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AUTORADIOGRAPHIC VISUALIZATION OF [^{3}H]KAINIC ACID RECEPTOR SUBTYPES IN THE RAT HIPPOCAMPUS

M. BERGER and Y. BEN-ARI*

Laboratoire de Physiologie nerveuse, CNRS, F-91190 Gif-sur-Yvette (France)

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Unfixed, slide-mounted tissue sections from the rat forebrain have been incubated in the presence of 20 and 100 nM [^{3}H]kainic acid ([^{3}H]KA). For the last 2 min of incubation, 10 μM unlabelled KA was added to displace [^{3}H]KA from binding sites with high on–off rate. Washed and dried slices were exposed on [^{3}H]Ultrofilm for 178 days. Our results confirm the high density of KA receptors in the terminal field of the hippocampal mossy fibre system which is shown to be due to receptors with slow dissociation rate. Furthermore, the concentration dependency of the specific labelling, as quantified by microdensitometry, allows some suggestions concerning the local binding affinities involved.

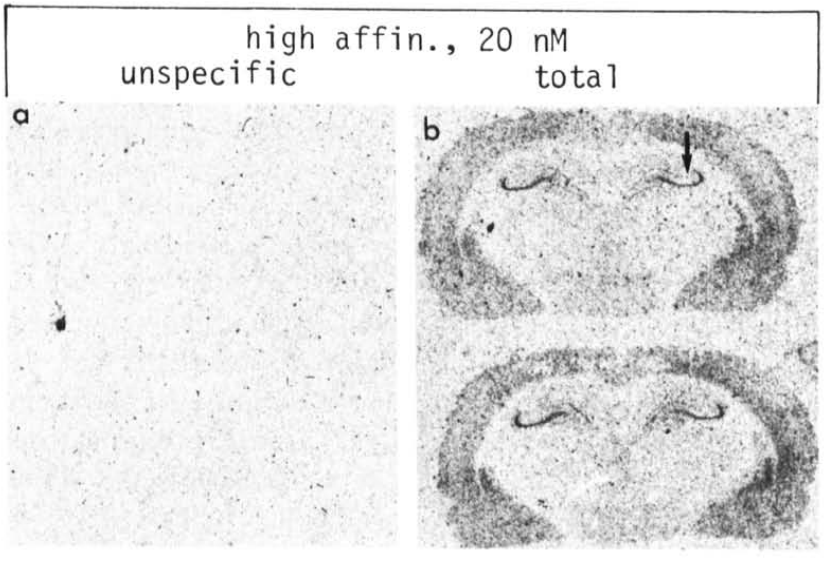
Intracerebral injection of kainic acid (KA) in a number of brain structures, notably in the amygdaloid complex, produces a limbic seizure brain damage pattern [3, 11] that is characterized by the presence of local damage at the site of injection, due to the well described cytotoxic action of KA [8], and distant damage [13, 14] which, at least in the CA3 region of the Ammon's horn, is causally related to propagation of paroxysmal discharge per se [2, 16]. Since this syndrome bears some resemblance in both its aetiology and pathological outcome to human temporal lobe epilepsy [1, 10], a better understanding of the mechanism of the cytotoxic and epileptogenic actions of KA is of more than theoretical interest. The presence of specific binding sites in the mammalian brain with high affinity for KA [7, 15] may offer some promising strategies. Very recent observations indicate that there may exist at least 3 different KA receptor subtypes: low affinity receptors with high rates of association and dissociation, and at least two types of high affinity receptors, with low association and dissociation rates (Berger, submitted). In the present investigation, we tried to find out if one of these subtypes could be correlated with the limbic seizure syndrome produced by KA. We have chosen the autoradiographic

*Author for correspondence.

'in vitro' slice technique [20] and selective conditions to visualize separately high and low affinity KA receptors [6] in the rat forebrain.

Adult male Wistar rats were perfused with saline and their brains processed for the preparation of slide-mounted tissue sections by conventional techniques [19]. Slices were incubated in the presence of [^{3}H]KA as described in the legend of Fig. 1. Our major objective was to label selectively high affinity KA binding sites, and we also made an attempt to visualize exclusively low affinity ones. In a series of biochemical pre-experiments, [^{3}H]KA bound to slices was quantified by direct scintillation counting after wiping off the slices with small glass fibre filters. Scatchard analysis resulted in affinity constants of 12 and 54 nM for high and low affinity receptors, respectively, in agreement with a recent publication [17], with an unspecific binding of 18 and 41% at a ligand concentration of 20 nM. The autoradiographs obtained showed the highest amount of [^{3}H]KA bound in the CA3 region of the hippocampus, confirming recent studies [9, 17]. After selective elimination of [^{3}H]KA dissociating readily from low affinity receptors, the radioligand that remained bound was highly concentrated in the hippocampal terminal field of the mossy fibres and, to a lesser extent, to a narrow band of binding sites outlining the dentate gyrus (Fig. 1b). At this rostro-caudal level, the rest of the hippocampal formation appeared almost devoid of specific [^{3}H]KA receptors with high affinity. Other areas with strong labelling were the cerebral cortex (especially the deep layers in vicinity of the rhinal fissure, e.g. Fig. 1d), the striatum, and the amygdala. Using 20 nM [^{3}H]KA, the unspecific binding was indistinguishable from film background (Fig. 1a). The maximum density of silver grains in the stratum lucidum was about 10 times higher than in the immediately adjacent hippocampal layers, as determined by quantitative densitometry (Fig. 2a). Slices incubated in 100 nM [^{3}H]KA showed more intense labelling in all regions (Fig. 1d), but also the unspecific binding became distinguishable from film background (Fig. 1c). The relative density of silver grains in the terminal field of the mossy fibres was higher by 25% than that obtained with 20 nM [^{3}H]KA (Fig. 2b). A similar rise by 20% was also found in the very medial part towards the hilus in response to the higher ligand concentration. This would agree with a local affinity constant of 6 nM. No relative change of labelling was seen in the pearlstring-like band of sites in the dentate gyrus (9 areas scanned on 5 different slices, window 40 × 40 μm).

These local rises of silver grain densities in response to elevated ligand concentrations may be of some relevance, since we observed in hippocampal membrane suspensions that, even after the elimination of rapidly dissociating [^{3}H]KA, the radioligand still remained bound to more than one receptor population (Berger, submitted): 90% of these high affinity binding sites showed an affinity constant of 6–7 nM; the remaining 10% exhibited an extremely high affinity for [^{3}H]KA, with an affinity constant of 0.2–0.3 nM. The high predominance as well as the affinity constant of the first binding site agrees with its autoradiographic localization in the hippocampal CA3 region. On the other hand, the only candidates in our autoradio-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a
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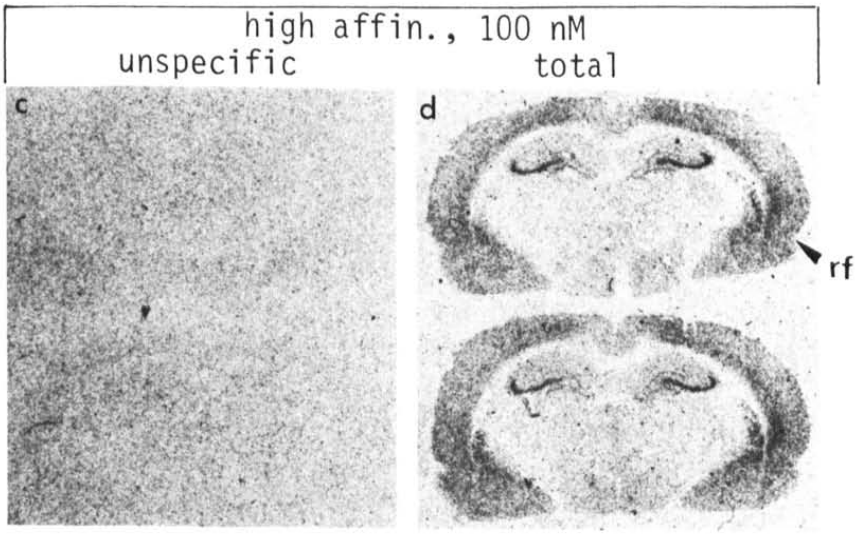
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unspecific
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c
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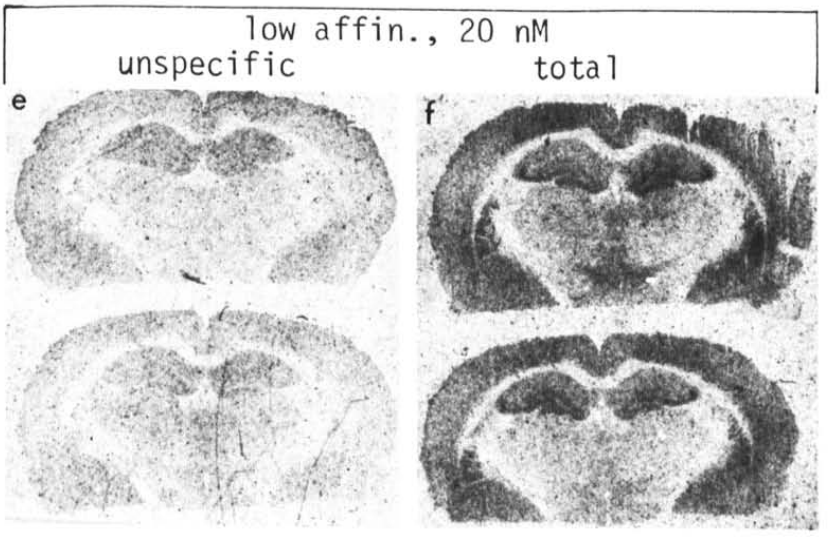
low affin., 20 nM
unspecific
total
e
f

graphs for the localization of [^{3}H]KA receptors with very high affinity seem to be the granule cells in the dentate gyrus.

The binding of [^{3}H]KA to sites not occupied by slowly dissociating unlabelled KA is visualized in Fig. 1e, f. Quantitative densitometry revealed an overall contribution of unspecific binding in keeping with the biochemical pre-experiments. Specific binding was distributed over the whole hippocampal cross-section, also in parts where, at a ligand concentration of 20 nM, practically no high affinity sites were labelled. The high labelling in the mossy fibre terminal field and in the dentate gyrus also under low affinity conditions, must not necessarily be due to the presence of low affinity KA receptors in these regions, since [^{3}H]KA may have diffused during the drying procedure after the last washing step, changing place from low to high affinity receptors in the still wet sections. So, we cannot draw any definitive conclusions on the particular distribution of low affinity KA receptors in the hippocampus from the autoradiographs we obtained. Their kinetic properties suggest some extrasynaptic localization, but our results do not provide compelling evidence for this issue.

On the other hand, our results provide strong evidence for the identification of the KA receptors in the terminal field of the mossy fibres as receptors with slow association and dissociation rates, and with an affinity constant of about 6 nM. Their very discrete localization as well as their kinetic properties suggest that they are perhaps situated in synaptic clefts [4]. The fact that they are highly concentrated in exactly the same brain structure which shows by far the highest susceptibility against local [5, 11] and 'distant' KA brain damage [2, 12], raises the possibility that they may serve as physiological recognition sites for some endogenous substance contained in the mossy fibre nerve terminals, with KA-like properties.

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Fig. 1. Autoradiographic visualization of [^{3}H]KA receptors on slide-mounted tissue sections from the rat forebrain. Sections were incubated in plastic cuvettes with 3 ml chambers. After a 15 min preincubation at 37° C in 50 mM Tris-acetate buffer (pH 7.0), slices were left for 60 min in Tris-acetate buffer (4°C) containing 20 and 100 nM [^{3}H]KA (2 Ci/mmol, Amersham). Thereafter, they were transferred to chambers containing 10 μM unlabelled KA for 2 min to eliminate binding of easily displaceable [^{3}H]KA, and finally immersed in fresh buffer for 5 min (a–d). To label predominately low affinity receptors, a second series of slices received, after 15 min at 37°C, another preincubation in the presence of 100 nM unlabelled KA, on ice. After 60 min, the sections were washed for 30 sec and transferred for 20 min to cuvettes containing [^{3}H]KA, to label sites not masked by very slowly dissociating unlabelled KA, and finally washed in buffer two times for 5 sec (e and f). Unspecific binding was assayed under both conditions in the presence of 10 μM unlabelled KA. For autoradiography, slides were dipped in distilled water for 1 sec, dried at 4°C, and exposed to [^{3}H]Ultrofilm (LKB) for 178 days. Arrow in b shows direction of densitometric scans; rf, rhinal fissure.

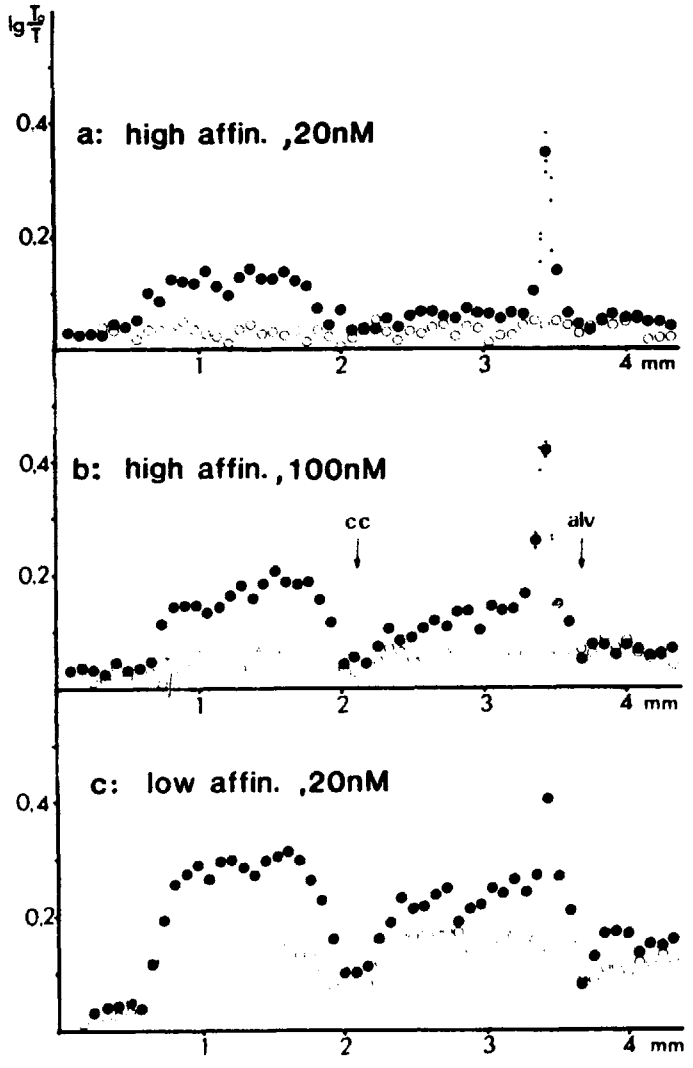

Fig. 2. Light transmittance scans taken from the autoradiographs presented in Fig. 1, by a computer-assisted Leitz microphotometer. A beam of light with rectangular cross-section (40 × 400 μm) was adjusted parallel to the zone of intense staining in the hippocampal CA3 region and moved along a path indicated by arrow in Fig. 1b. Light transmittance (T) was recorded in 40 μm steps and the logarithmic relation to film background (T_o) plotted against the distance covered. Only each second point is illustrated (mean of 3 scans). Open symbols refer to unspecific binding. Dots in peaks of a and b indicate individual values. cc, corpus callosum; alv, alveus.

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