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기 초

Serotonin 1A Receptor Agonist(8 OH DPAT) Reduces Tissue Lesion Volume in the Rat Middle Cerebral Artery Occlusion Model of Ischemia

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= Abstract =

We studied the effectiveness of serotonin 1a agonist(8 OH DPAT) on the 24 hour ionic lesion volume produced by permanent occlusion of the middle cerebral artery(MCAo) in rats. A 4-hour intravenous infusion of 30μmg/kg/hr of 8 OH DPAT were given starting at 10 minutes after occlusion. Tissue concentrations of Na, K, and water at infarct and peri-infarct zone were measured by atomic absorption spectroscopy and by wet-dry weight measurements 24 hours after occlusion. Compared with vehicle treatment, 8 OH DPAT treatment reduced tissue water accumulation by 10% and 55% in the frontopyriform cortex(L1) and frontoparietal cortex(L2), sodium accumulation by 20% at L1 and 47% at L2, potassium loss by 24% at L1 and 44% at L2, cell volume fraction loss by 24% at L1 and 47% at L2. Finally the treatment reduced overall lesion volume by about 37%. All these changes were statistically significant at $p < 0.05$. Our findings suggest strongly that 8 OH DPAT is neuroprotective in the rat MCAo model of focal cerebral ischemia.

KEY WORDS : Middle cerebral artery occlusion · Serotonin · 8 OH DPAT · Brain ischemia · lesion volume.

Introduction

Protecting the human brain from ischemic damage is an important clinical problem and it has been reported that numerous pharmacological agents were effective in the treatment of ischemic stroke[9)14)26)29)35)52)]. But, ischemic cerebrovascular disease still remains one of the most difficult problem to be prevented and treated. Serotonin(5-HT) has been described as one of the potent vasoconstrictor that may be involve in the pathogenesis of arterial spam after subarachnoid hemorrhage and migraine[6)7)24)25)]. In early study, Osterholm et al suggested that 5-HT causes tissue damage in brain[28)]. It was also suggested that abnormal 5-HT release from ischemic tissue might be responsible for the progression of cerebral infarction and extensively investigated its role in secondary injury to central nervous system(CNS)[24)49)50)]. The relatively high levels of serotonin detected following injury are consistent with its having a role in secondary damage. It is well known that spinal cord injury also releases large amounts of 5-HT into extracellular space[1)21)38)41)44)]. The mechanisms by which 5-HT causes secondary tissue damage, are not well understood. It appears that release of 5-HT in the brain is mostly probably related to a failure of the neuronal energy stores and the membrane transport following vascular occlusion[24)]. Serotonin may enhance injury either by direct effects on neurons or by reduction in blood flow caused by constriction of blood vessels or via both mechanisms[7)]. 5-HT is predominantly located in raphe neurons[45)]. The rat cerebral cortex receives a prominent serotoninergic innervation originating in the midbrain raphe nuclei and exhibits a high density of serotonin binding sites[27)]. It has been well know that multiple types of 5-HT receptors exist in the central nervous system[15)53)]. The highest density of $5HT_1$ binding sites in rat brain is found in the hippocampus, striatum, and cerebral cortex and the highest density of 5-HT_2 sites in the frontal parts of the cortex[10)13)20)30)31)53)]. Recent studies have shown that 5-HT_{1a} and 5-HT_2 receptors mediate opposing responses on membranc

excitability in rat cortex[5)22)32)]. In other studies, we have shown that both 5-HT_{1a} and 5-HT_2 receptors are also present in spinal axons and that these receptors appear to have opposing effects on axonal excitability, the former significantly reduces excitability of axons[40)]. 5-HT_2 antagonist, have long been recognized as being effective in the prophylaxis of migraine[25)], also reported to be neuroprotective in acute spinal cord injury[34)39)54)] and protective actions in models of middle cerebral artery occlusion(MCAo) were reported for the 5-HT_2 blocker ketanserin[19)26)]. Recent our study also suggests that 5-HT_{1a} agonists should depress axonal excitability more than 5-HT2 antagonist[40)]. 8 OH DPAT is a member of the aminotetraline family and one of the most site-selective 5-HT_{1a} agonists known[13)53)]. As a consequence, 5-HT_{1a} agonist, 8 OH DPAT may also exert neuroprotective effects, perhaps more strongly than mianserin. Thus, we studied the effects of 8 OH DPAT on changes in regional brain lesion volume in the rat MCAo model.

Materials and methods

1. Animal preparation

We anesthetized 28 adult male Long-Evans hooded rats weighing 260~350g(319±3.9g) with 60mg/kg i.p. pentobarbital which was sufficient to anesthetize the rat for 4-5 hours. These rats were randomized to two treatments groups(vehicle and treatment). The right femoral vein and tail artery were cannulated with small diameter polyethylene catheters for drug infusion and blood pressure and gas monitoring. The skin over the left temporal skull was incised. To expose the MCA, we removed the temporalis muscle and the zygoma to allow access to the infratemporal skull. Small craniectomy was made with 2.5 mm diameter trephine anterior to foramen ovale. The dura was opened with a hooked 25 gauge needle. The MCA was coagulated with bipolar radiofrequency currents applied with fine forceps 1~2mm below the rhinal fissure and was devided with microscissors to ensure complete occlusion. The area was carefully inspected for hemostasis and washed with sterile saline, The skin was then closed with 3~0 silks. At 10 minutes before and after MCAo, 0.1 ml arterial blood samples were withdrawn to measure Arterial blood gas values(pH, $PaCO_2$, PaO_2, H_2CO_3, base excess, and O_2 saturation). In addition, blood pressures were continuously measured at 10 minutes before, shortly after occlusion, and 10 minutes after occlusion, after the drug infusion has started. The maximum and minimum blood pressures were noted. Rectal temperatures were maintained at 37±0.5℃ with a heating pad during surgery. The arterial catheter was removed at 15 minutes after occlusion. The rats were placed in the temperature-controlled incubator with free access to water and food for 24 hours. Rats were decapitated 24 hours after surgery and the brains were removed just after decapitation and cooled in a deep freezer. A 3mm thick coronal slice of brain was then cut, centered at the point that the middle cerebral artery crosses the rhinal fissure with a double razor blade. The brain tissues were sampled by coring with a hollow 3mm i.d. metal tube with sharp edges. The areas sampled included the pyriform cortex(L1), frontoparietal cortex(L2), Parasagittal cortex(L3), and basal ganglia(L4). In general, the infarct center is located at L1 and extends into L2. Tissue samples were obtained from both hemispheres in each rat. The hemisphere on the operated side will be referred to as ipsilateral and the other contralateral(Matching contralateral tissue samples were called C1, C2, C3, and C4). These tissue samples were analyzed by atomic absorption spectroscopy for regional tissue Na^+ and K^+ concentrations and for water content. In addition, blood and plasma samples were obtained and analyzed for plasma Na^+ and K^+.

2. Experimental groups and treatment

The drug 8 OH DPAT was provided by Miles Laboratory(Bayer Pharmaceutical) in power form. We first prepared the vehicle solution by dissolving 50mg of citric acid, 10mg of sodium citrate, and 425mg of mannitol into 250ml of normal saline. We then dissolved 1mg of 8 OH DPAT into 50ml of this solution with a final concentration of 0.002 mg/ml. The drug solution was prepared shortly before the experiment and infused intravenously at the rate of approximately 0.5ml/hour starting shortly after MCAo. The volume was adjusted so that the rats received 30μmg/kg/hour over a four hour period. A syringe pump was used to deliver the drug solutions, The rats were randomized to two treatments groups (vehicle and treatment). At 10 minutes after occlusion, one group(A) received a continuos infusion of 2 ml of vehicle solution for four hours starting shortly after occlusion. The other group(B) received a 4-hour intravenous infusion of 30μmg/kg/hr of 8 OH DPAT dissolved in the vehicle solution. The identity of the drugs was marked from the experimenter, the tissue analysis team, and the data analyst until all data has been entered and initial

phases of the analyses were completed.

3. Tissueion and Water Analyses

Immediately after decapitation, the brains were removed and cooled at −85 ℃ until for cutting. The sampling method has been described in detail[14)36)51)52)]. The samples were weighed(=0.1 mg) to obtain wet weight(W), then dried overnight in a vacuum chamber(30 mm Hg) at 100℃ and reweighed to obtain dry weight(D). we analyzed these samples for Na^+ and K^+ by air-acetylene flame atomic absorption spectroscopy as described previously. In addition, we collected blood from the decapitation site into test-tubes coated with Na-free heparin to prevent clotting. The blood was centrifuged to obtain plasma. Both whole blood and plasma were analyzed by atomic absorption analysis for Na and K. We divided tissue Na and K contents by wet weight to obtain concentration units of mmoles/g of wet tissue([Na]w and [K]w). Tissue water concentrations were calculated from the formula : (W − D)/W. Since W − D represents the weight of water in the tissue and 1ml of water weighs 1 gm, water concentrations are given in ml/gm of wet tissue so that ionic and water concentration units are consistent. Units of blood([Na]b and [k]b) and plasma([Na]p and [K]p) concentrations are expressed as mol/gm of blood and mol/ml(or mM of plasma) respectively. To correct for bound or sequestered ions in the tissue, we normalized spinal cord [Na]w and [K]w to plasma levels by multiplying with([Na]p and [K]p)/([Na]w and [K]w) to obtain [Na]t and [K]t in units of mM. This correction assumes that tissue fluids are isotonic with plasma. In most cases, values of [Na]t and [K]t were 5% to 6% lower than [Na]w and [K]w, suggesting that a fraction of [Na]t and [K]t may be bound or sequestered. We used [Na]t and [K]t, as well as [Na]w and [K]w, to assess spinal cord damage. Because [Na]t and [K]t more accurately represent soluble tissue Na and K concentrations, we used lesion volumes calculated from [Na]t and [K]t.

4. Cell volume fraction determinations

Tissue cell volume fractions are linearly related to tissue Na and K concentrations. The relationship stems from isotonicity of intracellular and extracellular fluids. Small differences in tonicity generate huge pressures.

For example, according to the Van Hoff't equation, 1mM of ionic osmolarity difference will generate 19. 7mmHg of pressure. because Na, K, and associated anions exert greater than 95% of tissue fluid osmolarity, sums of Na and K concentrations should be approximately equal in intracellular([Na]i and [K]i) and extracellular([Na]e and [K]e) fluids. Likewise, since macromolecules in plasma compensate for blood pressure differences, sums of Na and K concentrations should be similar in plasma and extracellular fluids. Therefore, isotonicity can be expressed by the following equation :

$$[Na]i+[K]i=[Na]e+[K]e=[Na]p+[K]p \quad (1)$$

If so, transmembrane Na and K gradient(G) values also should be equal ; that is,

$$G=[Na]i-[Na]e=[K]e-[K]i \quad (2)$$

By definition, tissue ionic contents equal sums of intracellular and extracellular ionic contents,

$$[Na]t\ Vt=[Na]i\ Vi+[Na]e\ Ve \quad (3)$$

and

$$[K]t\ Vt=[K]i\ Vi+[K]e\ Ve \quad (4)$$

where Vt, Vi and Ve are tissue intracellular and extracellular volumes respectively. Subtracting Equation 4 from Equation 3 yields :

$$([Na]t-[K]t)\ Vt=([Na]e-[K]e)\ Ve+([Na]i-[K]i)\ Vi \quad (5)$$

Since [Na]i=G+[Na]e and [K]i=[K]e − G, substitution into Equation 5 gives :

$$([Na]t-[K]t)Vt=([Na]e-[K]e)\ Ve+([Na]e-[K]e+2G)\ Vi \quad (6)$$

Rearranging terms gives :

$$[Na]t-[K]t=([Na]e-[K]e)(Vi+Ve)/Vt+2G\ Vi/Vt \quad (7)$$

Since Vt=Vi+Ve, the equation simplifies to :

$$[Na]t-[K]t=[Na]e-[K]e+2G\ Vi/Vt \quad (8)$$

Equation 8 states that [Na]t − [K]t is linearly related to Vi/Vt with a slop of twice the gradient and a y intercept of [Na]e − [K]e. The ratio of cell to tissue volume(Vi/Vt) is equivalent to the cell volume fraction(CVF) of the tissue.

Cell volume assessment

Vi/Vt can be directly calculated from [Na]t − [K]t, G, and [Na]e − [K]e.

[Na]w − [K]w should closely approximate [Na]t − [K]t. To obtain [Na]t − [K]t with units to mol/ml, we extended the isotonicity assumption in equation 1 to include :

$$[Na]t+[K]t=[Na]p+[K]p \quad (9)$$

We normalized [Na]w and [K]w to plasma levels, as fellows :

$$[Na]t-[K]t=([Na]w-[K]w)([Na]p+[K]p)/[Na]w+[K]w \quad (10)$$

To estimate [Na]e − [K]e, we extended the isotonicity assumption to include

[Na]e+[K]e=[Na]p+[K]p.

Ion − selective microelectrode recordings have shown

that [Na]e and [K]e approach [Na]p and [K]p within 30 minutes of injury. Therefore, [Na]e − [K]e=[Na]p − [K]p

Therefore, substitution and rearrangement of above equation give

$$Vi/Vt=\{([Na]t-[K]t)-([Na]p-[K]p)\}/2G \quad \text{------- (11)}$$

calculated Vi/Vt values are expressed as percentages.

Alternatively, we assumed that [K]e=4 mM.

[Na]t+[K]t − 2[K]e=[Na]t+[K]t − 8=[Na]e+[K]e − 2[K]e=[Na]e − [K]e.

Substituting [Na]t+[K]t − 8 for [Na]e − [K]e in above equation gives :

$$Vi/Vt=\{([Na]t-[K]t)-([Na]t+[K]t-8)\}/2G=4-[K]t/G \quad \text{---------- (12)}$$

We will use Vp to refer to Vi/Vt values calculated from equation(11), Vk refer to Vi/Vt values calculated from equation(12), and cell volume estimated from equation $Vi/Vt=\{([Na]t-[K]t)-([Na]p+[K]p-8)\}/2G$ will be called Vi.

Vp, Vk and Vi tend to be close to each other. Vk is useful when plasma [Na]p and [K]p are not available or blood samples are inadvertently hemolyzed. If accurate [Na]p and [K]p measurements are available, Vp is usually less variable than Vk.

Changes of ionic and water concentrations, cell volumes, and lesion volumes

Since treatment of MCAo may affect general brain ion concentrations in individual rats, we expressed changes in ionic concentrations in the lesioned(left) hemisphere due to MCAo by subtracting the corresponding ionic concentrations in homologous areas of the contralateral(right) hemisphere(Δ Na, Δ K, Δ Vk, Δ Vp, Δ Vi, etc.) reducing experiment-to-experiment variations in ion measurements or drug effects independent of MCAo. Change in water content Δ Hw was calculated by subtracting water content in the lesioned hemisphere from that in homologous areas of the contralateral hemisphere.

5. Statistical analysis

All data were entered and initially calculated on a spreadsheet program and then transferred to a statistics program(Stat view, Super Anova 1.1 by Abacus Concepts, Berkeley, CA) for statistical analyses on Macintosh computers. Mean and standard deviation of ionic concentrations, water concentration, and cell volume fractions were calculated for each area in each group.

Individual outcome variables such as arterial blood pressure and gases, rectal temperatures, body weight, and blood and plasma Na^+ and K^+ concentrations, and lesion volumes were compared by analysis of variance(ANOVA). These are straight forward comparisons with only one factor(Rx), i.e. vehicle vs. 8 OH DPAT treatment groups, and yield results equivalent to a two-tailed t-test.

Multi-site outcome variables such as tissue ionic and water concentrations, cell volume fractions, and changes were compared by repeated measures ANOVA. This analysis treats the multi-site outcome variables as linked sites and the reduced degrees of freedom. In addition to providing F-values and p values for differences due to treatment(Rx), the analysis also provides F-values for differences among sample sites(Sites) and interactions between treatment and sample sites(Rx*Sites). A p-value of 0.05 served as the criterion for statistical significance. All values and error bars given in the text and figures are represent standard errors of means.

Results

1. Systemic Variables

Almost all animals lost body weight after MCAo with mean values ranging 8.7±1.6%. Temperature did not differ significantly between the two treatment groups. Rectal temperatures at 10 minutes before occlusion were virtually identical at 37.1±0.4℃ in the vehicle and treated groups respectively(F=0.083, p=0.9281). At 10 minutes after occlusion, rectal temperatures were likely virtually identical at 37.3±0.1℃ and 37.3±0.1℃(F=0.026, p=0.8731). Blood pressures also did not differ significantly in the two treatment groups. Preocclusion systolic pressure were 108.6±2.5 and 107.8±2.6mmHg in vehicle and treated groups respectively(F=0.039, p=0.8458). Pre-occlusion diastolic pressure were slightly different in vehicle and treated groups, i.e. 80.0±2.6 and 74.3±2.0 respectively, but the difference did not reach statistical significance(F=3.059, p=0.0921). At the time of occlusion, systolic pressures were 107.9±2.4 and 106.4±2.0 in vehicle and treated groups respectively(F=0.206, p=0.6541) and diastolic pressure were 78.9±2.5 and 72.9±2.4(F=3.4, p=0.0781). At 10 minutes after occlusion, systolic pressures were 108.9±2.2 and 106.4±2.7(F=0.510, p=0.4815) and diastolic pressures were 77.1±1.9 and 73.6±2.5(F=1.3, p=0.2674). Because the diastolic difference was present before the treatment, the slight difference is not due to treatment. Pre-occlusion arterial blood gases did not differ significantly between the vehicle and treated groups. All the values

were within normal range for pentobarbital anesthetized rats. Pre-occlusion pH values were 7.402±0.007 and 7.394±0.010 in vehicle and treated groups respectively(F=0.47, p=0.5011). At 10 minutes after occlusion, after treatment was started, pH values were slightly higher at 7.411±0.007 and 7.415±0.009 in vehicle and treated groups respectively(F=0.128, p=0.7235). pre-occlusion PaCO2 values were 36.2±1.2 and 35.2±1.5 in vehicle and treated groups respectively(F=0.271, p=0.6074) while post-occlusion PaCO2 values were 36.1±1.5 and 32.4±1.1(F=3.6, p=0.0710). Pre-occlusion PaO2 values were 79.5±3.6 and 82.9±2.3 in vehicle and treated groups respectively(F=0.65, p=0.4284) and post-occlusion PaO2 values were 83.5±3.5 and 80.8±2.2 in vehicle and treated groups respectively(F=0.46, p=0.5022). Treatment, however, appeared to change arterial bicarbonate(HCO_3) levels. Pre-occlusion HCO_3 values were 23.5±0.4 and 22.5±0.5 mM in vehicle and treated groups respectively(F=2.9, p=0.0994) while post-occlusion HCO_3 values were 23.9±0.5 and 22.2±0.5 in vehicle and treated groups respectively(F=5.0, p=0.0345), suggesting that the treated group had slightly lower arterial bicarbonate levels. However, pre-occlusion base excess values were similar in the vehicle and treated groups, respectively −1.6±0.5 and −2.4±0.6(F=1.0, p=0.3180) while post-occlusion base excess values were likewise similar, respectively −1.2±0.8 and −2.8±0.7(F=2.4, p=0.1393).

In summary, the vehicle and treated groups has virtually identical rectal temperature, arterial pH, $PaCO_2$, or PaO_2 levels at 10 minutes before and 10 minutes after occlusion. Likewise, arterial systolic or diastolic blood pressures were very similar in the two groups. Arterial blood gases were very similar in the vehicle and treated groups except that the latter had slightly lower bicarbonate level after occlusion.

2. Blood and Plasma Na and K and Hematocrits

At 24 hours after MCAo, plasma and blood Na^+ and K^+ concentrations did not differ significantly between the vehicle and treated groups. Plasma Na concentrations([Na]p) were 140.4±0.8 and 139.4±1.0mM(F=0.677, p=0.4181) in vehicle and treated groups respectively whereas plasma K concentrations([K]p) were 4.5±0.4 and 4.1±0.4 mM(F=0.446, p=0.5101). The sum of plasma Na^+ and K^+ ([Na]p + [K]p) were 135.9±0.9 and 135.3±0.8 in the vehicle and treated groups respectively(F=0.30, p=0.5885). Blood Na^+ concentrations([Na]b) were 93.2±2.2 and 91.6±2.4(F=0.267, p=0.6099) while blood K^+ concentrations ([K]b) were 50.6±1.5 and 51.4±1.9(F=0.11, p=0.7458) and [Na]b+[K]b values were 143.3±1.4 and 143.0±1.2(F=0.029, p=0.8663). Hematocrits were calculated from [Na]b-[K]b and [Na]-[K]p, assuming that G is -120 nM. The calculated hematocrits from blood obtained at 24 hours after MCAo were 38.7±1.3 and 39.6±1.3 in vehicle and treated groups respectively, within normal range and not significantly different(F=0.122, p=0.7300).

3. Tissue Water Concentrations

MCAo significantly increased tissue water concentrations([H]w) at the infarct site(L1), peri-infarct zone (L2), and surrounding cortical and subcortical tissues. In vehicle-treated animals, as shown in Fig. 1, mean [H]w rose from normal values of 0.76~0.78 to 0.82~0.85ml/

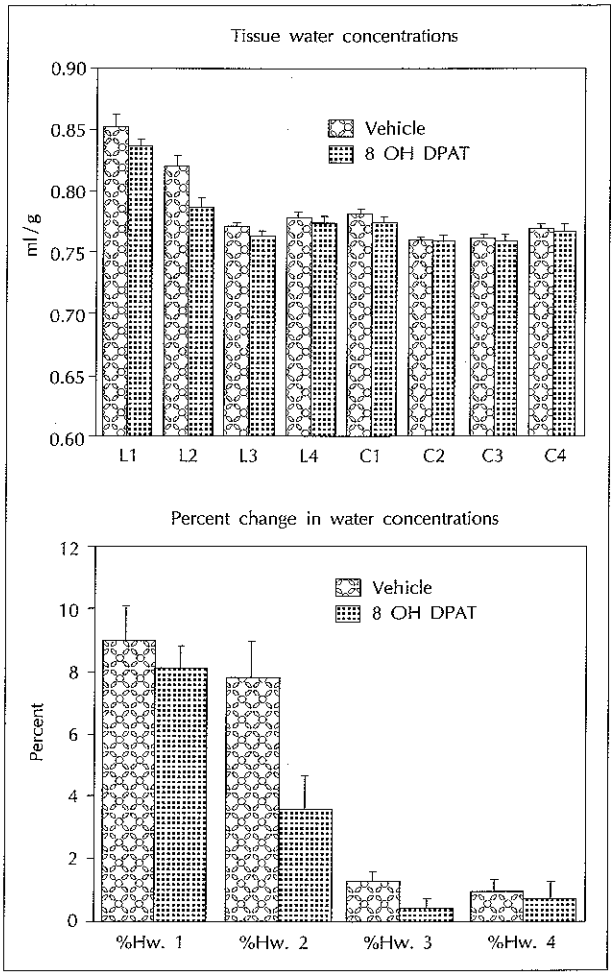

Fig. 1. Mean and standard errors of tissue water concentration([H]w, top graph) and percent tissue water concentration change(%[H]w, bottom graph). The sample sites at the infart center in frontopyriform cortex(L1), peri-infarct zone in frontoparietal cortex(L2), parasagittal cortex(L3), and subcortical regions(L4) are indicated on the abscissa.

Table 1. Ion and water concentrations by hemisphere and area in rats subjected to middle cerebral artery occlusion

	Lesioned hemisphere				Contralateral hemisphere			
	Area 1	Area 2	Area 3	Area 4	Area 1	Area 2	Area 3	Area 4
Group A								
Water(ml/g)	0.85±0.010	0.82±0.110	0.77±0.005	0.78±0.004	0.78±0.004	0.76±0.003	0.76±0.003	0.77±0.003
Na(μmol/g)	109.5 ±5.7	96.8 ±7.5	54.3 ±1.7	52.3 ±1.6	49.5 ±0.6	48.8 ±0.3	49.6 ±0.5	48.5 ±0.3
K(μml/g)	40.3 ±6.0	49.7 ±8.7	93.3 ±2.3	95.4 ±1.8	103.5 ±1.0	106.8 ±0.9	101.9 ±0.9	99.8 ±0.8
Group B								
Water (ml/g)	0.84±0.007	0.79±0.010	0.76±0.006	0.77±0.007	0.77±0.005	0.76±0.005	0.76±0.006	0.77±0.005
Na(μmol/g)	93.8 ±4.2	72.8 ±6.5	50.2 ±1.1	48.7 ±0.9	48.6 ±0.4	48.6 ±0.4	48.4 ±0.6	47.9 ±0.5
K(μmol/g)	58.6 ±3.9	75.6 ±7.5	98.8 ±1.6	99.1 ±0.8	105.0 ±0.7	106.6 ±0.7	102.3 ±0.7	100.6 ±0.5

Data are mean±SEM. Group A, vehicle control : Group B, OH-DPAT treated. Area 1, frontopyriform cortex : Area 2 frontoparietal cortex : Area 3, parasagital cortex : Area 4, subcortical regions. Group B significantly different from Group A in tissue Na(ANOVA, p=0.0007), K(p=0.01). Water concentration did not differ significantly between groups(ANOVA, p=0.1683), but suggests significant effects of treatment on [H]w at some site(ANOVA, Rx*Sites, p=0.0020).

g at L1 and L2. Treatment reduced [H]w from 0.85(0.010 to 0.84(0.007ml/g at L1 and 0.82±0.011 to 0.79±0.010ml/g at L2. Repeated measures ANOVA indicated that treatment effects on [H]w were not statistically significant across all the sample sites(Rx, F=2.01, p=0.1683). The analysis, however, indicated very significant differences in [H]w among sample sites(Sites, F=96.1, p=0.0001) and a significant interaction effect of treatment and sample sites(Rx*Sites, F=3.39, p=0.0020) on [H]w. Thus, the data suggests significant effects of Rx on [H]w at some sample sites. Repeated measures ANOVA of Δ[H]w indicated very significant effects of treatment(Rx, F=4.4, p=0.0448), sample site(Sites, F=59.9, $p<0.0001$), and interaction of treatment and sites(Sites*Rx, F=3.3, p=0.0249). Expressed as a percent of contralateral hemisphere, %[H]w rose by 8~9% at L1 and L2 in vehicle treated animals and only 0.5~1.0% in L3 and L4, consistent with edema in cortex at the infarct and peri-infarct regions and less edema in the surrounding cortex and subcortical regions. Treatment reduced%[H]w from 9.0(1.1% to 8.1(0.7% at L1 and from 7.8(1.2% to 3.5(1.1% at L2. Repeated measures ANOVA of%[H]w indicated a significant effect of treatment(Rx, F=4.3, p=0.0470), sample sites(Sites, F=58.2, $p<0.0001$), and interaction of treatment and sites (Sites*Rx, F=3.3, p=0.0236).

In summary, MCAo markedly increased water accumulation at the infarct center and peri-infarct cortex by 8~9%. Treatment significantly reduced edema, particularly in the peri-infarct cortex where it reduced tissue water accumulation by more than half and virtually eliminated edema in the subcortical regions but did not significantly reduced water accumulation at the infarct center or parasagittal cortex.

Table 2. Percent changes in Ion and Water concentrations on Different Area of both groups.

	Area 1	Area 2	Area 3	Area 4
Group A				
% Hw	9.0 ± 1.1	7.8 ± 1.2	1.3 ± 0.4	1.0 ± 0.3
% Na	121.9 ±12.1	98.3 ±15.3	9.4 ±73.5	7.7 ± 2.9
% K	−60.7 ± 6.2	−53.5 ± 8.0	−8.5 ± 1.9	−4.4 ± 1.7
% Vi	−64.7 ± 6.5	−55.4 ± 8.9	−6.8 ± 2.0	−4.6 ± 1.7
Group B				
% Hw	8.1 ± 0.7	3.5 ± 1.1	0.5 ± 0.3	0.7 ± 0.6
% Na	93.1 ± 8.8	50.8 ±14.3	4.0 ± 2.5	1.6 ± 1.6
% K	−44.1 ± 3.8	−29.0 ± 7.2	−3.4 ± 1.5	−1.4 ± 0.9
% Vi	45.5 ± 4.1	27.3 ± 7.4	2.5 ± 1.3	1.0 ± 0.0

Data are mean±SEM.Group A,Vehicle control : Group B, 8 OH-DPAT treated. Area 1, frontopyriform cortex : Area 2, frontoparietal cortex : Area 3, parasagittal cortex : Area 4, subcortical regions. Group B significantly different from Group A in tissue % Hw(p=0.0470), % [Na]w(p=0.0188), %[K]w(p=0.0132), and% Vi/Vt(p=0.0078)

4. Tissue sodium concentrations

MCAo significantly increased tissue sodium concentrations([Na]w) from normal values of 50mM in the contralateral hemisphere to 96~110 mM at L1 and L2, as shown Fig. 2. Treatment reduced [Na]w from 109.5±5.7 to 93.8±4.2μmol/g at L1 and from 96.8±7.5 to 72.8±6.5μmol/g at L2. Repeated measures ANOVA indicated a significant effect of treatment on [Na]w(Rx, F=8.6, p=0.0071), large differences among sample sites(Sites*Rx, F=4.5, p=0.0001). Treatment reduced percent change of [Na]w(%[Na]w) from 121.9±12.1% to 93.1±8.8% at the infarct center at L1 and from 98.3±15.3% to 50.8±14.3% in peri-infarct zone at L2. Repeated measures ANOVA of % [Na]w indicated very significant effects of treatment(Rx, F=6.3 p=0.0188). Sample site(Site, F=82.2, p<

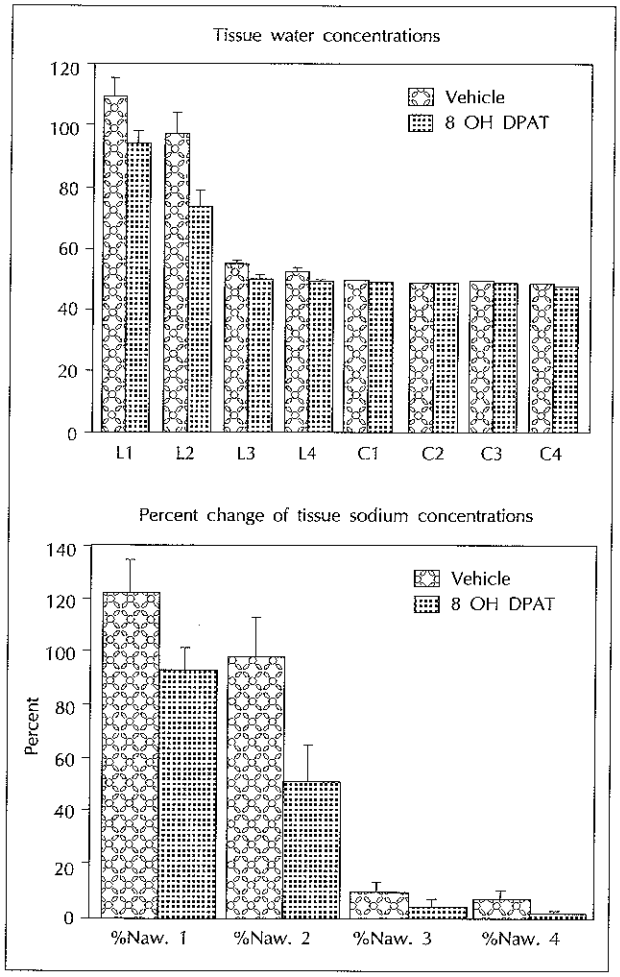

Fig. 2. Mean and standard errors of tissue sodium concentration ([Na]w, top graph) and percent tissue water concentration change(%[Na]w, bottom graph). The sample sites at the infarct center in frontopyriform cortex(L1), peri-infarct zone in frontoparietal cortex(L2), parasagittal cortex(L3), and subcortical regions(L4) are indicated on the abscissa.

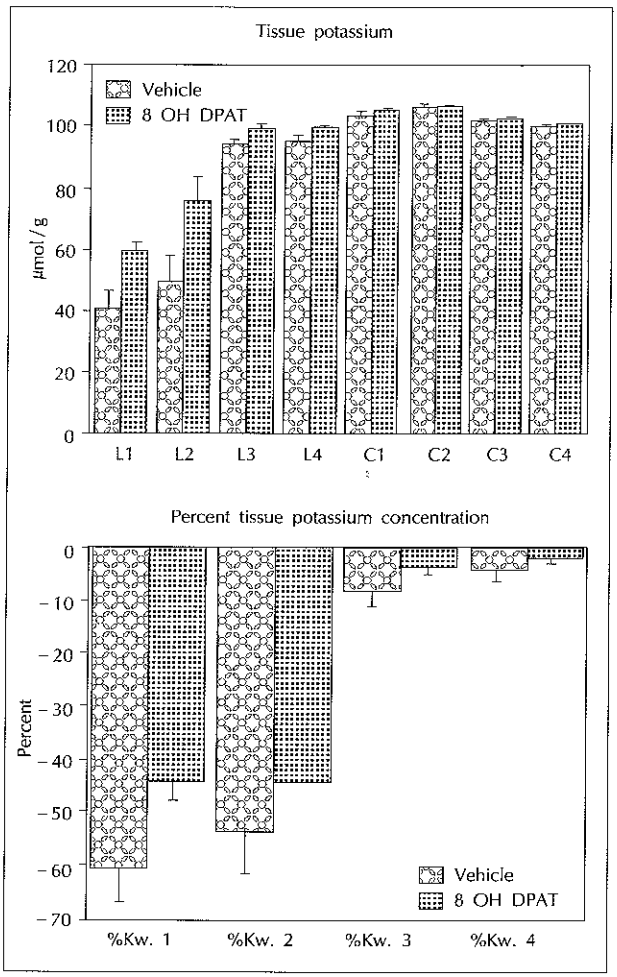

Fig. 3. Mean and standard errors of tissue potassium concentration ([K]w, top graph) and percent tissue potassium concentration change(%[K]w, bottom graph). The sample sites at the infarct center in frontopyriform cortex(L1), peri-infarct zone in frontoparietal cortex(L2), parasagittal cortex(L3), and subcortical regions(L4) are indicated on the abscissa.

0.0001), and interactive effects of treatment and sites(Rx* Sites, F=3.3, p=0.0264). Thus MCAo markedly increased [Na]w at L1 and L2 by 90~110% and treatment significantly reduced sodium accumulation at the ischemic hemisphere, particularly at L2 where treatment reduced % [Na]w by nearly a half.

5. Tissue Potassium Concentrations

MCAo markedly reduced tissue potassium concentrations([K]w) from normal values of 100~110μmol/g in the contralateral hemisphere to 40~50μmol/g at L1 and L2 in vehicle-treated rats. Treatment increased [K]w from 40.3±6.0 to 58.6±3.9μmol/g at L1 and from 49.7±8.7 to 75.6±7.5μmol/g at L2 as shown in Fig. 3. Repeated measures ANOVA of [K]w revealed a significant effect of treatment(Rx, F=7.7, p=0.0102), sample site(Sites, F=87.10, $p<0.0001$). and interaction of treatment and site(Rx*Sites, F=4.5, p=0.0001). Compared with matched sample sites in the contralateral hemisphere, treatment altered the percent change of tissue potassium concentrations(%[K]w) from −60.7±6.2% to −44.1±3.8% at L1 and from −53.5±8.0% to −29.0±7.2%. Repeated measures ANOVA of %[K]w indicated a significant effect of treatment(Rx, F=7.1, p=0.0132), sample site(Sites, F=80.5, $p<0.0001$), and interaction between treatment and sites(Rx*Sites, F=3.4, p=0.0223). Thus, MCAo caused marked potassium loss at the infarct center and peri-infarct cortex and treatment prevented potassium loss in the ischemic hemisphere. At the peri-infarct zone, treatment reduced potassium loss by nearly a half.

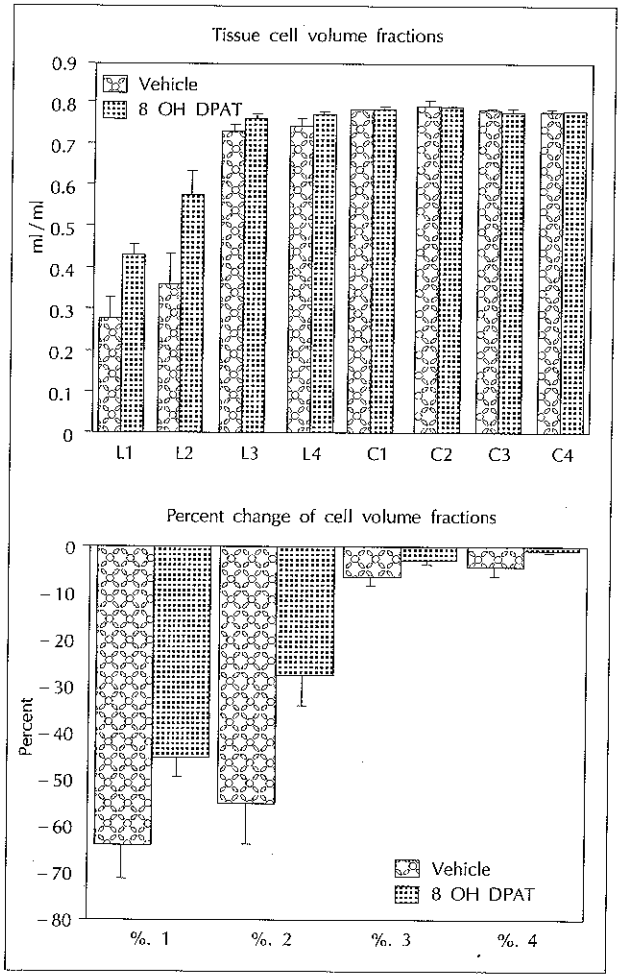

Fig. 4. Mean and standard errors of tissue cell volume fraction(Vi/Vt, top graph) and percent tissue cell volume fraction(%Vi/Vt, bottom graph). The sample sites at the infarct center in frontopyriform cortex(L1), peri-infarct zone in frontoparietal cortex(L2), parasagittal cortex(L3), and subcortical regions(L4) are indicated on the abscissa.

6. Tissue Cell Volume Fractions

MCAo markedly reduced tissue cell volume fractions(Vi/Vt) from normal values of 0.78~0.79 to 0.31~0.38 at L1 and L2 in vehicle-treated rats. Treatment increased Vi/Vt from 0.277±0.049 to 0.427±0.031ml/ml at L1 and from 0.358±0.072 to 0.574±0.059ml/ml at L2, as shown in Fig. 4. Repeated measures ANOVA revealed a significant effect of treatment(Rx, F=5.5, p=0.0277), sample sites(Sites, F=88.4, $p<0.0001$), and interaction between treatment and sites(Rx*Sites, F=5.6, p=0.001). In terms of percent change from contralateral hemisphere, treatment reduced the percent loss in Vi/Vt from −64.7±6.5% to −45.5±4.1% at L1 and −55.4±8.9% to −27.3±7.4% at L2. Repeated measures ANOVA indicated a significant effect if treatment(Rx, F=8.4, p=0.0078), sample sites(Sites, F=76.8, $p<0.0001$). and interaction between treatment and sites(Rx*Sites, F=4.1, p=0.0101).

7. Lesion Volumes

To estimate lesion volumes, we multiplied ΔVi/Vt with W to obtain the volume of cell loss per tissue sample and then summed the volume of cells lost in the four tissue sample from the lesioned hemisphere. Calculated ionic lesion volumes were 26.8±3.4 and 17.0±2.5μliters in vehicle and treated groups respectively, significantly different(F=5.3, p=0.0290). Fig. 6 shows a box plot of lesion volumes in the two groups. Although mean values were distinct, there was substantial overlap in the lesion volumes in the two groups. It appears some animals showed relative small lesion volumes at 24 hours after MCAo but the vehicle-treated group had a group of animals with substantial greater lesion volumes. to rule out outlier effects, we carried the Mann-Whitney U-test and this analysis showed that the ranks of the grps differed significantly from each other(p=0.0308, A summed ranks=17.86 and B summed ranks=11.143).

Thus, MCAo eliminated about 26.8μliters of cells from the 3-mm coronal brain slice centered in the occlusion site and 8 OH DPAT treatment reduced average of 37%. Fig. 6 shows a notched box plot of the lesion volumes in the two groups. Lesion volumes were significantly different both on parametric ANOVA(p=0.0290) and nonparametric Mann-Whitney U(p=0.0308, Z=−2.16) tests.

Discussion

Since our MCAo model spares the lenticulostriate branches of the middle cerebral artery, infarcts were largely localized to the cerebral cortex, especially in the frontopyriform(L1) and frontoparietal cortex(L2)[11)12)14)16)17)36)37)45)51)52)]. Parasagittal cortex(L3) was relatively well preserved because this area has some collateral circulation from anterior cerebral artery[8)48)]. Our results strongly indicate that 8 OH DPAT, administered intravenously at a dose of 30 μg/kg/hr for four hour period starting shortly after MCAo, reduced 24 hour ionic shift and lesion volume at the infarct site in the rat. 8 OH DPAT had little or no effect on systemic variables such as body temperature, blood pressure, or blood gases, except for a slight tendency to reduced blood bicarbonate levels by 1~2 mM at 10 minutes after MCAo. The drug had no significant effect

on plasma or blood Na and K levels at 24 hours after injury but significantly reduced tissue edema and ionic shifts at the infarct center(L1) and peri-infarct cortex(L2) at 24 hours. 8 OH DPAT reduced tissue water accumulation by 10% at L1 and 55% at L2, sodium accumulation by 20% at L1 and 47% at L2, potassium loss by 24% at L1 and 44% at L2, cell volume fraction loss by 24% at L1 and 47% at L2. Finally the treatment reduced overall lesion volume by about 37%. All these changes were statistically significant at $p < 0.05$.

Since the water accumulation in the brain reflects edema, and the tissue Na, K shift at the lesion site indicates cell loss[14)36)51)52)] our results suggest that 8 OH DPAT protected the infarct site. Decreased ionic shifts may results paradoxically from a decrease in blood flow. If blood flow is reduced, ionic shifts should be correspondingly[14)]. But our data suggest that decreased blood flow is not adequate explanation of the reduced ionic shifts. Because 8 OH DPAT with 30μg/kg/hour had no effects on systemic blood pressure. Moreover, decreased perfusion should affect any site of infarct area equally, but 8 OH DPAT had a different effect on each sites. The effect was more prominent in frontoparietal zone(L2) compare to infarct center(L1).

We have not definitely established how these drugs produce their protective effects against cerebral infarction. The role and effects of 5-HT_{1a} receptor agonist are not known in the central nervous system, but depressive effects of 5-HT on neuronal membrane and blood flow are reasonable possibility. It might inhibit neuronal excitatory action of serotonin as well as increased blood flow.

Serotoninergic neurons are located in the raphe nuclear complex of the midbrain and the serotoninergic terminals innervate cortical neurons and arteries[13)30)31)45)]. It was not well known that 5-HT actions in the vascular wall and CNS. But, serotonin receptors are abundant on both vascular and neuronal membrane, serotonin may affect on both neuronal membrane permeability and blood flow in the brain[2)7)]. The relatively high levels of serotonin detected following central nervous system injury are consistent with its having a role in secondary damage[24)49)]. Recent studies has also shown that spinal cord injury releases large amounts of 5-HT into extracellular space[1)21)38)41)42)] Serotonin concentrations quickly rise following injury and take 30~45 minutes to return to control level in traumatized spinal cord[21)]. The major source of serotonin after injury is in nerve cell bodies of the spinal cord gray matter and in serotoninergic pathways located in the white matter and serotonin may be also released from blood platelets activated by injury [3)38)]. An increased release or accumulation of serotonin might well induce vascular and cellular changes which may be reflected in altered water content and edema of the tissue. There are some evidence that serotonin is implicated in the reduction of blood flow which is an essential part of the changes occurring within an traumatized brain and spinal cord segment[3)29)]. It has been also postulated that serotonin release after ischemia may aggravate the ischemic insult by constricting cortical arteries.

The 5-HT_2 antagonist, ketanserin, has been shown to reverse vasoconstriction of large pial arteries due to serotonin application[7)] Ischemic core in the cortex are usually surrounded by a relatively wide zone of moderately reduced flow[26)46)47)48)]. This penumbral areas were originally defined as those having a reduction in CBF but can maintain membrane potentials and gross cellular ion hemostasis and ischemic damage in this area is believed to be reversible and has been a popular target of therapeutic intervention[17)23)33)43)]. But, it was known that this zone had shrunk to a very narrow zone 1 to 2 mm wide, and ischemic damage was already evident adjacent to this narrow area by 4 hours after MCAo[26)48)] that means penumbral conditions may exists only few hours after onset of focal ischemia. Since our treatment effect is more prominent in frontoparietal cortex compare to infarct center, there is some possibility that 8 OH DPAT may improve collateral blood flow in this peri-infarct zone. Because, 8 OH DPAT treatment decreased Na accumulation and K loss almost equally, this pattern of ionic shifts may be explained by improved collateral blood flow in the peri-infarct zone. But, It has been also reported that serotonin has dual effect on cerebral blood vessels, constriction of large and dilatation of small arteries and did not effect on cerebral blood flow[7)]. Therefore, further investigation for cerebral blood flow will be required to determine the effectiveness of 8 OH DPAT on blood flow.

Significant neuroprotective effects has been reported for the 5-HT_2 antagonist in the spinal injury model as well as brain ischemic model[19)39)54)]. These observations also let us consider that serotonin takes part in the early response of CNS ischemia and trauma of the spinal cord.

It has been reported that 5-HT_{1a} and 5-HT_2 receptors mediate opposing responses on membrane excitability in rat cortex, Membrane hyperpolarization is mediated by a

receptor of the $5HT_{1a}$, whereas membrane depolarization was mediated by 5-HT_2 receptor[5)22)32)]. It has been known that the serotonin-induced hyperpolarizing and inhibitory responses by receptors of the 5-HT_{1a} is generally mediated by the increases in potassium conductance[4)]. And it has been also suggested that majority of pyramidal neurons possess both 5-HT_{1a} and 5-HT_2 receptors in different combinations, these finding that 5-HT has dual excitatory and depressive effects suggests a mechanism by which neurons can regulate axonal excitability by varying ratios of 5-HT_{2a}/5-HT_{1a} receptors[5)]. These results indicated that if the density of 5-HT_{1a} receptor is increased, the ability of the stimuli to elicit spiking might be impaired due to the membrane hyperpolarization. Therefore 5-HT_{1a} agonist can prevent cellular excitation and do effectively function as an 5-HT_2 receptor antagonist like ketanserin. Local administration of 8 OH DPAT inhibits glutamate-evoked firing of motor neurons, in contrast to the facilitory effects of iontophoretically applied 5-HT[18)]. We have shown that both 5-HT HT_{1a} and 5-HT_2 receptors are also present in spinal axons and that these receptors appear to have opposing effects on axonal excitability, the former significantly reduces excitability of axons[40)]. Our study also suggests that 5-HT_{1a} agonists should depress axonal excitability more than 5-HT_2 antagonist, These finding let us to consider that 8 OH DPAT effectively prevents 5-HT-induced excitation of neuron in addition to restore blood flow in ischemic brain injury, perhaps more strongly than mianserin. Therefore, both a reduced energy demand and increased cerebral blood flow might be considered as neuroprotective mechanism of 8 OH DPAT.

In conclusion, regardless of mechanism, our result indicate that 8 OH DPAT is a highly neuroprotective and can reduce neurological damage caused by ischemic stroke. In preliminary studies, we have found that this treatment protocol does not alter ionic shifts and lesion volumes in our rat spinal cord injury model. We suspect that the lack of effect probably is due to different dosage requirements for trauma. Further investigation will be required to determine the most useful doses and time courses for administration of this drugs.

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쥐의 중대뇌동맥 폐쇄후 Serotonin 1A Agonist(8 OH DPAT) 의 치료효과

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=국문초록=

저자들은 쥐의 중대뇌동맥을 폐쇄시킨후 serotonin 1a agonist인 8 OH DPAT의 치료효과를 연구하였다. 중대뇌동맥 폐쇄 10분 후 부터 4시간 동안 30㎍mg/kg/hr의 8 OH DPAT을 정주하였으며 24시간 후에 대뇌 허혈부위에서 Na, K, H_2O의 함량을 측정하였다. 8 OH DPAT는 frontopyriform cortex와 frontoparietal cortex의 수분함량을 대조군에 비하여 각기 10%, 55% 감소시켰으며, Na 축적은 20%, 47% 방지하였고, K손실은 24%, 44% 줄일 수 있었고 결국 lesion volume을 37% 줄일 수 있었다. 이 모든 변화는 모두 통계학적으로 의의가 있었다. 저자들의 이러한 결과는 8 OH DPAT가 쥐의 중대뇌동맥 폐쇄 후 발생하는 신경학적 손상을 현저히 줄일 수 있다는 것을 암시한다.