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The Acoustic Sensorimotor Gating Predicts the Efficiency of Hypoxic Preconditioning. Participation of the Cholinergic System in This Phenomenon

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ABSTRACT

Moderate one-off hypobaric hypoxia (HBH) provokes preconditioning and prolongs the resistance (T, the time before apnoea) to severe hypobaric hypoxia (SHBH). Hypoxic preconditioning has therapeutic potential; however, the efficiency of hypoxic preconditioning varies greatly and the methods for its preliminary evaluation are absent in both animals and humans. This rodent study evaluates the dependence of SHBH resistance, initiated by HBH, on the rate of sensorimotor gating estimated in the model of the acoustic startle prepulse inhibition (PPI). A stable negative correlation was found between PPI and T. Low doses of the *α7* nicotinic receptor agonist, PNU-282987 (PNU), and more pronouncedly dimethyl sulfoxide (DMSO) (a PNU solvent), inverted the correlation between PPI and T from negative to positive. The DMSO and PNU effects were reversed at PPIs of 0.36 - 0.40 (36% - 40%). DMSO increased T values by 52.2% ± 9.7% in the region of lower HBH efficiency (PPI ≥ 0.40) and reduced it by 35.2% ± 9.3% in the region of higher HBH efficiency (PPI < 0.40). PNU reduced both DMSO effects. The involvement of the central cholinergic mechanisms was substantiated in both DMSO and PNU influences on HBH. In conclusion, 1) PPI can be used to predict the efficiency of hypoxic preconditioning and to study its mechanisms, 2) two opposite cholinergic PPI-related mechanisms participate in the preconditioning effects of HBH, 3) the sensitivity of rats to DMSO and PNU diverges when the PPI is 0.36 - 0.40,

and 4) DMSO can enhance resistance to severe hypoxia in the region of the lower preconditioning efficiency of HBH at PPI ≥ 0.4.

1. INTRODUCTION

Hypoxic and ischaemic preconditioning, in which hypoxia is the main adaptive factor, mobilise the body's protection and thereby prevent or reduce the pathogenesis of severe hypoxia or ischaemic stroke in organs and tissues [1-4]. Moderate one-off hypobaric hypoxia (HBH; 11% O_2 for 60 min) provokes preconditioning and increases the resistance to severe hypobaric hypoxia (SHBH; 4.5% O_2). In other words, it prolongs the time (T) before the onset of apnoea in SHBH conditions [5-7]. Recent rodent studies have shown high rat-to-rat variability in the SHBH resistance initiated by HBH and that the efficiency of HBH does not depend on the innate resistance to the direct action of SHBH (*i.e.* it is formed on its own, independent of SHBH mechanisms) [7, 8].

Thus the problem appears to be the absence of methods for the preliminary evaluation of the efficiency of hypoxic preconditioning. The efficiency of the preconditioning effects of HBH could be based on different mechanisms, and knowledge of these different mechanisms could be significant for both fundamental and practical medicine.

The sensorimotor gating of the startle reflex is estimated by the magnitude of prepulse inhibition (PPI) in acoustic sensorimotor startle reaction, also briefly called the PPI of startle. It is a well-known model that was developed in the second half of 20th century for neurobiology, especially psychiatry [9]. The PPI is decrease of the amplitude of the reaction to an intense sudden stimulus under conditions when a signal of lower intensity precedes it with a small interval (<500 ms). PPI characterises the mechanisms sensorimotor information filtration (gating), which depends on the functional state of several brain structures and the activity of many neurotransmitter systems [10]. A reduced PPI is a biomarker of predisposition to schizophrenia and other psychiatric disorders [9-13]. At the same time, low levels of PPI are observed in healthy individuals, and the biological benefits of high levels of PPI are unclear [11]. The mechanisms underlying the low level of PPI as a manifestation of biological diversity may differ from the mechanisms that lead to its abnormality in pathological processes [11, 14].

Reports suggest that there is a possible association between the sensorimotor gating and hypoxic preconditioning due to the existence of common links in their regulatory mechanisms. One such mechanism may be the NF-κB signalling pathway [15-17]. In this rodent study, the PPI rates and HBH preconditioning effects were compared and the PPI model was proposed as a test for predicting HBH efficiency.

Acetylcholine, via nicotinic receptors (nAChRs), is involved in hypoxic or ischaemic preconditioning in the brain and other organs, and this is most convincingly demonstrated for the homomeric *α7* nAChRs [18-21]. The preconditioning (as well as postconditioning) *α7* nAChRs effects are closely related to neuronal adaptive and neuroimmune antiinflammatory mechanisms in which *α7* nAChRs are particularly important in protecting against hypoxic damage in peripheral organs [22-25] and the central nervous system [19, 20, 22, 26-28]. Functional *α7* nAChRs have been detected in neurons [9, 29-33] and in cytokine-producing cells of the immune system in both the brain [20, 27, 29] and other organs [22, 24-26, 34, 35]. In addition, *α7* nAChRs of the hippocampus and cortex also play important roles in cognitive function in norm and pathology [29, 30, 36-39], and there is evidence of a relationship between PPI and cognitive disturbances in patients with schizophrenia [9, 10, 40]. There are also single direct data that *α7* nAChRs selectively mediate the stimulating effect of nicotine and other selective agonists on PPI [41, 42].

Taken together, the data suggest that there is an interconnection between *α7* nAChRs, hypoxic preconditioning and PPI. This study investigates the cholinergic nicotinic mechanisms of HBH preconditioning in rats using pharmacological experiments with the *α7* nAChRs selective agonist, PNU-282,987 (PNU), in a solution of dimethyl sulfoxide (DMSO) in the PPI model. PPI measures were compared with the HBH-initiated SHBH resistance and with the effects of PNU on the HBH preconditioning efficiency.

2. MATERIALS AND METHODS

2.1. Animals and Ethical Approval

Experiments were performed on male outbred albino laboratory rats aged 2 - 2.5 months (weight 220 - 300 g). The rats were supplied by the animal nursery at Light Mountains (Russian Federation) and kept in the vivarium of the Institute of General Pathology and Pathophysiology.

All animal care and experimental procedures were conducted in accordance with the official regulations of the European Communities Council Directive on the use of laboratory animals (86/609/EEC). All protocols were performed under a license from the Institute of General Pathology and Pathophysiology (institute order number 38-P, 2 June 2014) and were approved by the institute's ethical committee. All efforts were made to minimize the number of animals used and their suffering.

The rats were housed in a temperature-controlled room (20˚C - 24˚C) with 5 - 7 animals per cage. They had free access to food and water and were maintained with a 12 h light-dark cycle. The rats were handled for at least 2 consecutive days prior to being placed in the pressure chamber. At the end of the experiment, the animals were euthanized via inhalation of CO_2 using euthanasia apparatus (AE0904, Open Science, Russian Federation).

2.2. Model of PPI of Startle

The equipment for testing PPI is described in detail else where [43]. The rats were tested in a specialised transparent round plexiglass chamber with a diameter of 160 mm and a height of 140 mm. The camera was equipped with a load cell, which fixed the amplitude of the startle reaction, and a personal computer. Broadband noise with duration of 100 ms and loudness of 110 dB was used as the main stimulus. The background-masking sound signal had a broadband noise with a loudness of 72 dB, and the prestimulus had a signal duration of 40 ms and a loudness of 85 dB. Animals were placed in the chamber, and after 5 min acclimatisation were exposed to a total of 12 trials. The first two trials were pulse-alone trials. The remaining 10 trials were presented in pseudo-random order and included five pulse-alone trials and five pulse trials with a preceding prepulse of 100 ms in the lead-off interval. The inter-trial intervals ranged from 10 to 20 with a mean value of 15 s.

The PPI value was estimated from the formula $Am - Ap/Am$, where Am is the average reaction amplitude in the samples without a prestimulus (n = 7) and Ap is the average reaction amplitude in the samples with a prestimulus (n = 5). The PPI values were not presented as percentages, as is customary, but as the relationships that used to demonstrate on the graphs.

2.3. Hypoxic Models

The same hypoxic models were used as previously described [7, 8]. Varying severities of hypoxia were created in a pressure chamber. The barometer of the chamber (altitude gauge) was calibrated to an altitude above sea level. The rats in the chamber were "raised" at a speed of 50 m/s to the adaptive altitude of 5000 m (HB = 3.0 Pa, equivalent to 11% O_2 for 60 min) or to the critical altitude of 11,500 m (SHBH = 1.2 Pa, equivalent to 4.5% O_2). In the critical altitude test, the resistance to hypoxia was recorded with respect to the endurance under SHBH conditions, which was the time (T, s) until agonal inspiration (apnoea) in combination with a loss of voluntary control of body tone.

2.4. Drugs

PNU (MW = 269.25, Tocris Bioscience, Bristol, UK) is a selective agonist of the $\alpha 7$ subtype of nAChRs. DMSO (MW = 78.13, LLC "Tula Pharmaceutical Factory", Tula, RF) is a bipolar aprotic solvent for hydrophobic drugs. Rats given the drugs received a single intraperitoneal (I.P.) injection of 7 or 70 μg/ kg (26 or 260 nmol/kg) PNU in 3% DMSO (PNU groups) or 3% DMSO only (DMSO group).

2.5. Experimental Protocol

The scheme of the experiment is presented in Figure 1. All rats were tested in the acoustic sensorimotor startle reaction model, and the values of PPI were estimated. After that the rats were subdivided into the HBH group (n = 19), PNU group (n = 35), DMSO group (n = 16) and SHBH group (n = 20). At 2 - 4 days after PPI testing, animals in the HBH, PNU and DMSO groups were subjected to a single HBH session, and 4 min after the end of the session they were subjected to SHBH and the values of T were estimated. Animals in the SHBH group were directly exposed to SHBH, bypassing the HBH session.

Rats in the PNU groups received a single I.P. injection of PNU (26 nmol/kg, n = 23; 260 nmol/kg, n = 12) in 3% DMSO. Rats in the DMSO group received a single I.P. injection of 3% DMSO. Rats in the HBH and SHBH groups received a single I.P. injection of physiological saline. Both drugs and saline were injected 10 - 15 min before HBH or SHBH (in the SHBH group) session. The time of treatment was evaluated from the experimental data [36, 37].

All data were generated in a double-blind manner. Data in all rat groups were obtained from two independent experimental rat samples.

2.6. Statistics

Data were calculated using the nonparametric one-sided Fisher's exact test and the r-criterion of the Pearson's correlative test in Microsoft Excel, with the formula being adjusted to account for the small number of observations (n = 4 - 15) [44]. Differences were considered to be statistically significant if $P < 0.05$. Using the Bonferroni correction, differences were considered to be statistically significant if $P < 0.025$ for four selections. In this case, a significance level of $P < 0.05$ was considered a strong tendency for the validity of events and was taken into account in the presence of other comparisons.

When performing correlation analyses, the presence of "outliers" in the experimental groups was taken into account. It should be noted in almost every data set in which the correlation dependence is examined, individual values represent the "outliers" in accordance with statistical terminology (*i.e.* they are atypical sharply-allocated observations of unknown aetiology). "Outliers" are usually calculated as values

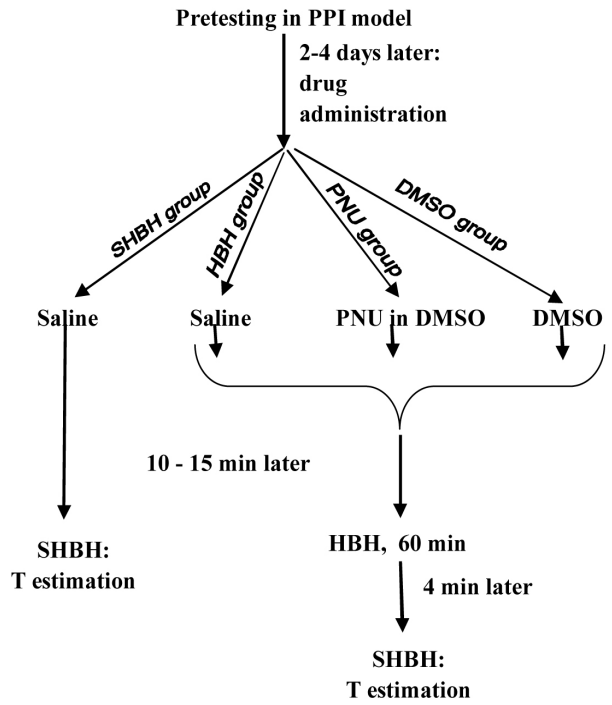

Figure 1. Scheme of experiments.

that go beyond the mean by two or more standard deviations. Since these "outliers" can artificially increase or decrease the existing correlation, they were excluded from the calculation.

3. RESULTS

3.1. HBH Group

Hypoxic preconditioning initiated a range of resistance to SHBH with T values from 4 min 20 s to 28 min 25 s. Such a range was received immediately in the first experimental sample. Correlation analysis revealed a significant negative correlation between T and PPI values (r = −0.764, n = 10, $P < 0.02$). In this sample, the PPI range was 0.062 - 0.876 (6.2% - 87.6%). In the second experimental sample, the PPI range advanced towards the animals with a weak or even complete absence of inhibition (PPI = −0.122 - 0.711 or −12.2% - 71.1%). T values in the second sample also showed a negative dependence on the PPI values (r = −0.791, n = 8, $P < 0.02$). In this sample, one rat was an exception: it showed a higher resistance to SHBH after HBH than was expected from the results of testing of rats in the first sample (PPI = 0.711, T = 1380) and disturbed the correlation between T and PPI values (r = −0.178, n = 9, $P > 0.05$). This T value goes beyond the mean by one and a half standard deviation and according to some experts it can be counted as "outlier" (the description of "outliers" is provided in the statistics subsection of the materials and methods). However, a T value higher than expected does not violate the idea of the preconditioning effect of HBH, and in the combined HBH group of rats, including the exception, the general data array confirmed the negative correlation between T and PPI indices (r = −0.548, n = 19, $P < 0.02$) (Figure 2(a)).

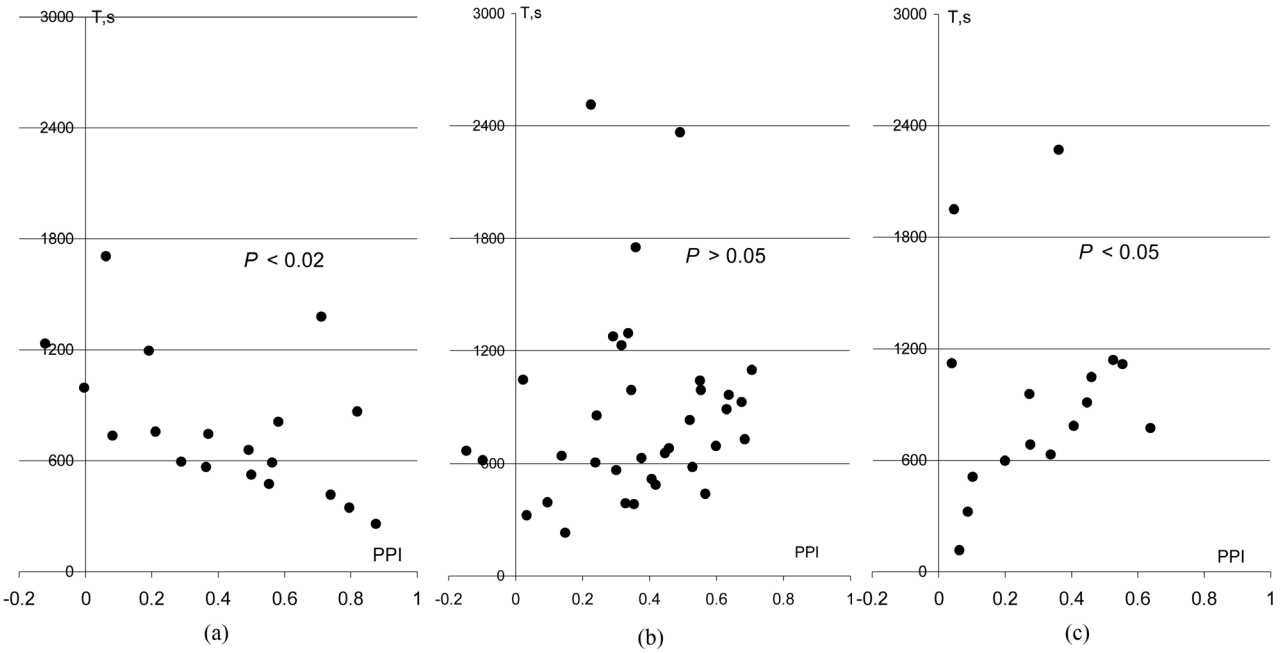

Figure 2. Graphs showing the dependence of the HBH preconditioning efficiency (T) on the rate of PPI under the different experimental loads. The significant negative correlation between T and PPI (r = −0.548, $P < 0.02$) after HBH (a) became positive under the influence of PNU in DMSO (r = 0.232, $P > 0.05$) (b) or DMSO alone (r = +0.592, $P < 0.05$) (c) using the Pearson's r-criterion test. The two largest T values in both of the PNU (b) and DMSO (c) groups were calculated as "outliers". (a) The HBH group; (b) The PNU group; (c) The DMSO group; *round markers*, individual values of T corresponding to own PPI; T, a time before apnoea.

3.2. SHBH Group

The rats exposed to direct SHBH showed a usual wide-spectrum of innate resistance to severe hypoxia from 49 s to 21 min 26 s [7]. The indicators of innate SHBH resistance were not correlated with PPI values in either of the experimental samples (sample one: r = −0.179, n = 8, $P > 0.05$; sample two: r = 0.165, n = 12, $P > 0.05$) and in the general data array (r = 0.122, n = 20, $P > 0.05$).

3.3. PNU and DMSO Groups

It was noted that the two largest T values in both the PNU and DMSO groups were "outliers". These were the T values 2364 and 2514 s in the PNU group and 1951 and 2271 s in the DMSO group. Although these T values are shown in the figures, they were excluded from all calculations.

In the PNU group, the *α7* nAChRs agonist was injected at both 26 and 260 nmol/kg doses in both the first and second experimental sample, and no dose-dependent differences were observed in either sample. Therefore the data for these two doses were combined.

In the PNU group of the first experimental sample, a significant correlation was observed between T and PPI, which was in opposition to that found in the HBH group (r = 0.567, n = 13, $P < 0.05$). However, the values in the DMSO group showed a distribution of T values similar to the PNU group with a medium-strength correlation between T and PPI (r = 0.465, n = 7, $P > 0.05$). Therefore an additional experiment was performed.

In the PNU group of the second experimental sample, the inverse directivity of T with respect to PPI was confirmed; however, this did not reach significance in the second sample or in the general data array (sample two: r = 0.060, n = 19, $P > 0.05$; general array: r = 0.232, n = 33, $P > 0.05$) (**Figure 2(b)**). Conversely, additional data in the DMSO group revealed a positive correlation between T and PPI in the general data array (r = 0.592, n = 14, $P < 0.05$) (**Figure 2(c)**).

3.4. The PPI-Related Separation of DMSO and PNU Effects

Based on the results, the DMSO group was divided into two subgroups when PPI = 0.36, or even better when PPI = 0.40 (because the value of PPI = 0.362 corresponded to an "outlier"). With respect to the HBH group, higher T values prevailed in the DMSO subgroup with PPI values ≥ 0.36 - 0.4 and lower T values prevailed in the subgroup with PPI values < 0.36 - 0.4 (**Figure 3**). At the same separating points, a similar ratio was observed between DMSO and PNU in the region of PPI ≥ 0.36 - 0.4, while in the PPI region <0.36 - 0.4, the T values were more mixed (**Figure 4**).

The mean T values were very similar in each experimental rat subgroup at both PPI = 0.36 and PPI = 0.4, and the statistical results were completely identical. Therefore, PPI = 0.4 was arbitrarily chosen to demonstrate the data. In rats with a PPI ≥ 0.4, DMSO significantly increased the T value by an average of 52.2% ± 9.7% ($P < 0.025$) compared to HBH alone. PNU significantly reduced this effect by an average of 20.3% ± 5.5% ($P < 0.05$) and did not show a significant difference from the data in the respective HBH subgroup (**Figure 5(a)**). Conversely, in rats with a PPI < 0.4, DMSO significantly reduced the T values by an average of 35.2% ± 9.3% ($P < 0.05$) compared with the mean value in the HBH subgroup. Similarly, the mean T value in this PNU subgroup fell between the average values in the HBH and DMSO subgroups but did not differ significantly from either of them (**Figure 5(b)**).

4. DISCUSSION

4.1. The Selective Relationship between the Rate of PPI and HBH Efficiency (The PPI-T Test)

The PPI model has previously been used to investigate sensory-motor information and the integrity of the brain structure involved [9-11, 13, 40]; however, it has not yet been used to estimate the efficiency of hypoxic preconditioning. Severe hypoxia in early ontogenesis was shown to reduce PPI in adult mice and rats [45, 46]. In adult rats, no correlation was found between PPI and innate SHBH resistance both before and 2 - 3 weeks after SHBH (Storozheva ZI, unpublished data). The experiments in this study, performed

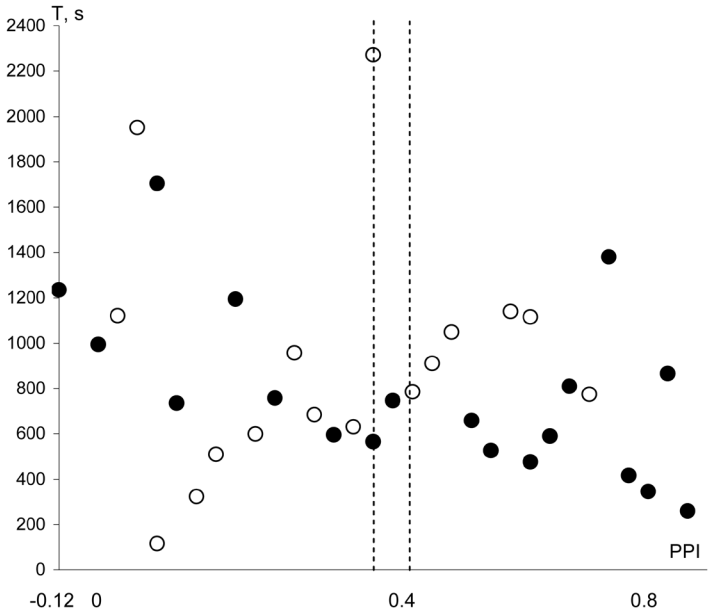

Figure 3. Combined graphs of the dependence of the HBH preconditioning efficiency (T) on the rate of PPI in the HBH and DMSO groups. *Vertical dotted lines* indicate the values of PPI 0.36 and 0.40. The figures under the x-axis are given for orientation and denote the location of the corresponding values of PPI on the axis. The relationship between the T values in the compared groups differs on the opposite sides of PPI 0.36 - 0.40. *Round markers*, the individual values of T: *T*, as in Figure 1; *black markers*, HBH group; *white markers*, DMSO group.

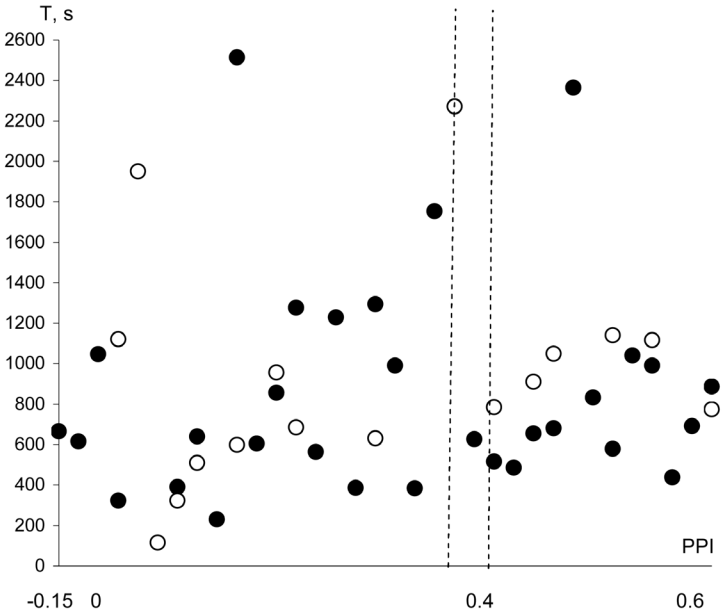

Figure 4. Combined graphs of the dependence of the HBH preconditioning efficiency (T) on the rate of PPI in the PNU and DMSO groups. *Black markers*, PNU group. All remaining symbols are the same as Figure 3.

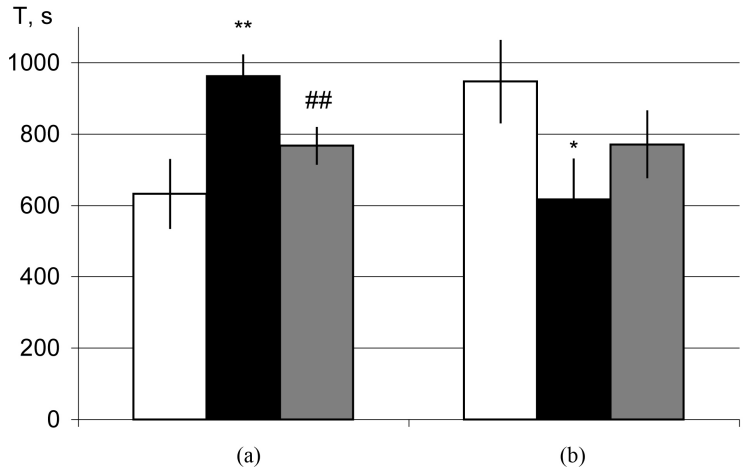

Figure 5. Influence of DMSO and PNU on the HBH preconditioning efficiency in (a) subgroups with PPI > 0.4 and (b) PPI < 0.4. Values are expressed as means ± SE. *P < 0.05 and **P < 0.025 compared with T values in the relevant HBH groups, $^{\#\#}P$ < 0.025 compared with T values in the relevant DMSO group using Fisher's exact test. *White bars*, HBH group; *black bars*, DMSO group; *grey bars*, PNU group.

in accordance with the first scheme (*i.e.* pretesting of PPI), confirmed the absence of correlation between the rate of PPI and innate SHBH resistance in adult rats; however, there was a correspondence between the values of PPI and T initiated by HBH, and the HBH efficiency was reliably and negatively correlated with PPI. The discrepancy in the relationship between PPI-HBH-SHBH versus PPI-SHBH reinforces previous data that showed that HBH has its own mechanisms of resistance to SHBH [7, 8].

These are the first direct experimental data to report the relationship between PPI and hypoxic preconditioning pathways (RF patent no. 2571603). Such a link has previously only been assumed from indirect data. For example, components of the NF-κB signal transduction pathway were found to be oxygen dependent [47-49] and involved not only in pathological proinflammatory processes but also in the preconditioning mechanisms of brain tolerance to hypoxia and ischaemic stroke [15, 17]. Downregulation of the NF-κB pathway was also detected in patients with schizophrenia, and the correlation between NF-κB signalling component abnormalities and reduced PPI in healthy individuals [16] led to the speculation of a relationship between NF-κB (and other regulatory factors) and PPI in pathology and adaptation to it in the wider sense of these concepts.

In accordance with the data presented in this study, PPI indications can serve to predict the efficiency of hypoxic preconditioning. It is recommended that only one PPI-T test be performed in the experimental animals. A calibration PPI-T line should be created from the lowest values of T and this should be used in further experiments to estimate the PPI values in the animals. This highly-reliable line guarantees a minimum resistance to hypoxic loads in an animal with known PPI. A calibration line for adult albino outbred rats is presented in **Figure 6**.

One advantage of PPI is that it can be measured across species, from humans to rodents, and the PPI model can be used in both experimental and clinical practice [13, 40, 50, 51]. Therefore the PPI-T test has the ability to be used in many areas of practical medicine.

4.2. PPI-Related Bidirectional Effects of DMSO and PNU

In this study, the PPI-T test was used to investigate the cholinergic mechanisms of hypoxic preconditioning using the *α7* nAChRs agonist PNU. Some unexpected results were obtained, such as the more pronounced effects of HBH under the influence of the solvent DMSO and not PNU. At the given doses,

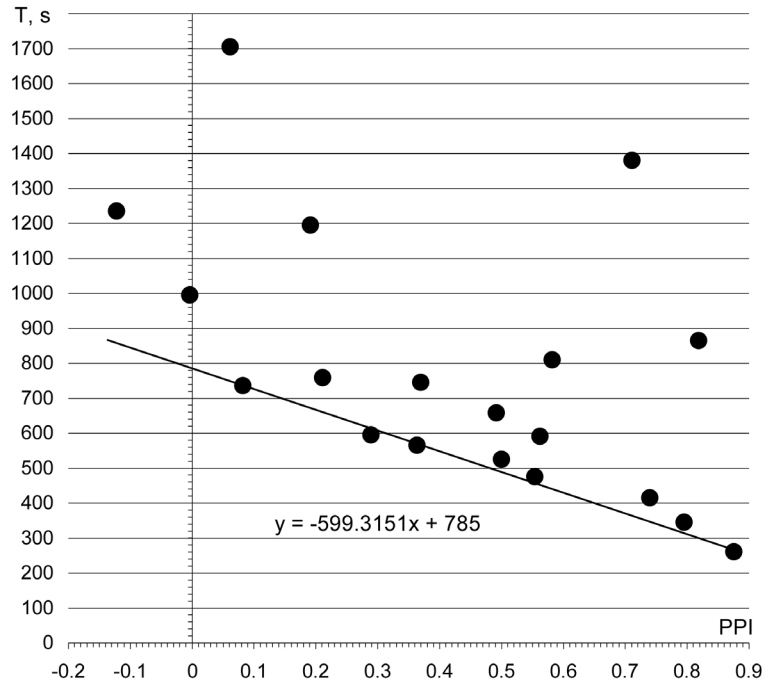

Figure 6. The PPI-T test showing the lower limit of SHBH resistance after HBH in the male outbred albino laboratory rat. The line is drawn along the lower boundary of the T values, which serves as a calibration line for determining the lower limit of T values in rats with a known value of PPI. *Black round markers*, individual values of T in the HBH group; under the line is *the formula of line*.

only DMSO authentically corrected the efficiency of HBH and completely reversed the relationship between the PPI and T values. These data indicate that DMSO operated on HBH in two bidirectional pathways: 1) the drug potentiated the HBH preconditioning effects in the DMSO subgroup with PPI ≥ 0.36 - 0.40 and 2) reduced the preconditioning effects in the subgroup with PPI < 0.36 - 0.4. PNU reduced the significance of the DMSO effects on HBH, approximating the mean values from both own subgroups to those of the HBH subgroups.

The mechanisms behind the opposite effects of DMSO and PNU on HBH efficiency on either side of the PPI boundary remain unknown. It should be noted that there are no data of such a phenomenon in healthy individuals; however, numerous observations indicate the existence of such a boundary in psychiatric practice, with PPI < 50% reflecting pathology across different patients [40]. Electrophysiological studies on neuronal cultures and slices showed a high variability in the reactions of the same types of neurons to the same doses of nAChRs agonists [30, 38, 52, 53], including PNU at the low doses (30 and 300 nM) [36]. Therefore it is possible that the features of neuronal interactions underpin the functional differences in the subgroups of rats used in this study. Data from this study will allow more definitive conclusions to be drawn about the mechanisms of DMSO and PNU action on HBH preconditioning at the examined doses.

4.3. DMSO Can Regulate the Efficiency of HBH Preconditioning as an Anticholinesterase Agent

DMSO has a broad spectrum of biological activity and is increasingly used in medicines due to its antiinflammatory, trophic and conductive properties [54]. The mechanisms of DMSO action have mostly been studied in neurobiology [55] where the drug was shown to exhibit anticholinesterase action [56], block the reuptake of choline and glutamate [57, 58] and modulate neuronal activity (including choliner-

gic function) [55, 58, 59].

In this study, DMSO was used I.P at a concentration of 3% (1 mL/250g). DMSO can easily penetrate into any body tissue and can cross the blood-brain barrier. Therefore, the concentration of DMSO in the brain was 0.006% - 0.007% (0.75 - 0.9 mM) based on its uniform distribution in the body and did not exceed ~0.04% (4.8 - 5.5 mM) based on the maximum 5% penetration of a substance into the brain. According to the literature, such low concentrations of DMSO would only result in anticholinesterase activity, as additional effects require much larger doses. This suggests that the cholinergic system participates in the preconditioning mechanisms of HBH in both DMSO subgroups. The central action of DMSO was confirmed by the PNU influences on its effects.

The preconditioning effect of acetylcholinesterase inhibitors, which are used in the treatment of Alzheimer's disease, was shown to protect rats against ischaemic injury in the cortex and hippocampus or to protect cortical neuronal cultures [60-62]. These data coupled with the data presented here suggest that all anticholinesterase agents, including DMSO, have possible preconditioning effects.

4.4. The Desensitising Action of PNU Is Opposite to the Action of DMSO on HBH Efficiency

PNU is a potent and selective agonist of neuronal *α7* nAChRs and rapidly penetrates into the brain [36, 37, 63]. In a rodent study, the peripheral antiinflammatory effect of PNU on the blood-brain barrier permeability was recorded by stimulation of the *α7* nAChRs on splenic macrophages using large agonist doses (30 mkg or ~113 nmoles injected into the spleen) [34].

This study aimed to reveal the effects of low concentrations of PNU on HBH since the agonist is known to cause negative side effects [37]. At similar low concentrations, a selective agonist of the *α*4β2 subtype of nAChRs (RJR 2304) and an antagonist of non-*α7* nAChR subtypes (mecamylamine) were able to influence the learning of normal and ischaemic rats following subchronic systemic administration [64]. This study used two doses of PNU (7 and 70 μg/kg equating to 26 and 260 nmoles/kg), initially assuming that 5% of the agonist would penetrate the brain. Assuming that the rat brain weighed approximately 2 g and had a volume of 4 ml (measurements from this laboratory), PNU concentrations in the brain would be approximately 80 and 800 nM, respectively. Different effects were expected with the different doses of PNU [36].

However, the effects of both doses of PNU were identical in the subgroups of rats with both lower and higher HBH efficiency. Therefore the concentrations of the agonist penetrating the brain are believed to have been much lower. According to a calculation based on the comparative data of effective PNU doses in vivo and in vitro [36], the PNU concentrations in the brain were approximately 2 and 20 nM with the low and high doses, respectively. The same concentrations, or almost half the value (1.2 - 12 nM), were obtained from a calculation based on recent data regarding the concentration of PNU in mouse brain 1 h after systemic administration, and from comparative data on the permeability of PNU and FRM-17874 (another agonist *α7* nAChRs) in mouse brain and the permeability of FRM-17874 in mouse and rat brain [38, 63].

PNU is thought to exhibit desensitising properties at such low concentrations [36, 38, 52]. The desensitising effect of PNU is opposite to that of DMSO, the anticholinesterase action of which is manifested in an increase in the acetylcholine concentration in the synaptic cleft and, accordingly, the activation of cholinergic function. To achieve the desensitising effect, an ineffective dose of an *α7* nAChRs agonist must precede the subsequent action of acetylcholine or another *α7* nAChRs agonist in an effective dose [52]. However, PNU was administered simultaneously with its solvent in this study. Nevertheless, it is possible that PNU, as a highly selective and potent agonist of *α7* nAChRs, reached the receptors earlier than DMSO blocked acetylcholinesterase, thus enabling the conditions for preincubation of *α7* nAChRs to be met. This could explain the antagonistic action of the two drugs.

4.5. Another Possible Pathway of Interaction between PNU and DMSO

Investigation of the preconditioning mechanisms of acetylcholinesterase inhibitors revealed that these

inhibitors not only possess an anticholinesterase action but also manifest neuroprotective properties that are independent of acetylcholinesterase inhibition. The protective effects of these inhibitors were antagonised using selective nAChRs antagonists, and the neuroprotection was shown to be carried out through the modulation of the expression levels of the *α7* and *α4* subunits of nAChRs [62]. This agrees with the results presented in this study and suggests a closer interaction of DMSO and PNU through *α7* nAChRs. The action of PNU versus DMSO in low doses is believed to manifest itself as an antagonist of *α7* nAChRs.

5. CONCLUSIONS

Using the PPI model for preliminary evaluation of the acoustic sensorimotor gating, a PPI-T test was developed to predict the efficiency of HBH preconditioning in animals. Also, the PPI-T test has the potential to be used in medical practice because the PPI is measured in both animals and humans.

The PPI-T test allowed the mechanisms of hypoxic preconditioning to be studied and substantiated that the central cholinergic mechanisms were involved in HBH preconditioning. The efficiency of HBH preconditioning was significantly negatively correlated with PPI values. Pharmacological experiments with the *α7* nAChR agonist, PNU, revealed that PNU and solvent DMSO modulate the preconditioning effects of HBH. Two PPI-related rat subgroups were shown to have opposite actions of PNU and DMSO on the HBH effects. DMSO potentiated the preconditioning effects of HBH in rats with PPI values ≥ 0.36 - 0.40, while it reduced the HBH effects in rats with PPI < 0.36 - 0.4. PNU reduced the effects of DMSO on HBH in both subgroups of rats. Thereby, DMSO has the useful ability to enhance resistance to severe hypoxia in the region of the lower preconditioning efficiency of HBH.

Principal reasoning is that the PNU and DMSO experiments generally demonstrate the validity of the proposed PPI-T test. It is the distribution of T in accordance with the PPI values that has made it possible to identify two subgroups with opposite effects of DMSO and PNU on HBH preconditioning. It is noteworthy that these effects would not have been seen if the data were not indexed in the PPI model.

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