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Neuroreceptor Bindings and Synaptic Activity in Visual System of Monocularly Enucleated Rat

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Purpose: To study the changes in the distribution of postsynaptic benzodiazepine (BDZ) and presynaptic adenosine A_1 (AA_1) receptors in the superior colliculus (SC) and visual cortex (VC) of rats following monocular enucleation.

Methods: The right eyes of 6-week-old Long-Evans rats were enucleated and ex vivo autoradiography was performed on the SC and VC obtained at different times up to 8 weeks after the enucleation. [^{14}C]deoxyglucose was used to detect glucose metabolism, and [^{11}C]flumazenil and [1-methyl-^{11}C]8-dicyclopropylmethyl-1-methyl-3-propylxanthine ([^{11}C]MPDX) were used to map BDZ and AA_1 receptors, respectively. The receptor-specific binding for ^{11}C was determined, and ^{11}C and ^{14}C activities were evaluated separately in the same tissue by a double tracing method.

Results: The uptake of [^{14}C]deoxyglucose in the SC was depressed immediately after enucleation and gradually recovered. The binding of [^{11}C]flumazenil to BDZ receptors in the contralateral SC was increased at week 2, and then returned to the pre-enucleation levels. The uptake of [^{11}C]MPDX by the AA_1 receptors in the contralateral SC decreased by about 67% on day 5 after enucleation and remained low thereafter. In the contralateral VC, the uptake of [^{14}C]deoxyglucose decreased immediately after the enucleation followed by a gradual recovery, whereas the uptake of [^{11}C]flumazenil and [^{11}C]MPDX was not altered.

Conclusions: The axon degeneration related decrease of the AA_1 receptor density resulted in a transient up-regulation of postsynaptic BDZ receptor density in monocularly enucleated adult rats. These results suggest that these radioligands can be used to study the distribution of the postsynaptic BDZ and presynaptic AA_1 receptors in the visual system and can probably be applied to the human visual system for positron emission tomography. **Jpn J Ophthalmol 2001;45:264–269**

Key Words: Benzodiazepine and adenosine A_1 receptors, enucleation, ex vivo autoradiography, glucose metabolism, superior colliculus, visual cortex.

Introduction

The rat has been widely used to study the effects of enucleation on the visual pathways, and conventional anatomical methods have been used to determine the projection of the retina.[1–6] More recently, immunohistochemical and autoradiological methods have been used to study biochemical activity in the cortex. Thus, monocular enucleation resulted in an immediate reduction of cerebral glucose metabolism in the contralateral superior colliculus (SC), lateral geniculate body (LGB), and the visual cortex (VC).[1,7] A reduction of adenosine A_1 (AA_1) receptors in the contralateral SC and LGB was demonstrated by in vitro autoradiography (ARG) using [^{3}H]cyclohexyladenosine ([^{3}H]CHA)[2,6] and by a membrane-binding assay.[8] These findings led to the hy-

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pothesis that AA_1 receptors are located on the presynaptic membrane and are recognized as presynaptic markers for retinal input to the SC and LGB.[2,9]

We recently proposed the ^{11}C-labelled xanthine derivatives, [1-propyl-^{11}C]8-dicyclopropylmethyl-1, 3-dipropylxanthine ([^{11}C]KF15372)[10,11] and [1-methyl-11C] 8-dicyclopropylmethyl-1-methyl-3-propylxanthine ([^{11}C]MPDX),[12] as new positron-emitting ligands that have the potential for in vivo mapping of AA_1 receptors by positron emission tomography (PET). We detected a reduction of AA_1 receptor binding by ex vivo ARG in the contralateral SC of monocularly enucleated rats after an intravenous injection of these radioligands.[11,12]

Adenosine is an endogenous modulator of synaptic activity in the central nervous system. The effect is mediated by at least four subtypes of receptors: A_1, A_{2A}, A_{2B}, and A_3.[13] The AA_1 receptors are mainly located on the presynaptic nerve endings of excitatory neurons and modulate excitatory transmission. A relatively high receptor density has been demonstrated in the hippocampus, cerebral cortex, thalamic nucleus, cerebellar cortex, and SC of the postmortem human brain by in vitro ARG.[14–16]

Monocular enucleation of rodents results in a temporary increase in the binding capacity of central BDZ receptors in the contralateral SC and VC using [^3H]flumazenil as a radioligand[17] and by in vitro ARG with [^3H]Ro 15-4513.[18] High percentages of the central BDZ receptors are located on the postsynaptic membrane of the SC and VC.[17,19] These receptors can serve as markers for the postsynaptic component of visual input to the SC and VC.[20]

In this study, we determined the changes in the distribution of BDZ and AA_1 receptors in the SC and VC by ex vivo ARG following unilateral enucleation. We used a double tracer ARG method with short (^{11}C) and long half-life (^{14}C) radioisotopes to visualize the receptor density and glucose metabolism simultaneously in the same tissues. A part of the present study has been presented previously.[21]

Materials and Methods

[^{14}C]deoxyglucose (392 GBq/mmol) was purchased from Research Biochemicals International (Amersham, Buckinghamshire, England). [^{11}C]flumazenil and [^{11}C]MPDX were prepared, as previously described, for mapping BDZ receptors and AA_1 receptors, respectively.[12,22]

Male Long-Evans rats were supplied by Charles River Japan (Yokohama). This study was conducted in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research, and was approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Gerontology.

Six-week-old, male Long-Evans rats were anesthetized with intraperitoneal pentobarbital injection and the right eye was enucleated. At various times up to 8 weeks after the enucleation, the animals were sacrificed and the brain removed for ex vivo ARG. One group of 25 rats was given [^{14}C]deoxyglucose (3 MBq/kg; half-life of ^{14}C = 6,000 years) 30 minutes before the intravenous injection of [^{11}C]flumazenil (0.5 GBq/kg; half-life of ^{11}C = 20 minutes) and a second group of 17 rats received an intravenous injection of [^{11}C]MPDX (1 GBq/kg). Both groups of rats were kept alert and sacrificed by decapitation 15 minutes after the injection of the ^{11}C-labeled tracers. The brain was rapidly removed, frozen, and cut coronally into 20-μm-thick sections using a cryotome at −15°C. The sections containing the SC and VC[23] were mounted on glass slides, dried on a hot plate at 60°C, and apposed to imaging plates (IP-BAS TR; Fuji Film, Tokyo). In the single ^{11}C-labeled tracer ARG, the imaging plates were exposed to the brain sections until the ^{11}C had decayed (> 4 hours). In the double tracer ARG studies, the first exposure time was 1 to 2 hours to visualize the distribution of ^{11}C. Four to 24 hours later, more than 10 times the half-life of ^{11}C, the brain sections were apposed to another imaging plate to visualize the distribution of ^{14}C. The second exposure time was 19 to 22 hours. The regional distribution in the brain was visualized with a bioimaging analyzer, type BAS 3000 (FUJIX, Fuji Film).

After visualization of the distribution of radioactivity, the brain sections were stained with cresyl violet. Brain sections that contained the SC and VC were selected, and the regions of interest (ROIs) were determined on the superficial layers of the SC and VC based on the histological images. The radioactive density (total uptake) in the ROIs was calculated as the photo-stimulated luminescence (PSL) value per injected dose (PSL/mm^2/MBq), in which the injected dose was decay-corrected at the contact time. For the double tracer ARG, the PSL value for ^{14}C in each ROI was determined as the PSL value of the second exposure subtracted from the PSL value of the first exposure to calculate the PSL value based on ^{11}C.

Nonspecific uptake of receptor ligands was assessed by a blockade study using normal, 8-week-old Long-Evans rats. ^{11}C-labeled tracer was injected with and without an excess amount (700 mmol) of a selec-

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tive AA_1 antagonist, 8-dicyclopropylmethyl-1,3-dipropylxanthine (KF15372),[24] for [^{11}C]MPDX or of nonradiolabeled flumazenil for [^{11}C]flumazenil. Fifteen minutes after the injection, ex vivo ARG was performed as described.

The percentage of receptor-specific uptake of each ligand was calculated from the total uptake in the control and that in the blocked group ($n = 4$). In the individual eye-enucleated rats, the nonspecific uptake was calculated from the total uptake on the ipsilateral side of the brain. The receptor-specific binding of radioligands in the SC and VC was determined as the difference between the nonspecific uptake and total uptake on the contralateral side. The specific binding ratio of the contralateral to the ipsilateral ROI was also calculated. The time course of the logarithm of the left-to-right ratio was statistically analyzed for the presence of an increasing or decreasing trend and for left-to-right differences. When the time course appeared biphasic, the trend was tested for each phase.

Results

In normal rats, the uptake of [^{11}C]flumazenil and [^{11}C]MPDX by the SC was clearly visualized by ex vivo ARG. A combined injection with an excess amount of a blocking agent or a carrier compound led to a significant decrease in the uptake of both tracers. In normal rats, the receptor-specific uptake for [^{11}C]flumazenil 15 minutes after the injection was estimated to be 91% and 92% of the total uptake in the SC and VC, respectively. The corresponding values for [^{11}C]MPDX were 48% and 60%.

Two weeks after enucleation, the uptake of [^{11}C]MPDX and [^{14}C]deoxyglucose was lower in the contralateral than in the ipsilateral SC (Figure 1). On the other hand, the uptake of [^{11}C]flumazenil was higher in the contralateral (left) than in the ipsilateral (right) SC. In the left VC, the uptake of [^{14}C]deoxyglucose was decreased but the uptake of [^{11}C]MPDX and [^{11}C]flumazenil was not altered.

Time Course of Receptor-specific Binding

The time course of the left-to-right ratios of the [^{14}C]deoxyglucose and that of the receptor-specific binding of [^{11}C]flumazenil and [^{11}C]MPDX in the SC are plotted in Figure 2. The left-to-right ratios of [^{14}C]deoxyglucose uptake were lower on day 1 after enucleation. The deafferented left SC was labeled significantly less than the control right side ($P < .001$). Thereafter, a weak increasing trend was detected ($P = .08$) (Figure 2A). The [^{11}C]flumazenil binding, on the other hand, increased for the first 2 weeks after a lag time of several days ($P < .02$) and returned to the pre-enucleation level (Figure 2B). The ratio for the [^{11}C]MPDX binding decreased by about two thirds for 2 weeks ($P < .01$) and remained low until 5 weeks ($P < .001$) (Figure 2C).

In the VC, the left-to-right ratio of [^{14}C]deoxyglucose uptake was significantly lower ($P < .001$) and gradually increased throughout the study period ($P < .001$) (Figure 3A). No alteration in the left-to-right ratio of receptor-specific binding of [^{11}C]flumazenil (Figure 3B) or [^{11}C]MPDX (Figure 3C) was found.

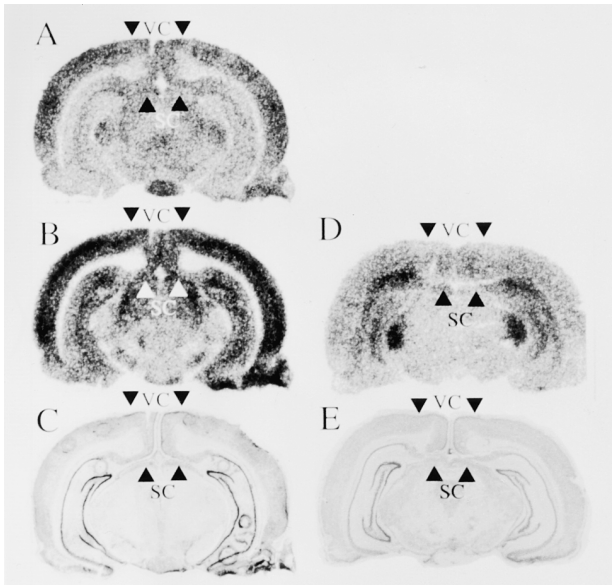

Figure 1. Autoradiograms and histological sections of rat brain two weeks after enucleation of right eye of rat. (**A**) Autoradiogram obtained with [^{14}C]deoxyglucose in rat brain 2 weeks after enucleation. [^{14}C]deoxyglucose uptake in denervated left superior colliculus (SC) and visual cortex (VC) (right side of image) was lower than in right side. (**B**) Autoradiogram obtained from same section but with [^{11}C]flumazenil. Denervated left SC shows 33% higher ^{11}C uptake by image analysis. This indicates up-regulation of postsynaptic benzodiazepine receptors. (**C**) Same section as (**A**) and (**B**) stained with cresyl violet. (**D**) Autoradiogram obtained with [1-methyl-^{11}C]8-dicyclopropylmethyl-1-methyl-3-propylxanthine for presynaptic adenosine A_1 (AA_1) receptors, showing loss of presynaptic AA_1 receptors. (**E**) Same section as (**D**) stained with cresyl violet.

Discussion

Using Positron-emitting Ligands and Imaging Plate for Ex Vivo ARG

Positron-emitting ligands specific for neuroreceptors have been used to evaluate the receptor density

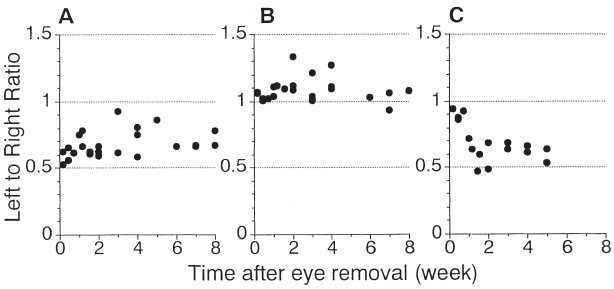

Figure 2. Time course of left-to-right ratios of [^{14}C]deoxyglucose uptake (**A**), receptor-specific binding of benzodiazepine ligand [^{11}C]flumazenil (**B**), and adenosine A_1 ligand [1-methyl-^{11}C]8-dicyclopropylmethyl-1-methyl-3-propylxanthine (**C**) in superior colliculus.

on the surface of neural cells.[25] They are more suitable because of their high specific activity and short half-life than radionucleides with a long half-life. Quantitative, repetitive, and noninvasive measurements of receptor density can be performed by PET with positron-emitting ligands. Recently, a high-resolution PET scanner with a maximum resolution of 1.8 mm has been proposed.[26] However, even such a scanner is not sufficient to evaluate intracerebral structures, such as the SC and VC in rats. Our results show that ARG with positron-emitting ligands provides good resolution and can be quantified. The use of an imaging plate enhances the sensitivity to ARG compared to traditionally utilized noncoated x-ray autoradiographic film for tritium. The use of an imaging plate is reasonable because the positron emitter has a short half-life in the order of minutes. This means that the results of ARG can be obtained within several hours. Thus, quantitative measurement of neuroreceptor density in a living brain has been possible for the first time with the use of a positron ligand

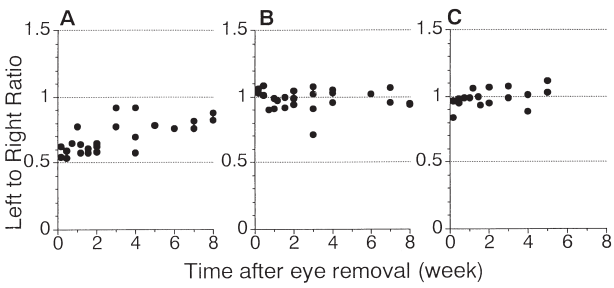

Figure 3. Time course of left-to-right ratios of [^{14}C]deoxyglucose uptake (**A**), receptor-specific binding of benzodiazepine ligand [^{11}C]flumazenil (**B**), and adenosine A_1 ligand [1-methyl-^{11}C]8-dicyclopropylmethyl-1-methyl-3-propylxanthine (**C**) in the visual cortex.

and imaging plate. All of the data shown here were obtained by this newly arranged combination of techniques.

There are, however, some disadvantages to this technique. The entire procedure must be done fast enough to minimize the loss of radioactivity due to decay. This is the reason why the source of brain slices was limited to the SC and VC. Another disadvantage of the short-half life of ^{11}C is that a relatively high radioactivity level must be injected to obtain good images. The specific binding of [^{11}C]flumazenil to BDZ receptors was more than 90%. Therefore, we omitted the nonspecific binding from the total uptake of receptor ligands and evaluated the degenerative loss of the receptors as the right-to-left ratio of specific binding.

On the other hand, judging from our previous data,[12] the carrier amount of [^{11}C]MPDX slightly exceeded the optimum binding conditions, which may result in a 10% to 20% underestimation of the specific binding. This may partially explain the high nonspecific binding of [^{11}C]MPDX observed. The high nonspecific binding may also be explained by the metabolism of [^{11}C]MPDX and the blocking agent KF15372, which may not completely block the tracer uptake.[12]

Double Tracer Autoradiography

Two images representing different physiological information can be obtained from the same brain sections with the use of two radioisotopes that have different half-lives. Thus, information on neurotransmitter receptor density, metabolism of substrates such as glucose and amino acids, and blood flow can be obtained. In this experiment, ^{11}C- and ^{14}C-labeled tracers reflected the density of BDZ receptors and glucose metabolism, respectively. The use of a positron-emitting ligand with a very short half-life enhanced its efficacy for animal experiments.[27] With an imaging plate, sensitivity to radioactivity was greatly improved, and the exposure time for both ^{11}C and ^{14}C could be shortened to 2 days with a minimum of 24 hours.

Alterations of Radiotracer Uptake Following Unilateral Enucleation

[^{14}C]deoxyglucose uptake. It has been reported that disruption of visual input by the destruction of the anterior visual pathway causes a depression of glucose consumption in vision-related structures in the brain.[1,7] This depression was shown in this study

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by a rapid reduction of ^{14}C-deoxyglucose uptake in the SC and VC followed by a gradual recovery.

BDZ receptor binding of [^{11}C]flumazenil in the SC. A delayed increase of BDZ receptor binding of [^{11}C]flumazenil in the SC was observed. Binding in the VC did not increase although Mardar et al[17] reported increased BDZ receptor binding of [^{3}H]flumazenil in the VC. This discrepancy may possibly be due to differences in methods. We do not know whether this increase is due to increased receptor density (B_{max}) or to altered receptor affinity (Kd) because increased receptor ligand binding can occur in either situation. Pinard et al[18] reported an enhanced Kd for BDZ in the SC bilaterally by in vitro ARG 7 days after enucleation using [^{3}H]Ro15-4513 as a ligand. This phenomenon is called an up-regulation of the receptor density, a compensating mechanism for reduced input.[1] An increase in receptor ligand binding has also been reported for some neurotransmitters or neuromoderators after unilateral enucleation.[2,3]

Delayed decrease of presynaptic AA_1 receptors in the SC. Our results demonstrated that the maximal reduction of AA_1 receptors in the superior colliculus was approximately 50%, although the level of AA_1 receptors later than 6 weeks after the enucleation was not examined in this study. This result may be interpreted to mean that only half the optic nerve endings had disappeared in the SC within 3 weeks after contralateral enucleation in the rat, if all the AA_1 receptors were present on the presynaptic membrane of retinal ganglion cells projecting into the SC.[2] On the other hand, this may suggest that all the AA_1 ligand binding sites in the superficial layer of the SC are not always located on the presynaptic membrane. Alternatively, projections from the cortical areas or nuclei other than the SC or an intrinsic connection of the SC may have been present and continued to function after monocular enucleation.

Wan and Geiger[8] reported that unilateral enucleation reduced the [^{3}H]cyclohexyladenosine ([^{3}H]CHA) binding to AA_1 sites by 51% and the AA_1 component of the [^{3}H]N-ethylcarboxamidoadenosine ([^{3}H]NECA) binding by 42% in the contralateral SC by conducting an in vitro binding assay. The binding of [^{3}H]CGS21680 or [^{3}H]NECA to adenosine A_2 sites was not significantly affected by the enucleation procedure. On the other hand, no significant reduction in adenosine receptor binding was found in the SC of rats placed in the dark for 3 weeks. These findings suggest that the AA_1 sites are located presynaptically on retinal projections and are vulnerable to deafferentation but not to functional deprivation. Chalmers and McCulloch[2] reported a 50% reduction of AA_1 receptors in the SC and an absence of reduction in the VC using in vitro ARG. During the first 5 days after enucleation, they found no significant alterations in AA_1 receptor binding of [^{3}H]CHA in visual structures of the visually deprived hemisphere. However, at 10 days postlesion, a significant reduction (50%) was found in [^{3}H]CHA binding in the visually deprived SC, but not in the dorsal LGB or VC. These results are comparable to our finding of a 50% reduction of receptor-specific binding of [^{11}C]MPDX in the SC and no reduction in the VC even though the ligands and measurement techniques differed. The absence of an alteration of AA_1 binding of [^{11}C]MPDX in the VC is explained by the fact that AA_1 binding is unaffected by a functional suppression of transsynaptic input. There is also a possibility that sufficient numbers of AA_1 receptors were not localized on the presynaptic terminals from the LGB.

A 1-week delay in the decrease of AA_1 receptor density in the SC in our experiment was shorter than the reported time for the appearance of neuronal degeneration in the SC after monocular enucleation in adult rats examined by electron microscopy.[28] This suggests that the detection of a degenerative process by ex vivo ARG was earlier than detection based on morphological changes. In addition, the absence of recovery of receptor density in our study supported the idea that this decrease was related to the loss of presynaptic components of the synapses. This supports an earlier suggestion of this phenomenon by an in vitro binding assay.[8]

Similar density decreases were also reported for alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptors,[2] 5-hydroxytriptamine-1 receptors and γ-aminobutyric $acid_A$ (GABA) receptors,[1] and angiotensin-2 receptors[29] in the SC and/or in the LGB. They are considered to represent a presynaptic receptor loss.

Our results indicated that neuroreceptor imaging by PET, especially for the comparison of pre- and postsynaptic components in the visual structures such as the primary visual cortex in hemianoptic patients, will provide useful information on blindness.

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References

1. Chalmers DT, McCulloch J. Alterations in neurotransmitter receptors and glucose use after unilateral orbital enucleation. Brain Res 1991;540:243–54.
2. Chalmers DT, McCulloch J. Selective alterations in glutamate receptor subtypes after unilateral orbital enucleation. Brain Res 1991;540:255–65.
3. Nakagawa Y, Yanai K, Ryu JH, Kiyosawa M, Tamai M, Watanabe T. Marked increase in [^{3}H](R)alpha-methylhistamine binding in the superior colliculus of visually deprived rats after unilateral enucleation. Brain Res 1994;643:74–80.
4. Kumar A, Schliebs R, Bigl V. Postnatal development of NMDA, AMPA, and kainate receptors in individual layers of rat visual cortex and the effect of monocular deprivation. Int J Dev Neurosci 1994;12:31–41.
5. Wakabayashi S, Freed LM, Bell JM, Rapoport SI. In vivo cerebral incorporation of radiolabeled fatty acids after acute unilateral orbital enucleation in adult hooded Long-Evans rats. J Cereb Blood Flow Metab 1994;14:312–23.
6. Kiyosawa M, Dauphin F, Kawasaki T, et al. Unilateral eyeball enucleation differentially alters AMPA-, NMDA- and kainate glutamate receptor binding in the newborn rat brain. Neurosci Res 1996;26:215–24.
7. Cooper RM, Thurlow GA. Depression and recovery of metabolic activity in rat visual system after eye removal. Exp Neurol 1985;89:443–64.
8. Wan W, Geiger JD. Effects of enucleations and visual deprivation on adenosine A_1 and A_2 receptor binding in superior colliculus of rat. Neurosci Lett 1990;117:160–4.
9. Goodman RR, Kuhar MJ, Hester L, Snyder SH. Adenosine receptors: autoradiographic evidence for their location on axon terminals of excitatory neurons. Science 1983;220:967–9.
10. Ishiwata K, Furuta R, Shimada J, Ishii S. Synthesis and preliminary evaluation of [^{11}C]KF15372, a selective adenosine A1 antagonist. Appl Radiat Isot 1995;46:1009–13.
11. Furuta R, Ishiwata K, Kiyosawa M, et al. Carbon-11 labeled KF15372; a potential CNA adenosine A1 receptor ligand. J Nucl Med 1996;37:1203–7.
12. Noguchi J, Ishiwata K, Furuta R, et al. Evaluation of carbon-11 labeled KF15372 and its ethyl and methyl derivatives as a potential CNS adenosine A_1 receptor ligand. Nucl Med Biol 1997;24:53–9.
13. Collis MG, Hourani SMO. Adenosine receptor subtypes. Trends Pharmacol Sci 1993;14:360–6.
14. Fastbom J, Pazos A, Palacios JM. The distribution of adenosine A_1 receptors and 5′-nucleotidase in the brain of some commonly used experimental animals. Neuroscience 1987; 22:813–26.
15. Fastbom J, Pazos A, Probst A, Palacios JM. Adenosine A1 receptors in the human brain: a quantitative autoradiographic study. Neuroscience 1987;22:827–39.
16. Onodera H, Kogure K. Differential localization of adenosine A_1 receptors in the rat hippocampus: quantitative autoradiographic study. Brain Res 1988;458:212–7.
17. Madar I, Scheffel U, Frost JJ. Transient increase in the in vivo binding of the benzodiazepine antagonist [^{3}H]flumazenil in deafferented visual areas of the adult mouse brain. Synapse 1994;18:79–85.
18. Pinard R, Segu L, Lanoir J. Transient increase in [^{3}H]Ro 15-4513 specific binding in the superficial gray layer of the rat superior colliculus induced by visual deafferentation. Brain Res 1991;543:287–95.
19. Matus A, Pehling PJ, Wilkins D. Gamma-aminobutyric acid receptors in brain postsynaptic dendrites. Neurobiology 1981;12:67–73.
20. Pinard R, Richards JG, Lanoir J. Subcellular localization of $GABA_A$/benzodiazepine receptor-like immunoreactivity in the superficial gray layer of the rat superior colliculus. Neurosci Lett 1990;120:212–6.
21. Ishiwata K, Noguchi J, Kiyosawa M, Endo K, Suzuki F, Senda M. Alteration of pre-synaptic adenosine A_1 and post-synaptic benzodiazepine receptor densities in the rat superior colliculus after eye enucleation assessed by ex vivo autoradiography. Cereb Blood Flow Metab 1997;17(Suppl 1):S5.
22. Ishiwata K, Itou T, Ohyama M, et al. Metabolite analysis of [^{11}C]flumazenil in human plasma: assessment as the standardized value for quantitative PET studies. Ann Nucl Med 1998;12:55–9.
23. Paxinos G, Watson C. The rat brain in stereotaxic coordinate, 2nd ed. New York: Academic Press, 1986.
24. Shimada J, Suzuki F, Nonaka H, Ishii A. 8-Polycycloalkyl-1,3-dipropylxanthines as potent and selective antagonists for A_1-adenosine receptors. J Med Chem 1992;35:924–30.
25. Volkow ND, Brodie JD, Gomez-Mont F. Applications of positron emission tomography to psychiatry. In: Reivich M, Alavi A, eds. Positron emission tomography. New York: Alan R Liss, 1985:311–27.
26. Chatziioannou AF, Cherry SR, Shao Y, et al. Performance evaluation of micro PET: a high-resolution lutetium oxyorthosilicate PET scanner for animal imaging. J Nucl Med 1999;40:1164–75.
27. d'Argy R, Ullberg S, Stalnacke CG, Langstrom B. Whole-body autoradiography using ^{11}C with double-tracer applications. Int J Appl Radiat Isot 1984;35:129–34.
28. Pinard R, Benfares J, Lanoir J. Electron microscopic study of GABA-immunoreactive neuronal processes in the superficial gray layer of the rat superior colliculus: their relationships with degenerating retinal nerve endings. J Neurocytol 1991;20:262–76.
29. Michels KM, Heemskerk FM, Saavedra JM. Selective changes in angiotensin II AT_1 and AT_2 receptor subtypes in the rat superior colliculus following eye enucleation. Neuroscience 1994;58:835–44.