EFFECTS OF NEW AND POTENT NITRIC OXIDE SYNTHASE INHIBITORS ON SLEEP/WAKING STAGES AND EEG POWER SPECTRUM IN THE RAT

E. Dzoljic, R. de Vries and M.R. Dzoljic
Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands

SUMMARY

In this study we examined the effects of three potent inhibitors of neuronal nitric oxide synthase (NOS), 7-nitro indazole (7-NI), 3-bromo-7-nitro indazole (3-Br-7-NI) and S-methyl-L-thiocitrulline (S-Me-TC) on vigilance stages and EEG power spectra in the rat. The results show that 3-Br-7-NI (but not S-Me-TC) decreases slow wave sleep (SWS) and rapid eye movement (REM) sleep. 7-NI and 3-Br-7-NI reduced the EEG power density in all frequency bands, suggesting a depression of central neuronal activity. This effect of 7-NI was more prominent during the day than during the night, indicating a circadian variation in the NOS response to NOS inhibitors. A decrease of the EEG power was the most prominent in the high frequency range (7-9 Hz) of rhythmic slow activity (theta rhythm). These results suggest that nitric oxide (NO) exerts a stimulatory effect in the central neuronal structures involved in the regulation of vigilance.

INTRODUCTION

NO is a highly reactive molecule produced by the enzyme NOS. Three NOS isoforms have been isolated, neuronal NOS (n-NOS or brain NOS), endothelial NOS (e-NOS) and inducible NOS (i-NOS). It seems that all three isoforms are present in the brain. The e-NOS is expressed in endothelial cells and in the hippocampal neurons (Lowenstein, 1995), while i-NOS was identified in astrocytes (Simmons and Murphy, 1992). The n-NOS have been identified in a distinct population of central neurons (Bredt et al., 1990), but also in the peripheral nervous system and in the fast-twitch fibres at the sarcolemma of skeletal muscles (sarcolemmal n-NOS, Nakane et al., 1993).

Related to the vigilance, we observed that a relatively weak and non specific inhibitor of NOS, L-NMMA had a sleep promoting effect in the rat (Dzoljic et al. 1994). However, a relatively specific and potent inhibitor of the n-NOS, 7-NI induced a prominent central depression, associated with reduced motility and disrupted sleep architecture in rats (Dzoljic et al. 1996). We suggested that NOS inhibitors inducing mild sedation may facilitate sleep, while in case of a prominent central depression, they can disrupt the sleep pattern.
In order to further determine the role of NO in the central nervous system, we examined the effects of three new and potent inhibitors of the n-NOS, 7-NI, 3-Br-7-NI and S-Me-TC on the sleep/waking pattern and on the EEG power spectrum in rats. 3-Br-7-NI is a more potent inhibitor of rat neuronal NOS than 7-NI (Bland-Ward and Moore, 1995), while S-Me-TC is the most potent NOS-inhibiting agent described to date (Narayanan and Griffith, 1994) and represents a new class of NOS inhibitors with strong pressor activity.

MATERIALS AND METHODS

Animals and implantation procedure. Experiments were performed on male Wistar rats (300-350 gr). The animals were anaesthetized with pentobarbital (60 mg/kg, i.p.) and implanted with two epidural stainless steel screw electrodes over the parietal cortices and two electrodes inserted into the neck muscle. The rats were allowed 6 days for recovery.

EEG and EMG recording. During the last days of the recovery period, the animals were habituated to the recording cables for 3-4 hrs daily. The EEG and EMG signals were recorded and amplified by a polygraph (Grass 78, Grass Instruments Co., Quincy, Massachusetts), connected to a 386 microcomputer. Control EEG/EMG recording following administration of vehicle started on the 7-th day after operation and consisted of a 4 hrs session from 10.00-14.00. The 1-st hr of the session was used as an adaptation period to ensure the stability of the EEG recording. The following day (8-th day after operation) the NOS inhibitor was administered.

Sleep scoring. Scoring of sleep and wakefulness (W) was based on visual observation of EEG and EMG. The records were read by an experienced investigator and each 10 sec epoch was classified visually being W, slow wave sleep (SWS) or rapid eye movement (REM) sleep. Stages of vigilance were scored according to criteria of Ursin and Larsen (1983). SWS latency or REM sleep latency were defined as a time (min) from the drug injection to the first 10 sec period of SWS or REM sleep respectively.

EEG power spectral analysis. EEG spectral analysis was performed by Fast-Fourier-Transformation. Signals were recorded with a Multi Channel Registration Program (CAID, Dijkzigt, Rotterdam) and sampled at a frequency of 150 Hz. The EEG data were collapsed into the bins 0.5 to 20.0 Hz. Due to considerable intraindividual variations in the absolute power densities, the power values for each rat were expressed relative to control. In order to observe the effect of 7-NI in the rat during the active period (night) and the sleep period (day) the measurements of EEG power in vehicle (control) pretreated animals started during the night (23.00-2.00) and continued the next day (11.00-14.00). The measurements of EEG power were repeated after 5 days, but than the animals were pretreated with 7-NI (50 mg/kg).

Statistical analysis. Data were analyzed with statistical software by comparing mean power values in each frequency band, using multiple analysis of variance (MANOVA). Significant differences between treatments were further evaluated with paired t-test.
Ethical approval. The experiments and protocol of this study were approved by the Faculty Commission for experiments, handling and care of animals.

Drugs. 7-NI (Lancaster) was suspended in arachis oil by sonification. 3-Br-7-NI (Alexis) was dissolved in DMSO, while the S-Me-TC was dissolved in saline. Drug solutions/suspensions were prepared before each experiment. All drugs were administered intraperitoneally (i.p.).

RESULTS

Similar to our earlier observations, 7-NI disrupted sleep architecture (Dzoljic et al., 1996). In this study, the EEG power was suppressed by 7-NI (50 mg/kg, n = 5) in each frequency band. The maximum decrease in the EEG power was seen in high theta frequency bands (7-9 Hz). This effect was dose related (15-50 mg/kg, n = 4-5 for each dose) and more pronounced during the dark (23.00-2.00) period than during the light (11.00-14.00) period.

3-Bromo-7-NI (30 mg/kg, n = 5) decreased SWS significantly, while the SWS latency was increased (75 ± 19 min, versus 23 ± 5.4 min in the controls). REM sleep was nearly abolished, therefore the EEG power of REM sleep was not examined. In the non-sleeping animal a low amplitude EEG, normal EMG and depressed behaviour dominated (flat body posture, decreased locomotion and occasional ptosis and loss of righting reflex). The EEG power decreased in each frequency range, but particularly in the frequency band 7-9 Hz, two hours following drug administration.

S-Me-CT (30 mg/kg, n = 6) had no effect on SWS or REM sleep. The EEG power densities during wakefulness and SWS were decreased, but not significantly.

DISCUSSION

The major finding of this study is that the NOS inhibitors, 7-NI, and 3-Br-7-NI decreased sleep stages and EEG power. The arousal EEG/EMG pattern (low EEG amplitudes and normal EMG) induced by 3-Br-7-NI was, however, not associated with wake behaviour, since behavioral depression dominated (reduced motor activity, ptosis and loss of righting reflex) and EEG power was decreased. Thus, 3-Br-7-NI decreased the intensity of sleep, but did not increase wakefulness. The generalized depression of EEG power (particularly prominent in the high theta frequency band, 7-9 Hz) is a reflexion of depressed neuronal activity in various brain regions, leading to the reduction of cortical and hippocampal input. The reduced input to the hippocampus results in decreased theta activity, which is associated with a decrease of locomotion (Depoortere, 1987). Diminished cortical afferent activity causes a decrease of EEG power in other frequency bands. A decrease of the EEG power induced by indazole-derived NOS inhibitors is similar to the reduced EEG power induced by high doses of alcohol (Ehlers et al., 1992) or benzodiazepines (Glatt et al., 1983) in the rat.
It is of interest to note that the depression of the EEG power by 7-NI is less prominent during the night (active period of rat) than during the day period. The reason is not known, but it has been reported that NOS activity in the rat brain is higher during the dark phase, compared to the light period (Kapás et al., 1995). Although the S-alkyl-L-thiocitrullines are the most potent inhibitors of NOS in in vitro experiments (Narayanan et al., 1995), the effect of S-Me-TC in the rat in vivo is less prominent, compared to 7-NI or 3-Br-7-NI. The reason is not known. However, in contrast to indazole-derivatives, S-Me-TC has a strong pressor activity (Narayanan et al., 1995). This might be of importance, since the rise in blood pressure stimulates wakefulness (Fevell and Johnson, 1984; Ebenezer, 1994). In addition, the vasoconstriction and reduction of local cerebral blood flow (due to inhibition of e-NOS, Tanaka 1991) may significantly reduce oxygenation and perfusion of the neuronal tissue. This may affect the neuronal reactivity in the CNS and the development of the corresponding vigilance stage. These data imply that the effect of a relatively non-specific NOS inhibitor, such as S-Me-TC on vigilance and EEG can be mediated by activity of both, the brain NOS (n-NOS) and peripheral e-NOS.

CONCLUSION

Besides the quantitative differences between 7-NI, 3-Br-7-NI and S-Me-TC, the common effect of these NOS inhibitors is a decrease of EEG power as a result of central depression of the neuronal activity. This can lead to the disruption of normal sleep architecture. All these data indicate that NO exerts an excitatory role in the structure involved in the regulation of vigilance.

REFERENCES