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学位論文名	Neuroprotective Effect of Hypothermia at Defined Intraischemic Time Course in Cortical Cultures （ラット大脳皮質一次培養モデルを用いた脳虚血に対する低体温療法の神経保護効果）
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論文内容の要旨

〔目的〕

Cerebral infarction following temporary ischemia is a critical pathogenic event in many clinical conditions. Many experimental and clinical studies have shown that hypothermia confers cerebroprotective benefits against ischemic insults. Because of the many conflicting reports on hypothermic neuroprotection, we undertook the present study in cultured neurons to quantify the protective effect of mild to profound hypothermic temperatures during exposure to hypoxia at 6 hr intervals for 24 hr.

However, no reports on cell cultures have examined the protective effects of hypothermia during prolong intra-ischemic period that closely approximate the time course of ischemic insults associated with many cardiovascular and neurosurgical procedures. Furthermore, much of this knowledge has arisen from *in vitro* studies, as the findings in the *in vivo* experiments were inconsistent. Thus, a study on primary neuronal cultures has been considered for the analysis of the outcome of suitable temperatures for cerebral protection.

〔方法ならびに成績〕

Cell culture : Rat foetuses at embryonic day 16 were removed surgically from anaesthetized pregnant Wistar rats and their brains were isolated under the microscope. Cerebral cortical neurons were dissected in phosphate buffered saline (PBS), and the neurons were treated with 0.25% trypsin at 37℃ for 20 min and triturated with a Pasteur pipette. The dissociated cells were diluted to a concentration of 1 X 10^6 cells per ml in Dulbecco's modified Eagles's medium (DMEM) with 8% foetal calf serum (FCS) and 4% horse serum (HS), 50 μ g/ml streptomycin and 50 IU/ml penicillin. This suspension was placed on poly-L-lysine-coated 35 mm diameter/2 mm grid tissue culture dishes (1.5 ml/well). Cultures were maintained in 100% humidified 5% CO_2/95% air atmosphere at 37℃. When the neurons reached 4 days *in vitro*, non neuronal cell division was arrested by 3 days exposure to 5 μ g ml^{-1} Of 5-fluoro-2'-deoxyuridine (5-FU) and cultures were subsequently fed twice weekly with medium. Experiments were carried out between 13 and 14 days *in vitro*.

Experimental procedures : The cultures were exposed to hypoxic atmosphere in a special anoxia incubator with

controlled environment at 37℃. The cell culture dishes were studied in five groups according to the grades of temperatures during ischemia : 37℃ (normothermia), 32℃ (mild hypothermia), 27℃ (moderate hypothermia), 22℃ (deep hypothermia) and 17℃ (profound hypothermia). For each group we exposed batches of five to seven culture dishes to four different time intervals : 6 hr, 12 hr, 18 hr and 24 hr. Each group, exposed to hypoxia for different time courses and temperatures, was later returned to normal atmosphere (37℃ & 20% O_2) for the remaining period of 30 hr. Thereafter the survival rate of neurons were evaluated.

As a control study, cultures were treated at different hypothermic temperatures similarly to the above mentioned experiment but in normal aeration (O_2 of 20%) for 24 hr and then returned to normal atmosphere (at 37℃) for the remaining period of 30 hr (that is for 6 hr).

Cytotoxicity : Three or four photomicrographs were made of each well shortly before exposing the cells to hypoxia and at the end of the experiment according to the grid arrangement of the dish. The cells were exposed to 0.4% trypan blue with PBS to stain non-viable cells, and photomicrographs were taken again at the same area as before the experiment. Approximately 500−1000 viable neurons per culture dish were subjected to manual counting. Survival rates were calculated with the following formula ; 100 X (non-stained cells at the end of the experiment)/(whole cells shortly before the experiment).

Results : The survival rates were almost similar in all four hypothermic groups at 6 hr but tended to be reduced significantly in normothermic group. Cultures exposed to 32℃, 27℃ and 22℃ for 12 hr and 18 hr of hypoxia did not show significant differences in survival rates, but differed significantly from 37℃ and 17℃ cultures (12 hr : 37℃−56.1±2.1%, 17℃−85.9±2.5% ; 18 hr : 37℃−34.2±1%, 17℃−74.7±3.7% ; $P<0.01$). Furthermore, at 24 hr the mean survival rate of neurons was 84.1 ±1.6% at 22℃ and was significantly higher compared to other groups ($P<0.001$). The mean survival rate of neurons at 37℃ was consistently low in the 24 hr compared to all four hypothermic temperatures ($P<0.001$).

〔総括〕

Deep cerebral hypothermia are more advantageous than mild, moderate and profound hypothermia when prolonged ischemic insults are expected. Mild and moderate hypothermia confers much neuroprotection as deep hypothermia against brief episodes of cerebral ischemia. Profound hypothermia does not offer significantly more protection to the brain than mild, moderate or deep hypothermia during ischemia. The potential for hypothermia of 17℃ to cause additional hypothermic injury in hypoxic neurons will require further investigations.

論文審査の結果の要旨

低体温が脳虚血に対して保護作用を有することはよく知られている。しかし、低体温による脳保護効果の至適温度についてはよく分かっていない。そこで今回、培養脳神経細胞を用いて低酸素誘発性神経細胞死に対する低温環境の至適温度を調べた。妊娠16周ラットより胎児大脳皮質を採取し、インキュベーターで14日間培養した。培養神経細胞を37、32、27、22、17℃環境のインキュベーターに移し、6、12、18、24時間の無酸素状態に暴露後、細胞生存率を測定した。その結果、神経細胞生存率は無酸素暴露時間に比例して低下した（6時間 80.3±2.7% ; 12時間 56.1±2.1% ; 18時間 34.2±1% ; 24時間 18.1±2.2%）。また、22℃までは温度低下に比例して細胞生存率が上昇したが、17℃では22℃よりも細胞生存率は有意に低値となった。さらに、6−18時間の無酸素暴露条件下では32−22℃の低温度群の細胞生存率に差がみられなかったが、24時間の無酸素暴露下では22℃群の細胞生存率が他の低温度群に比べて有意に高値であった。以上より、低温環境は低酸素誘発性神経細胞死に対して著明な保護効果を有し、その至適温度は22℃付近にあることが示唆された。

本研究は、低酸素誘発性神経細胞死に対する低体温の保護効果とその至適温度を in vitro で証明したものであり、学位の授与に値するものと考えられる。