A New Model of Global Postischemic Reperfusion in Rabbit

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Received January 30, 1990; Accepted October 19, 1990

ABSTRACT

An ideal model of global ischemia in rabbits has not yet been developed. The present study describes a new model of global post-ischemic reperfusion (GPIR) in the rabbit, characterized by lack of systemic hypotension. The experimental procedure involves reversible occlusion of the bilateral internal carotid arteries (ICA) and bilateral external carotid arteries (ECA) for 60 min combined with permanent ligation of bilateral vertebral arteries (VA). This grouping is called 6-artery occlusion (6AO). Sixty minutes after the occlusion, bilateral ICA and bilateral ECA were released for 120 min at which time the experiment was terminated. The results revealed severely depressed EEG activity; Water content of brain tissue increased to $80.33 \pm 1.20\%$ (control $78.28 \pm 0.59\%$, $p < 0.01$); K, Mg, and Zn decreased ($p < 0.05$ or $p < 0.01$), and were negatively correlated with tissue water content. Na increased ($p < 0.05$) and correlated with water content of brain. No significant changes were observed in lipid peroxide (LPO) levels, but the activity of superoxide dismutase (SOD) of brain tissue decreased ($p < 0.01$), and was negatively correlated with water content ($r = -0.5808$, $p < 0.05$). These results were compared with those obtained with the model of 4-artery (bilateral common carotid arteries (CCA) and VA) occlusion (4AO) and sug-

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gested that the brain damage be more severe with 6AO than with 4AO.

Index Entries: Global ischemia; 4-artery occlusion; 6-artery occlusion; reperfusion; rabbit.

INTRODUCTION

A satisfactory model of global posts ischemic reperfusion (GPIR) should be: (a) ability to induce a condition of severe cerebral ischemia, (b) simple and reliable reproduction, (c) the model uncomplicated by systemic hypotension, and (d) acceptable animal species with respect to size, ease of supply, and without excessive costs. Although many models of global cerebral ischemia have been reported in gerbils (Yoshida et al., 1980), rats (Pulsinelli and Brierley, 1979; Todd et al., 1986), cats (Paschen et al., 1983), dogs (Jackson and Dole, 1979), and the primates (Branston et al., 1980), we consider that the rabbit should be chosen as the best candidate for the model of CPIR because the pattern of brain vessels in the rabbit is more similar to that in the primates and man than that in dogs and cats (Aguilar, 1963; Gu, 1983). In addition, the rabbit's size is bigger than rats, so it is easier to measure intracranial pressure, blood pressure, and to take more blood samples. Moreover, the cost of rabbits is cheaper and they are more easily supplied than dogs, cats, and the primates. Unfortunately, an ideal model of CPIR in the rabbit has not yet been developed. Those models that had been reported usually involved 4-artery occlusion, bilateral common carotid arteries (CCA) and bilateral vertebral arteries (VA) occlusion (i.e., 4AO), and had to drop simultaneously the systemic blood pressure induced either by bleeding (Li et al., 1987) or by drugs (Kolata, 1979; Mchedlishvili et al., 1976), or to impede cerebral venous return (Kowada et al., 1968). However, systemic hypotension can result in damage to heart, kidney, and other vital organs and thus is an undesirable complicating factor of studies of ischemic brain. The present study describes a new model of GPIR induced by reversible occlusion of the bilateral internal carotid arteries (ICA) and external carotid arteries (ECA) combined with permanent ligation of bilateral VA (i.e., 6AO) in rabbits. The model is not complicated by systemic hypotension and does not impede cerebral venous return.

MATERIALS AND METHODS

Native white rabbits, 30 male and 40 female, weighing 1.6 ± 0.2 kg, were divided into four groups: Group A, Sham-operated; Group B, 60 min of 6AO; Group C, 120 min of bilateral ICA and ECA reperfusion after 60 min of 6AO; and Group D, 120 min of bilateral CCA reperfusion after 60 min of 4AO. Rabbits were anesthetized intravenously through an ear

Fig. 1. Schematic representation of position of 6AO ICA, internal carotid artery; ECA, external carotid artery; CCA, common carotid artery.

marginal vein with 25% urethane (1 g/kg). A stainless steel screw was mounted 3 mm posterior to coronal suture, and 3 mm right lateral to midline to record the electroencephalogram (EEG, Polygraph Nihon, Japan). A reference electrode was fixed at the ipsilateral base of the ear. Bilateral VA were isolated at supraclavicular and the side of prominent vertebra where is the beginning of VA via a ventral midline cervical incision and ligated at the beginning of VA (Fig. 1). Bilateral CCA, ICA, and ECA were isolated and a suture was loosely placed around each artery without compromising its blood flow. Femoral arteries were cannulated bilaterally and injected with 0.2% heparin, one to record the mean artery blood pressure (MABP), and the other to sample blood for pH, pCO₂, and pO₂ (Blood gas analyzer, ABL-3 Denmark). The physiological state of the animals was assessed by measurements of rectal temperature and blood gases prior to and during the experiment. The data obtained from the 17 normal animals prior to the experiment were (average + SD): PaO₂, 88 ± 2 mmHg; PaCO₂, 31 ± mmHg; and pH, 7.37 ± 0.01. The body temperature was maintained at 37 ± 0.5°C with a heating lamp during the whole experimental period. The data were maintained in this range until the end of the experiment. The rabbits with global ischemia were in a similar physiological state. After 60 min of 6AO, or 4AO, either bilateral ICA and ECA (group C) or bilateral CCA (group D) were recirculated for 120 min. Animals were sacrificed by an overdose of urethane and their whole brain was rapidly removed. A 4 mm thick slice of 8 × 8 mm left parietal tissue was excised, weighed, and dried to constant wt at a temperature of 105°C in an electric oven. Brain water content was calculated as (%) = (fresh wt - dry wt) / fresh wt × 100%. The dried brain tissue samples were used to determine K,

Na, Ca, Mg, Fe, and Zn by atomic absorption spectrophotometry (HITACHI-180-70). The metabolic product of lipid peroxide (LPO) and malondialdehyde (MDA) in brain tissues was determined with thiobarbituric acid (TBA) (Yoshida et al., 1980). The activity of superoxide dismutase (SOD) was determined by the method of autoxidation of pyrogallol (Marklund and Marklund, 1974). An estimate of the effect of 6AO on brain perfusion was made in four other rabbits by injection into the ascending aorta of 30 mL normal saline containing 1% Evans blue at pressure 120 ± 10 mmHg. A craniotomy was immediately performed and the whole brain was removed to reveal the distribution of dye.

RESULTS

Survival of Rabbits

In 6AO group, 26 of 38 rabbits (68%) survived and they were sacrificed at 120 min after reperfusion. In the 4AO group, 12 of 22 rabbits (54%) survived and they were sacrificed at 120 min after reperfusion. But this difference was not statistically significant. All deaths occurred during the early period of ischemia and arrest of their respiratory occurred prior to arrest of their heart.

Mean Artery Blood Pressure

The MABP increased 18 ± 14 mmHg in average in group B, 15 ± 11 mmHg in average in group C, and 24 ± 5 mmHg ($p < 0.05$) in group D during 5 min of ischemia. The increase of MABP was the most remarkable in group D. The decrease of MABP was more in group D (average decreased 37 ± 21 mmHg) than in group C (average decreased 16 ± 14 mmHg) at 5 min after reperfusion, $p < 0.01$ (Table 1; Fig. 2).

EEG Activity

Table 2 shows that the EEG activity was severely depressed in all animals of group B (6AO) and C (6AO + reperfusion) and 9 of 10 in group D (4AO + reperfusion) during 60 min ischemia. The EEG depression was more severe in group C than in group D within 120 min reperfusion (Table 2; Fig. 3).

Water Content

The average water content of brain samples was $78.28 \pm 0.59\%$, $78.96 \pm 0.79\%$, $80.33 \pm 1.20\%$, and $79.42 \pm 1.29\%$ in groups A, B, C, and D respectively (Fig. 4). The water content was highest in group C ($p < 0.01$).

Table 1
The Changes of MABP During Ischemia and Reperfusion

Groups	Ischemia (min.)			Reperfusion (min.)		
	0	5	60	5	60	120
A(n=11)	104± 8	104± 8	98± 9	98± 9	89± 14	84± 14
B(n=12)	101± 8	119± 14	81± 32			
C(n=12)	97± 9	112± 9	91± 12	75± 13	73± 15	61± 15
D(n=10)	102± 10	125± 15 ^a	107± 16	70± 18 ^{b,c}	68± 11	61± 15

Values are mmHg, mean ± SD. Numbers of animals are given in parentheses.

^aStatistically different from 5 min of ischemia: $p < 0.05$.

^bStatistically different from 60 min of ischemia: $p < 0.05$.

^cStatistically different from the decrease of BP 5 min after reperfusion between group C and group D.

(by two-tailed Student's *t*-test and with Bonferroni modification)

Fig. 2. The rise in blood pressure after 4AO (A) was greater than after 6AO (B). Conversely, the fall in blood pressure after bilateral CCA release (C) was greater reperfusion with bilateral ICA and bilateral ECA release (D). O: occlusion; R: release.

Estimation of Brain Perfusion

Injection of 1% Evans blue dye into the ascending aorta revealed well-perfused areas of the whole brain to be stained dark blue in the control (no occlusion), bilateral VA, or bilateral CCA occlusion animals. Although 6AO resulted in a marked decrease in perfusion of the cerebral

Table 2
The Changes of EEG Activity During Brain Ischemia
and Reperfusion in Rabbits

Scale	Groups (60 min ischemia)				(120 min reperfusion)		
	A	B	C	D	A	C	D
I	0	10	11	8	0	8	4
II	0	2	1	1	0	3	3
III	0	0	0	0	2	1	3
IV	11	0	0	1	9	0	0
H value*		32.55				20.85	
p value		<0.01				<0.01	

Note: Scale I: The voltage was less than 25% initial level or isoelectric; Scale II: 50–25% initial level; Scale III: 75–50% initial level; Scale IV: more than 75% initial level or no observed change.

*Kruskal Wallis test.

hemispheres, it did not produce a complete ischemia because of collateral supply (Fig. 5).

Lipid Peroxide (LPO), Superoxide Dismutase (SOD), and Ionic Content

Table 3 shows that the difference of LPO was not significant from group to group. The lowest activity of SOD occurred in group C, and was significant when compared with groups A and B ($p < 0.01$). Moreover, there was a negative correlation between the changes in activity of SOD and water content of brain, but there was no correlation between LPO and water content of brain (Fig. 6).

The detection of ionic content revealed changes of K, Na, Mg, and Zn in group C as either significant ($p < 0.05$) or highly significant ($p < 0.01$), when compared with either group A or B (Table 3). There was a positive correlation between Na and water content, and there was a negative correlation between water content and K, Ca, Mg, and Zn (Fig. 6). No significant changes were observed on K, Na, Ca, Mg, Fe, or Zn in group B (ischemia only).

DISCUSSION

The previous methods that produced global ischemia were usually used by bilateral CCA occlusion. The method of bilateral CCA could result in the rise of systemic blood pressure due to the reflex of carotid sinuses. Thus the hypertension could open the collateral circulation

Fig. 3. The time course of EEG activity comparing group A (control), with group C (6AO) and group D (4AO). Depression of EEG activity is more severe in group C than group D during ischemia (I) and reperfusion (RP).

between anterior spinal arteries and cerebral arteries (Hossmann and Zimmermann, 1979). On the other hand, the drop of pressure of carotid sinus baroreceptor itself also could result in the compensable increase of cerebral blood flow (Ponte and Purves, 1974). So, those methods that resulted in global ischemia have to associate with a systemic hypotension induced by bleeding (Li et al., 1987) or drugs (Boulu et al., 1981; Kolata, 1979; Mchedlishvili et al., 1976), even to prevent venous drainage

Fig. 4. The water content of brain tissue (mean $\pm$ SD). A. Sham-operated, $n = 16$; B. 60 min of 6AO, $n = 17$; C. 120 min of reperfusion after 60 min of 6AO $n = 16$; D. 120 min of reperfusion after 60 min of 4AO, $n = 10$. **compared with group A $p < 0.01$; † compared with group B $p < 0.05$, †† $p < 0.01$; Δ compared with group D $p < 0.01$.

from the head (Kowada et al., 1968). However, sustained systemic hypotension could result in the damage to heart, kidney, and other vital organs. A significant feature of the new method described is avoidance of the pressure of bilateral carotid sinuses dropping abruptly, thus maintaining normal tension in the bilateral carotid sinuses and normal systemic blood pressure. So, the new model might avoid the compensable increase of cerebral blood flow. Consequently, it did not have to make a systemic hypotension, thus avoided the damage to vital organs induced by hypotension.

Our data on survival rate (68%) in 6AO group after cerebral reperfusion are compatible with the results reported by Kolata (70%, Kolata, 1979), but are remarkably higher than results reported by others using rabbits (40%, Kowada et al., 1968) or dogs (more than 40%, Jackson and Dole, 1979). We consider the survival rate might be increased in our model if artificial ventilator would be used in each animal, because our data showed that in all deaths arrest of their respiratory was prior to arrest of their heart.

The depression of EEG activity is an important feature of brain ischemia, and correlates strongly with the degree of brain ischemia. The changes of EEG did not happen until the regional cerebral blood flow (rCBF) decreased to $20 \text{ mL} \cdot 100\text{g}^{-1} \cdot \text{min}^{-1}$; and the EEG did not become

Fig. 5. Dorsal and ventral views of whole brains in rabbits injected via the ascending aorta with 1% Evans blue. A. control; B. bilateral VA occlusion; C. bilateral CCA occlusion, whole brains were well perfused. D. 6AO; slightly perfused with dye in cerebellum, basilar artery, and circle of Willis.

Table 3
LPO, SOD, and Ionic Content in Brain Tissue of Ischemic Rabbit Brain

Group	N	LPO	SOD	K	Na	Ca	Mg	Fe	Zn
A	6	225 ±40	469 ±28	94.5 ±3.3	52.1 ±2.3	1.32 ±.28	6.69 ±.24	309 ±35	214 ±11
B	7	216 ±57	431 ±55	87.8 ±5.1	52.7 ±5.9	1.22 ±.07	6.53 ±.42	300 ±11	196 ±7
C	6	227 ±38	^{a,c} 335 ±62	^{a,c} 72.1 ±15.8	^{b,d} 63.0 ±9.2	1.50 ±.42	^{b,d} 5.98 ±.57	359 ±9	^a 192 ±15

Values are $\mu\text{g/g}$ dry wt (SOD), nmol/g dry wt (LPO, Fe, Zn), or $\mu\text{mol/g}$ dry wt (K, Na, Ca, Mg), mean $\pm$ SD.

^aStatistically different from group A: $p < 0.01$.

^bStatistically different from group A: $p < 0.05$.

^cStatistically different from group B: $p < 0.01$.

^dStatistically different from group B: $p < 0.05$.

(by two-tailed Student's *t*-test and with Bonferroni modification)

Fig. 6. The correlation between water content and the activity of SOD, and ions during ischemia and reperfusion in brain tissue of rabbits. Each point came from the same sample of group A.B.C. $n = 17$ in K, Na, Mg, and SOD; $n = 11$ in Ca, and Zn.

isoelectric until rCBF decreased to $15\text{--}18\text{ mL}\cdot 100\text{g}^{-1}\cdot\text{min}^{-1}$ (Rehncrona, 1986; Sundt et al., 1974; Trojaborg and Boysen, 1973). This model demonstrated that the activities of EEG were depressed markedly in all animals with 6AO. It suggested that rCBF be decreased strongly during 6AO.

Brain edema is another important feature of brain ischemia (Klatzo et al., 1984). The changes of water content of brain also correlate strongly with rCBF. Iannotti and Hoff (1983) and Crockard et al. (1980) measured the relationship between brain edema and rCBF in their model with the gerbil. Their results showed that brain edema did not occur during zero

rCBF (e.g., "no flow no edema") and the most severe brain edema occurred with rCBF less than $10 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, and that it was not easily reabsorbed after reperfusion. Brain edema was mild during rCBF values in the range of $10\text{--}14 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, and it was easily reabsorbed after reperfusion. The water content of brain tissue was increased markedly in our 6AO model ($p < 0.01$). It also suggested that the rCBF be decreased strongly in the 6AO model.

It is not yet well understood why brain edema can sometimes deteriorate rather than ameliorate during the reperfusion of ischemic brain tissue. It has been thought that the accumulation of metabolic substances and cellular energy exhaustion might be responsible for the formation of brain edema in the ischemic period (Iannotti, 1983; Siesjo and Wieloch, 1985), but during reperfusion, free radical release might be responsible. (Braugher and Hall, 1989; Hall and Braugher, 1989; McCord, 1988; Yang, 1987). Yoshida (1980) and Yamamoto et al. (1983) reported that the metabolic product of LPO (MDA) was increased during cerebral reperfusion in gerbils or rats. Our results failed to show that the increasing of MDA at 120 min of reperfusion; however, the activity of SOD was decreased markedly, and the decrease correlated negatively with water content of brain. The decrease of activity of SOD might be due to increased SOD consumption caused by an excess of superoxide free radicals and brain tissue anoxia.

With regard to ionic homeostasis during brain reperfusion, Kumar et al. (1987) studied the changes of K, Na, Ca, Mg, and Fe levels of the brain tissue during resuscitation after 15 min of cardiac arrest, and found that Na and Ca were increased, K decreased, but no significant changes occurred in levels of Mg and Fe levels. Our results showed that the changes of Na and K were similar to Kumar's, Mg and Zn were decreased with statistically significant, and the changes were not statistically significant in Ca and Fe.

ACKNOWLEDGMENTS

We are indebted for excellent technical assistance provided by Mr. P. Zou, Mrs. S.A. Zhang, Mr. D.C. Liu, Mr. S. Tan, and Mr. Y.G. Zhou of Hunan Medical University; and for the assistance of Mr. Michael McHugh and Professor Hanna M. Pappius of Montreal Neurological Institute in preparation of the manuscript.

The project was supported by National Natural Science Foundation of China and The Public Health Department of Human Province.

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