Short communication

Does fucose or piracetam modify the effect of hypoxia preconditioning against pentylenetetrazol-induced seizures?

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Abstract

To clarify the question whether the duration of hypoxia exposure has an influence on the point in time or the strength of hypoxic preconditioning, hypoxia exposure of rats lasting 1 and 8 h was tested regarding the modification of susceptibility to acute pentylenetetrazol-induced seizures. Following the short-lasting (1 h) hypoxia, the maximum level of preconditioning action was observed 7 days after hypoxia, whereas the longer-lasting hypoxia (8 h) produced the maximum level of protection 14 days after hypoxia. We investigated the influence of fucose and piracetam on the effect of hypoxia preconditioning by the application of the substances 20 min before the beginning of hypoxia exposure. Fucose did not modify the result of hypoxia preconditioning. But after the treatment with piracetam, the preconditioning effect was prevented following hypoxia lasting 1 and 8 h. We suggest that the radical scavenger properties of piracetam are responsible for the absence of protection against pentylenetetrazol-evoked seizures. © 2000 Elsevier Science B.V. All rights reserved.

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Moderate hypoxia reduces the susceptibility of mice to pentylenetetrazol (PTZ)-induced seizures [13] and protects rats against the neurotoxicity of kainic acid [4,11]. Inversely, it can be assumed that this phenomenon of ‘hypoxic preconditioning’ may be involved in the protection from neuronal death following carotis artery ligation combined with hypoxia which is triggered by kainic acid- or flurothyl vapour-induced seizures [20]. However, it is becoming evident that the mammalian brain also possesses adaptive processes that can confer tolerance to seizures or hypoxia/ ischemia-induced neuronal damage which have not yet been clarified. During hypoxia or the ischemic period, or both, an excessive glutamate release and an increased stimulation of different glutamatergic receptors and the subsequent enhanced formation of free radicals are possibly involved in the mediation of protection against the different noxes [21].

The protective mechanisms are plastic-adaptive processes of transient duration [13,14]. Whether there is a connection between the duration or strength of preconditioning or the event producing ischemic tolerance and the consequence for hypoxic preconditioning or ischemic tolerance, was not investigated. For this reason, our first question is whether the process of hypoxic preconditioning is dependent on the duration of hypoxia exposure. For this reason we exposed one group of rats to hypoxic conditions for 1 h and a second group for 8 h.

Another question is whether preconditioning may be modified by treatment with nootropic drugs intervening in plastic-adaptive processes such as learning and memory or long-term-potentiation (LTP). In the present studies we investigated fucose (FUC), a substance improving learning and memory and LTP [7] and piracetam (PIR) facilitating learning and memory [10] and influencing LTP in different manner [16]. Both nootropic drugs were studied at doses facilitating plastic adaptive processes.

The experiments were performed using 8-week-old male Wistar rats. The animals were kept under a lighting regime of light/dark 12:12 (light on at 6.00 a.m.), temperature 20±2°C, and air humidity 55–60%. They had free access to food and water. For all procedures followed, ethical approval was obtained prior to the experiments according
The following groups were prepared. Group 1: naive controls which were not treated; group 2: sham-exposed controls which were exposed to room air in the hypoxia chamber. Group 3: hypoxic animals which were exposed to 9% O₂ and 91% N₂.

A mixture of 9% O₂ and 91% N₂ streamed continuously through the chamber. Accurate mixing of O₂ and N₂ was achieved by controlling the air pump by an oxygen sensor (Oxopac RE, Draeger, Germany). Sham-exposed controls were exposed to the same chamber which was ventilated by room air. Every five rats were placed into the hypoxia chamber and exposed to the hypoxic conditions for 1 or 8 h.

Experiments to test the susceptibility to seizures induced by pentylenetetrazol (PTZ) were done 1, 4, 7, 14 or 21 days after hypoxia or sham exposure. Different groups were investigated at each time point. To estimate the influence of drug treatment on hypoxic preconditioning, the animals were injected with PTZ, 7 days following hypoxia lasting 1 h and 14 days following hypoxia lasting 8 h, respectively. The rats of all groups received an intraperitoneal application of 55 mg/kg PTZ dissolved in physiological saline solution. After administration of PTZ, the behavior of the animals was observed for a period of 20 min. The resultant seizures were classified as follows: Stage 0: no response. Stage 1: ear and facial twitching. Stage 2: convulsive waves though the body. Stage 3: myoclonic jerks, rearing. Stage 4: turn over onto side position. Stage 5: turn over onto back position, generalized tonic-clonic seizures. The experimenter was unaware of the pretreatment of the animals.

To test the influence of fucose (FUC) or piracetam (PIR) on hypoxia-induced changes in the susceptibility to seizures evoked by PTZ, both substances were intraperitoneally injected at a dose of 100 mg/kg body weight 30 min before the start of hypoxia exposure. Saline controls received 10 ml NaCl/kg body weight.

Statistical differences were determined using one-way-ANOVA followed by a modified LSD (Tukey) with a significance level of 0.05 for post hoc comparisons. Data are presented as mean±S.E.M. for each treatment group.

As shown in Fig. 1, the hypoxia preconditioning lasting 1 or 8 h causes a significant reduction in susceptibility to PTZ if the observed seizure stage of hypoxia exposed animals were compared with that of naive or sham-exposed control animals at day 7 or 14 after hypoxia. The prolongation of hypoxia duration from 1 to 8 h could not improve the quantity of protection regarding the susceptibility to PTZ-induced seizures. It could be observed that there was a postponement of the maximum level of influence only from day 7 to day 14 after hypoxia. Several studies have recently indicated that protection against neuronal cell death caused by cerebral ischemia can be observed if the animals were pretreated with a sublethal ischemia or hypoxia [5]. This phenomenon of so-called ‘ischemic tolerance’ is correlated with the expression of several genes and transcription factors [18]. Furthermore, the preconditioning effects are connected with the selective synthesis of a number of specific proteins [2,3], brain-derived neurotrophic factor (BDNF) [6] or nerve growth factor (NGF) [8]. It is conceivable that both, protective and damaging mechanisms were induced by hypoxia preconditioning. Following hypoxia lasting 1 h the harmful events are shorter-lived and the maximum level of protective action can still be observed after 7 days. On the other hand, it might be speculated that following a longer-lasting
hypoxia, the damaging processes are more continuously course of protection nor the strength of PTZ-induced
and pronounced and so the protection can be established at seizures 7 or 14 days after hypoxia exposure.
a later point in time.

Fig. 2 demonstrates that the pretreatment of rats with The molecular action of FUC is different from that of
100 mg/kg fucose (FUC) was not able to modify the PIR. FUC increases the fucosylation of brain glycoproteins.
susceptibility to PTZ independently of the duration of The effect is thought to play a crucial role in the late hypoxia preconditioning. In contrast to this, the application phase of LTP and influences memory trace processing of 100 mg/kg piracetam (PIR) prevented significantly of [1,9]. In this connection, it was speculated that the late the effects of preconditioning following hypoxia exposure phase of glycoprotein synthesis coincides with the modula-
lasting 1 or 8 h, 7 or 14 days later. FUC injected 20 min tion of the cell–cell adhesion processes, reflecting the before the beginning of hypoxia influenced neither the time section and stabilization of synapses which maintain an enduring representation of long-term memory [15,17].
from our data it may be concluded that increased fucosylation and the resulting alteration of synaptic connectivity have no importance for the induction of processes leading to a protection against PTZ seizures.

Different mechanisms were taken into consideration for the learning improvement by PIR. An involvement of glutamate receptors in the mediation of protection as was discussed for the implement of facilitating effects on learning and memory [22] can not explain this action. Hypoxia causes the formation of reactive oxygen species [14]. The generation of oxygen species has been usually considered a sign of oxidative stress that puts cells on the course to apoptotic cell death. Reactive oxygen species may initiate multiple signalling events such as the expression of several genes that either protect against neurodegeneration and seizures, respectively, or are involved in a cascade of events mediating cell injury [19].

The radical scavenger properties of PIR [12] may be a possible mechanism which inhibit the protective effect of hypoxia as described for the spin trap reagent N-t-butyl-α-phenylnitrone (PBN), too [14].

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References

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