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Carbon-11 *N*-methyl alkylation of L-368,899 and in vivo PET imaging investigations for neural oxytocin receptors

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ABSTRACT

Compound L-368,899 was successfully alkylated with [^{11}C]iodomethane to generate the oxytocin receptor selective (2*R*)-2-amino-*N*-((2*S*)-7,7-dimethyl-1-(((4-(*o*-tolyl)piperazin-1-yl)sulfonyl)methyl) bicyclo[2.2.1]heptan-2-yl)-*N*-[^{11}C]methyl-3-(methylsulfonyl)propanamide ([^{11}C]**1**) with very high radiochemical purity and high specific activity. PET imaging studies were performed with [^{11}C]**1** to investigate brain penetration and oxytocin receptor uptake using rat and cynomolgus monkey models. For rat baseline scans, brain penetration was observed with [^{11}C]**1**, but no specific uptake could be distinguished in the brain region. By administering a peptide oxytocin receptor selective antagonist for peripheral blocking of oxytocin receptors, the uptake of [^{11}C]**1** was amplified in the rat brain temporarily to enable some visual uptake within the rat brain. A baseline scan of [^{11}C]**1** in a cynomolgus monkey model resulted in no detectable specific uptake in anticipated regions, but activity did accumulate in the choroid plexus.

The oxytocin (OT) system modulates a wide variety of social behaviors, including maternal care, social recognition, and social bonding.[1–3] It has been suggested that alterations in the OT system may contribute to the core social deficits in psychiatric disorders such as autism.[4–8] Furthermore, several studies suggest that intranasal administration of OT or other drugs that stimulate the OT system may be useful for enhancing social functioning in autism spectrum disorders.[9–12] In recent years there has been a significant increase in the number of studies examining the effects of intranasal administration of OT on human behavior.[13] The development of a ligand for positron emission tomography (PET) which has a high affinity and selectivity for the oxytocin receptor (OTR) would provide a non-invasive method for localizing and generating quantitative in vivo data of OTR density, data that can only be obtained currently via post mortem techniques. Furthermore, in a clinical setting, an OTR PET ligand may eventually be useful as a biomarker for psychiatric disorders in which social dysfunction may be attributed to altered neural OTR expression. It could also serve as a pharmacodynamic tool to be used for validation of new OT selective, brain penetrant pharmaceuticals. We have recently reported our first preliminary investigations toward the development of a PET imaging agent which is selective for human OTR.[14] Although all of our candidates were selective for human OTR over human vasopressin receptor subtypes, the blood–brain barrier and/or P-glycoprotein pump prevented our first set of tracers from successfully imaging the neural OTR in vivo in rat models. While we remain interested in further developing these molecules, we became interested in another class of reported molecules bearing high affinity for OTR (Fig. 1), especially since one of the derivatives, L-368,899, was reported to penetrate the blood–brain barrier and used in behavior studies involving the monogamous marmoset model.[15–17] The inhibition constants of L-368,899 and its methylamino (1) derivatives for the rodent OTR and vasopressin receptors are included in Table 1 as was reported by Williams et al., Pettibone et al., and with data obtained from NIH's Psychoactive Drug Screening Program (PDSP) using human cell lines.[18] As

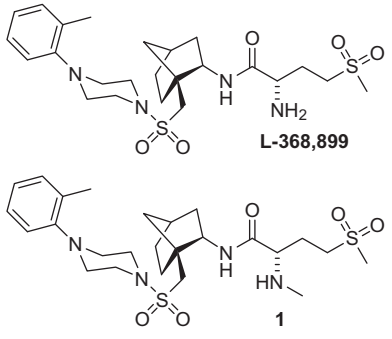

Figure 1. Reported compounds developed through Merck which are selective for OTR.

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Table 1
Inhibition constants of L-368,899 and **1** for OT and vasopressin receptors and their binding affinity (K_i in nM) for the respective human receptors

Compound	Rodent[a]			Human[b]			
	IC_{50} OT	IC_{50} V1a	IC_{50} V2	K_i OTR	K_i V1a	K_i V1b	K_i V2
L-368,899	8.9	370	570	13	180	—	590
1	9.2	320	350	5.2	620	1300	181

[a] The inhibition constants are measured in nanomolar values and were derived from rat uterus (OT), rat liver (V1a), and rat kidney (V2) as previously reported by Williams et al (Ref. 15).

[b] The K_i values for L-368,899 are as reported by Pettibone et al (Ref. 17) and measured from the human uterus (OTR), liver (V1a), and kidney (V2). K_i values for **1** were measured in nanomolar values and were derived from human receptor assays generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA.

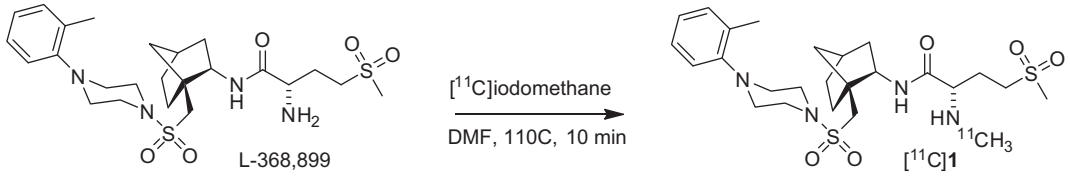
suggested by the tabulated data, these compounds all have desired selectivity and potency for the OTR in rodents for a PET ligand. It should be noted that the methylamino derivative 1 has nearly identical potency and only slightly less selectivity as the brain penetrating L-368,899. Therefore, carbon-11 methyl alkylation of L-368,899 seemed an obvious route to generate an adequate radiotracer to evaluate this class of molecules as PET imaging radiotracers for neural OTR. Since the selectivity of respective ligands may vary from rodent to human species, **1** was investigated via the PDSP for human OTR and vasopressin receptor affinity. The data suggests increased potency for human receptors while the selectivity remains adequate and with slight improvement across species, which encouraged us to proceed in the development of its positron emitting analogue. We report here the carbon-11 methyl alkylation of L-368,899 and its in vivo evaluation via PET imaging of female rat and male cynomolgus monkey models.

The labeling reaction used to generate [^{11}C]**1** is outlined in Scheme 1. Both L-368,899 and the cold standard of **1** were synthesized in our laboratories using the previously reported procedures.[15] Although L-368,899 is commercially available as a hydrochloride salt, the free base is the desired precursor due to the possible formation of [^{11}C]chloromethane with the presence of chloride ions in the reaction mixture. After multiple trials, our standard dose production of [^{11}C]**1** was conducted by preparing a mixture of 2 mg of L-368,899 precursor in 200 μl DMF in a small 1 ml V-vial and cooling it to 0 °C in an ice bath. A stream of helium and [^{11}C]iodomethane generated from a GE PETtrace MeI Microlab was then bubbled through the mixture. Once activity maxed, the vial was placed in a preheated 110 °C oil bath for 10 min. The vial was then cooled quickly in its original ice bath, diluted with 500 μl of 50:50:0.1 ethanol: water: triethylamine (HPLC solvent), and injected on an 19 × 100 mm Waters XTerra RP18 prep HPLC column with a 5 μm bed. Using the above mentioned HPLC solvent, [^{11}C]**1** was eluted with a retention time of 16 m 20 s at 6 ml/min. After being diluted 500% with water, the eluted fractions containing [^{11}C]**1** were loaded on a waters tC-18 cartridge, rinsed with 30 ml saline, and product was eluted off with 1.5 ml ethanol into a sterile vial containing 13.5 ml saline. The dose was then passed through 1 μm and 0.2 μm filters using high argon pressure. A dose would normally be prepared in 65 min from EOB and result in approximately 5% uncorrected yields of [^{11}C]**1** with a specific activity of 1 Ci/μmol as calculated from a calibration curve. Radiochemical purity was >99% and no trace of the starting material (which elutes just before the product) was observed.

To estimate the potential of [^{11}C]**1** for crossing the blood–brain barrier, its log$P_{7.4}$ was measured and determined to be 2.62 using a previously reported method.[19] This value was deemed satisfactory to achieve brain penetration.

To validate the functionality of [^{11}C]**1** as a PET tracer for imaging neural OTR, the compound was investigated in vivo via PET imaging using Sprague-Dawley rats (n = 4; weighing 180–200 g). The rats were subcutaneously injected with estradiol benzoate (10–12 μg in 0.2 ml sesame oil) once a day for 3 days prior to the day of the scan to maximize OTR expression in the hypothalamus. After being anesthetized with a ketamine/xylazine cocktail (100 and 10 mg/kg, respectively, via ip) the rats were affixed with a tail vein catheter and strapped in the prone position on the microPET bed. A 10 min transmission scan was performed using a cobalt-57 source to provide both attenuation correction and anatomical details. At exactly 30 s after emission scanning began, 500 μCi of [^{11}C]**1** was injected into the subjects and a 45 min emission scan was performed. Animals were euthanized with compressed CO_2 following the scan.

The images generated from the sum of the 45 min baseline scan are shown in Figure 2. After thorough examination of the data at various time points, we concluded there was no specific uptake of [^{11}C]**1** within the rat brain during the baseline scan despite some observed penetration into the brain. The similar patterns of the time–activity curves generated for both the brain region and muscle region suggests the possibility of brain penetration through the entirety of the scan (Fig. 4a), but there is clearly lower overall uptake within the brain region. Significant uptake was observed in the vicinity of the pituitary gland as indicated by these time–activity curves and the arrows in Figure 2. To confirm if this uptake was specific to oxytocin and in an attempt to amplify the amount of [^{11}C]**1** reaching the brain region, periphery blocking studies were performed by injecting 5 mg/kg of desGly–NH_2,d$(CH_2)_5$[Tyr(Me)^2, Thr^4] ornithine vasotocin (OVT), an oxytocin selective peptide antagonist, 10 min prior to the injection of [^{11}C]**1**.[20] There were no visual changes observed in the pituitary uptake after administration of OVT suggesting uptake in the pituitary was not blocked. The time–activity curves shown in Figure 3b clearly confirm that the uptake in the pituitary was still significant. This suggests the observed uptake of [^{11}C]**1** within the pituitary is not due to specific OTR binding. This could be attributed to affinity to another receptor as the structure of **1** does contain a known pharmacophore in its structure, 4-(2-methylphenyl)piperazine. These time–activity curves also suggest the overall uptake in the muscle and pituitary was slightly lower when the periphery OTR was blocked with OVT. Interestingly, when OVT was administered, the uptake within the brain increased during the first 10 min of the scan. To determine whether there was improved localization of [^{11}C]**1** in specific areas during this time frame, the sum of the first 10 min of the scan were examined. As can be seen in Figure 4, uptake appears in regions



Scheme 1. Radiolabeling reaction of L-336,899 with [^{11}C]iodomethane.