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Comparison of three PET dopamine D_2-like receptor ligands, [^{11}C]raclopride, [^{11}C]nemonapride and [^{11}C]*N*-methylspiperone, in rats

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We studied the tracer kinetics of three dopamine D_2-like receptor ligands, [^{11}C]raclopride ([^{11}C]RAC), [^{11}C]nemonapride ([^{11}C]NEM) and [^{11}C]*N*-methylspiperone ([^{11}C]MSP), in anesthetized rats by tissue dissection, *ex vivo* ARG and PET in order to clarify their characteristics for PET imaging. The *in vivo* affinity of the three ligands for the striatum ([^{11}C]MSP > [^{11}C]NEM > [^{11}C]RAC) obeyed the *in vitro* affinity for dopamine D_2 receptors. The affinity of [^{11}C]RAC and [^{11}C]MSP for the cerebellum was very low, but the affinity of [^{11}C]NEM for the cerebellum was compatible to that for the cortex and was not to be ignored. Also the affinity of [^{11}C]MSP for the cortex was relatively high. [^{11}C]RAC showed the highest selectivity. The striatal PET image with [^{11}C]RAC was clearer than that with [^{11}C]NEM or [^{11}C]MSP, but the activity decreased much faster than that measured by tissue dissection because of the partial volume effect. The striatal activity with [^{11}C]NEM remained high and that with [^{11}C]MSP gradually increased. [^{11}C]RAC and [^{11}C]MSP, but not [^{11}C]NEM, showed a high accumulation in the periorbital region.

Key words: raclopride, nemonapride, *N*-methylspiperone, dopamine D_2-like receptor, rat, PET

INTRODUCTION

[^{11}C]Raclopride ([^{11}C]RAC) is a standard *in vivo* ligand for mapping dopamine D_2 receptors by positron emission tomography (PET) because of its high selectivity.[1,2] A higher affinity ligand [^{11}C]*N*-methylspiperone ([^{11}C]MSP) has also been used since the first demonstration of the human dopamine D_2 receptors.[3] [^{11}C]Nemonapride ([^{11}C]NEM, [^{11}C]YM-09151-2), a benzamide compound like [^{11}C]RAC, has been developed as an other candidate.[4–8] Nevertheless, the *in vivo* binding properties of these three ligands are different from each other, because [^{11}C]MSP and [^{11}C]NEM have higher but less selective affinity for D_2-like receptors than [^{11}C]RAC. Several *in vitro* and *in vivo* studies with ^{3}H-labeled ligands have shown different binding properties of these ligands.[9–12]

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It is well known that [^{11}C]MSP binds serotonin 5-HT_2 receptors in the cortex.[13] This property can be used to measure the 5-HT_2 receptor occupancy by neuroleptics in human beings with PET.[14–16] Radiolabeled NEM is widely used as a representative dopamine D_2-like receptor ligand in pharmacological studies. Seeman and co-workers suggested the D_4-like binding sites as the cause of the difference between the density of the [^{3}H]NEM binding site and the density of the [^{3}H]RAC binding site, because [^{3}H]NEM has similar affinity for each of the D_2-like receptor subtypes (D_2, D_3 and D_4),[17–19] whereas [^{3}H]RAC lacks the affinity for the D_4 subtype. They found increased D_4-like binding sites in postmortem striatum from patients with schizophrenia using [^{3}H]NEM and [^{3}H]RAC,[17,20] although inconsistent findings were also reported.[21,22] On the other hand, Helmeste et al. and Ujike et al. argued that NEM had an affinity for sigma receptors as well as for dopamine D_2-like receptors in an *in vitro* binding assay.[23–25] We recently confirmed that [^{11}C]NEM is specifically bound *in vivo* and *in vitro* to the cortex and cerebellum of rats besides dopamine D_2-like receptors in the striatum[26] and that the binding of [^{11}C]NEM was

blocked by sigma receptor ligands (unpublished data).

With a recent improvement in the spatial resolution of PET,[27–30] [^{11}C]RAC and [^{11}C]MSP were also applied to experimental PET studies of pharmacokinetics and receptor function in small laboratory animals such as rats. Hume et al. clearly demonstrated a marked reduction in receptor binding of [^{11}C]RAC in the unilaterally ibotenic acid-lesioned rat striatum by PET.[31] The reduction was recovered after transplantation of embryonic striatal grafts,[32] but the time-activity curve for the normal rat striatum measured by PET was considerably different from that measured by the tissue dissection method. PET showed the highest radioactivity level in the striatum immediately after the tracer injection, followed by a rapid decrease.[31] On the other hand, the radioactivity level in the striatum measured by the tissue dissection method increased for the first 15 min and then gradually decreased.[2] Hume et al. indicated that the PET striatum signal is markedly reduced compared with that directly recovered from the tissue because of the partial volume effect due to the limited spatial resolution of PET.[31] Higher affinity ligands than [^{11}C]RAC may provide a larger imaging time-window and may be more suitable to detecting changes in dopamine D_2-like receptors in the small animal brain by PET. Tsukada et al. used [^{11}C]MSP to detect the changes in dopamine D receptor binding after pharmacological intervention.[33,34]

So far the potentials of [^{11}C]RAC, [^{11}C]NEM and [^{11}C]MSP as PET ligands have not been directly compared *in vivo* either in humans or in experimental animals. In the present study, the tracer kinetics of the three ligands was examined in rats by the tissue dissection method and by PET. The regional brain distribution of the three tracers was also compared by *ex vivo* autoradiography (ARG).

MATERIALS AND METHODS

Materials

Desmethyl compounds of RAC and NEM were kindly supplied from Astra Arcus AB (Södertälje, Sweden) and Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Spiperone hydrochloride was purchased from Research Biochemical, Inc. (Natick, MA, USA). [^{11}C]RAC, [^{11}C]MSP[35] and [^{11}C]NEM[8] were prepared by ^{11}C-methylation of the respective desmethyl compounds with [^{11}C]methyl iodide in the presence of NaH. The preparation method for [^{11}C]RAC will be described elsewhere.

Animal studies

The animal studies were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of

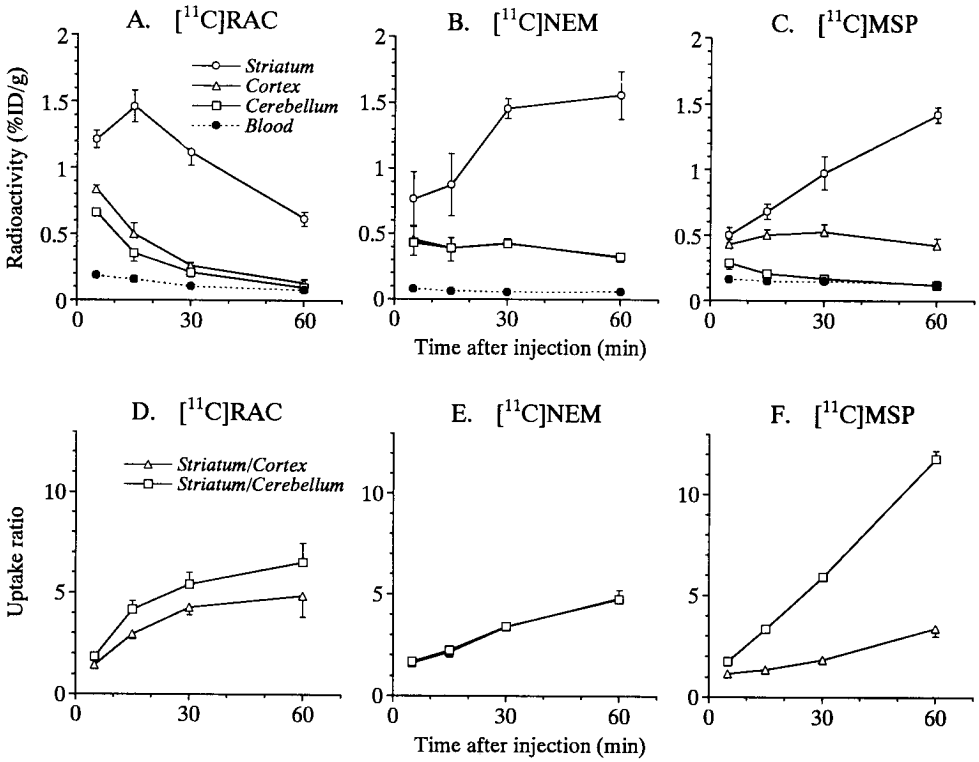

Fig. 1 Regional brain distribution of radioactivity (A–C) and uptake ratios of striatum to cortex and striatum to cerebellum (D–F) measured by the tissue sampling method after intravenous injection of [^{11}C]raclopride, [^{11}C]*N*-methylspiperone or [^{11}C]nemonapride into anesthetized rats. Mean ± SD (n = 4).

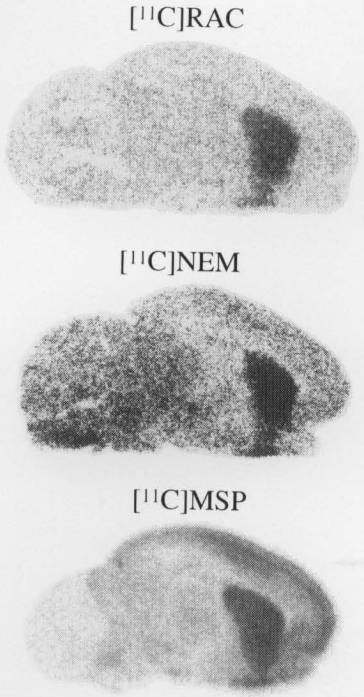

Fig. 2 *Ex vivo* autoradiograms of the sagittal rat brain section with [^{11}C]raclopride, [^{11}C]nemonapride or [^{11}C]*N*-methylspiperone. The *ex vivo* autoradiography was performed at 30 min after an intravenous injection of the ^{11}C-labeled tracer.

Gerontology. Male Wistar rats were obtained from Tokyo Laboratory Animals Company (Tokyo, Japan).

A regional brain distribution study of the three ligands by the tissue dissection method was performed in rats anesthetized with isoflurane. The rats (8 weeks old) were placed in a box ventilated with air containing 3–5% isoflurane. The ^{11}C-labeled tracer was intravenously injected into the anesthetized rats. The injected doses were 11.7 MBq/0.137 nmol for [^{11}C]RAC; 9.83 MBq/0.842 nmol for [^{11}C]NEM; and 10.2 MBq/0.518 nmol for [^{11}C]MSP. They were killed by cervical dislocation at 5, 15, 30 and 60 min after the injection (n = 4). The brain was removed and dissected into the striatum, cortex and cerebellum. The radioactivity in the tissues was measured with an auto-gamma counter. The uptake was expressed as a percentage of the injected dose per gram of tissue (% ID/g).

For *ex vivo* ARG, conscious rats were used. They were killed 30 min after intravenous injection of each of the three ^{11}C-labeled tracers. The injected doses were 610 ± 73 MBq/21 ± 8 nmol/kg for [^{11}C]RAC (n = 4); 490 ± 66 MBq/22 ± 10 nmol/kg for [^{11}C]NEM (n = 5); and 590 ± 250 MBq/21 ± 11 nmol/kg for [^{11}C]MSP (n = 4). 20 μm thick sagittal or coronal brain sections were prepared and ARG was performed with imaging plates and a Type BAS 2500 bioimaging analyzer, and the regional brain uptake of radioactivity was evaluated as the photo-stimulated luminescence value per mm^2 (PSL/mm^2) as described previously.[26]

PET measurement was carried out with a model SHR-2000 camera (Hamamatsu Photonics Co., Hamamatsu, Japan).[28] The camera consists of four-ring detectors and, in a Z-motion mode, provides a set of 14-slice images at center-to-center intervals of 3.25 mm with an image spatial resolution of 4.0 mm full width at half maximum (FWHM) and an axial resolution of 5.0 mm FWHM. The rat was anesthetized with isoflurane (2.0%) throughout the PET study. A catheter was inserted into the tail vein to inject radiotracers. The anesthetized rat was positioned prone on a stereotaxic head holder made of polymethyl methacrylate (Narishige, Tokyo, Japan). An incision was made on the scalp and the bregma was positioned at the 10th slice from the body. After a transmission scan to correct for photon attenuation, the ^{11}C-labeled tracer was injected through the catheter and time sequential tomographic scanning was performed for 60 min (20 frames by 30 sec and 50 frames by 1 min). The injected doses were 10.6 ± 0.6 MBq/0.40 ± 0.25 nmol for [^{11}C]RAC (n = 6), 10.8 ± 2.4 MBq/0.24 ± 0.05 nmol for [^{11}C]NEM (n = 5) and 10.4 ± 2.3 MBq/0.38 ± 0.22 nmol for [^{11}C]MSP (n = 7). Two or three PET scannings were performed successively at 90–120 min intervals on the same animal with different tracers. To measure the radioactivity in the striatum and cerebellum, regions of interest (ROI) were placed based on stereotaxic atlas of the rat brain[36] and a standard MRI images of the rat brain prepared in our laboratory (details described elsewhere). The 25 mm^2 striatal ROI was placed over hot spots observed in the 10th slice on the bregma. The cerebellar ROI (60 mm^2) was placed on the 7th slice at a distance of 10.25 mm from the bregma. The decay-corrected radioactivity value was expressed as a percentage of the injected dose per mL of tissue volume (% ID/mL).

RESULTS

Regional brain distribution measured by tissue dissection

Figure 1 shows the radioactivity levels (% ID/g) in the striatum, cortex and cerebellum after injection of [^{11}C]RAC, [^{11}C]NEM or [^{11}C]MSP into the rats. Initial uptake of [^{11}C]RAC by each region was higher than that of the other ligands. The striatal uptake of [^{11}C]RAC increased for the first 15 min after injection, followed by a gradual decrease. The radioactivity levels in the cortex and cerebellum rapidly decreased for 60 min. The level was slightly higher in the cortex than in the cerebellum. The striatal uptake of [^{11}C]NEM increased for the first 30 min and then remained constant. The cortex and cerebellum showed similar tracer kinetics, in which the radioactivity levels gradually decreased for 60 min. The striatal uptake of [^{11}C]MSP increased for 60 min. The radioactivity level in the cortex increased for the first 30 min and then gradually decreased, whereas that in the cerebellum decreased for 60 min. Consequently, the ratio of uptake of

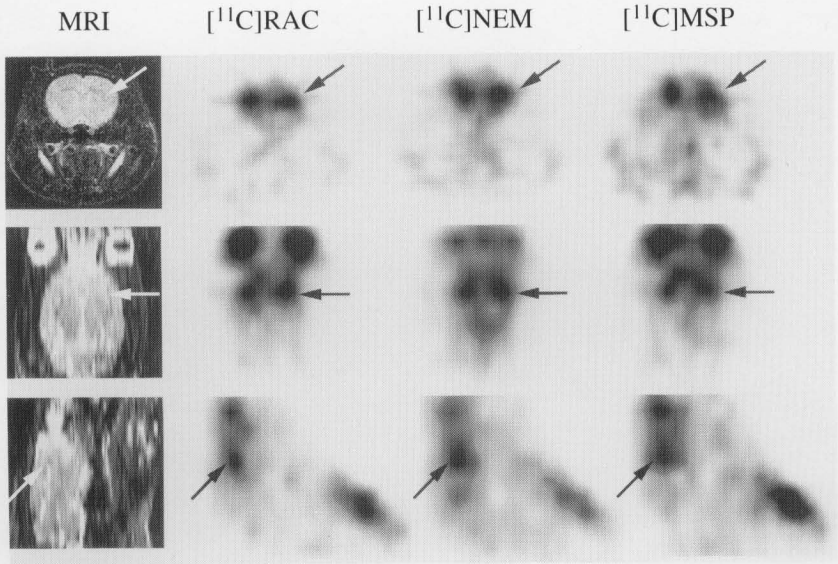

Fig. 3 PET images of the rat brain scanned with [^{11}C]raclopride, [^{11}C]nemonapride and [^{11}C]*N*-methylspiperone. The images were acquired for 30 min with [^{11}C]raclopride starting at 15 min after the tracer injection, and for 30 min with [^{11}C]*N*-methylspiperone or [^{11}C]nemonapride starting at 30 min after the tracer injection. Upper, middle and lower rows show coronal, horizontal and sagittal images, respectively. Arrows show the striatum. In horizontal images of [^{11}C]raclopride and [^{11}C]*N*-methylspiperone, a high accumulation of radioactivity was found in periorbital regions.

the three tracers in the striatum to uptake in the reference regions differed greatly (Fig. 1, D–F). The striatum to cerebellum ratio for [^{11}C]MSP was the largest and the striatum to cortex ratio was the smallest. The ratios for [^{11}C]NEM were all similar and relatively small. When the D_2-receptor specific uptake was assumed to be the difference between the striatal uptake and the cerebellar uptake, the maximal specific uptake value was 1.11 ± 0.06% ID/g for [^{11}C]RAC at 15 min, 1.23 ± 0.15% ID/g for [^{11}C]NEM at 60 min and 1.30 ± 0.05% ID/g for [^{11}C]MSP at 60 min.

Regional brain distribution measured by ex vivo *ARG*

Figure 2 shows the *ex vivo* ARG images of the sagittal brain sections of conscious rats at 30 min postinjection. A high ^{11}C density was observed in the striatum for each of the three ligands. The contrast of the striatum to the other regions was higher for [^{11}C]RAC than for [^{11}C]NEM and [^{11}C]MSP. The uptake of [^{11}C]NEM was slightly higher in the midbrain and medulla oblongata. [^{11}C]MSP accumulated in two layers in the cortex, and the uptake was lowest in the cerebellum.

The uptake ratios of striatum to cortex evaluated as the PSL/mm^2 were 4.06 ± 0.56 (n = 4) for [^{11}C]RAC, 2.37 ± 0.25 (n = 5) for [^{11}C]NEM and 1.97 ± 0.18 (n = 4) for [^{11}C]MSP. The uptake ratios of striatum to cerebellum were 5.9 (n = 1) for [^{11}C]RAC, 2.5 (n = 1) for [^{11}C]NEM and 11.7 (n = 1) for [^{11}C]MSP. The ratio of striatum to cortex for [^{11}C]NEM was significantly smaller than that measured by the tissue sampling (3.41 ± 0.15 in Fig. 1E, n = 4).

PET measurement of the rat brain

Figure 3 shows the brain images of rats acquired by PET with each ligand. In the coronal brain sections, the three ligands showed a pair of high radioactivity spots representing the striatal accumulation. In the horizontal images, the striatum was most clearly visualized with [^{11}C]RAC. The radioactivity also accumulated in the periorbital regions, being much higher with [^{11}C]MSP and [^{11}C]RAC than with [^{11}C]NEM.

The time-activity curves (% ID/mL) for the striatum and the cerebellum during the 60 min are shown in Fig. 4 (A, B and C). In the striatal region the radioactivity level of [^{11}C]RAC rapidly decreased, whereas that of [^{11}C]NEM was retained and that of [^{11}C]MSP gradually increased with time. The striatum-to-cerebellum uptake ratios are also shown in Fig. 4 (D, E and F). The ratio for [^{11}C]RAC rapidly increased for the first 20 min and then remained constant, whereas the ratios for [^{11}C]NEM and [^{11}C]MSP gradually increased with time. The ratio for [^{11}C]RAC was slightly larger than those for [^{11}C]NEM and [^{11}C]MSP.

DISCUSSION

The properties of [^{11}C]RAC, [^{11}C]NEM and [^{11}C]MSP as PET ligands for mapping dopamine D_2-like receptors were evaluated in rats by the tissue dissection method, *ex vivo* ARG and PET. [^{11}C]RAC penetrated most easily into the brain across the blood-brain barrier followed by [^{11}C]NEM and [^{11}C]MSP. The tracer kinetics showed that the three tracers had different characteristics.

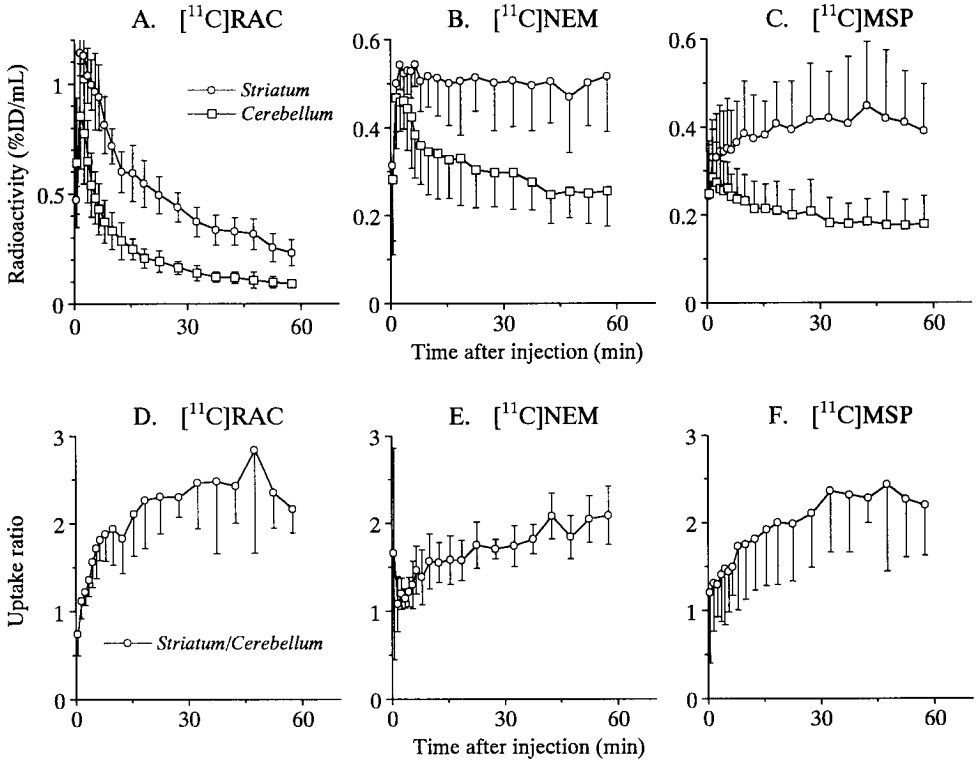

Fig. 4 Time-activity curves of the striatum and cerebellum (A–C) and uptake ratios of striatum to cerebellum (D–F) measured by PET after an intravenous injection of [^{11}C]raclopride, [^{11}C]nemonapride or [^{11}C]*N*-methylspiperone into the anesthetized rats. Mean ± SD (n = 5–7).

In the striatum the time-activity curves of the three tracers measured by tissue dissection reflect their affinity for dopamine D_2 receptors measured by *in vitro* binding assay.[12] The highest selectivity by [^{11}C]RAC for dopamine D_2 receptors was clearly demonstrated by *ex vivo* ARG. In the cerebellum where there are scarcely any D_2 receptors, [^{11}C]RAC and [^{11}C]MSP were rapidly washed out. Compared with these two ligands, the affinity of [^{11}C]NEM for the cerebellum was not to be disregarded. Therefore the cerebellum may be used as a reference region lacking D_2 receptors in PET studies with [^{11}C]RAC and [^{11}C]MSP, but not with [^{11}C]NEM. [^{11}C]MSP showed a higher affinity for the cortex, reflecting the serotonin receptor binding. It is pointed out that the radioactivity level of [^{11}C]RAC in the cortex was slightly higher than that in the cerebellum. This may reflect the presence of dopamine D_2 receptors in extrastriatal regions with lower density. [^{11}C]NEM was taken up by the cortex and cerebellum to a similar degree. Recently we found that the affinity of [^{11}C]NEM for the cortex and cerebellum reflects the sigma receptor binding (unpublished data). Therefore, it should be kept in mind that in PET studies on dopamine D_2 receptors the uptake of [^{11}C]MSP and [^{11}C]NEM partly reflects the binding to serotonin receptors and sigma receptors, respectively. In other words a combined use of two tracers, e.g. [^{11}C]RAC and [^{11}C]NEM, may permit evaluation of dopamine D_2 receptors and sigma receptors by PET, just in the same way as Tang et al. evaluated *in vitro* dopamine and sigma receptors in the postmortem human brain by using [^{3}H]NEM and [^{3}H]RAC.[24]

The PET imaging with the three ligands gave similar images of the striatal accumulation in the coronal brain section. In the horizontal brain sections, the striatum with [^{11}C]RAC was clearer than that with [^{11}C]NEM or [^{11}C]MSP. Probably because of the high affinity of [^{11}C]MSP for the cortex, the right striatum and left striatum were not separately visualized in the frontal brain region. A high radioactivity accumulation of [^{11}C]RAC and [^{11}C]MSP was found in the periorbital regions, whereas the accumulation of [^{11}C]NEM was low. The mechanism for this phenomenon was understandable but not explained by the presence of the specific binding site for dopamine D_2-like receptor ligands, because no blocking effect on the accumulation in the periorbital regions was found on co-injection of an excess amount of carrier ligand or *S*(+)-butaclamol (data not shown). It is well known that several PET tracers including receptor ligands and 2-deoxy-2-[^{18}F]fluoroglucose accumulate in the periorbital region of rats, probably in the Harderian glands.[31,37,38] Kuge et al. indicated that the high accumulation of 2-deoxy-2-[^{18}F]fluoroglucose in this region affected the measurement of glucose metabolism in the rat brain by PET.[37] The striatal activity of [^{11}C]RAC and

[^{11}C]MSP may also be affected by the high periorbital activity. These findings indicate that positioning is important in imaging the dopamine D_2 receptors of the rat striatum by PET.

The tracer kinetics measured by PET for each of the three ligands was not necessarily parallel to that measured by the tissue dissection method. Because of the low spatial resolution of PET for small brain structures of rats, the extrastriatal activity greatly influenced the time-activity curves in the striatum. For instance, in the striatum a high extraction but a rapid clearance of [^{11}C]RAC was found by PET without initial accumulation for the first 15 min. As for the striatum to cerebellum ratios, among the three ligands [^{11}C]RAC gives the most suitable contrast for the striatal imaging, but the ratios were approximately a half of those measured by tissue dissection. Although [^{11}C]MSP had an increasing time-activity curve, the striatum-to-cerebellum ratios for [^{11}C]MSP were lower than those for [^{11}C]RAC. This contrasts with the results obtained by tissue dissection and indicates the effects of extrastriatal and extracerebellar activity on the striatal and cerebellar ROIs, respectively. Anyhow, to quantitatively measure the striatal radioactivity of the three ligands, correction of the partial volume effect is required.

In the present study we compared the potential of three PET ligands and indicated their different properties. It should also be kept in mind that the distribution study was carried out on anesthetized rats with isoflurane for direct comparison of the PET data with the tissue sampling data, whereas *ex vivo* ARG was performed in conscious rats. The anesthesia affected the kinetics for [^{11}C]NEM. The striatum-to-cortex ratio for [^{11}C]NEM at 30 min post-injection was greatly enhanced by isoflurane anesthesia: 3.41 ± 0.15 (tissue dissection) vs. 2.37 ± 0.25 (*ex vivo* ARG) ($p < 0.001$). On the other hand, the difference was not significant for [^{11}C]RAC: 4.29 ± 0.39 (tissue dissection) vs. 4.06 ± 0.56 (ARG); or for [^{11}C]MSP: 1.85 ± 0.14 (tissue dissection) vs. 1.97 ± 0.18 (ARG). As for the effect of anesthetics, Onoe et al. reported that ketamine increased the striatal [^{11}C]MSP binding in the monkey brain but pentobarbital decreased it.[39] It is desirable to consider the characteristics of the ligands and use the most appropriate one in PET studies of humans and rats.

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