Analysis of fluorescently labeled substance P analogs: binding, imaging and receptor activation

ABSTRACT

The above results show that fluorescent labeling of SP altered the biological activity and the binding properties of the parent peptide. Oregon Green 488 and BODIPY FL-SP are the most useful fluorophores for labeling SP without affecting its biological activity. Given these results, these probes can now be utilized in further investigations of the mechanisms of SPR function, including receptor localization, internalization and recycling.

INTRODUCTION

Background Substance P (SP) is a peptide neurotransmitter that has been shown to play a role in nociception, smooth muscle control, allergic responses, inflammation and glandular secretion. The amino acid sequence of SP was determined in 1970 after being isolated from mammalian gastrointestinal tract in 1931. SP acts as an agonist at the SP receptor (SPR), known as the neurokinin-1 receptor (NK1) in mammalian systems. SP activation of the SPR, a G-protein-coupled receptor, has a variety of effects in the nervous system including inhibition of the M-type K+ current (IM). The mechanistic properties of the SPR have been extensively studied in receptor-expression systems. When transfected into Chinese hamster ovary (CHO) cells, activation of the SPR results in an increase in intracellular Ca++, accumulation of inositol phosphates and cAMP formation. The recent development of intense, photostable and pH-insensitive fluorophores, along with improvements in optical detection systems, has led to fluorophore labeling of many pharmacological agents. Fluorescent probes can be used to directly label ligand-binding sites without the use of radioactivity or antibodies. Receptor-labeling with a fluorophore-conjugated agonist provides advantages over these conventional methods. The production of antibodies to the receptor protein is not required, as the labeled agonist will bind directly to the receptor. Fluorescence rather than radioactivity can detect the labeled ligand, which provides more information on the localization of the receptor upon SP activation. In addition, labeled agonists can be used in live cells. Therefore, the use of fluorescently labeled agents may allow a more extensive investigation into various receptor functions. Molecular Probes, Inc. (Eugene, OR) has recently synthesized five fluorophore-conjugated analogs of SP as potential tools for direct labeling of the SPR. Alexa 488, BODIPY Fl, fluorescein, Oregon Green 488 and tetramethylrhodamine have been conjugated to the third amino acid of SP, Lys3. The amine group of Lys provides a convenient reactive group for labeling SP without altering its original amino acid sequence. Alexa 488, BODIPY Fl, fluorescein and Oregon Green 488 are green fluorophores, while tetramethylrhodamine is a red fluorophore. We have compared the receptor activation and labeling of five newly synthesized fluorescent analogs of SP. Each of the probes has been tested for: 1) ability to bind to the receptor, 2) receptor activation in both a heterologous expression system and in native neurons and 3) fluorescence labeling of the receptor. This study provides an extensive characterization of the new SP derivatives that will provide a basis for future studies involving the fluorescent conjugates. Oregon Green 488 was found to be the most useful fluorophore for labeling SP without altering its biological activity, whereas Alexa 488 drastically altered the binding and activation of SP.

CONCLUSION

Conclusions The results of our study show that there are dramatic differences in the function of SP labeled at the Lys3 position with different fluorophores. Alexa 488, which was the largest and added the most charged groups to SP, was unable to label the SPR and altered the biological activity of SP in live and cultured cells. Oregon Green 488 and BODIPY Fl, which are smaller and uncharged, appear to be the most useful fluorophores for labeling SP without altering the normal functions of SP at the SPR regardless of the expression system. These results suggest that Oregon Green 488-SP and BODIPY Fl-SP would be the most useful fluorescent ligands for future studies of the SPR.