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High-yielding $^{18}$F radiosynthesis of a novel oxytocin receptor tracer, a probe for nose-to-brain oxytocin uptake in vivo†

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A novel $^{18}$F labelled peptide tracer for PET imaging of oxytocin receptor has been accessed through a high radiochemical yield approach. This tracer showed comparable affinity and higher selectivity and stability compared to oxytocin, and was used to demonstrate direct nose-to-brain uptake following intranasal administration, a common yet controversial delivery route for oxytocin-based therapeutics.

The oxytocin (OT) system comprises the peptide hormone OT and the transmembrane G-protein coupled oxytocin receptor (OTR), which is expressed in organs including the uterus, heart, kidney and brain. OT, the endogenous ligand of OTR, is produced within the hypothalamus and stored in the posterior pituitary gland until release after stimulation such as hugging, breast-feeding and sexual intercourse. Consequently, OT has been termed the “love hormone”, as it facilitates reproduction, breast-feeding and sexual intercourse. The mechanism of putative OT nose-to-brain delivery is poorly understood, but is conjectured to occur either directly through olfactory and nerve fibre routes, or indirectly via absorption into blood capillaries. Currently no studies have quantified brain uptake or distribution of OT after i.n. administration, since measurements of this kind are typically invasive. We considered that a functional in vivo OT receptor expression tomography (PET) tracer could provide a useful biological tool for mapping OTR expression and enable a sensitive, non-invasive route to quantify brain uptake and distribution via i.n. administration of OT-based therapeutics.

$^{18}$F ($T_{1/2}$ 100 min, $\beta^+$ 0.635 MeV 97%) is the most frequently used isotope for PET owing to its almost ideal imaging properties, including low positron energy, an extended half-life and lack of side emissions. Fluoride labelling strategies most widely applied for PET tracer synthesis of biomolecules use nucleophilic or electrophilic chemistries to attach $^{18}$F to carbon as a “prosthetic group”, with these reagents requiring prior synthesis. In contrast, direct radio-labelling significantly simplifies and shortens radiosynthesis by late-stage incorporation of $^{18}$F, for example using NODA-derived chelation cages to irreversibly bind Al$^{18}$F in high yield. The core architecture of our tracer was design to maintain selective receptor binding while allowing direct [$^{18}$F]AlF labelling (Fig. 1A–D). Consequently the tracer core was based on dLVT, an OT analogue with reported to enhance OT stability in vivo.$^{16,17}$

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lysine 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (ivdde) allowed NODA conjugation on solid support via a methyleneacetic acid (MPAA) linker. Following conjugation, full deprotection and cleavage from the resin afforded crude peptide which was purified by RP-LCMS. Cyclisation was performed through DMSO-mediated oxidation, affording final product in 22% overall yield. To enable molar activity and Kᵦ determination, cold (non-radioactive) AlF-NODA-dLVT was also synthesised, alongside a library of OT peptide analogues (Table 1) to determine a structure–activity relationship (SAR) for OTR binding. Full sequences for these peptides can be found in ESL† Table S1.

Development of a highly specific ligand for OTR is challenging since endogenous OT and arginine vasopressin (AVP) peptides and their corresponding receptors share a related structure. OT analogues thus have the potential to bind to multiple members of the OT/AVP receptor family comprising the OTR and three AVP receptor subtypes, and therefore we determined AlF-NODA-dLVT’s binding profile across these potential targets.

OT analogues were screened in scintillation proximity assays that displace [1H]OT or [1H]AVP from the corresponding receptors. All peptides screened, including in a reduced state, preferentially bound human OTR with nanomolar affinity (Table 1). In line with previous studies, conjugation of a large group to position 8 afforded analogues with increased selectivity and improved affinity towards OTR† (approximately 60-fold for AlF-NODA-dLVT, and 7-fold for dLVT; Table 1 and ESL† Fig. S1). AlF-NODA-dLVT also demonstrated enhanced selectivity (approximately 60-fold) towards OTR compared to natural OT (approximately 16-fold). Remarkably, selectivity was improved when the tracer was in a reduced state (>240-fold), with similar affinity towards the OTR.

Encouraged by these results, a radiofluorination method for the preparation of [18F]AlF-NODA-dLVT was investigated. In the course of developing this reaction we identified a substantial loss of activity during concentration of the radionuclide. Following the reported procedure, [18F]fluoride is trapped on an anion exchange resin precondensed with sodium acetate buffer, and [18F] is concentrated into 100 µL fractions through elution with saline, at an optimal pH for subsequent peptide labelling.13 Under these conditions, 0.9% saline produced a broad Na18F elution profile (Fig. 2A).

Consequently only 53 ± 3% Na18F was isolated in the highest activity fraction. To overcome this loss, we narrowed the elution profile using higher saline concentration, and found that 20% saline increased isolation yields, allowing 76 ± 5% of total [18F] activity to be eluted in the highest activity fraction (Fig. 2A). These values are improved over previous reports that compensate for this loss by eluting Na18F in larger volumes from 2-fold13 to 5-fold.18 Dilution limits the activity used for radiolabelling since peptide concentration heavily influences RCY, resulting in overall RCY of 8.2% (from end of bombardment (EOB)), despite high radionuclide incorporation and purification (86%).18 In further optimisation experiments, radiofluorination was performed following 18F elution with the highest activity fraction eluted with either 0.9% or 20% saline, and with various molar equivalents of AlCl3 compared to peptide precursor. 20% saline consistently improved radiochemical conversion (RCC) compared to 0.9% saline under analogous conditions (Fig. 2B and C and ESL† Fig. S2). For example, with 10 nmol aluminium chloride a 1.5-fold increase in RCC of [18F]AlF-NODA-dLVT was observed with 20% saline (Fig. 2C). These yields are similar to those obtained when organic co-solvent is added to the mixture,19,20 and may offer an alternative method when DMSO or MeOH is not suitable for the precursor, for example for peptides sensitive to oxidation and for larger biomolecules. This marked improvement was translatable to an unrelated model peptide, NODA-GFAG, although the improvement in RCC was less pronounced (ESI† Fig. S3). Increased molar ratios of aluminium chloride to peptide gave higher conversion for [18F]AlF-NODA-dLVT, however, we found that lower concentrations of aluminium chloride were preferred to yield higher molar radioactivity in the isolated tracer; 60 nmol of aluminium chloride (1:1.2 aluminium chloride:peptide molar ratio) afforded [18F]AlF-NODA-dLVT in RCC of 90 ± 3% with a molar

Table 1 Kᵦ values (nM) of OT and peptide analogues for human OTR and vasopressin receptors

| Peptide       | OTR  | V₁a  | V₁b  | V₂   |
|---------------|------|------|------|------|
| OTc           | 1.2 ± 0.3 | 20 ± 3 | >6000 | >6000 |
| dOTc          | 1.6 ± 1.3 | 388 ± 157 | nd  | >6000 |
| dOTc          | 1.2 ± 0.9 | 362 ± 124 | nd  | >6000 |
| dLVTc         | 3.4 ± 1.0 | 24 ± 5  | nd  | 4844 ± 1670 |
| dLVTc         | 6.3 ± 3.6 | 31 ± 9  | nd  | 5819 ± 1704 |
| AlF-NODA-dLVTc| 2.6 ± 1.7 | 157 ± 26 | >6000 | >6000 |
| AlF-NODA-dLVTc| 3.3 ± 1.5 | 821 ± 217 | nd  | >6000 |

Values represent mean values of four different experiments, each performed in duplicate, with standard deviation. Key: c = cyclised; oc = open chain; OTR = oxytocin receptor.

Fig. 2 Optimisation of 18F radiolabelling of NODA-dLVT. (A) Elution profile of [18F]NaF with 0.9% (dashed) or 20% (solid) saline from anion exchange resin in 100 µL fractions, mean of 5 samples for each condition. Radiochemical conversion (RCC) for [18F]AlF labelling of NODA-dLVT (72 nmol) using 0.9% (B) or 20% (C) saline with different molar equivalents of aluminium chloride at 105 °C for 15 min. Values represent mean of 3 samples.
radioactivity of 39 ± 12 GBq μmol⁻¹, whereas 20 nmol (i.e. 1:3.6 aluminium chloride:peptide) afforded the tracer in 71 ± 5% RCC, but with a molar activity of 66 ± 8% GBq μmol⁻¹. Using optimised conditions, [⁸⁹F]AlF-NODA-dLVT was synthesised reproducibly and formulated within 70 min, with isolated RCY of 36% (decay corrected, from EOB), radiochemical purity (RCP) ≥ 95% and molar activity ≥ 58 GBq μmol⁻¹ (ESI† Fig. S4). Notably, the RCY reported here is significantly improved from previous reports using a similar labelling approach, which range from 8.2–24% (from EOB).¹⁸,²⁰–²²

To justify use of [⁸⁹F]NODA-dLVT in vivo, tracer lipophilicity and specific binding were determined through a standard shake flask assay and rat brain autoradiography, respectively. A log D₇,₄ of −0.54 ± 0.1 was established for [⁸⁹F]NODA-dLVT indicating a hydrophilic profile and potential for low non-specific binding. To investigate the feasibility of [⁸⁹F]NODA-dLVT for nervous system imaging, tracer was incubated at a concentration of 3 nM, either alone or in the presence of excess OT (10 μM) with brain tissue cut in the sagittal plane to determine total vs. non-specific binding (Fig. 3). This enabled calculation of % specific binding of [⁸⁹F]NODA-dLVT to regions of brain tissue (ESI† Section 4.2). [⁸⁹F]NODA-dLVT specifically bound regions known to express appreciable levels of OTR (Fig. 3),₂³–₂₆ including hippocampus (CA1: 53 ± 4%; subiculum, S: 60 ± 1%), central amygdala nucleus (ca: 56 ± 1%), olfactory systems (olfactory tuberle, Tu: 53 ± 5%; anterior olfactory nucleus, AO: 38 ± 2%), acumens nuclei (Acb: 43 ± 4%), and cortical regions (visual cortex, V1: 61 ± 4%; motor cortex, MC: 42 ± 4%). To investigate the plausibility of i.n. administration for OT-like therapies, given the similar structure and hydrophilic profiles and the extended apparent availability of OT following i.n. administration supports a longer timeframe for this study.²⁷,²⁸ Distribution was determined through gamma counting of radioactivity in isolated tissue. A large portion underwent excretion via kidneys (i.v. 4.6 ± 0.4%ID per g; i.n. 1.31 ± 0.7%ID per g) and urine (i.v. 2.5 ± 1.2%ID per g; i.n. 7.7 ± 2.2%ID per g), as expected from the hydrophilic character of the tracer, similar to previous results found with radiolabelled OT given i.v. (Fig. 4A).²⁹

[⁸⁹F]AlF-NODA-dLVT uptake in brain parenchyma was increased by i.n. administration, however exposure remained low (i.v. 0.05 ± 0.03%ID per g; i.n. 0.11 ± 0.03%ID per g, P = 0.044; Fig. 4B), which was mirrored in nanoPET scans over the course of 1.5 h (ESI† Fig. S6). Accounting for plasma volume contributions from the 5.2% of the brain assumed to be capillaries, the brain permeable material in this study is approximately 4.7 × 10⁻²%ID per g by i.v. administration.³⁰ A significant proportion was detected in the olfactory bulb following i.n. administration compared to the i.v. route (i.n. 2.30 ± 1.8 cf. i.v. 0.05 ± 0.02%ID per g, P = 0.026), implying nose-to-brain transfer via nerve fibres, as opposed to blood-to-brain transfer from indirect diffusion into the bloodstream.³¹,³² In addition, a large portion was detected in the cerebellum following i.n. administration in relation to parenchyma uptake (i.n. 0.31 ± 0.26; 2.8-fold cf. i.v. 0.05 ± 0.01%ID per g; 1.1-fold).

In summary, we have developed a novel OTR tracer NODA-dLVT radiolabelled with Al⁸⁹F, that binds OTR with nanomolar affinity, and exhibits an enhanced selectivity profile compared to OT in both oxidised and reduced states. During the radiosynthesis, we incorporated an improved methodology for aqueous Al⁸⁹F fluoride recovery which significantly enhances RCY, and is likely to be widely applicable to other radiotracers. [⁸⁹F]AlF-NODA-dLVT administered by i.n. and i.v. routes demonstrated low brain parenchyma uptake. Yet, i.n. administration gave a significant signal in the olfactory bulbs suggesting “direct nose-to-brain” uptake. However, it is important to note that the tracer was unable to penetrate...
deeper regions of brain tissue for specific binding during the timescale studied. Consequently, our findings suggest that the i.n. route is not significantly more efficient than i.v. for OT-based tracers. While i.n. administration lead to modest parenchyma delivery, this increase may be relevant for changes in behavioural and neural responses, as suggested in other studies. In addition, the presence of radioactivity in the cerebellum may suggest peptide degradation following absorption, and improve-ment in the stability and selectivity of OT agonists may prove beneficial for clinical efficacy.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

1 G. Gimpf and F. Fahrenholz, Physiol. Rev., 2001, 81, 629–683.
2 C. A. Pedersen, C. M. Gibson, S. W. Rau, K. Salimi, K. L. Smedley, R. L. Casey, J. Leserman, L. F. Jarssog and D. L. Penn, Schizophr. Res., 2011, 132, 50–53.
3 O. Penagarikano, M. T. Lazaro, X. H. Lu, A. Gordon, H. Dong, H. A. Lam, E. Peles, N. T. Maidment, N. P. Murphy, X. W. Yang, P. Golshani and D. H. Geschwind, Sci. Transl. Med., 2015, 7, 1–12.
4 E. Hollander, S. Novotny, M. Hanratty, R. Yaffe, C. M. DeCaria, B. R. Aronowizt and S. Mosovich, Neuropsychopharmacology, 2003, 28, 193–198.
5 D. S. Quinatana, A. J. Guastella, L. T. Westley and O. A. Andreassen, Mol. Psychiatry, 2016, 21, 29–38.
6 E. Andari, J. R. Duhamel, T. Zalla, E. Herbrecht, M. Leboyer and A. Sirigu, Proc. Natl. Acad. Sci. U. S. A., 2010, 107, 4389–4394.
7 T. Watanabe, O. Abe, H. Kuwabara, N. Yahata, Y. Takano, N. Iwashiro, T. Natsubori, Y. Aoki, H. Takao, Y. Kawakubo, Y. Kamio, N. Kato, Y. Miyashita, K. Kasai and H. Yamasue, JAMA Psychiatry, 2014, 71, 166–175.
8 A. J. Guastella, K. M. Gray, N. J. Rinehart, G. A. Alvare, B. J. Tonge, I. B. Hickie, C. M. Keating, C. Cacciotti-Sajia and S. L. Einfeld, J. Child Psychol. Psychiatry, 2015, 56, 444–452.
9 M. R. Dadds, E. MacDonald, A. Cauchi, K. Williams, F. Levy and J. Brennan, J. Autism Dev. Disord., 2014, 44, 521–531.
10 C. Dufes, J. C. Olivier, F. Gaillard, A. Gaillard, W. Court and J. M. Muller, Int. J. Pharm., 2003, 255, 87–97.
11 O. Jacobson, D. O. Kiesewetter and X. Chen, Bioconjuge Chem., 2015, 26, 1–18.
12 W. J. McBride, R. M. Sharkey, H. Karacay, C. A. D’Souza, E. A. Rossi, P. Laverman, C. H. Chang, O. C. Boerman and D. M. Goldenberg, J. Nucl. Med., 2009, 50, 991–998.
13 W. J. McBride, C. A. D’Souza, R. M. Sharkey, H. Karacay, E. A. Rossi, C. H. Chang and D. M. Goldenberg, Bioconjuge Chem., 2010, 21, 1331–1340.
14 S. Terrillon, L. L. Cheng, S. Stoev, B. Mouillae, C. Barberis, M. Manning and T. Durrouts, J. Med. Chem., 2002, 45, 2579–2588.
15 B. Chini, M. Chinol, P. Cassoni, S. Papi, A. Reversi, T. Marrocco, G. Paganelli, M. Manning and G. Bussolati, Br. J. Cancer, 2003, 89, 930–936.
16 D. Gazis, Proc. Soc. Exp. Biol. Med., 1978, 158, 663–665.
17 K. S. Sharma, G. Durand, F. Giusti, B. Olivier, A.-S. Fabiano, P. Bassacceo, T. Dahnman, C. Ebel, J.-L. Popot and B. Pucci, Langmuir, 2008, 24, 13581–13590.
18 N. Malik, B. Zlatopolyski, H.-J. Machulla, S. N. Reske and C. Solbach, J. Labelled Compd. Radiopharm., 2012, 55, 320–325.
19 C. A. D’Souza, W. J. McBride, R. M. Sharkey, L. J. Todaro and D. M. Goldenberg, Bioconjuge Chem., 2011, 22, 1793–1803.
20 S. Heskamp, P. Laverman, D. Rosik, F. Boschetti, W. T. van der Graaf, W. J. Oyen, H. W. van Laarhoven, V. Tolmachev and O. C. Boerman, J. Nucl. Med., 2012, 53, 146–153.
21 S. H. Hausner, N. Bauer and J. L. Sutcliffe, Nucl. Med. Biol., 2014, 41, 43–50.
22 S. Liu, H. Liu, H. Jiang, Y. Xu, H. Zhang and Z. Cheng, Eur. J. Nucl. Med. Mol. Imaging, 2011, 38, 1732–1741.
23 M. J. Freund-Mercier, M. E. Stoeckel, J. M. Palacios, A. Pazos, J. M. Reichhart, A. Porte and P. Richard, Neuroscience, 1987, 20, 599–614.
24 E. Tribollet, C. Barberis, S. Jard, M. Dubois-Dauphin and J. J. Dreifuss, Brain Res., 1988, 442, 105–118.
25 E. R. De Kloet, F. Rotteveel, T. A. M. Voorhuis and M. Terlou, Eur. J. Pharmacol., 1985, 110, 113–119.
26 E. Tribollet, S. Audigier, M. Dubois-Dauphin and J. J. Dreifuss, Brain Res., 1990, 511, 129–140.
27 O. Weismann, O. Zagoory-Sharon and R. Feldman, Psychoneuroendo- crinology, 2012, 37, 1582–1586.
28 I. D. Neumann, R. Maloumby, D. I. Beiderbeck, M. Lukas and R. Landgraf, Psychoneuroendocrinology, 2013, 38, 1985–1993.
29 I. Sjöholm and G. Rydén, Acta Endocrinol., 1969, 61, 432–440.
30 P. Cumming, A. Ase, M. Diksic, J. Harrison, D. Jolly, H. Kuwabara, C. LaliBerté and A. Gjedde, Biochem. Pharmacol., 1995, 50, 943–946.
31 R. G. Thorne, G. J. Pronk, V. Padmanabhan and W. H. Frey 2nd, Neuroscience, 2004, 127, 481–496.
32 S. R. Alcala-Barraza, M. S. Lee, L. R. Hanson, A. A. McDonald, W. H. Frey, 2nd and L. K. McLoon, J. Drug Targeting, 2010, 18, 179–190.