BM3h-8C8 Mutant of the heme domain of the bacterial cytochrome P450-BM3 (*Bacillus megaterium*)

BM3h-8C8

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Chemical name:	BM3h-8C8 Mutant of the heme domain of the bacterial cytochrome P450-BM3 (*Bacillus megaterium*)	
Abbreviated name:	BM3h-8C8	
Synonym:		
Agent Category:	Proteins	
Target:	Dopamine	
Target Category:	Others (neurotransmitter)	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	Fe	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

BM3h-8C8 is a mutant of the heme domain of the bacterial cytochrome P450-BM3 (BM3h) from *Bacillus megaterium*, and it was generated by Shapiro et al. with directed evolution of BM3h for magnetic resonance imaging (MRI) of dopamine (1).

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Dopamine is a catecholamine neurotransmitter produced mainly by the medulla of the adrenal glands and the nervous tissues, including the substantia nigra and the ventral tegmental area (2, 3). In neurons, synthetic dopamine is packaged into vesicles and then released into the synapse in response to presynaptic action potentials. Dopamine also acts as a neurohormone, inhibiting prolactin release from the pituitary gland (4). Dopamine is of further significance because of its roles in learning, reward, and motor coordination, and because of the dysfunction of dopaminergic systems underlying addiction and neurodegenerative diseases (5-8). Radionuclides have been investigated for imaging dopamine and its receptors *in vivo*, but both positron emission tomography and single-photon emission tomography have low spatial and temporal resolution in spite of high sensitivity (9-12).

Shapiro et al. developed a dopamine sensor for MRI using directed evolution of BM3h (1). BM3h, a 53-kDa moiety, contains a single iron(III) atom bound to a hemin prosthetic group (13). The iron ion is the site of oxygen binding; when oxygen is not bound, a water molecule fills the site with a weak bond. Interaction of the heme iron with exchanging water molecules at this axial site could promote T_1 relaxation and thus modulate MRI contrast (1). Studies by Shapiro et al. revealed an r_1 value (T_1 relaxivity; $1/T1$) of 1.23 ± 0.07 $mM^{-1}s^{-1}$ for BM3h in phosphate-buffered saline without ligands. Addition of a saturating quantity of arachidonic acid, a natural BM3h substrate, resulted in an r_1 value of 0.42 ± 0.05 $mM^{-1}s^{-1}$. This ligand-induced decrease in relaxivity, arising from the displacement of water molecules at the BM3h heme, enabled quantitative sensing of arachidonic acid with MRI. Further studies by Shapiro et al. showed that addition of 1 mM dopamine to BM3h induced a decrease in the r_1 value to 0.76 ± 0.03 $mM^{-1}s^{-1}$, indicating that dopamine directly replaces water as an axial metal ligand in the BM3h substrate-binding pocket. The apparent dissociation constant (K_d) for arachidonic acid was 6.8 ± 0.5 μM, and the K_d for dopamine was 990 ± 110 μM. These results suggest that BM3h could serve as a platform for molecular sensor engineering (1, 14, 15). Using the platform of BM3h, Shapiro et al. generated a series of MRI sensors for dopamine by decreasing the affinity for arachidonic acid, increasing the affinity for dopamine, and enhancing the relaxivity changes upon ligand binding with the directed evolution technique (1). Directed evolution is a molecular engineering method that utilizes successive rounds of mutagenesis and selection to generate proteins with novel functionality, starting from a molecule with some of the desired properties of the end product (14, 15). BM3h-8C8 is one of the BM3h-based mutants with the best combination of relaxivity change, improved affinity for dopamine, and decreased affinity for arachidonic acid (1).

Related Resource Links:

- Chapters for dopamine and dopamine receptor imaging on MICAD
- Bioassays for dopamine and dopamine receptors in PubChem BioAssay
- Dopamine-related articles in Online Mendelian Inheritance in Man (OMIM)

Synthesis

[PubMed]

Shapiro et al. generated BM3h-8C8 using directed evolution of BM3h (1). The wild-type (WT) BM3h with a C-terminal hexahistidine tag was housed in the pCWori vector. Mutant libraries were then produced through error-prone polymerase chain reaction (PCR) with the 5'-GAAACAGGATCCATCGATGCTTAGGAGGTCAT-3' (forward) and 5'-GCTCATGTTTGACAGCTTATCATCG-3' (reverse) primers. Input to each round of screening consisted of a library of BM3h mutants, each with an average of one to two amino acid substitutions, generated from the WT gene or a previously selected mutant. From each screen, eight to ten mutants were selected to determine their affinities for dopamine and arachidonic acid and to ensure the robustness of the ligand-induced changes in r_1. Between the fourth and fifth rounds of evolution, the mutation I366V was introduced *via* overlap extension PCR to improve protein thermostability.

The BM3h-8C8 mutant had an optically determined K_d values of 8.9 ± 0.7 μM for dopamine and 750 ± 140 μM for arachidonic acid. The r_1 values were 1.1 ± 0.1 $mM^{-1}s^{-1}$ in the absence of ligand and 0.17 ± 0.03 $mM^{-1}s^{-1}$ in the presence of 400 μM dopamine. BM3h-8C8 exhibited a dopamine concentration–dependent decrease in T_1-weighted MRI signal (up to 13% with 28.5 μM protein) with an estimated K_d value of 4.9 ± 2.7 μM. Specificity analysis with MRI showed that only dopamine, norepinephrine, and serotonin elicited substantial changes in the T_1 relaxation rate ($1/T_1$) from the potential ligands of norepinephrine, 3,4-dihydroxy-L-phenylalanine, serotonin, glutamate, glycine, γ-aminobutyric acid, acetylcholine, and arachidonic acid. The $1/T_1$ reductions produced by norepinephrine and serotonin were 0.0076 ± 0.0023 s^{-1} and 0.0041 ± 0.0020 s^{-1}, respectively, compared with 0.0182 ± 0.0006 s^{-1} for dopamine. The measured K_d values were 44 ± 3 μM and 80 ± 8 μM for norepinephrine and serotonin, respectively. Only monoamines had affinity for BM3h-8C8, whereas two catechols, epinephrine and 3,4-dihydrophenylacetic acid, both of which lack primary amines, showed no measurable affinity (data not shown). The primary amine in dopamine may serve as an axial ligand to the BM3h heme in the sensor-analyte complexes. BM3h-8C8 is specific for sensing dopamine at concentrations above 10 μM.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Shapiro et al. tested the ability of BM3h-8C8 to measure dopamine discharge from PC12 cells stimulated with extracellular K^+ (54 mM) (1). K^+ is a depolarizing stimulus to release dopamine, and K^+ itself has no effect on r_1 of the BM3h-8C8 (data not shown). PC12 is a cell line derived from a pheochromocytoma of the rat adrenal medulla and is useful as a cellular model of dopaminergic function. T_1-Weighted MRI images (spin echo TE/TR = 10/477 ms) showed that BM3h-8C8 had a 37 ± 2% reduction in r_1 in the supernatant of K^+-stimulated cells relative to Na^+ controls.

Animal Studies

Rodents

[PubMed]

Shapiro et al. tested the ability of BM3h-8C8 to measure dopamine concentrations in intact rats (n = 5-7/group) (1). T_1-Weighted MRI scans were performed continuously during paired infusions of BM3h-8C8 or control WT BM3h with and without dopamine *via* cannulae into the left and right striatum. Dopamine-dependent contrast changes were apparent in images obtained during and after infusion periods. Consistent with results obtained *in vitro*, addition of dopamine dampened the intensity enhancement by ~50% in the striatal regions of interest (P = 0.003). WT BM3h, which has a very low affinity for dopamine (K_d = ~1 mM), did not change the MRI intensity regardless of the presence or absence of dopamine (P = 0.8). Infusion of dopamine alone also produced no noticeable signal changes in an equivalent experiment (data not shown). These results indicate that the dopamine-dependent signal differences require the presence of a micromolar-affinity dopamine sensor and are not explained by physiological or biochemical effects of dopamine itself. On the basis of the relaxivity values measured for BM3h-8C8 *in vitro*, the estimated maximal concentrations were 89 ± 19 μM for BM3h-8C8 and 75 ± 28 μM for dopamine across the striatal regions. Histological analysis showed minimal evidence of toxicity due to these procedures.

Shapiro et al. then tested the ability of BM3h-8C8 to detect the release of endogenous dopamine in the rat brain (n = 6) (1). MRI data were acquired during co-infusion of BM3h-8C8 with elevated concentrations of K^+. As the control, WT BM3h with K^+ was infused into the opposite hemisphere. As additional controls, MRI signal changes in response to K^+ stimulation and to dopamine were also studied in the absence of BM3h-8C8. MRI signal decreased significantly in response to K^+ stimulation ($P < 0.01$), and the decrease was dopamine sensor–dependent. A discernable signal decrease of up to 3% was produced during each K^+ stimulation period. The first K^+ block evoked the largest response (presumably because of partial dopamine depletion over subsequent blocks) and elicited a clear spatiotemporal pattern of MRI signal change from baseline over the course of the stimulation period. The signal difference between low- and high-K^+ stimulation was also significant (0.07%, P = 0.0008) for BM3h-8C8, but not for the control (–0.02%, $P > 0.05$). The results were consistent with the expected suppression of MRI signal by dopamine release under high-K^+ conditions.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Shapiro M.G., Westmeyer G.G., Romero P.A., Szablowski J.O., Kuster B., Shah A., Otey C.R., Langer R., Arnold F.H., Jasanoff A. *Directed evolution of a magnetic resonance imaging contrast agent for noninvasive imaging of dopamine.* Nat Biotechnol. 2010;28(3):264–70. PubMed PMID: 20190737.
2. Missale C., Nash S.R., Robinson S.W., Jaber M., Caron M.G. *Dopamine receptors: from structure to function.* Physiol Rev. 1998;78(1):189–225. PubMed PMID: 9457173.
3. Nitsche M.A., Monte-Silva K., Kuo M.F., Paulus W. *Dopaminergic impact on cortical excitability in humans.* Rev Neurosci. 2010;21(4):289–98. PubMed PMID: 21086761.
4. Samson W.K., Taylor M.M., Baker J.R. *Prolactin-releasing peptides.* Regul Pept. 2003;114(1):1–5. PubMed PMID: 12763633.
5. Rogers R.D. *The roles of dopamine and serotonin in decision making: evidence from pharmacological experiments in humans.* Neuropsychopharmacology. 2011;36(1): 114–32. PubMed PMID: 20881944.
6. Arias-Carrion O., Stamelou M., Murillo-Rodriguez E., Menendez-Gonzalez M., Poppel E. *Dopaminergic reward system: a short integrative review.* Int Arch Med. 2010;3:24. PubMed PMID: 20925949.
7. Bromberg-Martin E.S., Matsumoto M., Hikosaka O. *Dopamine in motivational control: rewarding, aversive, and alerting.* Neuron. 2010;68(5):815–34. PubMed PMID: 21144997.
8. Gardian G., Vecsei L. *Medical treatment of Parkinson's disease: today and the future.* Int J Clin Pharmacol Ther. 2010;48(10):633–42. PubMed PMID: 20875369.
9. Tatsch K. *Positron emission tomography in diagnosis and differential diagnosis of Parkinson's disease.* Neurodegener Dis. 2010;7(5):330–40. PubMed PMID: 20616567.
10. Isaias I.U., Antonini A. *Single-photon emission computed tomography in diagnosis and differential diagnosis of Parkinson's disease.* Neurodegener Dis. 2010;7(5):319–29. PubMed PMID: 20616566.
11. Van Laere K., Varrone A., Booij J., Vander Borght T., Nobili F., Kapucu O.L., Walker Z., Nagren K., Tatsch K., Darcourt J. *EANM procedure guidelines for brain neurotransmission SPECT/PET using dopamine D2 receptor ligands, version 2.* Eur J Nucl Med Mol Imaging. 2010;37(2):434–42. PubMed PMID: 19838704.
12. Varrone A., Halldin C. *Molecular imaging of the dopamine transporter.* J Nucl Med. 2010;51(9):1331–4. PubMed PMID: 20720060.

13. Girvan H.M., Toogood H.S., Littleford R.E., Seward H.E., Smith W.E., Ekanem I.S., Leys D., Cheesman M.R., Munro A.W. *Novel haem co-ordination variants of flavocytochrome P450BM3.* Biochem J. 2009;417(1):65–76. PubMed PMID: 18721129.
14. Glieder A., Farinas E.T., Arnold F.H. *Laboratory evolution of a soluble, self-sufficient, highly active alkane hydroxylase.* Nat Biotechnol. 2002;20(11):1135–9. PubMed PMID: 12368811.
15. Li Q.S., Schwaneberg U., Fischer P., Schmid R.D. *Directed evolution of the fatty-acid hydroxylase P450 BM-3 into an indole-hydroxylating catalyst.* Chemistry. 2000;6(9): 1531–6. PubMed PMID: 10839169.