Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 2, pp. 553–556, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0076-G

BRIEF COMMUNICATION

Comparison of Benzodiazepine Receptor and Regional Cerebral Blood Flow Imaging of Epileptiform Foci in Hippocampal Kindled Rabbits: A Preliminary Report

ITSUKI JIBIKI,*[1] KENZO KUROKAWA,* HIROSHI MATSUDA,† TOSHINOBU FUKUSHIMA,* SHIRO TSUJI,† NARIYOSHI YAMAGUCHI* AND KINICHI HISADA†

*Department of Neuropsychiatry and †Nuclear Medicine, Kanazawa University School of Medicine, Takaramachi 13-1, Kanazawa, Ishikawa Prefecture, 920 Japan

Received 2 August 1993

JIBIKI, I., K. KUROKAWA, H. MATSUDA, T. FUKUSHIMA, S. TSUJI, N. YAMAGUCHI AND K. HISADA. *Comparison of benzodiazepine receptor and regional cerebral blood flow imaging of epileptiform foci in hippocampal kindled rabbits: A preliminary report.* PHARMACOL BIOCHEM BEHAV **48**(2) 553–556, 1994.—To clarify experimentally which of benzodiazepine (BZ) receptor or regional cerebral blood flow (rCBF) imaging is more sensitive in the detection of epileptic foci, we simultaneously examined the BZ receptor and rCBF distribution changes in hippocampal kindled rabbits with in vivo double tracer autoradiography using ^{125}I-labeled Ro 16-0154 (^{125}I-Iomazenil) and ^{99m}Tc-labeled hexamethyl-propyleneamine oxime (^{99m}Tc-HMPAO). In visual analysis of brain slices extracted after the intravenous injection of the double tracer following the kindling completion, ^{125}I-Iomazenil accumulation was more markedly and extensively decreased in the kindled right CA1 region mimicking a primary epileptic focus than ^{99m}Tc-HMPAO accumulation. Further, this decrease in ^{125}I-Iomazenil accumulation was not due to neuropathological abnormalities which consisted only of tissue damage corresponding to electrode track in the right CA1. These results suggest that BZ receptor imaging is more sensitive in the detection of epileptic foci than rCBF imaging and, therefore, that BZ receptor imaging is useful in clinical epilepsy.

Epilepsy Kindling Benzodiazepine receptor Regional cerebral blood flow Iomazenil HMPAO

RECENTLY, a new kind of neurochemical brain imaging, central-type benzodiazepine (BZ) receptor imaging has started to be performed on humans using positron emission tomography (PET) or single photon emission computed tomography (SPECT). From these studies, in patients with partial epilepsy, it is known that the BZ receptor distribution is decreased in epileptic foci in interictal periods (2,4,10,11). On the other hand, it has been established that regional cerebral blood flow (rCBF) determined by PET or SPECT is also decreased in the interictal foci, and that rCBF imaging is useful for the regional determination of epileptic foci (7). However, it is still controversial which of BZ receptor or rCBF imaging is more sensitive or useful for the detection of these foci (2,4,11). The present study aims at clarifying this problem experimentally.

Kindling is a suitable animal model of partial epilepsy, and is characterized by a virtual lack of histological cerebral damage. Using this experimental model of epilepsy, in the present study, we simultaneously examined BZ receptor and rCBF distribution changes with in vivo double tracer autoradiography using the central type BZ receptor antagonist or partial inverse agonist, ^{125}I-Ro 16-0154 (^{125}I-Iomazenil), and ^{99m}Tc-hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO), and deterimined which of these agents shows greater sensitivity for the detection of experimental epileptiform foci. At present,

[1] To whom requests for reprints should be addressed.

no reports are available on experimental epilepsy using this ^{125}I-Iomazenil. The present study may provide significant findings concerning the utility of neuroimaging for the regional determination of epileptic foci.

METHOD

Eleven adult male rabbits weighing 2.5–3.0 kg each were used. Surgical procedures were carried out under intravenous pentobarbital sodium anesthesia (20 mg/kg). According to our previous study (6), a tungsten microelectrode for recording (tip diameter: 1–2μm, resistance: 1–5 KΩ) and a concentric stimulating electrode (0.6 mm in diameter) were inserted into the right hippocampal CA1 region (2800–3950 μm below the pial surface) using an oil hydraulic microdrive while performing laminar analysis every 50 or 100 μm. For EEG recordings, skull screw electrodes were placed on the bilateral motor, parietal, and visual regions. Both the EEG recording and hippocampal depth recording were referred to a screw electrode placed on the frontal sinus. These electrodes were fixed to the skull with dental cement. After a postsurgical recovery period of 14 days, the threshold stimulus intensities to elicit an afterdischarges (AD) localized in the right CA1 region were determined, and kindling-inducing stimulus strengths (pulses of 1 ms duration, 60 Hz and 1 s in total duration, and 250–350 μA), which consisted of the suprathreshold stimulus intensities to induce ADs with 5–12 s durations, were decided. Thereafter, the kindling-inducing stimulations were repeated at 24-h intervals until generalized convulsions were induced a few times consecutively by the daily stimulations. Six animals received the daily stimulations until such a completion of the kindling was obtained (kindled group). The other three animals received 44 repeated trials of the daily stimulations with the current fixed at 150 μA, which induced no ADs on EEG throughout the trials (control group). This stimulus number of 44 was matched to the averaged stimulus number needed for the completion in the kindled group as mentioned later. In addition, the remaining two animals (sham group) received no daily stimulations, and only had electrodes implanted in the same manner as in the above two groups.

Autoradiographic study was started 14 days after the last daily stimulation in the kindled and control groups. This interval was taken to avoid the postseizure effect of the generalized convulsions in the kindled group, and matched to the kindled group in the control group. In the sham group, the autoradiographic study was started 72 days after the electrode implantation, including 14 days for the postsurgical recovery, to match the kindled and control groups. Two tracers, 18.5 MBq of ^{125}I-Iomazenil and 950 MBq of ^{99m}Tc-HMPAO, were intravenously injected simultaneously. The animals were killed by intravenous injection of KCl (20 ml) 2 h after the injection. It is known that ^{125}I-Iomazenil brain uptake reaches a plateau level at about 70 min after intravenous injection and remains stable for 3 h (5), whereas intracerebral ^{99m}Tc-HMPAO distribution is early decided at 2–3 min postinjection and remains stable for a long period of about 10 h (1). Therefore, the present 2 h interval is long enough to establish the intracerebral distribution of both agents. Thereafter, the brain was quickly extracted and frozen at -70°C in hexane and dry ice. Brain slices of 20 μm thickness were prepared at -20°C with a cryostat, mounted on coverslips, and dried for autoradiography. The first exposure of these slices was carried out for 15 h to obtain ^{99m}Tc-HMPAO imaging. Seven days later, which was a sufficiently long period to exclude the radioactivity of ^{99m}Tc-HMPAO, the second exposure of the same slices was performed for 14 days to obtain ^{125}I-Iomazenil imaging.

Autoradiograms were evaluated by visual inspection. The anatomical identification for the evaluation was performed in view of the identification in hematoxylin-eosin (H-E)-stained slices adjacent to the slices used for the autoradiograms according to Shek's atlas (12). Further, using the H-E-stained slices, histological examinations were performed to observe whether there was tissue damage due to the inserted electrodes and stimulations.

RESULTS

By the visual analysis in the sham group, ^{125}I-Iomazenil was highest in the gray matter of the frontal and temporal cortices and lowest in the white matter of the entire cortex. The accumulation in the dentate gyrus, hippocampus, amygdala, and thalamus was between that in the gray and white matters of the cortex. Also, ^{99m}Tc-HMPAO accumulation was highest in the frontal and temporal cortices and lowest in the white matter of the entire cortex. The accumulation in the dentate gyrus, hippocampus, amygdala, and thalamus was also between that in the gray and white matters of the cortex. However, among them, the thalamus showed the relatively high ^{99m}Tc-HMPAO accumulation. ^{125}I-Iomazenil was decreased corresponding to the electrode placement in the right CA1, whereas ^{99m}Tc-HMPAO accumulation showed no asymmetry in any of the cerebral regions including the CA1 region (Fig. 1, sham group).

In the control group, ^{125}I-Iomazenil accumulation was also decreased corresponding to the electrode placement in the right CA1. However, the extent of the decrease was larger than in the sham group. ^{99m}Tc-HMPAO accumulation showed almost no asymmetry in any of the cerebral regions including the CA1 region, as seen in the sham group (Fig. 1, control group).

In the kindled group, the stimulus number needed for the completion of kindling ranged from 38 to 54 (average, 44; standard deviation, 4). In the visual analysis, ^{125}I-Iomazenil accumulation was also decreased in the right CA1 as the stimulated site in comparison with the left CA1. However, the decrease was more marked and extensive beyond the electrode track than that in the control and sham groups. ^{99m}Tc-HMPAO accumulation was also decreased in the right CA1. However, both the degree and extent of the decrease were much slighter than those of the decrease in ^{125}I-Iomazenil accumulation (Fig. 1, kindled group).

In addition, histological examination showed that there was no tissue damage in any of the three groups except for the electrode track in the right CA1.

DISCUSSION

Both the present ^{125}I-Iomazenil and ^{99m}Tc-HMPAO distribution in the each cerebral region observed in the sham group is in rough agreement with those in normal human brain reported in the literature (5,13), reflecting normal central-type BZ receptor and rCBF distribution, respectively. The present visual analysis showed that ^{125}I-Iomazenil accumulation was slightly decreased in the right CA1 corresponding to the electrode track in the sham group. This decrease must have resulted from organic damage caused by the electrode placement. Further, the ^{125}I-Iomazenil accumulation in the right CA1 was more decreased in the control group than in the sham group. This difference suggests that the decrease in the ^{125}I-Iomazenil accumulation results from repeated electrical stimulations themselves, as well as the electrode placement. Moreover, the ^{125}I-Iomazenil accumulation in the right CA1

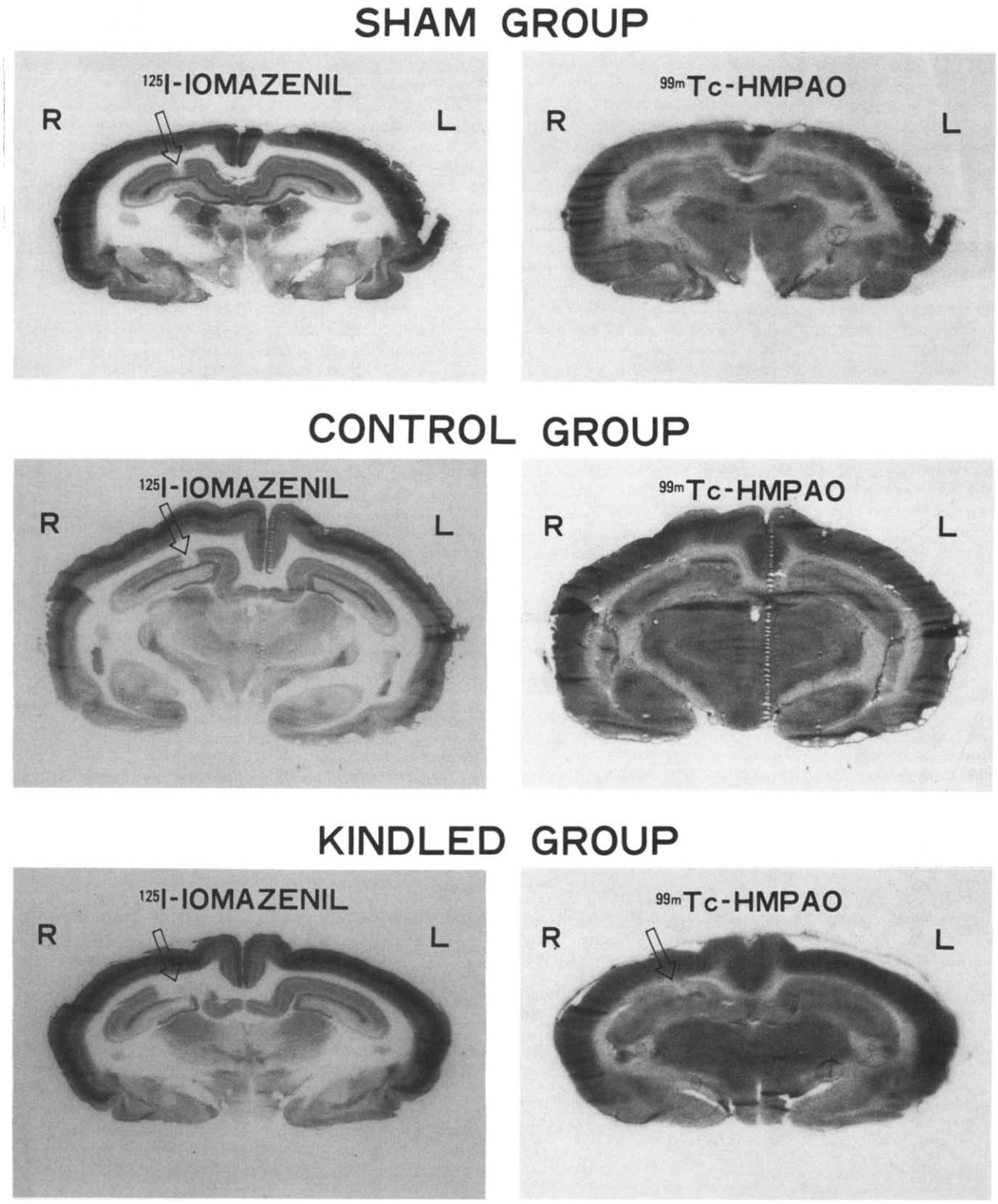

FIG. 1. Autoradiograms with ^{125}I-Iomazenil and ^{99m}Tc-HMPAO in sham, control, and kindled groups. They are enlarged 2.5-fold. R, right; L, left. Arrow marks indicate the sites of decreased accumulation of each tracer.

was more markedly and extensively decreased in the kindled group than in the control group. This marked difference suggests that the decrease results from the kindling effect itself, i.e., the effect of repeated epileptic activity. Such a decrease in ^{125}I-Iomazenil accumulation in the kindled group may be in agreement with past kindling studies that BZ receptor binding was decreased after the completion of kindling, although there are many conflicting studies (3).

On the other hand, the present study showed that ^{99m}Tc-HMPAO accumulation was slightly decreased in the right CA1 only in the kindled group. Natoli et al. have reported in a study with amygdaloid-kindled rats that rCBF was decreased in the hippocampus bilaterally, and the frontal cortex ipsilateral to the stimulated amygdala after the completion of kindling (9). However, no reports are available on decreases in kindled sites such as the present right CA1.

The most interesting finding was that the ^{125}I-Iomazenil accumulation was more markedly and extensively decreased in the right CA1 than the ^{99m}Tc-HMPAO accumulation. The right CA1 as the kindled site mimicks a primary epileptic focus in human epilepsy. This finding suggests that BZ receptor imaging is more sensitive for the detection of epileptic foci than rCBF imaging. Most SPECT studies comparing BZ receptor imaging with ^{123}I-Iomazenil and rCBF imaging with ^{99m}Tc-HMPAO or ^{123}I-IMP, etc. in human epilepsy have demonstrated that both imaging achieves almost equivalent detection of epileptic foci and, therefore, that BZ receptor imaging offers no advantage over the rCBF imaging (2,4), although a few studies have reported that the former was slightly superior to the latter (11). But, the present study suggests that the former is more sensitive in the detection of epileptic foci than the latter, although experimental results must be applied carefully in clinical situations.

In addition, a more recent autoradiographic study of the mesial temporal lobes excised from patients with intractable temporal lobe epilepsies showed that ^{125}I-Iomazenil accumulation was decreased only in the hippocampal area having neuron loss histologically (8). However, the present study showed that the decreased zone in the ^{125}I-Iomazenil accumulation extended largely beyond the area of tissue damage caused by the electrode placement in the histological examination. This finding may be of significance, indicating that the decrease in the ^{125}I-Iomazenil accumulation arises in areas without structural abnormalities, presumably on account of functional abnormalities themselvesin the areas; GABAergic inhibitory dysfunction coupled with with the BZ receptors.

In conclusion, the present study shows that BZ imaging with ^{125}I-Iomazenil is more sensitive in the detection of experimental epileptiform foci than rCBF imaging with ^{99m}Tc-HMPAO, suggesting that BZ imaging is useful for the determination of epileptic foci. After this, further detailed investigations with quantitative analysis in more animals should clarify whether changes in the BZ receptor and rCBF imaging are present not only in the kindled site mimicking the primary epileptic focus but also in the other cerebral regions to which epileptic discharges spread.

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