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Role of Medial Preoptic Area Beta Adrenoceptors in the Regulation of Sleep-Wakefulness

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SOOD, S., J. K. DHAWAN, V. RAMESH, J. JOHN, G. GOPINATH AND V. M. KUMAR. *Role of medial preoptic area beta adrenoceptors in the regulation of in sleep-wakefulness*. PHARMACOL BIOCHEM BEHAV **57**(1/2) 1–5, 1997.—The role of the medial preoptic area (mPOA) beta adrenergic receptors in the regulation of sleep-wakefulness (S-W) was investigated in this study. S-W was assessed on the basis of polygraphic recording of EEG, EMG and EOG, in free moving rats. Intracerebral microinjection of beta agonist, isoproterenol, into the mPOA produced arousal. The study was also conducted on another set of rats in which noradrenergic (NE) innervation to the mPOA was destroyed by injecting 6-hydroxydopamine into the ventral noradrenergic bundle, in the brain stem. Local application of isoproterenol, into the mPOA, in these animals, did not produce any significant change in S-W. Thus, the increase in awake period obtained on isoproterenol administration was the result of its action on the presynaptic NE terminals. Possible involvement of other responses in the isoproterenol induced increase in wakefulness, is discussed. © 1997 Elsevier Science Inc.

Medial preoptic area   Beta receptors   Sleep   Wakefulness   Isoproterenol   Sexual arousal
6-hydroxydopamine   Ventral noradrenergic bundle

INVOLVEMENT of the medial preoptic area (mPOA) in the regulation of sleep-wakefulness (S-W) is well documented (3,13,16). The mPOA receives afferent noradrenergic (NE) projections from the brain stem, mainly via the ventral noradrenergic bundle (VNA) (1,19). Both alpha and beta receptors are present in the presynaptic and postsynaptic regions of NE terminals. NE elicits different responses when it acts on the presynaptic and postsynaptic receptors in the mPOA. It was shown to induce sleep through its action at the postsynaptic receptors, whereas, through presynaptic receptors, it induced wakefulness (10). Beta receptor agonist, isoproterenol, produced arousal when applied at the mPOA (11). The present study was carried out to determine whether the wakefulness produced by isoproterenol is mediated through presynaptic or postsynaptic receptors. The effect of application of isoproterenol at the mPOA was studied in one set of normal rats. In another group of rats, NE projections from the brain stem to the mPOA was destroyed by injecting 6-hydroxydopamine (6-OHDA) at the VNA. The effect of application of the beta agonist, at the mPOA, on S-W was studied in these rats.

MATERIALS AND METHODS

Experiments were conducted on male Wistar rats (200–250g) divided into three groups of five each. The experimental rats were housed in separate cages in an animal house having a controlled temperature (26 ± 2°C) and lighting (05.00–19.00 h). Food and water were provided ad lib. Rats were anaesthetised with sodium pentabarbitone (40 mg/kg bw) and EEG, EMG and EOG electrodes were chronically implanted for assessment of S-W (7). Bilateral cannulae were also chronically implanted for injection of drugs into the mPOA, as per De Groot's atlas (5). Recordings were carried out after four days of surgery, when the animals had recovered from the surgical trauma.

Changes in S-W were assessed after injecting 0.2 μl sterile normal saline, at the rate of 0.1 μl/min, at the mPOA, in one group of rats (Group A). Isoproterenol hydrochloride (Sigma Chemicals, St. Louis, USA), 2 μg dissolved in 0.2 μl sterile saline, was injected into the mPOA in the second group (Group B). In the third group (Group C), 8 μg of 6-OHDA

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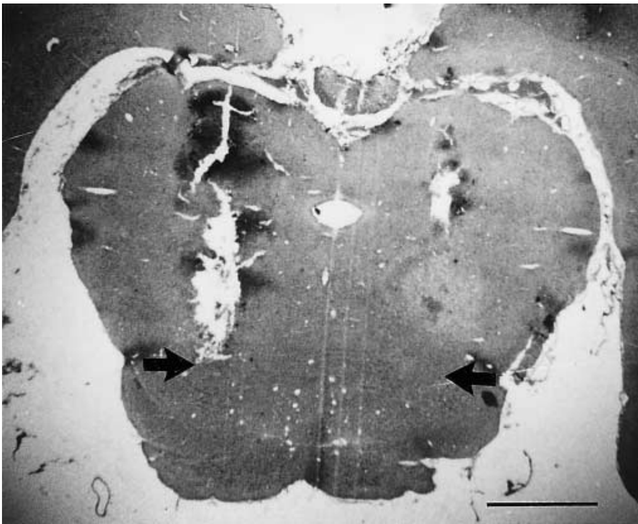

FIG. 1. Photomicrograph of rat brain section showing cannula tracts and the injection sites of 6-OHDA (indicated by arrows) at A1, L1.2, V-2 as per Konig and Klippel atlas (8), for destruction of the VNA. Scale bar = 1 mm. The 6-OHDA administered area was marked, at the end of the experiment, by ferric chloride injection. So the size of the stain does not indicate the extent of lesion. Effect of lesion was confirmed by histofluorescence as shown in Fig. 2.

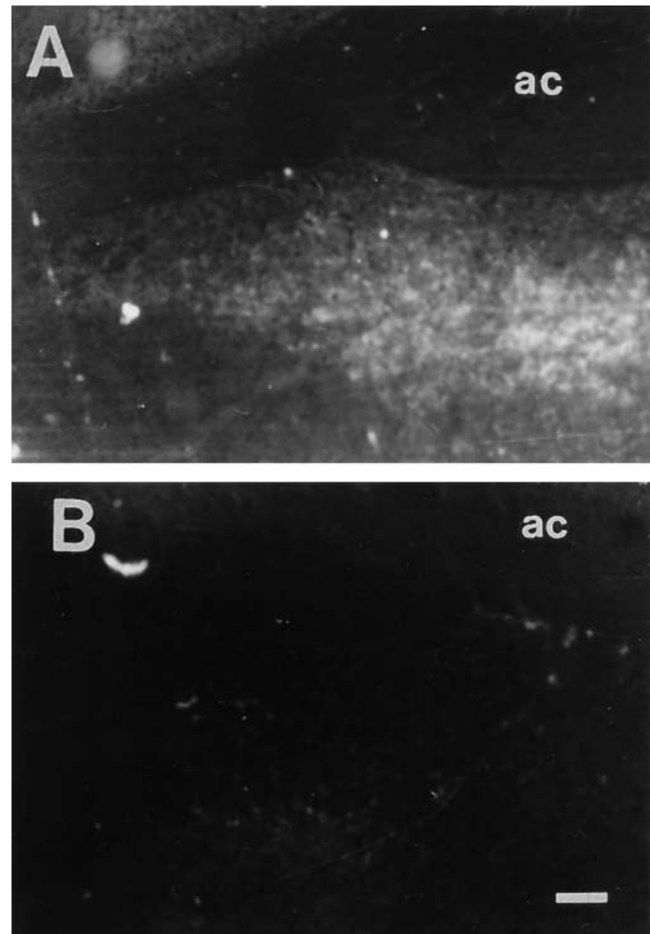

FIG. 2. Photomicrograph of rat brain section at the level of the mPOA, below the anterior commissure, showing fluorescent catecholaminergic terminals after the glyoxylic acid staining (A). This normal brain was stained along with the brain of the experimental animal which received bilateral injection of 6-OHDA at the VNA. Disappearance of florescence at the same location in the 6-OHDA treated rats indicates total destruction of catecholaminergic terminals (B); ac-anterior commissure. Scale bar = 50 μm.

dissolved in 1 μl saline, containing 0.1 mg/ml ascorbic acid, was injected into the VNA, at A1, L1.2, V2 as per Konig and Klippel atlas, (8), to destroy the ascending catecholamine fibres which project to the mPOA (Fig. 1). Isoproterenol (2 μg in 0.2 μl) was administered at the mPOA, three days after 6-OHDA injection in the VNA, and the changes in S-W were studied during the day time (11.30–17.30 h).

Rats were introduced into the recording cage 1 h prior to S-W recording and left undisturbed, with plugged cables. Food and water were provided ad libitum in the recording cage. S-W was assessed on the basis of polygraphic recording of EEG, EMG, EOG and behavioural observations. All the parameters were recorded for 90 min before, and 180 min after the injection of drugs at the mPOA. The S-W records were split into 30 s epochs, and visually scored as per the criteria described by Timo-Iaria et al (17). Sleep period was classified into two stages, namely slow wave sleep (SWS), and REM sleep (PS). EEG during wake period (W) had low amplitude, high frequency (desynchronized) waves. EMG activity was high, and it had gross body movement artifacts during activities like grooming, scratching, orienting and locomotion. The EOG record had eyeball movement artifacts, at times. SWS stage was characterized by low frequency, high amplitude slow (synchronized) EEG waves and marked reduction in EMG activity. The rats assumed a sleeping posture during this period. The PS was characterized by desynchronized EEG, drastic reduction in EMG and spiky waves in the EOG. The injection sites were confirmed histologically at the end of the experiment (2). In the third group, the extent of NE fibre degeneration was assessed by monoamine histofluorescence, by glyoxylic acid technique (18). A normal rat was sacrificed and processed simultaneously for histofluorescence to verify the staining procedure (Fig. 2).

The S-W records were divided into bins of 5 min each and the preinjection and postinjection data were statistically analysed. The preinjection data from each group was analysed by Friedman's two way analysis of variance to find out the possible significance of variation within the groups. The postinjection readings from each bin were compared with preinjection average values, using Friedman's multiple range test, to find out the effect of the control injection in group A animals. Wilcoxon's two-sample Rank (Mann–Whitney) test was applied for comparing each bin of group A (saline) with group B (isoproterenol) and group C (isoproterenol after VNA lesion). Preinjection (90 min) and postinjection (40 min immediately after injection) values of different stages of S-W, i.e. wakefulness (W), slow wave sleep (SWS), paradoxical sleep (PS) and total sleep, were compared by Wilcoxon's two-sample rank (Mann–Whitney) test.

RESULTS

There was no significant variation in S-W in the preinjection records obtained from different animals. The animals showed normal polycyclic pattern of sleep awake cycle during this period. Basal recordings from different groups of rats had

TABLE 1

PERCENTAGE CHANGES IN SLEEP-WAKEFULNESS

Group	Preinjection (90 min)				Postinjection (40 min)			
	W	SWS	PS	TS	W	SWS	PS	TS
A	51.2 ± 17.8	45.8 ± 18.6	3.20 ± 3.00	48.6 ± 17.8	60.4 ± 22.6	39.4 ± 22.0	0.40 ± 0.60	39.4 ± 22.6
B	34.6 ± 2.80	61.6 ± 3.40	3.00 ± 2.60	64.6 ± 2.40	96.4 ± 6.40**	3.60 ± 6.40**	0	3.40 ± 6.40**
C	59.8 ± 28.2	38.6 ± 27.0	2.20 ± 3.20	41.4 ± 28.6	71.8 ± 23.0	28.4 ± 23.0*	0	28.0 ± 23.0

TS = total sleep (SWS + PS). $*p < 0.05$; $**p < 0.01$.

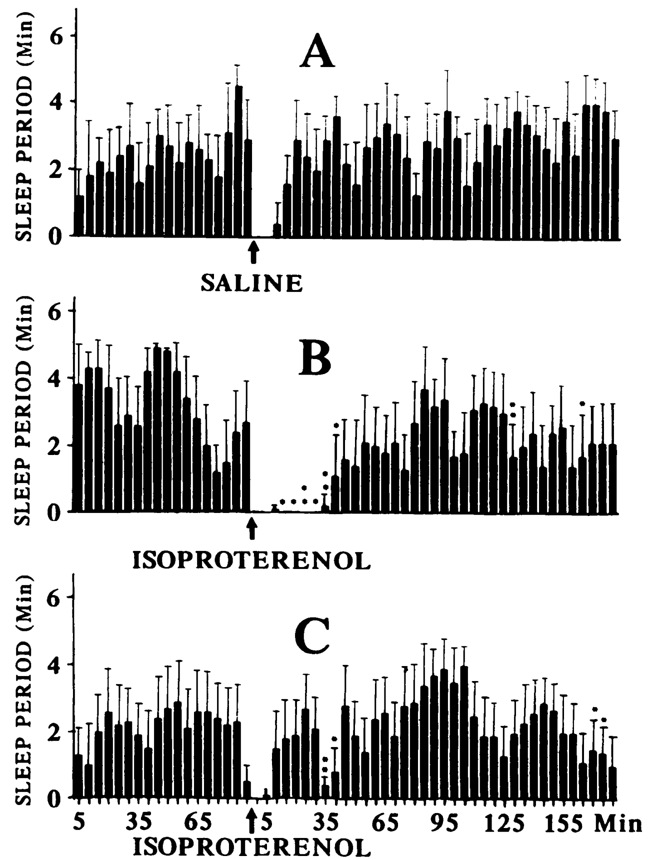

FIG. 3. The bar diagrams show the effect of application of saline (A) and isoproterenol (B and C), in the mPOA, on sleep-wakefulness. Injection point is indicated by an arrow. Application of isoproterenol produced an increase in wakefulness (decrease in sleep) when compared to the control (saline) in normal rats (B). The same drug did not produce any such injection bound change in S-W in the VNA lesioned rats (C). Y axis shows the sleep period expressed as min per bins of 5 min duration. Data are mean ± SD. $*p < 0.05$, $**p < 0.01$.

mean sleep time varying from 41.4 to 64.6% (Table 1). The sleep record was dominated by SWS, while PS was only occasionally observed.

Group A

Injection of saline during the day produced short-lasting arousal for about 15 min, probably due to the handling of the animals for injection (Fig. 3A). These rats reverted to the preinjection state after that period.

Group B

There was no significant variation in the preinjection record obtained from different animals. The SWS was significantly reduced, and W increased, during the 40 min after the injection of isoproterenol (Table 1, Fig. 3). Polygraphic record during this period was characterized by desynchronized EEG, high EMG and EOG artifacts. During this period, animals showed behaviour typical of active wakefulness such as exploration, scratching, grooming etc. No PS was observed during this period in any of the rats. Significant reduction in sleep was

also obtained at 130–135 min and 165–170 min bins after injection (Fig. 3B).

Group C

The preinjection record of this group of VNA lesioned rats was not significantly different from that of the other two groups of rats. Though the postinjection record did not reveal any significant change in sleep immediately after the injection, when compared to the control group, a reduction was seen at 35–45 min, and 160–175 min (Fig. 3C).

Injection sites were confirmed histologically. There was marked reduction in fluorescence (NE) in the mPOA indicating a near total destruction of the NE terminals (Fig. 2B). Reduction in the NE fluorescence was restricted to the regions below anterior commissure and the POA, in three rats. In two other animals it was reduced in the cortex as well. On the other hand, in the control animals, a high degree of fluorescence was observed at the dorsolateral part of the mPOA, below the anterior commissure (Fig. 2A). Fluorescence was also observed throughout the POA, especially in the medial part, near the ventricles and above the optic chiasma, in addition to regions like the cortex.

DISCUSSION

Isoproterenol (2 μg), when administered at the mPOA, induced injection-bound arousal in normal rats. This drug did not produce any such change in S-W in the VNA lesioned rats. Thus, it could be suggested from the present study that isoproterenol produces arousal by acting on the presynaptic receptors, as the response was not obtained after the removal of the NE nerve terminals (with the presynaptic receptors). At the same time, a postsynaptic site of action of this drug could also be suggested as the removal of NE afferents to the mPOA may result in a reduction in the endogenous release of NE. In this situation of decreased availability of endogenous adrenergic transmitter, in the VNA lesioned rats, the externally applied beta adrenergic agonist would be less effective in producing its arousal effect. Earlier studies have shown that the destruction of NE fibres in the mPOA produces an increase in wakefulness (10). But in the present study there was only a tendency towards an increased arousal, in the VNA lesioned rats (as was evident from the higher preinjection awake periods) though not statistically significant. The postsynaptic membranes are usually hypersensitive after denervation (6). So, it should have produced an arousal response with greater magnitude in the VNA lesioned rats, if isoproterenol was acting on the postsynaptic beta receptors. The lack of response to isoproterenol after VNA lesion could also be suggested as an argument against postsynaptic action of this drug.

The arousal produced by isoproterenol in the normal rats needs some explanation as the beta antagonist, when applied at the mPOA, does not produce any change in S-W, though it has a powerful inhibitory action on sex drive (4,12). Ohno et al. (14) have shown that the stimulation of presynaptic beta receptors in the hypothalamus caused an increased release of NE. It was shown that sleep was induced by the action of NE at the postsynaptic receptors, whereas through presynaptic receptors, it produced wakefulness (4,9,10,15). Even if it is argued that the presynaptic action of isoproterenol can account for the arousal, it must be kept in mind that the mPOA plays a key role in the regulation of several other functions like reproduction and thermoregulation. These functions are also influenced by NE administration. Beta receptors in the mPOA have been shown to be important in inducing sexual arousal (12). Sexual arousal would also cause generalized arousal. So, it is possible that in normal rats (without VNA lesion) the injected isoproterenol would have produced arousal through a stimulatory action on the sexual arousal system.

It may be hypothesized that there are different sets of neurons in the mPOA, controlling sleep, sex drive and other functions. It has been shown that the increased release of NE produces sleep (10). It could be possible that sleep inducing neurons are primarily stimulated through alpha receptors (10,15), whereas the neurons controlling the sex drive have beta receptors on their presynaptic NE terminals. Owing to the lack of anatomical segregation of these two types of neurons, isoproterenol injected into the mPOA may well act on both sets of neurons. The sexual arousal would be having an overriding influence on the hypnogenic effects (12).

The significance of S-W alterations obtained at 130–135 min and 165–170 min in group B, and 35–45 min and 160–175 min in group C, could be attributed to the coincidence of random changes due to the polycyclic sleep pattern of rats. However, they are not given importance as these significant changes were neither injection bound nor obtained on continuous bins (Fig. 3).

It is possible that isoproterenol may produce different responses at lower and higher doses. Though a dose response study would give a complete picture about the action of isoproterenol, the amount of drug used in this study is comparable to that used in the earlier report (11). In contrast to that report, in which isoproterenol induced arousal for 75 min, the results from the present study showed that the same dose induced arousal for 40 min only. Preinjection S-W was recorded for 90 min in this study to get a good basal reading. This value of awake period is close to the normal percentage of awake period in rats, during the day (7). On the other hand, in the earlier study, a preinjection record of only 30 min was taken. The high level of sleep in the preinjection record of the previous study would have exaggerated the postinjection sleep level.

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