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DISTRIBUTION OF DIACYLGLYCEROL LIPASE ALPHA, AN ENDOCANNABINOID SYNTHESIZING ENZYME, IN THE RAT FOREBRAIN

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Abstract—1,2-diacylglycerol lipase alpha (DAGLα) is responsible for the biosynthesis and release of 2-arachidonoyl-glycerol (2-AG), the most abundant endocannabinoid in the brain. Although its expression has been detected in discrete re-

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Abbreviations: ac, anterior commissure; aca, anterior commissure, anterior part; AcbC, accumbens nucleus, core; AcbSh, accumbens nucleus, shell; aci, anterior commissure, intrabulbar part; ACo, anterior cortical amygdaloid nucleus; ADP, anterodorsal preoptic nucleus; AHi, anterior hypothalamic area; AI, agranular insular cortex; AOM, anterior olfactory nucleus, medial part; AON, anterior olfactory nucleus; AOP, anterior olfactory nucleus, posterior part; AOV, anterior olfactory nucleus, ventral part; APir, amygdalopiriform transition area; Arc, arcuate hypothalamic nucleus; ArcL, arcuate nucleus, lateral part; ArcM, arcuate nucleus, medial part; ArcMP, arcuate hypothalamic nucleus, medial posterior part; Au, primary auditory cortex; AV, anteroventral thalamic nucleus; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; BLA, basolateral amygdaloid nucleus, anterior part; BLP, basolateral amygdaloid nucleus, posterior part; BMP, basomedial amygdaloid nucleus, posterior part; BSA, bovine serum albumin; BST, bed nucleus of the stria terminalis; BSTLD, bed nucleus of the stria terminalis, lateral division, dorsal part; BSTLV, bed nucleus of the stria terminalis, lateral division, ventral part; BSTMA, bed nucleus of the stria terminalis, medial division, anterior part; CA1/CA2/CA3, field CA1/CA2/CA3 of hippocampus; CB1/CB2, cannabinoid receptor type 1/2; cc, corpus callosum; Ce, central amygdaloid nucleus; Cg, cingulate cortex; Cl, claustrum; cp, cerebral peduncle, basal part; CPu, caudate-putamen (striatum); CxA, cortex-amygdala transition zone; DAGLα/β, 1,2-diacylglycerol lipase alpha/beta; DEn, dorsal endopiriform nucleus; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; DM, dorsomedial hypothalamic nucleus; ec, external capsule; Ect, ectorhinal cortex; EPl, external plexiform layer of the olfactory bulb; FAAH, fatty acid amide hydrolase; fi, fimbria of the hippocampus; fmi, forceps minor of the corpus callosum; gcl, granular cell layer of the dentate gyrus; GI, granular insular cortex; GrO, granular cell layer of the olfactory bulb; HDB, nucleus of the horizontal limb of the diagonal band; Hb, habenula; ic, internal capsule; ICj, islands of Calleja; IG, indusium griseum; IGL, intergeniculate leaf; IPl, internal plexiform layer of the olfactory bulb; ir, immunoreactive; La, lateral amygdaloid nucleus; LaDL, lateral amygdaloid nucleus, dorsolateral part; LaVL, lateral amygdaloid nucleus, ventrolateral part; LaVM, lateral amygdaloid nucleus, ventromedial part; LDdm, laterodorsal thalamic nucleus, dorsomedial part; LDvl, laterodorsal thalamic nucleus, ventrolateral part; LEnt, lateral entorhinal cortex; LGP, lateral globus pallidus; LH, lateral hypothalamic area; LHb, lateral habenular nucleus; LM, lateral mammillary nucleus; lo, lateral olfactory tract; LOT, nucleus of the lateral olfactory tract; LP, lateral posterior thalamic nucleus; LSD, lateral septal nucleus, dorsal part; LSI, lateral septal nucleus, intermediate part; LSV, lateral septal nucleus, ventral part; LT, lateral terminal nucleus of the accessory optic tract; lv, lateral ventricle; MAGL, monoacylglyceride lipase; Me, medial amygdaloid nucleus; ME, median eminence; MG, medial geniculate nucleus; MGD, medial geniculate nucleus, dorsal part; MGM, medial geniculate nucleus, medial part; MGP, medial globus pallidus; MGV, medial geniculate nucleus, ventral part; MHb, medial habenular nucleus; Mi, mitral cell layer of the olfactory bulb; ml, medial lemniscus; ML, medial mammillary nucleus, lateral part; MM, medial mammillary nucleus, medial part; MnPO, median preoptic nucleus; MT, medial terminal nucleus of the accessory optic tract; M1/M2, primary/secondary motor cortex; NBT, nitroblue tetrazolium; O, orbital cortex; opt, optic tract; PAG, periaqueductal gray; PBS, phosphate-buffered saline; Pe, periventricular hypothalamic nucleus; PF, parafascicular thalamic nucleus; PIL, posterior intralaminar thalamic nucleus; Pir, piriform cortex; pl, polymorphic cell layer of the dentate gyrus; PMCo, posteromedial cortical amygdaloid nucleus; PPT, posterior pretectal nucleus; PRh, perirhinal cortex; PrL, prelimbic cortex; PT, paratenial thalamic nucleus; PVA, paraventricular thalamic nucleus, anterior part; PVH, paraventricular hypothalamic nucleus; PVHmc, paraventricular hypothalamic nucleus, magnocellular part; PVHpc, paraventricular hypothalamic nucleus, parvocellular part; PVP, paraventricular thalamic nucleus, posterior part; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; RMS, rostral migratory stream; RS, retrosplenial cortex; RT, room temperature; Rt, reticular thalamic nucleus; S, subiculum; S1/S2, primary/secondary somatosensory cortex; SC, superior colliculus; SFi, septofimbrial nucleus; SHi, septohippocampal nucleus; SI, substantia innominata; sl-m, stratum lacunosum-moleculare; sm, stria medullaris of the thalamus; SNC, substantia nigra, pars compacta; SNRdm, substantia nigra, pars reticulata, dorsomedial tier; SNRvl, substantia nigra, pars reticulata, ventrolateral tier; so/or, stratum oriens; SO, supraoptic nucleus; SOR, supraoptic nucleus, retrochiasmatic part; sp/py, stratum pyramidale; sr/ra, stratum radiatum; st, stria terminalis; StA, strial part of the preoptic area; SuM, supramammillary nucleus; SVZ, subventricular zone; TBS, Tris–HCl buffered saline; TuDC, olfactory tubercle densocellular layer; TuPl, olfactory tubercle plexiform layer; TuPo, olfactory tubercle polymorph layer; V2L, secondary visual cortex, lateral area; V2M, secondary visual cortex, medial area; VDB, nucleus of the vertical limb of the diagonal band; VLG, ventral lateral geniculate nucleus; VM, ventromedial thalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area; VTT, ventral tenia tecta; 2-AG, 2-arachidonoyl-glycerol; 3v, third ventricle.

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gions, we showed here an integrated description of the distribution of DAGLα mRNA and protein in the rat forebrain using *in situ* hybridization histochemistry and immunohistochemistry. As novelty, we described the distribution of DAGLα protein expression in the olfactory system, the rostral migratory stream, neocortex, septum, thalamus, and hypothalamus. Similar DAGLα immunostaining pattern was also found in the brain of wild-type, but not of DAGLα knockout mice. Immunohistochemical data were correlated by the identification of DAGLα mRNA expression, for instance, in the somata of specific cells in olfactory structures, rostral migratory stream and neocortex, cells in some septal-basal-amygdaloid areas and the medial habenula, and magnocellular cells of the paraventricular hypothalamic nucleus. This widespread neuronal distribution of DAGLα is consistent with multiple roles for endocannabinoids in synaptic plasticity, including presynaptic inhibition of neurotransmitter release. We discuss our comparative analysis of the forebrain expression patterns of DAGLα and other components of the endocannabinoid signaling system, including the CB_1 receptor, monoacylglyceride lipase (MAGL), and fatty acid amide hydrolase (FAAH), providing some insight into the potential physiological and behavioral roles of this system.

Key words: diacylglycerol lipase alpha, cannabinoid, forebrain, rostral migratory stream, *in situ* hybridization, immunohistochemistry.

The identification of cannabinoid CB_1 and CB_2 receptors in the brain suggests the presence of enzymes that synthesize and release endogenous ligands for these receptors (Pertwee, 1997; Di Marzo et al., 1998; Gong et al., 2006; Suárez et al., 2009). Endogenous cannabinoid ligands, such as anandamide and 2-arachidonoyl-glycerol (2-AG), influence a broad range of functions, including learning, memory, cognition, pain perception, appetite, mood, endocrine regulation, and motor activity (for review see Breivogel and Childers, 1998; Piomelli, 2003). A sn-1-specific diacylglycerol lipase (DAGL) catalyzes the hydrolysis of DAG to 2-AG, the most abundant endocannabinoid in the brain (Sugiura et al., 1995; Stella et al., 1997; Bisogno et al., 2003). 2-AG is an intermediate in triacyl/diacylglycerol metabolism as well as a prominent signaling molecule, thereby linking cannabinoid signaling with lysophospholipids and the diacylglycerol-PKC signaling system. The standard model proposes that activation of metabotropic receptors coupled to phosphatidyl-inositol-specific phospholipase C and the diacylclycerol lipase pathway systematically leads to increases in 2-AG production (Stella et al., 1997; Piomelli, 2003). This hypothesis was confirmed by the cloning of two isozyme 1,2-diacylglycerol lipases, DAGLα and DAGLβ (Bisogno et al., 2003).

2-AG biosynthesis is dependent on intracellular Ca^{2+} concentration—it occurs on demand by receptor-stimulated cleavage of lipid precursor and is released from neurons immediately afterward. In the developing brain, DAGL activity is required for axonal growth and guidance (Brittis et al., 1996; Williams et al., 2003). In the adult brain, 2-AG is synthesized postsynaptically and acts as a retrograde messenger on presynaptic cannabinoid receptors, suppressing synaptic transmission in several brain areas, including cerebellum, hippocampus, striatum, and other brain regions (Kreitzer and Regehr, 2001; Maejima et al., 2001; Wilson and Nicoll, 2001; Piomelli, 2003; Tanimura et al., 2009). Several data in support of this was provided by the detection of DAGLα in the brain and the localization of DAGLα expression in dendritic spines of hippocampal and amygdaloid pyramidal neurons, and cerebellar Purkinje cells (Yoshida et al., 2006, 2011; Suárez et al., 2008). However, the first direct evidence supporting the loss of retrograde 2-AG signaling in DAGL knockout mice was provided by Tanimura et al. (2009) and Gao et al. (2010). 2-AG signaling is terminated through the action of a transporter and a reuptake mechanism that remains elusive, as well as by hydrolysis mainly by the monoacylglyceride lipase (MAGL; Dinh et al., 2002; Yoshida et al., 2011). In addition, we cannot exclude the participation of other hydrolases, such as alpha-beta-hydrolase domain 6 and 12 (ABHD6, ABHD12), and neurophaty target esterasa (NTE), in the termination of 2-AG signaling (Blankman et al., 2007; Marrs et al., 2010).

Determining the anatomical and cellular distribution of DAGLα, which is much more abundant in the brain than DAGLβ, identifies sites of 2-AG synthesis and possible release. Although there are several studies that describe DAGLα distribution in discrete brain regions (Katona et al., 2006; Yoshida et al., 2006, 2011), to date there is no report with detailed *in situ* hybridization and immunohistochemical analysis of DAGLα expression in the olfactory system, neocortex, septum, thalamus, and hypothalamus that could provide an integral view of DAGLα distribution in the entire rat forebrain. Here, we present a comprehensive description of DAGLα mRNA and protein localization in these and others regions of the rat forebrain. We compare this distribution pattern to the expression patterns of other components of the endocannabinoid signaling system, providing some insight into the potential physiological and behavioral roles of this system.

EXPERIMENTAL PROCEDURES

Animals

The maintenance of the animals as well as the experimental procedures followed the guidelines of the European Union (Council Directive 86/609/EEC) and according to the guidelines of the animal welfare committees of the University of Tokyo, Hokkaido University. This study was carried out in inbred adult (2–3 months old) male rats, and wild-type and DAGLα-knockout mice (4–6 weeks old, C57BL/6N strain; kindly donated by Professor Masahiko Watanabe). DAGLα-KO mice were generated by disrupting exon 3 and 4 of *DAGLα* gene, as was described by Tanimura et al. (2009). Rats and mice received food and water *ad libitum* and were kept in a 12-h light/dark cycles.

Tissue preparation

Animals were sacrificed 6 h after onset of light cycle. Rats and mice were deeply anesthetized with sodium pentobarbital (40 mg/kg i.p.) and briefly transcardially perfused with 0.1 M phosphate-buffered saline (RNase-free PBS; pH 7.4), followed by 4% paraformaldehyde (PFA) in RNase-free PBS at 4 °C for 30 min. Brains were dissected, postfixed by immersion in the same fixative overnight at 4 °C. Rat brains were cut into 30-μm-thick sections