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## Release of Fatty Acid Amides in a Patient With Hemispheric Stroke A Microdialysis Study

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**Background**—Excitotoxic insults such as stroke may induce release of fatty acid ethanolamides (FAEs), contributing to the downstream events in the ischemic cascade. We therefore studied release of FAEs such as anandamide, palmitylethanolamide (PEA), and oleylethanolamide (OEA) in the brain of a patient suffering from malignant hemispheric infarction treated with hypothermia.

**Case Description**—A patient with life-threatening hemispheric stroke was treated with moderate hypothermia (33°C) that was maintained for 3 days, followed by a 3-day rewarming period. Microdialysis was applied to measure glutamate, lactate, and glycerol by using a microdialysis analyzer. FAEs were measured by microdialysis coupled with high-performance liquid chromatography/mass spectrometry. Release of neuroprotective fatty amides occurred within the first day after ischemia and reached high concentrations for all 3 substances in tissue surrounding the primary ischemic lesion: anandamide up to 42 pmol/mL, PEA up to 120 pmol/mL, and OEA up to 242 pmol/mL. There was a significant correlation with elevation of lactate as early marker for the hypoxic insult.

**Conclusions**—This is the first report demonstrating release of FAEs in vivo during human stroke and may suggest contribution of the FAE signaling system to the pathophysiological events after ischemia. (*Stroke*. 2002;33:2112-2114.)

**Key Words:** amides ■ cannabinoids ■ cerebral ischemia ■ fatty acids ■ microdialysis ■ stroke

Amides of long-chain fatty acids with ethanolamine (fatty acid ethanolamides [FAEs]) are a family of ubiquitous signaling molecules that include the endogenous cannabinoid anandamide (arachidonylethanolamide), the analgesic and anti-inflammatory mediator palmitylethanolamide (PEA), and the anorexic mediator oleylethanolamide (OEA).<sup>1-5</sup> Though widely divergent in function, FAEs may share a common biosynthetic mechanism, which consists of 2 enzymatic reactions occurring in parallel. A phospholipase D activity may release FAEs by hydrolyzing the distal phosphodiester bond in *N*-acyl phosphatidylethanolamine (NAPE), a minor membrane phospholipid. Simultaneously, a Ca<sup>2+</sup>-regulated *N*-acyltransferase activity may resynthesize NAPE by translocating fatty acid from phosphatidylcholine to the amino group in phosphatidylethanolamine.<sup>6-8</sup> Intracellular Ca<sup>2+</sup> plays a key role in initiating these reactions, and Ca<sup>2+</sup>-mobilizing stimuli such as membrane depolarization or glutamate receptor activation are potent triggers for FAE and NAPE biosynthesis in neurons.<sup>2,7-9</sup>

The finding that FAEs are produced on demand in neurons via a Ca<sup>2+</sup>-dependent mechanism suggests that these compounds may participate in neural signaling. This possibility is

supported by a variety of evidence indicating a primary role for anandamide in cannabinergic neurotransmission and for other, noncannabinoid FAEs in the endogenous regulation of pain and feeding.<sup>3-5,10</sup> In addition to these physiological roles, FAEs may also contribute to the pathological response of brain tissue to postischemic damage. Results from in vitro and in vivo experiments suggest that anandamide and other FAEs are generated in brain neurons after excitotoxic insults such as traumatic brain injury and that these compounds exert significant neuroprotective effects that are in part linked to their ability to inhibit glutamatergic neurotransmission.<sup>11-14</sup> It is at present unclear whether FAEs endogenously occur in human brain and whether stroke triggers this release. To examine this possibility, we used microdialysis coupled with high-performance liquid chromatography/mass spectrometry to measure FAE release in the brain of a patient suffering from malignant hemispheric infarction. Our results show that anandamide, OEA, and PEA are detectable in the human brain in vivo. They also suggest that stroke triggers FAE release in tissue surrounding the primary ischemic lesion associated with elevations in extracellular lactate, an early marker of the hypoxic insult.

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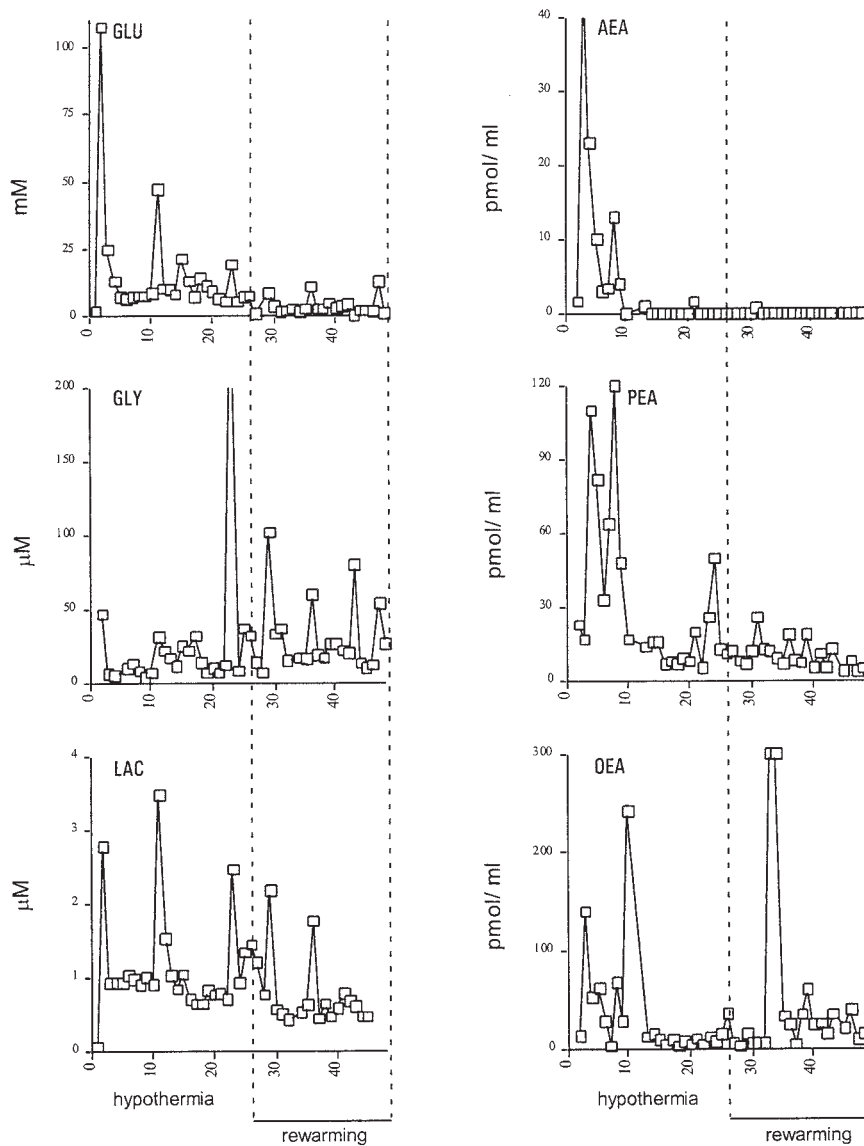
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Microdialysis studies of the penumbra of a 75-year-old patient with left-sided hemispheric infarction. Release of glutamate (GLU), glycerol (GLY), lactate (LAC), and the FAEs (anandamide [AEA], PEA, and OEA) during the first day after ischemia is shown. Note the significant co-release of lactate and anandamide ( $P < 0.01$ ) as well as lactate and PEA ( $P < 0.05$ ) (y axis: units of microdialysis measurements; x axis: probe interval 2 to 3 hours).

## Subjects and Methods

In a 75-year-old man with left-sided hemiplegia and drowsiness, CT and MRI analyses revealed an ischemic stroke that involved more than two thirds of the territory of the middle cerebral artery. We treated the patient with moderate hypothermia (33°C), following a protocol approved by the University of Heidelberg and described elsewhere.<sup>15</sup> Briefly, we induced hypothermia 8 hours after onset of symptoms and maintained it for 3 days, followed by a 3-day rewarming period during which mannitol was administered to prevent edema. An intracranial pressure-measuring probe (Spiegelberg AG) and a flexible microdialysis probe (length, 10 mm; external diameter, 0.5 mm; molecular size cutoff, 20 kDa; CMA) were inserted into the brain when hypothermia was initiated. The probe was aimed at the stroke penumbra by using diffusion- and perfusion-weighted MRI.<sup>16,17</sup> The microdialysis probe was perfused with a sterile isotonic solution (in mmol/L: Na<sup>+</sup> 147, K<sup>+</sup> 4.0, Ca<sup>2+</sup> 2.3, Cl<sup>-</sup> 156) at a flow rate of 0.3 mL/min. After a 60-minute equilibration period following probe implantation, we collected microdialysis effluent for 6 days over 2- to 3-hour periods. We measured the concentrations of glutamate, glycerol, and lactate in the microdialysis effluent by using a CMA 600 analyzer (CMA), following the manufacturer's instructions. We measured anandamide, OEA, and PEA by using an isotope-dilution high-performance liquid chromatography/mass spectrometry method previously described.<sup>18</sup> We

monitored body temperature continuously by using a probe inserted into the urinary bladder and documented other clinical variables such as blood gas and blood pressure. For statistical analysis, we used the hypergeometric model of co-occurrence, setting a probability value at  $< 0.05$ . The institutional review board at the University of Heidelberg reviewed and approved patient treatment and sample collection; the institutional review board at the University of California at Irvine approved the use of stored samples for FAE analysis. Patients' relatives were informed of the potential harmful effects of the hypothermic treatment and monitoring procedure and gave their informed consent.

## Results

As shown in the top panel of the Figure, glutamate level was increased (15 to 110  $\mu\text{mol/L}$ ) compared with values in normal patients (1 to 3.5  $\mu\text{mol/L}$ ).<sup>17,19,20</sup> This moderate increase of glutamate was much lower than glutamate measured in ischemic tissue (up to 200-fold), indicating that the probe was located in the penumbra.<sup>17</sup> Levels of glycerol as marker for neuronal disintegration and lactate as marker for metabolic stress were within normal ranges compared with their release under ischemic conditions.<sup>17,20</sup> Release of neuroprotective fatty amides occurred within the first day after

ischemia and reached high concentrations for all 3 substances measured: anandamide up to 42 pmol/mL, PEA up to 120 pmol/mL, and OEA up to 242 pmol/mL. Significant co-release occurred for lactate and anandamide ( $P < 0.01$ ) as well as lactate and PEA ( $P < 0.05$ ). Values then returned to lower levels for PEA (range, 4.1 to 50 pmol/mL) and OEA (range, 3.1 to 61 pmol/mL; 1 peak up to 300 pmol/mL occurred in the rewarming phase) or disappeared (anandamide). Intracranial pressure remained  $< 20$  mm Hg during the cooling and rewarming period. Body temperature was maintained at  $33.2 \pm 0.4^\circ\text{C}$  and increased by  $0.1^\circ\text{C}/4$  h to  $0.2^\circ\text{C}/4$  h during rewarming.

### Discussion

As shown here for the first time, neuroprotective FAEs such as anandamide, PEA, and OEA are detectable in humans and are released after stroke, as measured by in vivo microdialysis. This release occurred in penumbral tissue surrounding the primary ischemic lesion and was significantly associated with elevations in extracellular lactate, an early marker of the hypoxic insult. The release pattern with transient sharp increases of cannabinoids after stimulation with depolarizing substances or dopamine was comparable to that seen in our patient after cerebral ischemia, probably indicating receptor-stimulated cleavage of membrane lipid precursors and immediate release.<sup>10,18</sup> Release after ischemia may suggest an activation of the FAE signaling system recently suggested by Hansen et al<sup>11</sup>: *N*-methyl-D-aspartate exposure—or traumatic brain injury—induced release of the endocannabinoid precursors *N*-acylethanolamine phospholipids, probably catalyzed by the activation of the calcium-dependent *N*-acyltransferase. Furthermore, endogenous cannabinoids such as arachidonoyl glycerol were recently reported to be released after traumatic brain injury and to exert significant neuroprotective effects.<sup>14</sup>

Microdialysis is a safe and feasible monitoring technique in critically ill patients with subarachnoid hemorrhage, head trauma, and stroke.<sup>16,17,20,21</sup> Microdialysis monitoring includes bedside measurements of extracellular metabolic changes as indicators of the condition of the brain in the affected and nonaffected hemispheres and detects patient deterioration due to secondary ischemia earlier than other routine measures such as intracranial pressure, evoked potentials, or Doppler sonography.<sup>21–23</sup> Microdialysis monitoring may be further used to predict outcome in large hemispheric strokes or to guide therapy such as hypothermia.<sup>17,20</sup>

In conclusion, our results show that anandamide, OEA, and PEA are detectable in the human brain in vivo. Our results also suggest that stroke triggers FAE release in tissue surrounding the primary ischemic lesion associated with elevations in extracellular lactate, an early marker of the hypoxic insult. These findings point toward involvement of the FAE signaling system within the ischemic cascade. However, further studies are needed to fully understand the role and function of the endocannabinoid system after cerebral ischemia.

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