行政院國家科學委員會專題研究計畫　成果報告

水腦症腦內多巴胺代謝之研究:氧化胺 A,B,及類 D2 接受器之角色

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中文摘要

關鍵詞：鼠、水腦症、紋狀體、多巴胺、微透析、HPLC、DOPAC、HVA、MAO、mRNA

水腦症是小兒神經外科最常見的疾病，其造成之腦部功能障礙有的可以藉由腦脊髓液分流術（CSF diversion）來改善，因此一般學者認為水腦症造成之腦部功能障礙可能主要源於神經元之「功能」障礙（functional disturbances），而不只是腦部「結構」之破壞所造成的，因為後者引起的神經功能障礙往往是不易恢復的。過去的研究也証實水腦症發生時會活化腦部某些神經傳遞物系統。

本研究發現與過去研究不同，在急性水腦與某些尚未產生代償現象的慢性水腦，腦部神經傳遞物質多巴胺（dopamine）及其代謝產物DOPAC及homovanillic acid（HVA）之組織濃度反而是增加的，釋放CSF可獲得改善。在產生代償現象的慢性水腦，dopamine及其代謝產物DOPAC及HVA之組織濃度則是降低的，而且無法以釋放CSF改善之。至於負責代謝dopamine之酵素MAO的活性在第一種情形下是升高的，而且是可逆的變化。我們認為在急性水腦與某些尚未產生代償現象的慢性水腦，酵素MAO的活性增加，是因應dopamine上升的反應。dopamine上升會造成腦組織自我氧化而產生腦傷，酵素MAO扮演一個保護性的角色。

英文摘要

Keywords: Rat; Hydrocephalus; Dopamine; Striatum; Microdialysis; HPLC ; DOPAC; HVA; monoamine oxidase, mRNA

The dopaminergic system in brain alters in experimental hydrocephalus, however, the response of monoamine oxidase (MAO), the major metabolizing enzyme of dopamine, to hydrocephalus was not known. We induced hydrocephalus in neonatal rats by intracisternal injection of kaolin solution and examined the following changes in the striatum one week after. The tissue levels of dopamine and its metabolites and the activity of mRNA of MAO all increased in acute hydrocephalus. These changes could be reversed by release of cerebrospinal fluid, which also improved the release of dopamine and its metabolites from the presynaptic membrane. These data suggest that in acute hydrocephalus MAO plays a role in reducing dopamine, which can cause cytotoxicity to the brain.

前言與研究目的

Hydrocephalus is a pathological dilatation of the cerebral ventricles that results from oversecretion, obstruction of pathway, or malabsorption of cerebrospinal fluid (CSF). The presence of extrapyramidal signs in some patients with hydrocephalus

suggests an involevement of striatum, which may be similar to the condition occuring in patients with Parkinson's disease, a disease with constant decreases of dopamine in the striatum. Recently, monoamine oxidase (MAO) inhibitors are being investigated with the hope that they could be used for the treatment of Parkinson's disease. Though it has been shown that the concentrations of dopamine metabolites increased more or less in hydrocephalus, the role of MAO in hydrocephalus was never studied. It was speculated that CSF stasis during hydrocephalus may explain the accumulation of dopamine metabolites in the brainn. This study aimed to study the changes of dopaminergics system during hydroceohaplus and after release of CSF pressure. The tissue and extracellular levels of dopamine and its metabolites and the activity of mRNA of MAO-A and MAO-B were studied.

It is known that the evolution and severity of hydrocephalus in animal models varies in the species and mode of induction, which makes comparisons of the physiological system under investigation difficult between models. Like the study of Ishizaki et al., we found dichotomous changes of the ventricular size and the intracranial pressure in some animals with hydrocephalus [Ishizaki R, Tashiro Y, Inomoto T, Hashimoto N: Acute and subacute hydrocephalus in a rat neonatal model: correlation with functional injury of neurotransmitter systems. Pediatr Neurosurg 33:298-305, 2000]. This study excluded the hydrocephalic animals with extreme ventricular dilatation and moderate increase of ICP (< 100 mm H2O) to reduce the effect of parenchymal compensation for the CSF accumulation in the ventricular system.

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研究方法 (Subjects and Methods)

Young male Wistar rats (Charles River), 3-week-old were used for inducing hydrocephalus. Animals were anesthetized with 8% chloral hydrate (0.63 g/kg body wt, i.p.). To induce hydrocephalus, we used 25-gauge needle to inject 0.06 ml of sterile Kaolin suspension (25%, Sigma, St. Louis, MO) into the cisterna magna of the animal under microscopic view. Control rats received a sham injection with 0.9%

saline solution. Animals were further studied 1 week after intracisternal injection for the intracranial pressure measurement, the acute effect of hydrocephalus and CSF diversion on the dopamine metabolism and the expression of mRNA of MAO-A and MAO-B.

The technique of intracranial microdialysis was used to investigate the immediate effects of CSF diversion on the extracellular concentration of dopamine and it metabolites (metabolism of DA) in the striatum. Microdialysis probes were implanted into striatum. Striatal dialysate were analyzed for dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) by HPLC with electrochemical detection.

After the brain was removed, the lateral ventricle size was assessed at the level of foramen of Monro. Ventricle size index was determined by dividing the total width of ventricle/total width of cerebrum. All the kaolin-injected animals developed ventriculomegaly. The animals with the ventricle size index larger than 0.8 were excluded from the study.

1. The measurement of intracranial pressure (ICP) and CSF diversion

One week after intracisternal injection of kaolin or saline solution, anesthesia was again induced intraperitoneally with 8% chloral hydrate. The animals were mounted on a stereotaxic device, and allowed to breathe spontaneously during recording of the ICP. Following exposure of the skull, a small burr hole was placed with its center 2 mm lateral and 1mm posterior to the bregma. A No. 21 needle attached to polyethylene tubing was filled with physiological saline and placed stereotaxically into the lateral ventricle for 3.5-4 mm in depth. The phenomenon of CSF pulsation will be used as an additional confirmation of the location of the tip of the needle. The ICP was measured by the height of the physiological saline with the reference zero point at the external auditory meatus. Hydrocephalic animals with ICP less than 100 mm were excluded from this study.

For the group of hydrocephalus with CSF diversion, the CSF was released slowly till the normal ICP (40-50 mm) was reached.

2. Behavioral evaluation (Observation of Physiological Condition)

Following induction of hydrocephalus, animals were closely monitored for signs of neurological deficits, seizure-related or abnormal behaviors. Any of the behavioral manifestations will help in the interpretation of the results. Animals were fed food and water *ad libitum.*

3. Measurement of extracellular levels of dopamine and its metabolites (In vivo microdialysis study)

The technique of intracranial microdialysis was used to investigate the immediate effects of CSF diversion on the extracellular concentrations of dopamine and it metabolites in the striatum. Microdialysis probes were implanted into striatum. Striatal dialysate levels were analyzed for dopamine, DOPAC, and HVA by HPLC with electrochemical detection.

4. Measurement of tissue levels of dopamine and its metabolites

After euthanasia, the brains were cooled on ice immediately after removal. The striatum was dissected out and weighed. The tissue levels of dopamine and its metabolites were estimated using HPLC with electrochemical detection.

5. Tissue preparation for mRNA of MAO studies

For studies of mRNA of MAO, the brain was removed in control, hydrocephalus with and without CSF diversion. In the group of hydrocephalus with CSF diversion, the brain was removed 6 hour after release of CSF pressure. After removal, the brains were placed on ice immediately. The hippomcapus, striatum, thalamus, frontal and temporal cortex, and cerebellum were dissected out, then stored in -80℃ for further studies.

6. Expression of mRNA of MAO-A and -B in the brain

The mRNA levels of MAO-A and MAO-B are examined by RT-PCR. The upper-streamed primers for MAO-A and MAO-B are: 5’-CCGATTTTGACTGC CAAGATCC-3’ (806-827), and 5’-CCTGTTTTGGGCATGAAGATTCA-3’ (860-881), respectively. The down-streamed primer is common to both genes, 5’-ATTATGA AGAGAAGAACTGGTG-3’ (1179-1199 for MOA-A/1232-1252 for MAO-B).

The products will be 390 bp. The condition of RT-PCR is similar to our previous methods. Briefly, 5μg of total RNA from each sample is reversely transcribed by M-MLV reverse transcriptase. The reverse transcription mixture was finally diluted to 100 μl. Three microliters of the diluted reverse transcription mixture is added in the presence of 10 pmol of primers, 200 μM dNTPs, 1 mM $MgCl_2$, 2.5 U of Taq DNA polymerase (Gibco BRL), and 1X *pfx* DNA polymerase buffer with 1X Enhancer Solution (Gibco BRL). After denaturation at 96°C for 3', 35 cycles of amplification (96°C 30", 60°C 30", and 72°C 1') are performed.

If the mRNA levels are too little to be observed, southern blot will be done according to the pervious methods [Wu KD, et al., 2001]. The products of PCR are electrophoresed in 5% ployacrylamide gel of 0.5X TBE and then transferred to positively charged nylon membrane (Boehringer Mannheim) electrically. The membrane is denatured in 0.4N NaOH for 10 minutes, followed by rinsing in 2X SSCP for 5 minutes and UV crosslinking. Hybridization with digoxigenin-labeled cDNA probe was performed according to the manufacture's protocol (Boehringer Mannheim). The signal is detected by using alkaline phosphatase-conjugated antibody and CSPD® (disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo [3.3.1.1.^{3,7}] decan}-4-yl)phenyl phosphate). Digoxigenin-labeled cDNA probes are synthesized with Klenow enzyme according to the manufacture's protocol.

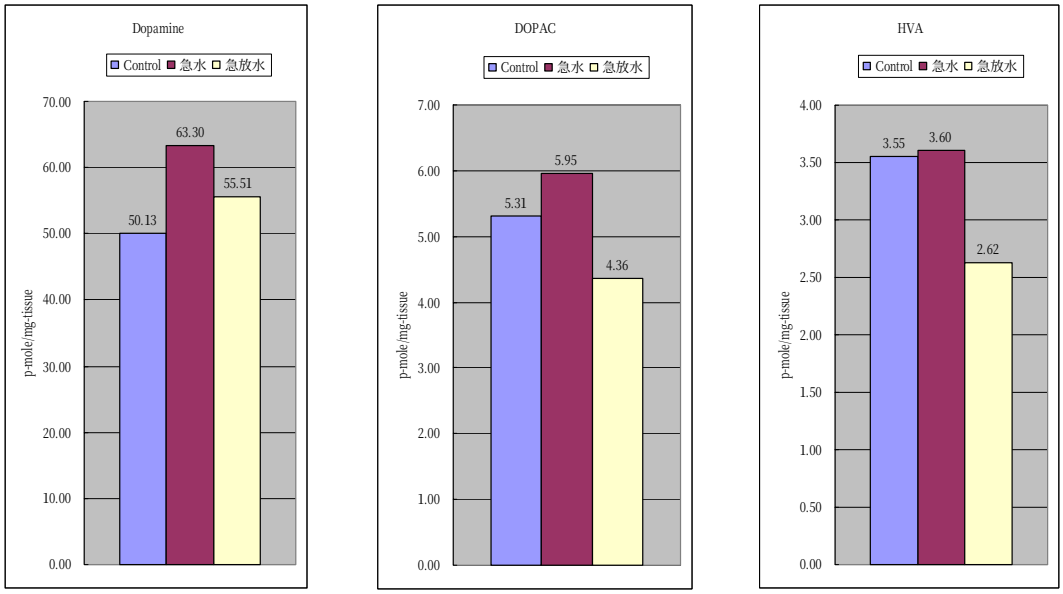
For quantitation of the mRNA levels, competitive RT-PCR will be performed according to our previously described methods [Wu KD, et al., 1995].

7. Statistical analysis

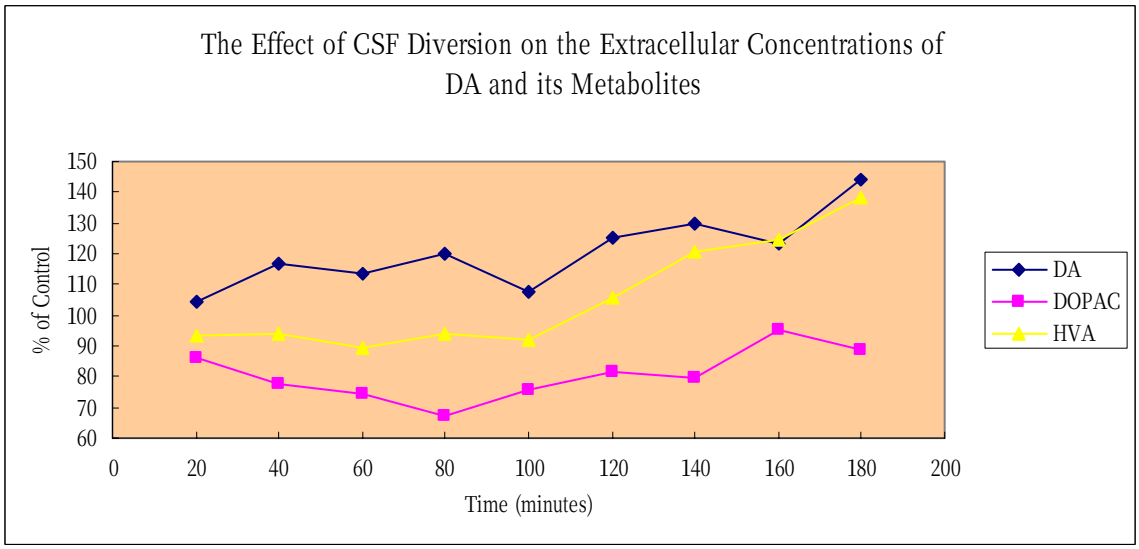
結果 (Results)

1. Tissue levels of dopamine, DOPAC, and HVA during acute hydrocephalus

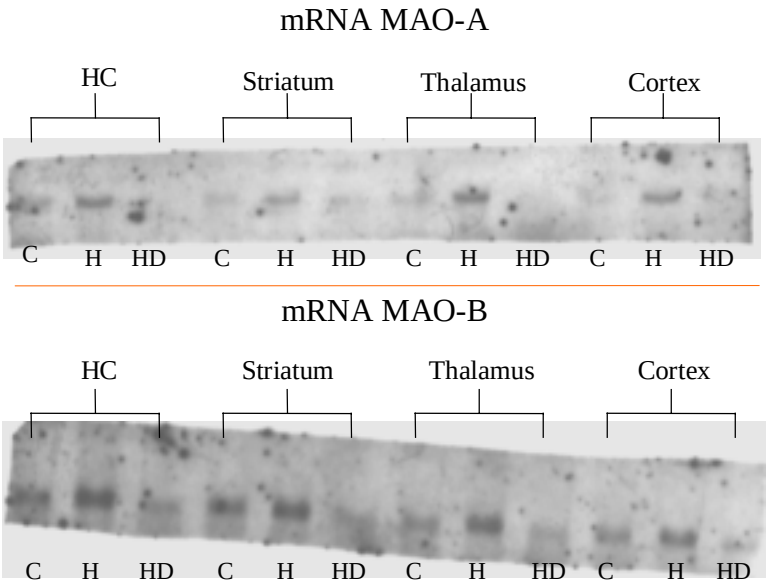
The dopamine and its metabolites increased during acute hydrocephalus. The changes were reversed by CSF diversion



2. The Effect of CSF diversion on the extracellular concentrations of dopamine and its metabolites during acute hydrocephalus



3. The expression of mRNA of MAO-A and MAO-B during acute hydrocephalus



4. The change of dopaminergic system during chronic hydrocephalus:

It was found that in chronic hydrocephalus, the changes of tissue levels and extracellular levels of dopamine and its metabolites varied with the ventricular size and the intracranial pressure. It can be divided into two conditions. If the frontal ventricular index was smaller than 0.8 or the ICP was higher than 100 mm H2O, the changes was similar to the changes during acute hydrocephalus. If the ventricular index was larger than 0.8 or the ICP was lower than 100 mmH2O, a picture quite similar to normal pressure hydrocephalus in adult and compensatory hydrocephalus in children, the tissue levels of dopamine and its metabolites decrease. The extracellular concentrations of dopamine and its metabolites did not change much after CSF diversion. The activity of mRNA of MAO-A and MAO-B had a similar picture to the response of dopaminergic system.

討論（Discussion）and 計劃成果自評（研究內容與原計劃相符程度、達成預期目標情況、研究成果之學術或應用價值）

This study clearly demonstrated that during hydrocephalus, the neurotransmitter system surrounding the ventricular system did change. Most of the animals with acute hydrocephalus had moderate enlargement of the ventricular size and marked increase of ICP. In chronic hydrocephalus, there are two types of changes according to the presence of parenchymal compensation or not. The chronic hydrocephalic animals without severe ventricular dilatation or low ICP had a picture similar to that of acute hydrocephalus. However, the animals with severe ventricular enlargement or low ICP had a different change of the neurotransmitter in the striatum. The changes could not be reversed by CSF diversion. It indicates that hydrocephalus without the development of marked parenchymal compensation may be benefit immediately from CSF diversion. On the contrary, CSF diversion may be less effective in cases with compensation to chronic hydrocephalus. This may explain the presence of neurological deficit, especially the presence of motor deficit in some patients with hydrocephalus even after shunting.

The activity of MAO increased during acute hydrocephalus or chronic hydrocephalus without compensation. It is speculated that the MAO plays an important role in reducing the dopamine concentration, which has cytotoxic effect to the brain through it auto-oxidation effect. Treatment with MAO inhibitor, like that is being used in Parkinson disease should not be considered.

In summary, this study confirmed that an early shunting is beneficial to the functional disturbances elicited by hydrocephalus. It also showed that early detection of parenchymal compensation in patients with a potential to develop hydrocephalus or having slow progression of hydrocephalus is important. The timing of surgery to prevent the development of permanent neurological defect is important.