星香正氣散이 低酸素 發作狀態의 고양이 大腦 皮質 切片의 이온 含量과 代謝에 미치는 效果

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Effects of Sunghyangchungisan(SHCS) on Cellular Ion Contents and Metabolism in Cat Brain Cortical Slices under Hypoxic Insult.

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목적: 고양이 대뇌 피질 절편을 사용하여 저산소 발작을 유발한 뒤, 성향정기산이 세포의 이온 환경과 대사의 변화와 관련하여 어떤 영향을 미치는지 연구하였다.
방법: 고양이의 대뇌 피질 절편에 저산소 발작을 유발한 뒤, flame photometry scintillation, method of Jorgensen and Skou, method of Fiske and Subbarow,oxygen monitor, luciferin-luciferase assay 등을 이용하여 세포내 이온함량과 세포대사를 측정하였다.
결과: 성향정기산은 저산소중으로 유발된 세포내의 K+와 Na+의 함량의 변화를 현저하게 지 연시켰다. 성향정기산은 Na-K-ATPase의 억제제인 와바인 또는 대사 억제제인 2,4-DNP로 유발된 세포내 K+ 함량의 변화에 어떤 효과도 보이지 않았다. 또한, 정상 상태의 절편뿐만 아니라 저산소 상태의 절편에서 분리된 과립체의 분설 에 있어서 Na-K-ATPase의 활동도에 영향을 미치지 않았다. 성향정기산은 저산소 발작하에서 산소 소비량과 세포의 ATP함량이 떨어지는 것을 현저하게 막았다. 또한 ATP를 생산하는 기능을 보호하는 저산소 조직의 사 립체를 돕는데 효과적이었다.
결론: 성향정기산은 대뇌 조직의 저산소 발작하에서 세포의 이온 환경과 대사를 보호하는 유익한 효과가 있음을 알 수 있다.

Key Word : 성향정기산, 저산소중, 세포내 K+와 Na+함량, Na-K-ATPase, 산소소비량, ATP

I. INTRODUCTION

Ischemia induced cell injury is associated with a variety of life threatening conditions such as myocardial infartion, cerebral stroke and renal failure. Tissue injury resulting from ischemia is mediated by the generation of reactive oxygen species.[1] In the brain, they have been implicated in the pathogenesis of a wide range of acute and long term neurodegenerative diseases including acute cerebral stroke, Parkinsons disease, Huntingtons disease, ischemic trauma, and seizures.[2,3] One of the major causes responsible for the neuronal dysfunction upon exposure to ischemia or hypoxia is deterioration of the ionic environment inside and around the cells.[4-7] In intact neurons, a significant fraction of available energy is used to maintain the concentration gradients of Na^+ and K^+. Na-K-ATPase or Na/K exchange pump, an ion-transporting ATPase, directly couple ATP hydrolysis to the transport these ions against an electrochemical gradient.[8] It transports Na^+ ions out of and K^+ ions into the cells.

When the aerobic production of ATP in a cell is inhibited experimentally by exposure to anoxia or metabolic inhibitors, the concentration of Na^+ and K^+ inside the cell gradually approaches that of the exterior environment.[4] This is caused by a slow leak of ions across the membrane down their electric and concentration gradients. In the brain, disturbance of these ionic environ-

ment leads to neuronal dysfunction by several ways. Failure of the Na-K-ATPase to pump out Na^+ ions out of cells leads to accumulation of these ions inside the cell, and as a result, the concentration of total ions inside the cell become to exceed that in the extracellular space as a consequence of Donnan equilibrium. It results in cellular swelling and finally leads to neuronal death.[9] Another impact is on the intracellular enzymes vital for the cellular function. Many cellular enzymes are specialized to function in a solution of low Na^+ ions and high K^+ ions.[8] Therefore, deterioration of these ionic environment in the cells may result in fatal dysfunction of these enzymes. In addition, membrane potential of the excitable cells such as neurons is easily affected by the changes of ionic concentration. Sunghyang-chungi-san (SHCS) is a well-known prescription in Korean traditional medicine. SHCS is first mentioned in "The Cardinal Principle of the symptoms and treatments(證治要訣)" written by Dai Sa Gong(戴思恭) is a mixed prescription of Sunghyang-san(星香散) and Kwaghyangchungi-san(藿香正氣散).[10] It has effects of regulating vital energy(理氣), eliminating phlegm(祛痰) and is a typical treatment used for the symtoms of apoplexy(中風), zhong qi(中氣), phlegm syncope(痰厥) and a syncope with eating and drinking(食厥) etc.[11,12] It is an emergency treatment for apoplexy marked by onset of fainting(中風昏倒), coma(人事不省) and abundant phlegm(痰涎壅盛).[13,14] It has been proven to be beneficial for the patients with cerebral stroke.

Therefore, this study was carried out to determine whether SHCS, is an emergency prescription for cerebral stroke, might have any beneficial effect on alterations of cellular ionic environment and metabolism under hypoxic stress using cat brain cortical slices.

II. MATERIALS and METHODS

1. SHCS extract preparation

SHCS was extracted with 3000ml distilled water at 100℃ for 3 hr and total extract was evaporated under reduced pressure to give 89.5g.

2. Preparation of brain cortical slices

Hybrid cats of both sexes (weight-1.5 to 2.5 kg) were housed in stainless steel cages at $22\pm2℃$, relative humidity of $50\pm10\%$, and 12 hr light and 12 hr dark cycle. Cats were fed catchow and deionized water adlibitum. After a one week quarantine period, cats were randomly used for this exiperiment. After anesthetizing animals by intravenous injection of pentobarbital sodium (30 mg/kg), the brain was promptly removed and placed in an ice-cold incubation buffer solution composed of (in mM) 120 NaCl, 5 KCl, 0.75 $CaCl_2$, 1.2 $MgSO_4$, 1 NaH_2PO_4, 24 $NaHCO_3$, 10 glucose, 10 Hepes/Tris (pH 7.4). Thin slices of cortical tissue (0.3 to 0.5 mm) were prepared using a Stadie-Riggs microtome.

3. Measurement of cellular K^+ and Na^+ concentration

Approximately 50 mg of cortical slices were incubated in 4 ml of the incubation buffer containing 1 Ci/ml

Table 1. Prescription of SHCS

Herbal name	Scientific name	Weight(g)
Agastaches Herba(藿香)	Agastache rugosa O. Kuntze	5.625
Perillae Herba(蘇葉)	Perillae frutescens var. acuta Kudo	3.750
Angelicae dahuricae Radix (白芷)	Angelica dahurica Bentham et Hooker	1.875
Arecae Pericarpium(大腹皮)	Areca catechu Linne	1.875
Poria(白茯苓)	Poria cocos Wolf	1.875
Machili Cortex(厚朴)	Machilus thunbergii S. et Z.	1.875
Atractylodis Macrocephalae Rhizom(白朮)	Atractylodis macrocephalae Koidzumi	1.875
Aurantii nobilis Pericarpium(陳皮)	Citrus nobilis Makino	1.875
Pinelliae Tuber (半夏)	Pinellia ternata Beit	1.875
Platycodi Radix (桔梗)	Platycodon glaucum Nakai	1.875
Glycyrrhizae Radix(甘草)	Glycyrrhiza uralensis Fischer et De Candolle	1.875
Zingiberis Rhizoma(生薑)	Zingiber officinale Roscoe	3.000
Zizyphi Fructus(大棗)	Zizyphus jujuba	3.000
Arisaematis Rhizoma(南星)	Arisaema ringens Schott	3.750
Inulae helenii Radix(木香)	Inula helenium Linne	3.750
	Total amount	39.750

[3H]-inulin. The incubation buffer was continuously bubbled throughout the incubation period with 95% O_2/ 5% CO_2 for proper oxygenation. In the experiments observing the effect of hypoxia, tissues were supplied with 95% N_2/5% CO_2 instead of 95% O_2/ 5% CO_2. After incubation, slices were blotted on a filter paper to remove the water around the tissue and weighed on a direct reading balance. Tissues were then dried overnight in a oven at 85°C, weighed again, and digested in 0.1 N HNO_3. Parts of digested tissue were taken and the flame photometry and scintillation spectrophotometry were used to measure tissue electrolyte and [³H]-inulin contents, respectively. Total tissue water content was calculated by the difference between the wet and dry tissue weights, and intracellular and extracellular water space was estimated by using the [³H]-inulin space as an index of extracellular water space. Cellular electrolyte concentration was expressed as mEq/ l of intracellular water space.

4. Measurement of Na-K-ATPase activity

After exposure to experimental protocols, brain cortical slices were homogenized and microsomal fraction was isolated by differential centrifugation method.[15] Na-K-ATPase activity was assayed according to the method of Jorgensen and Skou.[16] Microsomal fractions containing 0.25 mg/ml protein were preincubated in 2 mM EDTA, 25 mM imidazole, pH 7.0 in the presence of 0.6 mg/ml deoxycholate at 25°C. After 30 min, 100 μl aliquots were transferred to assay tubes at 37°C containing 1 ml of 130 mM NaCl, 10 mM KCl and 30 mM histidine, pH 7.5. After equilibration for 10 min reaction was initiated by the addition of ATP and $MgCl_2$ as the final concentration of 3 mM. After 10 min, the reaction was stopped by adding 0.2 ml of ice-cold 6% perchloric acid. The amount of inorganic phosphate released was determined by the method of Fiske and SubbaRow.[17] The enzyme activity was expressed as moles of Pi liberated per mg protein per hour. The protein concentration was determined according to the method described by Bradford with the bovine serum albumin as a standard.[18]

5. Measurement of oxygen consumption

Oxygen consumption of brain cortical slices was measured with an oxygen monitor (Yellow Spring Instrument, USA). Approximately 50 mg of slices were incubated in a reaction vessel containing 4 ml of the incubation medium saturated with oxygen at 25°C. Decrease in PO_2 in the medium was monitored using a Clark-type electrode for 15 min, and the rate of oxygen consumption was calculated.

6. Measurement of cellular ATP content

ATP levels in cells were measured by luciferin-luciferase assay.[19] Briefly, after exposure to the experimental protocol, brain cortical slices were homogenized with 4 ml of 0.1 M perchloric acid and 0.1% Triton X-100 and placed on ice. Then cellular extract was diluted with 10 mM potassium phosphate buffer containing 4 mM $MgSO_4$ (pH 7.4). 100 μl of 20 mg/ml luciferinluciferase was added to 10 μl of diluted sample and light emission was recorded for 20 sec with a luminometer (MicroLumat LB96P, Germany).

7. Isolation of mitochondrial fraction and measurement of ATP production

After exposure to experimental protocols, the brain cortical slices were homogenized in a sucrose buffer composed of (in mM) 50 sucrose, 200 mannitol, 5 KH_2PO_4, 1 EGTA, 10 HEPES, 0.1% bovine serum albumin and pH 7.15. Mitochondrial fraction was isolated from the homogenate by differential centrifugation and suspended in a KCl buffer composed of (in mM) 110 KCl, 5 KH_2PO_4, 5 succinate, 5 pyruvate, 10 HEPES, pH 7.15 to be a protein concentration of 30 mg/ml.[15] Time course of ATP synthesis in the KCl buffer containing 100 M ADP was monitored by measuring the changes of ATP concentration by the
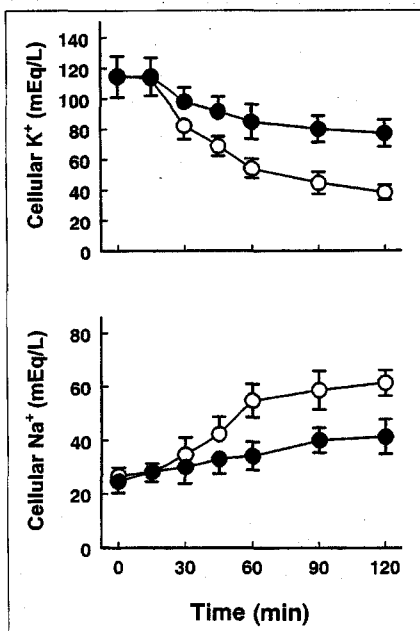
luciferin-luciferase assay as descri-
bed above.

8. Data analysis

Data are presented as means±SE.
When necessary, data were analyzed
by one-way analysis of variance
followed by Duncans multiple
comparison test. A value of P<0.05
was considered statistically significant.

III. RESULTS

### 1. Effect of hypoxia and SHCS on cellular K⁺ and Na⁺ contents

Fig. 1. Changes in cellular K⁺ and Na⁺ contents during exposure to hypoxia. The cat brain cortical slices were transferred to buffers gassed with 95% N_2/5% CO_2 and incubated for the indicated time periods in the absence (open circle) and presence (closed circle) of Sunghyangchungisan (1 mg/ml). Each point represents mean ±S.E. of 4 experiments.

Fig. 1 depicts the changes in
cellular K⁺ and Na⁺ contents in the
brain cortical slices subjected to
hypoxic stress. After equilibration in
the buffer gassed with 95% O_2/5%
CO_2 for 60 min, the brain cortical
slices were divided into normoxic
and hypoxic groups. Normoxic
group was further incubated in the
same buffer for the indicated time
periods. Hypoxic group was trans-
ferred to buffers gassed with 95%
N_2/5% CO_2 in the presence and
absence of SHCS (1 mg/ml), and
incubated for the indicated time
periods. The hypoxic buffer was pre-
saturated before the transfer of slices,
and continuously gassed throughout
the incubation period with 95%
N_2/5% CO_2 by vigorous bubbling.

In slices incubated in the buffer
gassed with 95% O_2/5% CO_2, there
was no significant alteration in
cellular K⁺ and Na⁺ contents by
further incubation for up to 120 min.
In these slices SHCS did not show
any effect on cellular K⁺ and Na⁺
contents (Data not shown). In
contrast, there were time-dependent
changes in cellular K⁺ and Na⁺
contents in slices subjected to
hypoxic stress. A significant decrease
in cellular K⁺ concentration was
observed in 30 min of hypoxic
incubation. The fall in K⁺ concen-
tration continued with further
incubation in the hypoxic buffer. In
120 min of hypoxic incubation,
cellular K⁺ concentration decreased
to 0.3 fold its normoxic control value

(114.8 to 38.4 mEq/ l). On the
contrary, cellular Na⁺ concentration
showed a time-dependent rise in
hypoxic slices. In 120 min of
hypoxia, cellular Na⁺ concentration
increased to 2.3 fold its normoxic
control value (26.8 to 61.4 mEq/ l).
Together with these results, it is
noteworthy that SHCS significantly
delayed the hypoxia-induced changes
in cellular K⁺ and Na⁺ contents.
When the values in 120 min of
hypoxia were compared, it blocked
50.3. and 58.9% of hypoxia-induced
fall and rise in cellular K⁺ and Na⁺
concentrations, respectively.

In Fig. 2, the effect of SHCS was
tested according to its concentration.
The brain cortical slices were
subjected to 120 min of hypoxia in
the presence of SHCS ranging from
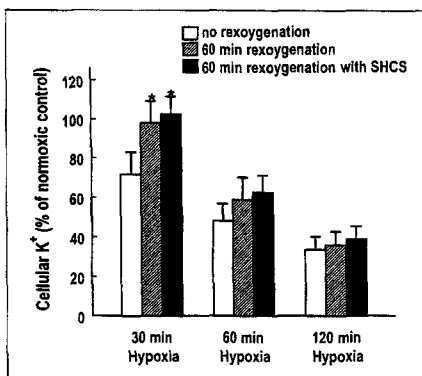0.1 to 10 mg/ml, and the effect was
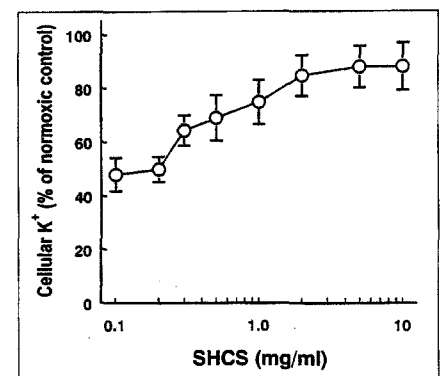
Fig. 2. Effect of various concentrations of Sunghyangchungisan on cellular K⁺ content after exopsure to hypoxia. The cat brain cortical slices were transferred to buffers gassed with 95% N_2/5% CO_2 and incubated for 120 min in the presence of indicated concentrations of Sunghyangchungisan (SHCS). Each point represents mean±S.E. of 4 experiments.

assessed by analyzing the change in cellular K^+ content. SHCS was effective at the concentration above 0.3 mg/ml to block the hypoxia-induced fall in cellular K^+ content. The effect of SHCS was dose-dependent up to 2 mg/ml.

2. Reversibility of hypoxic insults

In the next experiment, it was examined whether SHCS, when applied during reoxygenation period, is effective as well to reverse the hypoxia-induced changes in cellular K^+ content. The brain cortical slices were divided into 3 groups and incubated first in a hypoxic buffer gassed with 95% N_2/5% CO_2 for 30,


Fig. 3. Effect of reoxygenation and Sunghyangchungisan on cellular K^+ content after exopsure to hypoxia. The cat brain cortical slices were transferred to buffers gassed with 95% N_2/5% CO_2 and incubated for indicated time periods, and then reoxygenated with 95% O_2/5% CO_2 for 60 min in the absence and presence of Sunghyangchungisan (SHCS, 1 mg/ml). Each point represents mean±S.E. of 5 experiments. *P<0.05 vs. the value with no reoxygenation.

60 and 120 min. Slices were then reoxygenated by transferring and incubating for 60 min in a buffer gassed with 95% O_2/5% CO_2. Meanwhile, the effect of SHCS was examined by application into the reoxygenation buffer at the concentration of 1 mg/ml. As shown in Fig. 3, reoxygenation was effective to reverse the changes in cellular K^+ content in slices subjected to 30 min hypoxia. However, in slices subjected to 60 or 120 min hypoxia, cellular K^+ content was not reversed by reoxygenation. This result suggests that prolonged hypoxia for longer than 60 min results in an irreversible damage to the cellular mechanism to preserve cellular ionic environment. SHCS did not provide any beneficial effect to reverse the hypoxia-induced changes when applied during the reoxygenation period. This result indicates that, although SHCS is effective to delay or ameliorate the hypoxic insults, it does not help the damaged cell recover the mechanism to reverse the disturbed ionic environment.

3. Effect ouabain and 2,4-DNP

In Fig. 4, it was examined whether SHCS has any effect to block the fall in cellular K^+ content by direct inhibition of Na/K exchange pump or cellular metabolism. Brain cortical slices were incubated in an oxygenated buffer in the presence and absence of SHCS (1 mg/ml) with the
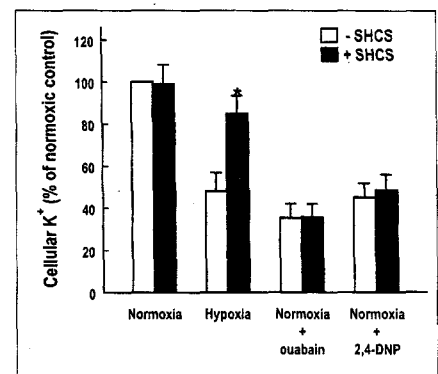

Fig. 4. Effect of Sunghyangchungisan on the changes in cellular K^+ content induced by inhibition of the Na-K-ATPase and cellular metabolism. The cat brain cortical slices were incubated for 60 min in buffers gassed with 95% O_2/5% CO_2 with ouabain (10-5 M) or 2,4-dinitrophenol (2,4-DNP, 10-4 M) in the absence and presence of Sunghyangchungisan (SHCS, 1 mg/ml). For a comparison, the hypoxia-induced alteration is presented together. Each point represents mean± S.E. of 4 experiments. *P<0.05 vs. the value without SHCS.

Na-K-ATPase inhibitor ouabain (10^{-5} M) or the metabolic inhibitor 2,4-dinitrophenol (2,4-DNP, 10^{-4} M).[20,21] As shown in Fig. 4, SHCS did not show any effect on the changes in cellular K^+ content induced by ouabain or 2,4-DNP. It suggests that the effect of SHCS to block the fall in cellular K^+ content did not result from the interaction with the passive leak mechanism.
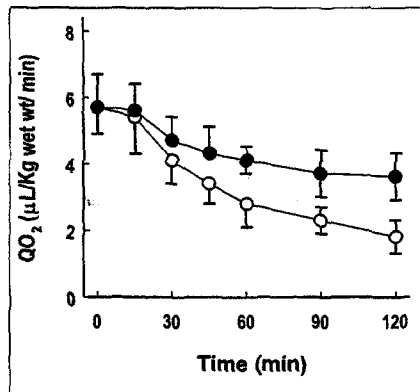
4. Effect on Na-K-ATPase activity

The Na/K exchange pump or the Na-K-ATPase, which opposes the passive leak of Na^+ and K^+, is the major determinant of cellular
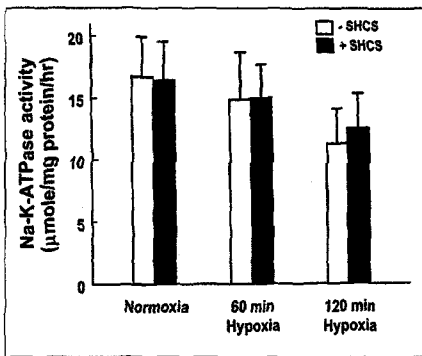
Fig. 5. Changes in the Na-K-ATPase activity during exposure to hypoxia. The Na-K-ATPase activity was determined in microsomal fractions isolated from the brain cortical slices incubated in the oxygenated and hypoxic buffers in the presence and absence of Sunghyangchungisan (SHCS, 1 mg/ml). Each point represents mean±S.E. of 4 experiments.

Fig. 6. Effect of Sunghyangchungisan on the changes in oxygen consumption after exposure to hypoxia. The cat brain cortical slices were transferred to buffers gassed with 95% N₂/5% CO₂ and incubated for *indicated time periods, in the absence (open circle) and presence (closed circle) of Sunghyangchungisan (1 mg/ml). Slices were then transferred to buffers saturated with 95% O₂/5% CO₂ and decrease in PO₂ in the medium was monitored using a Clark-type electrode for 15 min. Each point represents mean ± S.E. of 4 experiments.*

concentration of these ions. Therefore, it was examined whether SHCS exerts any direct interaction with the Na-K-ATPase. The results in Fig. 5 represent the Na-K-ATPase activity measured in microsomal fractions isolated from the brain cortical slices incubated in oxygenated and hypoxic buffers in the presence and absence of SHCS (1 mg/ml). As is shown in the figure, SHCS did not affect the Na-K-ATPase activity in hypoxic as well as normoxic slices. Together with this result, it is noteworthy that the Na-K-ATPase activity still remained unaltered in 60 min of hypoxia at which there was already a significant changes in cellular K⁺ and Na⁺ concentrations (refer to the results in Fig. 1). This result suggests that the Na-K-ATPase molecule itself remains intact after a considerable

period of hypoxia in spite that irreversible damages to some cellular mechanisms are already in progress.

5. Effect on oxygen consumption

In the experiments presented in Fig. 6, changes in oxygen consumption were assessed as an index of cellular metabolism. There was a drop in the oxygen consumption in tissues exposed to hypoxic insult. The longer the duration of the hypoxia, the bigger was the extent of decrease in the oxygen consumption. In the presence of SHCS (1 mg/ml), the drop in the oxygen consumption

was significantly delayed.

6. Effect on cellular ATP content

In Fig. 7, changes in cellular ATP content were determined as a function of incubation time in a hypoxic environment. As expected, the ATP content rapidly dropped in tissues subjected to hypoxic insult. In 120 min of hypoxia, cellular ATP content decreased to under 15% of its normoxic control value. In the presence of SHCS (1 mg/ml) the drop in ATP content was significantly attenuated. It blocked the decrease in cellular ATP content by 42.3% in tissues exposed to 120 min of hypoxic insult.
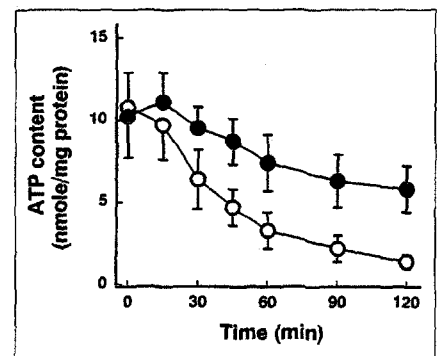
Fig. 7. Effect of Sunghyangchungisan on cellular ATP content during exopsure to hypoxia. The cat brain cortical slices were transferred to buffers gassed with 95% N₂/5% CO₂ and incubated for indicated time periods in the absence (open circle) and presence (closed circle) of Sunghyangchungisan (1 mg/ml), and then ATP content in the the tissue homogenate was determined by the luciferin-luciferase assay. Each point represents mean± S.E. of 4 experiments.

7. Effect on mitochondrial ATP production

To assess directly a protective effect of SHCS on the mitochondrial metabolic machinery, mitochondrial fractions were isolated from brain cortical slices which had been incubated under normoxia and hypoxia with or without SHCS (1 mg/ml), and the ability to generate ATP from ADP was determined. As is shown in Fig. 8, the rate of ATP production was significantly reduced in the mitochondrial fractions isolated from hypoxic tissues when compared with those from normoxic control tissues. It is also shown that
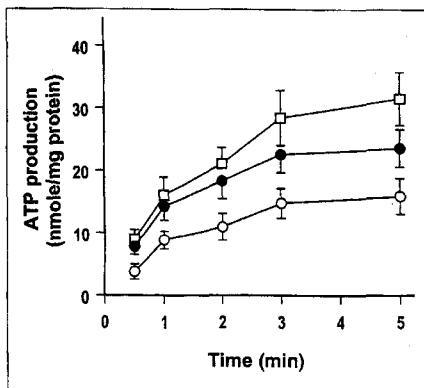
Fig. 8. ATP generation in mitochondrial fractions isolated from the brain cortical slices after exopsure to hypoxia. Mitochondrial fractions were isolated from the cat brain cortical slices which were incubated in a oxygenated buffer (open square) gassed with 95% O_2/5% CO_2 or a hypoxic buffer gassed with 95% N_2/5% CO_2 in the absence (open circle) and presence (closed circle) of Sung-hyangchungisan (1 mg/ml), and then tme course of ATP synthesis in a KCl buffer containing 100 M ADP was determined. Each point represents mean ± S.E. of 4 experiments.

SHCS might help the hypoxic tissues to preserve the mitochondrial function to produce ATP. This result, together with the those from Fig. 6 and 7, strongly suggests that SHCS provides a beneficial effect to protect the cellular metabolic machinery against irreversible damages under hypoxic insult.

IV. DISCUSSION

The function of the mammalian brain depends on a continuous supply of O_2 and glucose. when the brain no longer receives either of these substances, loss of function occurs quickly, and viability is endangered when the lack of substrates persists for more than a few minutes. [23-25] The dysfunction of the brain and of ion movements are believed to be linked, because normal brain function requires an intact ion distribution. In this study, alterations in cellular ion contents and metabolism were assessed in the brain under conditions in which energy metabolism of the brain is compromised.

In the cat brain cortical slices exposed to hypoxia there was a net gain of Na^+ and net loss of K^+ as expected. Ionic gradients across cell membranes are maintained by active processes that move the ions against an electrochemical gradient. The active processes are executed by membrane-bound ATPases. The dissipative processes do not take place to any significant extent via leak pathways in the lipid membrane but via transport systems composed of integral membrane proteins, i.e., ion channels. [25] Accordingly, the changes in cellular ionic concentration under hypoxia observed in this study can result from a reduction of the Na-K-ATPase activity or an activation of passive ion channels. Several lines of evidence in this study suggest that the disturbance in these ionic distribution is linked to insufficient generation of ATP fueling the Na-K-ATPase rather than to a direct damage to the Na-K-ATPase mole-cule. It was demonstrated in Fig. 5 that the Na-K-ATPase activity still remained unaltered in 60 min of hypoxia at which there was already a significant changes in cellular K^+ and Na^+ concentrations. It suggests that the Na-K-ATPase molecule itself remains relatively intact after a considerable period of hypoxic insult.

Sunghyangchungi-san (SHCS) is a well-known prescription in Korean traditional medicine. It has effects of regulating vital energy(理氣), eliminating phlegm(祛痰) and is a typical treatment used for the symtoms of apoplexy(中風), zhong qi(中氣), phlegm syncope(痰厥) and a syncope with eating and drinking(食厥) etc. [11,12] Let,s survey the phar-macological working of SHCS. Agastaches Herba(藿香) regulates vital energy(理氣), harmonizes middle-jiao(和中)

and treats exterior syndrome and interior syndrome(兼治表裏). Agastaches Herba(藿香), Angelicae dahuricae Radix(白芷) and Perillae Herba(蘇葉) dissipates cold pathogem(寒邪) by warming, benefits dysphagia(利膈), removes pathogenic factors attacking the exterior of the body, and eliminates wetness-evil(濁氣) with drugs of fragrant flavour(芳香). Platycodi Radix(桔梗), Machili Cortex(厚朴) and Arecae Pericarpium(大腹皮) regulates qi(調氣), removes mass of the abdomen(消脹) and transports water(行水). Pinelliae Tuber(半夏), Aurantii nobilis Pericarpium(陳皮) and Zingiberis Rhizoma(生薑) keeps the adverse energy(降逆), removes dempness(除濕), eliminates the phlegm(化痰), and resolves interior substance. Bai Poria(白茯苓), Atractylodis Macrocephalae Rhizoma(白朮) and Ziziphi Fructus(大棗) reinforces the spleen(健脾), dries damp(祛濕) and invigorates normal resistance of the body(正氣).[12,26] Arisaematis Rhizoma(南星) removes wind phlegm syndrome(風痰) and damp phlegm syndrome(濕痰) from the effect of drying damp(燥濕), eliminating the phlegm(化痰), dispelling wind-evil and spasmolysis(祛風解痙). Inulae helenii Radix(木香) resolves the stagnation of intestine,s vital energy from the effect of regulating qi(行氣), arresting pain(止痛), reinforcing the spleen(健脾), removing food retention and promoting digestion(消食).[12,26,27] SHCS is a prescription which adds

Arisaematis Rhizoma(南星) and Inulae helenii Radix(木香) to Kwaghyangchungi-san(藿香正氣散), and used for apoplexy(中風), zhong qi(中氣), phlegm syncope(痰厥) and a syncope with eating and drinking(食厥) as an emergency treatment.[11,12,14]

For the experimental studies about SHCS, Ahn,[28] Mun,[29] Kim[30] and Lee[31] once respectively reported the effects on the blood pressure, heart beat, cranial pressure, Ca^{2+}meta-bolism and oxidative injury with laboratory animal but there has been no report about effects of SHCS on cellular ion contents and metabolism in cat brain cortical slices under hypoxic insult.

The effect of SHCS in the present study to help the brain tissues preserve ionic environment under hypoxia is of great interest in view of the fact that it is a prescription proven to be beneficial for the patient with a cerebral stroke. It was effective only when applied before or during the hypoxic period. It did not provide any beneficial effect to reverse the ionic disturbance when applied to the tissue which was already damaged by hypoxic insult. Therefore, it is suggested that, although SHCS is effective to delay or ameliorate the hypoxic insults, it does not help the damaged cell recover the mechanism to reverse the disturbed ionic environment.

SHCS does not seem to interact directly with the passive leak mechanism or the Na-K-ATPase molecule itself. In the results

presented in Fig. 4, SHCS did not show any effect on the changes in cellular K^+ content induced by inhibition of the Na-K-ATPase by ouabain or metabolic inhibition by 2,4-DNP. If the effect of SHCS resulted from the interaction with the passive leak mechanism, it would be effective as well to attenuate the ouabain- or 2,4-DNP-induced fall in cellular K^+ content. It was also demonstrated in Fig. 5 that SHCS did not affect the Na-K-ATPase activity in hypoxic as well as normoxic slices.

The later part of the results in this study strongly suggests that the beneficial effect of SHCS might be linked to preservation of cellular metabolism under hypoxic insult. In the experiments in which the changes in oxygen consumption was assessed as an index of cellular metabolism, SHCS ameliorated significantly the drop in the oxygen consumption after exposure to hypoxic insult. Moreover, in the presence of SHCS, the fall in ATP content under hypoxic environment was significantly attenuated. A more direct demonstration was shown in the experiment using isolated mitochondrial fractions. It was shown that SHCS might help the hypoxic tissues preserve the mitochondrial function to produce ATP. Altogether, these results strongly suggests that SHCS provides a beneficial effect to protect the mitochondrial metabolic machinery against irreversible damages under

hypoxic insult.

The mechanism underlying the effect of SHCS to help the hypoxic tissue preserve the mitochondrial function is beyond description in the present study. Tissue injury resulting from hypoxia is mediated, in part, by the generation of reactive oxygen species and mitochondria is known to be easily damaged by these reactive oxygen species.[32,33] Unpublished observations in our laboratory suggested that SHCS is an effective scavenger of reactive oxygen species. It might provide a clue to explain the action mechanism underlying the clinical efficacy of SHCS. However, further extensive and carefully designed studies seem to be needed.

V. CONCLUSION

This study was carried out to determine whether SHCS might have any beneficial effect on alterations of cellular ionic environment and metabolism under hypoxic stress using cat brain cortical slices.

The result is as follows;

1. SHCS significantly delayed the hypoxia-induced changes in cellular K⁺ and Na⁺ contents.

2. SHCS was effective at the concentration above 0.3 mg/ml to block the hypoxia-induced fall in cellular K⁻ content.

3. SHCS did not provide any beneficial effect to reverse the hypoxia-induced changes when applied during the reoxygenation period.

4. SHCS did not show any effect on the changes in cellular K⁺ content induced by ouabain or 2,4-DNP.

5. SHCS did not affect the Na-K-ATPase activity in hypoxic as well as normoxic slices.

6. SHCS(1mg/ml) significantly delayed the drop in the oxygen consumption.

7. SHCS(1mg/ml) significantly attenuated the drop in ATP content.

8. SHCS might help the hypoxic tissues to preserve the mitochondrial function to produce ATP.

The results in this study show that SHCS provides a beneficial effect to protect the cellular metabolic machinery against irreversible damages under hypoxic insult.

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