Cell Tissue Res (2001) 306:391–398
DOI 10.1007/s004410100461

REGULAR ARTICLE

**Roberta Cesa · Ken Mackie · Massimiliano Beltramo
Maria Fosca Franzoni**

Cannabinoid receptor CB1-like and glutamic acid decarboxylase-like immunoreactivities in the brain of *Xenopus laevis*

Received: 10 April 2001 / Accepted: 10 August 2001 / Published online: 25 September 2001

Abstract Investigation of the cannabinoid system in a vertebrate group phylogenetically distant from mammals might improve understanding of its physiological role. Thus, in the present study, the distribution of the cannabinoid CB1 receptor has been investigated in the brain of *Xenopus laevis* (anuran amphibians) by immunohistochemistry, using both light and confocal laser-scanning microscopy. Immunostained neuronal perikarya and terminals were found in the olfactory bulb, dorsal and medial pallium, striatum, and amygdala. Varicosities and nerve terminals containing CB1-like immunoreactivity were also seen in the thalamus and hypothalamus. A number of stained cells were observed in the pars distalis of the pituitary gland. Positive nerve fibers were distributed throughout mesencephalic tegmentum, and in the cerebellum immunolabeling was observed in some Purkinje and possibly Golgi cells. The confocal microscopic analysis of CB1-like and glutamic acid decarboxylase-like immunoreactivities in both the medial pallium of the telencephalon and the olfactory bulbs showed a wide co-distribution of the two markers. The present results indicate that distribution of CB1 is conserved in the course of phylogeny. Furthermore, the close relationship between CB1-like and glutamic acid decarboxylase-like immunolabelings point toward the existence of a functional link between cannabinergic and GABAergic innervations also in amphibian brain.

Keywords Cannabinoid receptor CB1 · Glutamic acid decarboxylase · Confocal microscopy · *Xenopus laevis* (Anura)

Work supported by MURST (MFF), NIDA grants DA11322 and DA00286 (K.M.), Fondazione Cavalieri Ottolenghi (fellowship to R.C.)

R. Cesa · M.F. Franzoni (✉)
Dipartimento di Biologia Animale e dell'Uomo,
Università degli Studi di Torino, via Accademia Albertina 13,
10123 Turin, Italy
e-mail: mariafosca.franzoni@unito.it
Tel.: +39-011-6704679, Fax: +39-011-6704692

K. Mackie
Department of Anesthesiology, University of Washington,
Seattle, WA 98195, USA

K. Mackie
Department of Physiology and Biophysics,
University of Washington, Seattle, WA 98195, USA

M. Beltramo
Schering-Plough Research Institute,
San Raffaele Biomedical Science Park, Milan, Italy

Present address:
R. CesaRita Levi Montalcini Center for Brain Repair,
Dipartimento di Neuroscienze, Università degli Studi di Torino,
C.so Raffaello 30, 10125 Turin, Italy

Introduction

The main psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (THC), produces a number of behavioral and physiological effects in human and rodents. These effects are mediated by receptor activation as demonstrated by cloning and pharmacological characterization of two cannabinoid receptor subtypes, CB1 (Matsuda et al. 1990) and CB2 (Munro et al. 1993), which are located in the mammalian central nervous system (CNS) and in cells of lymphoid or myeloid origin involved in immune and inflammatory reactions. The discovery of putative endogenous ligands such as anandamide (Devane et al. 1992) and 2-arachidonylglycerol (2-AG; Mechoulam et al. 1995) suggests the existence of an endogenous cannabinoid system in the rodent brain. However, the localization of the cannabinergic neurons (i.e., the neurons producing anandamide and 2-AG) faces several difficulties. Because of their lipid nature, these molecules have poor antigenic properties, hampering a direct immunocytochemical approach. Furthermore, the enzymes involved in anandamide and 2-AG formation have been only partially characterized (Desarnaud et al. 1995) and consequently no antibodies against them have been produced. Thus, the information available on the localization of the cannabinergic neurons has been mainly obtained from receptor-binding autoradiography

(Herkenham et al. 1990) and in situ hybridization (Matsuda et al. 1993) studies. Recently, additional information has been gathered on the organization of the rat brain cannabinoid innervation by using antibodies directed against either the CB1 receptor (Tsou et al. 1998a) or the enzyme involved in anandamide degradation (anandamide amidohydrolase; Tsou et al. 1998b). The cannabinoid system is present in many cerebral regions (cortex, hippocampus, basal ganglia, cerebellum, etc.), where it probably modulates cognitive as well as sensorimotor and homeostatic functions (Piomelli et al. 1998; Elphick and Egertova 2001). To better elucidate the functional meaning of the central cannabinoid receptor, relationships between CB1 and other neurotransmitters have been investigated. For example pharmacological and physiological results have suggested the possibility that CB1 may be present in some neuronal populations containing GABA (Maneuf et al. 1996; de Miguel et al. 1988; Szabo et al. 1998; Tsou et al. 1998a; Katona et al. 1999). All these results were collected from studies performed in rat and human brains. Nevertheless, several observations suggest that the cannabinergic system may be phylogenetically very ancient. Receptors with molecular and/or pharmacological characteristics similar to those of CB1 have been described in both nonmammalian vertebrates (Howlett et al. 1990; Van der Kloot 1994; Yamaguchi et al. 1996; Soderstrom et al. 2000) and invertebrates (Elphick 1998).

In the present report, we used an antibody directed against rat CB1 (Tsou et al. 1998a) to map the CB1 immunoreactivity (IR) in the CNS of the toad *Xenopus laevis*. In addition, we compared the distribution of CB1-like IR with that of glutamic acid decarboxylase (GAD, an enzyme involved in GABA biosynthesis) in some areas of the *Xenopus* prosencephalon by using double immunostaining techniques. Some sections processed for double immunofluorescence (IFL) were analyzed by both fluorescence and confocal laser-scanning microscopy (CLSM).

Materials and methods

The experiments were performed under the guidelines established by the Italian law for animal welfare. Adult *X. laevis* (*n*=4) of both sexes were deeply anesthetized with 1:1,000 tricaine methanesulphonate (MS222; Sandoz, Basel, Switzerland) and transcardially perfused with Zamboni's fixative. Brains were removed, postfixed overnight, cryoprotected, and frozen in liquid isopentane.

ABC and double ABC/IFL techniques

Coronal sections (12 µm thick), obtained with a cryostat, were mounted onto gelatinized slides and then incubated overnight with an affinity-purified CB1 receptor primary polyclonal antibody raised in rabbit (1:1,000–2,000 dilutions in 0.01 M PBS+0.1% Triton-X100). The sections were then incubated with a biotinylated goat anti-rabbit antibody (1:200 dilution) for 1 h followed by avidin-biotin complex (ABC; Vector Laboratories, Burlingame, Calif.), and immunoreactivity was revealed by treating the sections with diaminobenzidine (DAB) as a chromogen. Some of these sections were then processed for IFL by using a polyclonal primary anti-GAD antibody, raised in sheep (1:500; courtesy of Dr. Tappaz, INSERM U433, Laennec, Lyon, France), which mainly recognizes the isoform GAD_{65} (Oertel et al. 1981). The sections were incubated with a secondary biotinylated horse anti-goat antibody (1:200, Vector Laboratories, Burlingame, Calif.), for 1 h at room temperature. The immunoreaction was revealed by Texas red-avidin (1:400; Vector Laboratories, Burlingame, Calif.) and photographed with a fluorescence photomicroscope (Orthoplan Leitz equipped with a filter set I2).

Double IFL

Selected sections, incubated overnight with a mixture (1:1) containing both the anti-CB1 (1:500) and anti-GAD (1:500) antibodies, were treated for 1 h at room temperature with a mixture (1:1) of the following secondary antibodies (Battaglia et al. 1995): a swine anti-rabbit coupled to tetramethylrhodamine (TRITC, 1:40; Dako, Denmark) plus a donkey anti-goat coupled to fluorescein isothiocyanate (FITC, 1:60; Chemicon International, USA) or a swine anti-rabbit coupled to fluorescein isothiocyanate (FITC, 1:60; Chemicon International, USA) plus a donkey anti-goat coupled to tetramethylrhodamine (TRITC, 1:40 dilution; Dako, Denmark). The sections were observed and photographed with a fluorescence photomicroscope (Orthoplan Leitz equipped with a filter set I2). Selected sections were observed with a CLSM by using standard protocol with a TSC-4d confocal imaging system (Leica Instruments, Heidelberg, Germany), equipped with an argon-krypton ion laser (excitation wavelengths, 488/568 nm) allowing simultaneous scanning of two fluorescent dyes. Processing of the images was done with the software Adobe Photoshop 5.0.

Controls

Specificity of the antibody anti-CB1 was assessed in rat (Tsou et al. 1998a) and confirmed in *X. laevis* by the complete lack of immunostaining in sections treated with the CB1 antibody previously adsorbed with the immunizing fusion protein. Specificity of the antibody GAD antibody had been demonstrated previously in rat (Oertel et al. 1981) and in *Xenopus* (Barale et al. 1996).

Results

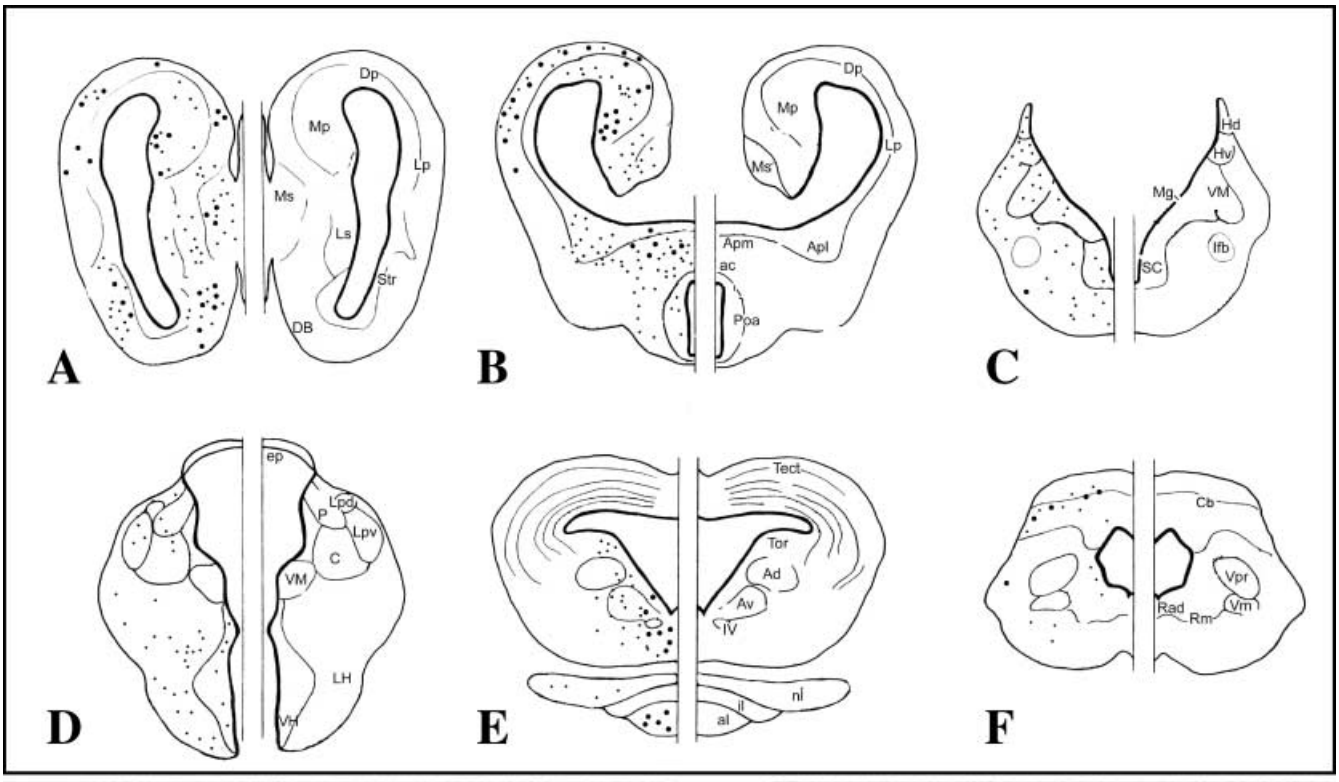
CB1-like IR in the *Xenopus* CNS was abundant in defined regions of the forebrain, although immunoreactive cell bodies were also observed in the brain stem and cerebellum. The CB1-IR distribution is shown in drawings of representative coronal sections of the *Xenopus* brain (Fig. 1A–F, modified according to Gonzalez and Smeets 1992).

The specificity of the CB1 antibody was confirmed in the telencephalic dorsal pallium of *X. laevis* by the complete lack of immunostaining in a section treated with the CB1 antibody adsorbed with the immunizing fusion protein (Fig. 1G). For comparison a consecutive section incubated with the nonpreadsorbed CB1 antibody is shown in Fig. 1H.

CB1-like IR in the forebrain

Olfactory bulbs

Fine immunoreactive puncta were found throughout all the layers of the olfactory bulbs even they were more abundant in the fibrous and glomerular layers. In the latter a number of small and medium-sized perikarya, pos-



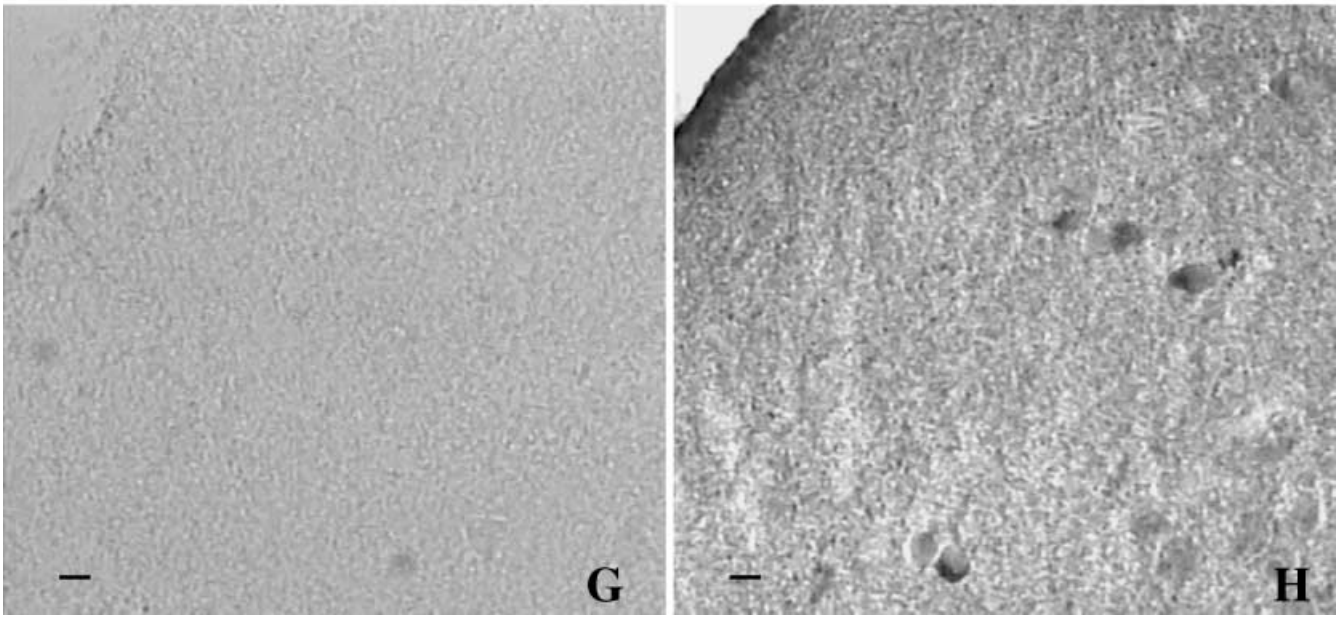


Fig. 1 A–F Sites of CB1-like immunoreactivity (IR) in coronal sections of the *Xenopus* brain (modified after Gonzalez and Smeets 1992). *Black dots*, immunoreactive cell bodies; *black points*, immunoreactive nerve fibers and terminals. **G, H** CB1 IR in coronal sections of the telencephalic hemispheres of *Xenopus* brain. **G** No CB1-like IR was detected in the dorsomedial pallium after preincubation of the primary antiserum with the immunizing protein. **H** In a consecutive section, incubated with the CB1 antibody, some CB1-like immunostained piriform neurons and terminals are found in the same area (ABC technique). *(ac* Anterior commissure, *Ad* anterodorsal tegmental nucleus, *al* anterior lobe of the pituitary gland, *Apl* amygdala pars lateralis, *Apm* amygdala pars medialis, *Av* anteroventral tegmental nucleus, C nucleus centralis thalami, *Cb* cerebellum, *DB* diagonal band, *Dp* dorsal pallium, *ep* epiphysis cerebri, *Hd* dorsal habenular nucleus, *Hv* ventral habenular nucleus, *lfb* lateral forebrain bundle, *il* intermediate lobe of the pituitary gland, *LH* lateral hypothalamus, *Lpd* lateral thalamus pars dorsalis, *Lpv* lateral thalamus pars ventralis, *Ls* lateral septum, *Mg* magnocellular preoptic nucleus, *Mp* medial pallium, *Ms* medial septum, *nl* neural lobe of the pituitary gland, *P* nucleus posterior thalami, *Poa* anterior preoptic nucleus, *Rad* raphe dorsal nucleus, *Rm* nucleus reticularis superior, *SC* suprachiasmatic nucleus, *Str* striatum, *Tect* tectum, *tor* torus semicircularis,, *VH* ventral hypothalamus, *VM* nucleus ventromedialis thalami, *Vm* trigeminal nerve, motor nucleus, *Vpr* trigeminal nerve, principal sensory nucleus, *IV* trochlear nerve.) *Bars* **G, H** 10 μm

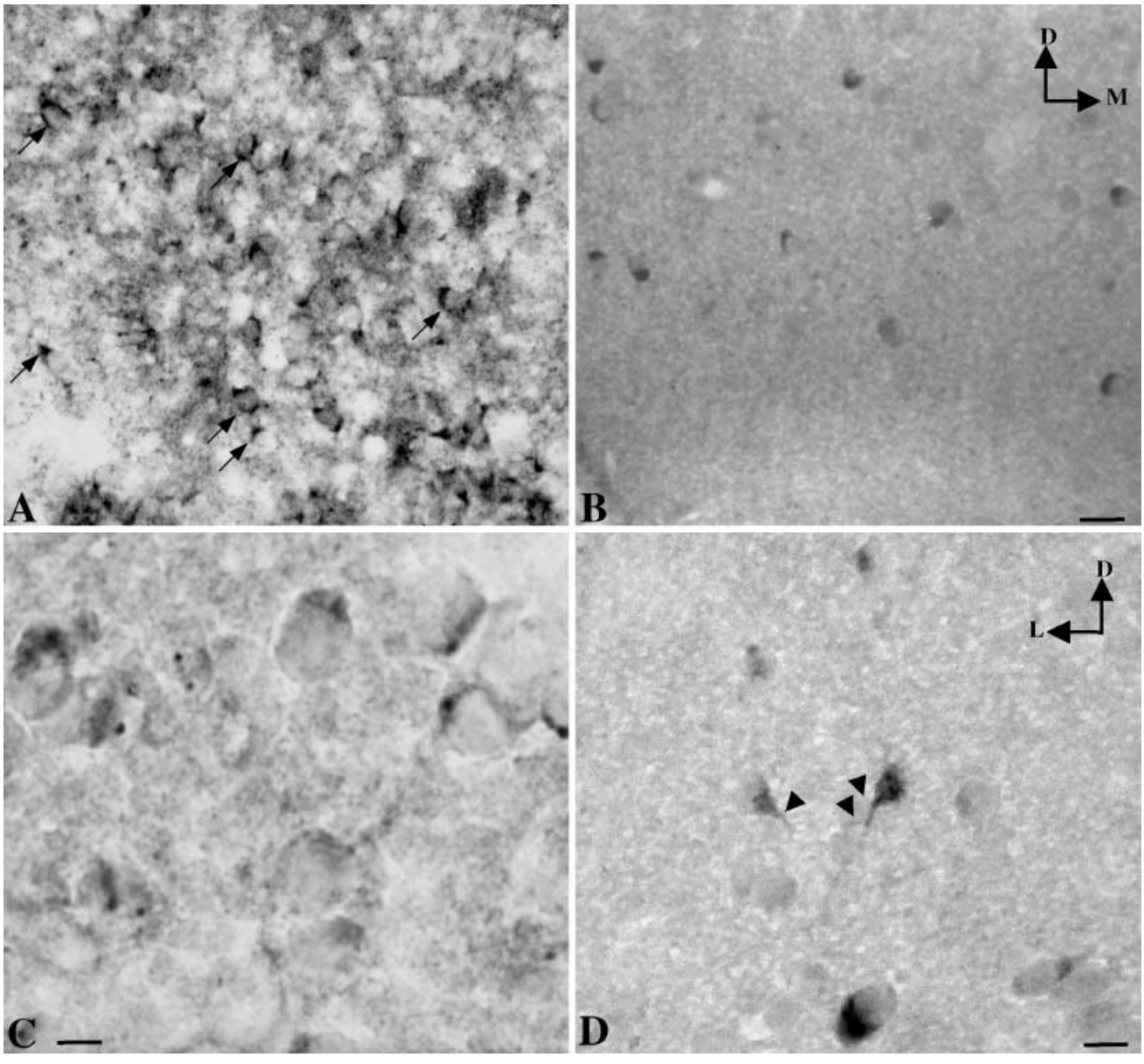


Fig. 2A–D CB1 IR in the *Xenopus* brain. **A** Longitudinal section of the *Xenopus* olfactory bulb showing the glomerular layer. Immunostained cell bodies, possibly belonging to periglomerular cells (*arrows*) together with dot-like nerve terminals were seen. **B–D** Coronal sections of the *Xenopus* telencephalon. **B** Piriform immunolabeled cell bodies and punctuate terminals in the medial pallium. **C** Immunopositive cell bodies in the dorsal pallium. **D** Amygdala, pars medialis: unipolar "tufted" neurons showing a strong CB1-like IR in both perikarya and dendritic stumps (*arrowheads*). ABC technique. *Bars* **A, B** 20 µm; **C** 5 µm; **D** 10 µm

sibly belonging to periglomerular neurons, were immunolabeled (Fig. 2A).

Telencephalic hemispheres

Immunostained piriform neurons were distributed throughout the walls of the telencephalic hemispheres, namely in the dorsolateral and the medial pallium (Figs. 2B, C). The number of the labeled pallial neurons increased in the caudal third of the hemispheres. Beaded nerve fibers and fine dots were particularly abundant in the medial pallium. Labeled polymorphic neurons, many of which belonging to the "tufted" type, were found within the amygdala, pars medialis (Fig. 2D), and in the striatum and septum. The immunoreactivity of these cells frequently displayed a triangular cap-like shape located at one end of the perikaryon and extending into the proximal dendritic stump (Fig. 2D).

Diencephalon

Besides the positive nerve terminals distributed throughout the habenular complex, a rich CB1-like-immunoreactive innervation was observed in the lateral wall of the dorsal and ventral thalamus (Fig. 3A). Positive fibers

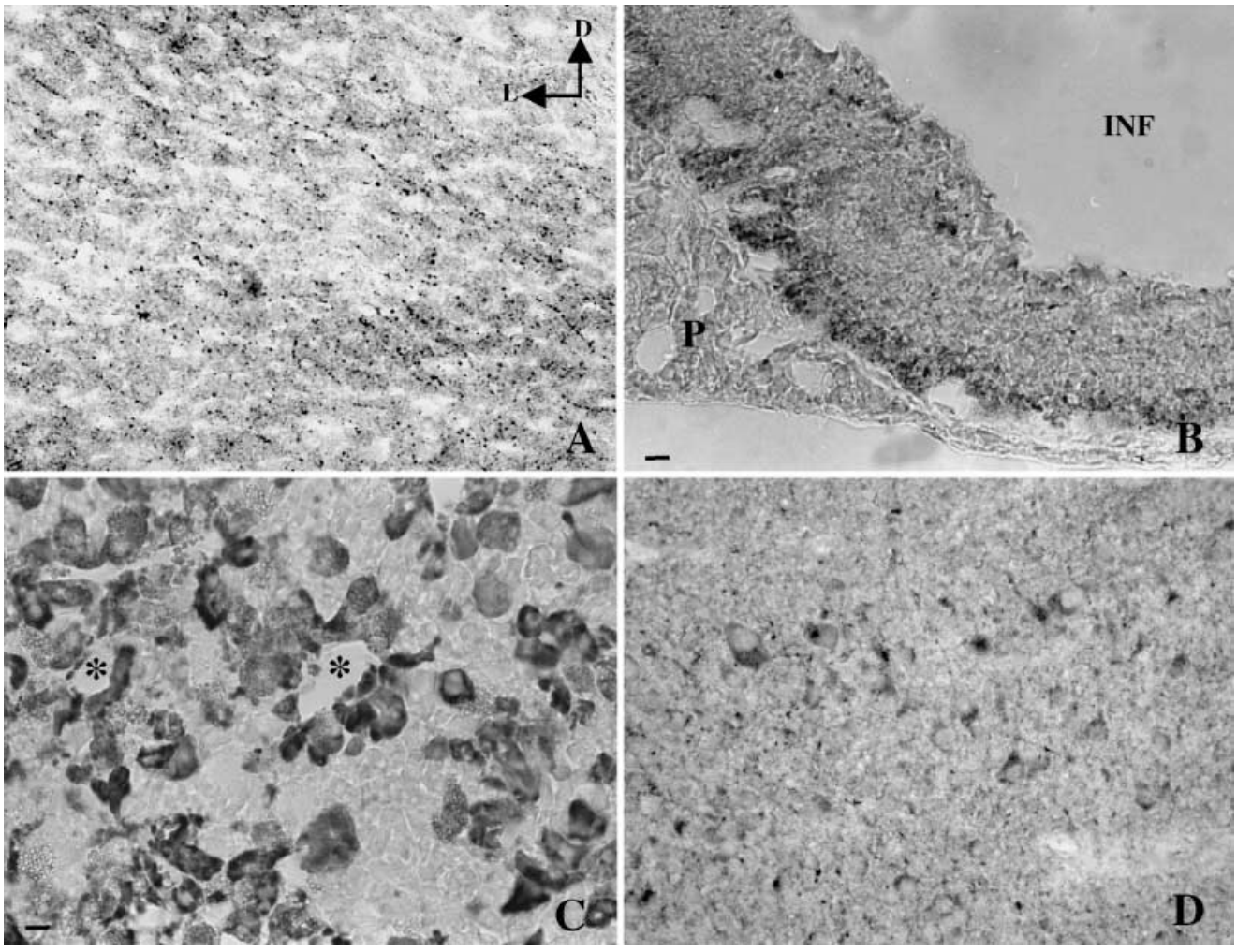


Fig. 3A–D CB1 IR in coronal (**A, C, D**) and sagittal (**B**) sections of the *Xenopus* brain. **A** Immunolabeled nerve terminals and fibers in the lateral wall of the ventral thalamus. **B** Numerous CB1-immunopositive nerve fibers were found in the palisade zone of the external layer of the median eminence. The pituitary (*P*) region adjacent to the median eminence was completely devoid of CB1-like IR (*INF* infundibular recess). **C** Numerous CB1-immunopositive endocrine cells in the adenohypophysial pars distalis. *Asterisks* blood capillaries. **D** Immunolabeled cell bodies of variable size together with nerve terminals in the ventral tegmentum of the mesencephalon. ABC technique. *Bars* **A, B** 20 µm; **C, D** 10 µm

were found throughout the hypothalamus and they became very abundant in the palisade zone of median eminence external layer (Fig. 3B). Numerous strongly labeled cells occurred within the pars distalis of the pituitary gland (Fig. 3C) except for the ventrorostral region, closely adjacent to the median eminence, where the adrenocorticotropic hormone (ACTH)-secreting cells are mainly concentrated (Fig. 3B).

CB1-like IR in the hindbrain

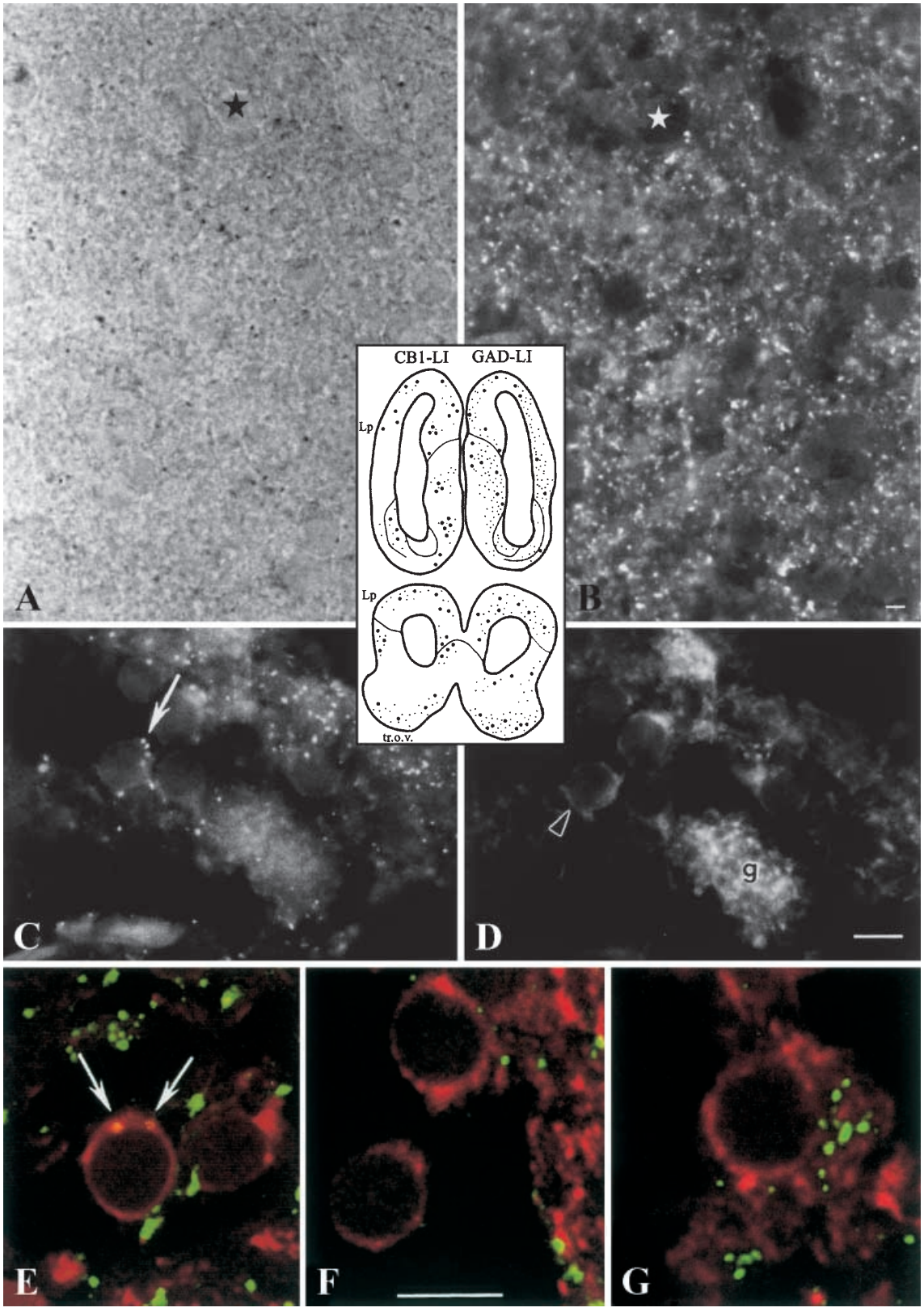
The CB1-like IR was generally less abundant in the hindbrain than in the forebrain. Positive nerve fibers and neurons were found in the periventricular gray of the ventral mesencephalic tegmentum (Fig. 3D). Caudally, a few immunostained cells were localized in the lateral isthmus and medulla oblongata.

In the cerebellum, CB1-like IR was observed in some small and medium-sized neurons that were located in the lateral part of the corpus cerebelli below the Purkinje neuron layer and that possibly represent Golgi cells (data not shown). Some Purkinje cells seemed to be stained.

CB1/GAD-like IRs in the forebrain

A rich GAD-like-immunolabeled network of nerve terminals and fibers is characteristic of some pallial regions of the *Xenopus* telencephalon such as the dorsolateral and medial pallium (Fig. 4, inset, upper part). As shown by double immunolabeling, a considerable number of nerve terminals containing CB1-like IR and GAD-like-immunoreactive dots were codistributed in these regions (Fig. 4A, B).

The olfactory bulbs contained many CB1- and GAD-like immunoreactive nerve fibers (Fig. 4, inset, lower part). In particular, the glomerular layer comprised a conspicuous CB1-like immunostaining with punctuate


A
B
CB1-LI
GAD-LI
Lp
Lp
tr.o.v.
C
D
g
E
F
G

appearance (Fig. 4C). The immunoreactive dots were observed either in close contact to the plasma membrane of GAD-like immunoreactive interneurons, possibly corresponding to periglomerular cells (Fig. 4D) or tightly intermingled with GAD-immunopositive nerve fibers and terminals. Immunofluorescence and CSLM showed that CB1-IR actually coexisted with GAD-like labeling in some periglomerular neurons (Fig. 4E). More frequently, CB1-positive terminals were intermingled with GAD-containing cell bodies or in close proximity to their plasma membrane (Fig. 4E–G).

Discussion

In the present study we have analyzed the distribution of CB1 receptors in the CNS of *Xenopus laevis* by means of immunohistochemistry and a specific primary antibody raised against the amino-terminus of the CB1 receptor. The specificity of this antibody had been assessed previously (Tsou et al. 1998a) and the complete lack of immunostaining in the brain sections of *Xenopus* after the preabsorption of the CB1 antibody with the fusion protein has confirmed that the immunoreaction was specific for the amino-terminus sequence of the CB1 receptor. Recently the pharmacological and molecular characterization of the CB1 in the brain of a urodele amphibian, *Taricha granulosa* (Soderstrom et al. 2000), has demonstrated that CB1 of this species is very similar in ligand-binding specificity, expression levels, and signal transduction to that of mammals. Moreover, the comparison between the amino acid sequence alignments of *Taricha* and rat CB1 (Soderstrom et al. 2000) has shown a high degree of homology (84% of identity) between the primary structures.

Our immunohistochemical findings have shown that an abundant CB1-like IR is present in the *Xenopus* forebrain, especially in the olfactory bulbs, lateral and medial pallium, amygdala, septum, and striatum. Furthermore the ventral thalamus, and the tuberal hypothalamus and median eminence were innervated by positive nerve fibers and terminals. By contrast, in the hindbrain, the immunoreaction was present in cell bodies and nerve fibers of the mesencephalic ventral tegmentum and in Golgi cells of the cerebellum. These data indicate that, at least in some regions of the amphibian forebrain, CB1-IR shows a distribution comparable with that described in mammals, suggesting that the cannabinoids may subserve similar functions in these two vertebrate groups. This may be the case, for example, in the *Xenopus* medial pallium and hypothalamus, which are considered homologous to the mammalian limbic system. The abundant CB1-like IR observed in there regions suggests that endocannabinoids could be involved in the control of some neuroendocrine functions. Influence of cannabinoids on pituitary hormone regulation has indeed been reported in rodents (de Miguel et al. 1988; Weidenfeld et al. 1994; Wenger et al. 1995; Fernandez-Ruiz et al. 1997) and attributed to interactions with hypothalamic neurotransmitters such as norepinephrine (Murphy et al. 1990) and GABA (de Miguel et al. 1988). On the other hand, the presence of CB1-like IR in the median eminence (palisade zone of the external layer) and a number of endocrine cells in *Xenopus* pituitary pars distalis may suggest that, in this species, the cannabimimetic action could be accomplished at both hypothalamic and pituitary levels.

The relationships between cannabinoids and classic neurotransmitters have been inferred mainly from pharmacological and physiological investigations (de Miguel et al. 1988; Murphy et al. 1990; Maneuf et al. 1996; French et al. 1997; Pettit et al. 1998), and it has been suggested that CB1 receptors are expressed by tuberoinfundibular (de Miguel et al. 1988), striatal (Maneuf et al. 1996), and cortical (Tsou et al. 1998a) GABAergic neurons. In the present study double immunohistochemical techniques were used to investigate the morphological relationships between CB1- and GAD-like IRs in the telencephalic medial pallium and olfactory bulb, which are both characterized by dense networks of CB1-containing nerve fibers and GABA (Franzoni and Morino 1989). With regard to the glomerular layer of the olfactory bulb, our results point out that a few GABAergic interneurons seem to contain also CB1-like IR but, more frequently, CB1-immunolabeled terminals are in tight apposition to GABAergic cell bodies. These observations suggest the possibility of a modulation by CB1 receptors on terminals presynaptic to GABA neurons.

In conclusion, similarities of CB1-like IR distribution in mammalian and amphibian CNS confirm that the cannabinoid system is phylogenetically ancient and may be involved in the control of relevant physiological activities, possibly through interactions with classic neurotransmitters. However, the comparison between *Xenopus* and mammals has revealed some discrepancies. CB1 IR is, for example, abundant in *Xenopus* olfactory bulbs and, in general, in all centers functionally connected to the olfaction (olfactory nuclei and amygdala), as well as

◀ **Fig. 4** **A, B** Double immunolabeling of the same coronal section of the telencephalic medial pallium showing **A** the CB1-like IR innervation (ABC technique) codistributed with **B** glutamic acid decarboxylase (GAD)-like IR (immunofluorescence, IFL). The *stars* indicate the same cell. *Inset:* CB1-like IR (*LI, left*) and GAD-LI (*right*) in two schematic coronal sections of the telencephalic hemispheres (*upper part of the inset*) and olfactory bulbs (*lower part of the inset*). *Black dots* neurons; *black points* nerve terminals. **C, D** Coronal section of the olfactory bulb double-labeled with the CB1 antibody (tetramethylrhodamine, TRITC; **C**) and the GAD antibody (fluorescein isothiocyanate, FITC; **D**). Punctuate CB1-immunoreactive terminals are seen in close apposition (*arrow*) to GAD-like-immunopositive periglomerular cell (*arrowhead*). **G** Glomeruli. **E–G** CLSM analysis of neurons stained with the CB1-antibody (FITC) and the GAD antibody (TRITC) in the olfactory bulb of *Xenopus*. Some GAD-like-immunopositive neurons and CB1-like-immunoreactive nerve terminals and processes were seen. *Yellow* (*arrows* in **E**) indicates coexistence of the two markers in the same cell. CB1-like-immunopositive punctuate nerve terminals are also in close contiguity with GAD-like-positive neurons (**E–G**). (*Lp* Lateral pallium, *tr.o.v.* ventral olfactory tract.) *Bars* **A, B** 5 μm; **C–G** 10 μm

in the lateral pallium, where polysensory information is integrated (ten Donkelaar 1998). In the rodent brain, by contrast, a considerable amount of CB1-IR was found in areas related to motor activity (such as basal ganglia and cerebellum; Tsou et al. 1998a). These observations suggest that endocannabinoids play a modulatory role in amphibian sensory and integrative centers and that this signaling system may implement its function through different mechanisms over the course of the phylogeny. Further analysis integrating pharmacological, physiological, and behavioral studies of the amphibian cannabinoid system will help to shed light on this interesting aspect of cannabinoid neuromodulatory function.

Acknowledgements We thank Prof. A. Calas (Institut de Neurosciences, Université Paris VII, Paris) for the CLSM availability; Mr. Claudio Gendusa and Dr. Daniela Donna for help with image processing.

References

Barale E, Fasolo A, Girardi E, Artero C, Franzoni MF (1996) Immunohistochemical investigation of γ-aminobutyric acid ontogeny and transient expression in the central nervous system of *Xenopus laevis* tadpoles. J Comp Neurol 368:285–294

Battaglia AA, Feuilloley M, Mulatero B, Beltramo M, Thibault J, Franzoni MF, Calas A, Vaudry H, Fasolo A (1995) Confocal microscopy analysis of NPY and TH immunoreactivities in the hypothalamo-hypophysial system of the frog. Neuroreport 6: 645–649

Desarnaud F, Cadas H, Piomelli D (1995) Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. J Biol Chem 270:6030–6035

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum D (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946–1949

Donkelaar HJ ten (1998) Anurans. In: Nieuwenhuys R, Donkelaar HJ ten, Nicholson C (eds) The central nervous system of vertebrates, vol 2. Springer, Berlin Heidelberg New York, pp 1151–1314

Elphick MR (1998) An invertebrate G-protein coupled receptor is a chimeric cannabinoid/melanocortin receptor. Brain Res 780:170–173

Elphick MR, Egertova M (2001) The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc Lond B Biol Sci 356:381–408

Fernandez-Ruiz JJ, Munoz RM, Romero J, Villanua MA, Makriyannis A, Ramos JA (1997) Timecourse of the effects of different cannabimimetics on prolactin and gonadotropin secretion: evidence for the presence of CB1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. Biochem Pharmacol 53:1919–1927

Franzoni MF, Morino P (1989) The distribution of GABA-like-immunoreactive neurons in the brain of the newt, *Triturus cristatus carnifex*, and the frog, *Rana esculenta*. Cell Tissue Res 255:155–166

French ED, Dillon K, Wu X (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. Neuroreport 8:649–652

Gonzalez A, Smeets WJAJ (1992) Distribution of vasotocin- and mesotocin-like immunoreactivities in the brain of the South African clawed frog, *Xenopus laevis*. J Chem Neuroanat 5:465–479

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, Costa BR de, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci USA 87:1932–l936

Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M (1990) The cannabinoid receptor: biochemical, anatomical and behavioral characterization. Trends Neurosci 13:420–423

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, F Freund T (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19:4544–4558

Maneuf YP, Crossman AR, Brotchie JM (1996) Modulation of GABAergic transmission in the globus pallidus by the synthetic cannabinoid WIN 55,212-2. Synapse 22:382–385

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564

Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327:535–550

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almong S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83–90

Miguel R de, Romero J, Munoz MR, Garcia-Gil L, Gonzalez S, Villanua MA, Makriyannis A, Ramos JA, Fernandez-Ruiz JJ (1988) Effects of cannabinoids on prolactin and gonadotropin secretion: involvement of changes in hypothalamic γ-aminobutyric acid (GABA) inputs. Biochem Pharmacol 56:1331–1338

Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365: 61–65

Murphy LL, Steger RW, Smith MS, Bartke A (1990) Effects of Δ9-tetrahydrocannabinol, cannabinol and cannabidiol alone and in combinations, on luteinizing hormone and prolactin release and hypothalamic neurotransmitters in the male rat. Neuroendocrinology 52:316–321

Oertel WH, Schmechel DE, Tappaz ML, Kopin IJ (1981) Production of a specific antiserum to rat brain glutamic acid decarboxylase by injection of an antigen-antibody complex. Neuroscience 6:2689–2700

Pettit DAD, Harrison MP, Olson J M, Spencer RF, Cabral GA (1998) Immunohistochemical localization of the neural cannabinoid receptor in rat brain. J Neurosci Res 51:391–402

Piomelli D, Beltramo M, Giuffrida A, Stella N (1998) Endogenous cannabinoid signaling. Neurobiol Dis 6:462–473

Soderstrom K, Leid M, Moore FL, Murray TF (2000) Behavioral, pharmacological and molecular characterization of an amphibian cannabinoid receptor. J Neurochem 75:413–423

Szabo B, Dorner L, Pfreundtner C, Norenberg W, Starke K (1998) Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. Neuroscience 85:395–403

Tsou K, Brown S, Sanudo-Pena C, Mackie K, Walker JM (1998a) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411

Tsou K, Nogueron MI, Muthian S, Sanudo-Pena MC, Hillard CJ, Deutsch DG, Walker JM (1998b) Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. Neurosci Lett 254:137–140

Van der Kloot W (1994) Anandamide, a naturally occurring agonist of the cannabinoid receptor, blocks adenylate cyclase at the frog neuromuscular junction. Brain Res 649:181–184

Weidenfeld J, Feldman S, Mechoulam R (1994) Effect of the brain constituent anandamide, a cannabinoid receptor agonist, on the hypothalamo-pituitary-adrenal axis in the rat. Neuroendocrinology 59:110–112

Wenger T, Toth BE, Martin BR (1995) Effects of anandamide (endogen cannabinoid) on anterior pituitary hormone secretion in adult ovariectomized rats. Life Sci 56:2057–2063

Yamaguchi F, Macrae DA, Brenner S (1996) Molecular cloning of two cannabinoid type 1-like receptor genes from the puffer fish *Fugu rubripes*. Genomics 35:603–605