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This study was partly supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism

DIFFERENCES IN BINDING SITE CONFORMATIONS BETWEEN SUBSTRATE-BOUND AND INHIBITOR-BOUND DOPAMINE (DAT), SEROTONTIN (SERT) AND NORADRENALIN (NET) TRANSPORTERS

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Introduction

The dopamine (DAT), serotonin (SERT) and noradrenalin (NET) transporters are molecular targets for different classes of psychotropic drugs. Cocaine and the SSRI S-citalopram block neuro-transmitter reuptake competitively, but while cocaine is a non-selective reuptake inhibitor, S-citalopram is a selective SERT inhibitor.[1] Site directed mutagenesis studies indicate that cocaine and S-citalopram bind in the same binding site as the substrate.[2-4]

Aim

Investigation of

- Differences in binding site conformations of occluded and open-outward neurotransmitter transporter models.
- Putative binding modes of cocaine and S-citalopram in DAT, NET and SERT.

Methods

The crystal structures of *Aquifex aeolicus* $LeuT_{Aa}$, complexed with a substrate (leucine) in an occluded conformation[5] (PDB id 2a65), and with an inhibitor (tryptophane) in an open-to-out conformation[6] (PDB id 3f3a), were used as templates for molecular modeling of DAT, SERT and NET using the ICM version 3.6.[7] ICMPocketFinder was used to investigate differences in binding site size and shape between the occluded conformation and open-to-out conformation. Cocaine and S-citalopram were docked into DAT, SERT, and NET.

Results & Discussion

The occluded and open-outward DAT models, with substrate binding pockets displayed, are shown in Figure 1. Two ligand binding pockets ("S1"(substrate binding site) and "S2" (putative extracellular binding site) were detected in the occluded conformation, while in the open-to-out conformation the binding sites where "fused" and overlapping, yielding one large ligand binding pocket. A conserved tyrosine and a conserved phenylalanine formed a gate between the putative binding pockets in the occluded conformation, and the distance between these two amino acids was longer in the open to out conformation (Figure 2).

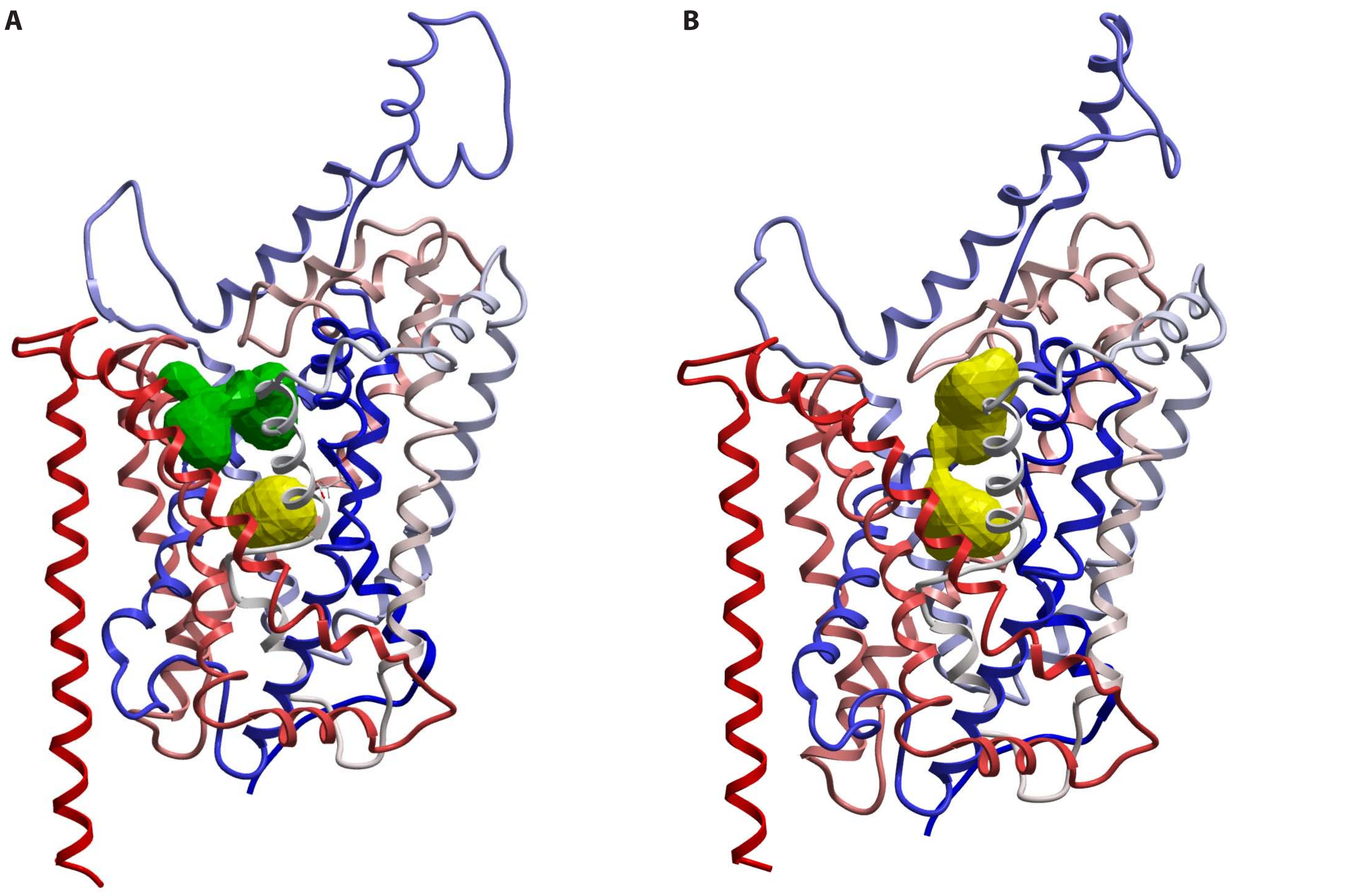

Figure 1

Backbone Cα-traces of occluded DAT model (A) and Open-to-out DAT model (B) viewed in the membrane plane, cytoplasm downwards. Binding sites as detected by ICMPocketFinder are displayed in yellow (S1) and green (S2) (A); and in yellow (B). Coloring of the C-alpha traces of the model is blue via white to red from N-terminal to C-terminal.

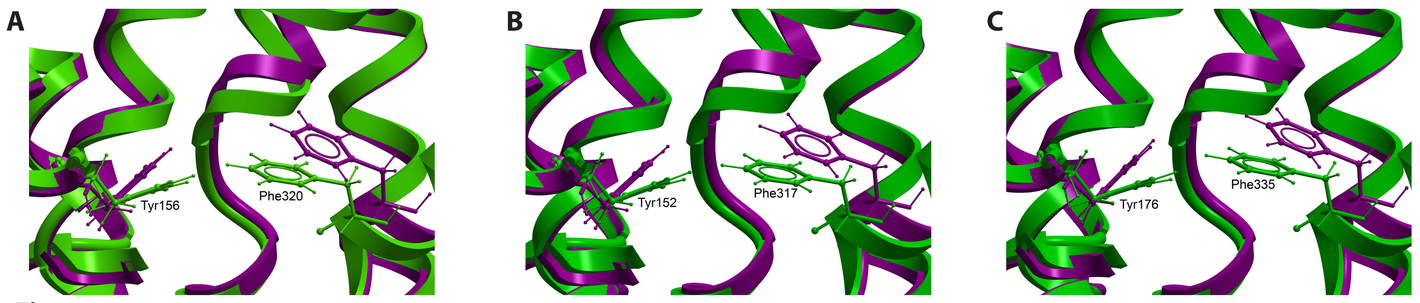

Figure 2

Close-up view of differences in the "Tyrosine-Phenylalanine Gate" between the occluded (green) and open-to-out (purple) DAT (A), NET (B), and SERT (C) models.

Figure 3 shows cocaine and S-citalopram docked into the open-to-out conformations of DAT, NET and SERT. The majority of the amino acids in the substrate binding sites of DAT, NET and SERT consist of conserved amino acids. However, there are differences in TMH1 and TMH8 (DAT: Phe75, Ala423, Gly425; NET: Phe72, Ser420, Gly422; SERT: Tyr95, Thr439, Ala441). In the docking studies, the nitril group of S-citalopram interacted with these three amino acids in each of the transporters. In contrast, the cocaine molecule did not reach this area of TMH8 in the binding site.

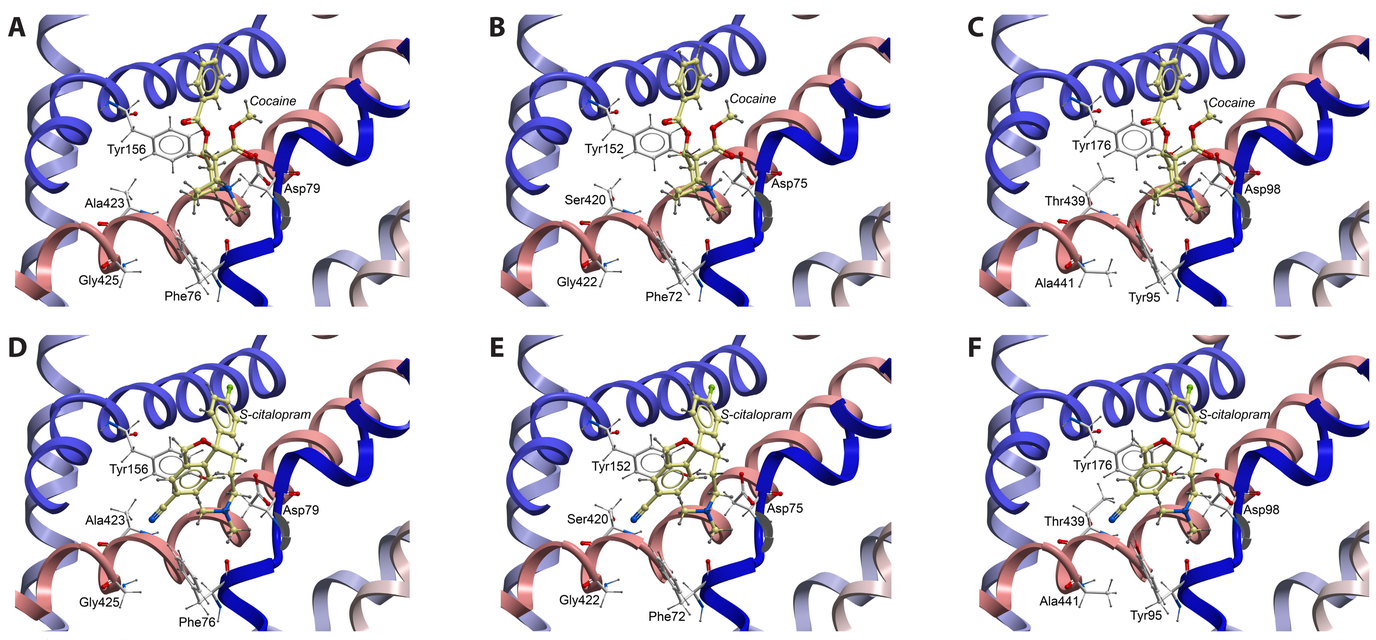

Figure 3

Cocaine and S-citalopram docked into DAT, NET and SERT models. A. Cocaine docked into DAT; B. Cocaine docked into NET; C. Cocaine docked into SERT; D. S-citalopram docked into DAT; E. S-citalopram docked into NET; F. S-citalopram docked into SERT. Non-conserved amino acids in TMH1 and TMH8, and the conserved aspartate in TMH1 and tyrosine in TMH3, are displayed as sticks. Color coding: C-alpha traces blue via white to red from N-terminal to C-terminal; sticks colored according to atom type (C = white (protein), yellow (ligand); H = grey; O = red; N = blue; F = green).

Conclusion

Our studies indicate that

- A "Tyrosine-Phenylalanine Gate" separates the S1 and S2 binding sites in the occluded conformation. When the transporter opens towards the extracellular side, these two amino acids part and the two binding sites merge into one large ligand binding area.
- Non-conserved amino acids in a peripheral region of the binding site (TMH1 and TMH8) may, at least partially, explain differences in binding selectivity between S-citalopram and cocaine.

References

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