高次神経機能制御部門（客員部門）

客員教授　野村靖幸　（薬学博士）

本部門では，脳におけるニューロン・ニューロン間，およびグリア細胞・ニューロン間の情報伝達機能とその分子機構，ならびにそれらへの和漢薬をはじめ諸種薬物の作用を研究している。すなわち，グリア細胞における NO 合成酵素（NOS）誘導の細胞内機構の解析と，NO のニューロンへの作用をニューロン死およびノルアドレナリン放出作用の観点から検討を行っている。また，和漢薬や薬物の脳神経系機能への行動薬理学的効果とその神経化学的作用機序に関する研究を行っている。さらにニューロンの各種モデル細胞における受容体刺激応答機構と薬物作用を研究している。

本年度の研究内容と成果は下記の通りである。

Ⅰ．グリア細胞における NO 合成酵素誘導機構と NO のニューロンへの作用

ラット脳グリア細胞に対し，リポ多糖（LPS）は転写因子の NF-κB を介し，一方 IFNγ は JaK 2 型のチロシンキナーゼを介し，NOS を誘導することを示した。またグリア細胞で誘導された NOS は，kyotorphin（L-tyrosyl-L-arginine）を基質とし得ることを示した。こうして生成された NO は，ニューロンに作用しアポトーシス様の細胞死を惹起すること，この作用に glyceraldehyde-3-phosphate dehydrogenase が関与することを示唆した。一方 NO は cysteine と反応し nitrosothiol となって，ラット海馬切片より noradrenaline 放出を引き起こすことも示した。なお，NO の惹起するニューロンのアポトーシスに関連し，アポトーシス抑制因子 Bcl-2 蛋白質の発現がＣキナーゼによって促進され，Ａキナーゼによって抑制されることも示した。

Ⅱ．和漢薬をはじめ薬物の神経精神機能への作用とその機序に関する研究

抗精神分裂病薬の長期投与がラット脳 D_2 ドパミン受容体誘起のアデニル酸シクラーゼ活性抑制を消失させること，また抗うつ薬 rolipram の脳シナプス膜画分への結合，および phosphodiesterase 活性抑制が加齢に伴い減少することを示した。さらに M_1 ムスカリン性受容体遮断薬や dantrolene の記憶障害誘起効果から，記憶に M_1 受容体や細胞内 Ca^{2+} 動態が関わることを示した。丹参のメタノール抽出物が老化促進モデルマウスの記憶障害を改善することを明らかにした。抗痴呆薬 VA-045 の脳シナプス膜画分への結合の諸性質を示すとともに，運動障害モデルとしての自然発症振戦ウズラ（quail）脳におけるＧ蛋白質活性の異常を示した。

Ⅲ．ニューロンのモデル細胞やリンパ球における受容体応答機構に関する研究

ニューロンのモデルとしての PC12 細胞において，蜂毒ペプチド masptoparan がノルアドレナリン遊離を引き起こすこと，ならびにこれに百日咳毒素感受性Ｇ蛋白質は関与しないことを見い出した。P_2 プリン受容体刺激に伴うアデニル酸シクラーゼ活性ならびに細胞内 Ca^{2+} 濃度（$[Ca^{2+}]_i$）の変化を示し，これらのノルアドレナリン遊離機構との関連性を示した。ニューロンのモデル NG108-15 細胞において，セロトニン刺激は細胞内ニトロソ体より NO を生成させ，その結果 cGMP レベルを上昇させること，また Jurket 細胞ではヒスタミン H_1 受容体刺激により $[Ca^{2+}]_i$ が上昇することを示した。NG108-15 細胞をＣキナ

ーゼとAキナーゼで同時処理すると発現する神経突起進展因子TA20mRNAの，発育期ラット脳における部位別発現の変化を示した。

以上の研究は北海道大学薬学部薬効学教室との共同研究として行われたものである。

Ⅰ．グリア細胞におけるNO合成酵素誘導機構とNOのニューロンへの作用

Ⅰ-1. Possible Involvement of Tyrosine Kinase Activation in Lipopolysaccharide-Induced Expression of Ca^{2+}-Insensitive But Calmodulin-Coupling Nitric Oxide Synthase in Rat Glial Cells

To clarify the properties of an inducible type of nitric oxide synthase (i-NOS) in the brain, we examined whether lipopolysaccharide (LPS) induces NOS in glial cells cultured from neonatal rats. NOS activities (NO_2^- accumulation and L-[^{14}C] citrulline formation) were detected by treatment with LPS at 10 μg/ml for 6-72 hr. L-[^{14}C] citrulline formation by LPS-induced i-NOS was inhibited by N^G-monomethyl-L-arginine (a NOS inhibitor) and diphenyleneiodonium (a flavoprotein inhibitor). The activity was not markedly changed in the presence or absence of Ca^{2+}. The induction of i-NOS by LPS was abolished by cycloheximide, actinomycin D, or dexamethasone. In addition, the induction was inhibited by herbimycin A (a tyrosine kinase inhibitor), but was not by staurosporine, W-7, or FK-506. After LPS stimulation, 130 kDa proteins were reacted with anti-rat liver i-NOS antibody 5-72 hr. i-NOS induced from glial cells coupled tightly with endogenous calmodulin (CaM) even in the absence of Ca^{2+}. These results suggest that LPS induces expression of 130-kDa i-NOS through an activation of tyrosine kinase, after which i-NOS couples with CaM, and that NO is formed for 6-72 hr in glial cells.

Ⅰ-2. Possible involvement of Janus kinase Jak2 in interferon-γ induction of nitric oxide synthase in rat glial cells

To clarify the induction pathway of inducible nitric oxide (NO) synthase in the brain, we examined the effects of interferon-γ and lipopolysaccharide on the induction of inducible NO synthase in glial cells cultured from neonatal rats, compared to those in the macrophage cell line RAW264.7 which was derived from Abelson leukemia virus-induced BALB/c lymphocytic lymphoma. NO synthase activity (NO_2^- accumulation) and 130 kDa protein of protein of inducible NO synthase were induced 24 h after treatment with interferon-γ or lipopolysaccharide in both glial cells and RAW264.7 macrophages. These induction activities were inhibited by a tyrosine kinase inhibitor, herbimycin A. Immunoprecipitation assay using antibodies against Janus kinases, and the signal transducer and activator of transcription-1 (STAT1), revealed that interferon-γ induced tyrosine phosphorylation of the just another kinase-2 (Jak2) and STAT1α but did not induced the phosphorylation of Jakl, the non-receptor tyrosine kinase-2 (Tyk2) and STAT1β. Tyrosine phosphorylation of Jak2 and STAT1α induced by interferon-γ was also inhibited by herbimycin A, while lipopolysaccharide did not induce any tyrosine phosphorylation of Janus kinases and STAT1 at all. These results suggest that the interferon-γ-induced inducible NO synthase induction involves activation of Jak2-STAT1α pathway in both glial cells and macrophages.

Ⅰ-3. Kyotorphin (L-tyrosyl-L-arginine) as a possible substrate for inducible nitric oxide synthase in rat glial cells

L-Arginine (L-Arg) is an endogenous substrate for nitric oxide synthase (NOS). In the present study, we examined whether L-tyrosyl-L-Arg (kyotorphin), an endogenous analgesic neuropeptide, might be a substrate for inducible NOS (iNOS) in the brain. Both kyotorphin and L-Arg caused an accumulation of nitrites in lipopolysaccharide (LPS)-treated glial cells cultured from infant rat brains. However, such accumulation of nitrites was not induced by N^G-nitro-L-Arg (a NOS inhibitor), L-tyrosyl-D-Arg (D-kyotorphin) or D-Arg. L-Leucyl-L-Arg (an antagonist for kyotorphin receptors) or bestatin (an inhibitor for kyotor-

phin-hydrolyzing peptidase) did not inhibit the kyotorphin-induced accumulation of nitrites in LPS-treated cells. On the contrary, L-Leucyl-L-Arg caused an accumulation of nitrites in a concentration-dependent manner. The results indicate that nitric oxide (NO) is produced in LPS-treated glial cells directly from kyotorphin through the catalytic action of iNOS.

I-4. Neuronal Apoptosis by Glial NO : Involvement of Inhibition of Glyceraldehyde-3-Phosphate Dehydrogenase

In primary cocultures of neurons and glial cells prepared from the neonatal rat brain, lipopolysaccharide (LPS) reduced the numbers of neuronal cells but the effects were markedly inhibited by N^G-monomethyl-L-arginine, indicating the involvement of NO and LPS-induced NO synthase in neuronal death. LPS stimulated the expression of inducible NOS (iNOS) in preparations of primary cultured microglias/astrocytes, but not in primary cultured neurons. In addition, LPS caused DNA fragmentation only in NG108-15 cells but not in primary cultured astrocytes as well as astrocytes in cocultures of the two cell types, suggesting that NOS induces the apoptosis of neurons but not glial cells. We then examined the NO-induced neuronal death in NG108-15 cells using NO donors. SNP, an NO donor, caused NO_2^- accumulation in the reaction medium and lactate dehydrogenase (LDH) leakage from NG108-15 cells. Although SNP stimulated guanylyl cyclase and accumulated cGMP, cGMP analogs did not affect LDH leakage. In addition, SNP induced chromosomal condensation and fragmentation of nuclei in NG108-15 cells. Gel electrophoretic analysis of cellular DNA extracted from SNP-treated cells, confirmed the internucleosomal DNA fragmentation typical of apoptosis in this culture. SNP increased the amount of radiosotopic labeled glyceraldehyde-3 phosphate dehydrogenase (GAPDH) in the presence of [^{32}P] NAD and inhibited the enzyme activity. The results suggested that SNP-induced cell death is partly due to the NO-induced inhibition of GAPDH, perhaps by stimulating the binding of NAD to GAPDH.

I-5. Sodium nitroprusside stimulates noradrenaline release from rat hippocampal slices in the presence of dithiothreitol

It is becoming apparent that nitrogen monoxide (NO) such as nitric oxide has regulatory roles for neuronal cell functions. We examined the role of NO using NO donors on [^{3}H]noradrenaline (NA) release from prelabeled rat hippocampal slices. Sodium nitroprusside (SNP), which had no effect by itself, stimulated [^{3}H]NA release in a dose-dependent manner (ED_{50}=0.5 mM) in the presence of dithiothreitol (DTT). The stimulatory effect of SNP with DTT, but not high-K^+, was observed in an extracellular Ca^{2+}-free buffer. The maximal effect of SNP was obtained after incubation for 1-2 h with DTT in buffer at physiological pH (7.4). The simultaneous addition of SNP and DTT to the slices induced a small effect, and the effect of SNP declined after 3.5 h. SNP stimulated cyclic GMP accumulation in the slices without DTT. $NaNO_2$ and 1-hydroxy-2-oxo-3,3-bis (2-aminoethyl)-1-triazene (a generator of nitric oxide), which stimulated cyclic GMP accumulation by themselves, did not stimulate [^{3}H]NA release in the presence and absence of DTT. 3-Morpholinosydnonimine HCl (a generator of peroxynitrite) had no effect on the release. The stimulatory effect of SNP and DTT on NA release was inhibited 40 % by nitric oxide scavengers such as oxyhemoglobin and 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide, although cyclic GMP accumulation induced by NO donors was completely inhibited. These findings suggest that SNP reacts with DTT to produce unknown active species, and that cyclic GMP is not a mediator for SNP-stimulated NA release.

I-6. NO Donors Stimulate Noradrenaline Release From Rat Hippocampus in a Calmodulin-Dependent Manner in the Presence of *L*-Cysteine

Nitrogen oxides (NO) such as nitric oxide have been suggested to potentiate neurotransmitter release in a variety of neuronal cells. In this study, we showed that NO donors stimulate the release of noradrenaline (NA) from rat hippocampus both in vivo and in vitro. Co-addition of NO donors (sodium nitroprusside [SNP] or S-nitroso-N-acetylpenicillamine [SNAP]) and thiol compounds (dithioth-

reitol [DTT] or *L*-cysteine) stimulated [^{3}H]NA release from prelabeled hippocampal slices. Microdialysis in freely moving rats was used to ascertain the role of NO in control of NA release from the hippocampus in vivo. Co-addition of SNAP and *L*-cysteine stimulated endogenous NA release within 30 min. The concentration of NA peaked between 30-60 min to almost 3 times basal level. Another thiol compound, glutathione, had no effect on [^{3}H]NA release in the presence of SNP or SNAP. In the presence of SNAP, the effect of *L*-cysteine was much higher than that of the *D*-isomer, although SNAP did not show stereospecificity. The effect of SNAP/*L*-cysteine was rapid and the maximal increase in [^{3}H]NA release was attained 0-1 min after application, which was similar in time course to the effect of KCl. Unlike the release by KCl, SNAP/*L*-cysteine-stimulated NA release was independent of extracellular $CaCl_2$. However, pretreatment with the calmodulin antagonists W-7 or trifluoperazine significantly reduced the SNAP/*L*-cysteine-stimulated [^{3}H]NA release. Formation of nitric oxide and activation of guanylate cyclase by nitric oxide were not responsible for SNAP/*L*-cysteine-stimulated NA release. These findings suggest that NO donors stimulate NA release from the hippocampus in the presence of thiol compounds such as *L*-cysteine in vivo and in vitro in a calmodulin-dependent, Ca^{2+}-and cyclic GMP-independent manner. The physiological roles of thiol compounds such as *L*-cysteine or glutathione as intermediates of NO are discussed.

I-7. Regulation of Bcl-2 Protein Expression in Human Neuroblastoma SH-SY5Y Cells : Positive and Negative Effects of Protein Kinases C and A, Respectively

The regulatory mechanism of Bcl-2 protein expression was investigated in SH-SY5Y cells, the human neuroblastoma cell line that expresses natively Bcl-2 proteins. When the cells were treated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) or retinoic acid, the level of Bcl-2 protein was increased compared with the control. These effects were inhibited by pretreatment with a protein kinase C (PKC) inhibitor, staurosporine or calphostin C. The level of Bcl-2 protein was also increased by treatment with carbachol, a muscarinic acetylcholine receptor (mAChR) agonist, and the effects were also inhibited by pretreatment with staurosporine or calphostin C. In addition, a carbachol-induced increase in Bcl-2 protein levels and a transient elevation of $[Ca^{2+}]_i$ were inhibited by pretreatment with 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine), an m_3 mAChR antagonist. In contrast, the level of Bcl-2 protein was decreased by treatment with dibutyryl cAMP (diBu-cAMP), forskolin, or cholera toxin, and the effects of diBu-cAMP were inhibited by pretreatment with a protein kinase A (PKA) inhibitor, H-89. From these results, we suggest that the expression of Bcl-2 proteins is regulated by PKC and PKA in positive and negative manners, respectively, in SH-SY5Y cells. Furthermore, the nucleosomal DNA fragmentation induced by serum depletion for 4 h was obsereved in SH-SY5Y cells when the level of Bcl-2 protein was down-regulated by treatment with 1 mM diBu-cAMP for 3 days, although the DNA fragmentation by serum depletion for 4 h was not observed in nontreatment cells, indicating that Bcl-2 proteins whose expression is regulated by PKC and PKA play important roles in serum depletion-induced apoptosis.

II. 和漢薬をはじめ薬物の神経精神機能への作用とその機序に関する研究

II-1. Long-term neuroleptic treatments counteract dopamine D_2 agonist inhibition of adenylate cyclase but do not affect pertussis toxin ADP-ribosylation in the rat brain

We have investigated the response of adenylate cyclase to GTP and to dopamine (DA) in striatal membranes of rats treated for 3 weeks with chlorpromazine or haloperidol, and further measured the level of Gi (an inhibitory GTP-binding protein) or Go (a similar GTP-binding protein of unknown function) in 3 areas (cerebral cortex. striatum and hippocampus) utilizing pertussis toxin-catalyzed ADP ribosylation. In saline-treated control membranes, GTP exerted a biphasic effect on basal and DA-stimulated enzyme activity-peak levels of stimulation by DA plus GTP were observed at 1 μM

GTP. Conversely, dopaminergic inhibitory effects at 10-100 μM GTP were completely attenuated in chlorpromazine or haloperidol-treated membranes. D_2 inhibition of adenylate cyclase by the selective D_2 agonist PPHT was also attenuated due to these neuroleptic treatments, while an increase in D_2 receptor binding was observed. The pertussis toxin ADP-ribosylation of G-proteins (Gi/Go) did not differ significantly in any area. This indicates that long-term neuroleptic treatments increased D_2 receptor binding, but attenuated D_2 inhibition of adenylate cyclase, and exercised no influence on pertussis toxin ADP-ribosylation.

II-2. Influence of Aging on Roliopram-Sensitive Phosphodiesterase Activity and [^{3}H]Rolipram Binding in the Rat Brain

To clarify the quantitative and qualitative changes in type IV phosphodiesterase (PDE IV) with aging, phosphodiesterase (PDE) activity and [^{3}H] rolipram binding in the cytosolic fraction from the brains of young and aged rats were examined. In all areas of the aged (100-week-old) rat brain except for hippocampus, the PDE activity was decreased by about half that the young (10-week-old) animals. However, inhibition % by 100 μM rolipram and by 100 μM isobutylmethylxanthine (IBMX) was not significantly different between the aged and young rats. On the other hand, [^{3}H]rolipram binding and rolipram-sensitive PDE activity did not change with aging in the hippocampus, although both were decreased in other brain regions. These results suggest that PDE IV does not decrease with aging and maintains its cAMP degrading activity in the hippocampus. It may be involved in the dysfunction of the hippocampus with aging.

II-3. Effects of Selective Muscarinic Antagonists, Pirenzepine and AF-DX 116, on Passive Avoidance Tasks in Mice

To clarify the physiological roles of muscarinic acetylcholine (mACh) receptor subtypes, M_1 and M_2, on learning and memory, we examined the effects of three antagonists, atropine (non-selective), pirenzepine (M_1 selective) and 11-[[2-[(diethylamino) methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido [2,3-*b*][1,4]benzodiazepine-6-one, AF-DX 116 (M_2 selective), on step-through passive avoidance tasks in mice. During acquisition tests, mice were trained repeatedly until they achieved criterion latency (300s). In all experiments, drugs or vehicles were intracerebroventricularly administered. Pre-training (5 min before) administration of atropine (1-40 nmol) and pirenzepine (10 and 40 nmol) shortened the response latency in retention tests at 14d after acquisition training. Pre-test (5 min before) and post-training (immediately after the acquisition training) administration of atropine slightly but not significantly impaired retention scores. The administration of AF-DX 116 did not apparently affect the scores in any of tests. Thus, the M_1 receptor subtype coupling systems seem to be more important in the acquisition-consolidation process rather than in the retrieval process.

II-4. 1-[[[5-(4-Nitrophenyl)-2-furany]methylene]imino]-2,4-imidazolidinedione (Dantrolene), an Inhibitor of Intracellular Ca^{2+} Mobilization, Impairs Avoidance Performance and Spatial Memory in Mice

Effects of the intracerebroventricular administration of 1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]imino]-2,4-imidazolidinedione (dantrolene, an inhibitor of intracellular Ca^{2+} mobilization) on learning/memory were investigated in mice using step-througt passive avoidance and radial-arm maze tests. In the passive avoidance test, the administration of 6 nmol of dantrolene shortened the response latency in the retention test. The number of times of acquisition training required to achieve the criterion latency (300s) did not change in the acquisition test. Ten nmol of administration of dantrolene increased the number of times of acquisition training required to achieve the criterion latency in the acquisition test and shortened the response latency in the retention test. In the radial-arm maze tests, 20 nmol of administration of dantrolene disrupted maze-choice accuracy and increased error numbers. These results suggest that intraneuronal Ca^{2+} mobilization plays important roles in learning and memory.

II-5. Ameliorating Effects of Dan-Shen Methanol Extract on Cognitive Deficiencies in Senescence-Accelerated Mouse

Effect of the long-term treatment with a Dan-Shen (*Salviae miltiorrhizae radix*) methanol extract (DME) on memory and learning in scenescence-accelerated mouse was investigated by means of Morris's water maze task. DME treatment significantly decreased the escape latency and floating time in the P8 strain of senescence-accelerated mouse (SAMP8), which strain spontaneously develops learning deficits. These results suggest that DME treatment improved spatial learning and activated emotional function. In neurochemical investigation, although no effect on choline acetyl transferase activity or [^{3}H]QNB (quinuclidinyl benzilate) binding was observed, DME treatment caused increases in [^{3}H]MK-801 ((+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, b]-cyclohepten-5,10-imine maleate) binding to the cortical membrane fraction and [^{3}H]NNA (N^G-nitro-L-arginine) binding to the cytosolic fraction of the brain stem, and decreased [^{3}H]PDBu (phorbol 12,13-dibutyrate) binding to membranes from the hippocampus, striatum, and cerebellum. Thus, our data suggests that long-term DME treatment improves spatial learning and emotional functions in SAMP8. As to the mechanism, DME treatment enhances cortical glutamatergic excitatory neurotransmission and nitric oxide synthase-related functions in brain stem, and affects hippocampal and striatal functions mediated by protein kinase C.

II-6. Partial Characterization of Binding Sites of VA-045, a Novel Apovincaminic Acid Derivative, in Rat Brain Membranes

We characterized the binding sites of VA-045 [(+)-eburunamenine-14-carboxylic acid (2-nitroxyethyl) ester] in the rat brain. VA-045 showed no affinity for various types of well-known neurotransmitter-related receptors or channels. However, radiolabeled VA-045 ([^{3}H]VA-045) bound to rat brain membranes in a saturable and reversible manner. The K_d and B_{max} values of [^{3}H]VA-045 binding were 58.2 nM and 2685 fmol/mg of protein, respectively. The largest specific binding of [^{3}H] VA-045 was observed in the cerebellum, among seven brain regions, and in subcellular synaptosomes. Specific binding of [^{3}H] VA-045 was inhibited by VA-045 (K_i=0.06 μM), a levorotatory enantiomer of VA-045 (VA-213) and its structural analog, vinpocentine. Moreover, compounds with calmodulin antagonistic activity inhibited the [^{3}H] VA-045 binding. These results suggest that VA-045 binds to specific sites, which may resemble calmodulin, on synaptic membranes in the brain.

II-7. Changes of GTP binding proteins, not neurofilament-associated proteins, in the brain of the neurofilament-deficient quail "Quiver"

A neurofilament (NF)-deficient mutant of the Japanese quail was named "Quiver", as it showed generalized quivering as a clinical sign. NF consists of three major subunits ; low, middle and high. We previously reported that the noradrenaline and 5-hydroxytrptamine content in the neostriatum of the Quiver's brain was different from that in the normal quail, although disappearance of the three NF proteins occurred in all areas of the Quiver's brain. Thus, NF-related proteins may show considerable changes in the specific sites of Quiver's brain. In this study, an examination was made of the changes in NF-related proteins in the Quiver, by immunoblotting analysis. The amounts of cyclin-dependent kinase 5 (cdk5), which phosphorylates NF proteins, and tau which is a substrate of cdk5, in the neostriatum of the Quiver, were essentially the same as those in the normal quail, although NF proteins could not be detected in the Quiver. The amount of α-tubulin in the Quiver's brain was similar to that in the normal quail. Next, we investigated the changes of GTP binding (G) proteins in the Quiver's brain, because cytoskeletal components such as tubulin and F-actin bind with G proteins. [^{32}P]ADP-ribosylation of G proteins (G_S by cholera toxin and G_i/G_o by pertussis toxin) in the neostriatum of the Quiver increased significantly, although α subunits of G proteins showed no change by immunoblotting analysis. The ratios of the trimer form in G proteins thus appear to increase more in the NF-deficient Quiver brain than in the brain of the normal quail. The G proteins-mediated adenylate cyclase activities were the same in the brain of both the Quiver and the normal quail. Possible interactions between NFs and G proteins are discussed.

Ⅲ. ニューロンのモデル細胞やリンパ球における受容体応答機構に関する研究

Ⅲ-1. Pertussis Toxin-Insensitive Effects of Mastoparan, A Wasp Venom Peptide, in PC12 Cells

Recent studies have shown that mastoparan, an amphiphilic peptide derived from wasp venom, modifies the secretion of neurotransmitters and hormones from a variety of cell types. Mastoparan interacts with heterotrimeric guanine nucleotide-binding proteins (G proteins) such as G_i and G_o, which are ADP-ribosylated by pertussis toxin (PTX) and thereby uncoupled from receptors. Previously, some of the effects of mastoparan including secretion were reported to be modified selectively by PTX but not by cholera toxin (CTX). In the present study, we examined the influence of bacterial toxins on the effects of mastoparan in PC12 cells. Mastoparan stimulated [^{3}H]noradrenaline (NA) release from prelabeled PC12 cells in the absence of $CaCl_2$, although high K^+ or ATP stimulated the release in a Ca^{2+}-dependent manner. Pretreatment with CTX, not PTX, for 24 h inhibited mastoparan-stimulated [^{3}H]NA release. Mastoparan inhibited forskolin-stimulated cyclic AMP accumulation in a dose-dependent manner, although mastoparan had no effect by itself. Pretreatment with PTX completely abolished the inhibitory effect of carbachol via G_i on cyclic AMP accumulation and partially reduced the effect of mastoparan. However, the inhibitory effect of 20 μM mastoparan was not modified by pretreatment with PTX. Thus. we investigated the effect of mastoparan on CTX-catalyzed [^{32}P]ADP-ribosylation of proteins in PC12 cells. A subunit of CTX (CTX-A) catalyzed [^{32}P]ADP-ribosylation of many proteins in the cytosolic fraction of PC12 cells. One of these was a 20 kDa protein, named ADP-ribosylating factor (ARF). The addition of mastoparan to assay mixtures inhibited ADP-ribosylation of many proteins including ARF and CTX-A in the presence of the cytosolic fraction. In the absence of the cytosolic fraction, however, mastoparan slightly enhanced ADP-ribosylation of bovine serum albumin and auto-ADP-ribosylation by CTX-A. Mastoparan did not inhibit ADP-ribosylation of the α subunit of G_s in the membrane fraction. These findings suggest that 1) mastoparan interacts with PTX-insentitive and CTX-sensitive factor(s) to stimulate NA release, and 2) mastoparan interacts with ARF inhibiting its activity to enhance the ADP-ribosylation reaction by CTX. ARF, may be an exocytosis-linked G protein.

Ⅲ-2. P_2 purinoceptor-mediated stimulation of adenylyl cyclase in PC12 cells

PC12 pheochromocytoma cells have P_2 purinoceptors which are activated by ATP and coupled to Ca^{2+} influx and catecholamine release. Also PC12 cells have adenosine receptors coupled positively to adenylyl cyclase, and cyclic AMP regulates cell functions such as catecholamine release. The effects of ATP and ATP analogs on cyclic AMP accumulation in PC12 cells were investigated in this study. ATP and adenosine 5'-*O*-(3-thiotriphosphate) stimulated cyclic AMP accumulation at low concentrations up to 300 μM but showed inhibitory effects above this concentration. 2',3'-*O*-(4-Benzoyl) benzoyl ATP and 2-methylthio ATP showed similar effects, although the responses were very limited. Addition of adenosine 5'-*O*-(2-thiodiphosphate) (ADPβS) or β,γ-methylene ATP, but not α,β-methylene ATP, stimulated cyclic AMP accumulation markedly without causing an inhibitory phase. The effects of ATP, ADPβS and β,γ-methylene ATP were not inhibited by adenosine deaminase or specific antagonists to A_1 and A_2 adenosine receptors. Neither ADPβS nor β,γ-methylene ATP showed any effect on Ca^{2+} influx or noradrenaline release. Suramin, a P_2 receptors antagonist, had no inhibitory effect against ATP analog-stimulated cyclic AMP accumulation, although reactive blue 2 inhibited the β,γ-methylene ATP-stimulated reaction but not that up-regulated by ADPβS. These findings suggest that the pharmacological characteristics of these ATP receptors coupled to adenylyl cyclase are clearly different from those of ligand-gated ion channels defined by P_{2X} purinoceptors, which have been cloned and shown to be coupled to Ca^{2+} influx and catecholamine release in PC12 cells. The existence of a new type of P_2 purinoceptor-mediating stimulation of adenylyl cyclase is

proposed in PC12 cells.

III-3. Go Protein Does Not Regulate ATP-Stimulated [Ca^{2+}]$_i$ Elevation or Noradrenaline Release in PC12 Cells

The roles of Go, a heterotrimeric GTP binding (G) protein with a 40-kDa α subunit and which is localized predominantly in neuronal cells, in exocytosis have been discussed recently. PC12 pheochromocytoma cell line is a convenient model in which to study the Ca^{2+}-dependent mechanisms of the neurosecretory process. The stimulation of ATP receptors or addition of KCl stimulated an elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) and [^{3}H]noradrenaline (NA) release in PC12 cells. In this study, we investigated the roles of Go in ATP- and KCl-stimulated reactions. Nerve growth factor treatment for 2 days and transfection of PC12 cells with cDNA of subunit of Go (Goα) had no effect on ATP-stimulated [^{3}H]NA release, although both treatments increased levels of the Goα and its trimeric form by about twofold over those in unstimulated cells. The $[Ca^{2+}]_i$ rise induced by ATP in NGF-treated cells was similar to that in control cells, although the maximal response was slightly smaller. Cholera toxin treatment for 2 days inhibited ATP- and KCl-stimulated NA release, although this treatment caused an approximately twofold increase in the level of Go. Pertussis toxin treatment, which ADP ribosylated over 90 % of endogenous G proteins such as Go, had no effect on ATP-stimulated reactions. These findings show that Go does not directly regulate ATP-stimulated Ca^{2+} channels or the NA release process in PC12 cells. Cholera toxin-sensitive protein(s) may regulate exocytosis.

III-4. Cyclic GMP Elevation by 5-Hydroxytryptamine Is Due to Nitric Oxide Derived from Endogenous Nitrosothiol in NG108-15 Cells

To clarify the involvement of nitric oxide (NO) derived from nitrosothiols (RSNO) in 5-hydroxytryptamine (5-HT)-induced Ca^{2+}-independent cGMP formation (CIGF) in NG108-15 cells, we investigated the effects of 5-HT on intracellular contents of RSNO as well as of NO metabolites. 5-HT stimulation resulted in an increase in the intracellular contents of nitrate and cGMP. RSNO was detected in NG108-15 cells and was decreased by 5-HT stimulation. Furthermore, the time course of nitrate increase was coincident with that of RSNO decrease. Carboxy PTIO inhibited 5-HT-induced CIGF, whereas oxyhemoglobin failed to inhibit it. The data suggest that NO is stored in a stable form as RSNO and that 5-HT stimulates NO generation from endogenous RSNO, which is followed by elevation of cGMP via activation of cytosolic guanylyl cyclase by NO in NG108-15 cells. We suggest the existence of a novel 5-HT signal transduction pathway involved in NO generation in NG108-15 cells.

III-5. Regulation of $[Ca^{2+}]_i$ Rise Activated by Doxepin-sensitive H_1-Histamine Receptors in Jurkat Cells, Cloned Human T Lymphocytes

To clarify the presence of histamine receptor and its transmembrane mechanism in human T lymphocytes, we investigated the effects of agonists or antagonists of histamine receptor subtypes and bacterial toxins on intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$),[^{3}H] pyrilamine binding and c-*fos* mRNA expression in Jurkat cells, cloned human T lymphocytes. H_1-agonists (histamine and 2-methylhistamine) caused a transient rise of $[Ca^{2+}]_i$ and H_1-antagonists (pyrilamine and doxepin) inhibited the histamine-induced $[Ca^{2+}]_i$ rise more potently than the H_2-antagonist (cimetidine) on the H_3-antagonist (impromidine). Binding parameters of [^{3}H] pyrilamine binding were K_d=5.53 nM and B_{max}=2,647 sites/cell. Pretreatment with *B. pertussis,* V. cholera. or *C. botulinum* toxin did not influence histamine-induced $[Ca^{2+}]_i$ rise. Western Blot analysis using antibodies against subunits of GTP-binding proteins indicated that G_q/G_{11} richly existed in Jurkat cells. Histamine induced mRNA expression of an immediate early gene c-*fos*. Pretreatment with a protein kinase C activator, phorbol 12-myristate 13-acetate, caused almost complete inhibition of histamine-induced $[Ca^{2+}]_i$ rise, but did not do so by activators of cAMP- and cGMP-dependent protein kinases.

III-6. Expression of mRNA for a neuronal differentiation factor, TA20, in developing rat brains

In our previous study, a novel factor, TA20, was isolated from NG108-15 cells. The TA20 mRNA was increased by stimulation which also induced neuronal differentiation. Neuronal cells overexpressed with TA20 extended long neurites and stopped cell growth (Tohda et al., 1995, Neurosci. Res., 23 ; 21-27). We investigated the expression pattern of TA20 mRNA in developing rat brains to predict physiological roles of TA20. TA20 mRNA began to increase between embryonic days 13 and 16. TA20 mRNA was observed mainly in neocortical, hippocampal and precerebellar neuroepithelium on embryonic day 16. Although the level of TA20 mRNA in the cerebral cortex was higher before birth than after birth, the level in cerebellar Purkinje cells increased gradually even after birth. The high expression level of TA20 mRNA in the hippocampus was maintained before and after birth. Thus, TA20 was expressed highly in brain regions in which neurons were changing morphologically and qualitatively, suggesting that TA20 may be involved in neuronal formation in vivo.

◇ 原　著

1) Kitamura Y., Imaizumi R., Esumi H., and Nomura Y.: Possible involvement of tyrosine kinase activation in lipopolysaccharide-induced expression of Ca^{2+}-insensitive but calmodulin-coupling nitric oxide synthase in rat glial cells. J. Neurosci. Res., 43 : 235-245, 1995.

2) Kitamura, Y, Arima T., Kitayama Y., and Nomura Y.: Regulation of $[Ca^{2+}]_i$ rise activated by doxepin-sensitive H_1-histamine receptors in Jurkat cells, cloned human T lymphocytes. Gen. Pharmacol., 27(2) : 285-291, 1996.

3) Okada F., Ito A., Horikawa T., Tokumitsu Y., and Nomura Y. : Long-term neuroleptic treatments counteract dopamine D_2 agonist inhibition of adenylate cyclase but do not affect pertussis toxin ADP - ribosylation in the rat brain. Neurochem. Int., 28 : 161-168, 1996.

4) Hasegawa H., Murayama T., Takahashi A., Itakura C., and Nomura Y. : Change of GTP binding proteins, neurofilament proteins, in the brain of the neurofilament - deficient quail "quiver". Neurochem. Int., 28(2) : 221-229, 1996.

5) Tohda M., Murayama T., Nogiri S., and Nomura Y. : Influence of aging on rolipram-sensitive phosphodiesterase activities and [^{3}H] rolipram binding in the rat brain. Biol. Pharm. Bull., 19(2) : 300-302, 1996.

6) Shirasaki T., Tanaka M., Muramatsu M., Otomo S., Kitamura Y., and Nomura Y.: Partial characterization of binding sites of VA-045, a novel apovincaminic acid derivative, in rat brain membranes. Gen. Pharmac., 27(2) : 279-284, 1996.

7) Kitamura Y., Takahashi H., Nomura Y., and Taniguchi T. : Possible involvement of Janus kinase Jak2 in interferon-γ induction of nitric oxide synthase in rat glial cells. Europ. J. Pharmacol., 306 : 297-306, 1996.

8) Tohda C., Nagai S., Kuraishi Y., and Nomura Y. : Expression of mRNA for a neuronal differentiation factor, TA20, in developing rat brains. Neurosci. Res., 24 : 421-425, 1996.

9) Itano Y., Ito A., Uehara T., and Nomura Y.: Regulation of Bcl-2 protein expression in human neuroblastoma, SH-SY5Y cells : Positive and negative manners by protein kinase-C and -A, respectively. J. Neurochem., 67 (1) : 131-137, 1996.

10) Ohnuki T., and Nomura Y. : Effects of selective muscarinic antagonists, pirenzepine and AF-DX116 on passive avoidance tasks in mice. Biol. Pharm. Bull., 19 (6) : 814-818, 1996.

11) Satoh S., Murayama T., and Nomura Y. : Sodium nitroprusside stimulates noradrenaline release from rat hippocampal slices in the presence of dithiothreitol. Brain. Res., 733 : 167-174, 1996.

12) Ohnuki T., and Nomura Y. : 1-[[[5-(4-nitrophenyl)-2-furanyl]-methylene]imino]-2,4-imidazolidinedione (dantrolene), an inhibitor of intracellularmobilization, impairs avoidance performance and spatial memory in mice. Biol. Pharm. Bull., 19(8) : 1038-1040, 1996.

13) Oda H., Murayama T., and Nomura Y. : Go protein does not regulate ATP - stimulated $[Ca^{2+}]_i$ elevation or noradrenaline release in PC12 cells. Arch. Biochem. Biophys., 332 (1) : 1-7, 1996.

14) Satoh S., Kimura T., Toda M., Miyazaki H., Ono S., Narita H., Murayama T., and Nomura Y.: NO donors stimulate noradrenaline release from rat hippocampus in a calmodulin-dependent manner in the presence of L-cysteine. J. Cell Physiol., 169 : 87-96, 1996.
15) Arima T., Kitamura Y., Nishiya T., Takagi H., and Nomura Y.: Kyotorphin (L-tyrosyl-arginine) as a possible substrate for inducible nitric oxide in rat glial cells. Neurosci. Lett., 212 : 1-4, 1996.
16) Arima T., Ohshima Y., Mizno T., Kitamura Y., Segawa T., and Nomura Y.: Cyclic GMP elevation by 5-hydroytryptamine is due to nitric oxide derived from endogenous nitrosothiol in NG108-15 cells. Biochem. Biophys. Res. Commun., 227 : 473-478, 1996.
17) Arima T., Baba I., Hori H., Kitamura Y., Namba T., Hattori M., Kadota S., and Nomura Y.: Ameliorating effects of Dan-Shen methanol extract on cognitive deficiencies in senescence-accelerated mouse. Kor. J. Gerontol., 6 (2) : 14-21, 1996.
18) Nomura Y., Uehara T., and Nakazawa M.: Neuronal apoptosis by glial NO : involvement of inhibition of glyceraldehde-3-phosphate dehydrogenase. Human Cell, 9 (3) : 205-214, 1996.
19) Yakushi Y., Watanabe A., Murayama T., and Nomura Y.: P_2-purinoceptor-mediated stimulation of adenylyl cyclase in PC12 cells. Europ. J. Pharmacol., 314 : 243-248, 1996.
20) Murayama T., Oda H., and Nomura Y.: Pertussis toxin-insensitive effects of mastoparan, a wasp venom peptide, in PC12 cells. J. Cell Physiol., 169 : 448-454, 1996.

◇ 著　書

1）野村靖幸：生体内の情報伝達．薬理学．高折修二他編，103-127，廣川書店，東京，1996.
2）Nomura Y., Yamanaka Y., Kitamura Y., Arima T., Ohnuki T., Nagasima K., Ihara Y., Sasaki K., and Oomura Y.: Senescence accelerated mouse : Neurochemical studies on aging. Ann. New York Acad. Sci. 786, 410-418, 1996.
3）Oomura Y., Sasaki K., Li A., Yoshii H., Fukata Y., Yago H., Kimura H., Tooyama K., Hanai K., Nomura Y., Kitamura K., and Yanaihara N.: Protection against impairment of memory and immunoreactivity in senescence-accelerated mice by acidic fibroblast growth factor. Ann. New York Acad. Sci. 786, 337-347, 1996.
4）野村靖幸：黄芩，杜仲，鹿茸，五味子「漢方薬理学序説」高木敬次郎監修，木村正康編，南山堂，東京，1996.
5）野村靖幸：中枢神経疾患．「薬物治療学」百瀬弥寿徳，北田光一編，朝倉書店，東京，1996.
6）北村佳久，谷口隆之，野村靖幸：脳でのiNOS誘導の情報伝達「NOSとCOX：遺伝子から臨床へ」，61-70，メディカルビュー社，東京，1996.
7）野村靖幸，上原　孝：グルタミン酸．用語ライブラリー「アポトーシス」，38-39，羊土社，東京，1996.
8）上原　孝，野村靖幸：NMDA.用語ライブラリー「アポトーシス」，40-41，羊土社，東京，1996.

◇ 総　説

1）野村靖幸：ヒスタミンレセプター応答系の分子細胞薬理学的研究．Therapeutic Research 17 (1) : 75-81, 1996.
2）上原　孝，野村靖幸：ヒスタミンH1受容体を介する情報伝達系の解析．Therapeutic Reasearch 17 (1) : 143-146, 1996.
3）野村靖幸：IRS－1．生化学 68 (2) : 155, 1996.
4）野村靖幸：神経細胞のアポトーシスとBcl-2発現．神経研究の進歩 40 (2) : 263-271, 1996, 医学書院.
5）野村靖幸：代謝より見た脳の老化－ニューロン死とシナプス伝達低下．北海道医学雑誌 71 (3) : 309-314, 1996.
6）野村靖幸：神経系における細胞間，細胞内情報伝達系の最新の知見．最新医学 51 (7) : 1218-1228, 最新医学社，1996.
7）野村靖幸，平田結喜緒編：脳グリアiNOSの発現調節機構．医学の歩み別冊「NOのすべて」，129-133，医歯薬出版，1996.

◇ 学会報告

1）野村靖幸：和漢薬と老年痴呆．平成7年度富山県リカレント教育講座「和漢薬を暮らしにいかす」，1996，1，富山.
2）野村靖幸：老化とくに脳老化病態と薬物の作用．新適塾－21世紀の薬箱，1996，1，大阪.

3）野村靖幸：脳老化を考える：代謝よりみた脳の老化．北海道医学大会公開シンポジウム，1996，2，札幌．
4）野村靖幸：セロトニン－機能，受容体と薬物．第1回セロトニン研究会，1996，2，札幌．
5）有馬　隆，松野純子，堀　妃登美，難波恒雄，門田重利，服部征雄，野村靖幸：老化促進マウス（SAM）の空間認知機能障害に対するLithospermate Bの作用．第12回SAM研究発表会，1996，3，京都．
6）小田温子，村山俊彦，野村靖幸：ラット脳グリア細胞におけるinducible NOS（iNOS）誘導に対するendothelin（ET）の作用．第69回日本薬理学会年会，1996，3，長崎．
7）上原　孝，徳光幸子，野村靖幸：3T3-L1細胞におけるインスリンの脂肪細胞分化への誘導機構．第69回日本薬理学会年会，1996，3，長崎．
8）佐藤壮一，村山俊彦，野村靖幸：S-nitroso-L-cysteinによるラット海馬スライスからの[^{3}H]noradrenalineの放出．第69回日本薬理学会年会，1996，3，長崎．
9）徳光幸子，大坂康人，土屋正利，野村康幸：ラット脂肪細胞におけるvasoactive intestinal polypeptide（VIP）の糖取り込み促進作用．第69回日本薬理学会年会，1996，3，長崎．
10）鴨島　渉，北村佳久，野村靖幸，谷口隆之：神経芽細胞腫（SH-SY5Y）におけるNO誘発細胞膜の調節機構．第69回日本薬理学会年会，1996，3，長崎．
11）上原　孝，徳光幸子，野村靖幸：3T3-L1細胞におけるインスリンの脂肪細胞分化への誘導機構．日本薬学会第116年会，1996，3，金沢．
12）長井忍布，伊藤昭博，上原　孝，野村靖幸，東田千尋：NG108-15細胞の突起伸展，細胞死に及ぼす新規神経分化因子TA20の影響．日本薬学会第116年会，1996，3，金沢．
13）山田武宏，村山俊彦，野村靖幸：ラット胸腺細胞増殖能に及ぼすhydrocortisone，拘束ストレスおよびNO donorの影響について．日本薬学会第116年会，1996，3，金沢．
14）宮崎浩之，村山俊彦，野村靖幸，小野　哲，成田弘和：ラット大脳皮質スライス標品からのノルアドレナリン遊離に対する新規脳機能改善剤T-588の作用．日本薬学会第116年会，1996，3，金沢．
15）村山俊彦，上原　孝，川野裕美，徳光幸子，野村靖幸：細胞内情報伝達系とくにG蛋白質と$[Ca^{2+}]_i$に及ぼすリチウムの影響．第16回リチウム研究会，1996，4，東京．
16）村山俊彦，野村靖幸：ドパミンニューロンの生死と薬物の作用．第12回札幌神経科学研究会ミニシンポジウム「ドパミン」，1996，6，札幌．
17）松野純子，有馬　隆，野村靖幸：老化促進モデルマウス（SAM）の空間認知機能障害に及ぼすLithospermate Bの影響．第10回北海道薬物作用談話会，1996，7，札幌．
18）宮坂知宏，上原　孝，野村靖幸：脳虚血ラット脳におけるニューロン死とiNOS，サイトカイン発現．第10回北海道薬物作用談話会，1996，7，札幌．
19）野村靖幸，上原　孝，西屋　禎，村山俊彦：アストロサイト・ニューロン相互作用に関わるサイトカインとNO産生．第69回日本生化学会・第19回日本分子生物学会イブニングセミナー（ニューフロンティア）「ニューロンの生存・機能発現のためのグリア細胞との相互作用」，1996，8，札幌．
20）村山俊彦，小田温子，長沼朋佳，野村靖幸：PC12細胞からのノルアドレナリン放出－低分子量G蛋白ARFの関与．第69回日本生化学会・第19回日本分子生物学会，1996，8，札幌．
21）西屋　禎，上原　孝，枝松裕紀，上代淑人，伊東広，野村靖幸：グリア細胞における誘導型一酸化窒素合成酵素の発現機構．第69回日本生化学会・第19回日本分子生物学会，1996，8，札幌．
22）長田洋子，上原　孝，北村佳久，野村靖幸，堀池喜八郎：老化促進マウス脳におけるD-セリンレベルとNMDAレセプター/D-セリン結合部位の分布．第69回日本生化学会・第19回日本分子生物学会，1996，8，札幌．
23）北村佳久，鴨島　渉，野村靖幸，谷口隆之：NOドナーによるヒト神経芽細胞SH-SY5Yのアポトーシスは Bcl-2 発現抑制により増強される。第69回日本生化学会・第19回日本分子生物学会，1996，8，札幌．
24）Nomura Y.: The induction of glial iNOS and NO-induced neuronal apoptosis. International Congress of Human Cell and Cell Culture, 1996, 8, Tokyo.
25）松野純子，有馬　隆，堀妃登美，難波恒雄，服部征雄，門田重利，野村靖幸：老化促進モデルマウスの空間認知機能障害に対する丹参成分lithospermate Bの作用．第13回和漢医薬学会，1996，8，富山．
26）東田道久，中村　智，野村靖幸，渡辺裕司：補中

益気湯によるセロトニン2CmRNA発現促進作用．第13回和漢医薬学会，1996，8，富山．
27) 野村靖幸，宮坂知宏，馬場伊津子，上原　孝：脳虚血ラット脳におけるiNOS誘導，ニューロン死と薬物作用．第24回薬物活性シンポジウム，1996，9，京都．
28) Nomura Y., Uehara T., Nishiya T., and Kitamura Y.: The induction of iNOS and NO-induced neuronal apoptosis. Satellite Symposium of 12nd International Congress of Eye Research "Nitric Oxide and Free Radicals", 1996, 9, Kyoto.
29) 野村靖幸，上原　孝，西屋　禎：Involvement of glyceraldehyde-3-phosphate dehydrogenase in NO-induced neuronal apoptosis. 第39回日本神経化学会シンポジウム Nitric Oxide Synthase and Action of Nitric Oxide, 1996, 10, 横浜．
30) 小田温子，村山俊彦，佐々木幸生，岡田敏一，野村靖幸：ラット脳グリア細胞におけるinducible NOS（iNOS）誘導に対するendothelin（ET）の作用．第39回日本神経化学会，1996，10，横浜．
31) 長沼朋佳，村山俊彦，野村靖幸：PC12細胞におけるS-ニトロソシステインによる$[Ca^{2+}]_i$の上昇．第39回日本神経化学会，1996，10，横浜．
32) 大嶋義弘，有馬　隆，野村靖幸：NG108-15細胞における5-HT応答性Ca非依存性cGMP産生機構．第47回日本薬理学会北部会，1996，10，山形．
33) 村山俊彦，薬師義弘，渡辺あさ子，野村靖幸：PC12細胞におけるアデニル酸シクラーゼに関連したATP受容体の存在．第47回日本薬理学会北部会，1996，10，山形．
34) 伊藤昭博，上原　孝，野村靖幸：セラミドの神経芽細胞腫SK-N-MC細胞における細胞死と薬物作用．第47回日本薬理学会北部会，1996，10，山形．
35) 野村靖幸：遺伝子発現研究から創薬を展望へ．第16回細胞情報伝達系北海道研究会ミニシンポジウム「転写制御－機構，病態と創薬」，1996，10，札幌．
36) 西屋　禎，上原　孝，枝松裕紀，上代淑人，伊東広，野村靖幸：グリア細胞における誘導型一酸化窒素合成酵素の発現機構．第16回細胞情報伝達系北海道研究会．1996，10，札幌．
37) 宮坂知宏，上原　孝，野村靖幸：脳虚血モデルラットにおけるニューロン死惹起機構と薬物作用．第26回神経精神薬理学会年会．1996，10，東京．
38) 村山俊彦，佐藤壮一，野村靖幸：NO誘導体，S-ニトロソシステインによるラット海馬からのノルアドレナリン放出機構．第18回生体膜と薬物の相互作用シンポジウム，1996，11，徳島．
39) 上原　孝，西屋　禎，野村靖幸：グリア細胞におけるNO合成酵素誘導機構，第7回高次脳機能シンポジウム，1996，11，大阪．
40) 宮坂知宏，上原　孝，野村靖幸：一過性脳虚血に伴うNO合成酸素誘導とニューロン死．第2回北海道活性酸素・フリーラジカル研究会，1996，11，札幌．
41) 馬場伊津子，上原　孝，野村靖幸：各種ストレス刺激のC6グリオーマ細胞のケモカイン（CINC）産生応答．第107回日本薬学会北海道支部例会，1996，11，札幌．
42) Tohda M., Nomura Y., and H Watanabe.: Effects of imipramine on 5-HT2CR m RNA and a novel depression related gene (DRF24) expression. Society for Neuroscience, 1996, 11, Washington D.C.

◇ **そ の 他**

1）野村靖幸：ニューロン・グリア相関の病態生理的機構．科技庁成果集，1996．
2）野村靖幸：シナプスの形成と伝達の制御機構，文部省科学研究費・重点領域研究「中枢シナプス分子生物学」研究成果報告，350-353，1996．
3）野村靖幸，村山俊彦，有馬　隆，徳光幸子，上原孝：脳神経細胞のアデニル酸シクラーゼ，グアニル酸シクラーゼ系に対するLiClの作用．平成7年度厚生省精神・神経疾患研究委託費「感情障害の成因と治療に関する研究」による研究報告集，71-74，1996．
4）野村靖幸：平成7年度長寿科学総合研究報告書，分担研究，1996．
5）野村靖幸：神経免疫相関：細胞間，細胞内情報伝達機構の研究．（財）内藤記念科学振興財団の助成金による研究報告，1996．
6）松野純子，有馬　隆，堀　妃登美，難波恒雄，服部征雄，門田重利，野村靖幸：老化促進モデルマウスの空間認知機能障害に対する丹参成分lithospermate Bの作用，和漢医薬学雑誌，1996．