Neuroprotective Effect of Hypoxic Preconditioning: Phenomenon and Mechanisms

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 2, pp. 237-240, February, 2002
Original article submitted March 29, 2001

We developed a new model of hypoxic preconditioning improving tolerance of complete global cerebral ischemia. The role of adenosine receptors in the realization of this effect and in the mechanisms of hypoxic tolerance is demonstrated. Preconditioning decreases of body temperature, which correlates with the neuroprotective effect, but this effect does not directly result from hypothermia.

Key Words: *hypoxic preconditioning; cerebral ischemia; A-receptors; hypothermia*

Ischemic preconditioning, or preliminary short-term ischemia, improving the resistance of organs to subsequent severe ischemia attracted much recent interest, especially in cardiology [9,13]. The efficiency of preconditioning was demonstrated for the brain [1,14,15]. Episodes of transitory ischemia improved the resistance to subsequent ischemic stroke [11]. However, ischemic preconditioning of the brain in humans is hardly possible not only because of its difficult realization, but mainly because it is dangerous. On the other hand, hypoxic training is used in some diseases [8], but we found no publications concerning cerebral ischemia. None of the preconditioning variants was used in complete global cerebral ischemia (CGI). We developed a model of hypoxic preconditioning (HP) improving brain resistance to ischemia and elucidated the role of adenosine (A-) receptors and body temperature in the realization of this effect of HP.

MATERIALS AND METHODS

The study was carried out on 138 C57Bl/6 and random-bred mice of both sexes aged 2.5-4 months (16-25 g). Hypoxia was modeled by placing the mouse in an individual sealed chamber (100 ml) for 5-12 min. The procedure was repeated 1-4 times with 5-30-min intervals. CGI was induced 0-60 min after the last hypoxic exposure (Lowry's decapitation model) and the duration of gasping was evaluated [7]. CGI is the most rigid model of cerebral ischemia, but even on this model a high neuroprotective effect (NPE) of three classes of substances (A-receptor agonists, GABA- and α_2-receptors) was demonstrated [3]. Clinically CGI develops in severe cardiovascular disease (heart blockade, *etc*.) [3-5]. Body temperature was measured with a TPEM-1 electric thermometer at a depth of 3.5 cm. Theophylline in doses of 200 and 400 μmol/kg (40 and 80 mg/kg) was subcutaneously injected to two groups of mice 15 min before the first hypoxic exposure.

The series were compared using Mann—Whitney's test, qualitative parameters by the χ^2 test, and Spearman correlation coefficients (r) were evaluated [2]. The differences were considered significant at $p<0.05$.

RESULTS

The first stage of the study was development of the experimental model. The mean mouse life span in the chamber was 19 min (10-36 min). Comparison of different hypoxic protocols showed that HP significantly improved CGI tolerance from judging not

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0007-4888/02/1332-0202$27.00

only the mean values in the majority of experimental series, but also for in the majority of animals: gasping was higher than the upper threshold value in the control group in 57 (86%) of 66 mice. The optimal protocol of HP was 4 sessions of 12-min exposure with 10-min intervals without interval before ischemia. This protocol provided maximum NPE: CGI tolerance increased on average 2-fold (Table 1) in all mice of this series gasping duration surpassing the upper threshold value in the control. According to common terminology [12,14,15], our model corresponded to early or immediate (hours or minutes before ischemia) preconditioning.

When studying possible mechanisms of NPE, we showed that HP significantly reduced body temperature (to 31.8°C during the optimal exposure, *i. e*. by 7°C (Table 1). Moreover, the individual gasping duration and hypothermia before CGI closely correlated in 82 experimental and control mice: r=-0.78, p<<0.001 (Fig. 1) for body temperature before ischemia, r=-0.62, p<0.001 for its severity; r=-0.94, p<0.001 and r=-0.64, p<0.1 for the optimal protocol, respectively. The intensity of hypothermia effect (equal to r^2) was 0.61 and 0.38, respectively, for all series and 0.92 and 0.41 for the best series. Hence, CGI tolerance correlated with the development of hypothermia, as we previously showed for many types of exposures [3,5]. It is noteworthy that correlation with body temperature during development of ischemia was obviously stronger than with the intensity of its decrease.

We also studied the effect of A-receptor antagonist theophylline during the optimal regimen of HP. The results obtained in experiments with two doses (200 and 400 μmol/kg) were combined because the differences between them were negligible (presumably the lower dose was already saturating). The antagonist itself moderately reduced gasping duration (by 34% on average) in comparison with the control (Table 1). HP improved tolerance of

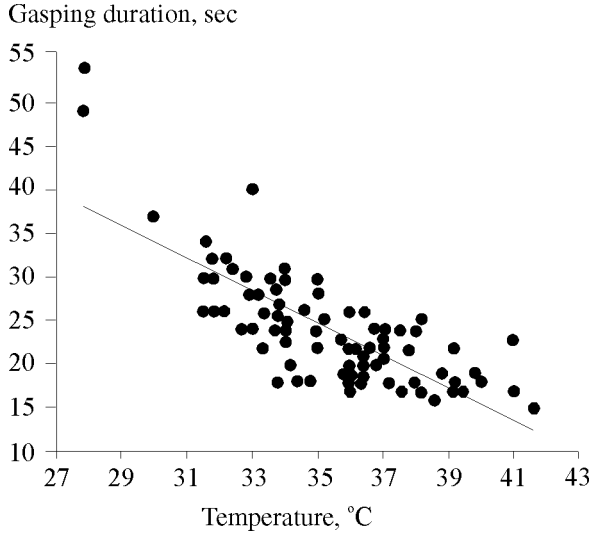

Fig. 1. Correlation of neuroprotective and hypothermic effects of hypoxic preconditioning (individual data for mice of all series; r=-0.78, p<<0.001).

ischemia by 94%, but only by 31% after theophylline injection (Fig. 2). This indicates that the A-receptor component contribute to both the natural resistance to ischemia and HP effect. This is in line with the data on the role of A-receptors in brain [12,14,15] and heart [9, 10,13] tolerance after ischemic preconditioning.

HP without theophylline caused death of some mice; in addition, some animals developed agonal state during the first hypoxic exposure (Table 2). After preinjection with theophylline 27% mice died and 33% developed agony (5.2 and 2.4 times more often, respectively, p<0.01 according to χ^2 test). In addition, two survivors from this series had repeated (2 or 4) agonies during subsequent hypoxic exposures, which was not observed in the series without theophylline. All this means that A-receptors blockade with theophylline not only decreases the duration of gasping in CGI, but also deteriorates animal status and survival during hypoxic exposure. Hence,

TABLE 1. Effect of Theophylline on Tolerance of Cerebral Ischemia and Body Temperature of Mice

Parameter	Control (n=11)	HP (n=8)	Theophylline (n=18)	Theophylline+HP (n=11)
Gasping duration, sec				
mean	17.2	33.4*	11.4*	22.6**
range of values	15.0-19.0	22.0-54.0	9.0-15.0	15.0-38.0
Final temperature, °C				
mean	38.8	31.8*	38.0	30.6*
range of values	36.0-41.4	27.8-34.6	31.8-42.0	24.6-33.8

Note. *p<0.002, **p=0.01 *vs.* the control.

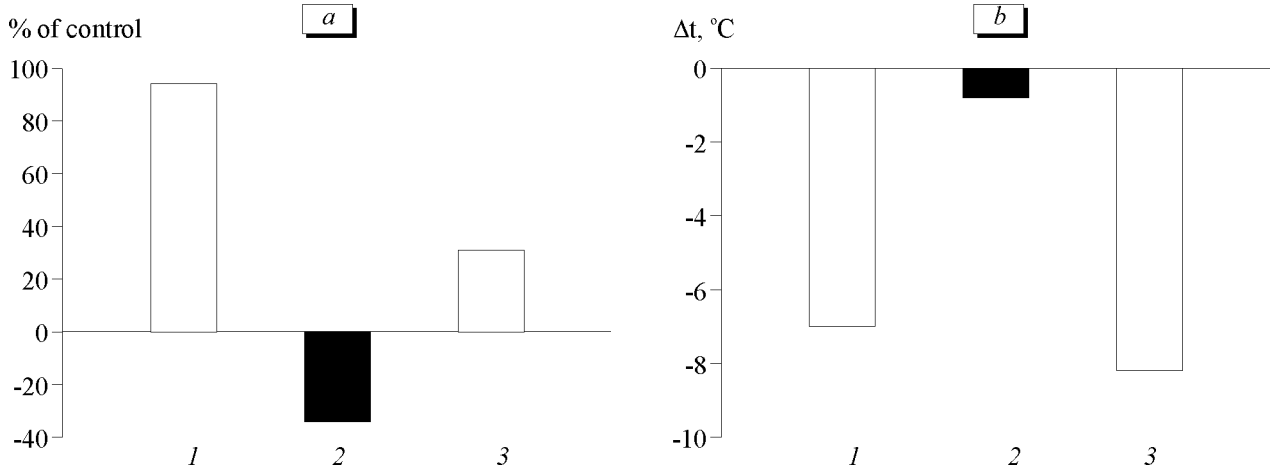

Fig. 2. Effect of hypoxic preconditioning (*1*), theophylline injection (*2*) and both (*3*) on gasping duration (*a*) and body temperature (*b*).

endogenous adenosine and A-receptors are involved in the mechanism of realization of both ischemia and hypoxia tolerance. This is confirmed by our previous data [3,4,6].

We hypothesized that A-receptor mechanism of HP effect was associated with the development of hypothermia, as hypothermia is typical of A-agonists and contributes to the development of their NPE [5,6]. However, theophylline had no effect on basal body temperature (Table 1) and on its decrease under the effect of HP ($p>0.2$). As a result, NPE and hypothermic effect of HP in animals injected with theophylline were clearly dissociated (Fig. 2). Moreover, these effects closely correlated in this series without theophylline, while after theophylline injection their relationship was negligible ($r=-0.51$, $p>0.1$ for temperature before ischemia and $r=-0.26$, $p>0.2$ for changes of temperature). Hence, in this series A-receptors did not mediate the development of hypothermia. In a wider sense, this means that though HP decreased body temperature and this correlated with its NPE, this latter effect was not a direct result of hypothermia, *i. e.* other mechanisms were involved in realization of NPE.

Hence, HP similarly to ischemic preconditioning notably improved early resistance to cerebral ischemia. This effect observed during CGI, the most rigid model of ischemia, indicates that HP protects not only the "semishadow" zone, but also the "core" of ischemic injury. Endogenous adenosine and A-receptors are involved in the realization of natural resistance to ischemia, HP effect, and hypoxic resistance. NPE correlates with hypothermia associated with HP, but is not its direct result. HP is clinically interesting and can be useful in complex prevention of cerebral circulation disorders.

TABLE 2. Effect of Theophylline on Survival after Hypoxic Preconditioning

Outcome	Without theophylline (n=58)	Theophylline (n=15)
Death	3	4
Survival		
agony	8	5
without agony	47	6

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