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RESEARCH ARTICLE

Change in the Binding of [^{11}C]BU99008 to Imidazoline I_2 Receptor Using Brain PET in Zucker Rats

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Abstract

Purpose: The imdazoline I_2 receptor (I_2R) has been found in the feeding centers of the brain, such as the hypothalamus, and certain I_2R ligands have been reported to stimulate food intake. Thus, it has been proposed that I_2R may play a role in feeding control. [^{11}C]BU99008 was developed as a positron emission tomography (PET) tracer for imaging of I_2R. [^{11}C]BU99008 displayed relatively high brain penetration and specific binding by brain PET studies in preclinical studies. Here, we evaluated a pathological condition caused by obesity related to I_2R function by quantitative PET study using [^{11}C]BU99008.

Procedures: PET scans were acquired in the Zucker (ZUC) lean and fatty rats, radioactivity and metabolites of plasma were measured, and the kinetic parameters were estimated.

Results: Radioactivity levels after the injection of [^{11}C]BU99008 in the hypothalamus of both ZUC lean and fatty rats were highly accumulated, and then gradually decreased until 60 min after the injection. The accumulated radioactivity from 30 to 60 min after the injection in the hypothalamus of the ZUC fatty rats was 1.3 times greater than that of lean rats. The volume of distribution (V_T) estimated by Logan graphical analysis in the hypothalamus of the ZUC fatty rats was 1.8 times greater than that in the ZUC lean rats. In metabolite analysis, the percentages of the unchanged form in the plasma of the ZUC fatty rats at 60 min after the injection (5.0 %) was significantly lower than that of lean rats (9.1 %).

Conclusions: By PET imaging using [^{11}C]BU99008, we demonstrated that the accumulated radioactivity and estimated V_T value in the feeding center of ZUC lean rats was lower than that in fatty rats. PET studies using [^{11}C]BU99008 may contribute to elucidate a pathological condition caused by obesity related to I_2R function.

Key words: [^{11}C]BU99008, Imidazoline receptor, I_2, Positron emission tomography, Obesity, Zucker rats

Introduction

The imidazoline receptors (IRs) were previously defined as the non-adrenoceptor for a group of structurally related compounds containing imidazoline or guanidium moieties,

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because it could not be accounted for by their interaction with adrenoceptors (ARs) [1, 2]. The IR have been classified into I_1 and I_2 subtypes on the basis of results obtained from radioligand binding studies: I_1R labeled with [^{3}H]clonidine and its analogs, and I_2R labeled with [^{3}H]idazoxan [3, 4]. Idazoxan which is an I_2R antagonist [5] has been reported to increase food intake in rats [6]. On the other hand, 2-ethoxy and 2-methoxy idazoxan derivatives, which are selective α_2-AR antagonists with negligible affinity for I_2R, have been reported to exhibit no effect on food intake [6]. Thus, it is considered that the effects on food intake by idazoxan are mediated by I_2R. In addition, certain I_2R ligands have also been reported to stimulate food intake [7–9]. Furthermore, I_2R have been found in feeding centers [10], such as the hypothalamus [11, 12]. Thus, it has been proposed that the I_2R may play a role in feeding control.

[^{11}C]BU99008 has high and selective affinity for I_2R (*K*i for I_2R, 1.4 nM) [13], and is a more potent positron emission tomography (PET) tracer for brain imaging than previously developed PET tracers [14–18]. [^{11}C]BU99008 displayed high uptake and specific binding in pig and rhesus monkey brains [19, 20]. Moreover, we previously reported that [^{11}C]BU99008 with high molar radioactivity exhibited high specific binding for I_2R in the hypothalamus of rats [21]. Thus, it is considered that a PET study using [^{11}C]BU99008 may detect small changes in the small regions with I_2R and that [^{11}C]BU99008 may be a representative PET tracer for imaging of I_2Rs. In this study, we evaluated a pathological condition caused by obesity related to I_2R function by quantitative PET study for Zucker (ZUC) fatty and lean rats using [^{11}C]BU99008.

Materials and Methods

General

[^{11}C]BU99008 was synthesized as described previously [21]. The synthesis of [^{11}C]BU99008 used an automated system developed in-house [22]. The molar radioactivity of [^{11}C]BU99008 at end of synthesis (EOS) was 24–206 GBq/μmol, and the radiochemical purity at EOS was > 98 %. Reagents and organic solvents were commercial products as described previously [21] and were used without further purification.

Male Zucker (ZUC) fatty rats (*Lepr^{fa}/Lepr^{fa}*; 8–9 weeks old [w.o.]; body weight 374 ± 40 g body weight [b.w.]) and ZUC lean rats (*Lepr^{fa}*/+ or +/+; 8–9 w.o.; 266 ± 32 g b.w.) were purchased from Charles River Laboratories Japan (Kanagawa, Japan). Both ZUC lean and fatty rats were allowed water and same food *ad libitum*. Rats were maintained and handled as described previously [23]. PET studies using rats were approved in our institute (the Animal Ethics Committee of the National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan).

PET Studies in ZUC Rats

PET studies using ZUC lean ($n = 5$) and fatty ($n = 5$) rats were performed according to the methods described previously [23]. [^{11}C]BU99008 (31–63 MBq/0.2–4.5 nmol/1.0 ml) was injected in the tail vein of the rat. Radioactivity was expressed as a standardized uptake value (SUV) as described previously [23].

Blood Sampling and Metabolite Analysis

Arterial blood (50 μl to 1.0 ml: total 2 ml) was sampled as described previously [23]. The radioactivity was counted using an automatic gamma counter (1480 Wizard, Perkin-Elmer, Waltham, MA, USA) and was corrected for decay. Blood samples were centrifuged to separate the plasma as described previously [23]. The plasma was collected in a tube, added an equivalent volume of formic acid, and then added threefold amount of 1 mol/l ammonium acetate solution. The plasma sample was analyzed as described previously [24]. The extraction eluent was used an aqueous 0.1 mol/l ammonium acetate buffer, and the capture column was used a Cadenza HS-C18 column (3 μm, 10 mm i.d. × 50 mm length; Imtakt, Kyoto, Japan). The extraction column was used an XSelect CSH Fluoro-Phenyl OBD Prep column (5 μm, 10 mm i.d. × 100 mm length; Waters, Milford, MA, USA). The analysis eluents were a mixture of 90 % acetonitrile solution and aqueous 0.1 mol/l ammonium acetate solution (0:100 → 100:0 gradient, *v*/*v*) at 3–11 min after the injection, and 90 % acetonitrile solution at 11–12 min after the sample loaded. The flow rate was 4.0 ml/min. The retention time of [^{11}C]BU99008 was 9.5 min.

The percentage of [^{11}C]BU99008 was calculated as described previously [23], and the metabolite-corrected plasma curve was generated as described previously [23].

Data Analysis

Summed PET images were reconstructed between 30 and 60 min after the injection of [^{11}C]BU99008 using ASIPro VM (Analysis Tools and System Setup-Diagnostics Tool/Siemens, Knoxville, TN, USA) and PMOD version 3.4 (PMOD Technologies, Zurich, Switzerland). Volumes of interest (VOIs) were drawn as described previously [25]. The respective tissue time-activity curves (tTACs) were derived as described previously [23]. The radioactivity was expressed as described previously [23].

A one-tissue compartment model (1-TCM), two-tissue compartment model (2-TCM), Ichise multilinear analysis (MA-1), and Logan graphical analysis (Logan GA) were assessed as previously described [23, 26] using a PMOD software version 3.4.

Ex vivo Metabolite Analysis in the Brain of ZUC Rats

[^{11}C]BU99008 (133–144 MBq/2.7–18 nmol) was intravenously injected into male ZUC fatty (8 w.o.; 325–372 g b.w.; $n = 3$) or lean rats (8 w.o.; 239–265 g b.w.; $n = 3$). Rats were euthanized at 30 min after the injection. The whole brain was treated as described previously [21]. The mixed supernatants were analyzed using radio-HPLC described above using a XSelect CSH Fluoro-Phenyl OBD Prep (5 µm, 10 mm i.d. × 100 mm length) as a column, a mixture of 90 % acetonitrile solution and aqueous 0.1 mol/l ammonium acetate solution (0:100 → 100:0, gradient, *v*/*v*) at 3–11 min after the injection, and 90 % acetonitrile solution at 11–12 min after the injection as an eluent, 4.0 ml/min as a flow rate, and 300 nm for UV detection. The retention time of [^{11}C]BU99008 was 8.5 min.

The distribution of radioactivities was measured, and radioactivity was estimated to percentages of the unchanged form as described previously [21].

Statistical Analysis

Quantitative data are expressed as the mean ± standard deviation (SD). Differences between the ZUC fatty and lean rats were examined using an unpaired *t* test and were considered significant at $P < 0.05$. The data were analyzed using SigmaPlot 13.0 software (Systat Software, San Jose, CA, USA).

Kinetic model selection was determined by three statistical results: AIC, MSC; and R square (see in supplemental material).

Results

PET Study in ZUC Rats

Typical coronal brain PET images acquired from 30 to 60 min after the injection of [^{11}C]BU99008 in the ZUC lean and fatty rats are shown in Fig. 1. In both lean and fatty rats, high radioactivity level after the injection was observed in the hypothalamus and hippocampus, which have high levels of I_2Rs. The radioactivity level after the injection in the ZUC fatty rat was higher than that in lean rat (Fig. 1).

Time-radioactivity curves (TACs) in the hypothalamus and hippocampus in ZUC lean and fatty rats are shown in Fig. 2. In both ZUC lean and fatty rats, the TACs gradually decreased from initial uptake until 60 min. The radioactivity concentration over 15 min after the injection in the ZUC fatty rats was slightly higher than that in the lean rats (Fig. 2).

The areas under the TACs ($AUC_{30–60\ min}$) values from 30 to 60 min after the injection of [^{11}C]BU99008 in the hypothalamus and hippocampus of the ZUC lean and fatty rats are shown in Fig. 3. In both brain regions, the radioactivity level after the injection in the ZUC fatty rats (51 for hypothalamus and 47 for hippocampus) was significantly higher than that in lean rats (39 for hypothalamus and 33 for hippocampus) (Fig. 3).

The TACs in the plasma after the injection of [^{11}C]BU99008 in the ZUC lean and fatty rats are shown in Fig. 4a. In the plasma, the radioactivity level after the injection of [^{11}C]BU99008 in the ZUC fatty rats slightly increased after the initial uptake, although that in the ZUC lean rats was maintained at a constant level after the initial uptake (Fig. 4a). The radioactivity level at 60 min after the injection in the plasma of ZUC fatty rats (0.36 SUV) was significantly higher than that in the lean rats (0.23 SUV) (Fig. 4a). The time course of percentages of the unchanged form in the plasma of the ZUC lean and fatty rats are shown in Fig. 4b. The percentages of the unchanged form of both rats declined over time for 60 min after the injection (Fig. 4b). The percentages of the unchanged form in the plasma of the ZUC fatty rats at 60 min after the injection (5.0 %) was significantly lower than that in lean rats (9.1 %) (Fig. 4b).

Based on the results of three statistical analyses for kinetic model selection, it was obvious that 2-TCM was the most reliable method for acquisition of distribution volume

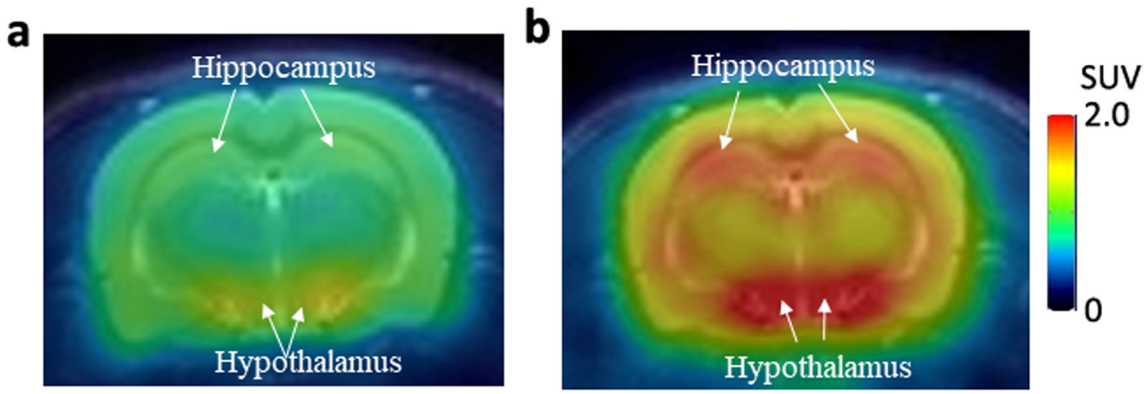

Fig. 1. Typical coronal PET/MRI images using [^{11}C]BU99008 (31–63 MBq/0.2–4.5 nmol) in the **a** ZUC lean and **b** fatty rat brain. Respective PET images were averaged during 30 to 60 min after the injection. Rats were anesthetized with isoflurane and placed in the prone position on the bed of the scanner. The scale of radioactivity is expressed as SUV. The MRI image was from a template using a representative normal rat.

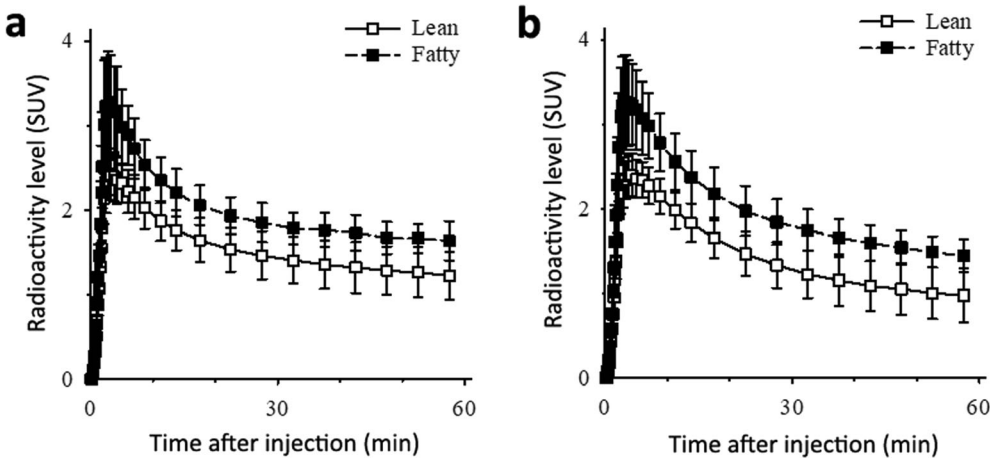

Fig. 2. Time-activity curves of the **a** hippocampus and **b** hypothalamus in the ZUC lean ($n = 5$) and fatty ($n = 5$) rats after the injection of [^{11}C]BU99008 (31–63 MBq/0.2–4.5 nmol). Radioactivity level is expressed as mean SUV.

(V_T) value. However, very high variation of V_T values in 2-TCM was shown due to low identifiability of parameters k_3 and k_4 (see in supplemental material). Therefore, we selected Logan GA as a simple method to obtain V_T value instead of 2-TCM. Results of V_T values in the brain regions of the ZUC lean and fatty rats are shown in Fig. 5. The rank order of V_T values was as follows: hypothalamus > hippocampus, thalamus, cortex, and striatum > cerebellum and pons, and was similar to the order of specific binding of I_2R ligands in these regions as previously described [12, 27]. The V_T value (25 for lean and 45 for fatty) in the hypothalamus showing the highest density of all rat brain regions in *in vitro* binding assay as previously described [12] was the highest among all investigated brain regions of both rats (21–24 for lean and 18–34 for fatty). The V_T values of all investigated brain regions except for the cerebellum and pons in fatty rats (30–45) were significantly higher than that in lean rats (19–25). The V_T values of the cerebellum and pons in the ZUC fatty rats (24 and 20, respectively) exhibited a tendency to be higher than those in lean rats (16 and 14, respectively).

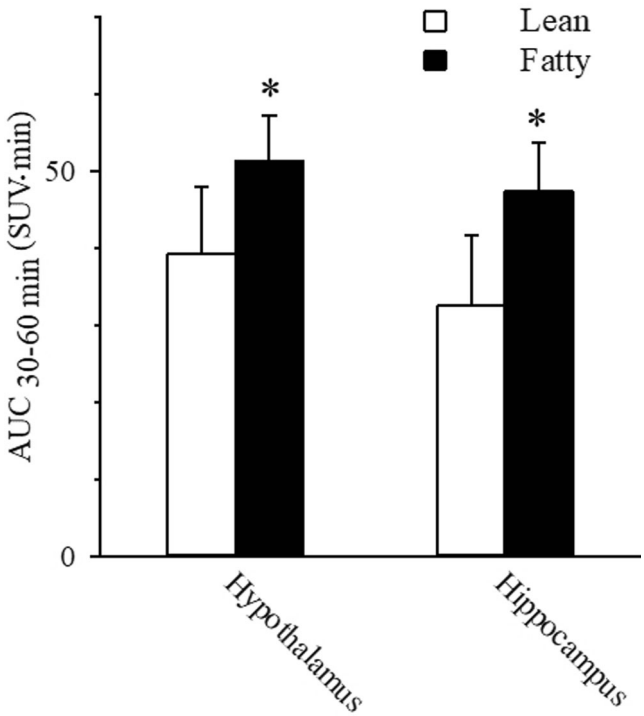

Fig. 3. Accumulated radioactivity ($AUC_{30–60\ min}$ [SUV × min]) in the hypothalamus and hippocampus of the ZUC lean ($n = 5$) and fatty ($n = 5$) rats after the injection of [^{11}C]BU99008 (31–63 MBq/0.2–4.5 nmol). *$P < 0.05$ (unpaired t test, compared to the ZUC lean rats).

The correlative relationship between radioactivity accumulation ($AUC_{30–60\ min}$) and the volume of distribution (V_T) in the hypothalamus and hippocampus using PET studies in ZUC lean and fatty rats are shown in Fig. 6. In the hypothalamus and hippocampus of ZUC rats, V_T values were strongly correlated with $AUC_{30–60\ min}$ values ($r^2 = 0.8480$; $P = 0.0012$ and 0.7510; $P = 0.0054$, respectively).

Metabolite Analysis of Brain in Rats

In the brain of ZUC lean and fatty rats, the unchanged radiolabeled form at 30 min after the injection of [^{11}C]BU99008 was 99.1 ± 0.9 and 97.9 ± 0.6 %, respectively.

Discussion

The ZUC rat is a typical rodent model of obesity characterized by leptin receptor deficiency [28]. To evaluate obesity-related brain function, several PET tracers for glucose metabolism, the dopamine D2 receptor, and the serotonin transporter have been evaluated using brain PET study in ZUC fatty and lean rats [29–32]. In this study, we indicated higher binding of [^{11}C]BU99008 by brain PET study in ZUC fatty rats compared to ZUC lean rats. [^{11}C]BU99008 is a typical PET tracer for imaging and determining the kinetics of I_2Rs. In a previous *in vitro* study, the density (B_{max}) and affinity (K_d) of I_2Rs did not exhibit significant differences in the hypothalamus of ZUC lean and

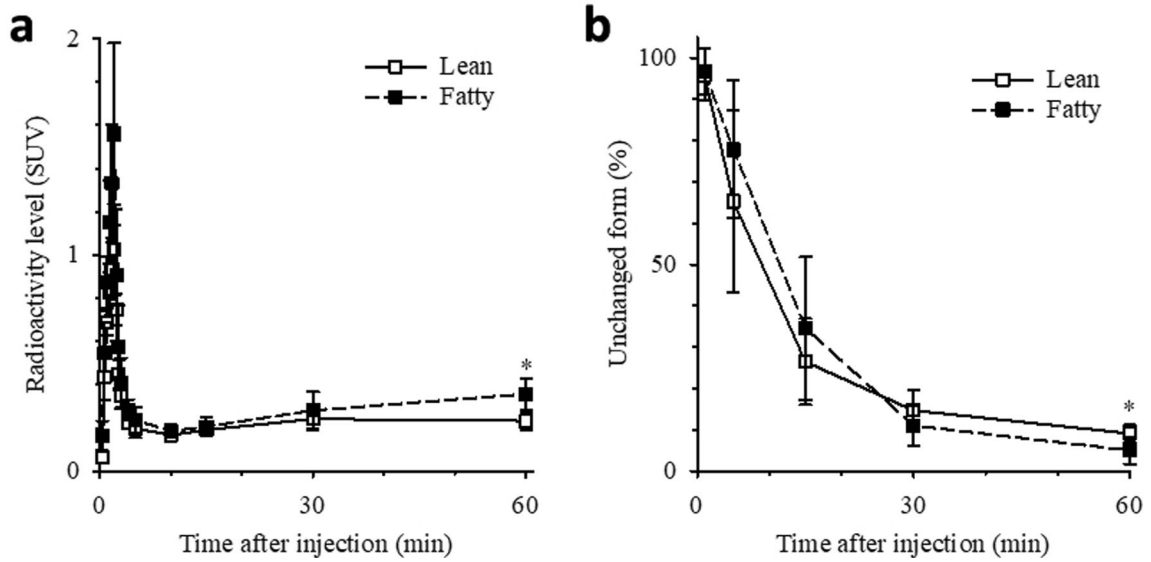

Fig. 4. Time-activity curves (TACs) of **a** the plasma and **b** percentage of the unchanged form of plasma in the ZUC lean ($n = 5$) and fatty ($n = 5$) rats after the injection of [^{11}C]BU99008 (31–63 MBq/0.2–4.5 nmol). Radioactivity level is expressed as mean SUV for plasma TACs, and as percentages of the unchanged form ([^{11}C]BU99008) for metabolite analysis of plasma. *$P < 0.05$ (unpaired t test, compared to the ZUC lean rats).

fatty rats [33], although previous *in vivo* studies of rodents demonstrated feeding effects by I_2R ligands [6–9]. Differences between *in vitro* and *in vivo* studies may be explained by multiple possibilities, such as the effects of other binding sites for I_2Rs, and the change of concentrations of the endogenous ligands of I_2Rs. Recently, it has been reported that several ligands which have high and selective affinity for I_2R have two different binding sites for I_2R, high affinity site and low affinity site [12, 34]. BU99008 has also these two different binding sites for I_2R, high affinity site (K_i = 1.4 nM) or low affinity site (K_i = 238.6 nM) [13]. Although function of the low affinity site for I_2R has not been confirmed, it has been shown that these several ligands also bind to monoamine oxidase (MAO) and are weak inhibitors for MAO [35, 36]. It is thus assumed that the low affinity site for I_2R may represent MAO, although further investigation is required [13]. MAO is one of major enzymes contributing to the metabolism of serotonin and dopamine, two neurotransmitter systems involved in the regulation of food intake [37] and obesity [38]. As for the other possibility, it is known that one of the endogenous ligands of I_2Rs is agmatine, which is the product of arginine

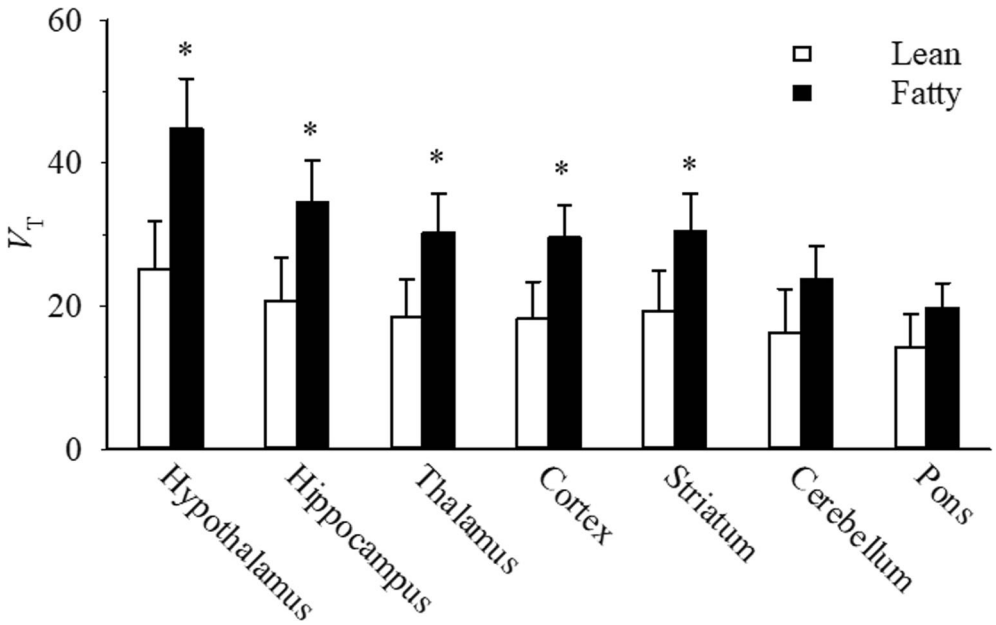

Fig. 5. The volume of distribution (V_T) values calculated by a Logan GA using PET studies of the brain in the ZUC lean ($n = 5$) and fatty ($n = 5$) rats after the injection of [^{11}C]BU99008. *$P < 0.05$ (unpaired t test, compared to the ZUC lean rats).

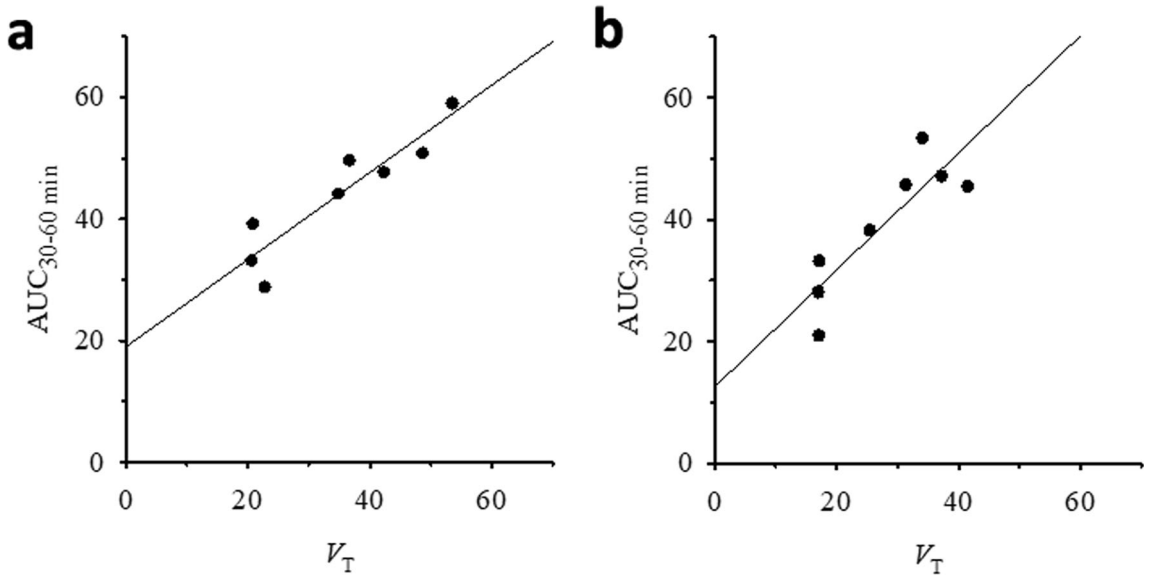

Fig. 6. Correlative relationship between radioactivity accumulation ($AUC_{30–60\ min}$) and the volume of distribution (V_T) in the **a** hypothalamus ($r^2 = 0.8480$; $P = 0.0012$) and **b** hippocampus ($r^2 = 0.7510$; $P = 0.0054$) using PET studies in the ZUC lean and fatty rats.

decarboxylation and influences multiple physiologic and metabolic functions [39, 40]. Previously, agmatine was demonstrated to improve pathological conditions of metabolic disease, such as decrease of fat mass, decrease of body weight, and improvement of insulin sensitivity [41]. Based on multiple effects of endogenous ligands for I_2Rs, such as MAO and agmatine, it is assumed that the concentration of endogenous ligands for I_2Rs in fatty rats may be lower than that in lean rats. Thus, it is considered that the binding of [^{11}C]BU99008 for I_2Rs in fatty rats was increased, because the blocking effect by relatively low concentrations of endogenous ligands on the binding in the fatty rats was smaller than the corresponding blocking effect in the lean rats. Previously, PET study using [^{11}C]DASB which is a serotonin transporter PET tracer was performed to measure changes in the concentration of endogenous serotonin [42]. Therefore, PET studies using [^{11}C]BU99008 may be helpful for estimating multiple changes of endogenous ligands for I_2Rs in the brain, because it is difficult to directly measure the change of endogenous ligands for I_2Rs for *in vivo* studies.

We also investigated the correlative relationship between the volume of distribution (V_T) and the accumulation of radioactivity ($AUC_{30–60\ min}$ [SUV × min]) in the hypothalamus and hippocampus, which have high expressions of I_2Rs. The $AUC_{30–60\ min}$ was well correlated with the V_T in the high levels of I_2Rs with the PET study using [^{11}C]BU99008. In addition, the accumulation of radioactivity in both ZUC rats at 30 min after the injection of [^{11}C]BU99008 was attributed to the almost unchanged form, as determined from the result of metabolite study. The binding parameters of V_T for I_2Rs in the brain from PET study of rats using [^{11}C]BU99008 could be represented by the accumulation of radioactivity after the injection of [^{11}C]BU99008.

In metabolite analysis of plasma in the ZUC rats, the percentages of the unchanged form in the ZUC lean rats at 60 min after the injection of [^{11}C]BU99008 was significantly higher than that in the ZUC fatty rats. This result suggests that [^{11}C]BU99008 in the ZUC fatty rat may be metabolized more easily than that in the ZUC lean rats, because it was found that cytochrome P450 enzyme 4A2 (CYP4A2) mRNA of the ZUC fatty rat was significantly higher than that of the ZUC lean rats [43].

Conclusion

Using PET study with [^{11}C]BU99008, we demonstrated that the accumulated radioactivity and estimated V_T value in the feeding center in ZUC fatty rats were higher than those in lean rats. The accumulation of radioactivity in the brain after the injection of [^{11}C]BU99008 can be used to reflect the binding parameters of V_T for I_2Rs in the brain. Dynamic or static PET studies using [^{11}C]BU99008 may contribute to elucidating pathological conditions caused by obesity related to I_2R function.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Bousquet P, Feldman J, Schwartz J (1984) Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. J Pharmacol Exp Ther 230:232–236
2. Parini A, Moudanos CG, Pizzinat N, Lanier SM (1996) The elusive family of imidazoline binding sites. Trends Pharmacol Sci 17:13–16
3. Michel MC, Inset PA (1989) Are there multiple imidazoline binding sites? Trends Pharmacol Sci 10:342–344
4. Michel MC, Ernsberger P (1992) Keeping an eye on the I site, imidazoline-preferring receptors. Trends Pharmacol Sci 13:369–370
5. Sánchez-Blázquez BMA, Olmos G et al (2000) Activation of I_2-imidazoline receptors enhances supraspinal morphine analgesia in mice: a model to detect agonist and antagonist activities at these receptors. Br J Pharmacol 130:146–152
6. Jackson HC, Griffin IJ, Nutt DJ (1991) The effects of idazoxan and other alpha 2-adrenoceptor antagonists on food and water intake in the rat. Br J Pharmacol 104:258–262
7. Nutt DJ, French N, Handley S et al (1995) Functional studies of specific imidazoline-2 receptor ligands. Ann N Y Acad Sci 763:125–139
8. Brown CM, MacKinnon AC, Redfern WS et al (1995) RS-45041-190: a selective, high-affinity ligand for I_2 imidazoline receptors. Br J Pharmacol 116:1737–1744
9. Polidori C, Gentili F, Pigini M, Quaglia W, Panocka I, Massi M (2000) Hyperphagic effect of novel compounds with high affinity for imidazoline I_2 binding sites. Eur J Pharmacol 392:41–49
10. Anand BK, Brobeck JR (1951) Localization of a "feeding center" in the hypothalamus of the rat. Proc Soc Exp Biol Med 77:323–324
11. French N (1995) α_2-adrenoceptors and I_2 sites in the mammalian central nervous system. Pharmacol Ther 68:175–208
12. Lione LA, Nutt DJ, Hudson AL (1998) Characterisation and localisation of [^{3}H]2-(2-benzofuranyl)-2-imidazoline binding in rat brain: a selective ligand for imidazoline I_2 receptors. Eur J Pharmacol 353:123–135
13. Tyacke RJ, Fisher A, Robinson ES et al (2012) Evaluation and initial in vitro and ex vivo characterization of the potential positron emission tomography ligand, BU99008 (2-(4,5-dihydro-1*H*-imidazol-2-yl)-1-methyl-1*H*-indole), for the imidazoline$_2$ binding site. Synapse 66:542–551
14. Roeda D, Hinnen F, Doll F (2003) Radiosynthesis of a 2-substituted 4,5-dihydro-1*H*-[2-^{11}C]imidazole: the I_2 imidazoline receptor ligand [^{11}C]benazoline. J Label Compd Radiopharm 46:1141–1149
15. Hudson AL, Tyacke RJ, Lalies MD et al (2003) Novel ligands for the investigation of imidazoline receptors and their binding proteins. Ann NY Acad Sci USA 1009:302–308
16. Kawamura K, Naganawa M, Konno F, Yui J, Wakizaka H, Yamasaki T, Yanamoto K, Hatori A, Takei M, Yoshida Y, Sakaguchi K, Fukumura T, Kimura Y, Zhang MR (2010) Imaging of I_2-imidazoline receptors by small-animal PET using 2-(3-fluoro-[4-^{11}C]tolyl)-4,5-dihydro-1*H*-imidazole ([^{11}C]FTIMD). Nucl Med Biol 37:625–635
17. Kawamura K, Yui J, Konno F, Yamasaki T, Hatori A, Wakizaka H, Fujinaga M, Kumata K, Yoshida Y, Ogawa M, Nengaki N, Yanamoto K, Fukumura T, Zhang MR (2012) Synthesis and evaluation of PET probes for the imaging of I_2 imidazoline receptors in peripheral tissues. Nucl Med Biol 39:89–99
18. Kawamura K, Shimoda Y, Kumata K, Fujinaga M, Yui J, Yamasaki T, Xie L, Hatori A, Wakizaka H, Kurihara Y, Ogawa M, Nengaki N, Zhang MR (2015) In vivo evaluation of a new ^{18}F-labeled PET ligand, [^{18}F]FEBU, for the imaging of I_2-imidazoline receptors. Nucl Med Biol 42:406–412
19. Kealey S, Turner EM, Husbands SM, Salinas CA, Jakobsen S, Tyacke RJ, Nutt DJ, Parker CA, Gee AD (2013) Imaging imidazoline-I_2 binding sites in porcine brain using ^{11}C-BU99008. J Nucl Med 54:139–144
20. Parker CA, Nabulsi N, Holden D, Lin SF, Cass T, Labaree D, Kealey S, Gee AD, Husbands SM, Quelch D, Carson RE, Nutt DJ, Huang Y, Tyacke RJ (2014) Evaluation of ^{11}C-BU99008, a PET ligand for the imidazoline$_2$ binding sites in rhesus brain. J Nucl Med 55:838–844
21. Kawamura K, Shimoda Y, Yui J, Zhang Y, Yamasaki T, Wakizaka H, Hatori A, Xie L, Kumata K, Fujinaga M, Ogawa M, Kurihara Y, Nengaki N, Zhang MR (2017) A useful PET probe [^{11}C]BU99008 with ultra-high specific radioactivity for small animal PET imaging of I_2-imidazoline receptors in the hypothalamus. Nucl Med Biol 45:1–7
22. Fukumura T, Suzuki H, Mukai K et al (2007) Development of versatile synthesis equipment for multiple production of PET radiopharmaceuticals. J Label Compd Radiopharm 50:S202
23. Yamasaki T, Fujinaga M, Yui J, Ikoma Y, Hatori A, Xie L, Wakizaka H, Kumata K, Nengaki N, Kawamura K, Zhang MR (2014) Noninvasive quantification of metabotropic glutamate receptor type 1 with [^{11}C]ITDM: a small-animal PET study. J Cereb Blood Flow Metab 34:606–612
24. Kawamura K, Hashimoto H, Ogawa M, Yui J, Wakizaka H, Yamasaki T, Hatori A, Xie L, Kumata K, Fujinaga M, Zhang MR (2013) Synthesis, metabolite analysis, and in vivo evaluation of [^{11}C]irinotecan as a novel positron emission tomography (PET) probe. Nucl Med Biol 40:651–657
25. Yui J, Hatori A, Kawamura K, Yanamoto K, Yamasaki T, Ogawa M, Yoshida Y, Kumata K, Fujinaga M, Nengaki N, Fukumura T, Suzuki K, Zhang MR (2011) Visualization of early infarction in rat brain after ischemia using a translocator protein (18 kDa) PET ligand [^{11}C]DAC with ultra-high specific activity. NeuroImage 54:123–130
26. Logan J, Fowler JS, Volkow ND, Wolf AP, Dewey SL, Schlyer DJ, MacGregor RR, Hitzemann R, Bendriem B, Gatley SJ, Christman DR (1990) Graphical analysis of reversible radioligand binding from time-activity measurements applied to [*N*-^{11}C-methyl]-(−)-cocaine PET studies in human subjects. J Cereb Blood Flow Metab 10:740–747
27. Robinson ES, Tyacke RJ, Nutt DJ et al (2002) Distribution of [^{3}H]BU224, a selective imidazoline I_2 binding site ligand, in rat brain. Eur J Pharmacol 450:55–60
28. Zucker LM, Zucker TF (1961) Fatty, a new mutation in the rat. J Hered 52:275–278
29. Thanos PK, Michaelides M, Piyis YK, Wang GJ, Volkow ND (2008) Food restriction markedly increases dopamine D2 receptor (D2R) in a rat model of obesity as assessed with in-vivo μPET imaging ([^{11}C]raclopride) and in-vitro ([^{3}H]spiperone) autoradiography. Synapse 62:50–61
30. Liistro T, Guiducci L, Burchielli S, Panetta D, Belcari N, Pardini S, del Guerra A, Salvadori PA, Iozzo P (2010) Brain glucose overexposure and lack of acute metabolic flexibility in obesity and type 2 diabetes: a PET-[^{18}F]FDG study in Zucker and ZDF rats. J Cereb Blood Flow Metab 30:895–899
31. Soto-Montenegro ML, Pascau J, Desco M (2014) Response to deep brain stimulation in the lateral hypothalamic area in a rat model of obesity: in vivo assessment of brain glucose metabolism. Mol Imaging Biol 16:830–837
32. Cumming P, Maschauer S, Riss PJ, Grill E, Pischetsrieder M, Kuwert T, Prante O (2015) Perturbed development of striatal dopamine transporters in fatty versus lean Zucker rats: a follow-up small animal PET study. Mol Imaging Biol 17:521–528
33. Miralles A, Ribas C, Olmos G, García-Sevilla JA (1993) No effect of genetic obesity and mazindol on imidazoline I_2 binding sites in the brain of Zucker rats. Eur J Pharmacol 243:305–308
34. Lione LA, Nutt DJ, Hudson AL (1996) [^{3}H]2-(2-benzofuranyl)-2-imidazoline: a new selective high affinity radioligand for the study of rabbit brain imidazoline I_2 receptors. Eur J Pharmacol 304:221–229
35. Alemany R, Olmos G, García-Sevilla JA (1997) Labelling of I_2B-imidazoline receptors by [^{3}H]2-(2-benzofuranyl)-2-imidazoline (2-BFI) in rat brain and liver: characterization, regulation and relation to monoamine oxidase enzymes. Naunyn Schmiedeberg's Arch Pharmacol 356:39–47
36. Ozaita A, Olmos G, Boronat MA et al (1997) Inhibition of monoamine oxidase A and B activities by imidazol(ine)/guanidine drugs, nature of the interaction and distinction from I_2-imidazoline receptors in rat liver. Br J Pharmacol 121:901–912
37. Schwartz MW, Woods SC, Porte D Jr et al (2000) Central nervous system control of food intake. Nature 404:661–671
38. Camarena B, Santiago H, Aguilar A, Ruvinskis E, González-Barranco J, Nicolini H (2004) Family-based association study between the monoamine oxidase A gene and obesity: implications for psychopharmacogenetic studies. Neuropsychobiology 49:126–129
39. Reis DJ, Li G, Regunathan S (1995) Endogenous ligands of imidazoline receptors: classic and immunoreactive clonidine-displacing substance and agmatine. Ann N Y Acad Sci 763:295–313
40. Raasch W, Schäfer U, Chun J, Dominiak P (2001) Biological significance of agmatine, an endogenous ligand at imidazoline binding sites. Br J Pharmacol 133:755–780

41. Nissim I, Horyn O, Daikhin Y, Chen P, Li C, Wehrli SL, Nissim I, Yudkoff M (2014) The molecular and metabolic influence of long term agmatine consumption. J Biol Chem 289:9710–9729
42. Lundquist P, Wilking H, Höglund AU, Sandell J, Bergström M, Hartvig P, Långström B (2005) Potential of [^{11}C]DASB for measuring endogenous serotonin with PET: binding studies. Nucl Med Biol 32:129–136
43. Enriquez A, Leclercq I, Farrell GC, Robertson G (1999) Altered expression of hepatic CYP2E1 and CYP4A in obese, diabetic ob/ob mice, and fa/fa Zucker rats. Biochem Biophys Res Commun 255:300–306