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Different sensitivities to competitive inhibition of benzodiazepine receptor binding of ^{11}C-iomazenil and ^{11}C-flumazenil in rhesus monkey brain

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The *in vivo* binding kinetics of ^{11}C-iomazenil were compared with those of ^{11}C-flumazenil binding in rhesus monkey brain. The monkey was anesthetized with ketamine and intravenously injected with either ^{11}C-iomazenil or ^{11}C-flumazenil in combination with the coadministration of different doses of non-radioactive flumazenil (0, 5 and 20 μg/kg).

The regional distribution of ^{11}C-iomazenil in the brain was similar to that of ^{11}C-flumazenil, but the sensitivity of ^{11}C-iomazenil binding to competitive inhibition by non-radioactive flumazenil was much less than that of ^{11}C-flumazenil binding. A significant reduction in ^{11}C-flumazenil binding in the cerebral cortex was observed with 20 μg/kg of flumazenil, whereas a relatively smaller inhibition of ^{11}C-iomazenil binding in the same region was observed with the same dose of flumazenil. These results suggest that ^{11}C-flumazenil may be a superior radiotracer for estimating benzodiazepine receptor occupancy in the intact brain.

Key words: ^{11}C-iomazenil, ^{11}C-flumazenil, benzodiazepine receptors, rhesus monkey, PET

INTRODUCTION

CARBON-11 labeled flumazenil and ^{123}I-labeled iomazenil have been used as selective radioligands for benzodiazepine (Bz) receptor mapping in emission tomography. As previously reported, a significant difference in apparent Bz receptor occupancy was observed between ^{125}I-iomazenil and ^{3}H-flumazenil in mice brain when the dose of flunitrazepam was varied.[1] The main purpose of this experiment was to clarify whether this phenomenon is also observed in primate brains.

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MATERIALS AND METHODS

^{11}C-iomazenil and ^{11}C-flumazenil (specific radioactivity 100 GBq/μmol) were synthesized by *N*-methylation according to a previously described method.[2] Briefly, *N*-desmethyl derivatives of iomazenil and flumazenil were reacted with ^{11}C-methyiodide and purified by liquid chromatography. After evacuation of the solvent, ^{11}C-labeled ligands were prepared in solution for injection. A rhesus monkey (7 kg body weight) was anesthetized with an intramuscular injection of ketamine (5 mg/kg) and subsequently seated on a modified monkey chair, which maintains the head in a fixed position.[3] The experimental protocol was approved by the Committee on the Safety and Ethical Handling Regulations for Laboratory Animal Experiments, National Institute of Radiological Sciences. About 0.4 GBq of ^{11}C-iomazenil or ^{11}C-flumazenil was intravenously injected with different doses of non-radioactive flumazenil (0, 5 and 20 μg/kg), and a PET scan was performed for 40 minutes with an animal PET camera (SHR2000, Hamamatsu Photonics, Hamamatsu, Japan)

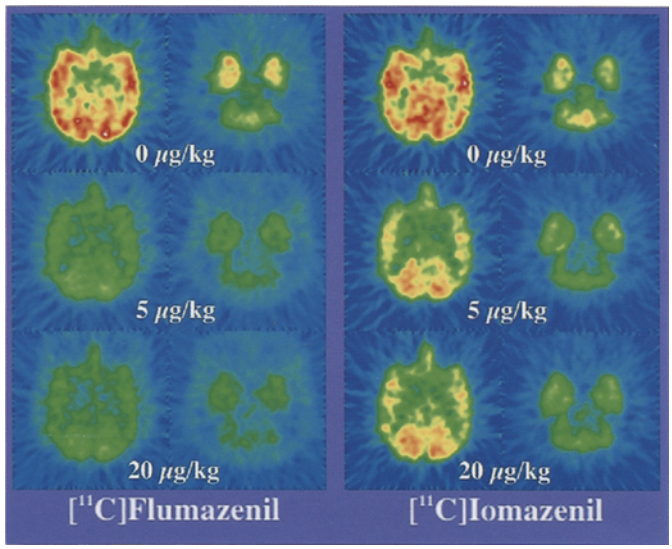

Fig. 1 PET images of ^{11}C-flumazenil or ^{11}C-iomazenil binding in the rhesus monkey brain. ^{11}C-flumazenil or ^{11}C-iomazenil was i.v. injected in coinjection with various amounts (0, 5, 20 μg/kg) of the competitor flumazenil. PET scans were performed for 40 minutes.

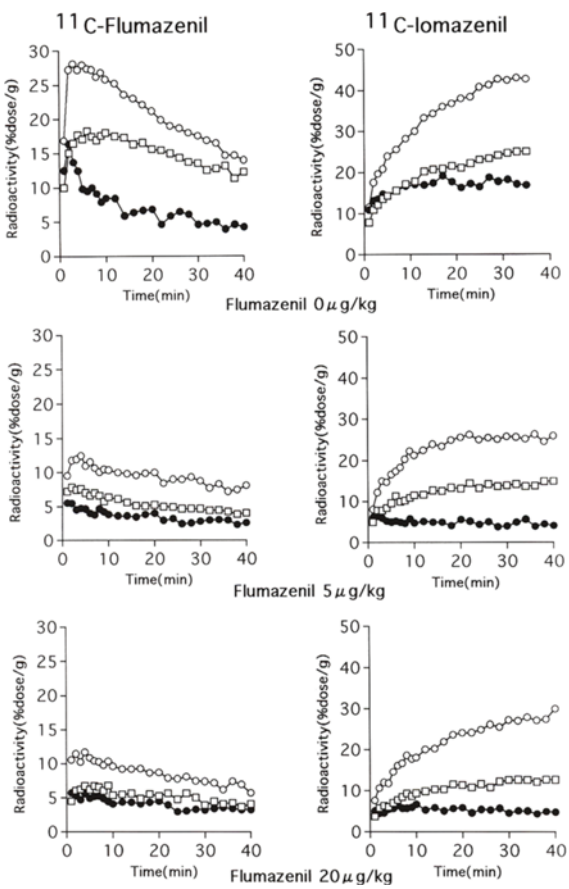

Fig. 2 The time course of radioactivity concentration in the cerebral cortex (○), cerebellum (□) and pons (●) following the injection of ^{11}C-flumazenil or ^{11}C-iomazenil in a rhesus monkey.

with transaxial resolution of 3.0 mm full-width at half-maximum (FWHM) and a center-to-center distance of 6.5 mm. Regions of interest (ROIs) were outlined by hand on reconstructed PET images. The time courses of radioactivity concentration in each region were then determined.

RESULTS

Figure 1 shows the resulting PET images for the injection of ^{11}C-iomazenil or ^{11}C-flumazenil and the coadministration of different doses of the competitor flumazenil. When the tracers were injected alone, similar regional distribution of ^{11}C-iomazenil and ^{11}C-flumazenil was observed in the slice, which contained the cerebral cortex, striatum and thalamus. On the other hand, a significant decrease in the accumulation of ^{11}C-flumazenil was observed in all ROIs when 5 μg/kg and 20 μg/kg of flumazenil were administered. Considerably smaller decreases in ^{11}C-iomazenil binding were observed for the same doses of flumazenil, as shown in Figure 1.

The time courses of the radioactivity concentration in each region are shown in Figure 2. The most important difference in the binding of ^{11}C-iomazenil and ^{11}C-flumazenil is their different kinetic properties. The kinetics of ^{11}C-flumazenil binding in rhesus monkey brain occurred very quickly, and binding within the cerebral cortex reached a maximum 5 minutes after the injection of tracer. With ^{11}C-iomazenil, however, binding occurred very slowly but increased continuously so that the apparent dissociation rate of ^{11}C-iomazenil appears to be negligible, and the association process can be measured by using ^{11}C-iomazenil for a period of at least 40 minutes after injection of the tracer. Another difference in the

binding properties of ^{11}C-iomazenil and ^{11}C-flumazenil was that a considerable amount of specific ^{11}C-iomazenil binding was observed in the pons, which has been used as a reference region for the quantitative analysis of ^{11}C-flumazenil binding.[4]

DISCUSSION

The most important finding in this study was that the sensitivity of ^{11}C-flumazenil to competitive inhibition by flumazenil is significantly higher than that of ^{11}C-iomazenil binding. Our rough estimations suggested that a dose of 5 μg/kg of flumazenil resulted in 70% occupancy of Bz receptors when measured with ^{11}C-flumazenil and 45% occupancy when measured with ^{11}C-iomazenil. This difference in receptor occupancy seems to be not due to changes in cerebral blood flow, since flumazenil has no significant pharmacological effect on the central nervous system. The present results were consistent with our previous data for mice with Bz agonist and inverse agonist as competitive inhibitors. It is of interest that Bz antagonist also showed a similar discrepancy in receptor occupancy. The reason for the discrepancy between the two radioligands in the apparent receptor occupancy seems to arise from the difference in their kinetic properties. ^{11}C-iomazenil binding probably reflects the association process because of its slow binding kinetics, and does not reach a state of pseudo-equilibrium within the period of PET measurement. Another possibility is that an increase in ligand concentration surrounding the receptors would increase the apparent association rate constant (kon) of ^{11}C-iomazenil binding (apparent positive cooperativity).[5] Since the environment surrounding the receptors in an intact brain is much more heterogeneous, interfacial or surface effects are more likely to significantly affect the available free ligand concentration. Such effects would probably be related to such physicochemical properties of the ligand as lipophilicity and electric charge.[6] In addition, the heterogeneity of brain capillaries should also be considered as a possible cause of the apparent discrepancy between ^{11}C-iomazenil and flumazenil in Bz receptor occupancy, as previously suggested.[7]

^{11}C-flumazenil seems to be a better radioligand than ^{11}C or ^{123}I-labeled iomazenil for estimating Bz receptor occupancy. Many patients with neurological or psychological disorders, who are candidates for SPECT imaging with ^{123}I-labeled iomazenil are sometimes medicated with benzodiazepines. The competitive inhibition effect of these drugs on SPECT images obtained during the association process of ^{123}I-iomazenil binding could be avoided, but our preliminary animal experiment indicated that Bz receptor occupancy could be estimated by means of a late SPECT image instead of an early SPECT image of ^{123}I-iomazenil binding. Both simulation studies and further animal experiments on the relationship between the kinetic properties of the radioligand and apparent receptor occupancy are in progress. This phenomenon is also observed in estimations of other types of receptor occupancy. For example, ^{11}C-raclopride seems to be better for estimating dopamine D_2 receptor occupancy than ^{11}C-*N*-methylspiperone[8] and ^{11}C-FLB457 because these substances have very low dissociation rate constants.

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