ELSEVIER

Brain Research 718 (1996) 145–150

BRAIN RESEARCH

Research report

Sleep and nitric oxide: effects of 7-nitro indazole, inhibitor of brain nitric oxide synthase

M.R. Dzoljic *, R. de Vries, R. van Leeuwen

Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Accepted 10 January 1996

Abstract

We examined the effect of 7-nitro indazole (7-NI, 2.5–50 mg/kg, i.p.), an inhibitor of central nitric oxide (NO) synthesis, on general behaviour and sleep. The results show that 7-NI induces ptosis, a loss of the righting reflex and decrease of the EEG amplitudes. Furthermore, a duration of slow wave sleep (SWS) and REM sleep decreased, while the latencies of SWS and REM sleep increased. The effects of 7-NI on general behaviour and sleep were partially antagonized by intraventricular administration of the NO precursor, L-arginine (600 μg). These findings indicate that 7-NI induces a state of prominent central depression associated with motor deficit and decrease in sleep stages and wakefulness. It further suggests that NO exerts a significant excitatory effect on the neuronal structure involved in the regulation of locomotion and vigilance.

Keywords: Brain nitric oxide synthase; Central depression; 7-Nitro indazole; Nitric oxide; Nitric oxide inhibitor; Sleep; Rat

1. Introduction

The synthesis of nitric oxide (NO) is catalysed by NO synthase (NOS) for which three distinct isoforms have been identified, neuronal NOS (n-NOS), endothelial NOS (e-NOS) and inducible NOS (i-NOS). The n-NOS isoform (also known as brain NOS) was originally isolated from brain but recently has been found also in the peripheral nervous system and skeletal muscle. The n-NOS can be activated by stimulation of excitatory amino acid receptors [10]. NO is also synthesized by e-NOS expressed mainly in the endothelial cells of blood vessels, but also in a variety of other cells (cardiac myocytes, platelets, some hypocampal neurons). Although i-NOS was originally found in macrophages (mac-NOS), it has been induced in other cells as well (hepatocytes, myocytes, epithelial cells, astrocytes and chondrocytes). n-NOS and e-NOS are calcium dependent, while i-NOS makes NO, even calcium levels are low [5,16,33].

The available NOS inhibitors, such as L-N^G-nitro arginine methyl ester (L-NAME), L-N^G-monomethyl arginine (L-NMMA) affect both n-NOS and e-NOS. Block of e-NOS results in a prolonged increase in blood pressure (BP) [27]. The hypertensive effect of these drugs is associated with a significant reduction in local cerebral blood flow in the rat [29], which might additionally affect the functional activity of the CNS. It has been reported that a short-lasting rise in BP in the cat, produced mechanically by an occlusion of the thoracic aorta or by rapid injections of saline, is immediately followed by an arousal reaction [3]. Systemic increase in BP by balloon inflation into the descending aorta of the lamb elicited an arousal from sleep [9]. Hypertensive agents also stimulate wakefulness. A rise in BP induced by vasopressin markedly increased the duration of wakefulness in cats [3]. Recently, it has also been shown that systemic administration of the pressor substance arginine-8-vasopressin (5–10 mg/kg, s.c.), which cannot cross the blood-brain barrier, increases the level of arousal and induces corresponding changes in the spectral power of the electroencephalogram (EEG) in rats [8]. It has been suggested that cortical arousal can be induced by afferent impulses from peripheral pressoreceptors [4].

All these data imply that effects of nonspecific NOS inhibitors on vigilance and EEG may be due also to the inhibition of e-NOS and corresponding hypertension and not exclusively to the inhibition of n-NOS. This may lead to misleading interpretations of the effects of nonspecific

* Corresponding author. Fax: +31 (10) 4366839.

0006-8993/96/$15.00 © 1996 Elsevier Science B.V.
PII S0006-8993(96)00102-3

NOS inhibitors and the role of central NO in the vigilance and neuronal activity.

Recently, it has been reported that 7-nitro indazole (7-NI) inhibits NOS in rat brain with a potency similar to or greater than that of L-NMMA or L-NAME [1]. This effect of 7-NI was not associated with an increase in BP [23], which is a characteristic of all other NOS inhibitors. Thus 7-NI provides the possibility of studying the role of central NO, without involvement of central side effects, caused by peripheral elevation of BP. In this respect 7-NI is a more appropriate experimental tool than other nonspecific NOS inhibitors. Therefore, in this study we used 7-NI in order to examine the role of NO on general behaviour and sleep in rats.

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats, weighing 300–350 g.

2.2. Ethical approval

The experiments and protocol of this study were approved by the Faculty Commission for experiments, handling and care of animals.

2.3. Behaviour: ptosis and righting reflex

The experimental rats were observed for ptosis and loss of the righting reflex. These two prominent changes in general behaviour were observed in preliminary experiments and evaluated in this study. 7-NI was used in a dose of 50 mg/kg, i.p., since this dose had a clear depressive effect on the amplitude of the electrocorticogram (ECoG) and on sleep. The experimental rats were tested for loss of the righting reflex every 30 min by placing the rat on its back. The righting reflex was regarded as lost if the rat placed on its back failed to right itself (with all four feet on the table, under the body) within 10 s. If the rat could not be placed on its back or did not remain on its back for more than 10 s, the righting reflex was considered as present (normal, not blocked).

2.4. Implantation procedure

The animals were anaesthetized with pentobarbital (60 mg/kg, i.p.) and implanted with two electrodes in the right and left parietal cortex for recording of the ECoG and two electrodes inserted into the neck muscles for recording of the electromyogram (EMG). A reference electrode was placed on the frontal skull. All electrodes were fixed in a socket and secured to the skull with dental cement. After surgery the rats were housed individually and allowed 6 days for recovery in a sound-proof and electrically shielded room. The animals had free access to food and water and remained in this room also during the days of the experiments (7th and 8th days after operation). The room was maintained on a 12 h light dark schedule (light on 07.30; light intensity in the cages was about 100 1 ×) with constant temperature (21°C) and humidity (55%). During the last 3 days of the recovery period the animals were habituated to the recording cables for 5–6 h daily.

2.5. EEG recording

Sleep recording started on the 7th day after operation and consisted of a 6 h daily session from 10.00–16.00 h. The first hour of the session was used as an adaptation period to ensure the stability of the ECoG recording. The following 5 h were evaluated for stages of vigilance. The EEG activities were amplified by a polygraph (Grass 78) connected to a 386 microcomputer. Signals were recorded with the Multi Channel Registration (MKR) program (CAID, Dijkzigt, Rotterdam). EEG signals were digitized with a sample frequency of 120 Hz. The half-amplitude frequency response was 1–100 Hz for the ECoG with a selective 50 Hz filter in each channel. The records were read by an experienced and 'blind' investigator and each 30 s of EEG record was classified visually as being wakefulness, slow wave sleep (SWS) or REM sleep, according to the criteria of Ursin and Larsen [30]. SWS-1 and SWS-2 were taken together as SWS. The SWS latency and REM sleep latencies were defined as the length of time (in min) between injection of the drug and appearance of the first 10 consecutive seconds of SWS or REM sleep respectively.

2.6. Drugs and experimental procedure

7-NI (Brunschwig Chemie) was suspended in arachis oil by sonification while L-arginine (Sigma) was dissolved in saline and adjusted with NaOH to pH 7.4. 7-NI was administered intraperitoneally (i.p.). L-Arginine was given intracerebroventricularly (i.c.v.), by slow infusion, 15 min following administration of 7-NI. This time-schedule was selected in order to coincide with the maximum effects of 7-NI and L-arginine. Maximum inhibition of n-NOS (80–85%) occurred 30 min following 7-NI (30 mg/kg, i.p.) treatment [19], while L-arginine (300 μg, i.c.v) produced ECoG desynchronization within 6 ± 2 min [21]. Drug solutions/suspensions were prepared on the morning of each experiment. The doses of 7-NI were selected on the basis of their inhibitory effect on neuronal NOS [1], dose-related antinociception [23] and our preliminary behavioral experiments. Vehicle of 7-NI (arachis oil, 4 ml) used as a control in the sleep study was administered i.p. on the 7th day after operation (one day before administration of 7-NI) and at the end of the first hour of the session (adaptation period). Vehicle of L-arginine (saline) was given by slow infusion (30 μl, i.c.v.) 15 min following 7-NI.

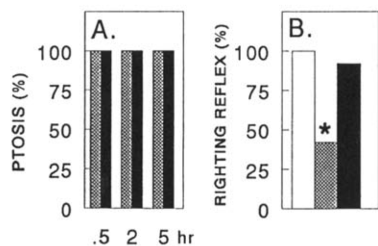

Fig. 1. Effect of 7-nitro indazole (7-NI, 50 mg/kg, i.p.,) on ptosis (A) and righting reflex (B) in rats. The 1st group of animals (N = 12) received vehicle of 7-NI (arachis oil, 4 ml, i.p.), followed by saline (30 μl, i.c.v. in slow infusion). Ptosis was not observed in this group (not shown), while the righting reflex was present in all animals (B: □). Two other groups (each 12 rats) were treated with 7-NI ($n = 24$), followed by saline (▩, 30 μl, i.c.v., $n = 12$) or L-arginine (■, 600 μg, i.c.v., $n = 12$). The time interval between drug administration was 15 min. The registration of ptosis and the righting reflex started 30 min after administration of 7-NI and was repeated each 30 min in the following 5 h. * Significance in respect to control (arachis oil). Note that 7-NI induced ptosis in all animals, while a loss of the righting reflex was seen in some of them (58%). Loss of the righting reflex (but not ptosis) was antagonized by L-arginine.

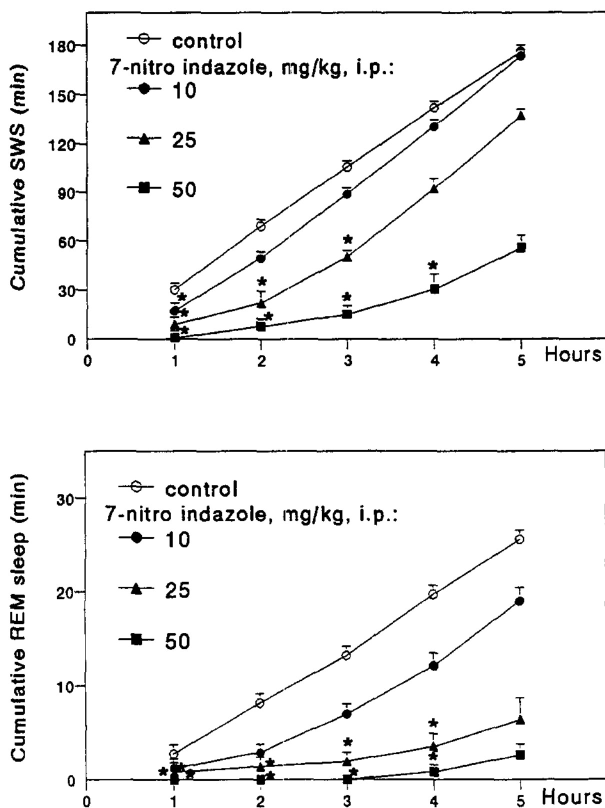

Fig. 2. Effect of 7-nitro indazole (7-NI, 10–50 mg/kg, i.p.) on cumulative slow wave sleep (SWS) and REM sleep (REM) time (mean ± S.E.M.) in rats during a 5 h observation period. The animals ($n = 14$) received vehicle (control, arachis oil, 4 ml, i.p.) on the 7th day after operation. Next day the same animals were divided into three nearly equal groups (4–5 rats/group). Each group received one of three doses of 7-NI. EEG registration started at the time of drug administration (0 time). Vertical bars represent ± S.E.M. * Significant difference from the control values in the corresponding time (hour) interval, following administration of vehicle in the same animals a day before. Note a significant and dose-related decrease of sleep stages in the first 4 h following 7-NI administration.

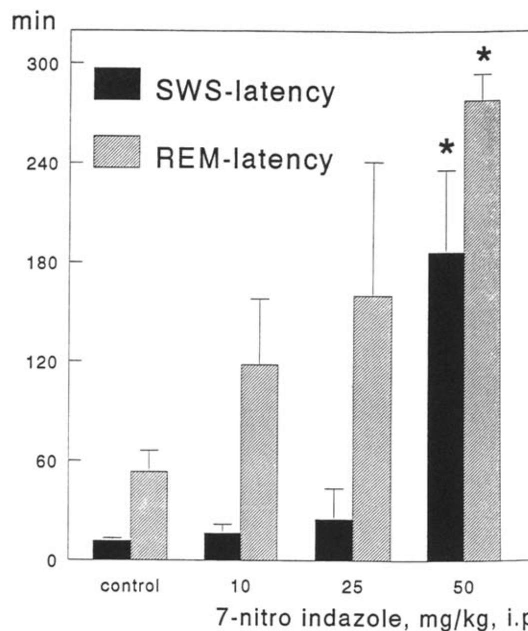

Fig. 3. Effect of 7-nitro indazole (7-NI, 10–50 mg/kg, i.p., the same groups and animals as in Fig. 2) on the latencies (min) of slow wave sleep (SWS) and REM sleep (REM) in rats ($n = 14$). Vertical bars represent ± S.E.M. * Significant difference from the control values in the same animals receiving vehicle (arachis oil, 4 ml, i.p.) a day before. Note an increase of latencies of SWS and REM sleep following 7-NI administration.

For observation of general behaviour 36 rats were used. Groups of animals (4–7 rats/each dose of 7-NI) were used in the sleep study. Each rat received 7-NI only once.

2.7. Data analysis

The data were statistically evaluated by using ANOVA and followed by Student's t-test for paired values in order to compare the sleep-waking parameters between vehicle and 7-NI. Chi-square test has been used for evaluation of effects on the righting reflex. A probability (P) value of 0.05 or less was taken to indicate a statistical significance.

3. Results

3.1. Behaviour: ptosis and righting reflex

Between 20–30 min following administration of 7-NI (50 mg/kg, i.p., $n = 24$), the rats showed ptosis, which lasted up to 5 h (Fig. 1A). Loss of the righting reflex was observed in many animals (58%) within the first 2 h following administration of 7-NI (Fig. 1B). This effect gradually declined and the reflex was completely normalized within 5 h after drug administration. Loss of the righting reflex (but not ptosis) was antagonized by L-arginine (600 μg, i.c.v., Fig. 1A,B). None of the animals treated with L-arginine (600 μg, i.c.v.) or with vehicles

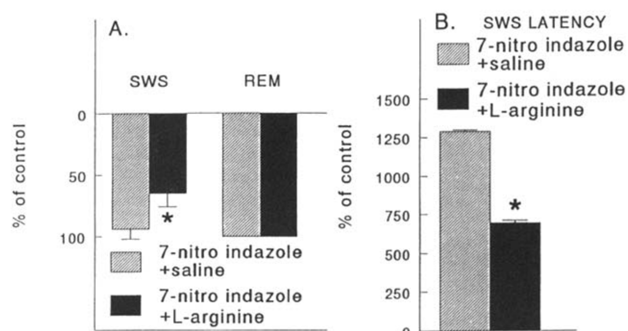

Fig. 4. Effect of 7-nitro indazole (7-NI, 50 mg/kg, i.p.) and L-arginine (600 μg, i.c.v.) on slow wave sleep (SWS) and REM sleep (REM) (A) and their latencies (B) in rats during a 5 h period. Changes in sleep stages and latencies are given as a percentage of control (0 line). Control animals ($n = 12$) were treated with vehicles (arachis oil, 4 ml, i.p., followed by saline, 30 μl, i.c.v.). The next day, the same animals were treated with 7-NI ($n = 12$), followed by saline ($n = 6$) or L-arginine ($n = 6$). The time interval between drug administration was 15 min. Vertical bars represent ±S.E.M. * Significant difference in respect to group of animals receiving 7-NI and saline. Note a partial and significant reversal of SWS and SWS latency (but not REM sleep) in animals treated with 7-NI and L-arginine, compared to animals treated with 7-NI and saline.

(saline, 30 μl, i.c.v.; arachis oil, 4 ml, i.p., Fig. 1B) showed signs of ptosis or loss of the righting reflex.

3.2. Sleep

7-NI (10–50 mg/kg, i.p., $n = 14$) caused a dose-related decrease in SWS and REM sleep, which lasted for 4 h (Fig. 2). SWS was completely abolished in the 1st h following injection of the highest dose of 7-NI (50 mg/kg), while REM sleep did not occur in the next 3 h. A low dose of 7-NI (2.5 mg/kg, i.p., $n = 7$) had no effect on SWS and REM sleep. The latencies of SWS and REM sleep were increased dose-dependently, reaching significance after the highest dose of 7-NI (50 mg/kg, Fig. 3). In an additional group of animals ($n = 12$), divided in two equal subgroups, we compared the changes in sleep parameters following administration of combinations of 7-NI (50 mg/kg, i.p.) with saline (i.c.v., $n = 6$) and 7-NI with L-arginine (600 μl, i.c.v., $n = 6$). The effect of 7-NI (7-NI + saline) on SWS and SWS latency (but not on REM sleep) was partially reversed by L-arginine (7-NI + L-arginine, Fig. 4).

4. Discussion

7-NI induced a long-lasting ptosis and a loss of the righting reflex in rats. The loss of the righting reflex used as an index of a hypnotic/narcotic effect [26] may indicate that 7-NI exerts a prominent depression of the CNS. Decrease in ECoG amplitudes and sleep stages, associated with increase in sleep latencies is suggesting that central depression induced by 7-NI is closer to the narcosis than to the sedation.

The mechanism(s) underlying the central depressive effect of 7-NI is unknown but some possibilities can be considered.

4.1. Cerebral blood flow

A neuronal excitability in the CNS can be affected by cerebral blood flow. Despite the apparent lack of peripheral vasoconstriction and increase in mean arterial BP, 7-NI is not devoid of cardiovascular effects. Heart rate and blood flow in various cerebral areas were significantly decreased, following administration of 25–50 mg/kg of 7-NI in rats [14]. However, in this respect there is no qualitative difference between 7-NI and other NOS inhibitors, since L-NAME reduced hippocampal blood flow as well [28]. Evidently, 7-NI and L-NAME have a similar effect on cerebral circulation but the behavioral effects are not the same. Although a role of the vascular factor could not be completely excluded, it seems unlikely that changes in the cerebral blood flow are of the essential importance for the central depression induced by 7-NI.

4.2. Decreased concentrations of excitatory neurotransmitters

NO increases the release of various neurotransmitters [18], including excitatory amino acids, L-glutamate (L-Glu) and L-aspartate (L-Asp) [11,17]. Several NOS inhibitors, including 7-NI, blocked the NMDA-mediated release of L-Glu and noradrenaline (NA) [22]. L-Glu, L-Asp and NA are three major excitatory neurotransmitters that may significantly affect neuronal excitability. It seems therefore logical to suppose that central depression of 7-NI is due to the inhibition of NO synthesis in the brain and to a corresponding decrease in release of excitatory neurotransmitters. Decreased levels of these three excitatory neurotransmitters in the close vicinity of central neurons, might be of crucial importance for decrease of neuronal excitability and development of central depression. This may explain the 7-NI-induced ptosis, loss of righting reflex (this study) and anticonvulsant effect [31]. It could also mean an attenuation of the activity of neurons involved in the initiation and/or maintenance of normal sleep. Regarding sleep the NO would act primarily as an endogenous stimulator of wakefulness, affecting a sleep pattern indirectly.

In order to prove an involvement of NO it is of importance to demonstrate a time correlation between observed phenomena in this study and inhibition of n-NOS. Recent work shows that 7-NI (25, 50 and 100 mg/kg, i.p.) decreased the NOS activity within cerebral cortex of rat by 40–60% when measured up to 120 min but not 240 min after administration [32]. Maximum inhibition of n-NOS (80–85%) occurs 30 min following 7-NI (30 mg/kg, i.p.)

[19]. The occurrence and duration of 7-NI-induced effects monitored in this study, correlate in time (except duration of ptosis) with data regarding the NOS inhibition. In addition, it is also of importance to demonstrate the ability of L-arginine to reverse the effects of 7-NI. This study shows that 7-NI-induced decrease of SWS and loss of the righting reflex (but not ptosis or REM sleep) can be partially antagonized by NO precursor, L-arginine. This indicates an involvement of NO in central depression induced by 7-NI. Although complete reversal has not been achieved, these need not necessary to be expected, since pharmacokinetic factors of L-arginine and/or a complex interaction between 7-NI and NOS could play a role. It has been shown that 7-NI is not only competitive with L-arginine but also with co-factor for NO synthesis, tetrahydrobiopterin (H_4 biopterin). Thus, 7-NI decreases the affinity of the enzyme not only for L-arginine but also for H_4 biopterin [15]. A recent in vitro study confirmed this possibility, since NOS inhibition due to 7-NI is partially reversed by L-arginine and partially reversed by H_4 biopterin [20].

Although we emphasized the role of the decreased NO synthesis and the corresponding derangement of neurotransmitter balance in the CNS as a main underlying mechanism of central effects of 7-NI, an involvement of some additional nonspecific effects of 7-NI, unrelated to the NO synthesis could not be ruled out. This possibility should be considered since we were not able to antagonize all effects of 7-NI by L-arginine. Furthermore, 7-NI-induced relaxation of rabbit aorta in vitro was unaffected by NOS blockade or endothelial removal [20]. This suggests that some peripheral effects of 7-NI are not mediated by inhibition of NOS. Although, there is no evidence of such unspecific effects of 7-NI in the CNS, this can still urge a caution in the interpretation of the effects of 7-NI and the role of NO in vigilance and other central phenomena.

4.3. 7-NI compared to other NOS inhibitors

Similarly to 7-NI, the NOS inhibitor L-NAME suppressed sleep in the rabbit and rat [12,13]. Although the effects of L-NAME on vigilance might due to the central excitation, particularly in cases of significant elevation of BP, the inhibitory/ depressive component should not be excluded. This is supported by recent data, showing that L-NAME reduces the sound-evoked arousal response in rats [2]. The possible presence of both, the inhibitory and excitatory components is reflecting in seizure phenomena as well. L-NAME exerted both anticonvulsant [24,25] and proconvulsant [6] effects in various experimental models of seizures. However, in contrast to 7-NI and L-NAME, the L-NMMA stimulates sleep pattern in rats [7]. This difference might due to the fact that L-NMMA is a weak NOS inhibitor, which may lead to the light sedation, associated with facilitation of sleep and reduction of the wakefulness [7].

The reason for controversial effects of NOS inhibitors on vigilance (and seizures) is not known. However, the NO deficit and corresponding neurotransmitter derangement may not be the same in various brain areas. The neuronal excitability altered by NOS inhibitor, can be additionally affected by several factors (such as doses, species, stage of vigilance), including the factors unrelated to the inhibition of n-NOS (for example, an elevation of BP by some NOS inhibitors). This could determine the neuronal excitability in the particular brain area involved in the development of corresponding behaviour.

Concluding, the behavioral effects (ptosis, loss of the righting reflex) and changes in sleep parameters (decreased ECoG amplitudes, decreased duration of sleep stages and increased sleep latencies), suggest that 7-NI-induced inhibition of n-NOS activity is associated with a prominent central depression but not with somnogenic effect. 7-NI-induced depression of CNS is more similar to the effect of narcotic/hypnotic/sedative agents used in high doses which disrupt the sleep and affect the locomotion (loss of righting reflex), then to the effect of the low doses of the same drugs which may promote sleep. Indirectly, these data may suggest that NO exerts at least in some brain regions an excitatory effect on central neurons. Regarding the sleep/wake pattern, the excitatory effect of NO in the brain, may primarily affect the neuronal structures regulating a wakefulness and indirectly the sleep.

References

[1] Babbedge, R.C., Bland-Ward, P.A., Hart, S.L. and Moore, P.K., Inhibition of rat cerebellar nitric oxide synthase by 7-nitro indazole and related substituted indazoles, *Br. J. Pharmacol.*, 110 (1993) 225–228.

[2] Bagetta, G., Iannone, M., Del Duca, C. and Nisticó, G., Inhibition by N_G-nitro-L-arginine methyl ester of the electrocortical arousal response in rats, *Br. J. Pharmacol.*, 108 (1993) 858–860.

[3] Baust, W. and Heinemann, H., The role of baroreceptors and blood pressure in the regulation of sleep and wakefulness, *Exp. Brain Res.*, 3 (1967) 12–24.

[4] Bowes, G., Townsend, E.R., Bromwley, S.M., Kozar, L.F. and Phillipson, E.A., Role of the carotid body and of afferent vagal stimuli in the arousal response to airway occlusion in sleeping dogs, *Am. Rev. Resp. Dis.*, 123 (1981) 644–647.

[5] Bredt, D.S., Hwang, P.M., Glatt, C.E., Lowenstein, C., Reed, R.R. and Sneider, S.H., Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase, *Nature*, 351 (1991) 714–718.

[6] Buisson, A., Lakhmeche, N., Verrechia, C., Plotkine, M. and Boulu, R.G., Nitric oxide: an endogenous anticonvulsant substance, *NeuroReport*, 4 (1993) 444–446.

[7] Dzoljic, M.R. and de Vries R., Nitric oxide synthase inhibition reduces wakefulness, *Neuropharmacology*, 33 (1994) 1505–1509.

[8] Ebenezer, I.S., The effects of subcutaneous administration of arginine-8-vasopressin on the electroencephalogram of conscious rats are mediated by peripheral vasopressin V1 receptors, *Methods Find. Exp. Clin. Pharmacol.*, 16 (1994) 315–321.

[9] Fevell, J.E. and Johnson, P., Acute increases in blood pressure cause arousal from sleep in lambs, *Brain Res.*, 311 (1984) 259–265.

[10] Garthwaite, J., Glutamate, nitric oxide and cell-cell signalling in the nervous system, *Trends Pharmac. Sci.*, 14 (1991) 60–67.

[11] Guevara-Guzman, R., Emson, C.P. and Kendrick, K.M., Modulation of in vivo striatal transmitter release by nitric oxide and cyclic GMP, *J. Neurochem.*, 62 (1994) 807–810.

[12] Kapás, L., Shibata, M., Kimura, M. and Krueger, J.M., Inhibition of nitric oxide synthesis suppresses sleep in rabbits, *Am. J. Physiol.*, 266 (1994) (Regulatory Integrative Comp. Physiol., 35): R151–R157.

[13] Kapás, L., Fang, J. and Krueger, J.M., Inhibition of nitric oxide synthesis inhibits rat sleep, *Brain Res.*, 664 (1994) 189–196.

[14] Kelly, P.A.T., Ritchie, I.M. and Arbuthnott, G.W., 7-Nitro indazole uncouples cerebral blood flow from metabolic demand in the conscious rat, *Br. J. Pharmacol.*, 112 (1994) Suppl. P348.

[15] Klatt, P., Schmid, M. Leopold, E., Schmidt, K., Werner, E.R. and Mayer, B., The pteridine binding site of brain nitric oxide synthesis. Tetrahidropteridine binding kinetics, specifity, and allosteric interaction with substrate domain, *J. Biol. Chem.*, 269 (1994) 13861–13866.

[16] Lamas, S., Marsden, P.A., Li, G.K., Tempst, P. and Michel, T., Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 6348–6352.

[17] Lawrence, A.J. and Jarrott, B., Nitric oxide increases interstitial excitatory amino acid release in the rat dorsomedial medulla oblongata, *Neurosci. Lett.*, 151 (1993) 126–129.

[18] Lonart, G., Wang, J. and Johnson, K.M., Nitric oxide induces neurotransmitter release from hippocampal slices, *Eur. J. Pharmacol.*, 220 (1992) 271–272.

[19] MacKenzie, G.M., Rose, S., Bland-Ward, P.A., Moore, P.K., Jenner, P. and Marsden, C.D., Time course of inhibition of brain nitric oxide synthase by 7-nitro indazole, *NeuroReport*, 5 (1994) 1993–1996.

[20] Medhurst, A.D., Greenlees, C., Parsons, A.A. and Smith, S.J., Nitric oxide synthase inhibitors 7- and 6-nitroindazole relax smooth muscle in vitro, *Eur. J. Pharmacol.*, 256 (1994) R5–6.

[21] Mollace, V., Bagetta, G. and Nisticò, G., Evidence that L-arginine possesses proconvulsant effect mediated through nitric oxide, *NeuroReport*, 2 (1991) 269–272.

[22] Montague, R.P., Gancayco, C.D., Winn, M.J., Marchase, R.B. and Friedlander, M.J., Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex, *Science*, 263 (1994) 973–977.

[23] Moore, P.K., Wallace, P., Gaffen, Z., Hart, S.L. and Babbedge, R.C., 7-nitro indazole, an inhibitor of nitric oxide synthase, exhibits antinociceptive activity in the mouse without increasing blood pressure, *Br. J. Pharmacol.*, 108 (1993) 296–297.

[24] Osonoe, K., Mori, N., Suzuki, K. and Osonoe, M., Antiepileptic effects of inhibitors of nitric oxide synthase examined in pentylenetetrazol-induced seizures in rats, *Brain Res.*, 663 (1994) 338–340.

[25] Przegalinski, E., Baran, L. and Siwanowicz, J., The role of nitric oxide in the kainate-induced seizure in mice, *Neurosci. Lett.*, 170 (1994) 74–76.

[26] Rall, T.W., Hypnotics and sedatives. In A.G. Gilman, T.W. Rall, A.J. Nies and P. Taylor, (Eds.), *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, Pergamon Press, New York, 1990, pp. 345–382.

[27] Rees, D.D., Palmer, R.M.J. and Moncada, S., Role of endothelium-derived nitric oxide in the regulation of blood pressure, *Proc. Natl. Acad. Sci. USA*, 86 (1991) 3375–3378.

[28] Rigaud-Monnet, A.S., Pinard, E., Borredon, J. and Seylaz, J., Blockade of nitric oxide synthesis inhibits hippocampal hyperaemia in kainic acid-induced seizure, *J. Cereb. Blood Flow Metab.*, 14 (1994) 582–590.

[29] Tanaka, K., Gotoh, F., Gomi, S., Takashima, S., Miharam, B., Shirai, T., Nogawa, S. and Nagata, E., Inhibition of nitric oxidase synthesis induces a significant reduction in local cerebral blood flow in the rat, *Neurosci. Lett.*, 127 (1991) 129–132.

[30] Ursin, R. and Larsen, M., Increased sleep following intracerebroventricular injection of the delta sleep-inducing peptide in rats, *Neurosci. Lett.*, 40 (1983) 145–149.

[31] Van Leeuwen, R., De Vries, R. and Dzoljic, M.R., Anticonvulsant effect of 7-nitroindazole, an inhibitor of neuronal nitric oxide synthase in pilocarpin model of epilepsy, *Eur. J. Pharmacol.*, 287 (1995) 211–213.

[32] Yoshida, T., Limmroth, V., Irikura, K. and Moskowitz, M.A., The NOS inhibitor, 7-nitroindazole, decreases focal infarct volume but not the response to topical acetylcholine in pial vessels, *J. Cereb. Flow Metab.*, 14 (1994) 924–929.

[33] Xie, Q.-W., Cho, H.J., Calaycay, J., Mumford, R.A., Swiderek, K.M., Lee, T.D., Ding, A., Troso, T. and Natham, C., Cloning and characterization of inducible nitric oxide synthase from mouse macrophages, *Science*, 256 (1992) 225–228.