Adrenergic and Cholinergic Modulation of Spontaneous and Brain Stem Reticular Formation Stimulation Induced Desynchronization of the Cortical EEG in Freely Moving Behaving Cats

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The brain stem reticular formation is responsible for EEG desynchronization. It bears, among others, adrenergic and cholinergic neurons. Neurons of this area project directly or indirectly to the cortex. Isolated studies have shown that both the adrenergic as well as the cholinergic inputs may induce and modulate cortical EEG desynchronization, however, their relative role was unknown. In this study, the differential influence of adrenergic and cholinergic inputs in cortical EEG desynchronization during spontaneous and brain stem reticular formation stimulation induced wakefulness was investigated in freely moving chronically prepared cats. The cats were chronically prepared for standard electrophysiologcial sleep-wakefulness recording and also with stimulating electrode in the midbrain reticular formation. After recovery, baseline recording of sleep-wakefulness was done. Thereafter, either adrenergic α1-adrenoceptor antagonist, prazosin (1 mg/Kg), α2-adrenoceptor agonist, clonidine (25 μg/Kg), α2-adrenoceptor antagonist, yohimbine (1 mg/Kg), β-adrenoceptor antagonist, propranolol (10 mg/kg) or, cholinergic muscarinic receptor antagonist, scopolamine (0.5 mg/Kg) was injected i.p. and the effect on spontaneous as well as midbrain reticular formation stimulation induced EEG desynchronization investigated. It was found that desynchronization of the EEG was modulated significantly by both, the cholinergic as well as by the adrenergic systems. The cholinergic action was mediated through the muscarinic receptor while the adrenergic through the α1-adrenoceptor. The effect of the former was relatively long lasting and it reduced the high frequency waves while the latter was more effective in increasing the rhythmicity of the EEG waves in the power spectrum record. These differences possibly have relevance in EEG desynchronization during wakefulness and REM sleep. (Sleep and Hypnosis 1999;1:14-21)

Key words : *adrenergic, agonist, antagonist, brain stem reticular formation, cholinergic, EEG*

INTRODUCTION

It is almost half a century that Moruzzi and Magoun (1), through their epoch making study, showed that the brain stem reticular formation (BSRF) is primarily responsible for cortical EEG desynchronization (DSYN). While the overall concept still holds good, the role played by different BSRF neurons and respective neurotransmitters for modulation of EEG continues to be under intense investigation. Stimulation of both adrenergic (2) and cholinergic (3) neuron rich areas in the brain are known to induce EEG DSYN. Local and systemic injections of noradrenergic and cholinergic agonist (2,4-6) have been reported to induce DSYN while their antagonist (5,7) induced synchronization (SYN) of the cortical EEG. Level of acetylcholine (ACh) was found to increase in the cortex during DSYN (8). However, the norepinephrine(NE)-ergic neurons are active during non-REM sleep state and cease firing during REM sleep although the EEG remains desynchronized during both the states. Similarly, there are certain group of cholinergic neurons which are active during REM sleep (9). Thus, it is likely that the adrenergic and the cholinergic systems have different roles to play in DSYN of the cortical EEG. Recently

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it has been reported that possibly separate BSRF neurons are involved in wakefulness and REM sleep (10). Interestingly, EEG remains desynchronized during two distinctly different states viz. wakefulness and rapid eye movement (REM) sleep. NE has been shown by invitro studies to depolarize the cholinergic neurons (11) which might be responsible for DSYN of EEG. Although localized regions in the BSRF are reported to be rich in either adrenergic (viz. locus coeruleus) or cholinergic (viz. laterodorsal tegmentum) neurons (12,13), such neurons are also scattered throughout the BSRF (14,15). Besides, the BSRF neurons project to the cortex relaying to several subcortical areas, containing neurons of various neurotransmitters. Hence, the BSRF stimulation induced DSYN of the EEG could at least partly be due to either the adrenergic or the cholinergic or both influences. Neurons containing other neurotransmitters including the peptidergic, are also scattered in the BSRF and may influence DSYN (16-19), however, for simplicity of investigation and understanding they have not been studied here. Although isolated studies have been conducted to investigate the role of adrenergic and cholinergic influence on cortical EEG changes, there was a lack of knowledge about their relative role specially with reference to BSRF stimulation induced DSYN. Hence, in this study, the BSRF was stimulated and the effect on DSYN of the EEG studied in the presence and absence of cholinergic and adrenergic antagonist in freely moving cats.

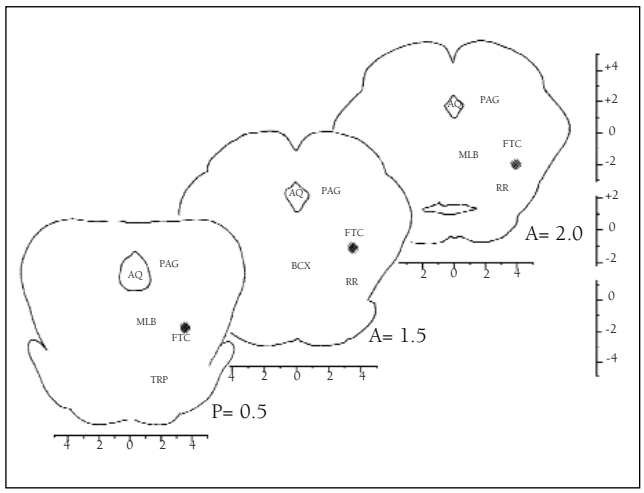
METHODS

Under surgical anaesthesia (Sodium pentobarbitone, 35mg/kg, i.v) 6 adult male cats (2.5-3.0 Kg) were chronically implanted with electrodes for standard sleep-wakefulness recording (20). Two small stainless steel screw electrodes were implanted bilaterally in the skull above the sensorimotor cortex for recording electroencephalogram (EEG). A pair of similar screw electrodes were implanted over the orbital bone to record bipolar electrooculogram (EOG). Flexible insulated wires were used to record bipolar electromyogram (EMG) from the dorsal neck muscles. Stainless steel tripolar electrode (150 μm diameter, approx 1.0 mm tip separation) was implanted at stereotaxic (21) co-ordinates of A1.0±1.0mm, L3.5±0.5mm, H1.5±0.5mm (22) for bipolar stimulation of the midbrain reticular formation (MRF). The other ends of the recording and the stimulating electrodes were connected to two separate female plugs and fixed on to the skull. The cats were recovered from surgical trauma with adequate post-operative care. During these recovery days the cats were acclimatized to the recording chamber.

Standard sleep-wakefulness recording began 10-14 days after surgery. On the day of recording the cat was left in a semi sound-proof recording chamber for at least one hour before the recording was started. Bipolar EEG, EOG and EMG were recorded in unrestrained freely moving cats. The recording was continued before and after 10+1sec bipolar electrical stimulation (100 Hz, 300 μsec, 200-400 μA) of the MRF. The stimulation strength was adjusted so that the stimulation induced DSYN of the EEG outlasted the period of stimulation without significant movement and activity of the animal. The behavior of the animal was monitored through an one way window without disturbing the animal. At least 3 episodes of MRF stimulation induced DSYN of the EEG (without injection of agonist and antagonist) were recorded. Thereafter either cholinergic antagonist, scopolamine (0.5 mg/Kg), adrenergic α2-agonist, clonidine (25 μg/Kg), α2-antagonist, yohimbine (1 mg/Kg), α1-antagonist, prazosin (1 mg/Kg) or β-antagonist, propranolol (10 mg/Kg) was injected intraperitoneally (i.p.) and recording continued. The dose of the chemicals used were based on previous reports (5,6). At the background of i.p. injection of adrenergic and cholinergic agonist or antagonist, at least 3 episodes of MRF stimulation induced effects on EEG were recorded. The MRF stimulation induced effects were studied between 30-60 mins of i.p. injection of all the chemicals except yohimbine. In case of yohimbine the effect was studied between 100 mins to 150 mins because yohimbine induced DSYN lasted for a mean period of 107 ±14.6 mins. All the chemicals (Sigma, USA), except prazosin which was dissolved in N,N,dimethyl- acetamide, were dissolved in distilled water and injected (i.p.) into the cats in a constant volume of 1ml. Handling the cats without any injection and injection of agonist and antagonist served control for each other. The effects of each of the chemicals were studied on three cats each at random. There was a gap of at least six days before a second injection was made into the same animal.

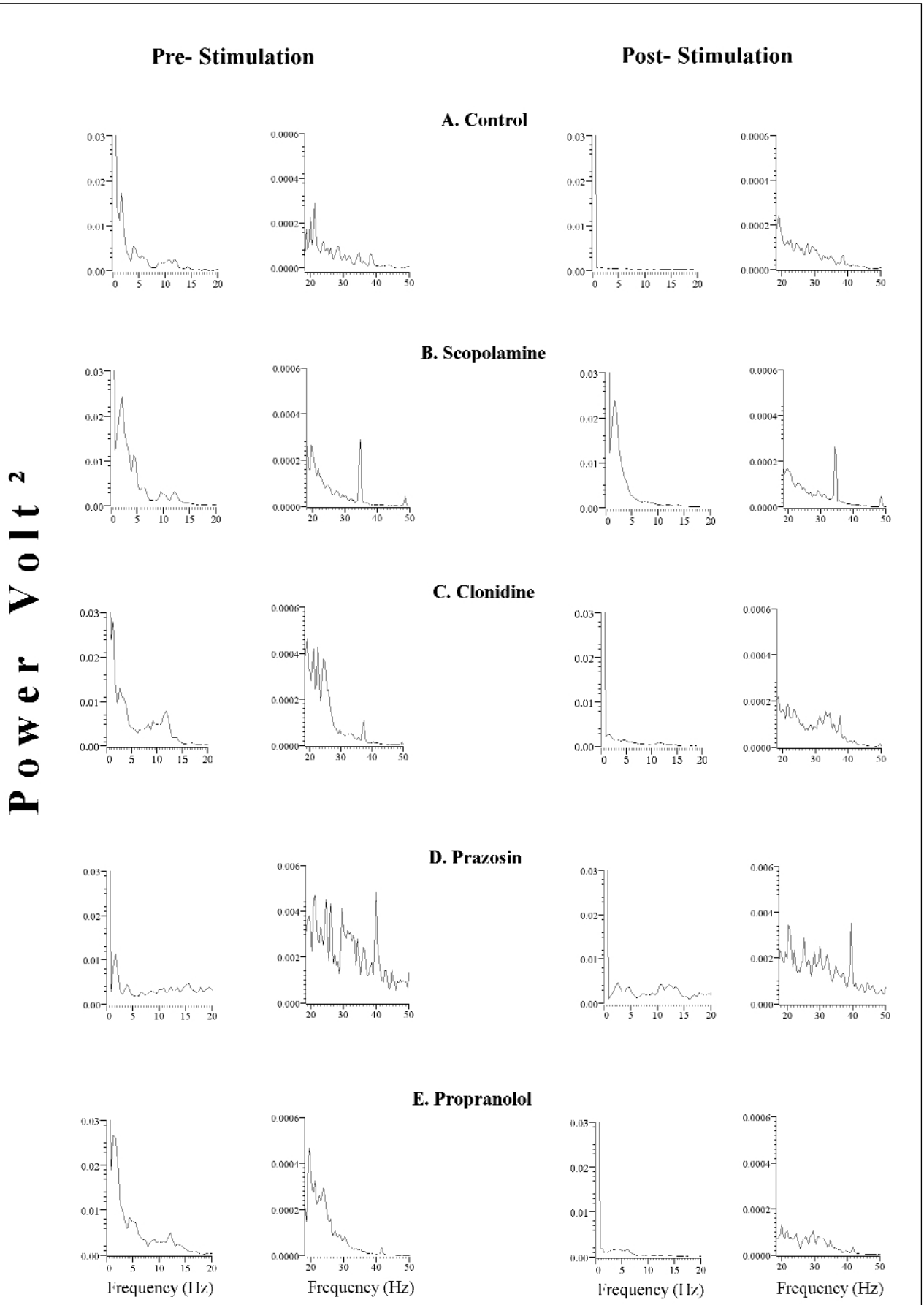
The effects of injection of the agonist and the antagonist on MRF stimulation induced changes in the EEG were statistically analyzed. For analyzing the MRF stimulation induced effects, the duration of uninterrupted DSYN of the EEG after the end of stimulation was measured. The effect during the period of stimulation was not considered to avoid the stimulation artifact. The mean of at least 2 episodes each from each of the 3 animals studied for every chemical was taken for the analysis. The mean values during pre-injection and post-injection periods were

Figure 1. A schematic representation of coronal sections of the brain stem of cat showing the site of stimulation of midbrain reticular formation (shaded region).



Abbreviations of anatomical terms: AQ, aqueduct; BCX, decussation of brachium conjunctivum; FTC, central tegmental field; MLB, medial longitudinal bundle; PAG, periaqueductal gray; RR, retrorubral nucleus; TRP, tegmental reticular nucleus pericentral division.

Figure 2. (Spectral analysis of the cortical EEG of cats (data acquisition by SPIKE2) under normal condition and after ip injection of cholinergic and adrenergic agonist and antagonist.) The left panel shows before (pre-stimulation) and the right panel shows after (post-stimulation) MRF stimulation. The power spectra shown are in two ranges one in 0-20 Hz and other 20 to 50 Hz. Detailed are mentioned in the results.



statistically compared by applying two way ANOVA coupled with the Newman-Keul's test and the significance levels determined. Representative samples of EEG were used for power spectral (SPIKE 2, CED) analysis for blocks of 120 seconds before and after MRF stimulation in control and treated cats. Each block was digitized at a sampling frequency of 100 Hz. At the end of experiment the stimulation site was electrolytically lesioned with 150 μA, 10 sec DC (Grass DCLM5 lesion maker). The brain was intracardially perfused with saline followed by 10% formolsaline containing 2% of potassium ferrocyanide. The lesioned site of stimulation was histologically identified. Schematic representation of the site of MRF stimulation is shown in Figure 1.

RESULTS

Effect of agonist and antagonist on spontaneous EEG :

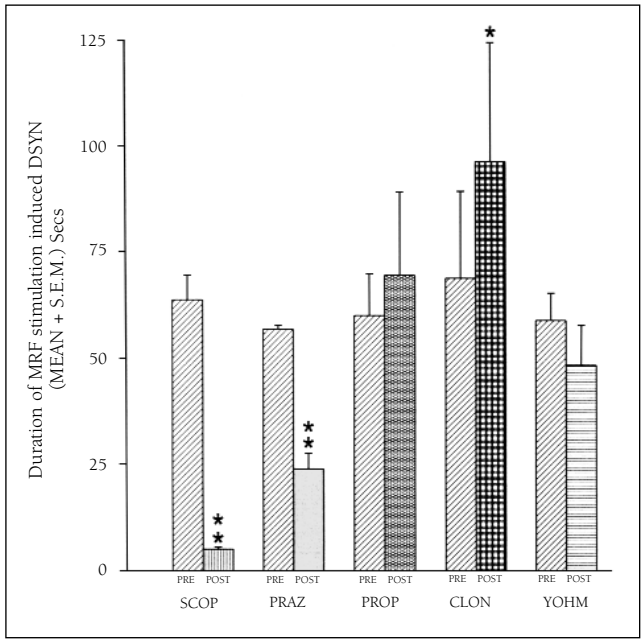
Normally, only handling the cats without any injection induced DSYN of the EEG and it took a mean latency of 33.3+3.52 mins for the appearance of SYN in the EEG uninterrupted for at least 15 sec. Intraperitoneal injection of scopolamine, a cholinergic antagonist, reduced the delay and precipitated relatively continuous SYN in the EEG at a mean latency of 4.66+0.33 mins. Prazosin, an α1-antagonist and clonidine, an α2-agonist induced SYN after at a mean latency of 12.0+1.52 mins and 22.0+2.88 mins, respectively. Propranolol, a β-antagonist was ineffective where the SYN reappeared after 37.0+2.88 mins of injection. However, yohimbine, an α2-antagonist delayed the process and induced SYN after a mean latency of 107.33+14.67 mins. The changes in EEG power spectra in control and after i.p. injection of chemicals are shown in Figure 2. The power spectra are shown in two panels one 0-20 Hz and other 20-50 Hz. The left two panels show before MRF stimulation while the right two panels after MRF stimulation. It may be noted that power and frequency of waves in the EEG were affected by both cholinergic and adrenergic inputs and corresponding receptors.

To appreciate DSYN the high frequency (100 Hz) stimulation of MRF was applied when the spontaneous EEG was not completely desynchronized. Such stimulation caused DSYN in the cortical EEG outlasting the period of stimulation. Since the physiological DSYN during the period of stimulation could not be separated from stimulation artifact, the effect of stimulation on the EEG was always considered from the end of stimulation. The stimulation intensity was so adjusted that there was DSYN without inducing active movement because the latter is known to induce DSYN of the EEG.

Effect of Scopolamine on MRF stimulation induced EEG desynchronization :

Before i.p. injection of scopolamine (i.e., control) the mean duration of MRF stimulation induced DSYN was 63.66+5.8 sec (Figure 3) which was significantly reduced to 5.0+0.57 sec ($p<0.01$, $F_{1,4}=85.8$) after scopolamine injection. The EEG was not DSYN even during eyes open and head movement of the animals. It may also be seen in the power spectrum where the MRF stimulation could not abolish the low frequency peak in the EEG (Figure 3). Interestingly, there was a peak ~35 Hz in scopolamine treated cats which persisted after stimulation of MRF. Thus MRF stimulation could hardly alter the EEG pattern in the

Figure 3. Mean duration (±SEM) of DSYN of the EEG inducing stimulation of MRF before and after i.p. injection of scopolamine (SCOP), prazosin (PRAZ), propranolol (PROP), clonidine (CLON) and yohimbine (YOHM) are shown in this figure.



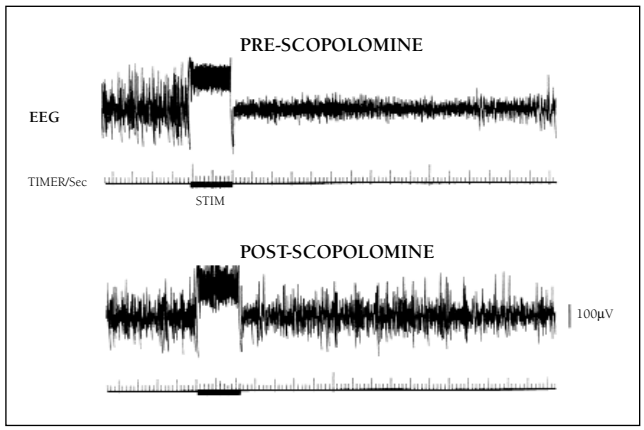
Significance levels between pre- and post-injection MRF stimulation induced DSYN is shown with asterisk.
* $p<0.05$ and ** $p<0.01$.

scopolamine treated cats. A sample raw data of MRF stimulation induced DSYN before and after scopolamine injection is shown in Figure 4.

Effect of Clonidine and Yohimbine on MRF stimulation induced EEG desynchronization :

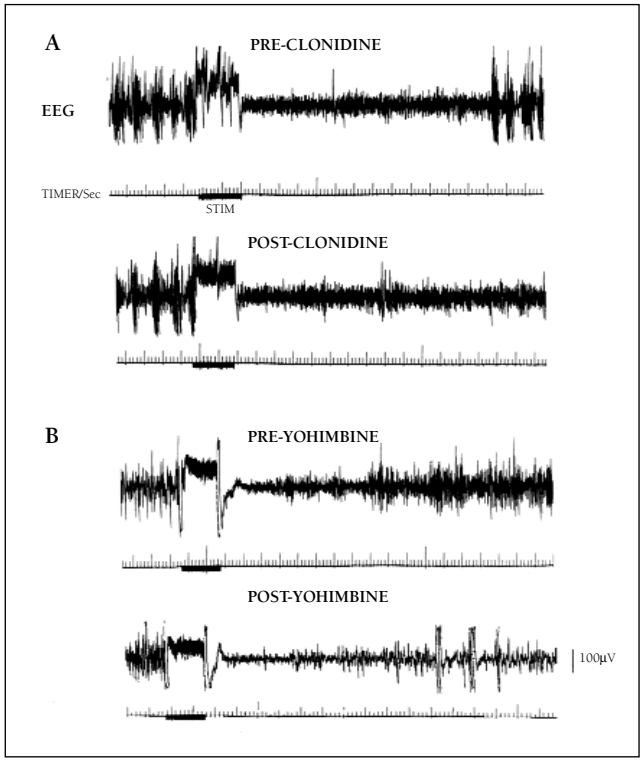
The mean durations of post-MRF stimulation induced DSYN before i.p. injection of α2-antagonist and agonist, yohimbine and clonidine, were 58.83+6.5 sec and 68.8+20.44 sec, respectively (Figure 3). After yohimbine

Figure 4. The duration of DSYN of the cortical EEG due to high frequency MRF stimulation before and after i.p. injection of scopolamine is shown here. It shows that scopolamine blocked MRF stimulation induced DSYN. STIM- Stimulation period is shown in timer channel.



injection the MRF stimulation induced DSYN was not affected significantly, which lasted for 48.41+9.23 sec. However, after clonidine injection, the MRF stimulation induced DSYN was significantly ($p<0.01$, $F1,4=1.66$) increased to 96.4+28.03 sec (Figure 3). Unlike scopolamine, the animals were responsive and showed DSYN during eye opening and movement of the head or parts of the body. Contrary to the effect of scopolamine, clonidine injection abolished the low frequency peak though the higher frequency peak persisted in the EEG power spectrum after stimulation of MRF (Figure 3). Raw signal recorded after MRF stimulation, before and after injection of clonidine and yohimbine, are shown in Figure 5 and 6, respectively.

Figure 5. EEG traces on high frequency stimulation of MRF before and after i.p. injection of clonidine (A) and yohimbine (B). The duration of stimulation is marked in the timer channel.



Effect of Prazosin and Propranolol on MRF stimulation induced EEG desynchronization :

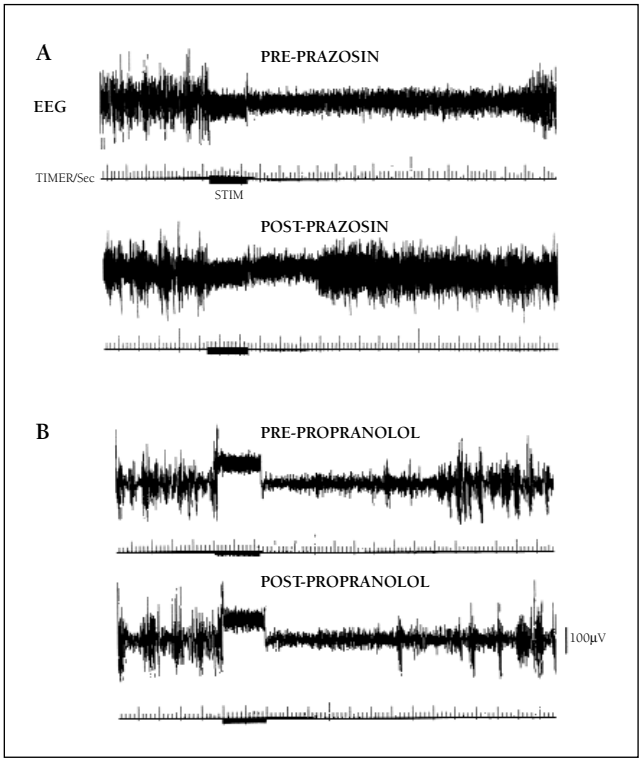
Power spectrum of the EEG after i.p. prazosin and propranolol injections with or without stimulation of the MRF are shown in Figure 2D and 2E, respectively. After prazosin injection there was a significant increase in the power density both in the low and the higher frequency range. The power density in both the frequency range (low and high) decreased after MRF stimulation. However, a prominent peak was seen at ~40 Hz after prazosin injection. The mean durations of MRF stimulation induced DSYN before and after prazosin injection were 56.8+0.9 sec and 23.93+3.64 sec, respectively (Figure 3).

On the other hand, after propranolol injection the power density of the high frequency range decreased with or without stimulation of the MRF. After i.p. injection of propranolol, the power of the high frequency bands in the EEG were comparable to that of controls. It was ineffective (Figure 6) in affecting the duration of MRF stimulation induced DYSN of the cortical EEG. The mean durations of post MRF stimulation induced DSYN of the EEG before and after propranolol injection were 59.93+9.86 sec and 69.53+19.57 sec, respectively (Figure 3). No apparent dissociation in the animal behavior and EEG was observed.

DISCUSSION

It was found in this study that both the cholinergic and the adrenergic antagonist caused SYN in the cortical EEG. The effects on DSYN during spontaneous wakefulness and MRF stimulation induced wakefulness were similar. The cholinergic antagonist was more effective than that of the adrenergic. The effective doses of the drugs used were based on previous reports (5,6). These drugs are reported to cross the blood brain barrier (5) and their physiological

Figure 6. A representative polygraphic traces of the MRF stimulation induced changes in the EEG before and after prazosin (A) and propranolol (B) treatment.



half life is reported to be long enough to exert the effects as observed in this study. Since, all the injections did not induce identical effects, the observed effects cannot be nonspecific responses. Besides, the drug effects were reversible suggesting that the injections did not produce any permanent damage to the brain. Since the injections were made intraperitoneal, the peripheral effect, if any, cannot be differentiated, from that of the central effect, and will be discussed later.

Scopolamine induced SYN and a reduction in duration of DSYN. This was reflected in the power spectrum of the EEG were the high frequency waves were lost or reduced by scopolamine. This is consistent with the observation that carbachol injection in the pontine area, for induction of REM sleep, increased high frequency waves in the EEG (unpublished observation). However, a peak ~35 Hz became prominent when cholinergic receptors were blocked. This peak (~35 Hz) persisted even after MRF stimulation. Its physiological significance is yet to be understood. Since SYN was obtained after injection of scopolamine, the cholinergic induction of DSYN must be mediated by its action on muscarinic receptors (23). It is

reasonable because muscarinic cholinoceptors are reported to be present in the cortex as well as in the subcortical structures (24,25). The effect of nicotinic antagonist has not been studied because there is a preponderance of muscarinic receptors in the CNS (26), and nicotine is reported to affect primarly cortical spindling (27). Our results also support earlier observations that ACh was increased in the brain during DSYN of the EEG (28) and its agonist as well as cholinesterase antagonist induced DSYN and wakefulness (29). The scopolamine, a parasympathetic blocker is likely to increase the blood pressure which is known to induce DSYN (30). Therefore, this effect (SYN) of scopolamine was unlikely to be mediated through changes in systemic blood pressure.

Yohimbine prolonged while clonidine reduced the delay of initiation of spontaneous SYN in the cats. Yohimbine is known to increase the secretion of NE (31) by acting on presynaptic autoreceptor (α2-adrenoceptor). This increased NE was likely to be responsible for significantly delayed appearance of spontaneous SYN after injection of yohimbine. Clonidine on other hand, is known to reduce release of NE (5). Hence, opposite effect of clonidine, compared to that of yohimbine is logical. Unlike scopolamine, where the effect was mediated through post-synaptic muscarinic cholinoceptor, the action of clonidine and yohimbine did not reflect the subtype of post-synaptic adrenoceptor involved in mediating the adrenergic action. Therefore, the effects of prazosin and propranolol, an α1 and α–adrenoceptor antagonist, respectively, were studied. The time taken for the appearance of spontaneous SYN in the polygraphic record after injection of propranolol was comparable to that of just handling the animals (without any injection) while that after prazosin injection spontaneous SYN started significantly sooner. Therefore, it may be said that NE induced duration of DSYN of the cortical EEG during spontaneous wakefulness is possibly mediated by its action on α1-adrenoceptor and not through the α2-adrenoceptor.

After propranolol injection, compared to control, there was no significant or marginal reduction in power density in the EEG power spectra before and after stimulation of the MRF. However, after prazosin injection there was an increased rhythmicity and an increase in the power density. Thus, NE acting on α1-adrenoceptors possibly break the rhythmicity in the EEG waves during wakefulness and thereby induces DSYN of the EEG. It may be argued that this effect of prazosin could be due to availability of more NE for -adrenoceptor (since α1-adrenoceptors were blocked) as reported earlier (32). However, this seems unlikely, because propranolol was ineffective. Since the durations of DSYN during spontaneous wakefulness and after MRF stimulation were affected by prazosin, it is likely to be involved in reverberatory neuronal circuitry responsible for continuation of DSYN. The role of α1-adrenoceptors in wakefulness induced DSYN may be supported by earlier reports (33,34) and also by the preponderance of such receptors in the cortex (35).

Apparently, α-adrenoceptor does not seem to be involved in wakefulness associated DSYN of the EEG. This is possibly true because it has been reported earlier that propranolol decreased deep sleep and REM sleep (23,36). It may be noted that, based primarly on DSYN of the EEG, NE has been reported to affect wakefulness (32), as well as REM sleep (5). Hence, it is possible that, DSYN during wakefulness is modulated by α1-adrenoceptors while that of during REM sleep by β-adrenoceptors. However, since REM sleep has not been studied here, it needs to be confirmed. This view may further be supported by our recent finding that possibly separate brain stem neurons are responsible for DSYN of the EEG during wakefulness and REM sleep (10). Thus, the two views may be synthesized as that the NE induced effect on DSYN during wakefulness is mediated through α1-adrenoceptors while that on REM sleep through α-adrenoceptors present on separate neurons. However, it needs to be confirmed if wake related neurons possess α1-adrenoceptors while REM sleep related neurons -adrenoceptor. Systemic administration of β-blocker, propranolol, is known to increase the blood pressure (32) and alteration in blood pressure affects EEG (30). Since the duration of DSYN was not affected by propranolol, in this study, the results obtained after intraperitoneal injections were unlikely to be mediated at least through changes in peripheral blood pressure.

Clonidine showed an increase while yohimbine was ineffective on the period of MRF stimulation induced DSYN. The clonidine and yohimbine are agonist and antagonist of presynaptic α2 receptor, respectively, and are known to reduce and increase spontaneous secretion of NE (31). Therefore, since the latter is likely to deplete the NE from the neuronal terminals, MRF stimulation induced further release of NE was not possible while NE was further released after MRF stimulation in clonidine treated animals. The results from this study suggest that NE plays a significant role in MRF stimulation induced DSYN and the effect was mediated by its action on α1-adrenoceptors. However, whether the brain stem neurons project directly to the cortex or indirectly by relaying to some other subcortical structure cannot be commented from this study. Nevertheless, it is reported that at least in the preoptico-anterior hypothalamic basal forebrain, NE induced wakefulness was mediated by its action on α-adrenoceptors (34,38,39). Since in this study the duration of DSYN of the EEG after stimulation of the MRF was not affected by propranolol, it was unlikely to be mediated by α-adrenoceptors. Thus it may also be said that the MRF stimulation induced adrenergic action was unlikely to be mediated at least through the basal forebrain area.

It has been seen that cholinergic and adrenergic system induce DSYN of the EEG. This may have relevance for DSYN of the EEG during REM sleep and wakefulness because the adrenergic locus coeruleus neurons cease firing (40) and the cholinergic laterodorsal and pedunculopontine tegmental neurons increase firing during REM sleep (9,13). It may be said that cessation of adrenergic neurons reduced the release of NE which probably is compensated by the increased activity of the cholinergic neurons during REM sleep for induction of DSYN during REM sleep. Thus, although cessation of adrenergic and activation of cholinergic neurons during REM sleep may be performing separate REM related functions, in the bargain, paradoxically, EEG remains desynchronized. In addition to the adrenergic and the cholinergic, the MRF also contains several neurons and projections including GABAergic (41,42) and glutamatergic (43,44). Their specific roles and the role of auto and hetero-receptors in DSYN of EEG need further investigation.

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