Changes in Lipid Metabolites and Enzymes in Rat Brain Due to Ischemia and Recirculation

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ABSTRACT

Thirty and 60-min ischemic insults resulted in an increase in free fatty acid and 1,2- diacylglycerol contents of rat forebrain. No significant changes were detected in phospholipids except phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-bisphosphate during ischemic insult. Phosphatidylinositol 4-monohosphate and phosphatidylinositol 4,5-bisphosphate contents decreased during ischemia. Although the increase in free fatty acid contents continued, 1,2-diacylglycerol did not show further increase after 30-min ischemia. These results suggest that there may be another pathway for the accumulation of free fatty acids in addition to phospholipase C coupled to di- and monoacylglycerol lipase.

Free fatty acid and 1,2-diacylglycerol contents increased transiently and thereafter decreased to control levels within 90 min after postischemic recirculation. The decrease in arachidonic acid content preceded those of other FFA. Phosphatidylinositol 4-monophosphate and phosphatidylinositol 4,5-bisphosphate contents gradually increased after the initiation of recirculation in ischemic brains. Lysophosphatidylcholine decreased gradually after temporary increase during 15 and 5-min recirculations in 30 and 60-min ischemic groups. Phospholipase A, phospholipase C, and di- and monoacylglycerol lipase activities did not show significant changes during

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entire course of recirculation. Total activities of lysophospholipase and acylation enzymes of lysophospholipid demonstrated 1.5-and 2.2-fold increase during 30-min recirculation.

Index Entries: Phospholipase; lysophospholipase; diacylglycerol and monoacylglycerol lipase; acylation enzymes of lysophospholipid; recirculation after ischemia.

ABBREVIATIONS

PC: phosphatidylcholine
PE: Phosphatidylethanolamine
PS: Phosphatidylserine
PI: Phosphatidylinositol
SM: Sphingomyelin
PIP: Phosphatidylinositol 4-monophosphate
PIP_2: Phosphatidylinositol 4,5-bisphosphate
LysoPC: Lysophosphatidyl-choline
LysoPE: Lysophosphatidyleth-anolamine

PLA: Phospholipase A
LPL: Lysophospholipase
PLC: Phospholipase C
DG + MG lipase: Di and Mono-acylglycerol lipase
1,2-DG: 1,2-Diacylglycerol
FFA: Free fatty acid
GroPCho: sn-Glycero-3-phosphocholine
GroPIns: sn-Glycero-3-phosphoinositol

INTRODUCTION

Brain ischemia causes an impairment of electrical activity, depletion of energy storage (Lowry et al., 1964), and an accumulation of free fatty acids (FFA) (Bazán, 1970), lysolecithin (Sun and Foudin, 1984), and 1,2-diacylglycerol (1,2-DG) (Abe et al., 1987).

Brain has a high content of lipid, mainly comprising phospholipids as the major constituents of the membranous structure. They also provide the membrane with suitable environment for the normal function of many receptors, channels, and membrane-bound enzymes. The release of FFA, lysolecithin, and 1,2-DG during ischemia is thought to be from phospholipids, and these phenomena may cause the disruption of biomembrane. Many enzymic mechanisms may be involved in the release of lipid metabolites. It has been demonstrated that the release of FFA and lysophospholipid are brought about by the increased activity of phospholipase A_2 coupled to lysophospholipase (LPL) (Bazán, 1976; Edgar et al., 1982; Sun and Foudin, 1984); Abe et al. (1987) have speculated that degradation of phosphoinositides via phospholipase C (PLC) and diacylglycerol lipase enzymatic pathway may be responsible for the release of 1,2-DG and FFA during early ischemia. Phospholipase A_1 coupled with LPL, or plasmalogenase coupled with LPL, may also be involved in the liberation of FFA from phospholipids (Van den Bosch, 1982; Horrocks

et al., 1984). Additionally, in a previous study, we have described that inactivation of reacylation enzymes of lysophospholipid contribute to the accumulation of FFA during ischemia (Hirashima et al., 1989). However, the relative contribution of these pathways during ischemia remains uncertain.

The fates of lipid metabolites during postischemic recirculation and the contributing mechanisms are also not well known. In this study, we have investigated the changes in FFA, 1,2-DG, phospholipids, including polyphosphoinositides and lysophospholipids, during postischemic recirculation using the rat 4-vessel occlusion ischemic model (Pulsinelli and Brierley, 1979). Additionally, in order to understand the mechanism of phospholipid breakdown, we have further examined the activities of related enzymes in mitochondrial, microsomal, and cytosol fractions of rat brain during recirculation. The sum of these three subcellular fractions was defined as total activities.

MATERIALS AND METHODS

Animal Preparation

Male Wistar LWH rats (Toyama Laboratory Animals Co., Toyama, Japan) weighing about 300 g were used in these experiments. Animals were fed ad libitum until the experiment.

Cerebral ischemia was induced by the method of Pulsinelli and Brierley (1979). Briefly, animals were anesthetized with thiopental (35 mg/kg, ip) and both vertebral arteries were occluded by cauterization. Both common carotid arteries were exposed by neck skin incision and then the wound was loosely sutured. For bipolar EEG recording, two pairs of burr holes were made on the parietal bones bilaterally (2-mm lateral from bregma) and electrodes were fixed with oxyphosphate cement. The reference electrode was inserted and sutured at the prefrontal bone. The next day, carotid arteries were exposed by gently pulling out the suture and then occluded with clips. After desired ischemia period, recirculation was started by removal of clips. EEG activities were recorded on magnetic tape (SONY NFR-3000). Analysis of the power spectrum of EEG activities was processed by BERG Fourier Analyzer (EEG TREND MONITOR OEE-7102, NIHONKODEN, Japan).

Following the ischemia or recirculation, the animals were killed by decapitation and the heads were immersed in liquid nitrogen. Then the brains were chiseled out with occasional irrigation with liquid nitrogen and stored at $-80°C$ until use.

Animals were divided into three groups as follows.
Group A; 30-min ischemia
    A–0 without recirculation
    A–5 5-min recirculation
    A–15 15-min recirculation

A–30 30-min recirculation
A–90 90-min recirculation
Group B; 60-min ischemia
B–0 without recirculation
B–5 5-min recirculation
B–15 15-min recirculation
B–30 30-min recirculation
B–90 90-min recirculation
Group C; control group
C–0 decapitation without operation
C–1 decapitation after sham operation
C–2 decapitation 150 min after sham operation and experimental procedure.

Forty-two animals of Group A and 40 of group B that showed the following EEG changes were used for this study. The EEG of Group A was completely suppressed within 5 min after ischemia, and the θ wave reappeared at 30 min and α wave at 90 min after recirculation. Group B's EEG was completely suppressed within 5 min after ischemia, and only the θ wave reappeared at 90 min after recirculation.

Preparation of Lipids from Rat Brain

Right cerebral hemisphere of frozen brain was weighed and homogenized with 3 vol of chilled 0.25M sucrose on ice. The lipid extract for assay of FFA and phospholipids was obtained from the homogenate by the method of Bligh and Dyer (1959). The lipid extracts of 1,2-DG and polyphosphoinositides was obtained by the procedures of Hauser et al. (1971) and Rehncrona et al. (1982).

Analyses of Lipids

FFA were isolated from the lipid extract by TLC using *n*-hexane/diethyl ether/acetic acid (80/20/1, by vol) as a solvent system. Conversion into methyl esters was via diazomethane derivatization. The methylated fatty acids were analyzed by GLC on a 15% DEGS column (0.3 × 200 cm) at 210°C with nitrogen gas as carrier gas. The amounts of the individual FFA were determined using nonadecanoic acid as an internal standard. Total FFA was defined as the sum of amounts of palmitic acid (C 16:0), stearic acid (C 18:0), oleic acid (C 18:1), arachidonic acid (C 20:4), and docosahexaenoic acid (C 22:6). Separation of phospholipids, except polyphosphoinositides, was performed according to the method of Dugan et al. (1986), using a Gilson HPLC system model with two solvent delivery pumps (Gilson Model 302). The injection port was Cotani California Rheodyne Model. The peak detection was performed at 205 nm with a variable wave length detector (Gilson Holochrome Model HM UV Moni-

tor). Dupont Zorbax SIL column (4.6 mm id × 25 cm, 5 nm particle) was used as the HPLC column. Each phospholipid was separated and quantified by the method of Rouser et al. (1970). 1,2-DG was separated by HPTLC with a solvent system composed of chloroform/acetone/methanol (96/4/1, by vol) (Keough et al., 1972) and the acyl groups were transmethylated with anhydrous HCl/methanol. Methylated FFA were analyzed by GLC. The 1,2-DG level was calculated from the expression and summary of fatty acid content divided by a factor of 2. Phosphatidylinositol 4-monophosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP$_2$) were separated by TLC using silica gel plates impregnated with 1% potassium oxalate (Gonzalez-Sastre and Folch, 1968). The spots were detected by lipid scanning using TLC scanner (Shimazu CS-930), scraped and phosphorus contents determined by the method of Rouser et al. (1966).

Enzyme Assays

Left cerebral hemisphere of brain was homogenized with 9 vol of ice-cold 0.25M sucrose. Subcellular fractions were prepared by the method of Shum et al. (1979), and protein was determined by the method of Lowry et al. (1951). The procedures for enzyme assays were described in detail in our previous communication (Hirashima et al., 1989). These procedures included the following: (1) incubation of exogenous radioisotope-labeled substrate with aliquot of the subcellular fraction (2) separation of product and substrate in the reaction mixture by TLC or another method, and (3) the measurement of radioactivity of reaction product. 1-Stearoyl-2-[1-^{14}C]arachidonyl-Gro$P$Cho, 1-[1-^{14}C]palmitoyl-Gro$P$Cho, 1,2-diacyl-Gro$P$[U-^{14}C]Ins, 1,2-di[1-^{14}C]palmitoyl-sn-glycerol, and [1-^{14}C]arachidonic acid were used as radioactive substrates. Individual substrates were appropriately employed for the assays of phospholipase A (PLA), LPL, phospholipase C (PLC), di and monoacylglycerol lipase (DG + MG lipase), and acylation enzymes of lysophospholipid. Separation of reaction products was carried out by TLC for the assays of PLA, LPL, DG + MG lipase, and acylation enzymes of lysophospholipid. For the assay of PLC, the product was recovered in the aqueous phase of the extraction mixture, and the radioactivity was measured after evaporation. The activities of enzymes in subcellular fractions from brain prepared after recirculation in group A were examined.

Statistical Analysis

Numerical values were statistically evaluated. We employed Dunnet's test (Dunnet, 1955) to compare the data of A-0, 5, 15, 30, 90, and B-0, 5, 15, 30, 90 vs C-1, and Wilcoxin U test (Wilcoxon, 1945) to compare the data of control groups.

Materials

Silica gel plates were purchased from Schleicher and Schuell, Bassel, FRG. HPTLC aluminum sheet silica gel plates (Art. 5547) for the assays of polyphosphoinositides were purchased from Merck Co., FRG. 1-Stearoyl-2-arachidonyl-GroPCho, 1,2-dipalmitoyl-GroPCho, 1-palmitoyl-2-lysoGroPCho, 1,2-diacyl-GroPIns, arachidonic acid, and other lipids were purchased from Funai Pharmaceutical Co., Tokyo, Japan. The radioactive reagents were products of Amersham International Ltd., Amersham, UK. Other chemicals and solvents in this study were of reagent grade.

RESULTS

Changes in the Levels of FFA

As summarized in Fig. 1, the amounts of palmitic acid (C 16:0), stearic acid (C 18:0), oleic acid (C 18:1), arachidonic acid (C 20:4), and docosahexaenoic acid (C 22:6) were 255 ± 48, 361 ± 37, 191 ± 46, 228 ± 40, and 5.2 ± 1.7 nmol/g brain (mean $\pm$ SEM), respectively, in the C-1 brain ($n = 5$), and 226 ± 24, 381 ± 59, 227 ± 59, 293 ± 86, 6.8 ± 2.5 nmol/g brain, respectively ($n = 4$), in the C-2 brain. There were no significant differences with respect to the levels of total and individual FFA between C-1 and C-2 brains. In C-0 brain, however, the amounts of individual FFA were 98 ± 13, 152 ± 14, 41 ± 13, 111 ± 13, and 2.2 ± 0.4 nmol/g brain, respectively ($n = 7$). The levels of total FFA and individual FFA, except docosahexaenoic acid and arachidonic acid, in the C-0 brain were lower than C-1 or C-2 brain. The total FFA and almost all individual FFA significantly increased by operation alone.

During ischemia, the level of FFA further increased in the brain. The amount of FFA accumulated after 30 and 60-min ischemia were about 1.60 and 2.14 times higher than those of C-1 brain. The increase in individual FFA contents continued for 60 min after initiation of ischemia. Further, the accumulation of stearic and arachidonic acids was higher than other FFA.

After transient increase, the levels of FFA during the initial period of recirculation gradually decreased. The increment of total FFA continued within 15 and 5 min in groups A and B, respectively. Individual FFA levels, except arachidonic acid, increased within 15 min after recirculation in group A. The decrease of arachidonic acid preceded that of other FFA. Arachidonic acid was the only fatty acid that began to decrease within 15 min of recirculation (Fig. 1.A). Changes of FFA level of group B also showed the similar trend. The decrease of arachidonic acid initiated within 5 min after recirculation, though other FFA still increased during this period (Fig. 1.B). The total and individual FFA levels recovered control levels within 90 min of recirculation in both groups A and B.

Fig. 1. Sequential changes in levels of FFA during ischemia and after reperfusion in rat brain. A: 30-min ischemia group (group A); B: 60-min ischemia group (group B). Data were expressed as means ± SEM (n = 4-6). * and ** denoted significance at $p < 0.05$ and $p < 0.01$, respectively, compared to C-1 group. ————, with ischemia; — — — —, without ischemia; ● O, C-0 group; ● O, C-1 group; ▲, C-2 group.

Changes in the Level of 1,2-DG and Its Acyl Group Composition

The levels of 1,2-DG in C-1 and C-2 were 69 ± 11 nmol/g brain (mean ± SEM, $n = 6$) and 87 ± 30 nmol/g brain (mean ± SEM, $n = 4$), respectively. These values were higher than that of C-0, 56 ± 4.3 nmol/g brain (mean ± SEM, $n = 5$). The level of 1,2-DG revealed 1.81 and 1.83-fold increase at 30 and 60 min of ischemia compared to C-1 value. The contents of stearic and arachidonic acids were predominant among acyl groups of 1,2-DG through ischemia (Fig. 2).

After initiation of recirculation, the content of 1,2-DG continued for 15 min followed by the decrease in both groups A and B. 1,2-DG contents were almost reversed within 90 min after recirculation. As for the acyl composition of 1,2-DG in the brain during recirculation, no changes could be detected compared to that of ischemic brain (Fig. 2).

Changes in the Levels of Phospholipids and Lysophospholipids

Regarding the level of individual phospholipids except poly-phosphoinositides, statistically significant differences were not observed among three controls, C-0, C-1, and C-2. Any significant changes of phospholipids except polyphosphoinositides also could not be detected during ischemia and recirculation, though fluctuation can be observed during early period after beginning of recirculation (Table 1).

On the other hand, the levels of polyphosphoinositides were significantly different among three controls. The levels of PIP and PIP_2 in C-1 and C-2 were lower than in C-0, suggesting that the operation caused breakdown of these phosphoinositides. The levels of PIP and PIP_2 at 30 and 60 min after ischemia appeared to be lower than C-1, and the decrease of these polyphosphoinositides continued within 5 min after the initiation of blood supply except PIP_2 level in 30-min ischemic group. Subsequently, these levels increased and normalized at 90 min after recirculation (Table 1).

There was an increasing trend in lysophospholipid levels during ischemia. After the initiation of recirculation, lysophosphatidylcholine (LysoPC) was significantly increased at 15 min (A) and 5 min (B), and decreased gradually thereafter (Fig. 3.A,B). Lysophosphatidylethanolamine (LysoPE) did not show marked changes (Fig. 3.C,D) compared to ischemic brain.

Changes of Enzyme Activities

PLA, PLC, and DG + MG lipase are Ca^{2+}-dependent (Hirashima et al., 1989). These enzyme activities were measured at optimal Ca^{2+} concentration. The data were statistically evaluated by the method of Dunnet (1955). According to this evaluation, there were no significant changes during entire course of recirculation in the total activities of PLA,
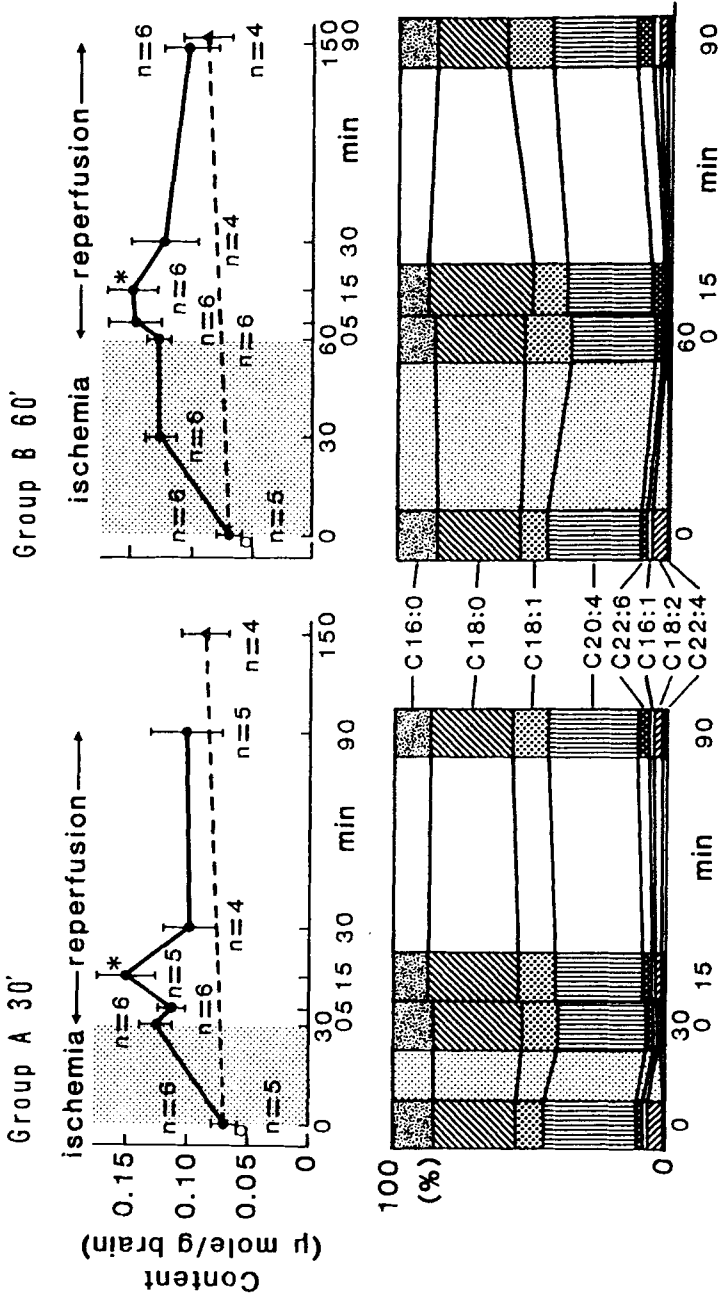
Fig. 2. Changes of amount and acyl group composition of 1,2-DG in ischemic and reperfused rat brain. A: 30-min ischemia group (group A); B: 60-min ischemia group (group B). Data were expressed as means ± SEM. * denoted significance at $p < 0.05$ compared to C-1 group. ○, C-0 group; ▲, C-2 group.

Table 1
Changes in Levels of Phospholipids Before Ischemia,
During Ischemia, and After Reperfusion in Rat Brain

		Pre-ischemia (n=4-6) Control	Ischemia (n=4-6)		Reperfusion (n=4-7)			
		C-0 / C-1 / C-2	A 30 (min)	B 60 (min)	5	15	30	90 (min) / 90 (min)
		(µ mol/g brain)						
PC	A	13.16±0.85	12.76±1.22		13.71±1.17	15.01±0.91	13.98±1.42	12.50±1.20
	B	13.40±1.40		11.13±1.27	13.85±0.62	13.21±0.72	11.31±0.92	11.77±0.62
		13.05±1.88						
PE	A	13.69±1.08	13.23±1.18		14.38±1.24	15.75±1.37	13.94±0.95	12.26±1.52
	B	14.63±1.01		11.71±0.65	14.82±1.24	12.70±0.55	11.96±0.46	10.49±0.39*
		13.24±1.77						
PS	A	3.61±0.32	3.67±0.25		3.15±0.23	3.98±0.41	3.27±0.15	2.84±0.34
	B	3.42±0.58		3.27±22.0	2.88±0.50	3.28±0.16	3.00±0.25	2.71±0.14
		3.86±0.64						
PI	A	0.59±0.06	0.70±0.10		0.55±0.10	0.88±0.13	0.76±0.12	0.78±0.09
	B	0.70±0.06		0.64±0.05	0.48±0.09	0.62±0.07	0.59±0.04	0.67±0.15
		0.67±0.09						
SM	A	1.82±0.09	1.82±0.16		2.03±0.28	1.97±0.16	1.63±0.20	1.39±0.07
	B	1.76±0.15		1.64±0.19	1.81±0.09	1.52±0.09	1.44±0.14	1.33±0.10
		1.69±0.19						
		(n mol/g brain)						
PIP	A	497.80±79.30	116.90±21.80		90.80±28.10	173.20±47.10	192.80±34.40	252.80±52.90
	B	223.50±41.70*		133.90±18.20	102.60±17.70**	152.20±36.80	147.60±37.80	191.20±14.10
		275.90±48.10*						
PIP$_2$	A	553.90±37.10	247.40±46.50		275.60±25.70	308.90±52.70	301.40±49.10	456.50±98.70
	B	368.10±76.50*		299.10±80.50	241.20±38.90	301.90±69.00	335.50±39.90	355.40±89.70
		480.30±76.90*						

*Denoted significance at $p < 0.05$ compared to C-0 value.
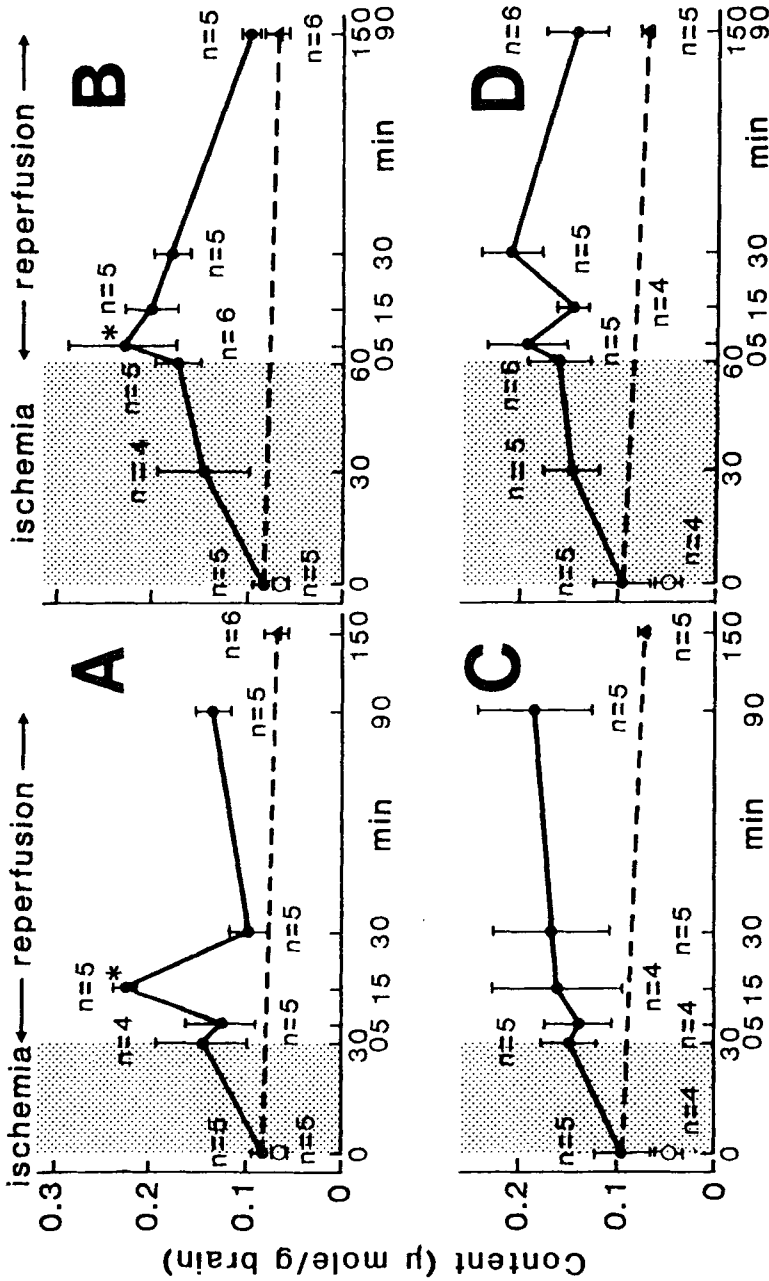**Denoted significance at $p < 0.01$ compared to C-1 value.

Fig. 3. Changes of lysophospholipid in ischemic and reperfused rat brain. A: the change of lysophosphatidylcholine in 30-min ischemia group; B: the change of lysophosphatidylcholine in 60-min ischemia group; C: the change of lysophosphatidylethanolamine in 30-min ischemia group; D: the change of lysophosphatidylethanolamine in 60-min ischemia group. Data were expressed as mean ± SEM. * denoted significance at $p < 0.05$ compared to C-1 group. ○, C-0 group; ▲, C-2 group.

LPL, PLC, and DG + MG lipase. But only acylation enzymes of lysophospholipid were activated at 30 min after recirculation (Fig. 4). Subcellular fraction study revealed changes in acylation enzymes of lysophospholipid and LPL during recirculation (data not shown). After the initiation of recirculation, the activities of these enzymes, except LPL of cytosolic fraction, increased gradually during 30 min, and decreased thereafter. The acylation enzyme activities of lysophospholipid in sub-cellular fractions increased 1.2–2.9 times at 30 min after recirculation compared to the end of ischemia (data not shown). The activities of LPL at that time-point were 1.5 and 2 times higher than 30-min ischemic brain in mitochondrial and microsomal fractions, respectively. However, the cytosolic activity did not show any changes (data not shown).

DISCUSSION

Lipid Metabolites in Control Brains

To determine the alteration in the amounts of metabolites during ischemia and recirculation, we need adequate control. We have com-pared their levels among three controls. The data of all metabolite con-tents in C-1 group agreed with C-2 group. However, there were differ-ences in FFA, 1,2-DG, and polyphosphoinositide contents between C-0 and C-1 or C-2 (Figs. 1, 2, Table 1). In spinal cord injury model, the FFA levels were increased after only laminectomy (Demediuk et al., 1985). It is possible that surgical trauma and anesthesia may cause an increase of the lipid metabolites in both spinal cord and brain, probably because of changes in neurotransmitters (Knepel and Meyen, 1986; Gecse et al., 1987). In this study, group C-1 was adopted as the control group in all comparisons.

Lipid Metabolites and Enzyme Activities
During Ischemia

Ischemia caused an increase in FFA and 1,2-DG levels and a decrease in polyphosphoinositides (Figs. 1, 2, Table 1). The composition of FFA and 1,2-DG liberated during ischemia were quite similar (Figs. 1, 2). The inositol-containing phospholipids has been reported to be rich in stearic and arachidonic acids (Abe et al., 1987). Our results agree with this. The action of PLC and diacylglycerol lipase on polyphosphoinositides may be important for the liberation of FFA (Abe et al., 1987). However, there were no differences in the contents of 1,2-DG and polyphosphoinositides between 30 and 60-min in ischemic brains (Fig. 2, Table 1). This means that the degradation of polyphosphoinositides may be small at the late period of ischemia. On the other hand, the liberation of FFA increased 1.5-fold between 30 and 60 min of ischemia. Judging from the above results, it seems that there may be another pathway for the liberation of
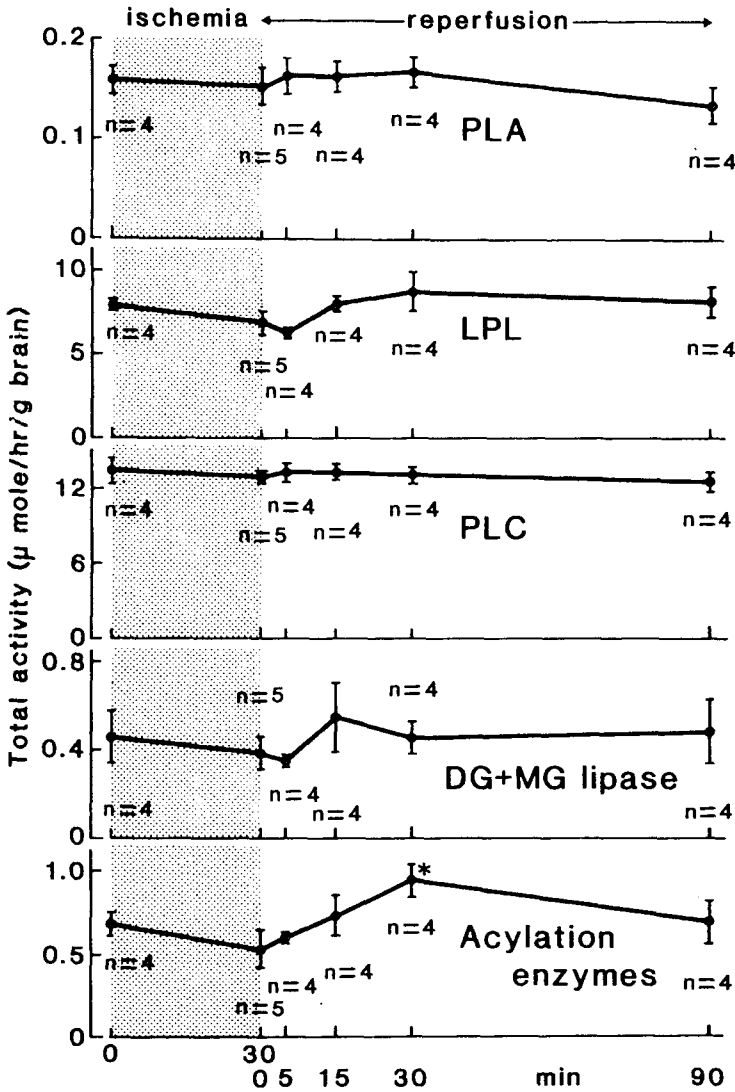
Fig. 4. Changes in total activities of individual enzymes during reperfusion in rat brain. Data were expressed as means ± SEM. * denoted significance at $p < 0.05$ compared to C-1 group.

FFA in addition to PLC coupled with DG + MG lipase, at least during late period of ischemia. No significant changes in the levels of individual phospholipids, except polyphosphoinositides, could be detected during ischemia (Table 1). In our previous report, the changes of FFA and phospholipid were measured in decapitated brain (Hirashima et al. 1989). These lipid changes agreed closely with the changes obtained from this 4-vessel occlusion model.

For the FFA pool in brain, the alteration of acylation activity also may be important. In a previous article, we reported that inactivation of acylation enzymes of lysophospholipid during ischemia and indicated that both ATP exhaustion and inactivation of these enzymes may be involved in the accumulation of FFA and lysophospholipids during ischemia (Hirashima et al., 1989). In the present study, we could not find a statistically significant increase in lysophospholipids during ischemia. However, there was a trend toward increased lysophospholipid content.

Lipid Metabolites and Enzyme Activities During Recirculation

Total FFA levels increased transiently during early period of recirculation, and thereafter decreased toward control values within 90 min. Interestingly, the decrease of arachidonic acid preceded those of other FFA (Fig. 1). Arachidonic acid has been known to be peroxidized enzymically during postischemic recirculation to produce prostaglandins and leukotrienes (Gaudet and Levine, 1979; Morskowitz et al., 1984). Polyunsaturated FFA, such as arachidonic acid, may also be peroxidized nonenzymically (Mead, 1976). It is possible that these pathways may be responsible for the decrease of arachidonic acid during recirculation. Recently, Yoshida et al. (1986) proposed the role of triacylglycerol, which can trap the excess of free arachidonic acid by acyl-exchange reaction. Rehncrona et al. (1982) also measured FFA and phospholipids during postischemic recirculation. However, they did not detect the increase of FFA during initial recirculation period, but demonstrated the same tendency in arachidonic acid during recirculation.

In our earlier study, the activity of acylation enzymes of lysophospholipid decreased during ischemia (Hirashima et al. 1989). This activity comprises the reactions catalyzed by two different enzymes, acyl-CoA synthetase, which is ATP-dependent, and AcylCoA:lysophospholipid acyltransferase. These enzymes may also play an important role in the decrease of FFA during postischemic recirculation. After the recirculation, the total activity of acylation enzymes of lysophospholipid gradually increase within 30 min. From the above result, it is possible that recovery of acylation enzymes themselves, as well as that of ATP, may contribute to the elimination of FFA during recirculation. LysoPC has been shown to inhibit the action of acylCoA:lysophospholipid acyltransferase by direct interaction (Wittels and Hurlbert, 1977; Sun et al., 1978; Weltzien, 1979). In the previous communication, we have speculated that the increase of lysocompounds may inhibit acylation enzymes during ischemia (Hirashima et al. 1989). In this study, total and some subcelullar lysophospholipase activities have been shown to increase during recirculation compared to the activity at the end of ischemia (data not shown). Although LysoPC level increased temporarily at initial recirculation period, thereafter, it decreased gradually. These

results suggest a possible mechanism recovery of acylation enzymes during recirculation, namely, the decrease of LysoPC probably as a result of activation of LPL during recirculation may release suppressed activity of acylation enzyme.

Recirculation after both 30 and 60 min of ischemia reversed the levels of 1,2-DG and polyphosphoinositides toward control levels within 90 min (Fig. 2, Table 1). 1,2-DG level in the brain declined rapidly after 15-min recirculation in both ischemic groups, whereas the polyphospho-inositide levels increased gradually during the entire course of recircula-tion (Fig. 2, Table 1). Yoshida et al. (1986) have demonstrated the trend toward increased phosphatidic acid during initial recirculation after pro-longed ischemia. Therefore, it is possible that the decrease of 1,2-DG may be related to the activation of DG kinase accompanied by the restoration of ATP. They have also indicated that not only kinases toward inositol-containing phospholipids, but also *de novo* synthesis may contribute to syntheses of polyphosphoinositides during postischemic recirculation (Yoshida et al., 1986). In this study, however, we could not obtain evidence that the *de novo* synthesis was via phosphatidic acid and CDP-diacylglycerol. The pathway catalyzed by choline or ethanolamine phosphotransferase should also be considered for the elimination of 1,2-DG during recirculation. Therefore, these enzymes may catalyze the reactions between CDP-amines and 1,2-DG (DeMedio et al., 1980) and decrease the contents of 1,2-DG that were accumulated during ischemia.

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