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MOZKOVÁ HEMODYNAMIKA A NEUROVASKULÁRNÍ COUPLING - ÚLOHA NO

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TABLE OF CONTENTS:

SUMMARY

Cerebral blood supply is critical to maintain high metabolic demands of nervous tissue. The blood flow is finally regulated by several molecular mechanisms affecting cerebral vascular tone. One of the most important pathways is production of nitric oxide (NO) by activated nitrinergic neurons. Nitric oxide is gaseous mediator which diffuses to the smooth muscle cell and by depression of intracellular calcium activity relaxes the vascular wall and thus regulates regional cerebral blood flow (rCBF). We have recently showed the importance of NO of neuronal origin also in the initiation of the seizures in vitro. The present study was set out to investigate the effect of neuronal NO on hemodynamic and neuronal responses to transcallosal electric stimulation in rats. Anaesthetics significantly influence cerebral blood flow and its regulation thus we performed our experiments on both conscious and anaesthetized rats.

Adult Wistar rats (200-300g, n=122) were anaesthetized with isoflurane or urethane and EEG electrodes were implanted over sensorimotor cortices. During spontaneous and epileptic activity rCBF was measured by Laser Doppler flowmetry so as arterial blood pressure, arterial blood gasses and EEG activity. Responses to electrical stimulation were measured before and after the administration of an intraperitoneal bolus ($25 mg kg^{-1}$) of 7-Nitroindazole (7-NI). In addition, behavioural tests were performed to elucidate the effect of 7-NI on motor behavior. 7-NI induced a rise in arterial blood pressure indicating a systemic effect and altered motor and emotional behaviour. Power and mean frequency of the spontaneous EEG were diminished following the i.p. administration of 7-NI. However, excitability of the tissue was significantly increased by means of numbers and durations of afterdischarges and lowering of the threshold of evoked potential response. 7-NI diminished rCBF responses to transcallosal stimulation.

Our findings demonstrates that NO contributes to the basal cerebrovascular tone, but the participation of NO to CBF responses to transcallosal stimulation remains uncertain.

Key words: cerebral hemodynamic response, brain excitability, neuronal nitric oxide synthase, 7-nitroindazole, rat.

SOUHRN

Zajištění stálého mozkového průtoku je nezbytné k pokrytí vysokých metabolických požadavků nervové tkáně. Změny v krevním průtoku jsou úzce spjaty s úrovní neuronální aktivity skrze složitý sled koordinovaných jevů. Důležitou roli v této problematice hraje oxid dusnatý tvořený aktivovanými neurony. Oxid dusnatý je biologický mediátor, který difunduje do buněk hladkého svalstva, kde snížením intracelulární koncentrace vápníku způsobuje relaxaci cévních stěn a tím reguluje regionální průtok krve mozkem. Cílem této práce bylo objasnit roli NO neuronálního původu na hemodynamickou a neuronální odpověd mozku na transkalosální stimulaci. Jelikož anestetika signifikantně ovlivňují průtok krve mozkem, byly experimenty provedeny jak na anestezovaných, tak i na neanestezovaných potkanech.

Experimenty byly provedeny na skupině 122 dospělých potkanů kmene Wistar (250 – 350 g). Potkanům byly pod anestezií implantovány stimulační a snímací elektrody k monitorování spontánního a evokovaného EEG. Během spontánní a evokované neuronální aktivity byly pomocí Laser Doppleru měřeny změny regionálního krevního průtoku . Odpověďi na elektrickou stimulaci byly měřeny před a po intraperitoneálním (i.p.) podání 7- nitroindazolu ((7- NI) v dávce 25mgkg $^{-1}$). Pro měření systémového tlaku krve a krevních plynů byl zaveden plastový katétr do arteria carotis communis. Dále byly použity motorické behaviorální testy k ozřejmění účinku 7-NI na motoriku.

7-NI způsobil nárůst arteriálního krevního tlaku, poukazující na jeho možné systémové působení a také ovlivnilo motorické i emocionální chování zvířat. Energie a frekvence spontánního EEG byla snížena po podání 7-NI. Jak však bylo patrné z nárůstu počtu i trvání následných výbojů a poklesu prahu k vyvolání evokovaných potenciálů, došlo k nárůstu excitability tkáně. 7-NI snížil odpověď regionálního průtoku krve mozkem po trankalosální stimulaci.

Výsledná data poukazují na významnou roli NO v regulaci cerebrovaskulárního tonu. Do jaké míry se zapojuje do neuronální aktivitou vyvolaných odpovědí krevního průtoku mozkem zůstává nejisté.

Klíčová slova: hemodynamická odpověď, mozková excitabilita, syntáza oxidu dusnatého, 7-nitroindazole, potkan.

INTRODUCTION

Nitric oxide (NO) is a key cellular signalling molecule and it can be found in most of the cells of the body. It is synthesized by a family of enzymes known as nitric oxide synthases (NOS). Four izoforms of this enzyme are recognized: neuronal NOS (nNOS), endothelial NOS (eNOS), inducible NOS (iNOS) and mitochondrial NOS (mtNOS). The nNOS and eNOS are Ca^{2+}-calmodulin-dependent. And they are constitutively expressed in mammalian cells. NO exerts its effects through many cellular signalling pathways. In the brain the main route is through the activation of the guanylate cyclase (GC) cascade resulting in a reduction of cytosolic calcium (Esplugues, 2002). Independently on soluble guanylate cyclase (sGC), NO by inhibiting complexes of the respiratory chain modulates oxidative phosphorylation in the mitochondria (Brown, 2001). This implies NO to be at least in part responsible for the regulation of energy generation in neurons. The action of this molecule is limitated by its biologic half-life of barely 1second *in vivo* and because of its capability to traverse only a relatively short distance.

Despite being a simple molecule, endogenous NO is involved in many biological processes and is a fundamental component in the fields of neuroscience (Garthwaite *et al.*, 1988;Gillespie *et al.*, 1989), physiology (Furchgott & Zawadzki, 1980), and immunology (Nathan & Hibbs, Jr., 1991). A critical involvement in the regulation of many functions in the CNS under physiological and pathophysiological states has been credited to NO ever since its first recognition as a signalling molecule in the central nervous system (Brozickova C & Otahal J., 2013, (Kovacs *et al.*, 2009)).

NO is regarded as an important regulator of regional cerebral blood flow (CBF). Since the brain functions are critically dependent on a continuous supply of blood, regional CBF must increase to balance oxygen demands during functional activation. The link between the regulation of cerebral blood flow (CBF) during brain activity is known as *neurovascular coupling* or *hemodynamic response* (Hoffmeyer *et al.*, 2007;Lindauer *et al.*, 1999). It involves the coordinated interaction of neurons, glia, and vascular cells. Such vasomotor effect is mediated mainly by NO and prostanoids with approximately same magnitude (Hoffmeyer *et al.*, 2007).

NO is associated in the processes ruling the pathophysiology of epilepsy. However, datas from *in vivo* experiments on the role of NO in the pathophysiology of epilepsy display controversies. nNOS inhibition was shown to have multiple effects varying from

anticonvulsive to proconvulsive (Del-Bel *et al.*, 1997a;Itoh & Watanabe, 2009) or from neuroprotective to toxic (Silverman, 2009;Calabrese *et al.*, 2007). Findings from our recent study support the significant role of NO of neuronal origin in seizure generation during SE induced by kainic acid in mice and its probable involvement in neurobiological changes associated with the development of chronic epilepsy (Beamer *et al.*, 2012). Additionally, nNOS inhibition by 7-nitroindazole (7-NI) in combination with a potent NO scavenger (c-PTIO) was able to delay the initiation of epileptic activity in a low-magnesium model of seizures in organotypic hippocampal slice cultures and acute slices from nNOS knockout mice (Kovacs *et al.*, 2009).

One of the reasons why results from acute in vivo studies display such variations might a shift in cerebral hemodynamics during pharmacologically induced alterations of NO system. Knowledge on the role for the intrinsic properties of blood and microvascular topography as determinants of the resistance to flow is still incomplete. Nevertheless, evidence has been provided on the dominant influence of vessel radius on resistance and flow. Due to technical aspects the knowledge from this field is up to now rather poor. Recordings in freely moving animals must be interpreted with caution because of the presence of frequent movement artefacts. Such noise in the signal may be partially avoided by performing measurements in anaesthetized animals. However, anaesthetics influence blood pressure as well as cerebral hemodynamics in a significant manner.

Besides vascular effects of NO in the brain the omnipresent localization of nNOS demonstrates its implication in a wide range of physiological processes, which could also cause such discrepancies in studies on the pathophysiology of epilepsy and seizures. Motor and emotional behaviour has been assumed to be modulated by NO (Araki *et al.*, 2001;West *et al.*, 2002;Del Bel *et al.*, 2005;Araki *et al.*, 2001); (Volke *et al.*, 2003;Miguel & Nunes-de-Souza, 2008). The alterations of motor and emotional behaviour by nNOS inhibition could influence the interpretation of results of epileptological studies where seizures are often detected by the appearance of clonic movements (Del-Bel *et al.*, 1997b).

In the present thesis we tried to elucidate the effects of NO on cerebral hemodynamics in response to spontaneous and evoked neuronal activity. To prevent misinterpretations of the outcomes, we needed firstly to identify the effect of selective nNOS inhibition on general physiological parameters, spontaneous brain activity, neuronal excitability and behaviour. Furthermore, since part of the experiments were conducted under urethane anaesthesia which is frequently used to study epileptic events in anaesthetised rats we had to prove its suitability for studies on cerebrovascular dynamics.

AIMS OF RESEARCH

The purpose of the present dissertation is to elucidate the role of NO of neuronal origin on cerebral hemodynamics and thus clarify the function of NO in vascular response to spontaneous and evoked neuronal activity.

We concentrated particularly on these questions:

1. What is the effect of NO produced by nNOS in regulation of CBF during spontaneous and evoked neuronal activity *in vivo*?
2. Does neuronal NO influence spontaneous and evoked neuronal activity *in vivo*?
3. Are physiological parameters influenced by nNOS inhibition *in vivo*?
4. Does nNOS inhibition influence motor and emotional behaviour?
5. Is urethane anaesthesia which is frequently used to study epileptic events in anaesthetised rats also convenient to study cerebrovascular dynamics?

EXPERIMENT 1

BEHAVIORAL AND SYSTEMIC RESPONSE TO A NEURONAL NITRIC OXIDE SYNTHASE INHIBITOR 7-NITROINDAZOLE IN ADULT RATS.

1. MATERIALS AND METHODS

1.1. Animals

Experiments were performed in eighty five adult male Wistar rats (280-350g), provided by the Institute of Physiology of the Academy of Sciences of Czech Republic. Specifically, 10 to monitor arterial blood pressure, 16 to monitor blood gas levels, 43 to measure spontaneous brain activity and brain excitability. A group of 16 rats were used asses changes in behaviour; therefore they were submitted to ladder rung walking tests and open field (OF) tests. Rats were housed in standard plastic cages in temperature-controlled environment (22±1˚C), humidity 50-60% with a 12-h light/dark cycle (lights on at 6 am) with free access to food and water.

1.2. Drug treatment

7-Nitroindazole was obtained from Sigma-Aldrich (Czech Republic) and dissolved in dimethyl sulfoxide (DMSO). All rats were given either 7-NI (25mg/kg) or its vehicle (DMSO) intraperitoneally in a total volume of 1 ml/kg body weight. The dose of 7-NI was selected on the basis of previous studies. Solutions were freshly prepared at the beginning of each experiment.

1.3. Experimental procedures

1.3.1. Continuous recording of blood pressure in conscious animals and blood gas analysis

To monitor arterial blood pressure (BP) and to obtain blood samples for blood gas analysis (BGA) a catheter was implanted into the common carotid artery. Anaesthesia was induced with 5% isoflurane and anaesthesia was further maintained with 1.5-2.5% isoflurane during the surgical procedure. From ventral midline neck incision a trigonum caroticum was carefully exposed to avoid any damage to glomus caroticum and its inervation. After arteriotomy a plastic catheter (PE50) was inserted into the central portion of common carotid

artery and fixed with ligations. The catheter was then passed under the skin and pulled out from a small nuchal incision After postsurgical recovery (one day for BGA and 4 hours for BP) animals were placed into a transparent plastic box and the catheter was washed with heparinised saline and connected to the pressure sensor (BLPR2, WPI, Germany) (Zicha *et al.*, 2008). Blood pressure was recorded during 5 minute sessions (10 min before, 30 and 180 min after drug application) and mean arterial pressure was calculated in Spike2 (CED, UK). To asses arterial blood gasses samples of arterial blood (150µl) were collected into a glass capillary (10 min before, 30, 180 and 240 min after drug application) and immediately analysed by ABL5 Blood gas system (Radiometer, Denmark).

1.3.2. Measurements of spontaneous EEG and brain excitability and seizure susceptibility

To monitor spontaneous and evoked cortical EEG, four recording and two stimulation epidural electrodes were implanted as showed in figure 1.

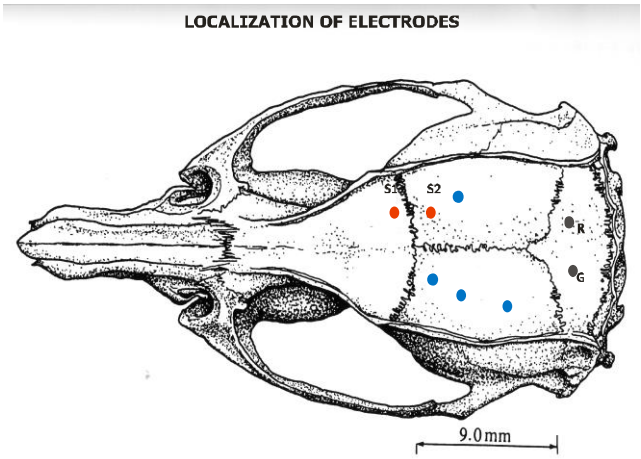

Fig.1 Localization of electrodes. Stimulation electrodes were placed over sensorimotor cortical area of the right hemisphere at coordinates AP +1 and –1; L 2 mm and are marked in red. Recording electrodes in blue were implanted over left hemisphere – sensorimotor area (AP -1; L 2.5 mm), parietal association area (AP 3; L 3 mm), occipital visual area (AP 6; L 4 mm) – and over the right hemisphere - parietal association area (AP 3; L 3 mm). Reference and grounding electrode were inserted into the occipital bone over cerebellum.

To assess the effect of 7-NI on spontaneous brain activity and on brain excitability three sessions of EEG recording were performed. Rats were placed in a transparent box (18 x 28 x 35 cm) and connected to a custom-made cable for EEG recordings. EEG data were acquired at 2 kHz and filtered at 2–500 Hz (RA16PA preamplifier and Pentusa Base Station; Tucker-Davis Technologies, Gainesville, FL, U.S.A.) (Tolner et al. 2011). Experiments were performed at room temperature in freely moving rats. First recording sessions were measured before drug application, second and third 30 or 180 min after drug application respectively. Each session consisted of spontaneous EEG recording (5min) and of a stimulation protocol to

obtain an input-output (I-O) curve. Evoked responses were evoked with 0.5 ms biphasic pulses ranging from 0.4–5 mA using a constant-current stimulator (AM Systems, Australia).

To investigate the effect of nNOS inhibition on seizure susceptibility a rat model for myoclonic seizures was used where epileptic afterdischarges (ADs) are elicited in freely moving rats by stimulation of the sensorimotor cortex. ADs (afterdischarges) were elicited by means of a 15 sec stimulation train of biphasic rectangular pulses (1 ms duration) at a frequency of 8 Hz. Biphasic constant current suprathreshold stimulus was applied to 7-NI treated and DMSO treated animals to elicit ADs. Absolute values of AD threshold intensities were designated for individual animals. Stimulation consisted of three sessions (preceding the drug administration, 30 min and 180 min after drug administration).

Power spectra and Shanon entropy was calculated from 30s epochs of the EEG offline using custom written scripts for MATLAB software (Mathworks, Inc., Natick, MA, U.S.A.). For analysis of single evoked responses, the amplitude from the first negative wave (N1) to the following positive wave (P2) was measured.

1.3.3. Behavioural measurement

Open field test (OF)

The test was performed 30 min (session 1) and 240 min (session 2) after the drug/vehicle administration for 5 min. The following behavioural variables were subsequently evaluated: locomotor activity (i.e. distance travelled), thigmotactic scanning (i.e. time spent locomoting along the walls of the open field), centre time (i.e. time spent in the central section of the open field), rearing (upright posture both against and away from the wall), self-grooming (including scratching, fur licking and face washing).

Ladder rung walking test

The horizontal ladder rung walking test was performed 90 min after the drug/vehicle administration. The time to cross the entire length of the ladder was assessed in a session with regular gaps and then in a second session with irregular gaps. In addition, the mean number of errors in foot placement was calculated; an error representing any kind of foot slip was evaluated from video recordings.

1.3.4. Statistical analysis

The OF data were analysed by a two-way repeated measure ANOVA with one between-group factor (DMSO, 7-NI) and one within subject factor (session 1, session 2). Always df value was (1,13). The data from the ladder walking test were analysed with one-way

ANOVA. As for error of foot placement, the data were expressed as the percentage of errors from the total number of steps. When appropriate, subsequent comparisons were performed with a Student- Newman-Keuls test. Remaining data were statistically evaluated using ANOVA for repeated measures and t-test where appropriate. All data are expressed as mean±standard error of mean (S.E.M.). The level of significance was set at $P< 0.05$. For statistical analysis the Sigma Stat3.5®SPSS package was used.

2. RESULTS

2.1. Effect of 7-NI on blood pressure in freely moving animals and blood gases

Following the injection of 7-NI a non-significant rise in BP occurred at both time points. To be precise, at 30 min after drug treatment the increase was 102, 77 % ± 2, 12 and at 180 min after the drug treatment it reached 102, 66 % ± 2, 11 when compared to baseline values. However, a statistically significant difference (P = 0.016) was detected between 7-NI and vehicle treated animals 180 min after the treatment.

The blood pH was non-significantly lowered at 180 min in both vehicle and 7-NI groups. The blood gas analysis revealed significant increase in pCO_2 at 180 min (114.42% ± 6.8710) and at 240 min (113.23% ± 4.3810) after the application of 7-NI when compared to values obtained 10 min before and 30 min after the 7-NI injection.

No significant alterations in blood gases or blood pressure were found after vehicle treatment.

2.2. Effect of 7-NI on spontaneous EEG, brain excitability and on seizure susceptibility

7-NI induced significant changes in spontaneous EEG and evoked EEG responses (Fig.2)

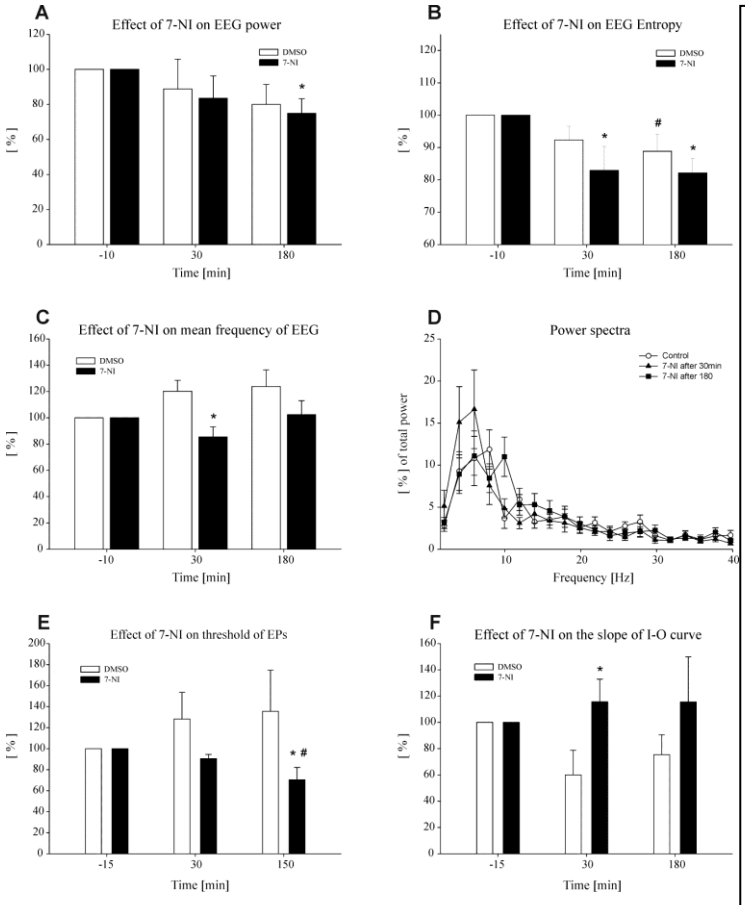

Fig.2 The power of the spontaneous EEG decreased after 7-NI injection with time (A). A significant decrease in EEG entropy occurred 30 and 180 min in 7-NI treated animals in comparison with basal values and also when compared to the DMSO group at 180min (B). 7-NI induced a significant decrease shift of the mean frequency of the EEG to the left 30min after the treatment (C, D).

Procentual changes in EPs thresholds in comparison with their magnitudes before 7-NI or DMSO treatment. The thresholds of the EPs underwent a significant drop in 7-NI treated animals. DMSO induced an increase over the cross of the experiment. This increase was not significant. (E) Procentual changes in the slope of the input-output curves (I-O curve) in comparison with their magnitudes before 7-NI or DMSO treatment. 7-NI induced an increase in slope of the I-O curve in comparison with the curve obtained from measuring before 7-NI treatment. DMSO induced a non significant decrease in the slope of the I-O curve. However, 30 min after the drug application a statistically significant difference in the value of procentual BP changes was observed between the 7-NI and DMSO treated animals. (*, $^{\#}$= P < 0, 05) (F)

In order to assess the effect of nNOS inhibition on seizure susceptibility a rat model for myoclonic seizures was used (Fig. 3) Cortical ADs were seen bilaterally and were paralleled by clonic seizures of forelimb muscles, however 7-NI reduced the behavioural seizures accompanying the ADs (data not shown).

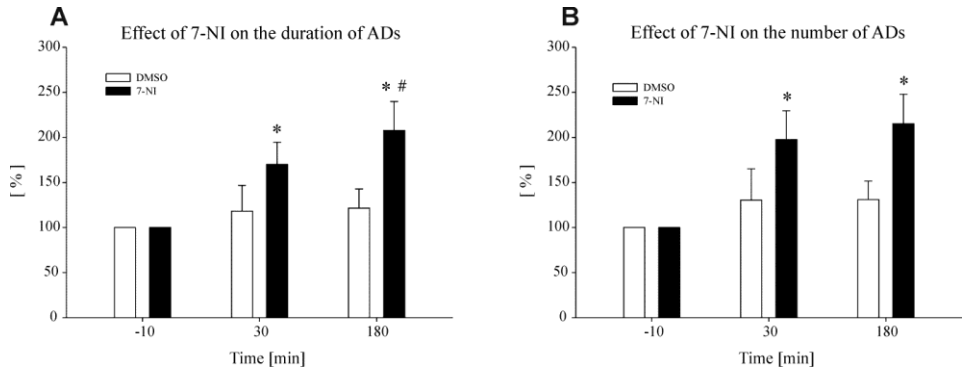

Fig.3(A)EEG monitoring revealed that the administration of 7-NI induced an increase in the duration of ADs, which was observed at both time points (30 and 180 min posttreatment) when compared to pretreatment values. Moreover, the duration of ADs was significantly longer in 7-NI treated animals than in DMSO treated animals 180 min after the drug injection. (B)Together with the prolongation of the duration of ADs a concomitant rise in the number of ADs was observed when compared to pretreatment values at both time points (30 and 180 min posttreatment). Data are expressed as the procentual changes in the duration and number of ADs of pretreatment values.

2.3. Effect of 7-NI in the Open field test and ladder rung walking test

Our results show that the selective neuronal NOS inhibitor, 7-NI in the dose of 25mg/kg decreased locomotion, exploratory rearing and grooming behaviour 30 min after systemic administration. In the test performed 240 min later the drug failed to produce a similar effect. These results demonstrate that the drug suppressed transiently the OF behaviour. With respect to ladder walking test, even though the 7-NI treated rats made more errors in foot placement, the animals were able to maintain balance and cross the entire length of the ladder irrespective of difficulty in rungs spacing. These findings strongly indicate that 7-NI affects the stepping and balance behaviour on the ladder

EXPERIMENT 2

EFFECT OF AN INHIBITOR OF NEURONAL NITRIC OXIDE SYNTHASE 7-NITROINDAZOLE ON CEREBRAL HEMODYNAMIC RESPONSE AND BRAIN EXCITABILITY IN URETHANE-ANESTHETIZED RATS

3. MATERIALS AND METHODS

3.1. Animals

Adult male Wistar rats (250-350 g, n =37) were used in our study. Rats were randomly divided into experimental groups (11 to monitor arterial blood pressure under urethane anaesthesia, 10 to monitor blood pressure in conscious animals and 16 to monitor spontaneous brain activity, brain excitability and changes of CBF). Rats were housed in standard plastic cages in temperature-controlled environment (22±1 ˚C), humidity 50-60 % with a 12-h light/dark cycle (lights on at 6 am) with free access to food and water. All experiments were performed in agreement with the Animal Protection Law of the Czech Republic, and the project was approved by the Animal Care and Use Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic. All efforts were made to minimize animal suffering and to reduce the number of animals used.

3.2. Drug treatment

7-NI obtained from Sigma-Aldrich (Czech Republic), was dissolved in dimethyl sulfoxide (DMSO). All rats were given either 7-NI or its vehicle intraperitoneally in a volume of 1 ml/kg body weight. The dose of 7-NI (25 mg/kg) was selected on the basis of previous studies and because it is the most widely used dose in neuroprotection studies. Solutions were freshly prepared at the beginning of each experiment. Urethane was obtained from Sigma-Aldrich (Czech Republic) and dissolved to 20% in distilled water. Heparin (Kentia, Czech Republic) was diluted to its final concentration 0.1% by sterile 0.01M phosphate buffered saline.

3.3. Experimental procedures

3.3.1. Continuous recording of blood pressure and heart rate

Under urethane anaesthesia (1.2 g/kg i.p.) a chronic catheter was implanted into the common carotid artery in order to measure systemic arterial blood pressure. This allowed us to measure BP in conscious animals. Heart rate was analyzed offline using power spectral algorithm in Spike2 software.

In order to assess the effect of urethane on cardiovascular dynamics same animals (n=10) were implanted carotid catheter under isoflurane anaesthesia (1.5-2 %) and then allowed to recover for 4 h. After recovery animals were placed into the plastic box for the acute recordings of blood pressure and heart rate.

3.3.2. Measurements of brain activity, brain excitability and cerebral blood flow

Epidural recording electrodes were stereotactically implanted over both cerebral hemispheres (L 3.0 mm, R-C 1.0 mm; 2x L 3.0 mm, R-C 3.0 mm; L 4.0 mm, R-C 5.5 mm) under urethane anaesthesia. Bipolar stimulation electrodes were placed over the right somatosensory cortex (L 2.5 mm, R-C +1.0 mm anterior and 1.0 mm posterior in relation to bregma, L = 2.5 mm). A reference and a ground electrode were placed over the cerebellum. For the CBF measurements skull was thinned to improve laser light transmition. Therefore skull was carefully drilled contralaterally to the stimulating electrodes until internal cortical lamina was reached. The Laser Doppler probe was then placed directly on internal cortical lamina of the remaining bone and connected to the LDF (LDPM – PF5010, Perimed, Sweden) and digitalized at 256 Hz. EEG data were acquired at 2 kHz and bandpass filtered at 2 to 500 Hz (RA16PA preamplifier and Pentusa Base Station; Tucker-Davis Technologies, Gainesville, FL, U.S.A.).

To assess cerebrovascular reactivity electric stimulation of right sensorimotor cortex was performed by gradually increasing stimulations (3, 5, 10 and 15 Hz, length 4s, intensity 3mA). Every stimulation was followed by a 3 min recovery period. The stimulation session was then repeated after 10 min and results were averaged for each frequency from both measurements. To assess the effect of 7-NI on spontaneous brain activity and brain excitability, the whole set of two stimulation sessions (see above) was repeated 30 min after treatment.

Data were analyzed offline using MATLAB software (Mathworks, Inc., Natick, MA, U.S.A.). For analysis of single evoked responses, the amplitude from the first negative wave (N1) to the following positive wave (P2) was measured. To evaluate the effect of treatment

on the brain plasticity the sum of all evoked responses during one stimulation train was calculated (∑ of EP). The baselines of CBF were measured in 10 s window preceding the stimulation. The CBF responses to the stimulations were assessed by measuring peak amplitude.

3.3.3. Statistical analysis

The data were tested for normality and statistically evaluated using ANOVA for repeated measures (in the case of more than two groups) and t-test where appropriate (two groups). All data are expressed as mean±standard error of mean (S.E.M.).

4. RESULTS

4.1. Effect of 7-NI on physiological parameters

- **Blood pressure**

During the experimental period, mean values of arterial blood pressure significantly increased after 7-NI treatment under urethane anaesthesia. The systemic blood pressure in 7-NI-treated rats was significantly higher 30 min after the drug injection in comparison to control animals (DMSO-treated): 83.95±4.84 mmHg vs. 71.79±5.50 mmHg.

Urethane itself significantly lowered arterial blood pressure (76.58±5.46 mmHg) in comparison with conscious animals (131.56±2.2 mmHg). However, 7-NI induced a nonsignificant increase in blood pressure 30 min after the treatment in conscious animals.

- **Heart rate**

Heart rate was not changed either by the application of DMSO or by 7-NI. However, a slight decrease in HR was observed following 7-NI injection.

4.2. Effect of 7-NI on cerebral blood flow

We tested the effect of the inhibition of nNOS by 7-NI on resting CBF. Regional CBF did not significantly change during nNOS inhibition by 7-NI, neither did it change in control animals.

4.3. Effect of 7-NI on neuronal excitability

To make sure that 7-NI does not cause changes in cortical excitability, cortical EPs were measured. There were no significant changes in the EPs evoked by transcallosal stimulation at any pharmacological situation. Analysis of rhythmic stimulation did not reveal any effect of 7-NI on synaptic plasticity. The sum of the EP during stimulation trains (3, 5, 10 and 15 Hz) linearly increased with frequency.

4.4. Effect of 7-NI on neurovascular coupling

Transcallosal stimulation of the contralateral cortex induces a frequency (dose) dependent rise in CBF. An application of 7-NI did not abolish the rise in CBF in response to the transcallosal stimulation. The amplitude of the hemodynamic responses increased with higher stimulation frequency in both groups. The dose response (the relation of CBF amplitude on stimulation frequency) demonstrates a linear relationship. However, we have observed an unsignificant decline of the dose-response curve after 7-NI treatment.

CONCLUSION

Up to now in vivo studies using NOS inhibitors on the role of NO in the pathophysiology of epilepsy have revealed inconsistent results. Such contradiction might be related to the systemic effects or behaviour modulations caused by the NO inhibitor itself or to the effect of pharmacologically induced alterations of the NO system on the regulation of cerebral blood flow.

Therefore, the goal of the first experiment was to elucidate the effect of the nNOS inhibitor 7-NI on behavioural and systemic parameters during spontaneous brain activity and in response to epileptiform activity after transcallosal electric stimulation in conscious rats. Our results show that in conscious rats 7-NI induced a rise in arterial blood pressure and significantly influenced levels of pCO_2 in arterial blood indicating a systemic effect. Taken together, results from performed behavioural tests indicate that the systemic administration of nitric oxide synthase inhibitor 7-NI in a dose of 25mg/kg produced disturbances in spontaneous locomotor, exploratory and grooming behaviour. Electrophysiological recordings demonstrated a suppression of the spontaneous EEG power following the i.p. administration of 7-NI. Further on, 7-NI had no effect on rhythmic potentiation or depression of EPs. However, the thresholds of the EPs underwent a significant drop in 7-NI treated animals and the slope of the I-O curve increased in comparison with the curve obtained from measuring before 7-NI treatment. According to these findings, we can assume that under the conditions of our study 7-NI increased cortical excitability.

The second experiment was carried out to identify the effect of 7-NI on neurovascular coupling in response to transcallosal electric stimulation in rats. To decrease movement artefacts in the acquired data, we conducted our experiment in urethane anaesthetised animals. Since anaesthetics influence blood pressure as well as cerebral hemodynamics, we had to prove the suitability of urethane anaesthesia to study cerebrovascular dynamics.

7-NI did not significantly alter basal cerebral blood flow and cortical excitability. Transcallosal stimulation of the contralateral cortex produced cortical epileptic afterdischarges which were paralleled by facial and fore limb clonic seizures and induced a frequency dependent rise in rCBF which was nonsignificantly reduced during nNOS inhibition. The dose response, which can be characterized as the dependency of the amplitude of the CBF response on stimulation frequency, shows only a slight decline from control measurements confirming the participation of nNOS in the hemodynamic response to

transcallosal stimulation. Our findings demonstrates that NO contributes to the basal cerebrovascular tone, but the participation of NO to CBF responses to transcallosal stimulation remains uncertain.

Finally, in urethane anaesthetised rats the inhibition of neuronal nitric oxide synthase by 7-NI influenced hemodynamic response yet it did not affect either systemic parameters or brain excitability. Consequently, our data suggest that urethane is apt to being used in the field of research of neurovascular coupling during epileptic events.

It will be of great interest to extend this study to elucidate the action of NO not only during experimentally induced seizures but also in status epilepticus and epileptogenesis. Since pharmacological research is incredibly progressive more sensitive and selective NO inhibitors are anticipated. More profound knowledge on the role of NO during in vivo seizures, epileptogenesis and epilepsy would presumably open new, highly specific targets for drug therapy of epilepsy and other pathologies of CNS. The elucidation of alterations of the neurovascular coupling in epilepsy might also highly improve diagnostic capabilities of methods which are based on detection of changes in CBF (fMRI- BOLD) where hemodynamic response functions of non-epileptic tissue are used.

The experiments are a component of a long-term research program at the Institute of Physiology, Academy of Sciences of the Czech Republic, Department of Developmental Epileptology.

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