招待講演

特別講演

IN VITRO AND IN VIVO PHARMACOLOGICAL PROFILE OF THE OP_4 RECEPTOR ANTAGONIST [Nphe^1]NOCICEPTIN(1-13)NH_2: FOCUS ON ITS ANTINOCICEPTIVE PROPERTIES

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Nociceptin/orphanin FQ (NC) and its receptor (OP_4) have been implicated in the control of various central functions (1). OP_4 selective antagonists are needed for clarifying the biological roles of this novel peptide/receptor system and possibly for defining the therapeutic indications of novel drugs interacting with OP_4 sites. [Nphe^1]NC(1-13)NH_2 (Nphe) was identified as the first pure and selective OP_4 antagonist (2). Its antagonist properties have been confirmed in a range of in vitro assays (cAMP accumulation, GTPgammaS binding, bioassays, neurotransmitter release and electrophysiological assays) showing pA_2 values in the range 6.0 – 6.7. In vivo experiments demonstrated that Nphe antagonizes NC effects on locomotor activity, learning and memory, food intake, ibotenate excitotoxicity, blood pressure and heart rate, and gastrointestinal transit (3). Nphe has been also evaluated in analgesiometric assays. It has been demonstrated that the compound antagonized the pronociceptive and antimorphine effects of supraspinal NC. In addition, Nphe elicits per se an antinociceptive effect which i) is dose-dependent and naloxone resistant, ii) does not undergo to tolerance iii) is no more evident in mice knocked out for the OP_4 receptor gene. Moreover, i.c.v. injected Nphe potentiated the analgesic effect of i) supraspinal morphine, ii) stress-induced analgesia and iii) systemic morphine in mice made tolerant to the alkaloid.

Collectively, these data indicate the utility of OP_4 receptor selective antagonists for the treatment of pain.

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Heterodimerization of opioid receptors: A role in signalling and trafficking

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Protein-protein interactions govern a large number of biological processes. It is well established that a variety of cell surface receptors interact with each other to form dimers and this is essential for their activation. A number of G-protein coupled receptors (GPCRs) including members of the rhodopsin, secretin and metabotropic glutamate receptor family have been recently shown to exist as dimers/oligomers (for review see Bouvier, 2001; Gomes et al., 2001). In some cases dimerization/oligomerization has been found to alter ligand binding, signalling and receptor trafficking properties (Bouvier, 2001; Gomes et al., 2001).

We have examined the dimerization of opioid receptors, which are members the rhodopsin subfamily of the GPCR superfamily. In a previous study we reported that δ and κ receptors exist as homodimers with distinct physical properties (Cvejic et al., 1997; Jordan and Devi, 1999). Expression of δ receptors with κ receptors leads to physical association between these receptors (Jordan and Devi, 1999). This heterodimerization also appear to change the pharmacological properties of these receptors (Jordan and Devi, 1999; Jordan et al., 2000).

A number of previous studies have reported that δ receptor selective drugs can enhance the potency of μ receptors (Jordan et al., 2000). In order to examine if this cross-modulation is due to heterodimerization, we expressed μ and δ receptors in heterologous cells. We find that co-expression of μ with δ receptors results in heteromerization as well as alterations in agonist binding and signalling properties of the receptors (Gomes et al., 2001). For example, treatment of cells expressing μ and δ receptors with extremely low doses of δ selective ligands results in a significant increase in the level of μ binding sites (Gomes, et al., 2001). Furthermore, treatment with the δ selective antagonist enhances both the potency and efficacy of the μ receptor signalling (Gomes, et al., 2001). Taken together, these studies show that heterodimerization

results in modulation of the ligand binding and signalling characteristics of receptors.

In a recent study we have examined if opioid receptors can heterodimerize with other members of the GPCR family. We chose β_2 adrenergic receptors that couple to stimulatory G-proteins and δ & κ opioid receptors that couple to inhibitory G-proteins. When co-expressed, β_2 receptors can associate with either δ or κ receptors (Jordan et al., 2001). This heteromerization does not significantly alter the ligand binding or coupling properties of these receptors (Jordan et al., 2001). However, their trafficking properties are altered (Jordan et al., 2001). δ receptors, in δ-β_2 cells, undergo isoproterenol-mediated endocytosis and β_2 receptors in these cells undergo opioid-mediated endocytosis (Jordan et al., 2001). Interestingly, β_2 receptors in κ-β_2 cells, undergo neither opioid-mediated nor isoproterenol-mediated endocytosis (Jordan et al., 2001). Treatment of these cells with a fairly high dose of isoproterenol is not able to mediate significant phosphorylation of the MAP kinases (Fig. 1, left). The opioid-mediated phosphorylation of MAP kinases is not significantly altered in these cells (Fig. 1, right) indicating that the ability to signal through opioid receptors is intact in these cells. These data suggest that dimerization with κ receptors leads to a significant loss of β_2 receptor-mediated activation of the MAP kinase pathway. Taken together, these results provide direct evidence for heterodimerization of GPCRs that couple to different types of G-proteins (G_i and G_s). Thus, dimerization/oligomerization of GPCRs appears to be a universal phenomenon that provides a mechanism for cross-talk between various members of the GPCR family.

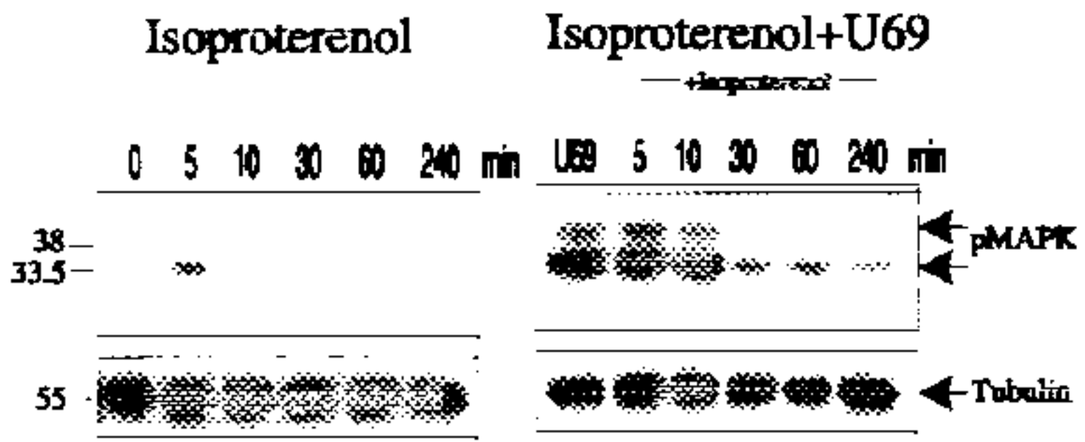

Figure 1. Heterodimerization with κ receptors affects the level of β_2 receptor mediated phosphorylation of pMAP kinase. Cells expressing κ–β_2 receptors were treated with 1 μM isoproterenol in the absence (left) presence (right) of 100 nM U69,593 for indicated time. The extent of phospho-MAP kinase (pMAPK) was determined by western blotting analysis with anti-phospho MAP kinase antibody; standardization was with MAPK or tubulin measured in the same blots using anti-MAPK or anti-

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MULTIRECEPTOR TARGETED ANALGESICS - HYBRIDS OF SUBSTANCE P AND OPIOID PHARMACOPHORES

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Substance P (SP) belongs to a family of chemically related tachykinin peptides that share a common C-terminal amino acid sequence. Anatomical, physiological and pharmacological data show that SP, originally isolated as an endogenous hypotensive compound, functions as a neuromediator. In the spinal cord, especially in dorsal horn neurons, SP plays an important role in the transmission of nociceptive signals from the periphery to the central nervous system. Intrathecal (i.t.) injection of SP and agonist analogues in rodents produces behavior consistent with nociception. SP antagonists block behavior evoked by peripheral noxious stimuli. Unfortunately, clinical trials of substance P antagonists as analgesics have been disappointing, and suggest the presence of more complicated mechanisms of action.

Numerous physiological and behavioral studies have indicated that substance P has a functional coexistence with endogenous opioid peptides. There are similarities in the distribution of SP and opioid peptides in the central and peripheral nervous system, including the dorsal horn. Most available data highlights the opposed roles of these systems in transducing nociceptive stimuli in the spinal cord. SP receptor activation enhances or induces nociceptive signals, whereas opioid receptor activation inhibits or suppresses such signals. Nevertheless, co-administration of small doses of SP potentiates opioid antinociception. Therefore, several mechanisms must be considered in any explanation of the functional interaction between SP and opioids. These mechanisms include opioid inhibition of presynaptic SP release by opioids, and postsynaptic interaction of SP and opioid receptors. Thus the functional interactions between SP and opioid systems seem to be multidimensional, and involve feedback mechanisms related to other neurotransmitter types (e.g. glutamic acid).

Ligands designed to target multiple receptors are a developmental area under exploration by our team. The cross involvement of SP and endogenous opioids in the formation and modulation of pain signals suggested the possibility of creating innovative multitargeted analgesics that interact with both SP and opioid systems.

The initial and obvious approach was to combine functionalities of SP-antagonism and opioid agonism. Our preliminary study in which a peptide SP-antagonist was co-administered with an opioid agonist (biphalin) led to observations that the SP-antagonist (i) if given alone has weak antinociceptive potency but also (ii) exhibits neurotoxicity at higher concentrations, and (iii) strongly enhances biphalin's analgesic activity when these two agents are co-administered [1]. These results served as a basis to develop new ligands that contained both opioid agonist and SP-antagonist pharmacophores. The initial compound of this series (AWL60) indeed expressed properties of both components, resulting in significant analgesia [2].

Tyr-Pro-D-Phe-Phe-D-Phe-D-Trp-MetNH$_2$

*****opioid********

**********SP-antagonist************

Amino acid sequence of AWL60

Nevertheless, the high hydrophobicity of AWL60 and its near insolubility in aqueous solution posed technical problems that halted further study for several years. The development of peptide and nonpeptide peptidomimetics with SP-antagonist potency, gives new importance to the development of useful ligands in which an SP-antagonist is hybridized with an opioid agonist. AA501 is a one of such compounds [3]. The compound expressed antinociceptive properties in both acute thermal as well as inflammatory pain. Further studies in this direction are in progress.

The rationale for hybridization of an SP agonist and opioid agonist is more speculative. As noted, it is generally accepted that SP is in functional antagonism to opioids. Indeed, hybridization of a potent SP fragment and an opioid peptide yielded a compound with a pronociceptive profile, albeit less intense than that of SP alone [4,5]. Blockade of the opioid moiety of this compound by naloxone increased its algesic. Nevertheless, it was subsequently reported that low doses of SP may enhance morphine analgesia, suggesting that SP is involved inautoregulatory mechanisms that stabilize nociceptive signaling. This prediction stimulated our search for ligands, which would hybridize a potent opioid agonist with a weak SP agonist. Consequently, two compounds were synthesized and more extensively studied (II and III).

Tyr-Pro-Phe-Phe-Gly-Leu-MetNH$_2$
Tyr-Pro-Phe-Phe-Pro-Leu-MetNH$_2$
******opioid*****

*******tachykinin agonist******

Amino acid sequence of peptide I and II

Both peptides hybridize an endomorphin portion with different C-terminal fragments of tachykinin agonists. These compounds expressed antinociceptive activity. Because of interfering effect of the SP agonist portion, antinociception from this compound did not display simple dose dependence. These compounds also slowed (or even reversed) the development of opioid tolerance [6,7]. In aggregate, the obtained data indicate that hybridization of SP agonists with opioids is a promising direction to develop new analgesics that may also be useful in the treatment of dependence and/or tolerance.

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カプサイシンの科学
－特にカプサイシン及び同族体の食品機能－

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Science of Capsaicin
Food Functionality of Capsaicin and Its Analogs

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Capsaicin is a major pungent principle of red pepper which is one of the most largely and widely consumed spices in the world. However, capsaicin has been considered as a "non-nutritent", and the information about the nutritional function is sparse. We have previously reported that capsaicin at a diet level enhances lipid catabolism and energy metabolism in rats via catecholamine secretion from the adrenal medulla through sympathetic activation of the central nervous system. Further studies indicated that capsaicin supplementation to the diet increased uncoupling protein contents in interscapular brown adipose tissue (IBAT) in rats. Direct measurement of IBAT temperature in anethetized rats revealed that a significant increase in temperature occurred following capsaicin administration in situ. A continuous oral administration of capsaicin was found to induce capsaicin-hydrolysing enzyme activity in rat liver. To examine the relationship between the pungency and thermogenic action, we synthesized a series of capsaicin analogs with various chain length of saturated acyl moieties. On pungency, there was a tendency that the longer the chain length of capsaicin analogs, the weaker was the pungency. Most pungent analogs except heptanoyl vanillylamide as well as nonpungent analogs except eicosanoyl vanillylamide exerted strong potency to stimulate adnenal catecholamine secretion. Recently, novel nonpungent capsaicin-like substances named "capsiate" and their analogs were isolated from the fruits of CH-19 Sweet, a nonpungent cultivar, selected and fixed from the pungent cultival, CH-19 Hot. Nonpungent capsaicin analogs with food functionality will be of great interest and useful in future.

カプサイシンは唐辛子の辛味の主成分であって、今日では世界中で最も大量に生産され利用されている香辛料成分である。しかしながら、カプサイシンは非栄養素であるので 1980 年まではその食品栄養学的研究はほとんど行われていなかった。

演者らはラットを用い、はじめてカプサイシンの吸収・代謝の検討から研究を進め、食品成分として摂取されたカプサイシンは、これまでの報告とは異なって胃および小腸上部でよく吸収され、血中では血清アルブミンと結合して存在し、肝臓中の酵素によって比較的速やかに分解代謝されることなどの体内動態を明らかにした。次いで、日常レベルのカプサイシン摂取は交感神経の活性化によって副腎髄質からのカテコールアミン分泌を亢進して体内における脂質代謝・エネルギー代謝を促進させることを明らかにし、1982～88 年にかけて報告してきた。

その後、さらに研究を進めた結果、カプサイシンを投与するとラットの肩甲間褐色脂肪組織の温度が上昇すること、及びカプサイシンを連続投与すると褐色脂肪組織中の uncoupling protein の含量が増加することを明らかにした。また、肝臓中にはカプサイシン分解酵素が存在し、この酵素活性はカプサイシンを経口的に連続投与することによって、誘導生成されることを見いだした。

次に、カプサイシンの示す辛味と体熱産生活性との関係を明らかにするためカプサイシンの脂肪酸アシル基の鎖長の異なる同族体を合成して検討した結果、辛味については鎖長の炭素数が9のノナノイルバニリルアミド（C_9-VA）が合成品中では最も辛く、これよりも鎖長が短くても長くても辛味は低下し、炭素数14以上の同族体では辛味は検出されなかった。一方、副腎からのアドレナリン分泌活性は、辛味のある C_9～C_{12}-VA ではカプサイシンと同様の強い分泌亢進活性が認められた。しかし、C_{14}～C_{18}-VA では無辛味であるのに著しいアドレナリン分泌活性を有することが見いだされた。

最近辛味種トウガラシ”CH-19 辛”から自殖選抜して固定された無辛味のトウガラシ”CH-19 甘”には新規の非辛味カプサイシン同族体の存在することが見いだされ、単離・同定してカプシエイトと命名した。このカプシエイトは動物実験において、カプサイシンよりは低いがアドレナリン分泌亢進活性を有することが認められている。

食品機能を有するこれらの無辛味のカプサイシン同族体は興味ある化合物であって、今後の活用が期待されるであろう。

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