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一氧化氮媒介之電針刺激-引發尿道反射增益現象及參與之細胞內訊息傳遞路徑

研究成果報告(精簡版)

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Calcium/calmodulin-dependent kinase II mediates NO-elicited PKG activation to participate in spinal reflex potentiation in anesthetized rats

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Chen G-D, Peng M-L, Wang P-Y, Lee S-D, Chang H-M, Pan S-F, Chen M-J, Tung K-C, Lai C-Y, Lin T-B. Calcium/calmodulin-dependent kinase II mediates NO-elicited PKG activation to participate in spinal reflex potentiation in anesthetized rats. *Am J Physiol Regul Integr Comp Physiol* 294: R487–R493, 2008. First published November 28, 2007; doi:10.1152/ajpregu.00600.2007.—Calcium/calmodulin protein kinase (CaMK)-dependent nitric oxide (NO) and the downstream intracellular messenger cGMP, which is activated by soluble guanylate cyclase (sGC), are believed to induce long-term changes in efficacy of synapses through the activation of protein kinase G (PKG). The aim of this study was to examine the involvement of the CaMKII-dependent NO/sGC/PKG pathway in a novel form of repetitive stimulation-induced spinal reflex potentiation (SRP). A single-pulse test stimulation (TS; 1/30 Hz) on the afferent nerve evoked a single action potential, while repetitive stimulation (RS; 1 Hz) induced a long-lasting SRP that was abolished by a selective Ca^{2+}/CaMKII inhibitor, autocamtide 2-related inhibitory peptide (AIP). Such an inhibitory effect was reversed by a relative excess of nitric oxide synthase (NOS) substrate, L-arginine. In addition, the RS-induced SRP was abolished by pretreatment with the NOS inhibitor, N^G-nitro-L-arginine-methyl ester (L-NAME). The sGC activator, protoporphyrin IX (PPIX), reversed the blocking effect caused by L-NAME. On the other hand, a sGC blocker, 1H-[1, 2, 4]oxadiazolo[4, 3-α]quinoxalin-1-one (ODQ), abolished the RS-induced SRP. Intrathecal applications of the membrane-permeable cGMP analog, 8-bromoguanosine 3′,5′-cyclic monophosphate sodium salt monohydrate (8-Br-cGMP), reversed the blocking effect on the RS-induced SRP elicited by the ODQ. Our findings suggest that a CaMKII-dependent NO/sGC/PKG pathway is involved in the RS-induced SRP, which has pathological relevance to hyperalgesia and allodynia.

spinal reflex potentiation; soluble guanylate cyclase; cyclic monophosphate sodium salt monohydrate; spinal cord; windup

ACTIVITY-DEPENDENT REFLEX plasticity, the dynamic regulation of reflex strength by ongoing neural activities, is a fundamental component of normal CNS functions. Long-term potentiation (LTP), a form of well-known activity-dependent reflex potentiation in synaptic responses that occurs in the CA1 area of the hippocampus, is considered the base for some forms of learning and memory (45). In the hippocampal CA1 region, LTP is induced by brief tetanic stimulation of afferent glutamatergic fibers and is typically dependent on activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors (3).

A key-initiating event in LTP induction is the activation of Ca^{2+}/calmodulin protein kinase II (CaMKII) (14, 44, 30; 54). An increase in the intracellular Ca^{2+} concentration, partly by the influx through NMDA receptor channels, activates calmodulin, which in turn triggers the activation of CaMKII, causing it to bind to the postsynaptic density. It is a well-known fact that nitric oxide (NO) stimulates soluble guanylyl cyclase, and, in turn, produces intracellular cGMP and subsequently activates the protein kinase G (PKG) to induced activity-dependent reflex potentiation (53). Brenman and Bredt (6) reported that NO can be activated by CaMKII. Several investigators revealed that NO plays a role in LTP as indicated by experiments showing that LTP is eliminated or significantly reduced by inhibitors of NO synthetase (4, 12, 20, 46).

Not only has activity-dependent reflex plasticity been found in the brain, but also in other areas, including the spinal cord (40, 41, 58, 62). C-fiber-evoked spinal LTP characterized by a potentiation on an evoked potential in the spinal dorsal horn (25–29) and spinal central sensitization, the enhancement of responsiveness to nociceptive afferent fibers following injury or inflammation (58, 59, 61) has both been thought to be involved in postinflammatory hyperalgesia and tactile allodynia. In our laboratory, we have recently discovered that low-frequency repetitive stimulations may elicit activity-dependent spinal reflex potentiation (SRP) (36–38). By using pharmacological manipulations, we established that induction of SRP shares a similar glutamatergic NMDA receptor-dependent mechanism with spinal LTP and central sensitization (9, 32, 34). However, whether the CaMKII-dependent intracellular NO/soluble guanylate cyclase (sGC)/PKG cascade is involved in SRP is not fully known thus far. The central role of the intracellular CaMK/NO/sGC/PKG cascade in the control of synaptic efficacy also needs to be further investigated.

We hypothesize that intracellular Ca^{2+}/CaMKII is involved in the activity-dependent SRP. The activation of Ca^{2+}/CaMKII stimulates nitric oxide synthase (NOS) to produce NO and, in turn, results in the activation of sGC to induce cGMP and, therefore, activates PKG to induce activity-dependent SRP. We

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tested this hypothesis and examined the effects of pharmacological agents involved in this pathway on the physiological properties of SRP. The aims of the present study, therefore, were to determine: *1*) whether the intracellular Ca^{2+}/CaMKII is involved in the activity-dependent SRP, and *2*) If the answer is yes, whether the stimulatory effect of Ca^{2+}/CaMKII on intracellular NO, sGC, and PKG participates in the mechanisms of SRP induction.

MATERIALS AND METHODS

Animal preparations. Adult Wistar rats weighing 200 to 300 g were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). Animal care and experimental protocol were in accordance with the guidelines of the National Science Council of Taiwan (1997), and the experimental protocol was approved by the committee of experimental animal research of Chung-Shan Medical University. All efforts were made to minimize both animal suffering and the number of animals used throughout the experiment. The trachea was intubated to keep the airway clear. A PE-50 catheter (Portex, Hythe, Kent, UK) was placed in the left femoral vein for the administration of anesthetics when needed. Body temperature was kept at 36.5 to 37.0°C by an infrared light. A midline abdominal incision was made to expose the pelvic viscera. A wide-bore cannula was inserted into the lumen of the urinary bladder at the apex of the bladder dome and was secured with cotton thread. The open end of the bladder cannula drained freely to avoid urine accumulation within the bladder. Both ureters were ligated distally and cut proximally to the ligation site. The left pelvic nerve was dissected carefully from the surrounding tissues and was transected as distally as possible for stimulation. The stimulated nerve and the electrodes were bathed in a pool of warm paraffin oil (37°C) to prevent drying. The rats were monitored for corneal reflex and response to noxious stimulation to the paw throughout the experiment. If either was present, a supplementary dose (0.4 g/kg) of anesthetic was given through the venous catheter. At the end of the experiment, the animals were euthanized by an intravenous injection of potassium chloride saturation solution under deep anesthesia.

Intrathecal catheter. The occipital crest of the skull was exposed and the atlanto-occipital membrane was incised at the midline with the tip of an 18-gauge needle. A PE-10 catheter was inserted through the slit and passed caudally to the sixth lumbar level of the spinal cord. The volume of fluid within the cannula was kept constant at 10 μl in all of the experiments. Each single 10-μl volume of drug solution was administered, followed by a 10-μl flush of artificial cerebrospinal fluid. At the end of the experiment, a laminectomy was performed to verify the location of the cannula tip.

Electromyogram activity. The oligo-unit recording technique was used in this study to record electromyogram activity. Epoxy-coated copper wire (50 μm; M. T. Giken, Tokyo, Japan) electromyogram electrodes were placed in the external urethra sphincter. This was performed using a 30-gauge needle with a hooked electromyogram electrode positioned at the tip. The needle was inserted into the sphincter ~1 to 2 mm lateral to the urethra and then was withdrawn, leaving the electromyogram wires embedded in the muscle. The activity of the external urethra sphincter electromyogram (EUSE) was amplified 20,000-fold and filtered (high-frequency cutoff at 3,000 Hz and low at 30 Hz, respectively) by a preamplifier (Grass P511AC, Cleveland, OH) and was then continuously displayed on an oscilloscope (Tectronics TDS 3014; Wilsonville, OR) and the recording system (Biopac, MP30; Santa Barbara, CA). Because the oligo-unit recording technique used in this study can differentiate each action potential evoked by the electric shock from background noise, we therefore analyzed reflex activity by directly counting the firing frequency rather than integrating the area under the curve after the raw activity had been rectified, a method that is commonly used in the field activity recording technique.

Drugs. The following drugs (all purchased from Sigma-RBI, Natick, MA) were used in this study: calmodulin kinase inhibitor, autocamtide 2-related inhibitory peptide (AIP; 100 μM, 10 μl); the substrate of NO synthetase, L-arginine (50 mg/ml, 10 μl); the NOS inhibitor, N^G-nitro-L-arginine-methyl ester (L-NAME; 100 μM, 10 μl); selective soluble guanylate cyclase (sGC) activator, N^G-nitro-D-arginine-methyl ester (D-NAME; 100 μM, 10 μl); an inactive isoform to L-NAME, protoporphyrin IX (PPIX; 100 μM, 10 μl), selective sGC blocker, 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ; 5 mg/ml, 10 μl), and cell-permeable cGMP analog, 8-bromoguanosine-3′,5′-cyclic monophosphate (8-Br-cGMP; 100 μM, 10 μl; Biolog Life Sciences, La Jolla, CA). All drugs were dissolved in artificial cerebrospinal fluid (in mM): 118 NaCl, 3 KCl, 25 $NaHCO_3$, 1.2 NaH_2PO_4, 1 $MgCl_2$, 1.5 $CaCl_2$, 10 glucose, pH = 7.4. The solution of identical volume to tested agents was dispensed to serve as the vehicle. At the end of the experiment, the location of the injection site was marked by an injection of Alcian blue (2%, 3 μl). The volume of drug injection into the spinal cord using this method has been reported to spread 0.2 to 0.5 mm from the site of injection (8–10). Therefore, a cannula positioned more than 0.2 mm from the intended site of injection was not included in the statistical analysis.

Experimental arrangement. The schematic arrangement of the EUSE recording, as well as the pelvic afferent nerve fiber stimulation, is shown in Fig. 1*A*. Once the electrodes' positions were optimized, recording of the reflex activity was started. An electric current of square wave pulse with a pulse duration of 0.1 ms was applied from a stimulator (Grass S88; Grass Instruments, Cleveland, OH) through a stimulus isolation unit (Grass SIU5B), and a constant current unit (Grass CCU1A) was applied to the afferent nerve by the stimulating electrodes. The protocol for assessing the effects of electrical stimulation and different kinds of reagents on the reflex activity was as follows. *1*) For test stimulation (TS), electric shocks at fixed suprathreshold strengths (10 to 15 V) were repeated at intervals of 30 s (TS). This frequency of stimulation was used to sample data for 10 min because it did not result in response facilitation. An electric intensity of stimulation that caused a single spike action potential in the reflex activity was used to standardize the baseline reflex activity. *2*) For repetitive stimulation (RS), after the baseline reflex activity had been established by the test stimulation, RS at 1 Hz for 10 min (repetitive stimulation, RS) with an electric intensity identical to the test stimulation was applied to induce SRP. *3*) For the agents test, after an equilibrium period (10 min), tested agents were injected via the intrathecal catheter and then the repetitive stimulation was used once again to induce SRP. The excitability of the reflex activity was assessed by recording the numbers of action potentials in the electromyogram under test stimulation or repetitive stimulation with or without intrathecal application of the tested agents.

Data analysis. All data in the text and figures are expressed as means ± SE. Statistical analysis of the data was performed by means of ANOVA. In all cases, a difference of $P < 0.05$ was considered statistically significant.

RESULTS

Baseline reflex activity and spinal reflex potentiation. The reflex excitability was assayed by recording the action potentials in the EUSE activities resulting from the pelvic afferent nerve stimulations with electrical shocks of pulses. Initial experiments were performed in an attempt to reproduce results showing that test stimulation evoked a baseline reflex activity, while repetitive stimulation produced reflex potentiation (36–38). The upper tracing in Fig. 1*B* demonstrates the recording showing that a baseline reflex activity with single action potentials was elicited by the TS (1/30 Hz for 10 min). The mean reflex time for the afferent nerve stimulations to evoke an action potential in the electromyogram was 55.48 ± 2.45 ms.

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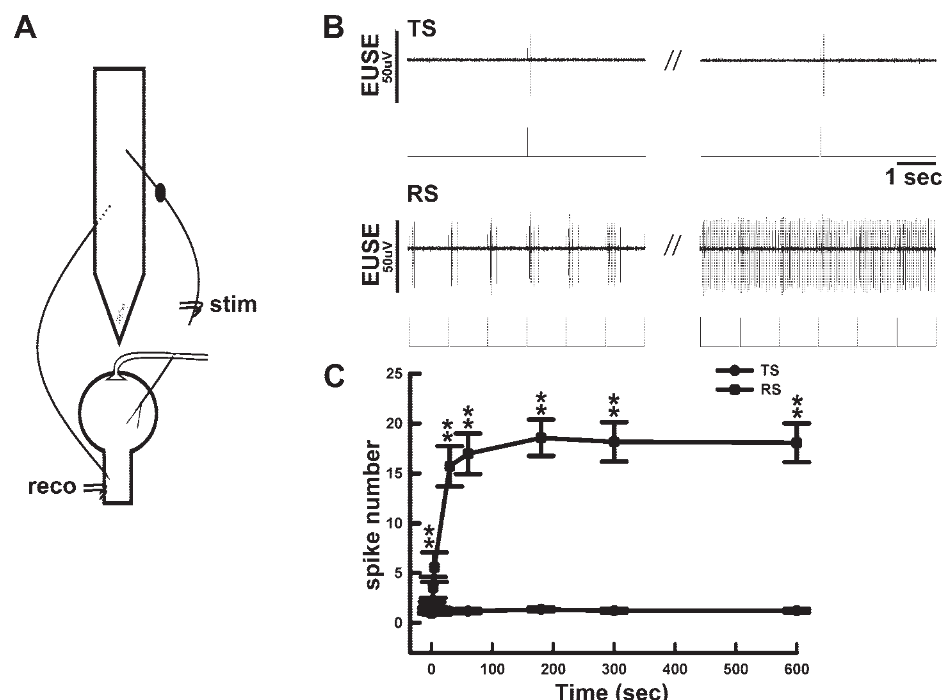

Fig. 1. A repetitive stimulation-induced spinal reflex potentiation. *A*: a schematic arrangement of the external urethra sphincter electromyogram (EUSE) recording (reco) in response to afferent pelvic nerve stimulation (stim). *B*: single pulses of the pelvic afferent nerve test simulation (TS; 1/30 Hz, marked by ticks at the bottom) evoked single action potentials, while a repetitive stimulation (RS; 1 Hz, marked by ticks at the bottom) induced reflex potentiation in EUSE. Tracings before and after the break symbols show the evoked reflex activity at and 10 min following the stimulation onset, respectively. *C*: summarized data showing the test stimulation (circle) evoked a constant spike number in the reflex activity, while the repetitive stimulation induced a reflex potentiation (square, $**P < 0.01$ to TS, $n = 54$).

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The summarized data in Fig. 1*C* show that the reflex activity evoked by the test stimulation varied little over the stimulating period. On the contrary, as shown in the lower tracing of Fig. 1*B*, a longer-lasting reflex potentiation was induced by the RS (1 Hz, for 10 min) with the identical intensity to the test stimulation during the stimulation period. The summarized data in Fig. 1*C* show that the evoked activity gradually increased following the repetitive stimulation onset, then reached a plateau at about 3 min, and then maintained this level until the cessation of stimulation. After the stimulation period, the evoked activity usually recovered to the baseline level within 1 min (ranging from 30 to 120 s, mean = 54.00 ± 17.49 s; n = 5) when the afferent fiber was continuously stimulated with the test stimulation following the repetitive stimulation offset. Mean spike numbers induced by RS increased significantly (18.07 ± 0.98 spikes/stimulation, counted at 10 min following stimulation onset, $**P < 0.01$, $n = 54$) compared with the baseline activities induced by the TS (1.22 ± 0.09 spikes/stimulation, counted at 10 min following stimulation onset).

Ca^{2+}/calmodulin kinase inhibitor. The involvement of Ca^{2+}/CaMKII in RS induced reflex potentiation was investigated by the intrathecal injection of a selective Ca^{2+}/CaMKII inhibitor, AIP, before repetitive stimulation onset. As shown in Fig. 2*A*, pretreated AIP abolished the repetitive stimulation-induced reflex potentiation. We then tested the possibility that the Ca^{2+}/CaMKII would activate NO synthases and, in turn, facilitate NO synthesis to mediate the repetitive stimulation-induced reflex potentiation. A relative excess of L-arginine, a substrate of NOS, was tested intrathecally after the repetitive stimulation-induced reflex potentiation had been blocked by AIP. The lower tracing in Fig. 2*A* shows that a relative excess of L-arginine reversed the blockage on repetitive stimulation-induced reflex potentiation caused by pretreated AIP (RS+AIP+ARG). Fig. 2*B* summarizes the mean spike numbers in the reflex activity counted 10 min following stimulation onset. The spike numbers decreased significantly in repetitive stimulation with pretreated AIP (RS+AIP, $\#\#P < 0.01$, $n = 7$) compared with repetitive stimulation alone (RS). In addition, the blocking effect on the spinal reflex potentiation elicited by AIP was reversed by the L-arginine (RS+AIP+ARG, $++P < 0.01$, $n = 7$).

NOS inhibitor. The involvement of NOS in RS-induced reflex potentiation was further investigated. We injected L-NAME, an NOS inhibitor, and D-NAME, an inactive isoform, before a repetitive stimulation-induced reflex potentiation had been established. Pretreatment of D-NAME exhibited no effect on the evoked activity (data not shown), while L-NAME blocked the repetitive stimulation-induced spinal reflex potentiation (Fig. 3*A*, RS+NAME). It was anticipated that stimulating NO would activate the sGC, which would lead to an increase in the endogenous cellular messenger, cGMP. We then tested this hypothesis by intrathecally applying the sGC activator, PPIX, after the repetitive stimulation-induced reflex potentiation had been abolished by pretreated L-NAME. As shown in the lower tracing in Fig. 3*A*, PPIX reversed the abolition of repetitive stimulation-induced reflex potentiation caused by pretreated L-NAME (RS+NAME+PPIX). Figure 3*B* summarizes the mean spike numbers in the reflex activity counted 10 min after stimulation onset. The spike numbers decreased significantly in RS with pretreated L-NAME (RS+NAME, $\#\#P < 0.01$, $n = 7$) compared with RS alone. In addition, the blocking effect on the spinal reflex potentiation elicited by L-NAME was reversed by PPIX (RS+L-NAME+PPIX, $++P < 0.01$, $n = 13$).

Soluble guanylate cyclase inhibitor. The involvement of sGC in repetitive stimulation-induced reflex potentiation was further investigated by intrathecally injecting ODQ, a sGC inhibitor, before the repetitive stimulation-induced reflex potentiation had been established. As shown in Fig. 4*A*, pretreated ODQ abolished the repetitive stimulation-induced re-

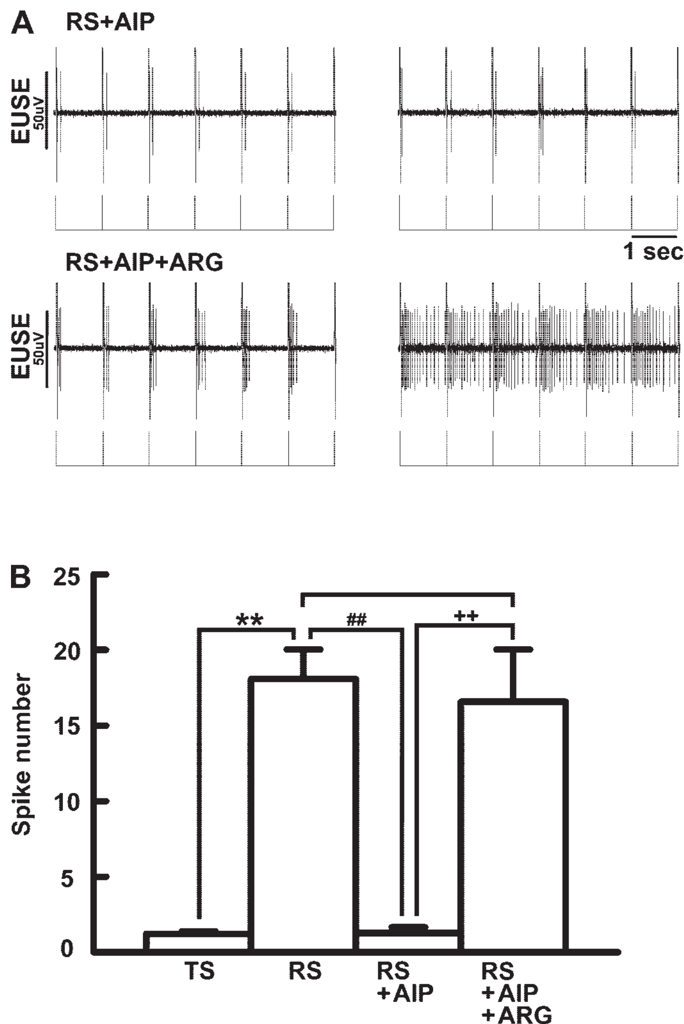

Fig. 2. Ca^{2+}/calmodulin protein kinase II (CaMK II) facilitate nitric oxide (NO) synthesis to mediate the RS-induced reflex potentiation. *A*: pretreated autocamtide 2-related inhibitory peptide (AIP) abolished the RS-induced reflex potentiation (RS+AIP). A relative excess of L-arginine, administered after the repetitive stimulation-induced reflex potentiation had been blocked, reversed the blockage caused by AIP (RS+AIP+ARG). Tracings before and after the break symbols show the evoked reflex activity at and 10 min following the stimulation onset, respectively. The ticks at the bottom indicate stimulation. ARG, L-arginine. *B*: summarized data show the mean spike numbers in the reflex activity counted 10 min after stimulation onset decreased significantly in repetitive stimulation with pretreated AIP (RS+AIP, ##$P < 0.01$ to RS, $n = 7$) when compared with RS alone. L-arginine reversed the blocking effect elicited by AIP (RS+AIP+ARG, ++$P < 0.01$ to RS+AIP, $n = 7$). **$P < 0.01$ to TS.

flex potentiation (RS+ODQ). It has been well established that sGC activation leads to an increase in endogenous cGMP and, in turn, activates PKG and therefore produces activity-dependent reflex potentiation. To test this hypothesis, we applied the membrane-permeable cGMP analog 8-Br-cGMP after the repetitive stimulation-induced reflex potentiation had been blocked by ODQ. As shown in the lower tracing in Fig. 4*A*, 8-Br-cGMP reversed the blocking effect on the repetitive stimulation-induced reflex potentiation elicited by ODQ (RS+ODQ+cGMP). Fig. 4*B* summarizes the mean spike numbers counted 10 min following the stimulation onset in the reflex activity evoked by RS alone, RS with pretreated ODQ (RS+ODQ), and RS with pretreated ODQ followed by 8-Br-cGMP. The spike numbers decreased significantly in repetitive stimulation with ODQ (RS+ODQ, ##$P < 0.01$, $n = 13$) compared with RS alone. In addition, the blocking effect on the reflex potentiation caused by ODQ was reversed by 8-Br-cGMP (RS+ODQ+cGMP, ++$P < 0.01$, $n = 11$).

DISCUSSION

This study addressed a fundamental prediction of the hypothesis that Ca^{2+}/CaMKII is involved in spinal activity-dependent SRP. In addition, an intracellular NO/sGC/PKG cascade serves as a signaling pathway in SRP induction, namely, the Ca^{2+}/CaMKII-dependent NO activity that activates the sGC/PKG pathway and plays a substantial role in the induction of spinal activity-dependent SRP.

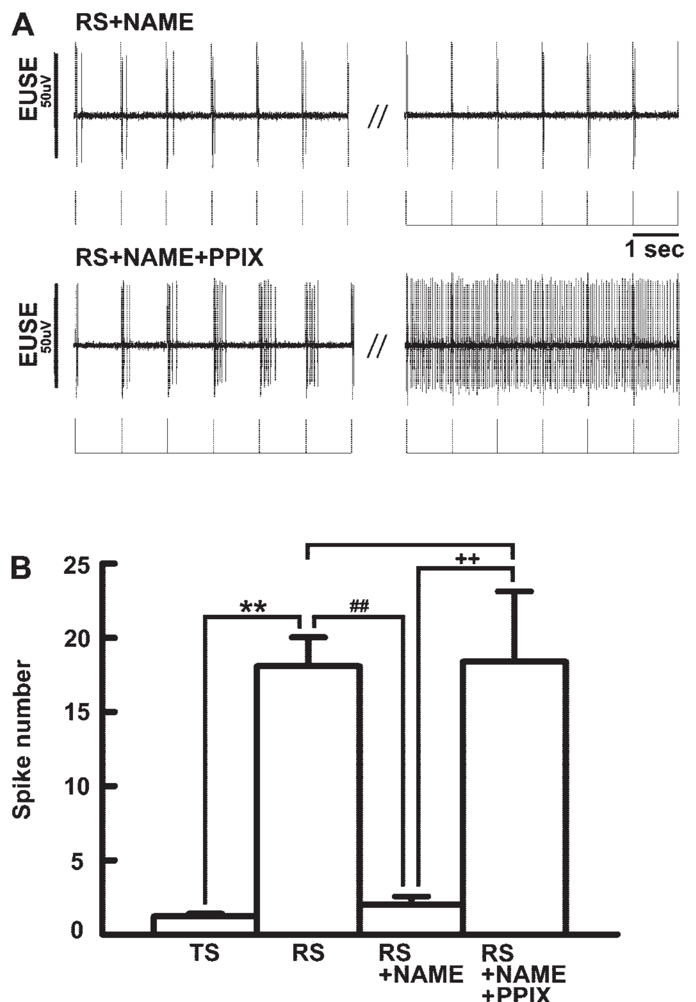

Fig. 3. NO activates the soluble guanylate cyclase (sGC) to mediate repetitive stimulation-induced reflex potentiation. *A*: pretreated N^G-nitro-L-arginine-methyl ester (L-NAME) blocked the RS-induced spinal reflex potentiation (RS+NAME). Intrathecal protoporphyrin IX (PPIX), after the reflex potentiation had been abolished, reversed the abolition caused by pretreated L-NAME (RS+NAME+PPIX). Tracings before and after the break symbols show the evoked reflex activity at and 10 min after the stimulation onset, respectively. The ticks at the bottom indicate stimulation. *B*: summarized data show the mean spike numbers in the reflex activity counted 10 min after stimulation onset decreased significantly in repetitive stimulation with pretreated L-NAME (RS+NAME, ##$P < 0.01$ to RS, $n = 7$) compared with RS alone. Whereas PPIX reversed the blocking effect elicited by L-NAME (RS+L-NAME+PPIX, ++$P < 0.01$ to RS+NAME, $n = 13$). **$P < 0.01$ to TS.

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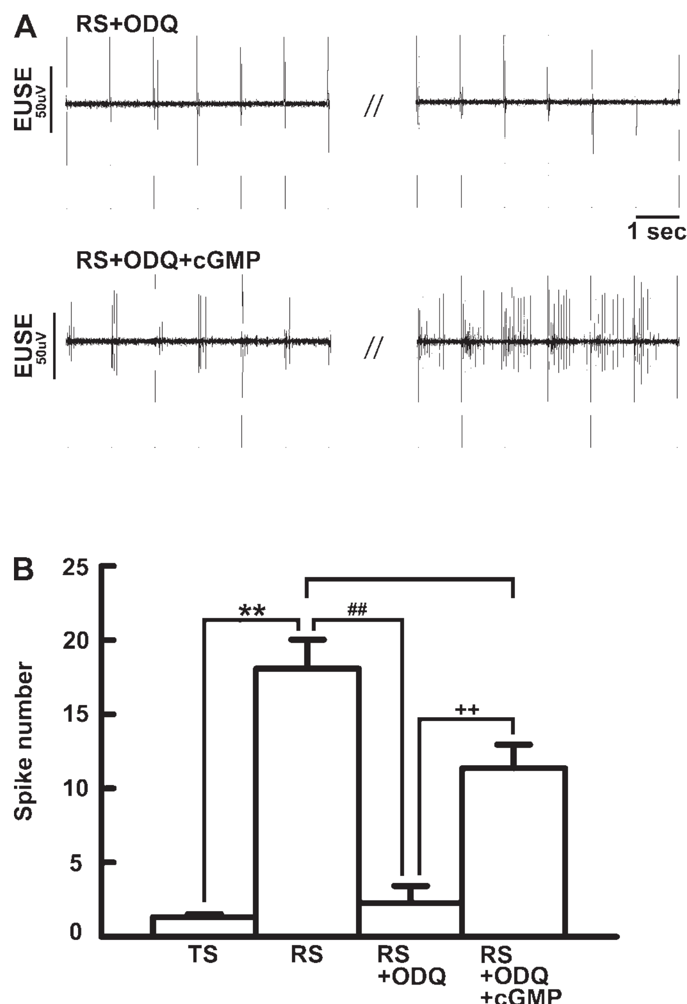

Fig. 4. sGC activation leads to an increase in endogenous cGMP to produce repetitive stimulation-induced reflex potentiation. *A*: pretreatment of 1H-[1, 2, 4]oxadiazolo[4, 3-α]quinoxalin-1-one (ODQ) abolished the repetitive stimulation-induced reflex potentiation (RS+ODQ). Intrathecal 8-Br-cGMP, after the reflex potentiation had been blocked, reversed the blocking effect elicited by the ODQ (RS+ODQ+cGMP). Tracings before and after the break symbols show the evoked reflex activity at and 10 min after the stimulation onset, respectively. The ticks at the bottom indicate stimulation. *B*: summarized data show the mean spike numbers in the reflex activity counted 10 min after the stimulation onset, evoked by the repetitive stimulation with ODQ, decreased (RS+ODQ; ##$P < 0.01$ to RS, $n = 13$) compared with RS alone. In addition, 8-Br-cGMP reversed the blocking effect on the reflex potentiation (RS+ODQ+cGMP).

Role of Ca^{2+}/CaMKII. It is widely accepted that glutamatergic NMDA receptor-dependent neural transmission underlies activity-dependent reflex plasticity (52). Stimulation of the NMDA receptor has been shown to mobilize internal calcium (Ca^{2+}) stores and, therefore, regulates signaling pathways downstream of the elevation in intracellular Ca^{2+} (15, 63). Among the intracellular molecules shown to play a critical role in reflex plasticity mechanisms is the enzyme Ca^{2+}/CaMKII (22). This protein is highly enriched in postsynaptic densities, and numerous observations indicate that it contributes to the increase in synaptic efficacy (2, 14, 18, 35, 39, 57). Several recent studies have provided plausible mechanisms by which CaMKII could enhance synaptic transmission and account for LTP (39, 55). Through the machinery responsible for the recycling of receptors, CaMKII, together with other kinases, probably regulates the number and function of receptors expressed at postsynaptic densities (2, 47, 51, 55).

Role of NO in spinal reflex potentiation. In addition to regulating receptor cycling, CaMKII may activate NOS signaling leading NO synthesis (49). The investigation of LTP using NO synthase inhibitors, NO scavengers, or NO synthase gene deletion to reflect the loss of an NO signaling pathway within neurons has shown an impaired LTP (21). On the other hand, an exogenous NO substrate generated a long-term potentiation in hippocampal slices in a guinea pig (66, 68) and a rat (43). Similar to these studies, our results showed that repetitive stimulation-induced SRP was completely blocked by the NO synthase inhibitor, L-NAME. On the other hand, in this in vivo study, the inhibition caused by the CaMKII blocker on SRP could be relieved by adding a relative excess of NO synthase substrate. These results provide the authentic molecule support for our proposal that the NO release, which is downstream from the CaMK, was responsible for SRP.

Role of sGC in spinal reflex potentiation. The intracellular pathway for the signal transduction of the gaseous messenger NO, in most forms of synaptic plasticity, is through sGC activation, leading to cGMP accumulation (7, 11, 16, 21). In the present in vivo experiments, we demonstrated that the sGC antagonist ODQ abolished the SRP induced by the repetitive stimulation. This result correlates with previous studies investigating LTP using hippocampus slices (50, 56, 67, 68). In addition, intrathecal PPIX, a selective sGC activator, reversed the blocking effect caused by L-NAME, indicating that NO may mediate the repetitive stimulation-induced SRP through sGC activation. Furthermore, in this study, a bolus of 8-Br-cGMP reversed the blocking effect elicited by ODQ. This result is consistent with previous studies that have demonstrated continuous perfusion with a cGMP analog overcomes the effect of NO synthase inhibition in hippocampal LTP (20). All these results implicate a sGC/cGMP/PKG-dependent mechanism engaged by NO-mediated repetitive stimulation-induced SRP.

There is direct evidence from hippocampal cultures that NO can potentiate synaptic transmission through a presynaptic mechanism involving cGMP and cGMP-dependent protein kinase (1, 53). At the same time, data from hippocampal slices indicate that NO can act postsynaptically to potentiate neurotransmission (31) and that its involvement in late LTP is mediated by cGMP-dependent activation of the transcription factor cAMP response element-binding protein in postsynaptic neurons (42). Also pertinent to a postsynaptic site of action, in situ hybridization suggests that the dominant subtype of the sGC expressed in the hippocampal pyramidal neurons is the α2β1 isoform (17), and this isoform associates with the postsynaptic density-95 protein (48), providing the substrate necessary for NO to act postsynaptically through cGMP. Bon and Garthwaite (5) investigated the role of NO on the LTP in hippocampal slices and suggested a presynaptic action may be responsible for an acute enhancement of synaptic efficacy caused by NO. Furthermore, a postsynaptic action may mediate the longer-term changes in the reflex plasticity. In the present study, the reflex potentiation was elicited gradually and plateaued at about 3 min following the repetitive stimulation onset. We conjecture that according to the latency necessary for a stable SRP to be established, this novel form of reflex plasticity

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demonstrated in this study may be mediated postsynaptically by NO. However, the detailed spinal mechanism needs further investigation using an intracellular recording technique to be elucidated, as our in vivo study was limited by the multiple-unit recording technique.

In the present study, the repetitive stimulation-induced SRP was characterized by a similar NO-dependent sGC/cGMP/PKG mechanism with LTP in the CA1 area of the hippocampus, indicating a mechanistic link between the repetitive stimulation-induced SRP and the hippocampal LTP (38). This assumption is in accordance with a report that shows that the strength of primary afferent transmission might potentiate following tetanic peripheral inputs (47a). However, LTP can last for several hours or even longer (45a, 47b), while the firing in the electromyogram in the present study usually lasted less than 1 s. In addition, the multiple-fiber recording technique used in this study was a limitation, so further investigation of the synaptic efficacy on dorsal horn neurons with the spinal cord is needed to discover whether this enhancement is mediated by an "LTP-like" synaptic transmission or not. On the other hand, a progressive increase in firing was noted during the early stage of the repetitive stimulation onset; therefore, the SRP presented in this study may be related to the windup phenomenon. However, the repetitive stimulation-induced SRP lasted for less than 10 min after the cessation of repetitive stimulation. This duration is quite short when compared with investigations on the spinal windup phenomenon (59, 60); therefore, the underlying mechanism and the physiological/pathological relevance of the repetitive stimulation-induced SRP need further investigation.

Perspectives and Significance

Neural plasticity, including LTP and central sensitization, are commonly considered relevant to postinflammatory hyperalgesia and tactile allodynia formation (23–29, 40, 41). A histological study investigating LTP in the spinal cord has demonstrated that NO synthesis is essential for induction of spinal LTP (23). Furthermore, electrophysiological studies of LTP induced by nociceptive C-fibers suggest that the involvement of NO production in the spinal cord is caused by NOS activation, as well as the participation of the downstream signal, cGMP, (65) in the spinal LTP (64). If so, and should NO be necessary for some types of neural plasticity mediating nociception processing at the spinal cord level, disruption of the NO signaling pathway should be one of the key strategies that offers a gateway to alleviate neuropathic and inflammatory pain.

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一、參加會議經過

第九十五屆的 Annual Meeting of the Japanese Urological Association （JUA）年會在日本神戶召開，由4月14日至17日共三日，其中4月14日主要的節目內容為workshop，15日～17日為正式議程，我們共投了一篇論文 TRPV1 mediates the uterine capsaicin-induced NMDA NR2B-dependent cross-organ reflex sensitization in anesthetized rats。被選為 poster。

二、與會心得

與會參加時，大會給予我們文章的評論如下：

Uterus-Urethral Cross Sensitization May Play Pain Role, Too

跨臟器敏感化（Cross-organ sensitization)，現今在間質性膀胱炎的研究上為一項熱門的研究議題，而其中主要是在探討直腸與膀胱間的交互影響關係。而此篇的生理學者們，主要是專注在尿道與膀胱間路徑的探討. 他們去測試以下這個想法的可行性：辣椒素（Capsaicin）活化來自於子宮的神經而使下泌尿道更敏感。辣椒素慢慢注入實驗動物的子宮會使股盤和尿道間的反射更活化。而這個現象會被前處理 Capsazepine（其為TRPV1的抑制劑)，於子宮內緩解。除此之外，給予 NMDA 及選擇性的 NMDA NR2B 的抑制劑也會阻斷使跨器官反射敏感化。承以上實驗結果，TRPV1的接受器在一個以glutamatergic NMDA依賴性，跨器官敏感化其發生在子宮和下泌尿道之間扮演重要的角色。

TRPV1 mediates the uterine capsaicin-induced NMDA NR2B-dependent cross-organ reflex sensitization in anesthetized rats. Am J Physiol Renal Physiol. 2008 Jul 16. [Epub ahead of print]

Peng HY, Chang HM, Chang SY, Tung KC, Lee SD, Chou D, Lai CY, Chiu CH, Chen GD, Lin TB.

Cross-organ sensitization has been a hot topic in basic IC research, mainly that between colon and bladder. These physiologists, however, are looking at pathways between the uterus and bladder. They tested the idea that exciting the “hot pepper” sensitive nerves from the uterus would sensitize the lower urinary tract. Capsaicin instilled into the uterus of experimental animals sensitized pelvic and urethra reflex activity. That was prevented by pretreating the uterus with capsazepine, which blocks the “hot pepper” (TRPV1) receptor. In addition, administering an NMDA and a selective NMDA NR2B blocker also thwarted the cross-organ reflex sensitization. These results show that TRPV1 plays a crucial role in the mediating the glutamatergic

NMDA-dependent cross-organ sensitization between the uterus and lower urinary tract, said the authors.