Structural Characterization of Y_1 and Y_2 Receptors for Neuropeptide Y and Peptide YY

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Two receptor subtypes for peptide YY (PYY) and neuropeptide Y (NPY) named Y_1 and Y_2 have been proposed to exist based on pharmacological studies.[1,2] In order to identify and structurally characterize Y_1 and Y_2 receptors we covalently cross-linked [^{125}I-Tyr^{36}]PYY to its receptors. Affinity labeling of the Y_1-receptor protein was analyzed in membranes from the MC-IXC human neuroblastoma cell line. Analysis by SDS-polyacrylamide gel electrophoresis and autoradiography resulted in labeling of a major band centered at M_r = 70,000, and a smaller band at 45,000. The labeled bands were unaffected by reducing agents (FIG. 1). The labeling of the M_r = 45,000 band was greater in the absence of protease inhibitors, suggesting that it is a degradation product of the larger band. For characterization of Y_2 receptors we utilized two different tissues; rat hippocampal membranes and rabbit kidney tubule membranes. In both tissues affinity labeling of PYY binding proteins resulted in labeling of a major protein centered at M_r = 50,000, which was unaffected by reducing agents (FIG. 2). For both the Y_1 and Y_2 receptors the binding to the receptors and the intensity of the labeled bands were inhibited in a parallel dose-dependent manner by increasing concentrations of unlabeled PYY.[3] Efficient labeling of Y_2-receptor proteins was obtained using a number of different homo- and heterobifunctional cross-linking reagents, whereas labeling of Y_1-receptor proteins was obtained only when N-5-azido-2-nitrobenzoyloxysuccinimide (ANBNOS) was employed for cross-linking followed by exposure to UV light. To determine whether the receptors were glycoproteins the detergent solubilized receptor-hormone complexes were exposed to different agarose-coupled lectins. The cross-linked Y_1 and Y_2 receptors were almost completely absorbed by wheat germ agglutinin agarose. The Y_1 receptor was partially absorbed by ricin communis II, and the Y_2 showed partial absorption to concanavalin A. These absorptions were in all cases blocked by the appropriate hapten sugar. These results indicate that the Y_1 and Y_2 subtypes of NPY and PYY receptors, previously characterized pharmacologically, are structurally distinct glycoproteins, not disulfide-linked to other subunits.

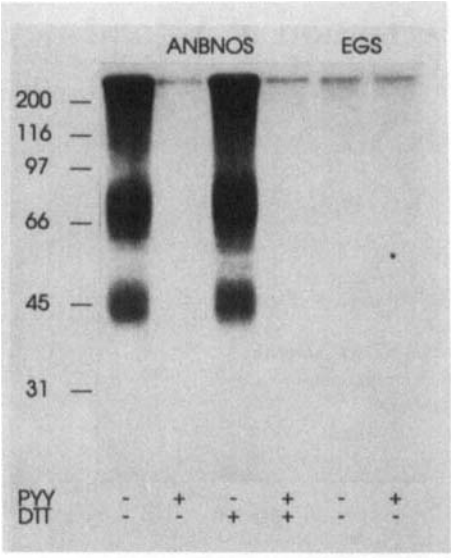

FIGURE 1. Affinity labeling of the Y_1 receptor in a human neuroblastoma cell line, MC-IXC, with radiolabeled PYY. Membranes (200 μg) were incubated with 250 pM radiolabeled PYY, washed and cross-linked with 0.1 mM ANBNOS or 0.5 mM EGS. Reducing agent (50 mM DTT) and unlabeled PYY (1 μM) were added as indicated. (From Sheikh and Williams.[3] Reprinted by permission from the *Journal of Biological Chemistry*.)

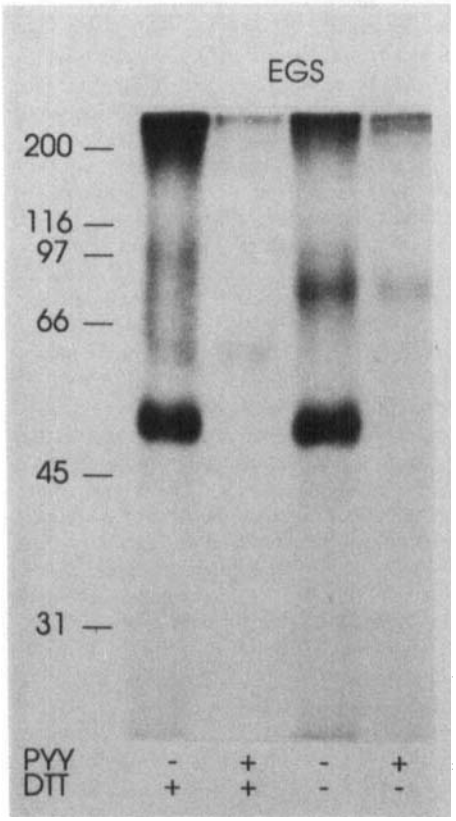

FIGURE 2. Affinity labeling of the Y_2 receptor in rat hippocampus with radiolabeled PYY. Membranes (200 μg/ml) were incubated with 200 pM radiolabeled PYY, washed free of unbound ligand, and cross-linked with 0.5 mM EGS. Unlabeled PYY (1 μM) and dithiothreitol (50 mM) were added as indicated. (From Sheikh and Williams. Reprinted by permission from the *Journal of Biological Chemistry*.)

REFERENCES

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