【サテライト国際シンポジウム】13:30-17:00
Neurobiology of Opioid Receptor Symposium
（オピオイド受容体の神経生物学シンポジウム）
［主催］
オピオイド受容体の神経生物学シンポジウム実行委員会
実行委員会委員長：植田弘師（長崎大・院）

13:30 Opening Remarks Hiroshi Ueda

[Chairmen] Shinobu Sakurada / Shiroh Kishioka

13:35 SS1 Involvement of the Endogenous Opioid System in Cannabinoid Rewarding Effects and Withdrawal Syndrome
R. Maldonado
Univ Pompeu Fabra, Barcelona, Spain 169

14:10 SS2 Opioid-Induced Synaptic Plasticity and the Alternation of the Sensitivity to Opioid Receptor-Mediated Responses under Chronic Pain State
Minoru Narita and Tsutomu Suzuki
Department of Toxicology, Hoshi University School of Pharmacy,
Tokyo, Japan 171

14:30 SS3 Opioid Receptor Genes Inactivated in Mice: The Highlights
Brigitte L. Kieffer
The Faculty of Pharmacy, University Louis Pasteur, Strasbourg, France 168

15:05 SS4 Neurobiology of Opioid and Plasticity – Toward Drug Discovery for the Pain Resistant to Morphine
Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience
Nagasaki University Graduate School of Biomedical Sciences,
Nagasaki, Japan 170

15:25 SS5 New Drug Targets for Opioid Receptors.
Christopher J Evans
Neuropsychiatric Institute, UCLA, LA, CA 90024, USA 167

16:00 SS6 Electrophysiological Characteristic of Corticoaccumbens Synapses in Rat Mesolimbic System Reconstructed Using Organotypic Slice Cultures
T. Maeda and S. Kishioka
Department of Pharmacology, Wakayama Medical University 166

16:20 Closing Remarks Masamichi Satoh

（国際シンポジウムの進行具合により以下時間変更の可能性あり)
17:10 世話人会報告

17:30 次期世話人代表挨拶
次期世話人代表 亀井淳三

17:40 閉会の辞
世話人代表 花岡一雄

ELECTROPHYSIOLOGICAL CHARACTERISTIC OF CORTICOACCUMBENS SYNAPSES IN RAT MESOLIMBIC SYSTEM RECONSTRUCTED USING ORGANOTYPIC SLICE CULTURES

T. MAEDA AND S. KISHIOKA
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The nucleus accumbens (NAc) is a component of the mesolimbic system involved in drug dependence. Activity of NAc neurons is increased by glutamatergic afferents from the medial prefrontal cortex (mPFC) and is decreased by dopaminergic afferents from the ventral tegmental area (VTA). In the present study, we reconstructed the mesolimbic system using organotypic slice cultures, and examined the effects of dopaminergic agents on synaptic activity in mPFC-NAc synapses. A slice of each of mPFC, NAc and VTA in newborn rat, was arranged on a multi-electrode dish filled with culture medium so that they contacted each other, termed 'triple culture'. Neurite outgrowth of mPFC to NAc in triple culture was identified by DiI. Immunohistochemical study with anti-tyrosine hydroxylase (TH) antibody revealed TH-positive cells in VTA and TH-positive fibers in both mPFC and NAc of triple culture. Extracellular recording using microelectrodes on a multi-electrode dish showed that a single electrical stimulation of mPFC evoked field excitatory postsynaptic potential (fEPSP), and that spontaneous population spikes (sPS) occurred in NAc of triple culture. Both fEPSP and sPS were suppressed by glutamatergic antagonists. The D_1-like receptor agonist SKF38393, but not the D_2-like receptor agonist quinpirole, reduced both the amplitude of fEPSP and frequency of sPS. Cocaine depressed fEPSP, and this depression was reversed by D_1-like receptor antagonist SCH23390, but not by D_2-like receptor antagonist sulpiride. These results suggest that glutamatergic synapses were regenerated in triple culture and were subject to exogenous and endogenous dopaminergic modulation that was similar to that shown in *in vivo*.

New Drug Targets for Opioid Receptors.

Chris Evans

Neuropsychiatric Institute, UCLA, LA, CA 90024

The variable actions of opioid agonists *in vivo* are generally attributed to a combination of factors including receptor selectivity and efficacy for signaling, pharmacokinetics, and ability to reach target receptors. Given these parameters, the unique attributes of buprenorphine will be discussed with regard to opioid receptor trafficking and identifying the pharmacology leading to the bell shaped dose-response/ceiling effect for buprenorphine analgesia. This study presents new insights into the clinical possibilities for buprenorphine like drugs. Inverse agonists as potential drug targets for opioid receptors have not been considered. Inverse agonists would be presumed to have opposite effects on signaling as agonists. However, with regard adenylate cycalse signaling we have recently found that chronic treatment with delta receptor inverse agonists mimics the action of acute treatment with delta agonists. The mechanism we propose is that the constitutive activity of the delta receptor induces cyclase supersensitivity in cells containing delta opioid receptors and this supersensitivity increases the cells response to adenyl cyclase stimulation via Gs coupled receptors or forskolin. This presents a new signaling rational for the design of inverse agonists of Gi/Go coupled receptors. Finally, there is accumulating evidence that different ligands may differentially trigger various signaling pathways via the same receptor, a phenomenon we term ligand-directed signaling. In support of this concept, data will be presented that inhibition of adenylate cyclase and activation of both MAPK and Akt can be regulated independently by different ligands following opioid receptor activation. Potential models will be presented to explain this observation.

Opioid receptor genes inactivated in mice: the highlights

Brigitte L. Kieffer

The Faculty of Pharmacy, University Louis Pasteur, Strasbourg, France

The opioid system controls nociception, stress responses and addictive behaviors. Exogenous alkaloid opiates and endogenous opioid peptides stimulate mu-, delta- and kappa-opioid receptors, whose activities have long been analyzed by pharmacological tools. Mice lacking opioid receptor and opioid peptide precursor genes have now been produced by gene targeting and salient phenotypes from these animals will be reviewed. Behavioral analysis of mutant animals in the absence of drug has highlighted a distinct role of opioid receptors or peptides in nociception and revealed an important role for delta receptors in emotional behaviors. Recent data on stress-induced analgesia from our laboratory will also be presented. The examination of responses to drugs has clarified involvement of each receptor as molecular targets for exogenous opiates *in vivo*. Those data have also demonstrated the critical role of mu-receptor in cannabinoid and alcohol reinforcement, and confirmed the involvement of kappa receptor in several dysphoric responses. On-going studies therefore help understanding the molecular basis of opioid-controlled behaviors, and will contribute to the development of novel therapeutics for pain, anxiety and drug abuse.

INVOLVEMENT OF THE ENDOGENOUS OPIOID SYSTEM IN CANNABINOID REWARDING EFFECTS AND WITHDRAWAL SYNDROME

R. Maldonado
Univ Pompeu Fabra, Barcelona, Spain

Several studies have shown functional relationships between the endogenous cannabinoid and opioid systems. However, acute delta^9-tetrahydrocannabinol (THC) pharmacological responses and physical dependence were not modified in knock-out mice with single deletion of mu, delta or kappa opioid receptors. To further investigate the neurobiological basis of cannabinoid dependence, we have evaluated THC responses in double mu and delta opioid receptor knock-out mice. Antinociception and hypolocomotion induced by acute THC administration remain unaffected whereas the acute hypothermic effects were slightly attenuated in these double mutants. During chronic THC treatment, knock-out mice developed slower tolerance to the hypothermic effects but the development of tolerance to antinociceptive and hypolocomotor effects was almost unaffected. The rewarding properties of THC were abolished in knock-out mice. Interestingly, the somatic manifestations of THC withdrawal were also significantly attenuated in mutant mice, suggesting that a cooperative action of mu and delta opioid receptors is required for the entire expression of THC dependence.

Neurobiology of opioid and plasticity

– toward drug discovery for the pain resistant to morphine

Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience
Nagasaki University Graduate School of Biomedical Sciences

The study for plasticity in the development of tolerance and dependence following chronic treatments with morphine or opioids is one of most important issues in the neurobiology. Our approaches are based on two parts, one is the mechanism at the single cell level, and the other is the one at the synaptic network in the brain. In both approaches we are in progress of visualizing the molecular mechanisms as one of goals. In cells expressing μ-opioid receptor (MOP-R), morphine and DAMGO shows differential MOP-R internalization, and protein kinase C (PKC) inhibition determines this selectivity. In the peripheral μ-opioid analgesia, similar PKC-regulated acute tolerance is also observed. On the other hand, chronic tolerance is mostly related to the synaptic plasticity involving the anti-opioid systems, which is observed in the central nervous system. NMDA-receptor and nociceptin/OFQ-receptor (NOP-R) are important candidates for such mechanisms. In such receptor knock-out mice, morphine tolerance is markedly reduced, and it is also found that both mechanisms play region-specific roles in the plasticity. Those selective antagonists could be candidates for adjuvants to delay the morphine tolerance. On the other hand, neuropathic pain would be another good target for the study of plasticity affecting morphine analgesia. Following sciatic nerve injury peripheral and systemic morphine analgesia are markedly reduced. In such mechanisms the functional switch of nociceptor fibers involved in pain perception is postulated. Some candidates as novel analgesics useful for neuropathic pain would be proposed.

Opioid-induced synaptic plasticity and the alternation of the sensitivity to opioid receptor-mediated responses under chronic pain state

Minoru Narita and Tsutomu Suzuki

Department of Toxicology, Hoshi University School of Pharmacy,

Tokyo, JAPAN

The stimulation of μ-opioid receptor by morphine can regulate a number of signaling pathways. These regulated pathways include inhibition of adenylate cyclase activity, activation of inwardly rectifying K^+ channels and blockade of Ca^{2+} entry through voltage-dependent Ca^{2+} channels. A considerable number of evidence indicates that the stimulation of μ-opioid receptors can activate phospholipase C (PLC)-dependent pathway via Gβγ proteins. We demonstrated that μ-opioid receptor agonists activate PLC, resulting in the activation of protein kinase C (PKC) and the increase in inositol (1,4,5) triphosphate (IP_3)-dependent Ca^{2+} signaling. In some cases, phosphoinositide 3-kinase (PI3K)-dependent PLCγ pathway can be involved in this event. We also found that PKC, especially PKCγ isoform, constitutes an essential pathway through which phosphorylation of μ-opioid receptors occurs. These findings indicate that PKCγ isoform may regulate the homologous negative feedback pathway for the μ-opioid receptor system. Furthermore, neuronal PKC and brain-derived neurotrophic factor (BDNF) are essential for the opioid-induced synaptic plasticity including the rewarding effect and the development of antinociceptive tolerance. Our recent analysis provides new insights that the sensitivity to opioid receptor-mediated responses under neuropathic pain state can be decreased. Sciatic nerve ligation leads to a long-lasting hyperalgesia, which is accompanied by the sustained increases in PKCγ, BDNF and c-Src family in the dorsal horn of the spinal cord. As well as the antinociceptive action, the morphine-induced rewarding effect is dramatically reduced by sciatic nerve ligation. We are currently investigating the effect of chronic morphine treatment on the nerve and astrocyte growth. A significant change in nerve growth associated with the activated astrocyte will be discussed.