

# Imaging the serotonin transporter with positron emission tomography: initial human studies with [<sup>11</sup>C]DAPP and [<sup>11</sup>C]DASB

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**Abstract.** Two novel radioligands, *N,N*-dimethyl-2-(2-amino-4-methoxyphenylthio)benzylamine (DAPP) and (*N,N*-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine (DASB), were radiolabeled with carbon-11 and evaluated as *in vivo* probes of the serotonin transporter (SERT) using positron emission tomography (PET). Both compounds are highly selective, with nanomolar affinity for the serotonin transporter and micromolar affinity for the dopamine and norepinephrine transporters. Six volunteers were imaged twice, once with each of the two radioligands. Both ligands displayed very good brain penetration and selective retention in regions rich in serotonin reuptake sites. Both had similar brain uptake and kinetics, but the cyano analogue, [<sup>11</sup>C]DASB, had a slightly higher brain penetration in all subjects. Plasma analysis revealed that both radiotracers were rapidly metabolized to give mainly hydrophilic species as determined by reverse-phase high-performance liquid chromatography. Inhibition of specific binding to the SERT was demonstrated in three additional subjects imaged with [<sup>11</sup>C]DASB following an oral dose of the selective serotonin reuptake blocker citalopram. These preliminary studies indicate that both these substituted phenylthiobenzylamines have highly suitable characteristics for probing the serotonin reuptake system with PET in humans.

**Keywords:** Positron emission tomography – Serotonin transporter – Substituted phenylthiobenzylamines – DAPP – DASB

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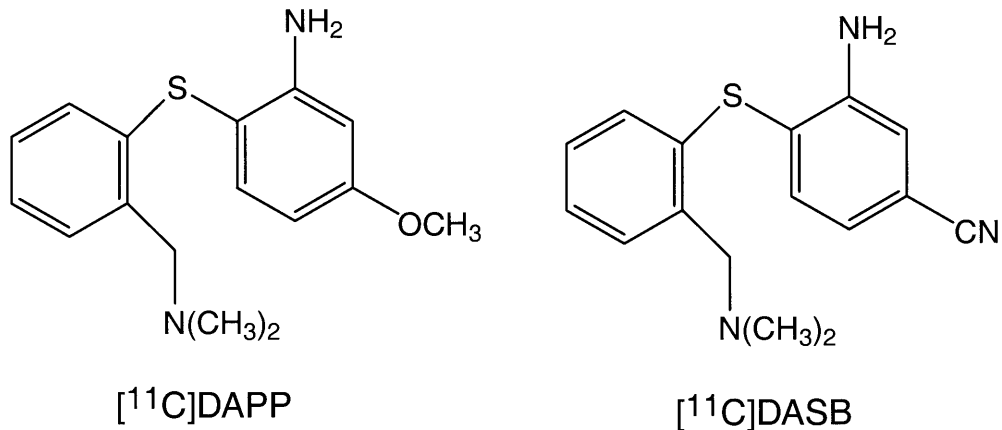
## Introduction

The serotonin neurotransmitter system plays an important role in many psychiatric and neurological disorders. As a modulator of extracellular serotonin levels and the site of action of many antidepressant drugs, the serotonin transporter (SERT) has been the focus of intense study for many years. Imaging the SERT *in vivo* either by positron emission tomography (PET) or by single-photon emission tomography (SPET) has been pursued actively by many groups but has met with limited success [1]. Most of the candidate radioligands, while possessing high affinity for the SERT *in vitro*, have not proven suitable for *in vivo* imaging. The most common shortcomings have been poor signal to noise ratios and a lack of selectivity for SERT over other monoamine transporters: dopamine transporter (DAT) and norepinephrine transporter (NET). Inappropriate pharmacokinetics, often with slow clearance of nonspecific binding, has been a common characteristic of these failed SERT radioligands. To date, the most useful SERT radiotracer for PET has been (+)-[<sup>11</sup>C]McN 5652. In human PET studies, this selective SERT radiotracer has shown a modest mid-brain to cerebellum ratio (1.8), and difficulties in quantitating its binding *in vivo* have limited its usefulness for clinical studies [2]. More recently, encouraging results have been obtained with a radioiodinated phenylthiobenzyl alcohol, [<sup>123</sup>I]IDAM, as a putative SERT ligand for SPET [3].

We have evaluated a series of substituted phenylthiobenzylamines radiolabeled with <sup>11</sup>C as potential PET radiotracers for imaging SERT [4, 5]. Four of these compounds displayed nano- or sub-nanomolar affinity for SERT, and a greater than 1,000-fold affinity in *in vitro* binding assays for cloned human SERT over DAT and NET. Biodistribution studies in rats showed a high brain uptake of radioactivity with a preferential distribution in brain regions rich in SERT. Pharmacological studies demonstrated that the uptake in the SERT-rich regions was both saturable and selective for SERT [5]. Two of the most promising radiotracers are the objects of the human PET studies that we report here. These two radioli-

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**Fig. 1.** Chemical structure of [ $^{11}\text{C}$ ]DAPP (left) and [ $^{11}\text{C}$ ]DASB (right)



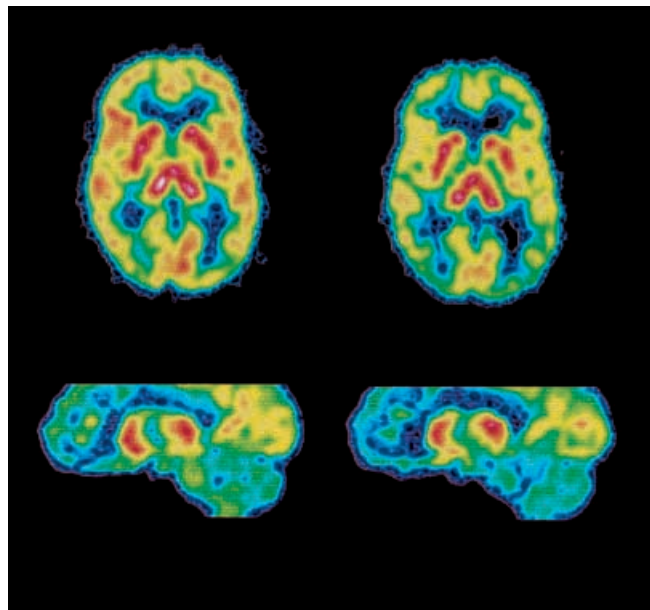
gands are: [ $^{11}\text{C}$ ]N,N-dimethyl-2-(2-amino-4-methoxyphenylthio)benzylamine (DAPP) and [ $^{11}\text{C}$ ]N,N-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine (DASB). Their chemical structures are shown in Fig. 1.

## Materials and methods

[ $^{11}\text{C}$ ]DAPP and [ $^{11}\text{C}$ ]DASB were synthesized by alkylation of their N-normethyl precursors using cyclotron-produced [ $^{11}\text{C}$ ]iodomethane in 30%–55% radiochemical yield (decay uncorrected from [ $^{11}\text{C}$ ]iodomethane) following high-performance liquid chromatography (HPLC) purification and formulation [5, 6]. The total synthesis times were 25–30 min from end of bombardment, and specific activities of 25–55 GBq/ $\mu\text{mol}$  at end of synthesis were obtained. Chemical and radiochemical purities of the final formulated radiotracers were >98% as determined by HPLC and thin-layer chromatography.

Nine healthy volunteers participated in the study after giving informed consent. The subjects were free of general medical, neurological, and psychiatric illness. They had no history of previous head injury or drug or alcohol abuse, nor had they taken any prescription or nonprescription centrally active drug for at least 8 weeks prior to the PET scans. Three subjects were women and six men. The age range was 23–51 years. The study was approved by the University of Toronto Human Subjects Review Committee. Six of the volunteers were imaged twice, once with each of the two tracers. Inhibition of specific binding was studied in three additional subjects who were scanned, with [ $^{11}\text{C}$ ]DASB, before and 3 h following a 40 mg oral dose of the selective serotonin uptake inhibitor citalopram.

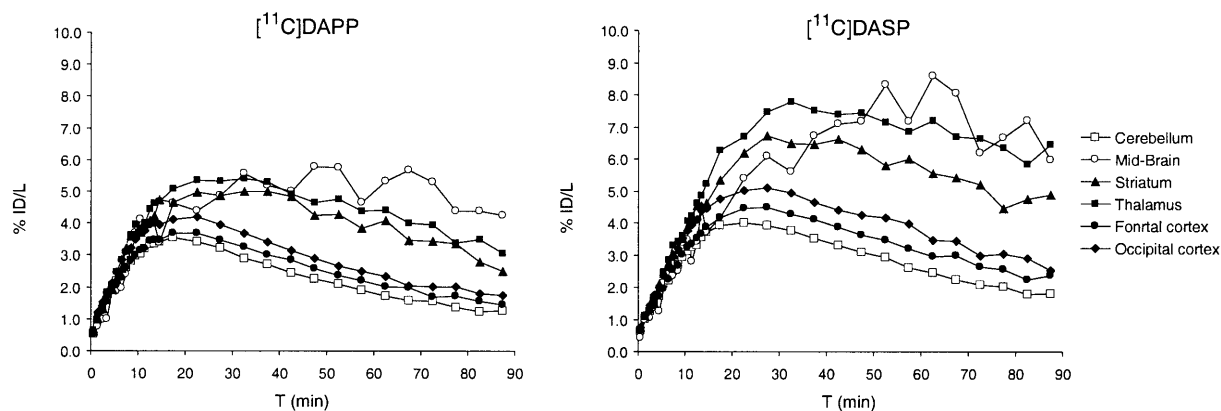
The radioligands were injected as an intravenous bolus (350 MBq). Sequential images of the brain were obtained over a 90-min period following injection with a Scanditronix/GEMS PC2048-15B brain PET scanner. After attenuation correction, the images were reconstructed by filtered back-projection. Regions of interest were drawn over the cerebellum, striatum, mid-brain, thalamus, occipital and frontal cortex to obtain time-activity curves for each region. Each curve was decay corrected to the time of injection. Ratios of radioactivity in those regions to that in the cerebellum were calculated using data from 55 to 90 min after injection. Blood samples (venous in six and arterial in three subjects) were obtained at several time points during each study. After centrifugation, metabolite analysis was carried out on the plasma by reverse-phase HPLC [5].



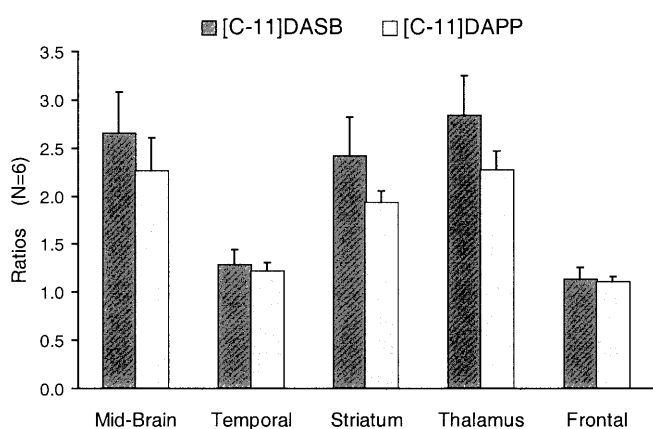
**Fig. 2.** Sagittal and transverse summed images for [ $^{11}\text{C}$ ]DAPP (left) and [ $^{11}\text{C}$ ]DASB (right). The transverse images are at the level of the striatum and the sagittal image plane passes through the head of the caudate

## Results

Figure 2 shows representative images obtained in the same subject with both radioligands. The highest uptake is in the mid-brain, thalamus, hypothalamus, and striatum. Time-activity curves from one of our subjects are shown for both tracers in Fig. 3. These curves are typical of those obtained in all our subjects. Both ligands have similar brain uptake and kinetics, but [ $^{11}\text{C}$ ]DASB has a slightly higher brain penetration in all subjects. The peak uptake occurs earlier for [ $^{11}\text{C}$ ]DAPP (20–30 min) than for [ $^{11}\text{C}$ ]DASB (30–40 min). In all subjects, the uptake in mid-brain and thalamus was similar, and that in the striatum only slightly less. Low levels of radioactivity were observed in the cerebellum. The uptake in the cerebral cortex was just above that in the cerebellum but dis-



**Fig. 3.** Time-activity curves for  $[^{11}\text{C}]\text{DAPP}$  and  $[^{11}\text{C}]\text{DASB}$  from the subject in Fig. 2



**Fig. 4.** Tissue to cerebellum ratios ( $\pm$  SD) in our six subjects

tinguishable in all cases. The washout of nonspecific activity from the cerebellum was similar for both compounds. The metabolism of both tracers is rapid, with approximately 50% of the parent compound remaining in plasma at 20 min post injection. The metabolites are mainly polar as determined by reverse-phase HPLC. The SERT-rich tissue to cerebellum ratios of radioactivity are shown in Fig. 4.

In all three subjects who received the oral dose of 40 mg of citalopram 3 h prior to the injection of  $[^{11}\text{C}]\text{DASB}$ , there was an  $80\% \pm 5\%$  reduction in specific binding in all SERT-rich tissues.

## Discussion

The results of the PET studies in our six normal subjects indicate that both  $[^{11}\text{C}]\text{DAPP}$  and  $[^{11}\text{C}]\text{DASB}$  bind reversibly to SERT in the living human brain. Both radiotracers have preferential uptake in SERT-rich regions and the rank order of uptake correlates well with regional SERT densities measured post-mortem in human brains using the selective reuptake inhibitor paroxetine [7, 8].

The tissue to cerebellum ratios in SERT-rich regions are higher than those of previous putative SERT radioligands. These ratios are estimates of the total-to-nonspecific binding since the cerebellum is relatively devoid of SERT sites [7, 8]. These high signal-to-noise ratios and the favorable kinetics of  $[^{11}\text{C}]\text{DAPP}$  and  $[^{11}\text{C}]\text{DASB}$  should facilitate the quantitation of regional SERT densities in pathological states or following drug treatment.

The specificity of  $[^{11}\text{C}]\text{DASB}$  binding to SERT was demonstrated by the marked decreases in brain radioactivity induced by the citalopram blockade in SERT-rich tissues. After pretreatment with an oral dose of 40 mg of citalopram we observed an 80% reduction in specific binding in SERT-rich regions. This dose is at the upper range of the usual clinical daily oral doses (20–40 mg) for the treatment of depression. This inhibition of specific binding indicates that this radioligand is well suited for occupancy studies of the SERT by serotonin reuptake inhibitors. It will provide a useful tool for the investigation of current and future drug therapies in depression and other psychiatric disorders.

In conclusion, both substituted phenylthiobenzylamines have highly suitable characteristics for imaging SERT in the living brain. Overall,  $[^{11}\text{C}]\text{DASB}$  has slightly higher specific binding than  $[^{11}\text{C}]\text{DAPP}$  (Fig. 4). Motivated by these encouraging preliminary results, we are continuing our work to fully characterize the pharmacokinetics of this promising new probe for the human SERT and to initiate PET studies in patients with psychiatric and neurological disorders.

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