR activation (Fig. VI. 2F). It is important to note that both activation and inactivation of alpha-1 AR failed to antagonize the sleep induced due to alpha-2 activation (Fig. VI. 2E).

ii) Beta agonist failed to induce hypothermia when alpha-1 was blocked (Fig. VI. 2D).

iii) Both alpha-2 and beta AR activation (both induced hypothermia when given individually) failed to induce hypothermia when alpha-1 was blocked (Fig. VI. 3D). If beta and alpha-2 are involved in thermoregulation, one would expect hypothermia after activation of alpha-2 and beta AR even when
alpha-1 was blocked. On the contrary it was hyperthermia, a characteristic of alpha-1 inactivation, which was induced (Fig. VI. 3D).

All these findings support that alpha-1 AR is predominantly involved in POA mediated influence of NA on thermoregulation. Though the explanations as discussed above fits in to a great extent, the failure of alpha-1 activation to induce hypothermia when beta was blocked does not fit in to its full extent (Fig. VI. 2D). This combination instead of inducing hypothermia, was ineffective in inducing any change in Tb for sometime and afterwards induced hyperthermia. Since, activation of alpha-1 along with the inactivation of beta was ineffective on Tb, the interaction between those two drugs/chemicals and their receptors may not be ruled out. Nevertheless it may be said that since the physiological system is unlikely to function only through any one pathway/mechanism (a complex phenomena), alpha-1 AR mediated response may not be the only exclusive pathway for Tb regulation, however, alpha-1 AR mediated response may be the most dominating pathway or mechanism for Tb regulation. The dominating effect of alpha-1 AR on Tb may be indirectly supported from the earlier report where yohimbine failed to nullify an induced hypothermia which prazosin could overcome (Van Oene et al., 1982).

The thermoregulatory role of alpha-1 AR can also be supported by some general observations. The alpha-1 AR densities show a circadian rhythmicity in different regions including the medial preoptic nucleus (Wirz-Justice, 1987; Weiland and Wise, 1990) which may be responsible for the
diurnal rhythmicity of the body temperature. The change in diurnal rhythm of the alpha-1 AR in the middle to old age group rats (Weiland and Wise, 1986; Klein et al., 1990) may be responsible for the tendency to lose the diurnal rhythmicity of body temperature regulation in the latter (Glotzbach and Heller, 1989).

At the end it is important to note that in this study no attempt has been made to comment on the exact receptor mediated changes by which these receptors induce the effect of NA on S-W and body temperature regulation, for it was not the aim of this study. It only provides experimental evidence of NA-induced POA mediated independent regulation of S-W and body temperature. The most significant finding of this study is that different responses are dependent on the activation and inactivation of different groups (subtypes) of ARs and their interactions. Similar mechanism may be operating in other areas/systems and for other neurotransmitter action as well. Besides, pharmacokinetics of the receptor- neurotransmitters or receptor- agonist/antagonist has not been taken into account. Those factors may be taken up in computer modeling and neural network study and this study can be used as a basis for such an attempt. Such phenomena, if exist in other systems, may help explaining precipitation of varied symptoms in a diseased condition which may affect different hormonal and biochemical parameters.