Implications of Intrathecal Pertussis Toxin Animal Model on the Cellular Mechanisms of Neuropathic Pain Syndrome

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Like opioid tolerance, neuropathic pain syndrome manifested by hyperalgesia and allodynia responds poorly to opioids. Hitherto, its development is still not clear and its treatment and prevention are still disputable. Pertussis toxin (PTX) which ADP-ribosylates the \( \alpha \)-subunit of inhibitory guanine nucleotide binding regulatory proteins (Gi/Go), is used to induce morphine tolerance through intrathecal (i.t.) injection. It decreases the antinociceptive effect of opioid receptor agonists, and produces a thermal hyperalgesia as well. With treatment of PTX the inhibitory Gi- and Go-proteins signal transduction is inactivated. Inhibition of the inhibitory system would likely lead to a predominance of the excitatory system. Intrathecal PTX administration has also been suggested as a model for study of the central mechanisms of neuropathic pain. In our previous studies, with intrathecal microdialysis and drug delivery techniques, we correlated the biochemical and pharmacological effects on the behavioral expressions of i.t. PTX-treated rats. Intrathecal PTX administration would induce thermal hyperalgesia in rats, with accompaniments of a prolonged increase in the concentrations of excitatory amino acids (EAAs), glutamate and aspartate, and a decrease in the concentration of the inhibitory amino acid (IAA) glycine in the spinal CSF dialysates. The PTX-induced thermal hyperalgesia peaked between day 2 and 4, but no cold allodynia is observed; i.t. administration of N-methyl-D-aspartate (NMDA) receptor antagonist, D-2-amino-5-phosphonovaleric acid (D-AP5), glycine and protein kinase C (PKC) inhibitor chelerythrine attenuated the thermal hyperalgesia. The PKC\(_\gamma\) content of both synaptosomal and cytosolic fractions were significantly increased in PTX-treated rats. In contrast, the levels of PKC\(_\alpha\), \( \text{PKC}_\beta\), or \( \text{PKC}_\delta\) isozymes in these fractions were unaffected. Infusion of NMDA antagonist D-AP5 prevented both the thermal hyperalgesia and the increase in PKC\(_\gamma\) expression in PTX-treated rats. Similar to our previous report, i.t. PTX reduced morphine's analgesic effect. PKC inhibitor chelerythrine attenuated this reduction of morphine's analgesia, and an inhibition of the morphine-evoked EAAs release was observed in PTX-treated rats as well. Taken together, i.t. PTX-induced neuropathic pain syndrome is accompanied by increasing of EAAs, decreasing of IAA release, and a selective increasing of PKC\(_\gamma\) expression in the spinal cord. PKC not only blocked thermal hyperalgesia, but also reversed the reduction of morphine's analgesic effect in PTX-rats. These results suggest that PTX-induced neuropathic pain syndromes are involved in EAAs, IAA and PKC alternations.

Key words: Pain. Pertussis toxin. Excitatory amino acids. Protein kinase C.
opioid receptor number, and (3) altera-
tion of opioid receptor high-affinity sites.
S binding of opioid receptors but not reduction of
opioid receptors function may be one of
subunit, along with 
opioid agonists on neuropathic
and five isoforms. Activation of G-protein
subunits. Both subunits are able
opioid, , and opioid receptor density and the content
opioid peptide PL017, in association with a
opioid receptor agonists would elicit bio-
mechanisms of neuropathic pain syndrome. In contrast to the
peripheral animal models, such as sciatic nerve ligation
spinal nerves transaction, central nociceptor sensitization
resulted from the peripheral treatments.

The possible mechanisms of reduction of opioids' antinociceptive effect are (1) down-regulation of functional c-opioid receptors (the high affinity state), (2) re-
duction of total μ-opioid receptor number, and (3) alteration of intracellular nociception signal transduction, such as increasing release of EAA and activation of PKC. Pervious studies demonstrated that PTX treatment did not affect total μ-opioid receptor density and the content of Gs-, Gi- and Go-proteins on the neuronal plasma mem-
brane from the brain and spinal cord of rats. Chen et al. demonstrated that the GTP_7 binding of μ-opioid receptor was significantly reduced in the spinal dorsal horn of diabetic neuropathic pain rats. Wong et al. also found that PTX injection decreased the antinociceptive effect of μ-opioid peptide PL017, in association with a
reduction of μ-opioid receptor high-affinity sites. This impairment of μ-opioid receptors function may be one of the mechanisms responsible for the reduction of μ-opioid agonists' analgesic effect in neuropathic pain animals.

Intrathecal PTX—A New Model for Neuropathic Pain Study

PTX was first introduced to research on receptor-coupled signaling by Katada and Ui. PTX is a hexa-
ermer (117,000 Da) of five dissimilar subunits that
were named in order of decreasing molecular weight (S_1-S_5). The S_1-subunit exhibits enzymatic activity of ADP-ribo-
syltransferase, which enters the cell together with the
bound glycoproteins by internalization with the help of
S_2-S_5 subunits. PTX has been known to inactivate
Gi/Go-proteins by ADP-ribosylation of the specific cystein on C-terminal, by disrupting the inhibitory signal transduction. Gi/Go-proteins regulate many intracel-
lar signal transduction and channel activity, such as
inhibiting adenylate cyclase activity, opening potassium
channels, closing calcium channels, and breaking down
inositol phospholipids. With heterotrimeric structure
all of the G-proteins acting as membrane signal transdu-
cers consist of nucleotide binding α-, β- and γ-subunits.
There are at least 16 types of α-subunit, along with at
least seven β and five γ isoforms. Activation of G-protein coupled receptors leads to the dissociation of G-protein into α- and βγ-subunits. Both α- and βγ-subunits are able
to activate G-protein-linked second messenger system.

For example, μ-opioid receptor agonists would elicit bio-
logical actions through decrease of production of cAMP,
opening Gi protein-mediated inwardly rectifying potas-
sium channels, and closing of voltage-dependent calcium
channels. PTX increases the release of excitatory amino acid (EAA) glutamate from chromaffin cells, cere-
bellar granule neurons, and dorsal root ganglion cells
by modulating Ca^{2+} channel activity. Durcan and Morgan also reported that PTX enhanced N-methyl-
D-aspartate (NMDA)-induced seizures in mice. When
i.t.-PTX-treated rats were used as a morphine tolerance
model, we observed both a reduction of opioids' analge-
sic effect and thermal hyperalgesia. A reduction of
μ-opioid receptor high-affinity sites was also observed
in the same study. NMDA receptor antagonists not
prevented the development of morphine tolerance but also inhibited the PTX-induced neuropathic pain syn-
drome. Activation of NMDA and down stream PKC
phosphorylation are crucial in the development of neu-opathic pain. Activation of PKC could down regulate
the opioid receptor activity, and might be responsible
for the reduction of the efficacy of morphine's antino-
iciceptive effect in either opioid tolerance or neuropathic
pain. PTX ADP-ribosylation disrupts the Gi/Go protein-
coupled signal transduction, may lead to a predomin-
ance of the spinal excitatory receptor system. Many
studies have suggested that i.t. PTX-induced hyperal-
gesia results from a direct central mechanism, which
differs from the effect of peripheral nerve damage.
Animal and human studies had demonstrated a decreasing analgesic effect of μ-opioid agonists on neuropathic
pain, and one of the mechanisms might be down-regula-
tion of functional μ-opioid receptors but not reduction of
receptor number. In chronic constriction injury (CCI)
of sciatic nerve rats, hyperalgesia was accompanied by a
rightward shift of opioid's antinociceptive dose response curve. Similarly, we found that i.t. PTX injection pro-
duced a thermal hyperalgesia as well as a reduction of
morpheine analgesic effect. Collectively, i.t. PTX injec-
tion can be used as an animal model to study the central
mechanisms of neuropathic pain syndrome. In combina-
tion with a microdialysis technique, a long-term CSF bio-
chemical changes can also be observed.

The Cellular Mechanisms of PTX-induced EAA Release

At least, three possible mechanisms are proposed for the PTX-induced EAA release. Firstly, PTX inacti-
vates a G-protein, which is normally negatively regulat-
ing Ca^{2+} channels. Dolphin and Scott had demonstrated
that PTX enhanced the Ca^{2+} channel activity in dorsal
root ganglia. Secondly, PTX ADP-ribosylates Gi/Go

\( \text{opioid} \)
proteins, and thus increases the number of function of Ca$^{2+}$. Intracerebroventricular (i.c.v.) injection of PTX increases the number of dihydropyridine (DHP) binding sites (L-type calcium channel).\textsuperscript{43} Huston \textit{et al.} also found that PTX induced glutamate release from cerebellar granule neurons via increasing in the presynaptic L-type Ca$^{2+}$-binding sites.\textsuperscript{35} Thirdly, PTX-induced EAA release might be via PKC activation. Activation of Ca$^{2+}$ channels results in an increasing of intracellular Ca$^{2+}$, which, in turn, results in Ca$^{2+}$-dependent glutamate exocytosis.\textsuperscript{44} All these changes may activate PKC and the activated PKC increases NMDA receptors activity.\textsuperscript{45-47} In a study of NMDA-induced seizures, Duncan and Morgan suggested that PTX induced glutamate release and PKC activation were via increasing of intracellular Ca$^{2+}$ concentration.\textsuperscript{36} PKC acts as a positive feedback to increase EAAs release that is responsible for the neuronal hypersensitivity and reduction of the nociceptive threshold. The ability of PTX to increase EAAs release indicates that there is a tonic G-protein-mediated, accompanied with Ca$^{2+}$ entry, inhibition of exocytotic process in the presynaptic terminal.

\textit{PTX-induced EAAs Receptor Activation Contributes to The Nociceptive Hypersensitization}

Previous studies proposed that the development of neuropathic pain syndrome is involved in EAAs release, and subsequently would activate EAAs receptors in dorsal horn of the spinal cord.\textsuperscript{7,48} In our study, we found that this increase of aspartate and glutamate concentration in the CSF after i.t. PTX injection was accompanied by a thermal hyperalgesia, and it could be blocked by i.t. NMDA antagonist D-AP5.\textsuperscript{52} Somers and Clemente also demonstrated that chronic sciatic nerve constriction injury induced neuropathic pain syndrome was accompanied by an increasing of EAAs content in the synaptosome prepared from spinal cord dorsal horn.\textsuperscript{49} Furthermore, Cui \textit{et al.} found that an increase of basal release of EAAs as well as a decrease of GABA release in spinal dorsal horn was observed in rats following partial ligation of the sciatic nerve.\textsuperscript{50} This elevation of EAAs likely plays an important role in the development of painful symptoms. The symptoms of allodynia and hyperalgesia induced by i.t. injection of inflammatory agents with accompaniment of EAAs release, were similar to those observed in neuropathic pain syndrome.\textsuperscript{51} Ample evidence has demonstrated that EAA receptors participate in the development and maintenance of neuropathic pain syndrome, hyperalgesia and allodynia. Spinal administration of NMDA or non-NMDA receptor antagonists inhibits the neuropathic pain induced by sciatic nerve ligation,\textsuperscript{52} spinal cord injury,\textsuperscript{53} or brachial plexus transection.\textsuperscript{54} Collectively, from our previous reports, we suggest that the PTX-induced EAAs release promotes nociceptive signal transduction, presented as the thermal hyperalgesia, by activating the pain facilitatory system, thereby enhancing the nociceptive response and reducing opioids' analgesic effect.\textsuperscript{42,55}

\textbf{The Effect of Inhibitory Amino Acids on PTX-induced Neuropathic Pain Syndrome}

Inhibitory neurotransmitter receptors (opioid, GABA$\beta$, and A1-adenosine) are linked with PTX-sensitive inhibitory G-proteins, and inactivation of these inhibitory G-proteins may contribute to the activation of excitatory receptors, such as the NMDA receptors. In our recent study, PTX treatment was accompanied by a decrease of CSF IAA glycine concentration, which might unmask the excitatory receptors activity, and further promote the development of thermal hyperalgesia.\textsuperscript{42} Glycine is a principal inhibitory neurotransmitter in the spinal cord widely distributed in spinal dorsal horn, and plays an inhibitory role in nociceptive transmission.\textsuperscript{56,57} Two major subtypes of glycine receptors (GlyR\textsubscript{A} and GlyR\textsubscript{A}) had been demonstrated in rats. The neonatal-type receptors (GlyR\textsubscript{A}) are only expressed in the spinal cord of the newborn and replaced by the adult-type receptors (GlyR\textsubscript{A}) 2 weeks after birth, which exist only in the spinal cord and the brain stem of adult animals.\textsuperscript{58} The inhibitory effect of glycine is different from its function as the co-agonist with glutamate on the NMDA receptors. Activation of glycine receptors results in chloride currents and produces a post-synaptic hyperpolarization.\textsuperscript{59} Numerous studies support that the function of glycine in the spinal cord is related to nociceptive transmission. Simpson \textit{et al.} found that i.t. administration of glycine and related compounds attenuated the neuropathic pain syndromes of thermal and mechanical hyperalgesia.\textsuperscript{60} A reduction of glycine receptors was observed in the dorsal horn of the spinal cord in chronic sciatic nerve constriction-injured rats.\textsuperscript{61} Furthermore, Ishikawa \textit{et al.} demonstrated that i.t. injection of the glycine receptor antagonist strychnine caused touch-evoked allodynia.\textsuperscript{62} Moreover, they found that this allodynia was reversed by NMDA antagonists, and suggested that loss of glycine inhibition might facilitate the release of EAAs, and then enhance the post-synaptic excitability of the EAAs system.\textsuperscript{62} In our study, we demonstrated that i.t. PTX injection, at doses of 1 and 2 $\mu$g, resulted in a decreasing of spinal glycine level between days 2 and 8, and this reduction correlated with the development of thermal hyperalgesia.\textsuperscript{42} Moreover, in the same study, we also found that glycine administration inhibited this PTX-induced thermal hyperalgesia.\textsuperscript{42} A significant increase in the aspartate/glycine and aspartate/GABA ratios is reported in clinical patients with neuropathic pain state.\textsuperscript{63} Woolf and Man-


The Possible Role of NO in PTX-induced Thermal Hyperalgesia

Glutamate is known to activate NMDA receptors and results in activation of the NMDA-NO cascade which is believed to contribute to the induction of neuronal wind-up and central sensitization in the spinal cord. Kitto et al. found that systemic or i.t. administration of the NOS inhibitor, L-NAME, inhibited NMDA-induced hyperalgesia.\(^{64}\) Wiertelak et al. suggested that the spinal NMDA-NO cascade is involved in central hyperalgesia development.\(^{65}\) Furthermore, an up-regulation of NOS expression is seen after sciatic nerve transection.\(^{66}\) We also demonstrated that NOS inhibitor prevents the development of neuropathic pain syndromes.\(^{64}\) I.t. injection of L-arginine was shown to produce thermal hyperalgesia.\(^{57}\) Pretreatment with NO scavenger hemoglobin delayed the development of thermal hyperalgesia in rats with sciatic nerve constriction injury.\(^{58}\) These findings suggest that NO also plays a role in thermal hyperalgesia following nerve injury. However, in our recent study, we failed to observe any changes in either NO release or expression of NOS isoforms in the spinal cord in PTX-rats.\(^{42}\) Moreover, Calcutt and Chaplan also failed to demonstrate the effect of L-NAME on allodynia in diabetic or nerve-injured rats.\(^{59}\) Luo et al. found that tight ligation of spinal L5/L6 roots in Harlan Sprague-Dawley and Holtzman rats increased nNOS mRNA levels in the dorsal root ganglion, but not in the dorsal horn of the spinal cord.\(^{70}\) In the same study, they found that mechanical allodynia was observed in Harlan Sprague-Dawley rats after nerve ligation, and systemic nNOS inhibitor 7-nitroindazole (7-NI) treatment failed to prevent or reverse the allodynia. Collectively, we suggest that NOS in the dorsal horn of the spinal cord may indirectly contribute to the neuropathic pain development.

Effect of PKC Isoforms on PTX-induced Thermal Hyperalgesia

In our recent study, we found that PTX treatment increased spinal PKC\(\gamma\) expression, but not \(\alpha, \beta_1, \text{ or } \beta_2\) isoforms.\(^{71}\) In contrast to the PKC\(\alpha, \beta_1, \text{ and } \beta_2\) isoforms which are widely distributed in the superficial dorsal horn, the PKC\(\gamma\) isoform is expressed mainly in the inner part of laminae II of spinal cord.\(^{72}\) Palecek et al. demonstrated that PKC\(\gamma\) is expressed only in the excitatory interneurons, but not inhibitory interneurons in rat spinal cord, and they suggested that PKC\(\gamma\) is a potential candidate contributing to the spinal nociceptor hyper sensitization.\(^{73}\) In PKC\(\gamma\) knock-out mice, a significant reduction in tactile allodynia was observed after partial sciatic nerve ligation.\(^{74}\) Moreover, an up-regulation of PKC\(\gamma\) protein expression was also observed in neuropathic pain animals after loose ligation of sciatic nerve.\(^{75}\) In our recent study, we provided an additional evidence that i.t. PTX-induced PKC\(\gamma\) up-regulation and translocation, via NMDA receptor activation, might be responsible for the development of thermal hyperalgesia,\(^{71}\) and thus we suggested that the increased PKC\(\gamma\) content in the dorsal horn of the PTX-treated rat spinal cord might be a consequence of NMDA receptor activation, which induced up-regulation and translocation of PKC\(\gamma\) from the cytosol to the plasma membrane. This PKC\(\gamma\) up-regulation/translocation modifies synaptic transmission in the spinal dorsal horn, and contributes to the nociceptive hypersensitization.

The Role of NMDA-PKC Signaling in Reduction of Opioid's Analgesic Effect

Many studies had demonstrated that activation of NMDA-PKC signal transduction cascade played an important role in neuropathic pain syndrome.\(^{52,56,77}\) Chen and Huang had demonstrated that activation of PKC down-regulated opioid receptor activity.\(^{45}\) PKC activation was found to phosphorylate the \(\mu\)-receptor-coupled G-protein, thereby decreasing opioids' ability to inhibit adenyl cyclase with resultant loss of morphine's antinociceptive effect.\(^{78}\) Activation of NMDA receptors and subsequent PKC activation reduces the effectiveness of morphine's antinociception in PTX-rat, via uncoupling of G-protein from opioid receptors and disrupting the extracellular signaling into the cells.\(^{71,79}\) In Shen and Grain's theory, activation of opioid receptors may activate either the inhibitory Gi/Go or the stimulatory G-proteins.\(^{80,81}\) Turning on the Gs-coupled opioid receptor signal transduction may increase the PKC activity and attenuate morphine's antinociceptive effect. I.t. PTX breaks the balance between opioid receptor coupled inhibitory and excitatory G-proteins, in which the stimulatory Gs-protein signal transduction pathway becomes prominent and results in an increasing of EAAs release, and thus reduction of morphine's analgesic effect. The up-regulation of excitatory opioid effect by PTX treatment might play an important role in the reduction of opioid's analgesic effect. Similarly, in our previous study, we found that the PTX-induced thermal hyperalgesia was accompanied by an increase of PKC\(\gamma\) expression in both the synaptosomal membrane and cytosolic fractions of...
the rat lumbar spinal dorsal horn and both effects were inhibited by NMDA receptor antagonist D-AP5. In the same study, the thermal hyperalgesia was accompanied by an increasing of glutamate and aspartate levels in spinal CSF dialysates, and it was attenuated by PKC inhibitor chelerythrine. Chelerythrine not only prevented the PTX-induced thermal hyperalgesia and CCI-induced neuropathic pain but also acutely reversed morphine's analgesia in tolerant rats. Recently, we found that PKC inhibitor chelerythrine was able to reverse the reduction of morphine's antinociceptive effect, while chelerythrine itself did not produce any antinociceptive effect in naive animals. Similar to our study, Smith et al. found that non-selective PKC inhibitors Go-7874 and sangivamycin had no effect on morphine's antinociception in naive mice, but could reverse morphine's antinociception in morphine tolerant rats. The evidence suggests that PKC may directly down-regulate opioid receptor function, and thus results in a reduction of opioid's analgesic effect. The rationale for the involvement of NMDA-PKC signaling for the poor response of opioids to neuropathic pain is that the increasing of pre-synaptic EAAs release and activation of post-synaptic NMDA receptors, initiated by PTX treatment, may trigger PKC activation, and thus reducing opioid's analgesic effect. This positive feedback regulation of NMDA-PKC cascade is involved in the enhancement of opioid receptor phosphorylation and the reduction of opioid receptor's function. Taken together, our data suggest that PTX-induced thermal hyperalgesia and the loss of morphine's analgesia share a common cellular mechanism, at least in part, activation of the EAAs-PKC system. The Ca\(^{2+}\)-regulated intracellular PKC is likely a linkage between hyperalgesia and reduction of opioid's analgesic effect in PTX-rats.

Summary

From our serial studies, we found that i.t. administration of PTX induced thermal hyperalgesia in rats, which was accompanied by a prolonged increase in EAAs concentration and a decrease in IAA glycine concentration in spinal CSF dialysates. The changes in CSF aspartate, glutamate, and glycine concentrations were paralleled to the thermal hyperalgesia development. Furthermore, we found an up-regulation/translocation of spinal PKC\(\gamma\) after i.t. PTX treatment. Infusion of NMDA antagonist D-AP5 prevented both thermal hyperalgesia and increase of PKC\(\gamma\) isoform expression in PTX-treated rats. I.t. PTX treatment did not affect NO concentration in the CSF as well as the nNOS or iNOS protein expression. The PTX-induced hyperalgesia was reversed by i.t. NMDA antagonist D-AP5, glycine and PKC inhibitor chelerythrine. Pretreatment with PKC inhibitor chelerythrine 1 h prior to morphine challenge on day 5 after PTX treatment could prevent the morphine-precipitated spinal glutamate and aspartate release and reverse morphine's analgesic effect in PTX-rats. These results suggest that PTX-induced neuropathic pain syndromes are involved in EAAs, IAA's and PKC alteration. The cellular mechanisms of PTX-induced spinal nociceptive hypersensitization are depicted diagrammatically in Fig. 1. (1) PTX is able to ADP-ribosylate the post-synaptic opioid receptor coupled \(\alpha\)-subunit of inhibitory G-proteins (Go/Gi). It disrupts the inhibitory Gi/Go protein-coupled signal transduction (such as \(\mu\) receptors), thus breaks the balance between Gs and Gi protein signal transduction, leading to a predominance of the spinal excitatory receptor system. (2) PTX inhibits the activation of \(\mu\)-opioid receptor signal transduction (opening Gi protein-gated potassium channels and closing voltage-gated calcium channels). (3) PTX also presynaptically induces the release of glutamate (Glu) and aspartate (Asp), via exocytosis by modulating the L-type Ca\(^{2+}\) channel activity at the pre-synaptic nerve terminals. (4) The released glutamate and aspartate activate postsynaptic NMDA receptors and open the NMDA ligand-gated Ca\(^{2+}\) channel with increases of Ca\(^{2+}\) influx into postsynaptic neurons, and (5) followed by activation of PKC-\(\gamma\) isofrom. (6) A transient increase in NO production via activating the NOS or PKC activity. (7) Inactivation of PKC by PKC inhibitor chelerythrine inhibits PTX-induced nociceptive hypersensitization. As we know, extracellular EAAs released from the pre-synaptic nerve terminals are counter-balanced by glutamate transporters in neurons/glial cells which terminate the glutamatergic signaling, and thus protecting neurons from EAAs toxicity. (8) Activation of PKC was found to down-regulate the glutamate transporter activity and expression, which results in an accumulation of synaptic EAAs concentration. (9) PKC phosphorylates the \(\mu\)-receptor-coupled G-protein and results in down-regulate opioid receptor activity. (10) Activated PKC results in an enhanced release of EAAs, and it further excites the spinal nociceptive responses. (11) Decreasing of glycine release results in a reduction of post-synaptic strychnine sensitive chloride channel (GlyR) flow and subsequently decreasing of the inhibitory mechanisms, thereby, disrupting the balance between inhibitory and excitatory receptor system. (12) NO, a reactive, diffusible, and short-lived neuromodulator may act retrograde as an initiator to provide a positive feedback regulation of pre-synaptic neuron activity.

From this review, we know that activation of EAA receptors, particularly the NMDA receptors, are involved in the PTX-induced neuropathic pain. Glutamate level released from nerve terminals in the synaptic junction is counterbalanced by glutamate transporters in neurons and glial cells, which terminate the glutamatergic signal-
ing, and thus protecting neurons from the glutamate toxicity and neuroplasticity. In our recent study, we found that the PTX-induced thermal hyperalgesia may be related to the PKC, whose activation was found to downregulate the glutamate transporter activity and expression, and phosphorylation of NR2B subunit of the NMDA receptors. Therefore, examining the role of glutamate transporters and NMDA receptors phosphorylation in the behavior expression of PTX-rats may be another research direction for the neuropathic pain management.

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椎管內注射百日咳毒素動物模式探討神經病理性疼痛之細胞學機轉

溫志宏，張貽真，汪志雄

如同類鴉片耐藥性，神經病理性疼痛(neuropathic pain)通常對於類鴉片藥物的鎮痛效果反應並不好，且伴隨著痛覺過敏(hyperalgesia)及觸感痛(alldynia)。有關神經病理性疼痛的發生及預防的機制到目前為止仍不清楚。在我們過去的研究發現椎管內給予百日咳毒素不僅可以造成嗎啡止痛作用的降低，同時也會誘發對溫度痛覺過敏(thermal hyperalgesia)之行為。百日咳毒素經由對抑制型Gi或Go之鳥嘌呤蛋白之ADP-ribosylation，造成抑制性訊息的傳遞受到抑制，進而使原始抑制性及興奮性兩系統間之平衡受到破壞，結果造成興奮性的訊息傳遞相對地提高。目前有研究報告認為，椎管內注射百日咳毒素可以用來當作一個研究中樞性的神經病理性疼痛之動物模式。在系列的研究中，我們利用椎管內微透析及給藥之技術，探討在大白鼠椎管內注射百日咳毒素後，以脊髓為研究範圍來探討神經病理性疼痛之細胞學機轉。結果發現，在大白鼠椎管內注射百日咳毒素可以誘發發溫度疼痛過敏，且伴隨著脊髓液中興奮性胺基酸麩胺酸(glutamate)及天冬胺酸(aspartate)釋放量之增加，然而抑制性胺基酸甘胺酸(glycine)則明顯下降。但百日咳毒素對其它胺基酸如絲胺酸(serine)、麸醯胺(asparagine)、牛磺酸(taurine)、精胺酸(arginine)及一氧化氮的釋放並沒有影響，由此結果判斷可以去除百日咳毒素對細胞之毒殺作用。而由百日咳毒素所誘發的疼痛過敏行為可以被NMDA接受器之拮抗劑D-AP5、甘胺酸及蛋白質激酶C(protein kinase C)抑制劑chelerythrine所抑制。同時我們也發現，椎管內注射百日咳毒素可以誘發脊髓中疼痛相關神經元的細胞質及細胞膜之蛋白質激酶C-γ含量增加，但對α、β及βII等其它異構(isoenzyme)卻沒有影響。而百日咳毒素所造成蛋白質激酶C-γ表現量之增加則受到椎管內D-AP5灌注之抑制，然而D-AP5對正常大白鼠脊髓中蛋白質激酶C-α、β、βII及γ異構則並無影響。由以上系列研究結果顯示，椎管內注射百日咳毒素除了會誘發對溫度痛覺過敏之行為外，並伴隨著脊髓中興奮性胺基酸釋放量增加、抑制性胺基酸減少及蛋白質激酶C表現量之增加。而百日咳毒素所造成之溫度痛覺過敏及嗎啡止痛作用降低之現象，可以受到椎管內給予蛋白質激酶C抑制劑所阻斷。這些實驗結果顯示百日咳毒素所誘發的神經病理性疼痛(溫度痛覺過敏)，包含蛋白質激酶C、興奮性及抑制性胺基酸是參與其中之機轉。

關鍵詞：疼痛。百日咳毒素。興奮性胺基酸。Protein kinase C。

三軍總醫院麻醉部，國防醫學院麻醉學科