Acta Biologica Hungarica 54 (1), pp. 45–53 (2003)

KAPPA-OPIOID RECEPTOR IN THE RODENT HIPPOCAMPUS: A COMPARATIVE IMMUNOCYTOCHEMICAL STUDY IN THE RAT, GUINEA PIG, HAMSTER AND GERBIL

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(Received: January 29, 2002; accepted: February 24, 2002)

Pre-embedding light microscopic immunocytochemistry, using a monoclonal antibody (mAb-KA8) raised against a frog brain kappa receptor preparation, recognising selectively the kappa-opioid receptor, was used for studying the occurrence, distribution, and species-specificity of the kappa-opioid receptor in the hippocampal formation of four rodent species (rat, guinea pig, hamster and gerbil). MAb-KA8 immunoreactivity was detectable in the rat, hamster and gerbil hippocampus, however the distribution of the labelled structures was heterogeneous. In the rat and hamster the hilus of dentate gyrus and the stratum oriens of the CA1 area contained immunoreactive cell bodies and proximal dendrites. In the gerbil mAb-KA8 immunopositive cell bodies were recognisable in the stratum radiatum of the CA1 and CA3 areas and in the subiculum. In the hamster varicose axon-like elements were also detected in the CA3 pyramidal layer. With the mAb-KA8 antibody there was no detectable kappa opioid receptor labelling in the hippocampus of the guinea pig. The results confirm the high degree of species-specific heterogeneity characterising the distribution of opioid peptides and their receptors in the hippocampal formation. The receptor was found in most cases postsynaptically, however in the hamster the immunopositive axons may refer to a presynaptic localisation.

Keywords: Hippocampus – interneurons – rodents – opioid receptor – immunocytochemistry

INTRODUCTION

The hippocampal formation is known to be one of the most important centres of learning and information processing. It contains relatively low amount of opioid receptors and the level of their endogenous ligands is also low [4]. A series of experiments has been shown that exogenous opioids applied to this area have a prominent disinhibitory effect on the principal cells [3, 14, 20]. It was also revealed, that specific kappa-opioid analgesic materials reduce the mortality and morbidity resulting from cerebral ischemia in rats and gerbils [19], and that kappa the opioid receptor agonists are highly effective against limbic seizures [18]. The hippocampal opioid dynorphin has a crucial effect on the spatial learning that is mediated through kappa-opioid receptors [17]. Thus, in spite of their sporadic appearance, the opioid recep-

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tors, especially their kappa subtype are strategically very important in the normal and pathological function of the hippocampal formation.

The distribution of opioid peptides and receptors was found to be diverse within the hippocampal formation, depending on the species and the method used for their visualization [12, 13, 15]. Arvidsson et al. [1], using immunocytochemistry, localised the kappa-opioid receptor primarily in postsynaptic position in the rat and guinea pig brain and spinal cord on neuronal somata and dendrites and rarely in axons. The antibody they used recognised primarily the kappa-1 subtype of the receptor. Others [12], applying receptor autoradiography, found this receptor to be the densest in and around the principal cell layers in the hippocampus. Drake et al. [5], used a rabbit antibody against a synthetic peptide from the carboxyl terminus of the cloned kappa receptor in the guinea pig hippocampus and found that immunopositive labelling was located in unmyelinated axons and asymmetric synapse-forming axon terminals. Another monoclonal antibody raised against a frog brain kappa receptor preparation recognising selectively the kappa receptor with preference for the kappa-2 subtype, that was used in this study as well, was produced and characterised by Maderschpach et al. [10]. With this antibody kappa-opioid receptor immunoreactivity was found to be associated to glial elements, postsynaptic densities of synapses and microtubules of dendrites in various brain areas of the rat and chicken brain [11]. In our previous study on the rat hippocampus, several interneuron-like cells were found to express mAb-KA8 immunoreactivity in the hilus and in the CA1 area [9].

The purpose of this experiment was to clarify and compare the distribution and species-specificity of the kappa-opioid receptor in the hippocampus of four rodent species based on light microscopic observation.

MATERIALS AND METHODS

The experiments were carried out on young adult Wistar rats, guinea pigs, golden hamsters and gerbils of both sexes. In this experiment all animal procedures were conducted in accordance with the guidelines set forth by the Animal Health and Welfare Institute of the Szent István University, and all efforts were made to keep animal stress, suffering, and discomfort to a minimum. The Local Animal Welfare Association permitted and controlled the experiment. The animals were deeply anaesthetised with pentobarbital, then perfused through the left ventricle with 0.9% NaCl for 10 min, followed by a mixture of paraformaldehyde (4%), picric acid (15%), and glutaraldehyde (0.1%) in 0.1 M phosphate buffer (PB, pH 7.4). Brains were removed and immersed in the same fixative for two hours. 60–80 μm thick coronal sections were cut from the hippocampal formation with a vibratome and processed for kappa-opioid receptor immunohistochemistry using the free-floating technique.

Sections were kept in 10, 20 and 30% saccharose in 0.1 M PB successively, then freeze-thawed in liquid nitrogen in order to increase the penetration of the antibodies. This was followed by three washes in 0.1 M PB, and treatment of 1% Na borohydride for 30 minutes. After a short rinse in three changes of 0.1 M PB, non-spe-

cific immunoreactivity was suppressed with 20% normal goat serum for 45 minutes at room temperature. The sections were incubated with the supernatant of KA8 hybridoma cell line diluted in 1 : 2 in TRIS-buffered saline (TBS) [10] for 48 h at 4 °C. Following several rinses in TBS, biotinylated rabbit anti-mouse IgG (DAKO, 1 : 50) was used as a secondary antibody for 5 hours at room temperature. This was followed by several rinses in 1% NGS and incubation in Avidin Biotin peroxidase Complex (1 : 100 for both components, Vectastain, Elite Kit) overnight. The immunopositive structures were visualised with 3,3-diaminobenzidine-tetrahydrochloride (DAB) after numerous washes in 0.05 M Tris buffer at pH 7.6.

The sections then were mounted on gelatine-coated glass slides, dehydrated in ascending ethanol series and xylene, and covered with cover slips in DePeX.

RESULTS

MAb-KA8-immunoreactivity (mAb-KA8-IR) was localised in the hippocampi of the four studied species, at least in some sublayers of the hippocampal formation (Table 1). The labelled neuron population was morphologically heterogeneous. Studying the hippocampal formation, the following species-specific morphological features could be revealed.

Rat

In accordance with our previous results [9] the mAb-KA8-IR was localised mainly postsynaptically within cell bodies and in their proximal dendrites in the subiculum (Fig. 1A) in strata pyramidale and oriens of CA1 region of the hippocampus (Figs 1B, 1C) and in the hilus of the dentate gyrus (Fig. 1D). The immunoprecipitate completely filled in proximal dendrites and interneuron-like cell bodies with a fusiform and/or multipolar shape in the majority of the labelled profiles. In some cases the immunoprecipitate was attached exclusively to the membrane of the labelled cells, whereas the rest of the cytoplasm was not labelled (Fig. 1A).

Guinea pig

In the guinea pig hippocampus no labelling of the kappa-2 subtype opioid receptor could be observed. Immunoreactive neuronal elements were not detected in any layers of the hippocampal formation (Fig. 1E, F).

Hamster

MAb-KA8-IR occurred in every single layer of the hippocampus and subiculum except the stratum lacunosum-moleculare of the CA1 area. The hippocampus of this species contained the strongest mAb-KA8-immunoreactivity in a variety of nerve elements. In the stratum oriens of the CA1 area (Figs 2A, 2C), in the dentate gyrus

(Fig. 2D) several – mainly fusiform – mAb-KA8-IR interneurons were detected. Among the pyramidal cells in the CA3 area varicose immunoreactive axon-like elements were observed (Fig. 2B). This type of labelling most probably represents presynaptically localised receptors.

Table 1
Occurrence of the kappa-opioid receptor in the hippocampal formation of four rodents

Species	Area	mAb-KA8-IR
Rat	GD	**
	CA1/subiculum	**
	CA1 oriens	***
	CA1 pyramidale	*
	CA1 radiatum	*
	CA1 lm	–
	CA3 oriens	–
	CA3 pyramidale	–
	CA3 radiatum	–
Guinea-pig	GD	–
	CA1/subiculum	–
	CA1 oriens	–
	CA1 pyramidale	–
	CA1 radiatum	–
	CA1 lm	–
	CA3 oriens	–
	CA3 pyramidale	–
	CA3 radiatum	–
Hamster	GD	***
	CA1/subiculum	**
	CA1 oriens	***
	CA1 pyramidale	**
	CA1 radiatum	*
	CA1 lm	–
	CA3 oriens	*
	CA3 pyramidale	*
	CA3 radiatum	*
Gerbil	GD	–
	CA1/subiculum	**
	CA1 oriens	**
	CA1 pyramidale	**
	CA1 radiatum	*
	CA1 lm	–
	CA3 oriens	–
	CA3 pyramidale	–
	CA3 radiatum	**

Abbreviations: DG = dentate gyrus; mAb-KA8-IR = kappa-opioid receptor immunoreactivity; lm = lacunosum-moleculare; – = not detected; * = few; ** = moderate; *** = many

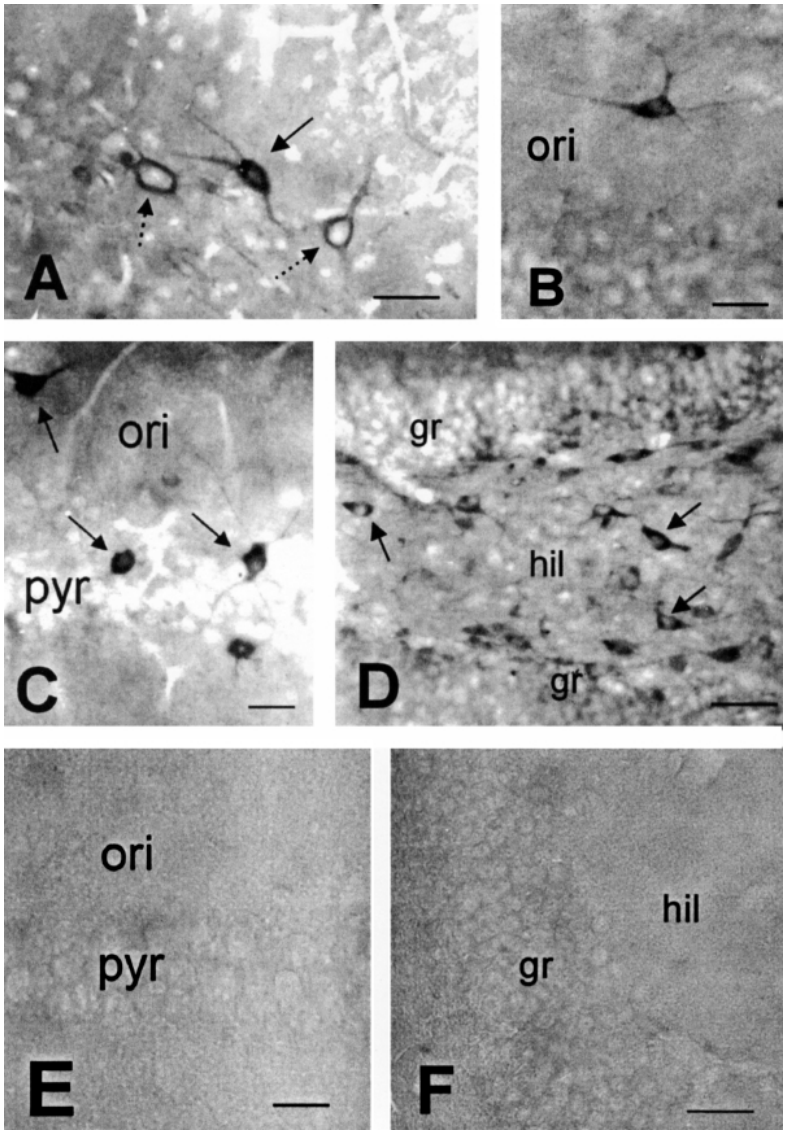

Fig. 1. Kappa-opioid receptor immunoreactive elements in the rat and guinea-pig hippocampus: A: Pericellular kappa-opioid receptor immunoreactivity surrounds two cell bodies (dashed arrows) in the rat subiculum. In the middle neuron (black arrow) the immunoprecipitate evenly fills the cell body and the main dendrites. Scale bar: 30 µm. B: A fusiform interneuron in the stratum oriens (ori) in the CA1 area of the rat hippocampus. Scale bar: 30 µm. C: Immunoreactive cell bodies in the pyramidal (pyr) and oriens (ori) layer of the CA1 area of the rat hippocampus (arrows). Scale bar: 32 µm. D: mAb-KA8-IR in the hilar (hil) neurons of the rat (arrows) (gr = stratum granulosum). Scale bar: 55 µm. E: Kappa receptor immunoreactivity can be observed neither in the pyramidal layer (pyr) nor in the oriens layer (ori) in the guinea pig hippocampus. Scale bar: 35 µm. F: The lack of immunoreactive elements characterises the guinea pig dentate gyrus as well (gr = stratum granulosum; hil = hilus). Scale bar: 43 µm

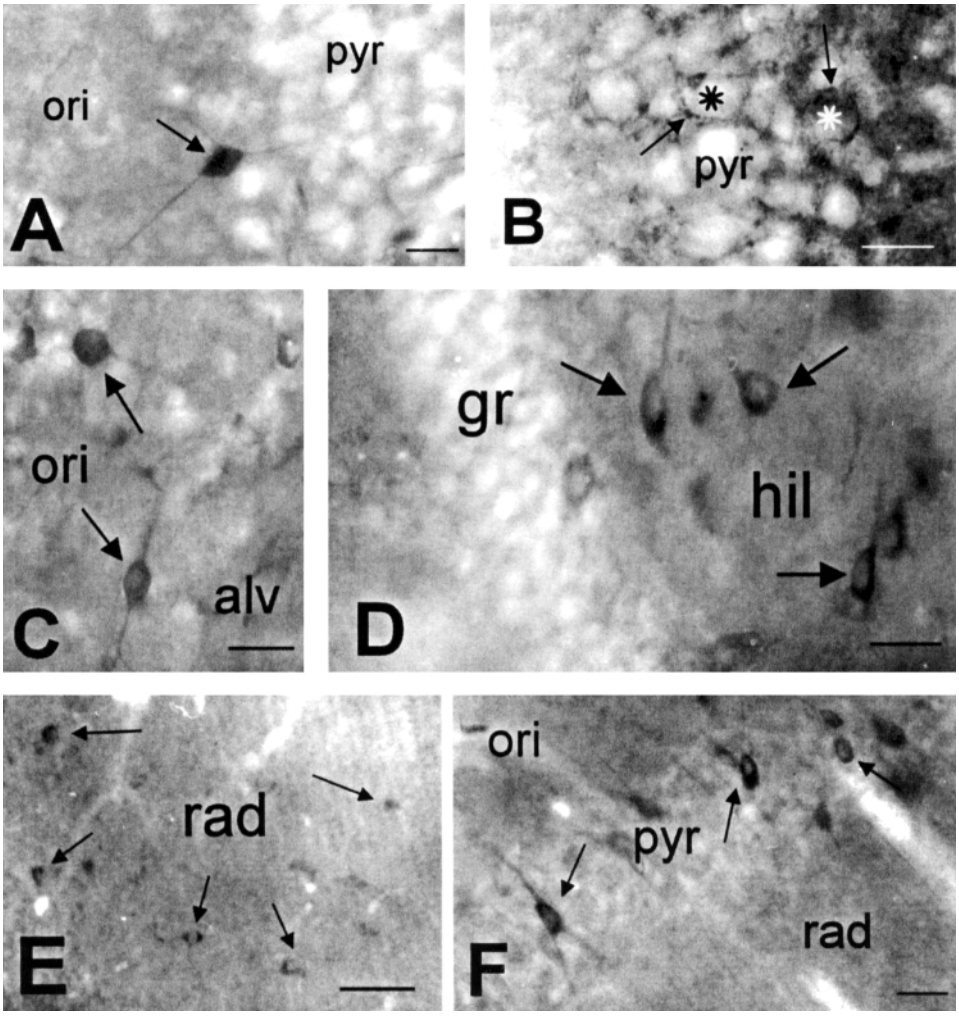

Fig. 2. Morphology of the kappa-opioid receptor immunoreactivity in the hamster and gerbil hippocampus: A: Kappa-opioid receptor immunopositive neuron in the oriens layer of the CA1 area of the hamster hippocampus (arrow). (ori = stratum oriens, pyr = stratum pyramidale) Scale bar: 30 µm. B: Varicose axons around the pyramidal cells (asterisks) in the CA3 area of the hamster hippocampus show mAb-KA8-IR (arrows). Scale bar: 32 µm. C: Two strongly immunostained interneurons at the oriens/alveus border (ori, alv) of the CA1 area of the hamster (arrows). Scale bar: 30 µm. D: mAb-KA8-immunopositive fusiform cell bodies (arrows) in the hilus (hil) of the dentate gyrus (gr = stratum granulosum) of the hamster. Scale bar: 33 µm. E: Kappa-opioid immunopositive neurons arrows) in the radiatum layer (rad) of the CA3 area of the gerbil hippocampus Scale bar: 80 µm. F: Cell bodies located in the CA1 subiculum border show immunoreactivity in the gerbil. Scale bar: 35 µm

Gerbil

MAb-KA8-IR was present primarily in the CA1 area in both the stratum oriens and the pyramidal layer (Fig. 2F), moreover in the subiculum. According to their morphological characteristics these cells represent a subset of the interneuron population of these areas. Some labelled cells resembling to basket- or axo-axonic cells were present in the pyramidal layer as well. A smaller amount of kappa-opioid receptor containing cells was present in the stratum radiatum of the CA3 area (Fig. 2E).

DISCUSSION

The present study is an assessment of the occurrence of the kappa-2 type opioid receptor in the hippocampal formation of four rodent widely used in laboratory experiments. The labelled neuronal elements can be characterised as follows.

Labelled cells were present in all species except the guinea pig in the stratum oriens of the CA1 region of the hippocampus. Since only GABAergic inhibitory interneurons have been published to be present in this layer [7], it can be hypothesised that these cells also represent a subpopulation of GABAergic interneurons. It is also observable that there is a fusiform cell population, which express the kappa-opioid receptor. The localisation and distribution of this type of cells is very similar to that of the somatostatin containing neurons. Our preliminary co-localisation studies revealed a subset of kappa-2 opioid receptor immunopositive interneurons containing somatostatin in their cell bodies as well [16]. Correspondingly in the dentate gyrus of the rat and hamster a kappa-2 opioid receptor immunopositive cell population exists that also represents morphological similarities to the hilar somatostatin containing neurons. Both cell population are in connection with the termination zone of the entorhinal efferents, which represents the main opioidergic input of the hippocampal formation. Thus, the location of the receptors suggests that they play a key role in the regulation of the neuronal transmission mediated by this input [7].

It is notable that a morphologically uniform kappa-opioid receptor immunopositive cell population exists in the subiculum of the hippocampus in all investigated species except the guinea pig. Recently, it has been proposed that the subiculum contains chemical sites at which drugs could act specifically to produce appropriate physiological effects, which is consistent with the hypothesis that the subiculum is a potential site of action for certain antipsychotic drugs [8]. The mAb-KA-8 immunopositive cells may modify or mediate the incoming and/or outgoing entorhinal information transmitted by dynorphin.

In the gerbil the majority of the mAbKA8-IR cells are located in this area, thus it can be proposed that these subicular neurons play a key role in the mediation of the protective effect of kappa agonists in forebrain ischemia resulting in a lower level of neuron loss.

We failed to detect mAb-KA8-IR elements in the guinea pig hippocampus. In contrast, in the rat, hamster and gerbil the kappa-2 receptor is known to be the predom-

inant subtype of kappa opioid receptor. Zukin et al. [21] have already shown that the U 69,593-sensitive, high-affinity kappa-1 site predominates in the guinea pig brain, and the U 69,593-insensitive, low affinity kappa-2 site predominates in the rat brain. Thus, being kappa-2 specific, with our antibody we did not manage to detect immunoreactivity in the guinea pig hippocampus.

It is relatively well known that opioids may influence glial cell activity, presumably related to glia-neuron communication. Previous electron microscopical studies [9, 11] have shown that the pericellular kappa opioid receptor immunolabelling around principal cells are indeed localised in a glial sheet that covers these neurons suggesting a very close glia-neuron interaction of opioids. In addition Eriksson et al. [6] demonstrated that kappa-opioid receptors are coupled to L-type Ca^{2+} channels on hippocampal astrocytes *in vitro.* This arrangement may represent a mechanism contributing to the depressant action of opioids on synaptic plasticity via the manipulation of the accessibility of the extracellular calcium necessary for presynaptic transmitter release. We could visualise this kind of glia-neuron arrangements in the rat and hamster hippocampus in many cases.

It is also notable that the hamster hippocampus not only contains the highest amount of opioid peptide [15] but also the kappa-opioid receptors are the most abundant, from among the other three species. This may have functional relationship to the fact, that the hamster is a hibernator. Changes in the amount of opioid peptides and receptors in this part of the limbic system were reported to be involved in hibernation and the adaptation of thermal stress in hibernating rodent species [2].

We can assess that the distribution of the kappa-opioid immunopositive elements in the rodent hippocampus is far from being uniform. The species-specific distribution of the kappa-opioid receptors suggests that this sort of receptors may mediate different effects or mediate similar functions through different networks.

ACKNOWLEDGEMENT

The authors are very grateful to Dr. Katalin Maderschpach, who kindly donated the primary antiserum mAb KA-8 used in this investigation.

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