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Development of Polyclonal Anti-D2 Dopamine Receptor Antibodies to Fusion Proteins: Inhibition of D2 Receptor–G Protein Interaction

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Abstract: Portions of the cDNA encoding the third intracellular loop (i3 loop) of the long and short isoforms of the rat D2 dopamine receptor were subcloned into the vector pNMHUBpoly and expressed in *Escherichia coli* as fusion proteins. The fusion proteins were gel-purified and used to immunize rabbits for the production of polyclonal anti-receptor antisera. The anti-fusion protein antisera recognized synthetic peptides corresponding to segments of the i3 loops of D2 dopamine receptors in a solid-phase radioimmunoassay. Antisera were tested in an immunoprecipitation assay using the reversible D2 antagonist [^{125}I]NCQ 298 and digitonin-solubilized extracts of canine and rat caudate. [^{125}I]-NCQ 298 bound reversibly and with high affinity (K_D = 0.14 n*M*) to receptors in solubilized extracts enriched by chromatography on heparin-agarose. The anti-UBI-D2i3$_L$ and anti-UBI-D2i3$_S$ antisera were able to immunoprecipitate quantitatively D2 dopamine receptors labeled with [^{125}I]NCQ 298 from solubilized rat caudate. The antibodies were tested for their ability to affect the coupling of D2 dopamine receptors to GTP-binding proteins in digitonin-solubilized rat caudate. Both anti-UBI-D2i3$_L$ and anti-UBI-D2i3$_S$ antisera were able to inhibit the high-affinity binding of the agonist *N*-propylnorapomorphine to digitonin-solubilized rat caudate. These findings indicate that the i3 loop of the D2 dopamine receptor is an important determinant for coupling of the G protein. **Key Words:** Anti-(D2 dopamine receptor) antibodies—Fusion proteins—Immunoprecipitation—[^{125}I]NCQ 298—Receptor–G protein coupling. **Boundy V. A. et al.** Development of polyclonal anti-D2 dopamine receptor antibodies to fusion proteins: Inhibition of D2 receptor–G protein interaction. *J. Neurochem.* **60**, 2181–2191 (1993).

Two subtypes of dopamine receptors were identified on the basis of pharmacologic and biochemical criteria (Kebabian and Calne, 1979). The application of molecular biology to the study of these receptors has resulted in the discovery of at least five genes encoding subtypes of dopamine receptors. These genes have now been grouped as D1-like or D2-like according to the pharmacologic profile of the expressed proteins and their nucleotide sequences. Genes have been isolated for two D1-like receptors, the D1 (Dearry et al., 1990; Monsma et al., 1990; Sunahara et al., 1990; Zhou et al., 1990) and the D5 (Sunahara et al., 1991), and for three D2-like receptors, the two alternate splice variants of the D2 gene, D2$_S$ (Bunzow et al., 1988) and D2$_L$ (Dal Toso et al., 1989; Giros et al., 1989; Grandy et al., 1989; Monsma et al., 1989), the D3 (Sokoloff et al., 1990), and the D4 (Van Tol et al., 1991). The D1b receptor (Tiberi et al., 1991) is believed to be the rat homologue of the human D5 receptor.

In the absence of either nucleotide or amino acid sequence information, the generation of antibodies against dopamine receptors was largely unsuccessful. Although several groups were able to raise anti-spiroperidol antibodies, cross-reacting anti-idiotypic antibodies were not obtained (Schreiber et al., 1983; Abbott and Strange, 1986; Luedtke et al., 1988). The cloning of dopamine receptor subtypes presented the possibility of using a molecular approach to the production of anti-receptor antibodies. The expression

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Abbreviations used: [^{125}I]IBZM, (*S*)-3-[^{125}I]iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide; i3 loop, third intracellular loop; [^{125}I]NCQ 298, (*S*)-3-[^{125}I]iodo-2-hydroxy-5,6-dimethoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide; NPA, *N*-propylnorapomorphine; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; pNM, pNMHUBpoly; SDS, sodium dodecyl sulfate; UBI, ubiquitin.