Iodine-123 labelled reboxetine analogues for imaging of noradrenaline transporter in brain using single photon emission computed tomography

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Running title: Imaging of NAT in brain using SPECT

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Abstract:

Preliminary investigation of the radioiodinated (*S*,*S*)-reboxetine analogue, ^{123}I-INER, in baboons showed this tracer to have promise for imaging the noradrenaline transporter (NAT) using single photon emission computed tomography (SPECT). More recently the radioiodinated (*R*,*S*)-stereoisomer of ^{123}I-INER , ^{123}I-NKJ64 has been synthesised and preliminary evaluation in rats has been reported. This paper reports the brain distribution and pharmacokinetic properties of ^{123}I-NKJ64 in baboons and compares results with ^{123}I-INER data in the same species. SPECT studies were conducted in two ovariectomised adult female baboons using two different protocols: (1) bolus of ^{123}I-INER or ^{123}I-NKJ64; and (2) bolus plus constant infusion of ^{123}I-NKJ64 with reboxetine (2.0 mg/kg) administration at equilibrium. Following bolus injection, both radiotracers rapidly and avidly entered the baboon brain. The regional brain accumulation of ^{123}I-NKJ64 did not match the known distribution of NAT in baboon brain, contrasting with previous results obtained in rats. Conversely, the regional distribution of ^{123}I-INER was consistent with known distribution of NAT in baboon brain. No displacement of ^{123}I-NKJ64 was observed following administration of reboxetine. This contrasts with previous data obtained for ^{123}I-INER, where 60% of specific binding was displaced by a lower dose of reboxetine. These data suggest that ^{123}I-NKJ64 may lack affinity and selectivity for NAT in baboon brain and ^{123}I-INER is the most promising iodinated reboxetine analogue developed to date for *in vivo* imaging of NAT in brain using SPECT. This study highlights the importance of species differences during radiotracer development and the stereochemical configuration of analogues of reboxetine *in vivo*.

Introduction:

Dysregulation of the noradrenergic system has been implicated in multiple psychiatric and neurodegenerative disorders, including: depression, post-traumatic stress, anxiety, attention-deficit/hyperactivity disorder, Alzheimer's disease and Parkinson's disease (Grandoso et al., 2004; Kiyono et al., 2004; Kung et al., 2004; Lakshmi et al., 2008; McConathy et al., 2004; Zeng et al., 2009). Non-invasive imaging using a selective noradrenaline transporter (NAT) radiotracer and either positron emission tomography (PET) or single photon emission computed tomography (SPECT) could be used for *in vivo* investigations of disease progression and treatment response. In addition a NAT radiotracer would enable studies investigating novel drugs targeted at the NAT, such as drug occupancy studies, thereby aiding the drug discovery process. A radiotracer specific for the *in vivo* assessment of changes in NAT density, is therefore desirable.

Multiple NAT-selective radiotracers have been developed over the years for *in vivo* and *in vitro* brain imaging, including: ^{11}C-desipramine (^{11}C-DMI) and its hydroxylated derivate (*R*)-^{11}C-OHDMI; ^{11}C-nisoxetine and its derivatives (^{125}I-INXT and ^{125}I-PYINXT); ^{11}C-thionisoxetine; ^{11}C-oxaprotiline; ^{11}C-lortalamine; ^{11}C-talopran; ^{11}C-talsupran; two ^{11}C-labelled analogues of mazindol; and ^{11}C-labelled, ^{18}F-labelled and ^{123}I-labelled analogues of reboxetine (McConathy et al., 2004; Schou et al., 2007; Schou et al., 2006; Zeng et al., 2009). The most promising PET radiotracers developed for *in vivo* imaging of the NAT are analogues of reboxetine and include: (*S*,*S*)-^{11}C-MeNER, (*S*,*S*)-^{18}F-FMeNER-D_2 and (*S*,*S*)-^{18}F-FRB-D_4 (Schou et al., 2007; Schou et al., 2006). For *in vivo* imaging of the NAT using SPECT, the most promising radiotracers developed to date are also reboxetine analogues, namely ^{123}I-INER (also denoted (*S*,*S*)-IPBM) (Kanegawa et al., 2006; Tamagnan et al., 2007) and ^{123}I-NKJ64 (Tavares et al., 2011a) (Fig. 1).

^{123}I-INER has a high affinity for the NAT in rat forebrain, with a K_D value of 1.30 ± 0.46 nM. In rat biodistribution studies, ^{123}I-INER displayed good initial brain uptake with maximum accumulation in the rat brain at 30 minutes post-injection (0.54% injected dose/g). The washout in rat brain was gradual and a high midbrain-to-striatum ratio was observed at 180 minutes post-injection (Kanegawa et al., 2006). In baboons, a slow washout from the brain, over more than 2 hours, was observed. Despite the longer physical half-life of ^{123}I being compatible with slower kinetics, the washout of ^{123}I-INER over 2 hours *in vivo* in baboons suggested improvement of NAT radiotracers for SPECT was desirable (Tamagnan et al., 2007). Thus, in order to develop a novel radiotracer for imaging of NAT in brain using SPECT with

improved kinetics, we investigated novel iodinated analogues of reboxetine (Jobson et al., 2008a; Jobson et al., 2009; Jobson et al., 2008b). Our lead candidate, NKJ64, a stereoisomer of INER was found to have high affinity for the NAT in rat brain (Ki of 8.4 ± 1.7 nM) (Jobson et al., 2009). Recently, we reported the radiosynthesis of ^{123}I-NKJ64 (Tavares et al., 2011b) and the evaluation of ^{123}I-NKJ64 in rat brain tissue revealed a nanomolar affinity for NAT (K_D of 4.82 ± 0.87 nM in cortical homogenates) (Tavares et al., 2011a). Additional evaluation of ^{123}I-NKJ64 in rats showed: 1) the regional distribution of ^{123}I-NKJ64 binding to be consistent with the known density of NAT in rodent brain; 2) a maximum uptake of 2.9% of the injected dose in brain; 3) a specific to non-specific ratio (locus coeruleus:caudate putamen) of 2.8 at 30 minutes post intravenous injection; 4) a relatively fast washout from whole brain with an effective half-life of 49 minutes (Tavares et al., 2011a). These data from rats indicated that further evaluation of ^{123}I-NKJ64 in non-human primates was warranted to determine its utility as a SPECT radiotracer for imaging of NAT in brain. In this paper the biodistribution and pharmacokinetic properties of ^{123}I-NKJ64 in non-human primate brain is reported. In addition, a comparative analysis between ^{123}I-NKJ64 and ^{123}I-INER data in non-human primate brain is presented.

Materials and Methods:

Animals

All procedures were conducted at Yale University, USA in accordance with institutional animal care protocols and in compliance with Federal regulations. Two ovariectomised adult female baboons (*Papio anubis*, 14.9 and 13.7 kg) were used for *in vivo* studies. One baboon was used to evaluate ^{123}I-NKJ64 distribution and pharmacokinetic properties and the other baboon was used for studies using ^{123}I-INER. Baboons were fasted for 24 hours prior to imaging studies. On the SPECT imaging day, anaesthesia was induced by intramuscular injection of ketamine (10 mg/kg) and glycopyrrolate (0.1-0.2 mg/kg). The animal was then transferred to the SPECT camera and immediately intubated with an endotracheal tube and anaesthesia was maintained by administering 2.5% isoflurane through a re-breathing circuit. A period of at least 2 hours between induction of anaesthesia and radiotracer injection was allowed, in order to stabilise the baboons under anaesthesia and minimise the effects of the initial administration of ketamine on radiotracer uptake, distribution and kinetics. An intravenous perfusion line was established in a femoral vein for injection of fluids for hydration and collection of blood samples for

metabolite analysis. In the contralateral leg, an additional venous line was used for injection of the radiotracer. Body temperature was maintained using a heated water blanket and monitored by rectal thermometer. Vital signs, including heart rate, respiration rate, blood pressure and body temperature, were monitored every 15 minutes.

General SPECT acquisition protocol

SPECT studies were performed using a Neurofocus SPECT camera (Neurophysics Inc., USA), with a ring of 12 wide-aperture pinhole collimator detectors. Data acquisition started immediately after radiotracer injection using consecutive dynamic SPECT scans, in which detectors moved side-to-side and in and out to completely sample each slice (Seibyl et al., 2002; Stoddart and Stoddart, 1992). The duration of each scan was approximately 20 minutes and a total of up to 18 slices were acquired. An energy window of 10% centred at 159 keV, 128×128×64 matrix, zoom of 1 and slice thickness of 5.0 mm were used for acquisitions starting at 0 minutes post injection. Acquisition duration was 240 minutes for bolus baseline experiments and 420 minutes for bolus plus constant infusion studies with displacement drug. Raw SPECT data were reconstructed using Neurofocus proprietary software (Neurofocus Inc., USA) and the manufacturer's recommended iterative reconstruction algorithm, which was based on maximum a-posteriori (MAP) reconstruction methods, similar to algorithms used for scanning microscopes (Seibyl et al., 2002). Injected radioactivity for bolus studies was 222.0 MBq and 432.9 MBq for ^{123}I-NKJ64 and ^{123}I-INER experiments, respectively; and for bolus plus constant infusion using ^{123}I-NKJ64 the injected radioactivity was 264.18 MBq. Venous blood was collected from a femoral vein at selected time points. The displacement study was conducted using a bolus plus constant infusion protocol. The bolus/infusion (B/I) ratio (or K_{bol}) was calculated to be 2.5 hours for ^{123}I-NKJ64 using PMOD 3.203 software (PMOD Technologies, Switzerland) and results from bolus baseline studies. A Gemini PC 1 (IMED Inc., USA) infusion pump with 60 mL syringes was used for infusion of radiotracer. Upon equilibrium, which was estimated to occur between 2.5 and 3 hours based on estimations using PMOD 3.203 software, a single bolus of 2.0 mg/kg of reboxetine was injected intravenously.

Plasma pharmacokinetic analysis

For studies using the radiotracer bolus paradigm, venous blood samples (4-5 mL) were obtained from the femoral vein prior to start of the study (- 5 minutes) and at 1, 3, 5, 10, 15, 30, 60, 120, 180 and

240 minutes post radiotracer injection. For displacement studies, where a bolus plus constant infusion paradigm was used, venous blood samples were collected at 15, 30 and 45 minutes prior to reboxetine injection and 15, 30, 45, 60 and 120 minutes following reboxetine administration. The samples were collected into ethylenediaminetetraacetic acid-coated tubes. Processing and analysis of blood was performed using previously established methodology (Baldwin et al., 1995; Baldwin et al., 1993; Gandelman et al., 1994; Zoghbi et al., 1992).

Image processing and co-registration with magnetic resonance (MR) imaging

Reconstructed scans were imported into PMOD 3.203 software and merged into a single file for image processing which was performed using PMOD tools, starting with decay correction. Motion correction was performed by creating an average image of consecutive scans with absence of motion, which was then used as a reference for rigid matching co-registration to all scans in the current study. Attenuation correction was performed by applying the Chang algorithm (attenuation coefficient = 0.011 mm^{-1}) to a semi-automatically drawn volume of interest (VOI) (Chang, 1978).

T1-weighted MR images were acquired with a GE Signa unit (General Electric, USA) at 1.5 T. The T1 sequence was a spoiled gradient recall protocol with the following settings: TR= 25 ms, TE=5 ms, NEX=2, matrix=256×256, field of view = 16 cm. T1-weighted MR images were reduced from an initial matrix of 256×256 with 112 axial slices (axial slice thickness = 0.7 mm) to a 128×128 matrix, with 20 axial slices (axial slice thickness = 2.8 mm). Each animal used for the current study had a brain MR scan for purposes of image co-registration and placement of VOIs for SPECT quantification. All VOIs were drawn bilaterally; however due to the lack of considerable differences between VOIs in left and right hemispheres, the results for the average VOI are reported. For each animal, a standard VOI template included the following brain regions: brainstem, midbrain, thalamus, caudate, putamen, frontal cortex, occipital cortex, cerebellum and subcortical white matter.

Averaged images of SPECT data were generated by averaging the scans presenting the highest radioactivity from cortical and subcortical brain structures. The averaged image was used to co-register to a MR template from the same animal using an automatic rigid matching tool or when necessary manual alignment of both imaging modalities, by adjusting translation and rotation of the images. The transformation matrix was saved and consequently applied to all individual dynamic scans of the

corresponding co-registered SPECT study. Finally, the MR-derived VOIs templates were applied to the final co-registered SPECT images for generation of time-activity curves.

Data analysis

Time-activity curves were generated for each brain region and standard uptake values (SUV) were calculated according to equation 1. The target-non target ratio was expressed as SUVr, i.e. SUV value of target region divided by SUV of occipital cortex, defined as reference region. Percentage washout from the brain was determined using equation 2.

$$\text{SUV}=\frac{\text{Concentration VOI target}}{\frac{\text{Injected dose}}{\text{Animal weight}}} \qquad \text{(Eq. 1)}$$

$$\%\ \text{Washout} = \frac{\text{Initial conc. VOI target- Final conc. VOI target}}{\text{Initial conc. VOI target}} \times 100 \qquad \text{(Eq. 2)}$$

where conc. = concentration. The initial and final concentrations were the radioactive concentrations at beginning and end of acquisition.

For the displacement study using the bolus plus constant infusion protocol, specific binding in different brain regions was obtained by subtracting the mean occipital cortex uptake. Percent specific binding displacement was calculated using equation 3:

$$\%\ \text{Displacement} = \frac{\text{SB prior displacement - SB post displacement}}{\text{SB prior displacement}} \times 100 \qquad \text{(Eq. 3)}$$

where SB is specific binding.

GraphPad Prism 4.0 (GraphPad Software, USA) was used for curve fitting.

Results:

SPECT images demonstrating the distribution of ^{123}I-NKJ64 and ^{123}I-INER in baboon brain are shown in Fig. 2a and 2b, respectively. A homogeneous distribution of radioactivity was observed following intravenous bolus injection of ^{123}I-NKJ64, such that the uptake in the brainstem and midbrain, the regions of baboon brain richest in NAT, was similar to that in other brain regions. Conversely, a markedly higher accumulation of radioactivity at the level of the brainstem and midbrain compared to the lower radioactivity accumulation in the cerebellum, caudate and occipital cortex was observed following intravenous bolus injection of ^{123}I-INER (Fig. 2b).

Following intravenous bolus injection of ^{123}I-NKJ64, whole brain uptake peaked at 20 minutes; the percentage of injected dose (%ID) in brain was 2.86%. At 240 minutes post-injection, 73% of whole brain initial uptake had been eliminated. The regional uptake of ^{123}I-NKJ64 was as follows: thalamus, caudate and putamen > midbrain, brainstem and subcortical white matter > frontal cortex, cerebellum and occipital cortex (Fig. 3a). Following intravenous bolus injection of ^{123}I-INER, whole brain uptake peaked at 20 minutes; the %ID in brain was 2.50%. ^{123}I-INER had a slower washout from the brain compared with ^{123}I-NKJ64, such that at 240 minutes post-injection 64% of whole brain uptake was eliminated. The regional uptake of ^{123}I-INER was as follows: brainstem > midbrain > thalamus, caudate, putamen, cerebellum and white matter > frontal cortex > occipital cortex (Fig. 3b).

Target:non target ratios determined following intravenous bolus injection of ^{123}I-NKJ64, expressed as target SUV relative to occipital SUV, were high in the thalamus, caudate and putamen and low in the frontal cortex and cerebellum (Fig. 4a). Conversely, intravenous bolus injection of ^{123}I-INER resulted in high target:non target ratios in brainstem and midbrain and a low ratio in frontal cortex (Fig. 4b). Bolus injection of 2.0 mg/kg of reboxetine during bolus plus constant infusion experiments using ^{123}I-NKJ64 did not reduce the radioactive concentration in any of the evaluated brain regions, including NAT-rich regions such as brainstem and midbrain (Fig. 5). This contrasts with previous bolus plus constant infusion experiments with ^{123}I-INER, which demonstrated that bolus injection of 1.0 mg/kg of reboxetine significantly reduced uptake in NAT rich regions (60% displacement) (Tamagnan et al., 2007).

The results from blood sampling following intravenous bolus injection of ^{123}I-NKJ64 or ^{123}I-INER are shown in Table I. At 60 minutes post-injection of ^{123}I-NKJ64, the parent fraction in venous blood was 32%, and declined over time reaching a value of less than 14% at 240 minutes post injection. At 60 minutes post-injection of ^{123}I-INER, the parent fraction in venous plasma was 31% and decline over time reaching a value of less than 13% at 240 minutes post-injection. Analysis of HPLC chromatograms from venous blood samples collected pre- and post- administration of reboxetine, during bolus plus constant infusion experiments using ^{123}I-NKJ64 or ^{123}I-INER, showed no differences in the metabolic profile of the parent compound for both radiotracers.

Discussion:

The pharmacokinetics and brain distribution of ^{123}I-NKJ64 in non-human primates was investigated in this project and it was compared with its stereoisomer, ^{123}I-INER. ^{123}I-NKJ64 rapidly and

avidly entered the baboon brain, reaching a peak %ID in whole brain of 2.86%. The whole brain uptake of ^{123}I-NKJ64 in baboon brain was similar to its stereoisomer, ^{123}I-INER (%ID in brain = 2.50% at 20 minutes post-injection). In comparison with other SPECT and PET radiotracers, whole brain uptake of ^{123}I-NKJ64 was either greater or similar (McConathy et al., 2004; Schou et al., 2003; Schou et al., 2007; Seneca et al., 2006).

Despite the high whole brain uptake, the distribution of ^{123}I-NKJ64 in baboon brain was inconsistent with known distribution of NAT in non-human primate brain (Smith et al., 2006). Analysis of ^{123}I-NKJ64 SPECT images obtained in baboons showed that the highest uptake was in the caudate and putamen, regions that are known to have low NAT density (Smith et al., 2006). Binding ratios determined for ^{123}I-NKJ64 where also highest in caudate and putamen. However, the uptake in the caudate and putamen was not due to specific binding to the NAT, since no displacement was observed in these regions following intravenous administration of the NAT blocking drug, reboxetine. The mechanism underlying the relatively high uptake in the caudate and putamen, remains unknown. However, some authors have suggested the existence of low-affinity NAT binding sites in the striatum as an explanation for the high striatal uptake frequently observed with NAT radiotracers (Ding et al., 2003). *In vivo* administration of a high dose of the reboxetine resulted in no displacement of ^{123}I-NKJ64 uptake in any of the other evaluated brain regions including the brainstem or midbrain. This suggests that the *in vivo* uptake of ^{123}I-NKJ64 in baboons is not due to specific binding of the radiotracer to the NAT and that ^{123}I-NKJ64 has a low affinity for the NAT *in vivo* in baboon brain. In contrast, ^{123}I-INER, a stereoisomer of ^{123}I-NKJ64, showed a distribution pattern in baboon brain consistent with known NAT densities in non-human primate brain. Moreover, 60% of ^{123}I-INER binding was displaced following intravenous injection of a lower dose of reboxetine than we used in the present study (Tamagnan et al., 2007). The lower *in vitro* affinity of ^{123}I-NKJ64 in comparison with ^{123}I-INER (K_D in rat frontal cortex of 4.8 nM and 1.3 nM, respectively) (Kanegawa et al., 2006; Tavares et al., 2011a) may explain the differences in distribution and kinetics between ^{123}I-NKJ64 and ^{123}I-INER *in vivo*. Although high *in vitro* affinity does not guarantee the success of a radiotracer *in vivo*, it is desirable, particularly when imaging low density molecular targets, such as the NAT. In 2009, Zeng et al. observed differences in the brain distribution and kinetics among different reboxetine analogues labelled with ^{11}C or ^{18}F with a range of affinities for the NAT. These authors suggested that radiotracers with low affinity for NAT will not enable the visualisation of a specific binding signal in NAT-rich regions using PET due to a low signal-to-noise ratio (Zeng et al., 2009).

Another contributory factor to the observed differences between ^{123}I-INER and ^{123}I-NKJ64 could be their relative selectivity for NAT over the transporters for serotonin (SERT) and dopamine (DAT). The calculated ratios of affinity for NAT relative to the other amine transporters were: SERT/NAT=51 and DAT/NAT=270 for ^{123}I-INER versus SERT/NAT=6 and DAT/NAT=63 for ^{123}I-NKJ64 (Jobson et al., 2009; Tamagnan et al., 2007). The binding of ^{123}I-NKJ64 to other aminergic transporters could contribute to the ^{123}I-NKJ64 uptake measured in non-target regions *in vivo* in non-human primate brain in comparison to ^{123}I-INER.

There have been previous reports of differences in the biodistribution and kinetics *in vivo* of stereoisomers of compounds other than ^{123}I-NKJ64 and ^{123}I-INER. For example, a study evaluating the distribution and kinetics of ^{11}C-labelled (*S*,*S*)- and (*R*,*R*)-MRB, two reboxetine stereoisomers, showed that only (*S*,*S*)-^{11}C-MRB presented the favourable characteristics for imaging of NAT *in vivo*. However, (*R*,*R*)-^{11}C-MRB did not exhibit regional specificity and was not blocked by administration of nisoxetine suggesting enantioselectivity of MRB *in vivo* (Ding et al., 2003). ^{123}I-NKJ64 is the first (*R*,*S*)-reboxetine analogue to be synthesised and investigated *in vivo* as an imaging agent. This study demonstrated ^{123}I-NKJ64 to be unsuccessful as a radiotracer for the visualisation of the NAT *in vivo* in baboons, and therefore any future development of radiotracers based on reboxetine may be best focussed on the (*S*,*S*)-stereoisomers that have higher affinity for the NAT.

The results presented here illustrate the effect that species differences can have on radiotracer performance *in vivo*. In contrast to the present study in baboons, our previous study in rats demonstrated that the brain distribution of ^{123}I-NKJ64 was consistent with known NAT density and moreover that ^{123}I-NKJ64 had a good target non-target ratio in rat brain (Tavares et al., 2011a). The differences between the way ^{123}I-NKJ64 behaves in rats and baboon could be due to numerous factors. These include the known differences in NAT density in rat and non-human primate brain. In non-human primate brain, the locus coeruleus has a NAT binding site density of around 220 fmol/mg, while the rodent locus coeruleus has a density of around 1500 fmol/mg (Smith et al., 2006; Tejani-Butt, 1992). Thus, rat brain has seven times more NAT binding sites than non-human primate brain. It is worth noting that that the density of NATs in human cerebral cortex is approximately nine times lower than in rodents (Schou et al., 2006). Consequently, the present results obtained with ^{123}I-NKJ64 in baboons, and the known differences in NAT densities across species, preclude the translation of ^{123}I-NKJ64 for use in humans.

It is unlikely that the quantification of ^{123}I-NKJ64 and ^{123}I-INER kinetics and distribution in baboon brain reported here was affected by radiolabelled metabolites generated in blood. Only a single metabolite was present in venous plasma and this was less lipophilic than the parent radiotracers. Since the metabolite is less lipophilic than the parent radiotracers it is unlikely to pass the blood-brain barrier and contribute to brain radioactivity. In addition, the time-activity curves obtained for all brain regions, after intravenous bolus injection of the radiotracer, had a single initial peak followed by continuous elimination over the duration of the study, supporting the hypothesis that there were no metabolites present in the brain over time. Both these observations provide support for the interpretation that the uptake in baboon brain was not due to metabolites generated either in tissue or in blood. The metabolic pattern of ^{123}I-NKJ64 in venous blood was similar to that of ^{123}I-INER. This suggests that differences in brain distribution and radiotracer pharmacokinetics *in vivo* between ^{123}I-NKJ64 and ^{123}I-INER were not due to differences in metabolism. Analysis of blood collected during displacement experiment showed no difference in the parent compound fraction in plasma taken post administration of reboxetine compared to plasma taken pre-administration of reboxetine. This suggests that there was no displacement of ^{123}I-NKJ64 binding in non-target organs indicating that there is no specific binding of ^{123}I-NKJ64 to peripheral organs. A similar observation was made in rats such that administration of reboxetine did not reduce ^{123}I-NKJ64 binding in any of the investigated peripheral organs (Tavares et al., 2011a).

In conclusion, the present data suggest that ^{123}I-NKJ64, may lack affinity and selectivity for NAT in baboon brain, and the high levels of non-specific binding could be obscuring any ^{123}I-NKJ64 specific binding that might be present *in vivo*. Despite promising preliminary evaluation in rodents, the data obtained from non-human primates do not support further studies with ^{123}I-NKJ64 and its translation for use in human imaging studies. Thus, to date, the most promising SPECT radiotracer developed for *in vivo* imaging of NAT in brain is ^{123}I-INER.

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References

Baldwin R, Zea-Ponce Y, Al-Tikriti M, Zoghbi SS, Seibyl J, Charney DS, Hoffer PB, Wang S, Milius R, Neumeyer J, Innis R. 1995. Regional Brain Uptake and Pharmacokinetics of [^{123}I]N-ω-Fluoroalkyl-2β-carboxy-3β-(4-iodophenyl)nortropane Esters in Baboons. Nucl Med Biol 22(2):211-219.

Baldwin R, Zea-Ponce Y, Zoghbi SS, Laurelle M, Al-Tikriti M, Sybirska E, Malison RT, Neumeyer J, Milius R, Wang S, Stabin M, Smith E, Charney DS, Hoffer PB, Innis R. 1993. Evaluation of the Monoamine Uptake Site Ligand [^{123}I]Methyl 3β-(4-Iodophenyl)-tropane-2β-carboxylate ([^{123}I]β-CIT) in Non-human Primates: Pharmacokinetics, Biodistribution and SPECT Brain Imaging Coregistered with MRI. Nucl Med Biol 20(5):597-606.

Chang L-T. 1978. A Method for Attenuation Correction in Radionuclide Computed Tomography. IEEE T Nucl Med 25(1):638-643.

Ding Y, Lin K, Garza V, Carter P, Alexoff D, Logan J, Shea C, Xu Y, King P. 2003. Evaluation of a New Norepinephrine Transporter PET Ligand in Baboons, Both in Brain and Peripheral Organs. Synapse 50:345-352.

Gandelman M, Baldwin R, Zoghbi SS, Zea-Ponce Y, Innis R. 1994. Evaluation of Ultrafiltration for the Free-Fraction Determination of Single Photon Emission Computed Tomography (SPECT) Radiotracers: β-CIT, IBF and Iomazenil. J Pharm Sci 83(7):1014-1019.

Grandoso L, Pineda J, Ugedo L. 2004. Comparative study of effects of desipramine and reboxetine on locus coeruleus neurons in rat brain slices. Neuropharmacology 46:815-823.

Jobson NK, Crawford AR, Dewar D, Pimlott SL, Sutherland A. 2008a. New iodoreboxetine analogues for SPECT imaging of the noradrenaline transporter. Bioorg Med Chem Lett 18:4940-4943.

Jobson NK, Crawford AR, Dewar D, Pimlott SL, Sutherland A. 2009. Design and synthesis of (2*R*,3*S*)-iodreboxetine analogues for SPECT imaging of the noradrenaline transporter. Bioorg Med Chem Lett 19:4996-4998.

Jobson NK, Spike R, Crawford AR, Dewar D, Pimlott SL, Sutherland A. 2008b. Stereoselective synthesis of (2*S*,3*R*)- and (2*R*,3*S*)-iodoreboxetine; potential SPECT imaging agents for the noradrenaline transporter. Organic and Biomolecular Chemistry 6:2369-2376.

Kanegawa N, Kiyono Y, Kimura H, Sugita T, Kajiyama S, Kawashima H, Ueda M, Kuge Y, Saji H. 2006. Synthesis and evaluation of radioiodinated (*S*,*S*)-2-(α-(2-iodophenoxyl)benzyl)morpholine for imaging brain norepinephrine transporter. Eur J Nuc Med Mol Imag 33:639-647.

Kiyono Y, Kanegawa N, Kawashima H, Kitamura Y, Iida Y, Saji H. 2004. Evaluation of radioiodinated (*R*)-N-methyl-3-(2-iodophenoxy)-3-phenylpropanamine as a ligand for brain norepinephrine transporter imaging. Nucl Med Biol 31:147-153.

Kung M, Choi S, Hou C, Zhuang Z, Foulon C, Kung HF. 2004. Selective binding of 2-[^{125}I]iodo-nisoxetine to norepinephrine transporters in the brain. Nucl Med Biol 31:533-541.

Lakshmi B, Kung M, Lieberman B, Zhao J, Waterhouse R, Kung HF. 2008. (*R*)-N-Methyl-3-(3-^{125}I-pyridin-2-yloxy)-3-phenylpropan-1-amine: a novel probe for norepinephrine transporters. Nucl Med Biol 35:43-52.

McConathy J, Owens MJ, Kilts CD, Malveaux EJ, Camp VM, Votaw JR, Nemeroff CB, Goodman MM. 2004. Synthesis and biological evaluation of [^{11}C]talopran and [^{11}C]talsupran: candidate PET ligands for the norepinephrine transporter. Nucl Med Biol 31:705-718.

Schou M, Halldin C, Sóvágó J, Pike V, Gulyás B, Mozley D, Johnson DP, Hall H, Innis R, Farde L. 2003. Specific *in vivo* binding to the norepinephrine transporter demonstrated with the PET radioligand, (*S*,*S*)-[^{11}C]MeNER. Nucl Med Biol 30:707-714.

Schou M, Pike VW, Sóvágó J, Gulyás B, Gallagher PT, Dobson DR, Walter MW, Rudyk H, Farde L, Halldin C. 2007. Synthesis of ^{11}C-labelled (*R*)-OHDMI and CFMME and their evaluation as candidate radioligands for imaging central norepinephrine transporters with PET. Bioorg Med Chem 15:616-625.

Schou M, Pike VW, Varrone A, Gulyás B, Farde L, Halldin C. 2006. Synthesis and PET evaluation of (*R*)-[S-methyl-^{11}C]thionisoxetine, a candidate radioligand for imaging brain norepinephrine transporters. J Label Compd Radiopharm 49:1007-1019.

Seibyl JP, Stobbart HA, Martin D, Smith E, Wisniewsk G, Stoddart HF. 2002. Evaluation of high resolution NeuroFocus SPECT device for small animal imaging. J Nucl Med 43(UNSP 202375|936).

Seneca N, Gulyás B, Varrone A, Schou M, Airaksinen A, Tauscher J, Vandenhende F, Kielbasa W, Farde L, Innis R, Halldin C. 2006. Atomoxetine occupies the norepinephrine transporter in dose-

dependent fashion: a PET study in nonhuman primate brain using (S,S)-[^{18}F]FMeNER-D_2. Psychopharmacology (Berl) 188:119-127.

Smith HR, Beveridge TJR, Porrino LJ. 2006. Distribution of Norepinephrine Transporters in the Non-Human Primate Brain. Neuroscience 138:703-714.

Stoddart HAS, Stoddart HF. 1992. New multidimensional reconstructions for the 12-detector, scanned focal point, single photon tomograph. Phys Med Biol 37(3):579-586.

Tamagnan GD, Brenner E, Alagille D, Staley JK, Haile C, Koren A, Early M, Baldwin RM, Tarazi FI, Baldessarini RJ, Jarkas N, Goodman MM, Seibyl JP. 2007. Development of SPECT imaging agents for the norepinephrine transporters: [^{123}I]INER. Bioorg Med Chem Lett 17:533-537.

Tavares A, Jobson NK, Dewar D, Sutherland A, Pimlott S. 2011a. ^{123}I-NKJ64: A Novel Single Photon Emission Computed Tomography Radiotracer for Imaging the Noradrenaline Transporter in Brain Synapse 65:658-667.

Tavares A, Jobson NK, Dewar D, Sutherland A, Pimlott S. 2011b. Development of the radiosynthesis of high-specific-activity ^{123}I-NKJ64. Nucl Med Biol 38:493-500.

Tejani-Butt SM. 1992. [^{3}H]Nisoxetine: A Radioligand for Quantitation of Norepinephrine Uptake Sites by Autoradiography or by Homogenate Binding. J Pharmacol Exp Ther 260(1):427-436.

Zeng F, Mun J, Jarkas N, Stehouwer JS, Voll RJ, Tamagnan GD, Howell L, Votaw JR, Kilts CD, Nemeroff CB, Goodman MM. 2009. Synthesis, Radiosynthesis, and Biological Evaluation of Carbon-11 and Fluorine-18 Labeled Raboxetine Analogues: Potential Positron Emission Tomography Radioligands for in Vivo Imaging of the Norepinephrine Transporter. J Med Chem 52:62-73.

Zoghbi SS, Baldwin R, Seibyl J, Al-Tikriti M, Zea-Ponce Y, Laruelle M, Sybirska E, Woods S, Goddard A, Malison RT, Zimmerman R, Charney DS, Smith E, Hoffer PB, Innis R. 1992. Pharmacokinetics of the SPECT Benzodiazepine Receptor Radioligand [^{123}I]Iomazenil in Human and Non-human Primates. Nucl Med Biol 19(8):881-888.

Table I. Results from metabolite analysis in venous blood following intravenous injection of ^{123}I-NKJ64 and ^{123}I-INER. Note the similar metabolic parent fraction in venous blood at selected time points following intravenous bolus injection of either radiotracer.

Metabolite analysis	**30 min. p.i.**		**60 min. p.i.**		**240 min. p.i.**	
	^{123}I-NKJ64	**^{123}I-INER**	**^{123}I-NKJ64**	**^{123}I-INER**	**^{123}I-NKJ64**	**^{123}I-INER**
Metabolite A	49.8	46.6	67.9	69.3	86.5	87.2
Parent	50.3	53.4	32.1	30.7	13.6	12.8

[1]p.i. = post-injection

Figure Legends

Fig. 1 Chemical structures of reboxetine and its analogues developed for PET and SPECT imaging of the NAT in brain.

Fig. 2 Brain SPECT images showing the distribution of ^{123}I-NKJ64 (a) and ^{123}I-INER (b) in baboons. Transverse, sagittal and coronal planes (left to right). The greatest accumulation of radioactivity is shown in red and the least in green. Note differences in brain distribution between SPECT images acquired using ^{123}I-NKJ64 compared with ^{123}I-INER. Uptake of ^{123}I-NKJ64 in brainstem (red arrows) is not different from other brain structures (a) while uptake of ^{123}I-INER in brainstem is greater than other brain regions (b).

Fig. 3 Time-activity curves derived from SPECT data acquired following intravenous bolus injection of ^{123}I-NKJ64 (a) and ^{123}I-INER (b). Note the higher uptake in brainstem and midbrain compared to other brain regions after injection of ^{123}I-INER, at later time points which is not seen after injection of ^{123}I-NKJ64.

Fig. 4 Binding ratios of ^{123}I-NKJ64 (a) and ^{123}I-INER (b) in different brain regions following bolus intravenous injection of the radiotracer. For ^{123}I-NKJ64 (a) binding ratios in NAT-rich regions, i.e. the brainstem and the midbrain, were similar to those of other regions but for ^{123}I-INER (b) binding ratios in NAT-rich regions were greater than other regions.

Fig. 5 Uptake of ^{123}I-NKJ64 in baboon brain pre- and post-administration of reboxetine. Time-activity curve obtained following bolus plus constant infusion of ^{123}I-NKJ64. The reboxetine bolus was administered 2.75 hours after radiotracer injection (indicated by black line and arrow). Note the absence of change in radioactive concentration post-administration of reboxetine in any of the evaluated brain regions.