Critical domains of C-terminal for targeting of dopamine 1 receptor to spines

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This study describes the role of the C-terminal of dopamine 1 receptor (D1R) for the targeting of the receptor to spines. Three weeks striatal organotypic cultures were Gene Gun transfected with wild type (WT), truncated or mutated D1R. Constructs were tagged with the fluorescent protein "venus" a modification of YFP, and studied under multiphoton confocal microscope. The full length C-terminal of D1R A332-T446 has interaction sites for NMDA subunits NR2A (S417-T446) and NR1A (L387-L416) as well as for the adaptor protein calcyon (S421-I435). Truncations were performed at K339, P392 and L420 leaving a C-terminal of 7 (C-7), 60 (C-60) or 88 amino acids (C-88). Strong WT D1R signal was observed in cell body, dendrites and a large number of spines. C-88 signal was observed in cell body, dendrites and spines. C-60 signal was observed in cell body and a few dendrites but not in spines. C-7 signal was strong in cell body, but could not be detected in dendrites and spines.

We recently reported that D1R are not only constitutively expressed in spines but can also be dynamically recruited to spines following activation of NMDA receptors (Scott et al 2002 PNAS 99(3):1661-4). Following 30 sec exposure to NMDA, WT D1R was observed to move as aggregates from cell body to spines and after 15 minutes the number of D1R positive spines was significantly increased (21%). Analys of putative phosphorylation sites revealed two excellernt consensus site (100% homology) for casein kinase but no good sites (>65%) for PKA and PKC phosphorylation. One of the casein kinase sites, Ser396 was mutated to alanin (S396A) and the mutation construct was tagged with the flouresent protein venus. The S396A was transfected to the organotypical culture and a venus signal was observed in cell body, dendrites and spines to a similar extent as was observed for venus tagged WT D1R. No NMDA induced translocation was however observed in neurons expressing S396A.

To further explore the mechanisms of D1R transport we performed live bleaching experiments. A section of a dendrite was bleached till no signal was recorded. The rate of signal return was recorded before and 6 minutes after NMDA exposure. Results suggest that the return rate before NMDA exposure was exponential and compatible with a diffusion process. In NMDA exposed cells, the return rate was more linear, compatible with the notion that D1R are trapped by the assembly to NMDA receptors.
Conclusion: The D1R C-terminal domains, that bind NMDA receptor and calcyon, is of crucial importance for the proper targeting of D1R to spines.