Type of Anesthesia Influences Positron Emission Tomography Measurements of Dopamine $D_{2/3}$ Receptor Binding in the Rat Brain

by ***Aage Kristian Olsen Alstrup[1,*], Mette Simonsen[1] & Anne M. Landau[1,2]***

[1]PET Centre, Aarhus University Hospital, Aarhus, Denmark

[2]Center of Functionally Integrative Neuroscience, Aarhus University, Aarhus, Denmark

Summary

Rats are often anesthetized prior to positron emission tomography (PET) brain imaging in order to prevent head movements. Anesthesia can be administered by inhalation agents, such as isoflurane (Forene), or injection mixtures, such as fentanyl-fluanisone-midazolam (Hypnorm-Dormicum). Unfortunately, anesthesia affects a variety of physiological variables, including those in the brain. The aim of this study was to compare the effects of inhalation and injection anesthesia on the binding potential of the dopaminergic $D_{2/3}$ tracer [^{11}C]raclopride used for PET brain imaging in human and animal studies. Male Lewis rats were assigned to either inhalation (isoflurane; N=4) or injection (fentanyl-fluanisone-midazolam; N=5) anesthesia. Isoflurane was given continuously, and fentanyl-fluanisone-midazolam was supplemented every 30-60 minutes when the tail reflex was positive. Catheters were surgically placed in femoral arteries and veins for blood sampling and tracer injection. After a short attenuation scan, the rats were PET scanned for 90 minutes after injection of [^{11}C]raclopride. We found that rats anesthetized with isoflurane had double the binding potential in the striatum compared with fentanyl-fluanisone-midazolam anesthetized rats. Our results are in agreement with other studies showing that anesthesia may have a major influence on brain imaging studies involving tracer kinetics in rats.

Introduction

Positron emission tomography (PET) brain imaging can be performed in humans and non-human primates in an awake state, however, this is much more difficult with small animals. Therefore, to prevent head movements when scanning rodents, PET scans are mostly performed under general anesthesia (*Toyama et al., 2004; Schiffer et al., 2007*). Anesthesia can be administered via different routes such as inhalation (ie. isoflurane), injection (ie. fentanyl-fluanisone-midazolam/Hypnorm-Dormicum), or continued infusion (ie. propofol) (*Colby and Morenko, 2004*). However, anesthesia affects a variety of physiological variables, as well as the outcome of studies performed in rodent brains (*LaManna and Harik, 1986*). This has been demonstrated by several studies showing that anesthesia affects tracer binding in animal brains (*Votaw et al., 2003; Momosaki et al., 2004; McCormick et al., 2006; Patel et al., 2008; Lee et al., 2010*), and is reviewed in Alstrup and Smith (2010).

Although some of the anesthesia-related effects can be avoided by fixation of the head, permitting scanning in an awake state (*Momosaki et al., 2004; Hosoi et al., 2005*), this is problematic from an animal welfare point of view as head fixation may induce stress (*Madrigal et al., 2006*). It is also important to note that stress can greatly influence the results of brain studies (*Senoo, 2000; Lu et al., 2003; Madrigal et al., 2006)*. In particular, handling and restraint of awake rats have been shown to influence the dopamine system (*Marsteller et al., 2002*). Patel

**Correspondence:* Aage Kristian Olsen Alstrup, DVM, Ph.D. PET Centre, Aarhus University Hospitals, 44, 10G Noerrebrogade, DK-8000 Aarhus C, Denmark
Tel +45 8949 4396
Fax +45 8949 3020
E-mail aage@pet.auh.dk

Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

and co-workers demonstrated that restraint of awake rats could significantly reduce binding potentials of the dopamine $D_{2/3}$ receptor antagonist [^{11}C]raclopride by 29 % (*Patel et al., 2008*). Therefore, it appears that performing raclopride studies in restrained awake rats is not ideal. Alternatively, the rats may be anesthetized with drugs with known effects on [^{11}C]raclopride binding. Patel and co-workers found approximately the same striatum to cerebellum ratio of [^{11}C]raclopride in xylazine-ketamine anesthetized rats as in free-moving awake rats (*Patel et al., 2008*)**.** However, the effects of two other commonly used anesthetics, isofluorane and fentanyl-fluanisone-midazolam, on [^{11}C]raclopride binding potentials have never been studied in rats. The aim of this study was to compare the effects of inhalation and injection anesthesia on the binding potentials of [^{11}C]raclopride in rat brains using PET imaging.

Materials and Methods

Animals

Animal experiments were performed in accordance with the Danish Animal Experimentation Act on a license granted by the Ministry of Legal Affairs. All housing and procedures were performed according to the Convention ETS 123 of the Council of Europe. The experiment was performed on 9 barrier-raised inbred male Lewis (LEW/Han) rats weighing 336 grams (± 37 grams) (Harlan, Horst, Holland). The rats had undergone health monitoring according to the Federation of European Laboratory Animal Science guidelines. The rats were maintained in groups of 2 - 3 in U-1400 cages (Techniplast, Buguggiate, Italy) on aspen bedding (Finn Tapvei, Kaavi, Finland) at 21 °C ± 2 °C, at a relative humidity of 55-85 %, and a light-dark cycle of 12 hours. The rats had free access to a standard laboratory pellet diet (Altromin 1324, Aarhus, Denmark) and tap water.

Animal preparation

The rats were assigned to receive either inhalation (N=4) or injection (N=5) anesthesia. Catheters were surgically placed in femoral arteries and veins for blood sampling and tracer injection. The rats were PET scanned for 90 minutes with [^{11}C]raclopride. After the end of the PET scan period the rats were killed by an overdose of pentobarbitone (100 mg/kg; IV).

Anesthesia

In the inhalation group, anesthesia was induced with 5 % isoflurane (Forene, Abbott, Solna, Sweden) in an induction chamber, and the anesthesia was maintained using a mask with 1.6 - 2.0 % isoflurane in 0.4 Liter / minutes oxygen and 1.5 Liter / minutes medical air. The isoflurane dose was reduced by 0.1 % at approximately each half hour during anesthesia to prevent hypoventilation. In the injection group, anesthesia was induced with a subcutaneous mixture containing 0.02 mg fentanyl, 0.75 mg fluanisone (Hypnorm, VetaPharma, Leeds, United Kingdom) and 0.38 mg midazolam (Midazolam, Hamelm, Herlev, Denmark). The anesthesia was maintained by injection of approximately 1/3 of the dose given for induction every 30-60 minutes during anesthesia when the tail reflex was positive. The rats were supplied with the same oxygen – medical air mixture as the inhalation group.

PET imaging

Imaging was performed using a small animal tomograph (microPET R4, CTI, Concorde). The spatial resolution (FWHM) of the scanner was 2 mm at the centre of the aperture, indicating a volume resolution of 8 mm^3. After the 10 minute attenuation scan was performed using a ^{68}Ge point source, the dynamic 90 minute emission recording was initiated after bolus injection of 17 (± 13) MBq [^{11}C]raclopride IV. The 90 minute emission recordings consisted of 26 frames increasing in duration from 15 seconds to 10 minutes. Arterial blood gases (partial arterial CO_2 concentration; $PaCO_2$, and partial arterial O_2 concentration; PaO_2) were measured before and after PET scanning. The absence of tail and interdigital pain reflexes were tested every 15 minutes. Changes in body temperature were automatically controlled by a warming lamp in a self-regulating

heating system, and the body temperature was set to remain between 36 and 37 °C.

Kinetic analysis

Summed emissions were manually registered to a rat brain atlas using the software Register (Montreal Neurological Institute (MNI), Montreal, Canada). Volume of interest templates for the striatum and cerebellum were identified in a stereotaxic atlas of the rat brain (*Paxinos and Watson, 1998*) and defined using the program Display (MNI, Montreal, Canada) as described previously (*Pedersen et al., 2007*). After re-sampling the templates to the native PET space, time activity curves for the striatum and cerebellum were extracted. The cerebellum was used as a reference tissue for the non-specific tracer binding. The distribution volume ratio (DVR) of the striatum was calculated relative to the cerebellum reference region using the linearization of the Logan simplified reference tissue kinetic model (*Logan et al., 1996*) for data recorded in the 30-90 minute interval of the scan. The binding potential was then obtained by subtracting 1 from the DVR.

Statistics

Differences in the striatal binding potential in the two groups of rats were assessed for statistical significance using a two-tailed student t-test. A paired t-test was used to compare blood gas measurements before and after PET scans. A two-tailed student t-test was used to compare blood gases between anesthesia groups. The threshold for significance was set at P=0.05.

Results

Effects on raclopride binding

Rats anesthetized with isoflurane had a significantly higher striatal binding potential than rats anesthetized with fentanyl-fluanisone-midazolam (Figure 1A). We found an average binding potential of 1.95 (+/- standard deviation 0.27) in isoflurane treated rats compared to an average of 0.74 (+/- standard deviation 0.24) in fentanyl-fluanisone-midazolam anesthesia injected rats (Figure 1B), (P<0.001).

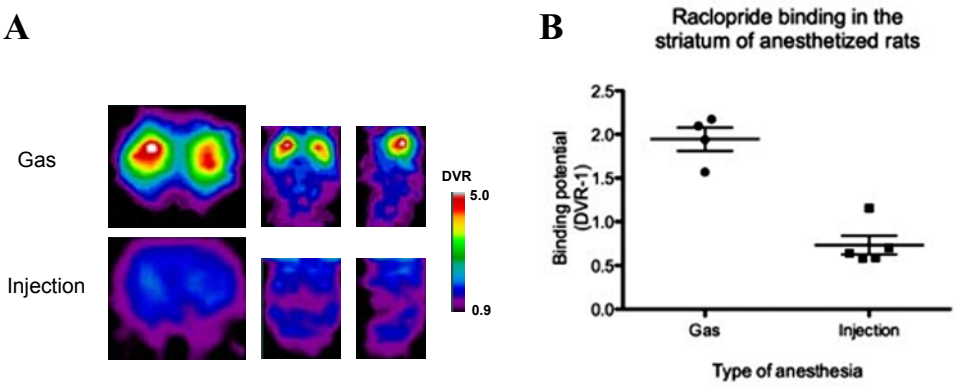

Figure 1. Isoflurane treated rats have significantly higher striatal [^{11}C]raclopride binding potential than fentanyl-fluanisone-midazolam treated rats. *(A) PET images are shown for [^{11}C]raclopride binding in one representative rat in each group. (B) Binding potential values for [^{11}C]raclopride are shown for each rat in the experiment, determined by subtracting 1 from the distribution volume ratio (DVR). Circles represent rats treated with isoflurane (N=4) and squares represent rats treated with fentanyl-fluanisone-midazolam (N=5). Binding potential was significantly greater in rats treated with isoflurane (2-tailed student t-test *P<0.001).*

Blood gases

The results of the blood gas analysis under different types of anesthesia are shown in Table 1. During the scans, $PaCO_2$ decreased significantly in the fentanyl-fluanisone-midazolam group (P=0.009), but not in the isoflurane group. However, $PaCO_2$ did not significantly differ between the two anesthesia groups before or after scanning. No significant changes in PaO_2 were observed during scanning in either group, but PaO_2 was significantly higher in the isoflurane group at both sampling times (p<0.001).

was roughly four times higher in anesthesized rats compared to awake rats. It is unknown whether the uptake of [^{11}C]raclopride in the isoflurane or the fentanyl-fluanisone-midazolam treated rats is similar to that in freely moving rats. Isoflurane increases cerebral blood flow (CBF) in rats (*Duong, 2007)*, and this may facilitate the delivery of [^{11}C]raclopride from plasma to brain tissue, resulting in higher brain uptake. The effects of fentanyl-fluanisone-midazolam on CBF have never been studied. However, in the current study PET data are collected during the 30-90 minute period of the scan, when the tracer

Table 1. Differences in blood gases (mean ± SD) before and after PET scanning in isoflurane and fentanyl-fluanisome-midazolam anaesthetized rats.

Parameters	**[units]**	**isoflurane**		**fentanyl-fluanisone-midazolam**	
		Before	**After**	**Before**	**After**
N	[]	4	3	5	5
$PaCO_2$	[kPa]	5.9 ± 0.5	5.2 ± 1.4	7.6 ± 0.6	5.0 ± 0.9^{A}**
PaO_2	[kPa]	20.1 ± 2.5	18.8 ± 6.4	9.2 ± 1.2^{B}***	12.9 ± 3.5^{B}***

*$PaCO_2$: partial arterial CO_2 concentration. PaO_2: partial arterial O_2 concentration. A: Significant lower $PaCO_2$ than before PET scanning. N: number of rats in each group. B: Significant lower PaO_2 than in the isofluorane group. A two-tailed student t-test was used to compare blood gases between anesthesia groups. *: P<0.05; **: P<0.01; ***: P<0.001.*

Discussion

We tested the effects of inhalation (isoflurane) versus injection (fentanyl-fluanisone-midazolam) anesthesia on the specific binding of [^{11}C]raclopride in rat brains. Our results strongly indicate that anesthesia may have a major influence on the binding potential of this dopamine $D_{2/3}$ receptor antagonist.

Effects on raclopride binding potentials

We found more than a two-fold higher level of raclopride binding in isoflurane anesthetized rats compared to rats anesthetized with fentanyl-fluanisone-midazolam. Patel and co-workers demonstrated that [^{11}C]raclopride striatum to cerebellum ratios were similar in xylazine-ketamine and freely moving rats 30 minutes after tracer injection (*Patel et al., 2008*). However, the uptake in stratum and cerebellum has reached a steady state between the concentration in the brain and plasma, and thus the potential contribution of blood flow is assumed to be minor.

We have previously shown a 19 % reduction in [^{11}C] raclopride striatal binding potential after amphetamine challenge in isoflurane anesthesized rats (*Pedersen et al., 2007*), which is smaller than the 30% reduction in both awake and xylazine-ketamine anesthesized rats in response to methamphetamine (*Patel et al., 2008*). McCormick and co-workers have tested the differential effects of isoflurane on the amphetamine sensitivity of three PET dopamine $D_{2/3}$ receptor tracers and found that isoflurane differentially increased receptor binding and amphetamine sensitivity of [^{11}C]PHNO and [^{11}C]NPA compared to [^{3}H]-raclopride (*McCormick et al., 2006*). Challenge effects on [^{11}C]raclopride binding

have never been studied during fentanyl-fluanisone-midazolam anesthesia. Based on our data, it may be expected that since the baseline binding potential of raclopride is lower in fentanyl-fluanisone-midazolam than in isoflurane anesthesia by about 50%, there may be large differences in the results of these types of challenge experiments due to the choice of anesthetic.

Anesthesia effects on [^{11}C]raclopride binding have been studied in other species such as cats and primates. In cats, [^{11}C]raclopride binding in striatum is unaffected by ketamine but is enhanced by halothane anesthesia compared to the awake condition. The effects were explained by an increase in CBF during halothane anesthesia (*Hassoun et al., 2003*). Tsukada and co-workers investigated whether isoflurane affects the outcome of drug challenges on [^{11}C]raclopride binding in the brains of *Macaca mulatta* monkeys and found that in both awake and anesthetized monkeys, methamphetamine caused a marked release of dopamine, whereas nicotine failed to affect dopamine release (*Tsukada et al., 2002*).

Blood gases

Most anesthetics are known to depress respiration, and this may increase $PaCO_2$. As a potent dilator, CO_2 may influence the CBF in rodents (*Broux et al., 2002; Sicard and Duong, 2005*). $PaCO_2$ should therefore be controlled and kept stable in brain studies, when animals are anesthetized, to avoid variations in CBF. In order to prevent accumulation of CO_2, the isoflurane concentration was reduced from 2.0 % to 1.6 % during the 90 minutes of the PET scans. In the fentanyl-fluanisone-midazolam anesthesized rats, anesthesia was maintained by injection of approximately 1/3 of the initial dose every 30-60 minutes. $PaCO_2$ was significantly higher at baseline in the fentanyl-fluanisone-midazolam group, but later on it was reduced to the same level as in the isoflurane group. This may indicate a respiration depression effect of the initial full fentanyl-fluanisone-midazolam dose. The PaO_2 was significantly lower in the fentanyl-fluanisone-midazolam than in the isoflurane group, which is consistent with a higher $PaCO_2$ concentration at baseline in this group suggesting hypoventilation. A non-significant increase in PaO_2 was observed in the fentanyl-fluanisone-midazolam group, and may indicate better ventilation.

Conclusion

In conclusion, our study indicates that inhalation (isoflurane) versus injection (fentanyl-fluanisone-midazolam) anesthesia may influence binding potential of [^{11}C]raclopride in rat brains. Our results are in agreement with other studies showing that anesthesia may have a major influence on brain imaging studies involving tracer kinetics in rats. However, more animal studies are needed to investigate which type of anesthesia is most representative of what occurs in freely moving rats.

References

Alstrup AKO & DF Smith: Anesthesia for PET neuroimaging in animals (in preparation).

Broux C, I Tropres, O Montogon, C Juliwn, M Decorps & J-F Payen: The effects of sustained hyperventilation on regional cerebral blood volume in thiopental-anesthetized rats. Anesth Analg, 2002, *95*, 1746-1751.

Colby LA & BJ Morenko: Clicical considerations in rodent bioimaging. Comp Med, 2004, *54*, 623-630.

Duong TQ: Cerebral blood flow and BOLD fMRI response to hypoxia in awake and anesthetized rats. Brain Res, 2007, *1135*, 186-194.

Hassoun W, M Le Cavorsin, N Ginovart, L Zimmer, V Gualda, F Bonnefoi & V Leviel: PET study of the [11C]raclopride binding in the striatum of the awake cat: effects of anesthetics and role of cerebral blood flow. Eur J Nucl Med Mol Imaging, 2003, *30*, 185-186

Hosoi R, A Matsumura, S Mizokawa, M Tanaka, F Nakamura, K Kobayashi, Y Watanabe & O Inoue: MicroPET detection of enhanced F-18-FDG utilization by PKA inhibitor in awake rat brain. Brain Res, 2005, *1039*, 199-202.

LaManna JC & SI Harik: Regional studies of blood-brain barrier transport of glucose and leucine in

awake and anesthetized rats. J Cereb Blood Metab, 1986, *6*, 717-723.

Lee MS, J Ko, AR Lee, IH Lee, MA Jung, B Austin, H Chung, S Nahm & K Eom: Effects of anesthetic protocol on normal canine brain uptake of 18F-FDG assessed by PET/CT. Vet Radiol Ultrasound, 2010, *51*, 130-135.

Logan J, JS Fowler, ND Volkow, GJ Wang, YS Ding & DL Alexoff DL: Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab, 1996, *16*, 834-840.

Lu L, JD Shepard, FS Hall & Y Shaham: Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev, 2003, *27*, 457-491.

Madrigal JL, B Garcia-Bueno, JR Caso, BG Pérez-Nievas & JC Leza: Stress-induced oxidative changes in brain. CNS Neurological Disorders – Drug Targets, 2006, *5*, 561-568.

Marsteller DA, MR Gerasimov, WK Schiffer, JM Geiger, CR Barnett, JS Borg, S Scott, J Ceccarelli, ND Volkow, PE Molina, DL Alexoff & SL Dewey: Acute handling stress modulates methylphenidate-induced catecholamine overflow in the medial prefrontal cortex. Neuropsychopharm, 2002, *27*, 163-170.

McCormick PN, N Ginovart, N Vasdev, P Seeman, S Kapur & AA Wilson: Isoflurane increases both the specific binding ratio and sensitivity to amphetamine challenge of [11C]-(+)-PHNO. Neuroimage, 2006, *31*, T33.

Momosaki S, K Hatano, Y Kawasumi, T Kato, R Hosoi, K Kobayashi, O Inoue & K Ito: Rat-PET study without anesthesia: Anesthetics modify the dopamine D_1 receptor binding in rat brain. Synapse, 2004, *54*, 207-213.

Patel VD, DE Lee, DL Alexoff, SL Dewey & WK Schiffer: Imaging dopamine release with positron emission tomography (PET) and 11C-raclopride in freely moving animals. Neuroimage, 2008, *41*, 1051-1066.

Paxinos G & Watson C. The rat brain in stereotaxic coordinates. California: Academic Press, 1998.

Pedersen K, M Simonsen, SD Østergaard, OL Munk, P Rosa-Neto, AK Olsen, SB Jensen, A Møller & P Cumming: Mapping the amphetamine-evoked changes in [11C]raclopride binding in living rat using small animal PET: modulation by MAO-inhibition. Neuroimage, 2007, *35*, 38-46.

Schiffer WK, MM Mirrione & SL Dewey: Optimizing experimental protocols for quantitative behavioural imaging with ^{18}F-FDG in rodents. J Nucl Med, 2007, *48*, 277-287.

Senoo H: Physiology of stress and starvation-like conditions, 447-460. In: GJ Krinke (ed) The laboratory rat. Academic Press, 2000, 756 pp.

Sicard KM & TQ Duong: Effects of hypoxia, hyperoxia, and hypercapnia on baseline and stimulus-evoked BOLD, CBF, and $CMRO_2$ in spontaneously breating animals. Neuroimage, 2005, *25*, 850-858.

Toyama H, M Ichise, J-S Liow, DC Vines, NM Seneca, KJ Modell, J Seidel, MV Green & RB Innis: Evaluation of anesthesia effects on [^{18}F] FDG uptake in mouse brain and heart using small animal PET. Nucl Med Biol, 2004, *31*, 251-256.

Tsukada H, K Miyasato, T Kakiuchi, S Nishiyama, N Harada & EF Domino: Comparative effects of metamphetamine and nicotine on the striatal [(11C)]raclopride binding in unanesthetized monkeys. Synapse 2002, *45*, 207-212.

Votaw J, M Byas-Smith, J Hua, R Voll, L Martarello, AI Levey, FD Bowman & M Goodman: Interaction of isoflurane with the dopamine transporter. Anesthesiology, 2003, *98*, 404-411.