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Neuroscience. Author manuscript; available in PMC 2009 April 9.

Published in final edited form as:
Neuroscience. 2008 April 9; 152(4): 1032–1039. doi:10.1016/j.neuroscience.2008.01.034.

REGIONAL DISTRIBUTION AND EFFECTS OF POSTMORTAL DELAY ON ENDOCANNABINOID CONTENT OF THE HUMAN BRAIN

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Abstract

Tissue levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) have been determined in 16 regions and nuclei from human brains, using liquid chromatography/inline mass spectrometry. Measurements in brain samples stored at −80°C for 2 months to 13 years indicated that endocannabinoids were stable under such conditions. In contrast, the postmortal delay had a strong effect on brain endocannabinoid levels, as documented in brain samples microdissected and frozen 1 to 6h *postmortem*, and in neurosurgical samples 0, 5, 30, 60, 180 and 360 min after their removal from the brain. The tissue levels of AEA increased continuously and in a region-dependent manner from one hour after death, increasing about 7-fold by 6h *postmortem*. In contrast, concentrations of 2-AG, which were 10 to 100-times higher in human brain regions than those of AEA, rapidly declined: within the first hour, 2-AG levels dropped to 25–35% of the initial ('0 min') value, where after they remained relatively stable. As analyzed in samples removed 1–1.5h *post mortem*, AEA levels ranged from a high of 96.3 fmol/mg tissue in the nucleus accumbens to a low of 25.0 fmol/mg in the cerebellum. 2-AG levels varied 8-fold, from 8.6 pmol/mg in the lateral hypothalamus to 1.1 pmol/mg in the nucleus accumbens. Relative levels of AEA and 2-AG varied from region to region, with the 2-AG:AEA ratio being high in the sensory spinal trigeminal nucleus (140:1), the spinal dorsal horn (136:1) and the lateral hypothalamus (98:1) and low in the nucleus accumbens (16:1) and the striatum (31:1). The results highlight the pitfall of analyzing endocannabinoid content in brain samples of variable postmortal delay, and document differential distribution of the two main endocannabinoids in the human brain.

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Keywords

anandamide; 2-arachidonoylglycerol; brain microdissection; neurosurgical dissections

INTRODUCTION

Arachidonoyl ethanolamide (anandamide), the first endogenous substance identified to have cannabinoid-like properties, was originally isolated from porcine brain (Devane et al., 1992), and a second endocannabinoid, 2-arachidonoylglycerol (2-AG), was also identified as a brain constituent (Sugiura et al., 1995). Although additional endogenous ligands that bind to cannabinoid receptors have also been identified, to date anandamide and 2-AG remain the most widely studied and best characterized members of this class. The generation of these two endocannabinoids in the mammalian brains and their possible physiological functions has been analyzed in countless studies, yet there is little information regarding their distribution in the human brain. Studies in the rat, analyzing the tissue levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in a limited number of brain regions, clearly documented their uneven distribution in the brain (Felder et al., 1996; Bisogno et al., 1999).

Studies in rodents have documented postmortal increases in brain levels of endocannabinoids (Schmid et al., 1995; Felder et al., 1996; Kempe et al., 1996; Sugiura et al., 2001; Patel et al., 2004). In the human brain, postmortal changes of cannabinoid receptors have been reported (Mato and Pazos, 2004), but similar changes in AEA and 2-AG have not yet been explored. This would be particularly important, because unlike in experimental animals, the time between death and the postmortal removal of brain tissue is often uncertain and much more difficult to control. Several factors may influence the concentrations of endogenous substances measured in samples taken from the *postmortem* brain, including gender, age at death, the agonal state, brain pH, postmortal delay, and the storage time and temperature (Perry and Perry, 1983; Mato and Pazos, 2004). In the present study, the effects of two of these factors, postmortal delay and sample storage time have been investigated on endocannabinoid levels in microdissected human brain areas and nuclei, as well as in cerebrocortical specimens collected during neurosurgical interventions.

EXPERIMENTAL PROCEDURES

Human tissues

Human brain samples were collected by the Human Brain Tissue Bank, Budapest, in accordance with the Ethical Rules for Using Human Tissues for Medical Research in Hungary (HM 34/1999) and The Code of Ethics of the World Medical Association (Declaration of Helsinki). Tissue samples were taken either during brain autopsy at the Department of Forensic Medicine of Semmelweis University, Budapest, or during brain surgery conducted at the Institute of Neurosurgery, Budapest and the Neurosurgical Department of Markusovszky Hospital, Szombathely, Hungary. Prior written informed consent was obtained from the next of kin, which included the request to consult the medical chart and to conduct neurochemical analyses. The protocol, including analyses of tissue samples, was approved by institutional Ethics Committees of Semmelweis University or Markusovszky Hospital.

Tissue samples from brain autopsy—Brain samples were collected from 25 subjects (16 males and 9 females) who died as a result of suicide (hanging) (n=6) or myocardial infarction in their home or in public place (n=19). All of these deaths were treated as forensic cases, and the exact time of death as well as the time of autopsy and removal of the brain were known and recorded. The age of the subjects ranged from 27 to 89 years. All subjects died without any known neurological or affective disorder. The medical history of the victims was

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obtained from medical or hospital records, interviews with family members and relatives, as well as from pathological and neuropathological reports. The protocol of tissue sampling and retrospective assessments were approved by the institutional review board of Semmelweis University, Budapest. Brains were removed from the skull with a postmortal delay of 1 to 6 h, rapidly frozen on dry ice and stored at −80°C until microdissection. All personal identifiers had been removed and samples coded before the analyses of tissue endocannabinoid levels.

The microdissection was performed by the punch technique (Palkovits, 1973): brains were cut as 1.0–1.5 mm thick coronal sections, and 16 individual brain regions and nuclei (Fig. 1) were removed bilaterally by special punch needles of an inside diameter of 1.0–3.5 mm, by using either a head magnifyer or a stereomicroscope. The microdissected samples were collected in 2.0 mm airtight plastic (Eppendorff) tubes and stored at −80°C until further use. The temperature of brain sections and the microdissected samples were kept under 0 °C during the whole procedure. The storage time of the samples varied between 2 and 155 months (see Table 1).

Brain tissue samples from neurosurgery—Samples were collected in the operating room from 2 male (ages 54 and 75) and 2 female patients (ages 37 and 41) undergoing brain resection for meningeoma, cerebral artery aneurism, and in 2 cases for glioblastoma. During penetration into deep brain regions, tissue fragments of 100–300 mg were collected from the frontal cortex. Because of their small size, the ratio of grey to white matter in these specimens was different from that in the larger cortical samples removed during autopsy. Tissue specimens were placed in a 6-well tissue culture dish lined with small pieces of aluminium foil in each well. The brain sample in the first well was frozen with powdered dry ice immediately after removal from the brain (0 min *postmortem* delay), while the other samples were covered by a drop of saline to prevent them from drying, and were left at room temperature for 5, 30, 60, 180, and 360 min, respectively, at which time they were frozen with powdered dry ice, packed airtight in the aluminium foil and kept at −80 °C until use.

Measurement of tissue endocannabinoid levels—Frozen tissue samples were rapidly weighed, placed into polypropylene tubes containing 0.5 ml of an ice cold solution of methanol/ Tris buffer (50 mM, ph 8.), 1:1 and 7 ng of $^{2}H_4$-anandamide (external standard), and rapidly homogenized in an ice bath, using a stainless steel Tissuemizer at 15,000 rpm. To each homogenate, 2 ml of ice-cold chloroform/methanol (1:1) and 0.5 ml of Tris buffer were added. The mixture was vortexed and centrifuged for 2 min at 4°C (500×g), the chloroform phase was recovered, and the water phase was extracted 2 more times with ice-cold chloroform. The combined chloroform phase was dried under a stream of nitrogen and reconstituted in 110 μl chloroform. The sample was deproteinized by the addition of 2 ml ice-cold acetone, followed by centrifugation at 1,800×g for 10 min to remove the precipitated proteins. The clear supernatant was removed, evaporated under nitrogen, the sample reconstituted in 50 μl of ice-cold methanol and analyzed by liquid chromatography/in line mass spectrometry using an Agilent 1100 series LC-MSD, as described in detail elsewhere (Wang et al., 2003). Values are expressed as fmol AEA or pmol 2-AG per mg frozen tissue.

RESULTS

Effect of storage time on AEA and 2-AG concentrations in human brain samples

Measurements using cortical samples from 11 brains (all of them with a 1–1.5 h postmortal delay and stored for various periods of time at −80°C) indicated that the storage time did not influence the concentrations of AEA and 2-AG in the microdissected brain samples. Concentrations, even after 10–13 years of storage, did not differ significantly from those in samples that had been stored for 2–8 months only (Table 1).

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Effect of postmortal delay on the concentrations of AEA and 2-AG in microdissected brain samples

The effect of the time elapsed between death and the removal and freezing of brain samples (postmortal delay) on AEA (Fig. 2) and 2-AG (Fig. 3) tissue levels was assessed in 6 brain regions: cerebral cortex (frontal, cingulate and somatosensory cortical samples combined), hypothalamus, hippocampus, amygdala, cerebellum and sensory spinal trigeminal nucleus (its pontine, medullary and rostral cervical spinal portions combined).

Concentration of AEA—A sustained, region-dependent increase in AEA concentration was detected in all 6 investigated brain as a function of increasing *postmortem* delay (Fig. 2). The concentrations measured in samples removed 2–3 h *postmortem* were almost doubled in all investigated brain regions relative to those measured in the 1–1.5 h *postmortem* samples. Further elevations in AEA levels were measured by 4–6 h *postmortem*, but the relative increases were region-dependent: a continued high increase was seen in the cerebral cortex, with relatively minor or no further increases in hippocampus, amygdala, spinal trigeminal nucleus and the cerebellum (Fig. 2).

Concentration of 2-AG—With the exception of the cerebellum, where 2-AG levels did not change between 1–1.5 and 4–6 h *postmortem*, a steady decline in tissue 2-AG levels was found in the other brain regions in the same interval (Fig. 3). This decline was relatively moderate, reaching ~50% by 6 h after death in the cerebral cortex, the hippocampus and the amygdala, but only 30% in the spinal trigeminal nucleus (Fig. 3).

Postmortal changes in the concentrations of AEA and 2-AG measured in neurosurgical brain specimens

Neurosurgical specimens were taken from the cerebral cortex, which were frozen at 0, 5, 30, 60, 180 and 360 min after surgical removal of the samples from the brain.

Concentration of AEA—A gradual, moderate elevation in AEA levels was detected in cortical samples left at room temperature for 30 min (25%) and 60 min (51%) after removal from the brain. With longer postmortal delays, AEA levels increased rapidly, reaching 350% by 3 h and 700% by 6 h relative to the samples frozen immediately upon removal (0 min *postmortem* samples, Table 2 and Fig. 4).

Concentration of 2-AG—In sharp contrast to AEA, 2-AG levels dropped dramatically during the first hour following removal from the brain in samples left at room temperature. By 30 min, the concentration of 2-AG was reduced by half and by 1 hour to one third of the levels measured in 0 min samples. There was only a minor further reduction by 3 h with somewhat higher levels again at 6 h *post mortem* (Table 2 and Fig. 4).

Comparison of values in microdissected *versus* neurosurgical cerebral cortical samples

A direct comparison of time-dependent changes in brain endocannabinoid levels in microdissected versus neurosurgical samples was hampered by the lack of microdissected autopsy samples with a less than 1 hour postmortal delay. Nevertheless, the trends of changing endocannabinoid levels were remarkably similar in the 1–6 hour postmortal time period, as illustrated in Fig. 4. Concentrations measured in both types of samples clearly showed the continuous elevation of AEA levels between 1 and 6 hours *postmortem*, and the initial rapid decline in 2-AG levels was also similar in the two types of cortical samples (Fig. 4). Interestingly, this phase of rapid decline was shifted to the right by about an hour in the microdissected samples, suggesting that there is little change in 2-AG levels in the 1st h after death, as long as the brain remains intact.

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Brain regional distribution of AEA and 2-AG

The regional distribution of AEA and 2-AG was analyzed based on measurements in microdissected autopsy samples with a 1 hour postmortal delay. AEA and 2-AG concentrations in 16 human brain regions/nuclei and the ratio of AEA to 2-AG levels in each region are presented in Table 3. There were marked differences in the regional distribution of the two endocannabinoids: for AEA, the highest concentrations were measured in the nucleus accumbens (96.3±13.0 fmol/mg tissue), followed by the medial (87.6±9.2 fmol/mg) and lateral hypothalamus (86.3±10.2 fmol/mg). Individual nuclei in the medial hypothalamus (paraventricular, ventromedial, dorsomedial, arcuate nuclei) all contained AEA in high concentration, whereas the lowest levels of AEA were detected in the cerebellum (25.0±1.9 fmol/mg).

In general, 2-AG brain concentrations were around two orders of magnitude higher than the concentrations of AEA, with the highest levels of 2-AG detected in the lateral hypothalamus (8.6±2.9 pmol/mg tissue), followed by the spinal dorsal horn (7.2±2.7 pmol/mg) and the sensory spinal trigeminal nucleus (6.0±1.4 pmol/mg), while the lowest values were found in various cerebrocortical regions (1.6–2.0 pmol/mg) and in the nucleus accumbens (1.1±0.1 pmol/mg). The difference in the regional distribution of AEA and 2-AG is also reflected in marked differences in their ratios in different brain regions. The 2-AG:AEA ratio was highest in the sensory spinal trigeminal nucleus (140±11), the spinal dorsal horn (136±34) and the lateral hypothalamus (98±27) and lowest in the nucleus accumbens (16±5) and the striatum (31±8).

DISCUSSION

Correlations between the brain concentration of neurochemical substances and pathological conditions are often used to gain insight into the mechanism of disease. Endocannabinoids, a novel class of lipid mediators, have been implicated in a growing number of neurological and psychiatric disorders, including schizophrenia, anxiety disorder, depression, Parkinson's disease, Alzheimer's disease, Huntington chorea and epilepsy, and have also been shown to have important neuroprotective effects in stroke and traumatic brain injury (reviewed in Pacher et al., 2006). Numerous studies have documented changes in the brain level of endocannabinoids in animal models for some of these diseases, such as Parkinson's (Di Marzo et al., 2000; Maccarrone et al., 2003), multiple sclerosis (Baker et al., 2001; Cabranes et al., 2005) or Huntington chorea (Bisogno et al., 2007), and in human brain tumors (Maccarrone et al., 2001). A tacit assumption in such studies is that the biochemical composition of the post-mortem brain reliably reflects *in vivo* conditions. However, there are many factors that influence the postmortem fate of various neurochemical substances in the brain, including gender, age, length of agony, brain pH, the time elapsed between death and the removal of brain samples (post mortem delay), storage time and temperature of frozen brain tissue (Perry and Perry, 1983). Among these factors, the effect of *post mortem* delay and the storage time of the brain samples were investigated in the present study.

Storage time

The effect of storage time on endocannabinoid levels in the human brain has not yet been investigated. The present findings in 11 human cerebrocortical samples that had been removed from the brain 1 hour after death and had been stored at −80°C for as little as 2 months or as long as 13 years clearly show that both AEA and 2-AG, the major endocannabinoids, are remarkably stable under such storage conditions. Mato and Pazos (2004) recently reported that CB_1 cannabinoid receptor density and the ability of the receptors to couple to G proteins decline by as much as 50% after 2 years of storing human brain samples at −25%. Whether this difference is due to the greater lability of a membrane protein compared to small lipid molecules

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or to the higher storage temperature in the latter study, is not known. The stability of endocannabinoids in brain samples stored at −25°C has not yet been determined.

Postmortal increase in brain AEA levels

The concentration of AEA in the mammalian brains increases rapidly after death (Schmid et al., 1995; Kempe et al., 1996; Bisogno et al., 1999; Patel et al., 2004). A seven-fold increase in AEA levels has been measured in the rat brain 24 h after death (Maccarrone et al., 2001). Increases of AEA levels in human cerebellum 2–24 h after death were reported by Felder et al. (1996), but systematic time- and region-dependent *postmortem* analyses in the human brain have not yet been performed. In the present study, a gradual, progressive increase in AEA levels was detected in 6 microdissected brain regions removed 1 to 6 h after death, and in neurosurgical samples with 0 to 360 min intervals between their removal and freezing. Although the time-dependent pattern of the elevation of AEA levels varied somewhat from region to region (Fig. 2 and Fig. 4), the increase was progressive and significant (up to 7-fold) in all brain regions, and values obtained in microdissected autopsy samples and in neurosurgical specimens were comparable at the different time points (Fig. 4).

AEA is generated in the brain from membrane phospholipid precursors in response to a rise in intracellular calcium or metabotropic receptor stimulation, and is believed to be released from post-synaptic neurons to act as a retrograde transmitter at presynaptic cannabinoid receptors (Pacher et al., 2006). The disposition of AEA involves cellular reuptake via a putative membrane transporter and subsequent degradation by the enzyme fatty acid amide hydrolase (FAAH, McKinney and Cravatt, 2005). Brain levels of AEA are 15-fold higher in $FAAH^{-/-}$ compared to wild-type littermates (Cravatt et al., 2001), and *in vivo* treatment of mice with a FAAH inhibitor results in marked increases in brain AEA content (Kathuria et al., 2003), indicating that degradation of AEA by constitutively active FAAH is the dominant mechanism in the control of steady state AEA levels in the brain. In view of the relative stability of AEA in intact brain tissue, this could then suggest that gradual degradation of the FAAH protein in the post-mortem brain may be responsible for the progressive increase in AEA levels. Indeed, Patel et al. (2005) recently reported that the elevated brain AEA content of $FAAH^{-/-}$ mice does not further increase but rather decreases for up to 24 hours postmortem, and a similar postmortem decrease in brain AEA was detected in wild-type mice treated in vivo with a FAAH inhibitor. Thus, postmortal degradation of FAAH, possibly through proteolytic mechanisms, is likely responsible for the associated increase in brain AEA levels.

Postmortal depletion of brain 2-AG levels

In sharp contrast to the postmortal gradual increase in AEA levels, brain 2-AG content was found to decline following death or surgical removal of samples. We quantified this for the first time in different human brain regions as a function of postmortal delay. Conspicuously, much of the rapid decline occurred within the first hour, as detected in brain samples removed during neurosurgery. This indicates that samples from brains removed through pathological dissections are not suitable to analyze postmortal changes in 2-AG levels, as in general the earliest brain dissections are at or over 2 hours after death, by which time brain levels of 2-AG have declined by 75–80% (Table 2 and Fig. 4). In blood plasma, 2-AG is much less stable than AEA (Jarai et al., 2000), and a similar greater lability in the brain may account for its rapid postmortal decline. Sugiura et al. (2001) have earlier reported that there is a very early postmortal increase in 2-AG levels in the rat brain that occurs within the first 30 seconds after decapitation, and that can be prevented by immediately freezing the brain in liquid nitrogen. A possible mechanism offered to account for that change is that decapitation leads to rapid breakdown of inositol phospholipids and the subsequent generation of diacylglycerol (Aveldano and Bazan, 1975) that can be further metabolized by diacylglycerol lipase to 2-AG. Although possible, it is unlikely that such a rapid early increase in 2-AG occurred in the

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neurosurgically removed samples, because removal of the samples is much faster than in animals following decapitation and the conditions are also different. It should be also noted that the time-dependent degradation patterns are also different in various human brain regions (Fig. 3), suggesting that chemical stability of 2-AG alone cannot account for the observed postmortal changes. A cytosolic serine hydrolase, monoacylglyceride lipase (MGL) is responsible for the in vivo hydrolysis of 2-AG in neurons (Dinh et al., 2002), and region-dependent differences in the distribution of MGL and in postmortal changes in its activity may also contribute.

Regional distribution of AEA and 2-AG in the human brain

There is scant information about endocannabinoid levels in the human brain (Felder et al., 1996; Maccarrone et al., 2001). We have described here for the first time the regional distribution of 2-AG in the human brain. In contrast to data reported about the similar rank order for the distribution of AEA and 2-AG in different brain areas of rats (Bisogno et al., 1999), their regional distribution is distinctly different in the human brain (Fig. 3, Table 3). In 16 human brain regions or nuclei (Fig. 1), the levels of AEA varied 4-fold, between 25 (cerebellum) and almost 100 fmol/mg tissue (nucleus accumbens). The regional differences in 2-AG concentrations were twice as high, displaying an 8-fold variation between 1.1 (nucleus accumbens) and 8.6 pmol/mg tissue (lateral hypothalamus, Table 3). In contrast to Maccarrone et al. (2001) who reported comparable amounts of AEA and 2-AG in the human brain with concentrations for both endocannabinoids in the high pmol/mg range, we measured 16–140-fold higher 2-AG than AEA concentrations in the investigated brain nuclei and regions, with AEA levels being about 3 orders of magnitude lower than in the above study (Table 3). The ratios as well as the absolute concentrations measured in the present study are in line with previous reports about AEA and 2-AG concentrations in the rat brain (Dinh et al., 2002).

The different regional distribution of AEA and 2-AG in the human brain may reflect different functional roles for these two endocannabinoids. Endocannabinoids acting at presynaptic CB_1 receptors play a key role in modulating various forms of synaptic plasticity, such as depolarization-induced suppression of inhibition or DSI. Recent evidence indicates that the mediator involved in this form of plasticity is 2-AG (Melis et al., 2004). Endocannabinoids have been shown to regulate appetite in the hypothalamus (Di Marzo et al., 2001), an effect recently localized in the lateral hypothalamus and attributed to a DSI of melanin-concentrating hormone (MCH)-containing neurons (Jo et al., 2005). Although not proving the role of 2-AG in this effect, the very high levels of 2-AG and high 2-AG:AEA ratio in the lateral hypothalamus (Table 3) is compatible with this possibility. In contrast, some endocannabinoid effects selectively unmasked by inhibitors of FAAH, such as a CB_1 receptor-mediated antianxiety effect (Kathuria et al., 2003), are thought to be mediated by AEA rather than 2-AG, because of the *in vivo* selectivity of FAAH for AEA. In this regard it is noteworthy, that brain regions thought to play a key role in anxiety-like behaviors, such as the striatum, hippocampus and prefrontal cortex (Matthew and Ho, 2006), have low 2-AG:AEA ratios (30–60). Further functional studies are obviously needed to explore these issues.

Acknowledgements

This research was supported by EU Grant FP6, BNEII No. LSHM-CT-2004-503036 to the Human Brain Tissue Bank, Budapest (to MP) and funds from the Intramural Program of the NIH (to GK)

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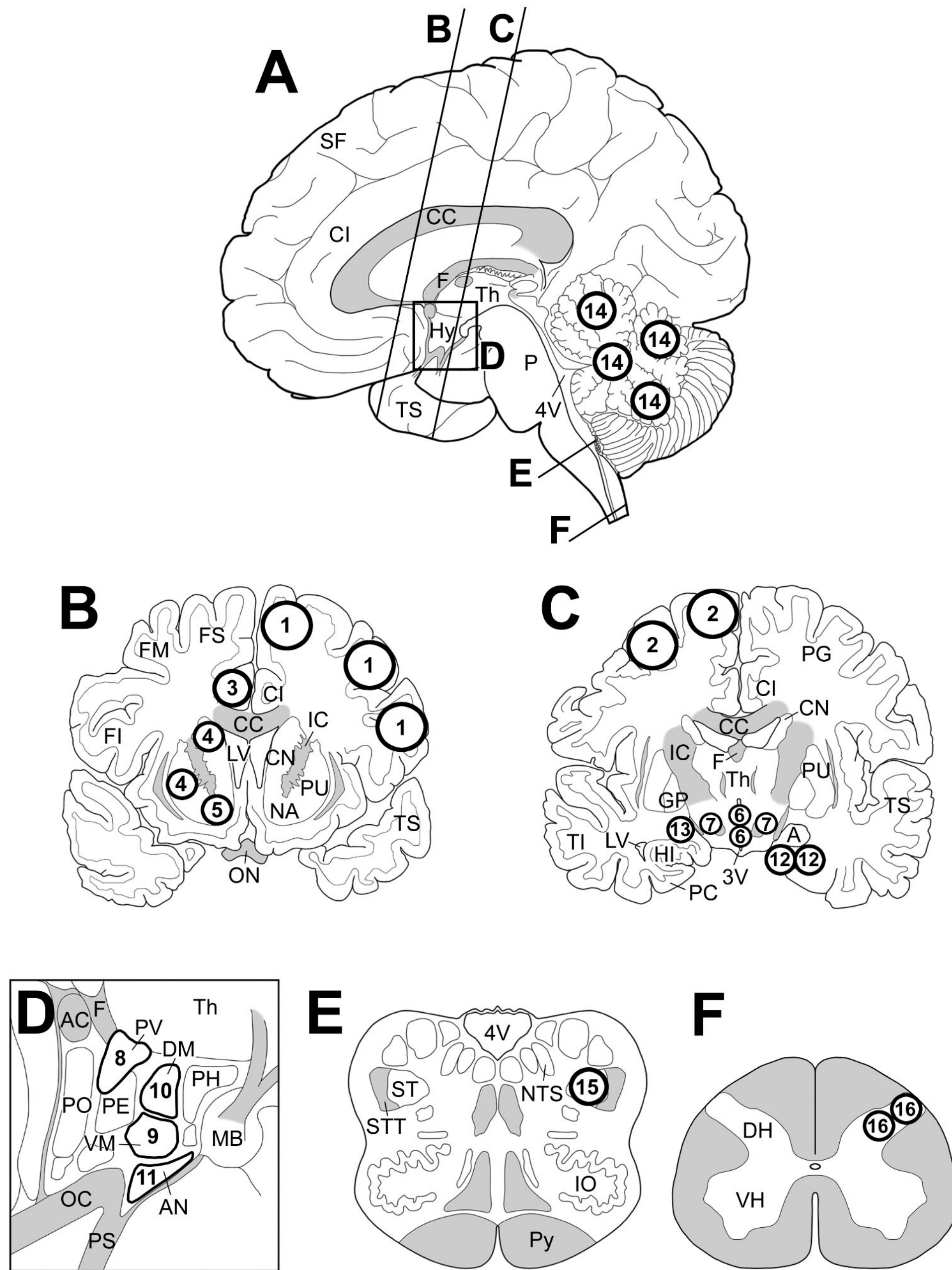

Fig. 1.

Sagittal (A and D) and coronal (B, C, E and F) drawings of the human brain with the topographical localization of the dissected brain areas and nuclei. B - Coronal section through the striatum and the nucleus accumbens. C - Coronal section through the amygdala and the hippocampus. D - Sagittal section with the topography of the hypothalamic nuclei, E - Coronal section through the medulla oblongata. F - Coronal section through the spinal cord. *Dissected brain areas and nuclei*: 1 - frontal cortex, 2 - somatosensory cortex, 3 - cingulate cortex, 4 - striatum, 5 - nucleus accumbens, 6 - medial hypothalamus, 7 - lateral hypothalamus, 8 - paraventricular nucleus, 9 - ventromedial nucleus, 10 - dorsomedial nucleus, 11 - arcuate nucleus, 12 - hippocampus, 13 - amygdala, 14 - cerebellum, 15 - sensory trigeminal nucleus,

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16 - spinal cord (dorsal horn). *Abbreviations*: A - amygdala, AC - anterior commissure, AN - arcuate nucleus, CC - corpus callosum, CI - cingulate cortex, CN - caudate nucleus, DH - spinal cord, dorsal horn, DM - dorsomedial nucleus, F - fornix, FI - inferior frontal gyrus, FM - middle frontal gyrus, FS - frontal superior gyrus, GP - globus pallidus, HI - hippocampus, Hy - hypothalamus, IC - internal capsule, IO - inferior olive, LV - lateral ventricle, MB - mamillary body, NA - nucleus acccumbens, NTS - nucleus of the solitary tract, OC - optic chiasm, ON - optic nerve, P - pons, PC - parahippocampal gyrus, PE - periventricular nucleus, PG - postcentral gyrus, PH - posterior hypothalamic nucleus, PO - medial preoptic nucleus, PS - pituitary stalk, PU - putamen, PV - paraventricular nucleus, Py - pyramidal tract, SF - superior frontal gyrus, ST - nucleus of the spinal trigeminal tract, STT - spinal trigeminal tract, Th - thalamus, TI - inferior temporal gyrus, TS - superior temporal gyrus, VH - spinal cord ventral horn, VM - ventromedial nucleus, 3V - third ventricle, 4V - fourth ventricle.

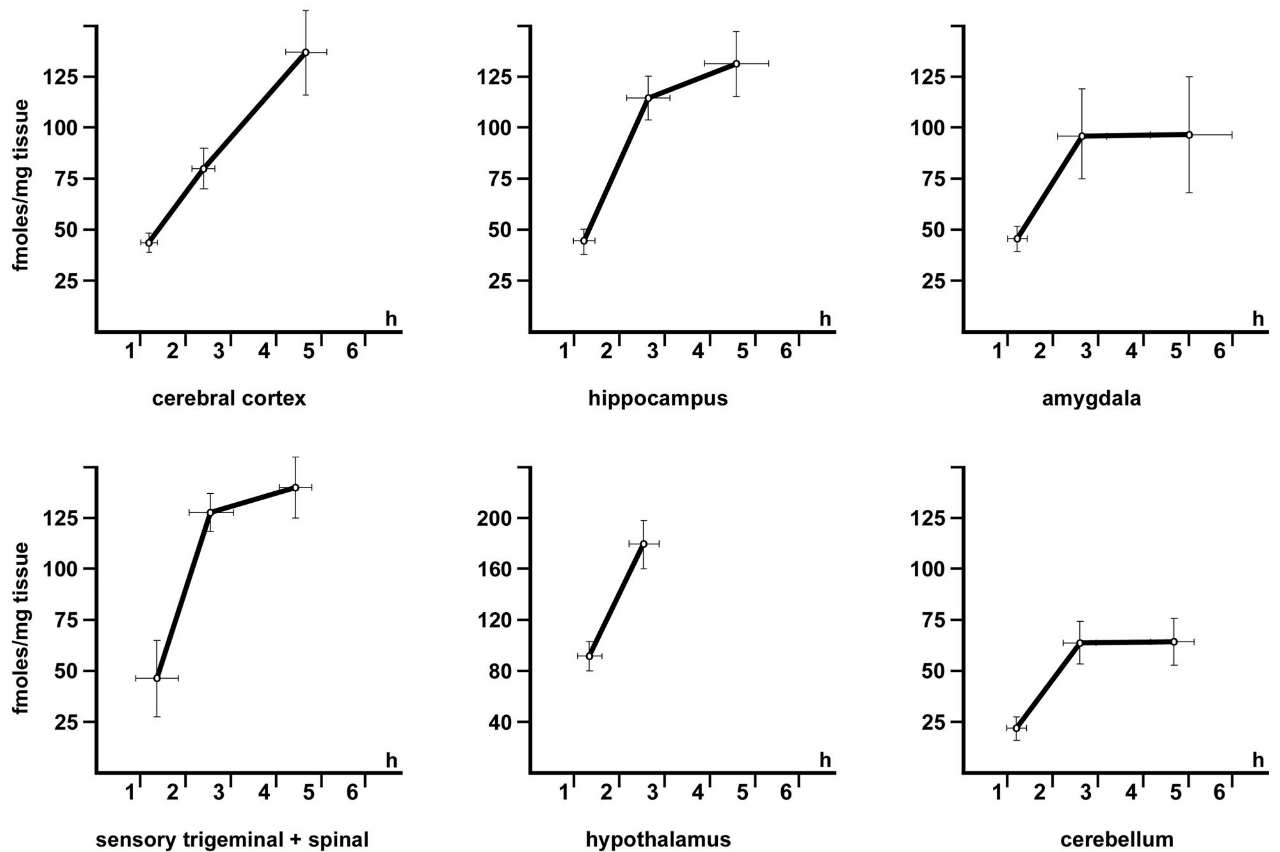

Fig. 2.
AEA levels in 6 human brain regions as a function of postmortal delay. Points and bars indicate means ± SEM, n=2–8. Cerebral cortex values reflect combined values from the frontal, cingulate and somatosensory cortex. Hypothalamus: medial and lateral hypothalamus combined.

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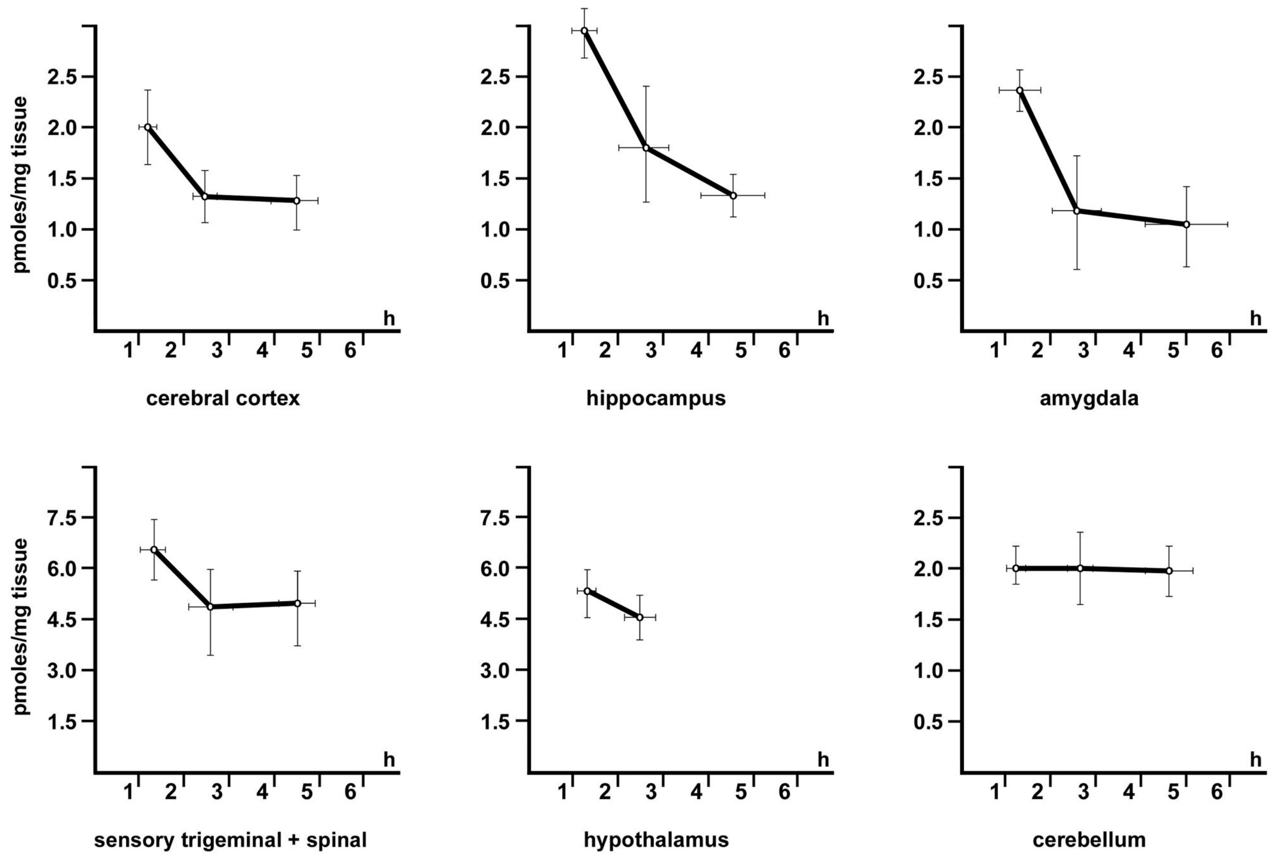

Fig. 3.
2-AG levels in 6 human brain regions as a function of postmortal delay. Points and bars indicate means ± SEM, n=2–8. Cerebellar cortex = combined values from the frontal, cingulate and somatosensory cortex. Hypothalamus = medial and lateral hypothalamus combined.

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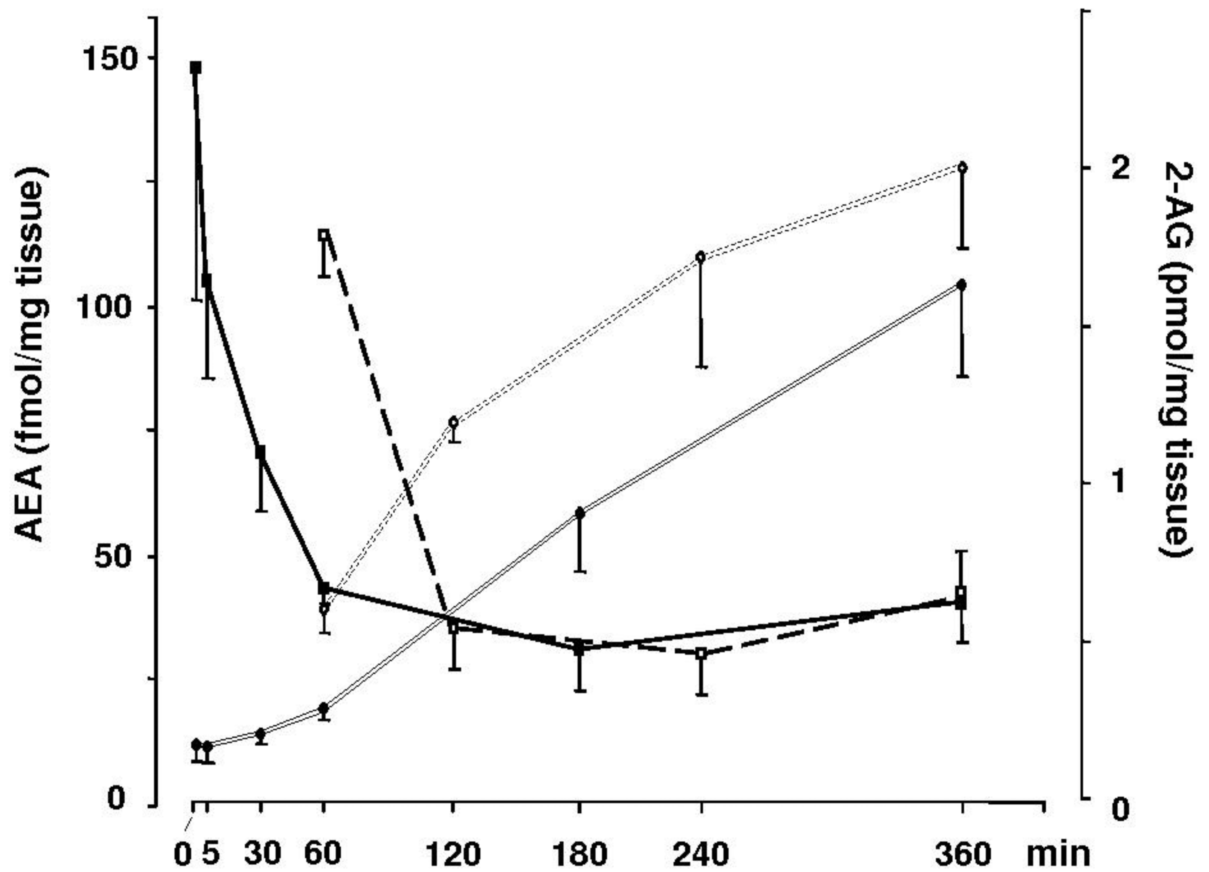

Fig. 4.
Comparison of postmortal changes in AEA and 2-AG levels measured in microdissected autopsy samples *versus* neurosurgical fronto-cortical specimens. Double lines: AEA; single lines: 2-AG; solid lines: neurosurgical samples; dashed lines: microdissected samples. Means ± SEM, n=3–6 for each time point.

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Table 1

Effect of the time of storage at −80 °C on the concentrations of AEA and 2-AG in human cerebrocortical specimens removed with a 1-1.5h postmortal delay.

Storage time in months	fmol AEA/mg tissue	pmol 2-AG/mg tissue
155	44.9	3.7
122	42.3	1.6
79	28.1	1.4
78	54.3	1.4
78	45.2	2.8
63	27.7	3.3
42	49.1	1.8
34	33.9	1.5
34	34.4	1.4
34	60.1	1.4
8	46.7	1.5
2	49.9	2.7

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Table 2

Postmortal changes in AEA and 2-AG concentrations in the frontal cerebral cortex measured in neurosurgical specimens

	AEA		2-AG	
	fmol/mg	**% of 0 min**	**pmol/mg**	**% of 0 min**
0 min	12.8	100	2.34	100
5 min	12.0	94	1.66	71
30 min	16.0	125	1.12	48
60 min	19.3	151	0.68	29
180 min	57.2	447	0.49	21
360 min	104.6	817	0.64	27

n = 4 for each time point

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Table 3

Concentrations of AEA and 2-AG in human brain regions and nuclei (1-1.5h postmortal delay).

	AEA fmol/mg	2-AG pmol/mg	2-AG:AEA ratio
cerebral cortex			
frontal cortex	39.9±4.9 (5)	1.8±0.3 (5)	50 ± 19
somatosensory cortex	39.1±8.5 (3)	2.0±0.4 (3)	45 ± 27
cingulate cortex	47.8±8.0 (4)	1.6±0.1 (5)	35 ± 11
striatum	68.1±8.2 (3)	2.3±0.1 (3)	31 ± 8
nucleus accumbens	96.3±13.0 (5)	1.1±0.1 (5)	16 ± 5
hypothalamus			
medial hypothalamus	87.6±9.2 (5)	5.9±1.8 (5)	77 ± 20
paraventricular nucleus	33.1– 80.6 (2)	2.4 – 7.3 (2)	72– 91
ventromedial nucleus	75.0– 95.1 (2)	3.7 – 8.0 (2)	50 – 84
dorsomedial nucleus	47.9–109.7 (2)	2.1 – 10.3 (2)	44 – 94
arcuate nucleus	71.9–106.0 (2)	4.3 – 6.9 (2)	60 – 65
lateral hypothalamus	86.3±10.2 (6)	8.6±2.9 (8)	98 ± 27
hippocampus	45.6±1.9 (7)	3.1±0.7 (8)	65 ± 16
amygdala	45.2±7.7 (5)	2.3±0.7 (5)	52 ± 14
cerebellum	25.0±1.9 (5)	1.9±0.3 (4)	83 ± 23
sensory trigeminal nucleus	42.7±9.3 (3)	6.0±1.4 (3)	140 ± 11
spinal cord (dorsal horn)	56.5±8.8 (3)	7.2±2.7 (3)	136 ± 34

values are means ± SEM, except for groups with n=2, where both values are shown. Number of samples in parentheses.