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Abstract

Nefiracetam is being studied as a novel cognition-enhancing agent; however, it has been suggested from studying its chemical structure that it has a potential anticonvulsive effect. We examined the antiepileptic effect of nefiracetam on kainic acid (KA)-induced seizures. KA was infused into the left basolateral amygdaloid nucleus, and focal limbic seizures were induced in 43 male Wistar rats. During status epilepticus, 10, 50, 100 or 200 mg/kg of nefiracetam was intravenously injected. Nefiracetam inhibited KA-induced limbic seizures at doses over 100 mg/kg while it had a sedative effect on the animals. In [^{14}C] deoxyglucose autoradiographic studies, the propagation of seizure-induced hypermetabolic areas was also suppressed dose-dependently. From the results, it was indicated that nefiracetam has an antiepileptic effect and that its application may suppress seizure propagation. Further study is required whether this agent is available as a novel anticonvulsant.

Key words: nefiracetam, antiepileptic effect, electroencephalogram, cerebral metabolism, experimental epilepsy, kainic acid

Introduction

Nefiracetam (N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide, DM-9384) is a pyrrolidone derivative and a cyclic analogue of gamma-aminobutyric acid (GABA). Both piracetam and levetiracetam are also pyrrolidone derivatives and have a similar chemical structure of nefiracetam. Piracetam has been used in some countries to treat mental disorders (Croisile et al., 1993, Richardson and Bereen, 1977). This drug has an antiepileptic effect and was indicated as add-on therapy for myoclonus (Obeso et al., 1988). A ethyl analogue of piracetam, levetiracetam, is effective in audiogenic, maximal electroshock, chemoshock and amygdala-kindled seizure models (Gower et al., 1992, Loscher and Honack, 1993, Loscher et al., 1998). Levetiracetam showed an antiepileptic effect in patients with complex partial seizures (Singh et al., 1992), and clinical studies are going on in Europe.

At first, Nefiracetam as a novel cognition-enhancing agent attracted the attention of pharmacologists (Sakurai et al., 1989). This drug improved the learning and memory functions in several experimental amnesia models and is currently undergoing experimental and clinical studies (Sakurai et al., 1990, Nabeshima et al., 1991, Luthman et al., 1992, Watabe et al., 1993, Abe et al., 1994, Gouliaev and Senning, 1994, Yoshii and Watabe, 1994, Huang et al., 1996, Yoshii et al., 1997, Nishizaki et al., 1998, Sakurai

et al., 1998). However, from its chemical structure, it seemed possible that nefiracetam had a potential anticonvulsive effect like piracetam and levetiracetam. In fact, nefiracetam inhibited maximal electroshock seizures in rats (Kitano et al., 1994). The maximal electroshock seizure may be a model of acute convulsion rather than an epilepsy model. Antiepileptic effect should be studied on various models. The aim of this study is to examine the antiepileptic effect of nefiracetam on kainic acid (KA)-induced seizures.

Material and Methods

1. KA-induced seizure model and treatment using nefiracetam

Twenty-five male Wistar rats weighing 250-380 g were used to study the electrographic and clinical effects of KA-induced seizures. The animals were immobilized on a stereotaxic apparatus under intraperitoneal pentobarbital anesthesia. A stainless steel tube, 0.6 mm in outer diameter and 20 mm in length, was prepared as an injection guide cannula. Bipolar depth electrodes were made with twisted Teflon-coated stainless steel wires (0.2 mm in diameter and 60mm in length). The cannula was fixed to the electrode by a adhesive, and a combined cannula/electrode was inserted into the left basolateral amygdaloid nucleus (A: +5.0, L: +5.0, D: -3.0) in accordance with the

information provided in the atlas of Pellegrino, et al (1979). The bipolar depth electrodes were also inserted into the right amygdala (A: +5.0, L: -5.0, D: -3.0) and bilateral hippocampus (A: +3.0, L: +/- 3.2, D: +1.5) for recording EEGs. The electrodes were connected with a socket, and the cannula, electrodes and socket were fixed to the skull with dental resin cement. The rats were kept for 7 days to allow recovery from the surgical damage. To prepare a KA solution, KA (Sigma, 1.0 mg) was dissolved in a phosphate buffer solution (0.2 M, pH 7.4, 1.0 ml). Seven days after the surgery, 1.0 μl of the KA solution was infused into the left amygdala via the cannula at a rate of less than 0.2 μl/min. EEG and behavior were recorded by a video-monitoring system. While in a limbic seizure status (60 to 100 minutes after KA infusion), 10, 50, 100 or 200 mg/kg of nefiracetam (Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan), which had been dissolved in a physiological saline (10 mg/ml), was intravenously injected via a tail vein in 19 animals (2 with 10 mg/kg, 3 with 50 mg/kg, 6 with 100 mg/kg and 8 with 200 mg/kg). For the control group of 6 rats, 1.0 ml of physiological saline was injected via the same route. Following the intravenous injection of nefiracetam or physiological saline, EEGs and behavior were recorded continuously for 8 hours, then intermittently after 24, 48 and 72 hours.

2. Measuring cerebral glucose metabolism

The cannula was implanted in the left amygdala (A: +5.0, L: +5.0, D: -3.0) of 18 male Wistar rats weighing 250-330 g. Seven days after this, a polyethylene catheter was inserted into the unilateral femoral artery and vein, and the lower body and limbs were immobilized with a plaster cast to prevent removal of the catheters. Following recovery from anesthesia 1.0 μl of KA solution (1.0 mg/ml) was infused via the cannula to induce seizures. During seizure status (90 minutes after KA infusion), 50, 100 or 200 mg/kg of nefiracetam or physiological saline was injected. Sixty minutes after the injection, 25 μCi of [^{14}C]-2-deoxy-D-glucose was injected intravenously. Arterial blood samples were collected 2, 3, 5, 10, 15, 20, 25, 30, 35, and 45 minutes following tracer injection. The animals were decapitated as soon as the last blood sample was collected. The brains were removed quickly, frozen instantly at –20℃, then serially sliced to 20 μm-thick coronal sections in a cryostat. The sections were dried immediately at 60℃. The dried sections and [^{14}C]-methyl methacrylate standards were laid consecutively on an x-ray film in a cassette and exposed for 7 days. Arterial blood samples were centrifuged, and plasma [^{14}C] radioactivity and glucose concentration were measured. The optical density of the autoradiograms was measured and the tissue [^{14}C] concentration was calculated by using an image-analyzing computer (Unigraphy UHG-101, Unique

Medical Co., Tokyo, Japan). Local cerebral glucose utilization (LCGU) was computed according to the equation described by Sokoloff, et al. (1977). LCGU in the brain structures was averaged over 2 consecutive slices (to reduce any error due to slice thickness) and comparatively analyzed by employing the Mann-Whitney U-test.

Results

1. Clinical and electrophysiological effects of nefiracetam

Continuous low-amplitude polyspikes were induced in the left amygdala by KA. The seizure activity developed into rhythmic spikes and propagated to the ipsilateral hippocampus and contralateral amygdala and hippocampus. Simultaneously, clinical seizures were observed as searching, motion arrest, hypersalivation and facial twitching. Soon these seizure activities appeared once in 5 to 10 minutes and developed into a limbic seizure at 60 to 100 minutes after KA infusion. The limbic seizures status and EEG change were seen in all animals.

During the seizure status, intravenous nefiracetam at a dose of 10 (in two rats) or 50 mg/kg (in three) caused no behavioral or EEG changes. However, when a dose of 100 mg/kg was administered, the animals became sedated immediately. The six rats that received this dosage closed their eyes and relaxed their limbs, showing no response to

noxious stimuli. The clinical seizures disappeared simultaneously but the electrographic seizure activities persisted in all animals. This sedative effect lasted for 30 to 60 minutes, then the clinical seizure returned. When a dose of 200 mg/kg was injected into 8 animals, both clinical and electrographic seizures were immediately suppressed for 5 minutes to 4 hours in 6 of them (Fig. 1). Spike activity from all electrodes vanished in 5 of the 6 and persisted only in the left amygdaloid focus of one. One of the 6 rats died 25 minutes after the injection; this may have been due to respiratory suppression. In the remaining 2 of the 8 rats that received 200 mg/kg of nefiracetam, electrical seizure activity persisted; but no clinical seizure was observed and they appeared drowsy for several hours. When the animals recovered from the sedative effect, clinical seizures occurred again, which were supported by EEGs. The recurrent seizures continued for 8 hours and only interictal discharge was observed following 24 hours. On 72 hours later, the interictal discharge became less frequent, and there was no abnormal behavior in the all animals. The duration of the acute seizure status was not different between the animals administrated the drug and only KA.

2. Change in cerebral glucose metabolism by nefiracetam

Autoradiograms of [^{14}C] deoxyglucose are shown in Fig. 2. During the limbic

seizures in the control animals, local glucose metabolism was increased in the following regions compared to our data of normal animals (Tanaka et al., 1990, Hashizume et al., 1998a, 1998b): bilateral amygdala, hippocampus and other limbic structures, left caudate-putamen, thalamus, substantia nigra, and sensorimotor cortex. These metabolic changes were similar to the previous report described by Tanaka et al. (1990). The animals that had received 50 mg/kg of nefiracetam showed a decrease in hypermetabolism in the contralateral (right) amygdala, hippocampus and neocortex. Nefiracetam at a dose of 100 mg/kg caused a reduction in hypermetabolism in all of the contralateral structures. In the animals that received 200 mg/kg, the hypermetabolic region was found in only the left amygdaloid focus. Accordingly, nefiracetam dose-dependently suppressed the propagation of seizure-related hypermetabolism. The values of LCGU and results of statistical analysis are shown in Table 1. The data were compared between nefiracetam-treated and just KA-treated on the same side.

Discussions

It is well known that KA is a potent neuroexcitatory agent and its systemic or local administration induces epileptic seizures in experimental animals (Ben-Ari et al., 1979, Lothman and Collins, 1981, Olney, 1981, Sperk et al., 1983, Berger et al., 1986,

Clifford et al., 1990). In rats and cats, a microinjection of KA into the unilateral amygdala induced limbic seizures in the acute stage and spontaneous limbic or secondarily generalized seizures in the chronic stage (Tanaka et al., 1988, 1990). These experimental seizures were similar to those caused by human temporal lobe epilepsy (Tanaka et al., 1992). KA-induced amygdaloid seizures are very severe. According to previous studies, the only antiepileptic that was capable of suppressing these seizures was zonisamide (Takano et al., 1995). However, it appeared that nefiracetam reduced limbic seizures when applied in dosages over 100 mg/kg. Clinical seizures were suppressed with 100 mg/kg of nefiracetam and the rats remained sedated in spite of exposure to a noxious stimulus. However, electrographic seizure activities persisted. When 200 mg/kg of nefiracetam was injected, both clinical and the electrically observed seizures disappeared; and an anesthetic effect was also observed. In the study of single oral dose toxicity of nefiracetam, LD50 value was 1200-1400 mg/kg in rats (Sugawara et al., 1994). Thus, 200 mg/kg of intravenous dose was relatively high but not toxic. Kitano et al. (1994) observed that over 60 mg/kg oral dose of nefiracetam decreased in alertness. From the change in glucose metabolism, it was found that nefiracetam dose-dependently suppresses propagation of areas of seizure-related hypermetabolism.

These results suggested that the mechanism by which nefiracetam exerts its

antiepileptic effect might be based on a reduction of seizure propagation. Additionally, nefiracetam reduced the neocortical glucose metabolism as compared with the normal animals. The results also indicated the affinity of nefiracetam to the neocortex, however, no receptors for this agent have been identified. The synaptic plasma membrane is a possible binding site of levetitacetam in antiepileptic effect (Noyer et al., 1995).

In the studies on a cognition-enhancing mechanism, it has been reported that nefiracetam facilitates cholinergic, GABAergic and monoaminergic neurotransmissions (Nabeshima et al., 1991, Luthman et al., 1992, Watabe et al., 1993, Abe et al., 1994, Yoshii and Watabe, 1994, Huang et al., 1996, Yoshii et al., 1997, Nishizaki et al., 1998). The dose-response curves of GABA-induced current were bell-shaped. Nefiracetam caused the GABA-evoked chloride currents at a low concentration of GABA to increase and caused a reduction at a high concentration (Huang et al., 1996). The submicromolar concentration of nefiracetam decreased acetylcholine-evoked currents and the micromolar concentration enhanced them (Nishizaki et al., 1998). It was suggested that these effects were caused by the regulation of protein kinase A, C and G-protein. However, the exact biochemical mechanism by which nefiracetam acts has not been elucidated.

In the present study, it was indicated that nefiracetam has a marked antiepileptic

effect and that its application may suppress seizure propagation. Further study is required to elucidate the antiepileptic mechanism and biochemical characteristics of nefiracetam and whether this agent is available as a novel anticonvulsant.

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References

Abe, E., Murai, S., Saito, H., Masuda, Y., Takasu, Y., Shiotani, T., Tachizawa, H., Itoh, T., 1994. Effects of nefiracetam on deficits in active avoidance response and hippocampal cholinergic and monoaminergic dysfunctions induced by AF64A in mice. J. Neural Transmission - General Section 95, 179-193.

Ben-Ari, Y., Lagowska, J., Tremblay, E., Le Gal La Salle, G., 1979. A new model of focal status epilepticus: intra-amygdaloid application of kainic acid elicits repetitive secondarily generalized convulsive seizures. Brain Res. 163, 176-179.

Berger, M.L., Charton, G., Ben-Ari, Y., 1986. Effect of seizures induced by intra-amygdaloid kainic acid on kainic acid binding sites in rat hippocampus and amygdala. J. Neurochemistry 47, 720-727

Clifford, D.B., Olney, J.W., Benz, A.M., Fuller, T.A., Zorumski, C.F., 1990. Ketamine, phencyclidine, and MK-801 protect against kainic acid-induced seizure-related brain damage. Epilepsia 31, 382-390.

Croisile, B., Trillet, M., Fondarai, J., Laurent, B., Mauguiere, F., Billardon, M., 1993. Long-term and high-dose piracetam treatment of Alzheimer's disease. Neurology 43, 301-305.

Gouliaev, A.H., Senning, A., 1994. Piracetam and other structurally related nootropics, Brain Res. - Brain Res. Reviews 19, 180-222.

Gower, A.J., Noyer, M., Verloes, R., Gobert, J., Wulfert, E., 1992. ucb L059, a novel anticonvulsant: pharmacological profile in animals. Eur. J. Pharmacol. 222, 193-203.

Hashizume, K., Tanaka, T., Yonemasu, Y., 1998a. Change in cerebral glucose metabolism during limbic seizures elicited from lateral septal nucleus. Epilepsy Res. 30, 167-176.

Hashizume, K., Tanaka, T., 1998b. Multiple subpial transection in kainic acid-induced focal cortical seizure. Epilepsy Res. 32, 389-399.

Huang, C.S., Ma, J.Y., Marszalec, W., Narahashi, T., 1996. Effects of the nootropic drug

nefiracetam on the GABAA receptor-channel complex in dorsal root ganglion neurons. Neuropharmacol. 35, 1251-1261.

Kitano, Y., Makino, M., Usui, C., Takasuna, K., Kasai, Y., Hirohashi, M., Tamura, K., Takayama, S., Kojima, H., Iizuka, H., Yanagita, T., 1994. General pharmacological profile of the new cognition-enhancing agent nefiracetam. Arzneim.-Forsch./Drug Res. 44, 199-210.

Loscher, W., Honack, D., 1993. Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. Eur. J. Pharmacol. 232, 147-158.

Loscher, W., Honack, D., Rundfeldt, C., 1998. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. J. Pharmacol. Exp. Therapeutics. 284, 474-479.

Lothman, E.W., Collins, R.C., 1981. Kainic acid induced limbic seizures: metabolic, behavioral, electroencephalographic and neuropathological correlates. Brain Res. 218,

299-318.

Luthman, J., Lindqvist, E., Dell'Anna, E., Kojima, H., Shiotani, T., Tachizawa, H., Olson, L., 1992. Effects of DM-9384, a pyrrolidone derivative, on ischemia-induced changes in the central monoamine systems, Pharmacol. Biochemistr. Behav. 41, 231-234.

Nabeshima, T., Tohyama, K., Murase, K., Ishihara, S., Kameyama, T., Yamasaki, T., Hatanaka, S., Kojima, H., Sakurai, T., Takasu, Y., 1991.
Effects of DM-9384, a cyclic derivative of GABA, on amnesia and decreases in GABAA and muscarinic receptors induced by cycloheximide. J. Pharmacol. Exp. Therapeutics 257, 271-275.

Nishizaki, T., Matsuoka, T., Nomura, T., Sumikawa, K., Shiotani, T., Watabe, S., Yoshii, M., 1998. Nefiracetam modulates acetylcholine receptor currents via two different signal transduction pathways. Molecular Pharmacol. 53, 1-5.

Noyer, M., Gillard, M., Matagne, A., Henichart J-P., Wulfert E., 1995. The novel

antiepileptic drug levetiracetam (ucb L059) appears to act via a specific binding site in CNS membranes. Eur. J. Pharmacol. 286, 137-146.

Obeso, J.A., Artieda, J., Quinn, N., Rothwell, J.C., Luquin, M.R., Vaamonde, J., Marsden, C.D., 1988. Piracetam in the treatment of different types of myoclonus. Clin. Neuropharmacol. 11, 529-536.

Olney, JW., 1981. Kainic acid and other excitotoxins: a comparative analysis. Advances in Biochemical Psychopharmacol. 27, 375-384.

Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J., 1979. A stereotaxic atlas of the rat brain, Plenum Press, New York.

Richardson, A.E., Bereen FJ., 1977. Effect of piracetam on level of consciosness after neurosurgery. Lancet 2, 1110-1111.

Sakurai, T., Hatanaka, S., Tanaka, S., Yamasaki, T., Kojima, H., Akashi, A., 1990. Protective effect of DM-9384, a novel pyrrolidone derivative, against experimental

cerebral anoxia. Jpn. J. Pharmacol. 54, 33-43.

Sakurai, T., Kato, T., Mori, K., Takano, E., Watabe, S., Nabeshima, T., 1998. Nefiracetam elevates extracellular acetylcholine level in the frontal cortex of rats with cerebral cholinergic dysfunctions: an in vivo microdialysis study. Neurosci. Lett. 246, 69-72.

Sakurai, T., Ojima, H., Yamasaki, T., Kojima, H., Akashi, A., 1989. Effects of N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide (DM-9384) on learning and memory in rats. Jpn. J. Pharmacol. 50, 47-53.

Singh, P., Sharief, M.K., Sander, J.W.A.S., 1992. A pilot study of the efficacy and tolerability of L059 in patients with refractory epilepsy [abstract]. Seizure 1 Suppl. A, P7/46.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O., Shinohara, M., 1977. The [^{14}C] deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values

in the conscious and anesthetized albino rat. J. Neurochemistry 28, 897-916.

Sperk, G., Lassmann, H., Baran, H., Kish, S.J., Seitelberger, F., Hornykiewicz, O., 1983. Kainic acid induced seizures: neurochemical and histopathological changes. Neuroscience 10, 1301-1315.

Sugawara, T., Kato, M., Furuhama, K., Inage, R., Suzuki, N., Takayama, S., 1994. Single dose toxity study of the new cognition-enhancing agent nefiracetam in mice, rats and dogs. Arzneim.-Forsch./Drug Res. 44, 211-213.

Takano, K., Tanaka, T., Fujita, T., Nakai, H., Yonemasu, Y., 1995. Zonisamide: electrophysiological and metabolic changes in kainic acid-induced limbic seizures in rats. Epilepsia 36, 644-648.

Tanaka, S., Kondo, S., Tanaka, T., Yonemasu, Y., 1988. Long-term observation of rats after unilateral intra-amygdaloid injection of kainic acid. Brain Res. 463, 163-167.

Tanaka, S., Sako, K., Tanaka, T., Nishihara, I., Yonemasu, Y., 1990. Uncoupling of local

blood flow and metabolism in the hippocampal CA3 in kainic acid-induced limbic seizure status. Neuroscience 36, 339-348.

Tanaka, T., Tanaka, S., Fujita, T., Takano, K., Fukuda, H., Sako, K., Yonemasu, Y., 1992. Experimental complex partial seizures induced by a microinjection of kainic acid into limbic structures. Prog. Neurobiol. 36, 317-334.

Watabe, S., Yamaguchi, H., Ashida, S., 1993. DM-9384, a new cognition-enhancing agent, increases the turnover of components of the GABAergic system in the rat cerebral cortex. Eur. J. Pharmacol. 238, 303-309.

Yoshii, M., Watabe, S., 1994. Enhancement of neuronal calcium channel currents by the nootropic agent, nefiracetam (DM-9384), in NG108-15 cells. Brain Res. 642, 123-131.

Yoshii, M., Watabe, S., Sakurai, T., Shiotani, T., 1997. Cellular mechanisms underlying cognition-enhancing actions of nefiracetam (DM-9384). Behav. Brain Res. 83, 185-188.

Figure Legends

Fig. 1: EEG after administration of nefiracetam 200 mg/kg

An injection of nefiracetam at a dose of 200mg/kg inhibited both clinical and electrographic seizure for 5 minutes to 4 hours. The EEG showed frequency of spike activity reduced gradually in all electrodes, which were 5-7 Hz before and 3-5 Hz after the injection. Mean spike amplitude decreased in RAM (300μV before and 120μV after) and RHIP (300μV before and 200μV after). LAM: left amygdala, RAM: right amygdala, LHIP: left hippocampus, RHIP: right hippocampus.

Fig. 2: [^{14}C]deoxyglucose autoradiograms during seizure status in nefiracetam treated and control rats

The brain was sectioned coronally. As compared with the control (line A), the animals with 50 mg/kg of nefiracetam showed a decrease of seizure-induced hypermetabolism in the contralateral (right) amygdala, hippocampus and neocortex (line B). At the dose of 100 mg/kg, nefiracetam reduced hypermetabolism in all of the contralateral structures (line C). In the animals received 200 mg/kg, the hypermetabolic area persisted in only the left amygdaloid focus (line D).

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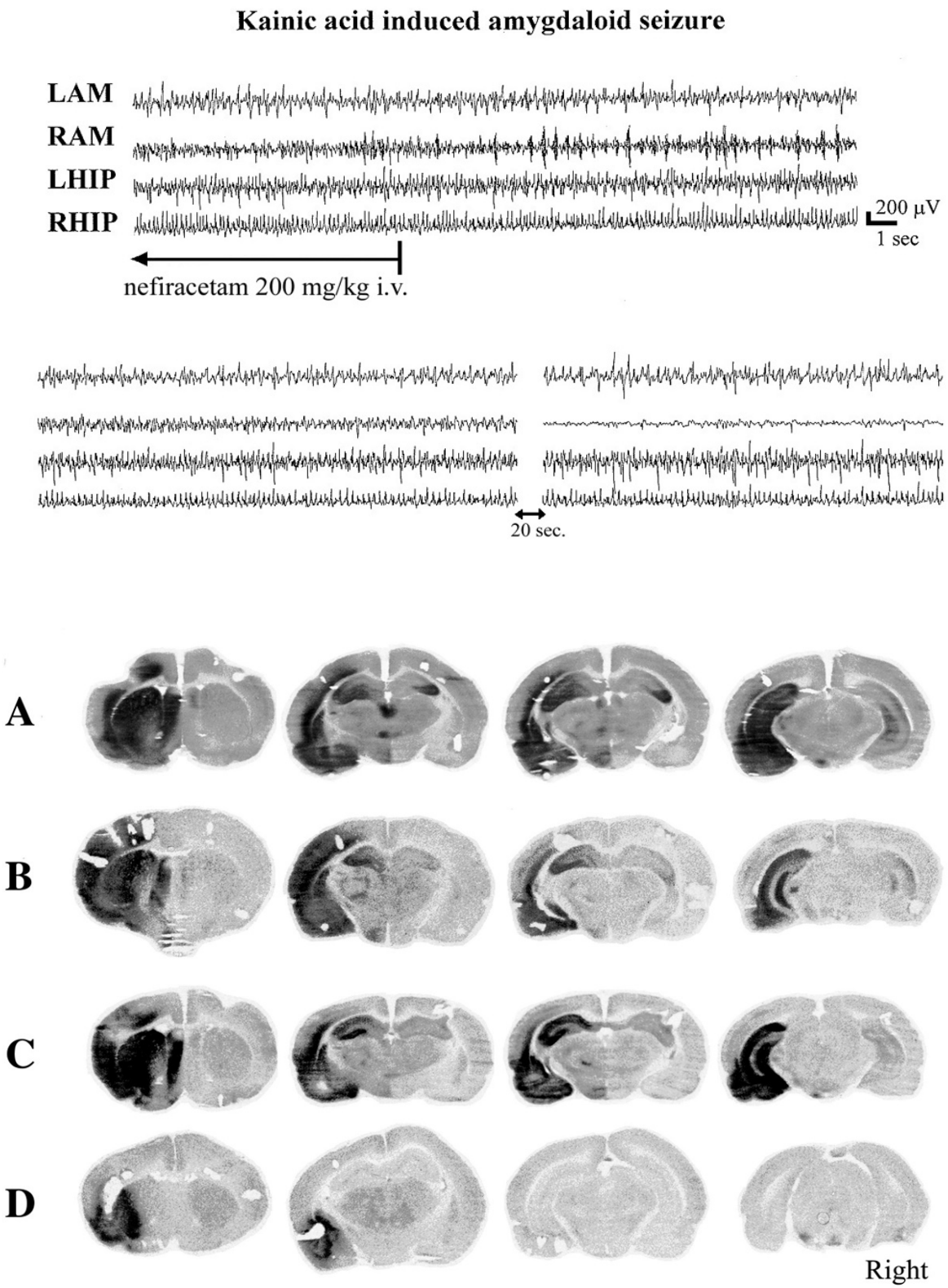
Kainic acid induced amygdaloid seizure
LAM
RAM
LHIP
RHIP
200 μV
1 sec
nefiracetam 200 mg/kg i.v.
20 sec.
A
B
C
D
Right