Increased phospholipase C activity after experimental brain injury

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Phospholipase C activity was measured in 1000 x G centrifuged cellular fractions isolated from cerebral cortical homogenates obtained from either control cats or cats subjected to experimental fluid-percussion brain injury. Phospholipase C activity was determined directly by measuring the Ca++-dependent conversion of membrane-bound, labeled phosphatidate to diacylglycerol or indirectly by measuring the diacylglycerol-dependent (brain diacylglycerol content) formation of phosphatidylcholine in the presence of labeled cytidine diphosphate (CDP) choline. Phospholipase C activity determined by either method was about two times greater in cell fractions isolated from animals subjected to brain injury than in controls (p < 0.01). The brain injury-induced rise in phospholipase C activity may be responsible, at least in part, for generating diacylglycerol that may be a source of free arachidonic acid that stimulates prostaglandin synthesis. These changes may account for the rise in brain prostaglandin levels that has been demonstrated earlier to occur after this type of brain injury.

KEY WORDS • prostaglandin • phospholipase • brain injury • vasodilation • cerebral arterioles • cerebral blood flow

EXPERIMENTAL fluid-percussion brain injury in cats causes cerebral arteriolar damage characterized by discrete destructive lesions in the endothelium and vascular smooth muscle of these vessels. These morphological lesions are associated with sustained vasodilation, reduced responsiveness to vasoconstrictor influences, and reduced oxygen consumption of the vessel wall. These abnormalities are either minimized or prevented by pretreatment with cyclo-oxygenase inhibitors or with free oxygen radical scavengers, suggesting that the immediate cause of these cerebral vascular abnormalities is the generation of free radicals in association with increased prostaglandin synthesis. An increase in prostaglandin concentration in brain occurs a few minutes after injury. In addition, the cerebral vascular abnormalities seen after this type of brain injury can be reproduced by topical application of arachidonate or of the cyclic endoperoxide G2 on the brain surface.

Since the concentration of free arachidonate, the precursor of prostaglandins, is generally quite low in tissues, it appeared likely that the increased prostaglandin synthesis following brain injury would be initiated by activation of phospholipases. For this reason, we investigated the activity of phospholipase C in brain tissue following brain injury.

Materials and Methods

Experiments were carried out in 14 cats weighing 2 to 3.5 kg. The animals were anesthetized with intravenous pentobarbital (30 mg/kg) and subjected to skeletal muscle paralysis with intravenous decamethonium bromide (0.5 mg/kg). The cats were ventilated passively with a positive-pressure respirator* attached to a tracheostomy tube. The end-tidal pCO2 was monitored continuously with a CO2 analyzer† and maintained constant throughout the experiment at approximately 30 mm Hg. Arterial blood pressure was monitored with a Statham strain gauge‡ connected to a catheter introduced into the aorta via the femoral

* Positive-pressure respirator manufactured by Harvard Apparatus Co., Inc., 150 Dover Road, Millis, Massachusetts.
† CO2 analyzer manufactured by Beckman Instruments, Inc., Electronic Instruments Division, 3900 River Road, Schiller Park, Illinois.
‡ Statham strain gauge manufactured by Statham Instruments, Inc., 254 Carpenter Road, Hato Rey, Puerto Rico.
artery. The microcirculation of the parietal cortex was visualized via an implanted cranial window described in detail before. Intracranial pressure was monitored with a Statham strain gauge connected to one of the outlets of the window. Vessel diameter was measured with a Vickers image-splitting device attached to a Leitz microscope. Fluid-percussion brain injury was carried out using a device described previously. This device induces a short pulse of increased intracranial pressure of controllable amplitude and of constant duration of 21 to 23 msec.

Phospholipase C activity was measured by minor modifications of methods described previously for rat liver. Hemispheric tissue was homogenized in buffer (0.25 gm/ml) containing 0.225 M sucrose, 0.05 M Tris-HCl at a pH of 7.5, and 2.5 mM ethylenediaminetetraacetic acid (EDTA) for 30 seconds at a setting of 4 on a Technicon homogenizer. Homogenates were centrifuged at 1000 × G for 10 minutes in a refrigerated RC-5 Sorvall centrifuge. The supernatant was decanted and the pellet was resuspended and homogenized in a volume of buffer that was equal to the original volume of the homogenate. This preparation was carried out on the day of the experiment. Aliquots of this preparation (0.1 to 0.5 mg of protein in 0.1 ml of buffer) were incubated with 0.5 mM sn-[1,3-14C]glycerol-3-phosphate (0.1 μCi), 0.6 mM ammonium palmitate, 0.7 mM dithiothreitol, 0.05 mM CoA, 3.3 mM CaCl2, 1.25 mg fatty acid-poor albumin, and 3.3 mM ATP in a total volume of 0.35 ml. Glycerolipid formation was initiated by the addition of cellular protein and terminated after a 30-minute period of incubation at 37°C by the addition of 4 ml of 1:1 CHCl3-MeOH containing 1% 1 N HCl. Lipids were extracted and separated into neutral lipids and phosphatidylcholine by silicic acid column chromatography. Neutral lipids and phosphoglycerides were separated into individual species by thin-layer chromatography, as described previously. Under these incubation conditions, the amount of phosphatidylcholine formation did not increase after 20 minutes. Phosphatidylcholine biosynthesis was terminated by the addition of 4 ml of 1:1 CHCl3-MeOH containing 1% 1 N HCl, and phosphatidylcholine was extracted and identified as described before. Under these conditions, the amount of phosphatidylcholine formed is directly proportional to the level of cellular DG. This conclusion is supported by the observation that 1000 × G centrifuged cell fractions which were incubated with exogenous (Clostridium perfringens) phospholipase C exhibited corresponding increases in DG content and methyl-14C-CDP choline incorporation into phosphatidylcholine. Therefore, the formation of phosphatidylcholine by cholinephosphotransferase was used to estimate changes in brain DG content.

The experimental design was as follows. The animals were first subjected to a period of arterial hypocapnia, induced through passive hyperventilation, to ascertain that all the vessels of the parietal cortex were normally responsive to this stimulus. Subsequently, the animals were divided into two groups randomly; one group served as controls, while the animals of the other group were subjected to brain injury. The controls were treated in identical fashion as the injured animals, except that no brain injury was induced. Arterial and intracranial pressures and vessel diameters were measured during a control period, 1 minute after injury, and 5 minutes after injury. At 8 minutes after injury, the cranial window was removed and a portion of the underlying cortex was excised rapidly, placed in chilled buffer solution, and processed further for biochemical analysis.

Results

Table 1 shows the vessel diameter for small (less than 100 μm in diameter) and large arterioles (larger than 100 μm in diameter), PaCO2, and mean arterial blood pressure during normal ventilation conditions.
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and during hypocapnia in the control animals and in the animals subjected to brain injury. The two groups were normally responsive to arterial hypocapnia. Intensity of brain injury averaged 2.7 ± 0.24 atm. The response to brain injury was similar to that we have found previously. There was a large increase in arterial blood pressure, which subsided within 5 minutes. The peak mean arterial blood pressure was 225 ± 13.6 mm Hg. The diameter of small arterioles increased by 23 ± 6.6% during the 1st minute, and by 15 ± 4.2% of the control diameter during the 5th minute after injury. The diameter of large arterioles increased by 18 ± 4.7% during the 1st minute, and by 13 ± 4.2% of the control diameter during the 5th minute following injury. These vascular changes are similar to those found earlier.

Figure 1 shows the amount of labeled membrane-bound phosphatidate that was converted into DG in the control animals and in the animals subjected to brain injury. The brain sample from one of the control animals was lost in processing. The phospholipase C-mediated conversion of phosphatidate into DG was significantly greater (p < 0.01, t-test) in the animals subjected to brain injury. Figure 2 shows that the diacylglycerol-dependent incorporation of labeled cytidine diphosphate (CDP) choline into phosphatidylcholine (PC) was significantly greater (p < 0.01, t-test) in the animals subjected to brain injury than in controls.

Discussion

These findings show the occurrence of increased activity of phospholipase C in cerebral cortical tissue following experimental brain injury. It is reasonable to expect that this increase in phospholipase C activity is responsible for generating diacylglycerol that is used to liberate free arachidonic acid. The latter is then used in the increased synthesis of prostaglandin, demonstrated in earlier experiments following this type of brain injury. The present findings, as well as the types of prostaglandins of which the concentrations increased following brain injury, suggest that the enhanced prostaglandin synthesis takes place in brain parenchyma. Whether the same occurs in cerebral vascular tissue is not known with certainty, because no independent examination of phospholipase activity and prostaglandin synthesis following this type of brain injury in cerebral vascular tissue has been carried out.

The mechanisms that initiate activation of phospholipase C activity following this type of brain injury are not known. A reasonable hypothesis is that the activation is due to the release from brain tissue or due to the entry from the blood stream into the parenchyma of substances which induce such activation. Bell, et al., found that human platelets contain a phosphatidyl inositol-specific phospholipase C and diacylglyceride lipase. They hypothesized that these enzymes are responsible for the liberation of free arachidonate from platelets during aggregation induced by thrombin. The same pathway was demonstrated by the same group of investigators in mouse fibrosarcoma cells. These investigators reasoned that phospholipase C activity in platelets was insufficient to account for the increase in free arachidonate required for prostaglandin and thromboxane synthesis.
during platelet aggregation. It remains to be seen whether diglyceride lipase or phospholipase A₂ activity is the enzyme immediately responsible for liberation of arachidonic acid in brain tissue after experimental brain injury or under other conditions where increased prostaglandin synthesis takes place. One may reasonably surmise, however, that the same factors that activate phospholipase C might also activate other phospholipases.

References


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