Neurochemical Research, Vol. 25, No. 2, 2000, pp. 269–276

Cortical Impact Injury in Rats Promotes a Rapid and Sustained Increase in Polyunsaturated Free Fatty Acids and Diacylglycerols

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(Accepted September 9, 1999)

Neurotrauma activates the release of membrane phospholipid-derived second messengers, such as free arachidonic acid (20:4n-6, AA) and diacylglycerols (DAGs). In the present study, we analyze the effect of cortical impact injury of low-grade severity applied to the rat frontal right sensory-motor cortex (FRC) on the accumulation of free fatty acids (FFAs) and DAGs in eight brain areas 30 min and 24 hours after the insult. At these times, accumulation of FFAs and DAGs occurred mainly in the damaged FRC. The cerebellum was the only other brain area that displayed a significant accumulation of DAGs by day one post-injury. By 30 min, accumulation of free AA in the FRC displayed the greatest relative increase (300% over sham value), followed by free docosahexaenoic acid (22:6n-3, DHA, 150%), while both 20:4-DAGs and 22:6-DAGs were increased 100% over sham values. At day one, free 22:6 and 22:6-DAGs showed the greatest increase (590% and 230%, respectively). These results suggest that TBI elicits the hydrolysis of phospholipids enriched in excitable membranes, targeting early on 20:4-phospholipids (by 30 min post- trauma) and followed 24 hours later by preferential hydrolysis of DHA-phospholipids. These lipid metabolic changes may contribute to the initiation and maturation of neuronal and fiber track degeneration observed following cortical impact injury.

KEY WORDS: Arachidonic acid; diacylglycerols; docosahexaenoic acid; free fatty acids; phospholipase A_2; phospholipase C; traumatic brain injury.

INTRODUCTION

Traumatic brain injury (TBI) activates injury cascades that initially include massive accumulation of extracellular potassium and release of neurotransmitters (e.g. glutamate) (1–6), followed by glutamate-mediated neuronal calcium overload through post-synaptic N-methyl-D-aspartate (NMDA) receptor-gated calcium channel activation (7,8). As a consequence, calcium-dependent enzymes (e.g. phospholipases, proteases and endonucleases) are activated and, in turn, participate in axonal and neuronal degeneration and cell death (9–12). Phospholipases are of particular interest because they generate several potent second messengers as they hydrolyze membrane phospholipids. Activation of phospholipase A_2 (PLA_2) and phospholipase C (PLC) releases free fatty acids (FFAs), particularly arachidonic acid (20:4n-6, AA) and diacylglycerols (DAGs), as an early response to brain insults (13,14), including TBI (15–19).

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0364-3190/00/0200-0269$18.00/0

We have developed a rat model of mild impact injury to the right sensory-motor cortex with minimal cardiorespiratory effects, low mortality and lasting behavioral effects (20). This technique minimizes changes in lipid metabolism generated by ischemia, hypoxia or hypotension that may overlap those lipid degradative pathways activated by cortical injury. The model employs a controlled pneumatic piston, which depresses the intact dura by one mm through craniectomy, thus damaging the whole fight sensorimotor cortex without tearing the dura. Neurologic/behavioral and histological correlates have been established (20–22). Neuronal damage throughout both hemispheres and fiber track degeneration associated with the right sensory motor system and within the cerebellum are consistently observed within 8 weeks after the focal insult (22). The hippocampus, however, which is highly sensitive to ischemia, is only minimally affected. One interesting observation is that this TBI model triggers a sustained increase in both FFAs and in DAGs that are highly enriched in AA and docosahexaaenoic acid (22:6n-3, DHA) by 4 and 35 days after the insult. These increases occur in cortical areas ipsilateral and contralateral to the damaged FRC and in the cerebellum, with no detectable changes in hippocampus (23). By 4 days following traumatic insult, DHA accumulates to similar or higher degrees than AA both in the FFA and in DAG pools. This is in contrast to the preferential accumulation of AA and stearic acid (18:0) observed in the brain following ischemia (24–27), seizures (28–31) and TBI (17,18).

In the present study, we focus on changes in brain FFAs and DAGs induced by TBI by 30 min and 24 hrs after the insult. We demonstrate that within 24 hrs after the insult, FFAs and DAGs accumulate in the damaged FRC with minimal changes in other cortical areas and in hippocampus. The profile of acyl group changes also suggests that the hydrolysis of DHA-phospholipids, which leads to the free DHA and DHA-DAG accumulation, peaks by 24 hr following the injury, while hydrolysis of AA-phospholipids prevails within the first 30 min after the insult. TBI-induced activation of PLA_2 and PLC may contribute to the simultaneous and/or sequential degradation of different polyunsaturated fatty acid (PUFA)-containing phospholipids highly enriched in synaptic terminals. This, in turn, may underlie alterations in excitable membranes affecting the functional activity of proteins (e.g. receptors, enzymes) and of other signaling pathways involved in synaptic activity.

EXPERIMENTAL PROCEDURE

Cortical Impact Brain Injury. Experimental protocols were approved by the institutional review committee and meet NIH guidelines. Sprague Dawley rats (300–400 g body weight) were kept under 12 hour light/12 hour dark conditions for two weeks prior to the experiment. Animals, anesthetized with isoflurane, were placed in a stereotaxic instrument and a 5 × 9 mm craniectomy was performed on the right side of the skull under sterile conditions. The craniectomy extended from 1 to 6 mm lateral of midline and 4.5 mm anterior and posterior to the bregma. While still in the stereotaxic frame, the rat was moved under a computer interfaced device fitted with a pneumatic piston (impact tip: 4 mm × 8 mm) with a mean impact velocity of 5.2 m/sec. This impact causes a 1 mm dural depression over the right sensorimotor cortex, which results in a left hemiparesis lasting at least 28 days (20). Control (sham) rats were subjected to similar craniectomy but were not injured. Thirty min and one day after the insult, rats were sacrificed by head-focused microwave irradiation. Brains were immediately removed and eight different regions were dissected on a frozen plate: frontal right cortex (FRC), frontal left cortex (FLC), occipital right cortex (ORC), occipital left cortex (OLC), right hippocampus (RH), left hippocampus (LH), cerebellum (CER) and brain stem (BS).

Lipid Extraction and Analysis. Lipids were extracted from each brain area and total phosphorus measured (32). FFAs and DAGs were separated by monodimentional thin-layer chromatography using silica gel GHL plates (Analtech, Newark, DE) and developed in hexane:ether:acetic acid (40/60/1.3, by vol). The plates were sprayed with 2′ 7′ dichlorofluorescein (0.005% in ethanol) and FFAs and DAGs spots visualized under UV light. Lipid bands were scraped off the plates, derivatized to their fatty acid methyl esters and analyzed by gas-liquid chromatography (GLC, 32). All lipid standards were obtained from Cayman Chemical (Ann Arbor, MI). The solvents (HPLC grade) and all other chemicals were from Fisher Scientific Products (Pittsburgh, PA).

Statistical analysis. Values (nmole/mg lipid phosphorus) are presented as mean ± SEM from 9–10 individual determinations. Statistical analysis was performed using the unpaired Student's *t*-test. Differences were considered significant when $p < 0.05$.

RESULTS

We measured the accumulation of FFAs and DAGs in eight different areas of the brain at 30 min and 24 hrs after cortical injury applied to the frontal right cortex (FRC) (Table I and II). Sham animals that were cranioctomized but did not receive the traumatic insult displayed changes in the levels of FFAs and DAGs between 30 min and 24 hrs after surgery in several brain areas. Higher levels of FFAs were only detected in the right and left hippocampus (RH, LH) in 24 hrs sham animals as compared to 30 min sham (Table I). The increase was significant ($p < 0.01$) in the LH due to high levels of free 16:0 and 18:0 (+50% and +80%, respectively) (data not shown) but not significant (NS) in the RH ($p < 0.06$). Twenty four hr sham

Table I. Free Fatty Acids Content in Different Rat Brain Areas after Cortical Injury

	30 min		24 hours	
AREA	SHAM	TRAUMA	SHAM	TRAUMA
	nMol/mg Lipid Phosphorus			
FRC	42.8 ± 5.1	71.4 ± 9.0*	35.3 ± 2.6	134.0 ± 38.0*
FLC	36.1 ± 2.1	38.5 ± 2.3	28.9 ± 3.3	30.2 ± 2.5
ORC	45.3 ± 3.7	62.1 ± 9.1	48.4 ± 5.4	63.3 ± 7.8
OLC	36.2 ± 2.3	36.3 ± 2.7	36.0 ± 3.1	38.9 ± 2.9
RH	46.8 ± 4.2	45.9 ± 4.3	61.7 ± 5.6	65.3 ± 5.8
LH	39.5 ± 2.4	37.7 ± 3.1	63.7 ± 6.7**	54.7 ± 6.1
CER	33.4 ± 1.4	33.5 ± 1.4	37.7 ± 3.6	34.6 ± 3.5
BS	38.5 ± 0.9	36.0 ± 1.7	37.2 ± 3.0	41.9 ± 4.5

Data are presented as mean ± SEM from 9–10 individual determinations. FFAs were quantified by GLC in different brain areas at 30 min and 24 hrs after fight cortical injury. FRC, frontal right cortex (injured area); FLC, frontal left cortex; ORC, occipital right cortex; OLC, occipital left cortex; RH, right hippocampus; LH, left hippocampus; CER, cerebellum; BS, brain stem. Asterisk denotes significant differences between TBI and sham values (*) and between 24 hour sham vs 30 min sham values (**) (Student's *t* test, $p < 0.05$).

animals displayed higher levels of DAGs in several brain areas as compared to 30 min sham values. This included the FRC (+40%, $p < 0.05$), ORC (+40%, NS $p < 0.08$), RH (+100%, $p < 0.03$), LH (+40%, NS $p < 0.3$) and BS (+60%, $p < 0.03$). As observed for FFAs in hippocampus of 24 hrs sham animals, only saturated fatty acids (16:0 and 18:0) contributed to the DAGs pool increase: 18:0-DAG in the FRC (+80%, $p < 0.01$), ORC (+60%, $p < 0.005$), RH (150%, $p < 0.03$), LH (+160%, $p < 0.02$) and 16:0-DAGs in the RH (+90%, $p < 0.002$) and BS (+80%, $p < 0.05$). Interestingly, in the FLC contralateral to where the craniotomy was performed (FRC), total DAGs were decreased by 20% ($p < 0.02$) in 24 hrs sham animals (Table II), due to lower content of 18:1n-9 (–40%, $p < 0.02$) and 20:4n-6 (–30%, $p < 0.002$). A similar decrease was also observed in 18:1- and 20:4-DAGs in the LH (data not shown). These changes indicate the high sensitivity of neural tissue to surgical manipulations, which differs from that induced by the traumatic insult as detailed below.

Within the first 24 hrs after TBI, activation of lipolytic pathways was circumscribed to the site of injury (FRC), with no significant increase in FFAs or

Table II. Diacylglycerol Levels in Different Brain Areas after Cortical Injury

	30 min		24 hours	
AREA	SHAM	TRAUMA	SHAM	TRAUMA
	nMol/mg Lipid Phosphorus			
FRC	51.5 ± 3.9	91.4 ± 6.3*	70.1 ± 6.4**	110.0 ± 12.7*
FLC	69.8 ± 8.0	69.7 ± 6.9	52.8 ± 8.1**	70.1 ± 9.3
ORC	57.2 ± 4.4	58.3 ± 2.5	79.9 ± 8.3**	93.1 ± 14.2
OLC	52.7 ± 6.8	46.5 ± 2.5	64.0 ± 6.5	57.9 ± 4.5
RH	48.0 ± 7.6	51.1 ± 4.2	95.0 ± 13.0**	84.2 ± 8.0
LH	69.2 ± 4.8	51.6 ± 1.6	93.8 ± 17.5	88.3 ± 10.0
CER	40.6 ± 2.7	40.8 ± 4.4	42.9 ± 2.9	73.7 ± 10.4*
BS	24.9 ± 2.0	28.6 ± 2.7	38.8 ± 4.5**	39.7 ± 5.7

Data are presented as mean ± SEM. DAGs were quantified by GLC in different brain areas at30 min and 24 hours after TBI. Asterisks denote significant differences between TBI and sham values (*) and between 24 hour sham vs 30 min sham values (**) (Student's *t* test, $p < 0.05$). Other details as in Table I legend.

DAGs observed in other areas of the brain other than DAGs in the cerebellum one day after the insult. Thirty min after the cortical injury, total FFAs were increased in the FRC by 70% over sham values, reaching by a 280% increase by 24 hr (Table I). From the 10 animals analyzed at 24 hr, 60% showed a moderate increase of FFAs (trauma: 63 ± 9 vs sham value: 35 ± 3 nmol/mg lipid phosphorus), similar to the FFA levels attained by 30 min after TBI (71 ± 9). Four of the 10 animals reached very high levels of FFAs in the FRC (240 ± 66) with no significant increase observed in the adjacent ORC. This indicates differences in the magnitude of the animals' response to the same traumatic insult.

DAGs were increased in the FRC by 80% and 60% at 30 min and 24 hrs, respectively, and by 70% in the cerebellum 24 hrs after TBI (Table II). Despite differences in the levels of FFAs attained by 24 hrs in the FRC, DAGs displayed similar values for both moderate and high FFA accumulation (97 ± 14 and 132 ± 13 nmole DAGs acyl groups / mg lipid phosphorus, respectively). This suggests the involvement of independent pathways in the FFA and DAG generation.

Changes in individual FFA and DAG-acyl groups in the FRC 30 min after TBI are shown in Fig. 1. In the FFA pool, 18:0 showed the highest accumulation (experimental minus sham value = 11.6 nmol/mg lipid P), followed by 18:l (5.7 nmol), 20:4n-6 (5.5 nmol) and 22:6n-3 (2.2 nmol). The highest relative increase was observed for 20:4n-6 (+300%), followed by 22:6n-3 (+160%). Stearic acid and oleic acid (18:l) showed a 80% increase while palmitic acid (16:0) remained unchanged. All DAG-acyl groups, including 20:4n-6 and 22:6n-3, were significantly increased above sham values (1.8-2-fold); 16:0-DAGs displayed the lowest increase (1.4-fold). The highest contribution to the DAG pool accumulation was given by 18:1 (13 nmol), followed by 18:0 (10 nmole) and 20:4n-6 (7.5 nmol).

By 24 hrs after TBI, all FFAs in the FRC showed a tendency to higher values; however, because of the large variation among the individual rats, differences were significant only for 22:6n-3 and 18:0 (600% and 300% increase above sham values, respectively) (Fig. 2). No changes were detected in individual FFAs in other brain areas except for a 100% increase of 22:6n-3 in the ORC (sham:l.2 ± 0.1; trauma: 2.6 ± 0.8, $p < 0.03$). Accumulation of DAGs in the FRC by 24 hrs showed that 18:1-DAG (10 nmol/mg lipid P) contributed the most to this accumulation, as observed by 30 min. The accumulation of 16:0-DAG was similar to 18:1-DAG, while 22:6n-3 increase (9 nmol/mg lipid P) was greater than that of 20:4 (7.4 nmol/mg lipid P). DHA-DAGs displayed the highest relative increase (+230%), while for 20:4n-6 the value was +70%. In the

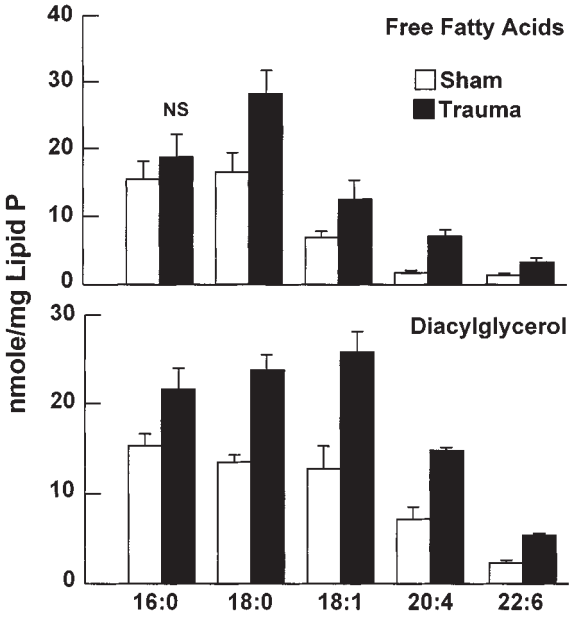

Fig. 1. FFAs and DAGs levels in the damaged frontal right sensorymotor cortex 30 min. after cortical impact injury. Mean ± SEM values are shown. All trauma values were significantly different from sham values except for free 16:0 (NS). Other details as in Table I legend.

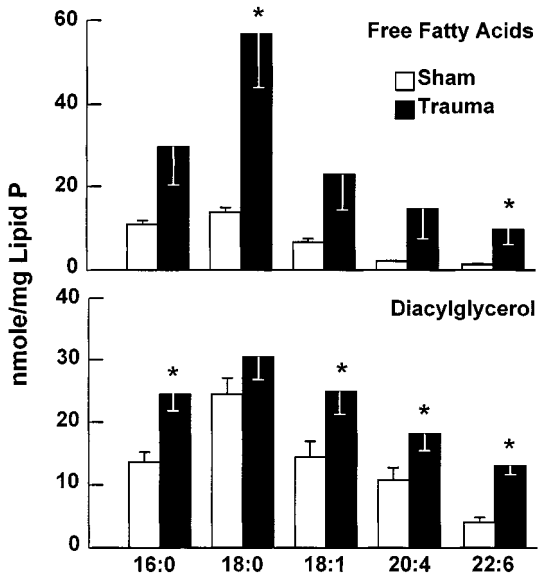

Fig. 2. FFAs and DAGs levels in the damaged frontal right sensorymotor cortex 24 hours after cortical impact injury. Mean ± SEM values are shown. Asterisk (*) denotes values significantly different from sham values (Student's *t* test, $p < 0.05$).

cerebellum, no individual FFA was increased 24 hrs after TBI (Fig. 3). In contrast, DAG-acyl groups showed a well-defined tendency to higher values, with a significant 100% increase in 18:1-DAGs ($p < 0.03$).

DISCUSSION

This study demonstrates that TBI stimulates phospholipase-mediated degradation of membrane 20:4- and 22:6-phospholipids, leading to an early accumulation of FFAs and DAGs enriched in PUFA. After 30 min and 24 hrs (**a**) FFAs and DAGs were increased primarily in the damage FRC; (**b**) free 20:4n-6 reached the highest value 30 min after TBI, while 22:6n-3 peaked by 24 hours; (**c**) 20:4-DAGs were similarly increased at 30 min and 24 hours; however, 22:6-DAGs displayed higher accumulation than 20:4-DAG by 24 hours. The overall profiles of FFAs and DAGs changes suggest that phospholipases activated by TBI hydrolyzed primarily 20:4-phospholipids within minutes of the insult, followed by a preferential hydrolysis of 22:6-phospholipids one day after TBI. These lipid changes that started in the damaged FRC later (4–35 days) affect the frontal and occipital cortices ipsi- and contralateral to the area of damage, including the brain stem (23).

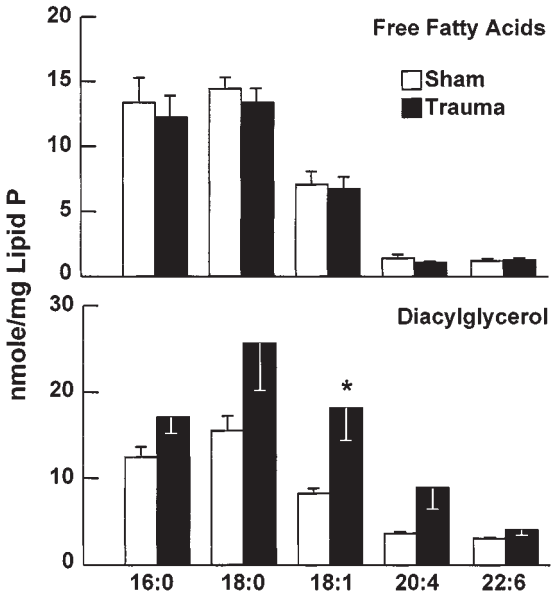

Fig. 3. FFAs and DAGs levels in the cerebellum 24 hours after controlled cortical impact injury applied to the frontal right sensorymotor cortex. Mean ± SEM values are shown. No changes were detected in individual FFAs. Asterisk (*) denotes value significantly different from sham values (Student's *t* test, $p < 0.05$).

In the present study, mild TBI applied to the right sensorimotor-cortex and head-focused microwave irradiation for tissue fixation (which immediately stops all enzymatic reactions) revealed a significant increase in FFAs and DAGs 30 min and 24 hours after TBI. Sham animals also displayed by 24 hours changes in these lipid pools with respect to 30 min values. In contrast to the increased levels of FFAs and DAGs induced by TBI, which involved unsaturated (18:1), polyunsaturated (20:4n-6, and 22:6n-3) and saturated (16:0 and 18:0) fatty acids, surgical trauma only increased free 16:0 and 18:0 in the hippocampus and 18:0- and 16:0-DAGs in hippocampus and other brain areas such as FRC, ORC and BS. After TBI, accumulation of FFAs and DAGs occurred mainly in the area of injury (frontal right cortex). Only the cerebellum showed increased levels of DAGs 24 hrs after TBI. Interestingly, the hippocampus, which is highly sensitive to ischemic damage, was not affected by the traumatic insult but was sensitive to surgical trauma. In fact, the levels attained by sham animals in RH and LH were not significantly different from injured animals (Tables I and II). This suggests that the hippocampus is highly sensitive to surgical trauma, yet remains unaffected by cortical impact injury, coinciding with histological examinations of the brain which consistently reveal time-dependent fiber track degeneration triggered by traumatic insult. Starting in the area of injury, the degeneration gradually progresses, reaching the corpus callosum and projecting into the striatum, thalamus and cerebellum and, finally, into the substantia nigra and pyramids (20,22). Despite this massive degeneration of fiber tracks associated with the right sensorimotor system, the hippocampus is spared histological damage. Our results differ from those of Dhillon et al. (17). Using a similar model of traumatic injury, they found an increase in FFAs in the injured area and also in the hippocampal region ipsilateral to the injury within 24 hours of the insult.

In the present study, we have also shown that TBI induces an early degradation of 20:4- and 22:6-phospholipids in the damaged FRC, with different profiles as a function of time, post trauma. In fact, by 30 min, free 20:4 and 20:4-DAGs displayed the greatest changes. Early activation of the phospholipases A_2 (15) and C, which are involved in polyphosphoinositide hydrolysis (18), contributes to the release of these bioactive lipids. A different profile of acyl groups was observed by 24 hrs. At this time, free 22:6 and 22:6-DAGs reached values even higher than those previously reported for later times (4–35 days post-trauma) (23). After mild and moderate fluid percussion brain injury, accumulation of free 22:6 in the injured cortex 72 hours after the

traumatic insult but not at earlier times has been recently reported (19). Also, it is noteworthy that the high accumulation of 18:1-DAG displayed the greatest contribution to the enlargement of the DAG pool both after 30 min and after 24 hrs. The amount of 18:1-DAGs was increased not only in the FRC, but also in the cerebellum where it was the only acyl group showing a significant increase by 24 hrs. This profile differs from that generated by brain ischemia or seizures, where accumulation of free 20:4 and 20:4-DAGs prevails (25–28). Mobilization of 22:6n-3 and 18:1 post-trauma is similar to the delayed second increase of FFAs reported in the gerbil brain after 5 min of ischemia followed by three days of reperfusion (33). In this case, accumulation of 18:1 and 22:6n-3 exceeds that of 20:4n-6 which prevails during the ischemic insult.

The lipid sources and lypolitic pathways activated by trauma and the cellular and/or subcellular level at which they occur are not defined at present. Activation of triacylglycerol (TAG) lipases leading to the enlargement of the FFA and DAG pools cannot be ruled out. However, during ischemia, the release of free 20:4n-6, 22:6n-3, and DAGs containing 20:4n-6 and 22:6n-3 does not originate from TAGs (25,26). Also, polyunsaturated molecular species of PC and PE containing 20:4n-6 and 22:6n-3 are actively degraded during long periods of ischemia, thus contributing to the accumulation of free PUFA (34). It is interesting to note that high accumulations of free 22:6 and 22:6-DAGs occur after cryogenic brain injury (35), another injury model in which the vasculature is greatly compromised and edema develops. This is also the case after traumatic insult (8,17,22). In this context, it is relevant that 22:6-containing molecular species of phospholipids are highly abundant in synaptic membranes (36,37) and also in neural capillary endothelial cells (38–40). Moreover, 22:6-phospholipids (e.g. phosphatidylserine) are essential to support optimal neural function (41). Thus, the activation of phospholipases after trauma may not only contribute to the release of second messengers that are modulators of neuronal activity and/or inflammatory mediators such as DAGs, 20:4, eicosanoids and PAF (42,43), but it may also alter membrane structure and function.

Differences in the time-course of FFA and DAG accumulation, as well as in their acyl group composition, strongly suggest that different degradative pathways contributed to their release immediately after injury and at later times (up to 35 days) in the damaged area and in other areas of the brain. Phospholipids enriched in PUFA and a high activity of PLA_2, PLC and PLD are characteristics of synaptic terminals (44–47). Among the different pathways that can contribute to FFA accumulation when activated by TBI are: **a)** PLA_2-mediated release of fatty acids from the *sn*-2 position of membrane phospholipids. Low molecular weight (~14 kDa) secretory PLA_2 ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$), both Ca^{2+}-dependent (~85 kDa) and Ca^{2+}-independent, could be activated by TBI and contribute to neurodegeneration (11,12). Furthermore, it has been shown that $sPLA_2$ potentiates glutamate excitotoxicity (48). Increased $sPLA_2$ and $cPLA_2$ expression after brain ischemia has been reported (49,50). In $cPLA_2$ deficient mice the involvement of this signaling pathway is implicated in the pathophysiology of ischemia-induced neuronal death (51). **b)** PLA_1-mediated release of fatty acids from the *sn*-1 position of membrane phospholipids, followed by the release of 20:4 by a lysophospholipase. These enzymes, present in the brain (52,53), may be a significant signaling pathway contributing to 20:4 release from PC (54). **c)** sequential degradation of DAGs and monoacylglycerols (MAGs) by DAG- and MAG-lipases (14). Accumulation of DAGs may involve: **a)** activation of PLC-mediated degradation of membrane phospholipids (e.g. PI, PC). PIP_2 hydrolysis after fluid percussion injury (18), seizures and ischemia (14) occurs in parallel with 20;4-DAGs and free AA accumulation in the brain. At early times, PLC-mediated degradation of PIP_2 appears to be the main contributor to both DAG and FFA release. **b)** activation of PLD pathway, which generates phosphatidic acid (PA) from PC and/or PE followed by its dephosphorylation by phosphatidate phosphohydrolase (PAP) with results in the conversion of PA→DAG (47,55). The early release of DAGs through the calcium-activated PLC after traumatic insult may lead to the activation of protein kinase C (PKC) which, in turn, activates the PLD pathway (47) leading to a sustained enlargement of the DAG pool. The fatty acid composition of DAGs accumulating in the brain after TBI, mainly their high content of 18:1 and 22:6, minor components of inositol lipids (18), strongly supports the notion that other signaling pathways, such as the PLD-pathway, may contribute to the enlargement of the DAG pool.

It is interesting to note that the increased levels of FFA in the brain after TBI and other insults, such as ischemia/reperfusion and seizures, depend upon both the rate of release from membrane phospholipids and the rate of re-esterification and clearance through the blood brain barrier and/or cerebral spinal fluid. Re-esterification of FFAs into membrane lysophospholipids, generated by trauma-activated PLA_2, involves

their activation to acyl-CoA. Acyl-CoA synthetases, which display high affinity for 20:4 n-6 and 22-6n-3, are present in the brain (56,57). Recent studies done in a gerbil model revealed an increased rate of lysophospholipids reacylation during reperfusion of the brain after an ischemic insult with 20:4n-6 and 18:0, displaying a preferential activation and incorporation into phospholipids (58,59). This preferential re-esterification of 20:4n-6 will favor its faster removal from the FFA pool. Thus, sustained release of fatty acids after the traumatic insult, coupled to a slower re-esterification of 22:6n-3 and 18:1 as compared to 20:4n-6, may contribute to the changes in the FFA profile observed between 30 min and 24 hrs after TBI.

In summary, this study shows that TBI induces the activation of signal transduction pathways, which results in the early release of bioactive lipids (free 20:4 and 20:4-DAGs) in the damaged area. The PLA_2-mediated signaling pathway controls arachidonate release and eicosanoid and PAF synthesis, potent metabolites involved in inflammatory responses (42) as well as in the pathophysiology of neurotrauma (14). This early hydrolysis of AA-phospholipids was followed by a preferential hydrolysis of 22:6-phospholipids that begins in the injured cortex and reaches other cortical areas and brain stem after 4–35 days (23).

ACKNOWLEDGMENT

This study was supported by U.S. Army Medical Research and Command contract DAMD-17-93-C-3008. Opinions expressed are the authors' and are not to be construed as an official Department of the Army position.

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