Abstract. Cats were trained to stay in a containment box, without developing any signs of behavioural stress, while their head was maintained in a position that allowed positron emission tomography (PET) experiments to be performed. The binding potential for $[^{11}C]$raclopride (BP raclo), a radioligand with good specificity for dopamine (DA) receptors of the D$_2$ type, was measured in the striatum and in three experimental situations: awake, anaesthetised with ketamine (50 mg kg$^{-1}$ h$^{-1}$; i.m.) and anaesthetised with halothane (1.5%). Non-specific binding was evaluated in the cerebellum. In the striatum of both sides, the BP$_{raclo}$ was unmodified by ketamine anaesthesia when compared with awake animals. In contrast, a large increase in BP$_{raclo}$ was observed under halothane anaesthesia. The non-specific binding of $[^{11}C]$raclopride, evaluated in the cerebellum, was also unchanged under ketamine anaesthesia but greatly increased under halothane anaesthesia. To evaluate whether changes in the cerebral blood flow (CBF) resulting from the different experimental situations could be at the root of these discrepancies, injections of $[^{15}O]H_2O$ were performed; measurements revealed a drastically increased CBF under halothane anaesthesia and a slight enhancement under ketamine anaesthesia, when compared with the waking state. These results are the first to be obtained on this topic in awake cats, and show that the BP$_{raclo}$ is greatly dependent on alterations in the CBF.

Keywords: Positron emission tomography – D$_2$ receptors – Animal – Anaesthesia

Introduction

Imaging techniques such as positron emission tomography (PET) or single-photon emission tomography (SPET) have been used over the past decade to evaluate acute fluctuations of neurotransmitter concentrations in the extracellular space [1, 2, 3]. This constitutes an important technological advance, allowing a non-invasive approach to assessment of the release of these substances, and permitting longitudinal studies in animals and humans. The method is based on competition for binding to receptors between the endogenous transmitter and an exogenous radioligand [4, 5]. Indeed, the binding potential (BP) of a radioligand is sensitive to the extracellular concentration of the corresponding neurotransmitter, the release of which may thus be quantified through the corresponding displacement of the pre-loaded radioligand [6, 7]. Such quantification has already been modelled using data obtained from human studies [8]. The use of this technique has also been confirmed in animals, by means of simultaneous evaluation of extracellular neurotransmitter concentrations with invasive techniques such as microdialysis [9, 10, 11].

Two main elements of this method are, however, subject to rapid changes that can produce bias in the observations: first, the affinity of the receptors and, second, the disposability of the radioligand delivered by the cerebral circulation. These alterations may be produced by the experimental conditions themselves, such as anaesthesia, waking states, stress etc. The BP of $[^{11}C]$raclopride (BP$_{raclo}$), a radioligand antagonist of dopamine (DA) receptors of the D$_2$ type, exemplifies this technical problem. In animals, a moderate reduction in BP$_{raclo}$ was observed after amphetamine administration (2 mg kg$^{-1}$) under ketamine anaesthesia [12, 13] but amphetamine administration had no effect on BP$_{raclo}$ under halothane anaesthesia [14]. However, amphetamine is known to produce a dramatic release of DA in these conditions [15, 16]. Conversely, in the urethane-anaesthetised rat,
complete displacement of the radioligand $[^{11}\text{C}]$raclopride was obtained by amphetamine [17]. It was therefore suggested, in the light of biochemical studies [18, 19], that this effect could be due to an anaesthesia-induced reduction in the affinity for DA receptors, as confirmed by an increase in the dissociation constant for DA receptors, measured under halothane anaesthesia [14].

Radioligand disposability must also be carefully considered in studies of neurotransmitter release using PET or other imaging methods. The disposability of the radioligand is a consequence of both the extracerebral metabolism and the amount of molecules delivered by the cerebral blood flow (CBF). When radioligands were administered at very low concentrations and under non-pharmacological conditions, alterations produced by changes in CBF were initially considered negligible, but recent experiments have suggested that this problem needs to be carefully addressed [7], and prompted the present study. To this end, two different conditions of anaesthesia (1.5% halothane, 50 mg kg$^{-1}$ h$^{-1}$ ketamine) were considered in cats, these conditions being chosen for their known ability to alter $BP_{raclo}$ [10, 14]. The values found for the $BP_{raclo}$ were compared with those found in awake animals and correlations between these changes and the CBF measured with the use of $[^{15}\text{O}]$H$_2$O were sought. An original experimental set-up was developed to allow PET scans on awake cats for comparison with anaesthetic situations and during test-retest experiments performed on the same day. Possible CBF changes were measured, taking advantage of the possible use of the $[^{15}\text{O}]$H$_2$O just before the evaluation of the $BP_{raclo}$.

Materials and methods

**PET system.** PET studies were performed on a Siemens ECAT Exact HR$^+$ used in three-dimensional mode. The system covers an axial distance of 15.5 cm [20]. The transaxial resolution of the reconstructed images is about 4.1 mm full-width at half-maximum (FWHM) in the centre. Transmission scans were acquired with three rotating $^{68}$Ge/$^{68}$Ga sources and used to correct the emission scans for the attenuation of 511-keV photon rays through tissue and head support. The $[^{11}\text{C}]$raclopride was synthesised as previously described by methylation of the desmethyl precursor using $[^{11}\text{C}]$methyl iodide [21]. $[^{11}\text{C}]$raclopride (2–2.2 mCi) was injected as a bolus for 10 s, immediately followed by a flush with 2 ml saline.

**Animals.** Animal studies were performed by licensed investigators in accordance with French (87–848, Ministère de l’Agriculture et de la Forêt) and European Economic Community (86–60, EEC) guidelines for care of laboratory animals and were approved by the regional ethical animal use committee. In this study, two European male cats weighing about 4 kg were obtained from Iffa-Credo, France.

**Surgery.** A single surgical procedure was performed under general anaesthesia, induced with halothane (4%). As soon as deep anaesthesia was obtained, endotracheal intubation was performed and anaesthesia was maintained by constant insufflation of 2.5% halothane in air. Carbon dioxide concentration in expired gases, heart rhythm and body temperature were continuously controlled during the surgical procedure [22]. A U-shaped piece of Plexiglas was stereotactically fixed to the skull with acrylic cement and screws. This head-holder permitted painless restraint of the animal’s head and ensured a reliable position of the head during PET measurement. During the week after surgery, the animals were treated daily with 50 mg kg$^{-1}$ sodium amoxicillin, an antibiotic.

**Experimental set-up and animal training.** After recovery, each cat was trained to lie down in a hammock, inside a box with a Plexiglas cover. It took about 2 months of daily training, based on kindness and alimentary motivation, to ensure that the animal remained quiet and in a waking state for the duration of the scanning procedure (1–2 h). The head movements of the animal were progressively restrained by attaching the head-holder to the cover of the box according to a previously described method [23]. The box was placed in the tomograph in such a way as to ensure that the head was in the centre of the field of view.

**Anaesthetic procedures.** Two anaesthesia protocols were employed. Ketamine (Panpharma, France) was administered intramuscularly as a single bolus 15 min before the beginning of the scan, yielding a dose of 50 mg kg$^{-1}$ h$^{-1}$. Halothane (Fluothane, Belamont) was 1.5% mixed in medical air supplied to the animal through a respiratory mask. The non-noxious restraint of the head of the animal allowed use of a low halothane concentration.

**Scan test procedures.** A catheter was first inserted in the cephalic vein for radiotracer injection. To measure the $BP_{raclo}$ each animal was first submitted to three simple scan tests (at least 1 week apart) under each experimental condition (awake, ketamine and halothane). A bolus injection of 1.5–2 mCi (55.5–74 MBq) of $[^{11}\text{C}]$raclopride was made via the catheter. Radioactivity was then measured in a series of sequential time frames of increasing duration from 30 s to 10 min. The total time for the measurement of the radioactivity in the brain was 67 min.

**Test-retest experiments.** Each animal was also submitted to three complex test-retest protocols entailing four radioligand injections. In the awake animal, a catheter was inserted in the cephalic vein for radiotracer injections. (1) A bolus injection of 1.5 mCi (55.5 MBq) of $[^{15}\text{O}]$H$_2$O was made via the catheter and the radioactivity was measured in a series of six sequential time frames lasting for 10 s and two frames lasting for 20 s. (2) Fifteen minutes later, a bolus injection of 1.5–2 mCi (55.5–74 MBq) of $[^{11}\text{C}]$raclopride was made according to the same protocol as for the simple test. (3, 4) Three hours later, on the same day, the animal was anaesthetised according to one of the described procedures, and the two aforementioned protocols (1 and 2) were carried out again.

**Data analysis.** The position of the cat in the PET system was such that the reconstructed images followed the frontal plane. Regions of interest (ROIs) for the right and left caudate nuclei and the cerebellum were drawn on horizontally reconstructed PET images (Fig. 1). The striatum was defined on three successive slices according to a cat brain atlas [24]. The regional radioactivity concentration (Ci ml$^{-1}$) was determined for each frame, corrected for decay and plotted versus time. ROIs of the same surface as used in the striatum were defined in the cerebellum, a region with a low density of D$_2$ receptors, and were used to estimate free radioligand concentration and non-specific binding in the brain (F). Specific ligand binding (B) was defined as the difference between the total