Hydrogen sulfide inhibits opioid withdrawal-induced pain sensitization in rats by down-regulation of spinal calcitonin gene-related peptide expression in the spine

Hai-Yu Yang*, Zhi-Yuan Wu and Jin-Song Bian
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Abstract

Hyperalgesia often occurs in opioid-induced withdrawal syndrome. In the present study, we found that three hourly injections of DAMGO (a μ-opioid receptor agonist) followed by naloxone administration at the fourth hour significantly decreased rat paw nociceptive threshold, indicating the induction of withdrawal hyperalgesia. Application of NaHS (a hydrogen sulfide donor) together with each injection of DAMGO attenuated naloxone-precipitated withdrawal hyperalgesia. RT-PCR and Western blot analysis showed that NaHS significantly reversed the gene and protein expression of up-regulated spinal calcitonin gene-related peptide (CGRP) in naloxone-treated animals. NaHS also inhibited naloxone-induced cAMP rebound and cAMP response element-binding protein (CREB) phosphorylation in rat spinal cord. In SH-SY5Y neuronal cells, NaHS inhibited forskolin-stimulated cAMP production and adenylate cyclase (AC) activity. Moreover, NaHS pre-treatment suppressed naloxone-stimulated activation of protein kinase C (PKC)α, Raf-1, and extracellular signal-regulated kinase (ERK) 1/2 in rat spinal cord. Our data suggest that H2S prevents the development of opioid withdrawal-induced hyperalgesia via suppression of synthesis of CGRP in spine through inhibition of AC/cAMP and PKC/Raf-1/ERK pathways.

Introduction

Opioid drugs are the most effective analgesics used to treat moderate to severe pain. However, chronic opioid-induced drug dependence remains a severe obstacle for clinic use. Abrupt abstinence or withdrawal from opioid drugs causes a series of severe adverse symptoms, which keep drug-dependent individuals craving continued opioids. One of the core withdrawal symptoms is pain sensitivity (pain sensitization or hyperalgesia), which has been well documented both in animal studies and in clinical reports (Lee et al., 2011). Unfortunately, very few drugs can be used to block these withdrawal symptoms.

It has been known that withdrawal hyperalgesia may be attributable to a relative enhancement of pain sensitivity of spinal nociceptive neurons, which may result from the release of pain-related neurotransmitters, such as calcitonin gene-related peptide (CGRP) (Trang et al., 2005). CGRP releases from the central termini of the sensory neurons and activates secondary dorsal horn neurons that, in turn, convey pain sensation to the brain (Benarroch, 2011). Chronic opioid treatment, such as morphine, significantly increases CGRP expression in the spinal dorsal horn of rats and may underlie opioid-induced pain sensitization (Ma et al., 2000; Gardell et al., 2002; Ossipov et al., 2003). More importantly, blockade of cAMP pathway or knockdown of spinal PKA activity with siRNA significantly attenuated the up-regulated spinal CGRP expression (Bie et al., 2005; Tumati et al., 2011), suggesting that the cAMP pathway tightly regulates the synthesis and release of CGRP.

Hydrogen sulfide (H2S), known as a noxious gas toxic to the central nervous and respiratory systems, has been recognized as a new biological gaseous transmitter alongside nitric oxide and carbon monoxide (Szabo, 2007). In mammalian cells, endogenous H2S is synthesized by two pyridoxal-5′-phosphate dependent enzymes: cystathionine-γ-lyase (CSE) and cystathione-β-synthase (CBS), and one pyridoxal-5′-phosphate-independent enzyme...
3-mercaptopuruvate sulfurtransferase (3-MST). H2S is mainly synthesized by CBS and 3-MST in the central nervous system. Different from CBS, which was found to be expressed in both astrocytes (Enokido et al., 2005) and neurons (Robert et al., 2003), 3-MST is mainly expressed in neurons (Shibuya et al., 2009). Since H2S presents in the brain at relatively high levels, it may function as a neuromodulator (Enokido et al., 2005; Ichinohe et al., 2005), highlighting the potential importance of endogenous H2S to the regulation of neuronal activity.

It has been reported that sodium hydrosulfide (NaHS), a donor of H2S, significantly suppressed adenylylate cyclase (AC) activity and therefore decreased forskolin-stimulated cAMP accumulation in different cell lines and tissues (Lim et al., 2008; Yong et al., 2008; Lu et al., 2010). We and other laboratories previously reported that application of NaHS significantly alleviated naloxone-precipitated withdrawal syndromes in opioid-dependent rodents via suppression of the AC/cAMP pathway (Jiang et al., 2012; Yang et al., 2013). In this study, we continued to investigate whether NaHS can be used to treat opioid withdrawal-induced pain sensitization in rats. To study the underlying mechanism, we examined the effect of NaHS on the expression of CGRP, a spinal pain neurotransmitter, and opioid withdrawal-induced rebound of cAMP production, phosphorylation of CREB and activation of related protein kinases in the spinal cord of opioid-dependent rats. The effects of NaHS on forskolin-stimulated cAMP production and AC activity were also detected in SH-SY5Y neuronal cells.

**Materials and method**

*Opioid withdrawal-induced pain sensitization rat model*

Male Sprague-Dawley rats (220–250 g) were randomly divided into the following groups as shown in Fig. 1 and described in the previous publication (Aley and Levine, 1997). [D-Ala2,N-Me-Phe4,Gly5-ol]-Enkephalin (DAMGO, a specific μ-opioid receptor agonist, 1 μg; Sigma), naloxone (200 ng, Sigma), NaHS (1.4 μg, Sigma) were dissolved in saline and administered intra-dermally in a total volume of 2.5 μl per paw. Whenever an antagonist was included, it was injected first. When drug combinations were used, they were administered from the same syringe in such a way that the first-mentioned drug reached the intradermal site first. The drugs were separated in the syringe by a small air bubble to prevent drugs mixing while in the syringe. The volume of each bubble was 2.5 μl. The nociceptive flexion reflex was quantified with a Basile Analgesymeter (Stoelting, USA), which applies a linearly increasing mechanical force to the dorsum of the rat’s hind paw. The measure was described previously (as determined by the Randall-Sellito paw-withdrawal test) (Aley and Levine, 1997). The baseline threshold from all rats before any drug treatment was determined as the mean of the six readings. The mechanical nociceptive threshold was re-determined at five timepoints (10, 15, 20, 25 and 30 min) after treatment. The mean of these four readings (10, 15, 20, 25 min) was considered the paw withdrawal threshold attributable to drug administration, and this value was used to calculate the percentage change from the baseline threshold. The animals were killed by decapitation 1 h after the behaviour experiments. The spinal cords were rapidly dissected out on an ice-cold glass Petri dish and used for Western blot analysis, reverse transcription-polymerase chain reaction (RT-PCR) and cAMP assay. The Institutional Animal Care and Use Committee of the National University of Singapore approved all animal experiments.

**Western blot assay**

Tissue samples were homogenized in tissue lysis buffer (1:10, w/v; Sigma). The lysate was shaken on ice for 1 h, then centrifuged at 12 000 g at 4 °C for 15 min. Epitopes were exposed by boiling the protein samples at 100 °C water for 5 min. Protein concentrations were determined with NanoDrop Spectrophotometer (NanoDrop technology, USA). Equal amounts of the protein samples were separated by electrophoresis using a 10% sodium dodecyl sulphate/polyacrylamide gel (SDS/PAGE) and transferred onto a nitrocellulose membrane (Whatman, UK). After being blocked in 10% milk with TBS-T (Tris buffer saline-Tween 20) buffer (10 mM Tris-HCl,
120 mM NaCl, 0.1% Tween-20, pH 7.4) at room temperature for 1 h, the membrane was incubated with various primary antibodies including Phospho-ERK1/2, total-ERK1/2, phospho-Raf-1, phospho-CREB, phospho-PKCα (1:1000, Cell Signalling, USA) or CGRP (1:1000, Santa Cruz Biotechnology, USA) at 4°C overnight. β-actin (1:5000, Santa Cruz) was used as a loading control. Membranes were washed three times in TBS-T buffer, followed by incubation with goat anti-rabbit or goat anti-mouse secondary antibodies (1:10000, Santa Cruz) at room temperature for 1h, and washed three times in TBS-T buffer. Visualization was carried out using ECL® (plus/advanced chemiluminescence) kit (GE Healthcare Life Sciences, UK). The density of the bands on Western blots was quantified by Image J software (USA).

Reverse transcription-polymerase chain reaction

Total RNA was extracted from rat spinal cord by the TRizol extraction method (TRizol Reagent, Invitrogen, USA). The RNA was then used to amplify fragments of the cDNA of CGRP by RT-PCR employing the One-Step RT-PCR kit (Bio-Rad, USA). The primers were adopted from previous publication (Pan et al., 2010). A positive control was performed by using primers specific for β-actin. One-step RT-PCR was performed with the following program: a reverse transcription reaction was initiated at 50°C for 30 min, PCR activation at 95°C for 10 min was followed by 35 cycles, each consisted of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and final extension time was set at 72°C for 10 min.

CAMP assay

Tissues were weighed and dropped into ten volumes of 0.1 M HCl, then homogenized on ice. After that, samples were centrifuged at 1500 g for 10 min and the supernatant was saved for determination of cAMP content. A cAMP enzyme immunoassay kit (Cayman Chemical, USA) was saved for determination of cAMP content. A cAMP enzyme immunoassay kit (Cayman Chemical, USA) was used to examine the concentration of cAMP. The reaction was stopped by incubation in 0.1 M HCl for 20 min, and then centrifuged at 1000 g for 10 min. The supernatant was saved for determination of cAMP content. Fifty micro-litres of samples were added into a 96-well plate followed by incubation with cAMP acetylcholine esterase tracer and cAMP acetylsine for 18 h at 4°C. Each sample was developed with Ellman’s reagent and the plate was read at a wavelength of 405 nm (Lu et al., 2010). cAMP concentration was calculated according to the cAMP standard and the protein was examined by Bradford Assay.

Cell culture and treatment

Human neuroblastoma SH-SY5Y cells were incubated under humidified 5% CO2 and 95% air at 37°C in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) containing 10% foetal bovine serum (FBS) and 1% streptomycin and penicillin (Invitrogen). Cells were plated onto 35 mm dishes and incubated overnight as it growing to 80–90% confluency. Regular medium was replaced with low-serum medium (0.5% FBS/DMEM) immediately before treatment. Cells were pre-treated with NaHS (0.1–1000 μM) for 30 min followed by the addition of forskolin (10 μM, 10 min, Sigma), cells were then collected to measure intracellular cAMP level. For the time-course study, NaHS (1.4 μg) co-injected with three hourly injections of DAMGO (1 μg) (S+D+Nal) but not by co-administration of NaHS (1.4 μg) with naloxone (200 ng) (D+Nal). (b) Timecourse for the effect of NaHS on naloxone-precipitated withdrawal hyperalgesia. Mean±S.E.M, n=8–12. **p<0.01, ***p<0.001, vs. basal; #p<0.05, ##p<0.01, ###p<0.001, vs. D+Nal; S, NaHS; D, DAMGO; Nal, naloxone; D+Nal: three hourly injections of DAMGO (1 μg) followed by naloxone injection at the fourth hour.

Cell fractionation and AC activity assay

The method for cell fractionation and AC activity assay was described in our previous publication.

Fig. 2. Effect of NaHS treatment on naloxone-precipitated withdrawal hyperalgesia in DAMGO-treated rats. (a) Naloxone-precipitated hyperalgesia could be reduced by NaHS (1.4 μg) co-injected with three hourly injections of DAMGO (1 μg) (S+D+Nal) but not by co-administration of NaHS (1.4 μg) with naloxone (200 ng) (D+Nal). (b) Timecourse for the effect of NaHS on naloxone-precipitated withdrawal hyperalgesia. Mean±S.E.M, n=8–12. **p<0.01, ***p<0.001, vs. basal; #p<0.05, ##p<0.01, ###p<0.001, vs. D+Nal; S, NaHS; D, DAMGO; Nal, naloxone; D+Nal: three hourly injections of DAMGO (1 μg) followed by naloxone injection at the fourth hour.
(Yong et al., 2008; Pan et al., 2010). AC activity assay was performed at 37 °C for 20 min in a 400 μl reaction mixture containing 1 mM ATP, 100 mM NaCl, 50 mM Hepes, 0.5 mM IBMX, 6 mM MgCl2, 1 μM GTP and 50 μl of membrane protein. In the forskolin group, samples were treated with forskolin (100 μM) for 10 min. In the NaHS-pre-treated group, NaHS (100 μM) was given 10 min before and during forskolin treatment. Reactions were stopped by the addition of 0.5 ml of trichloroacetic acid (10 %, w/v). The accumulation of cAMP was later detected by cAMP assay.

Statistical analysis

All data are presented as mean±S.E.M. Significance was assessed with one-way analysis of variance (ANOVA) followed by Bonferroni or Tukey test. For comparison between two groups, student t-test was used. Differences with p values less than 0.05 were considered statistically significant.

Results

NaHS treatment reduces naloxone-precipitated withdrawal hyperalgesia in rats

To examine the effect of NaHS on opioid withdrawal-induced pain sensitization (withdrawal hyperalgesia), rats received three hourly injections of DAMGO (1 μg) followed by naloxone (200 ng) administration intra-dermally at the fourth hour in the rat hind paw to induce naloxone-precipitated withdrawal hyperalgesia. The experimental protocol is shown in Fig. 1. The behavioural test showed that naloxone administration significantly decreased nociceptive threshold, indicating the induction of withdrawal hyperalgesia (D+Nal). NaHS (1.4 μg) alone had no significant effect on the baseline paw-withdrawal threshold (S). However, it attenuated naloxone-precipitated withdrawal hyperalgesia, when it was given together with three hourly injections of DAMGO (S+D+Nal). Co-administration of NaHS with naloxone (D+S+Nal) at the fourth hour failed to produce a significant effect (Fig. 2a). We also detected the time-course for the effect of NaHS on naloxone-precipitated withdrawal hyperalgesia. Figure 2b shows that hyperalgesia was observed from 10 to 30 min after injection of naloxone. Co-administration of NaHS with three hourly injections of DAMGO completely abolished withdrawal hyperalgesia.

NaHS treatment inhibits up-regulated mRNA and the protein level of CGRP in rat spinal cord

Repeated opioid treatment has been shown to produce paradoxical pain sensitization and causes an increase in spinal pain neurotransmitters, such as CGRP. Therefore, we examined the expression of CGRP in the spinal cord of opioid-dependent rats. As shown in Fig. 3, the CGRP expression levels of both mRNA (Fig. 3a, b) and protein (Fig. 3c, d) were markedly increased in the naloxone-precipitated withdrawal group (D+Nal). Co-injection of NaHS with DAMGO (S+D+Nal), but not that of NaHS together with naloxone (D+S+Nal), significantly attenuated the up-regulation of CGRP induced by naloxone-precipitated withdrawal in rat spinal cord (Fig. 3).
The spinal cord is the first relay site in the transmission of nociceptive information from the periphery to the brain and is also closely related to the development of nociceptive information from the periphery to the brain and is also closely related to the development of nociceptive information from the periphery to the brain and is also closely related to the development of nociceptive information from the periphery to the brain and is also closely related to the development of nociceptive information from the periphery to the brain. The development of opioid dependence and withdrawal.

The cAMP/CREB pathway plays a very important role in naloxone-precipitated withdrawal in rat spinal cord and CREB phosphorylation induced by NaHS treatment suppresses the activation of various protein kinases induced by naloxone-precipitated withdrawal in rat spinal cord.

As shown in Fig. 6a, the expression of p-ERK1/2 (Fig. 6a) and p-Raf-1 (Fig. 6b) was markedly increased in the naloxone-precipitated withdrawal group (D+Nal). Co-injection of NaHS with DAMGO (S+D+Nal), but not that of NaHS together with naloxone (D+S+Nal), significantly attenuated up-regulation of ERK1/2 and Raf-1 phosphorylation induced by naloxone-precipitated withdrawal. Naloxone precipitation also activated PKCa (Fig. 6c) in rat spinal cord. To a weaker extent, DAMGO also significantly stimulated the expression of PKCa.

Fig. 4. Effect of NaHS treatment on cAMP level and CREB phosphorylation in the spinal cord of DAMGO-treated rats. (a) Naloxone induced cAMP rebound (D+Nal) was abolished by S+D+Nal, but not by D+S+Nal. n=4-8. (b) Western blot showing up-regulation of CREB phosphorylation caused by naloxone-precipitated withdrawal was attenuated by both S+D+Nal and D+S+Nal. n=6. Mean±S.E.M. **p<0.01, ***p<0.001 vs. Con; ##p<0.01, ###p<0.001 vs. D+Nal. S, NaHS; D, DAMGO; Nal, naloxone.

**NaHS treatment inhibits forskolin-stimulated cAMP production and AC activity in SH-SY5Y cell**

To study the mechanisms for the inhibitory effect of NaHS on the cAMP pathway, we observed the effect of H2S on cAMP production in the SH-SY5Y neuronal cell line. As shown in Fig. 5a, pre-treatment with NaHS (10-100 μM) for 30 min significantly suppressed cAMP production in SH-SY5Y cells stimulated by forskolin (an AC activator, 10 μM, 10 min). Time-course study showed that NaHS induced a transient inhibition on forskolin-stimulated AC activity at 30 min and a sustained inhibition after application for 8-24 h (Fig. 5b).

To examine the effect of H2S on AC activity, we isolated cell membrane from SH-SY5Y cells to determine AC activity. As shown in Fig. 5c, forskolin significantly stimulated AC activity in the cell, and this effect was attenuated by pre-treatment with NaHS (100 μM) for 30 min. These data suggest that H2S may inhibit cAMP production by suppression of AC activity.

We also examined the effect of NaHS on cAMP production in differentiated SH-SY5Y cells. As shown in Fig. 5d, NaHS at 0.01-100 μM concentration-dependently attenuated forskolin-stimulated cAMP production. These data suggest that, compared with undifferentiated neuronal cells, the differentiated neuronal cells may be more sensitive to H2S treatment.

**NaHS treatment inhibits the up-regulation of cAMP and CREB phosphorylation induced by naloxone-precipitated withdrawal in rat spinal cord**

The cAMP/CREB pathway plays a very important role in the development of opioid dependence and withdrawal. The spinal cord is the first relay site in the transmission of noxious information from the periphery to the brain and is also closely related to the development of opioid withdrawal (Marshall and Buccafusco, 1985; Miyamoto and Takemori, 1993; D’Mello and Dickenson, 2008). We therefore determined cAMP level and CREB phosphorylation in rat spinal cord. As shown in Fig. 4a, naloxone-precipitated withdrawal markedly elevated the cAMP content in the rat spinal cord (D+Nal). Co-injection of NaHS with DAMGO (S+D+Nal) obviously attenuated the cAMP rebound induced by naloxone precipitation, while NaHS alone had no significant effect (S). However, co-administration of NaHS with naloxone (D+S+Nal) only slightly suppressed the elevated cAMP but without statistical significance. Similarly, naloxone induced marked phosphorylation of CREB.

Co-injection of NaHS with DAMGO significantly reduced the up-regulation of CREB phosphorylation induced by naloxone-precipitated withdrawal (Fig. 4b).
Both co-injection of NaHS with DAMGO (S+D+Nal) and co-administration of NaHS with naloxone (D+S+Nal) attenuated the activated PKCα (Fig. 6c).

Discussion

Repeated exposure to opioids leads to the development of dependence, which can be assessed by observing the emergence of a withdrawal syndrome following discontinuation of chronic opioid treatment or the administration of a competitive opioid antagonist such as naloxone (Maldonado and Koob, 1993). One of the key symptoms during opioid withdrawal is a state of sensitized pain. The commonly employed test is to examine the responses of rats, specifically the paw, to withdrawal tests after receiving multiple boluses of opioid (Sandkühler, 2009). In this study, we demonstrated for the first time that repeated administration of NaHS significantly alleviated naloxone-precipitated withdrawal hyperalgesia in DAMGO-dependent rats.

Chronic opioid-induced up-regulation of the cAMP/CREB cascade has been confirmed in many opioid...
dependence-related brain areas, including the spinal cord, and such a cAMP/CREB adaptation has been widely related to opioid dependence and withdrawal (Sharma et al., 1977; Shijun et al., 2009). Previous studies have shown that the cAMP/CREB pathway plays a major role in the regulation of pain-related neurotransmission such as CGRP synthesis and release (Freeland et al., 2000; Tumati et al., 2009). This is because there is a putative CRE gene sequence in the CGRP gene promoter (Watson and Latchman, 1995). More importantly, blockade of the cAMP pathway or knockdown of spinal PKA activity with siRNA significantly attenuated the up-regulated spinal CGRP expression and opioid-induced pain sensitization (Tumati et al., 2011). In our study, we demonstrated that administration of NaHS significantly inhibited opioid withdrawal-induced rebound of cAMP production, CREB phosphorylation and CGRP expression in rat spinal cord. To study the mechanism, we examined the effect of NaHS on cAMP production and AC activity in the SH-SY5Y neuronal cell line. Our data showed that H2S significantly inhibited forskolin-stimulated cAMP production and AC activity. These results suggest that the anti-hyperalgesia effect of NaHS is mediated by down-regulation of AC/cAMP pathway. Apart from suppression of the AC/cAMP pathway, the decreased CGRP expression and pain hypersensitivity may also be secondary to inhibition of the PKC/Raf-1/ERK pathway (Ji et al., 2009; Tumati et al., 2010). PKC plays an important role in the development of opioid dependence. Stimulation of an opioid receptor by an opioid agonist leads to activation of PKC (Mayer et al., 1995). Of all PKC isoforms, PKCα is demonstrated to be the main isoform involved in opioid-mediated signalling pathways, and to exert great activities in the mammalian brain together with PKCβ (Ventayol et al., 1997). In naloxone-precipitated withdrawal, PKCα was found to be highly activated in cellular or animal models of opioid dependence (Fundytus and Coderre, 1996). Activation of PKCα directly phosphorylates and activates Raf-1 (Kolch et al., 1993). The latter is one of the main effectors recruited by GTP-bound Ras protein to activate the MAPK/ERK pathway (Avron et al., 1994). Activation of MAPK/ERK pathway can phosphorylate multiple targets in the cytoplasm and in the nucleus (i.e. transcription factors such as CREB) (Williams et al., 2001). It was reported that inhibition of ERK with its selective inhibitors or molecular knockdown reduced opioid withdrawal-induced CREB phosphorylation in rat spinal cord (Grewal et al., 1999). In our previous study, we also reported that blockade of ERK1/2, with its specific inhibitor, attenuated naloxone-induced CREB phosphorylation in SH-SY5Y cells (Yang et al., 2014). In this study, we found that PKCα/Raf-1/ERK pathway was obviously up-regulated upon naloxone-precipitated withdrawal in the spinal cord of DAMGO-dependent rats. NaHS treatment significantly reversed these effects. Our data confirm that suppression of the PKCα/Raf-1/ERK pathway may also contribute to the therapeutic effect of H2S on withdrawal-induced hyperalgesia.

However, it is worthy of note that there are ‘cross talks’ between the AC/cAMP and PKC/Raf-1/ERK pathways (Fig. 7). It was reported that activation of PKC might modulate the cAMP pathway in a complex manner and eventually result in enhancement of AC/cAMP activity (Mayer et al., 1995). Blockade of Raf-1 with its selective inhibitors was also found to significantly attenuate chronic opioid agonist-mediated AC super-activation (Tan et al., 2001; Varga et al., 2002). In addition, MAPK/ERK can also be activated by PKA (Impey et al., 1999). Therefore, these two pathways may interact with each other to regulate CREB/CREG during opioid withdrawal-induced hyperalgesia.

In our cellular experiments, we found that NaHS produced significant effects at the concentration range of 10–100 μM in un-differentiated SH-SY5Y cells. However, the physiological concentration of H2S is now recognized to be at a nanomolar level (Liu et al., 2012). To observe whether H2S at physiological concentrations can also produce similar effects, we differentiated SH-SY5Y cells with retinoic acid. It was found that NaHS decreased forskolin-stimulated cAMP production in differentiated SH-SY5Y cells when its concentration is as low as 10 nM. Our data suggest that H2S may regulate cAMP production when its concentrations are at both physiological and pharmacological concentration ranges.
Interestingly, our study showed that NaHS could only block the opioid-induced withdrawal when it was given together with DAMGO. When NaHS was given together with naloxone, NaHS had a less significant effect on naloxone-induced rebound of cAMP and phosphorylation of CREB, ERK1/2, Raf-1 and PKCa. These results suggest that H2S may attenuate the development of opioid dependence by reversing the long-lasting alterations in neuronal plasticity, but not by suppression of the acute pathological changes caused by naloxone.

In conclusion, we demonstrated that H2S is important in the regulation of opioid dependence and withdrawal. Application of exogenous H2S may prevent the development of withdrawal-induced hyperalgesia via suppression of the AC/cAMP and PKCa/Raf-1/ERK pathways. These two pathways may further inhibit the phosphorylation of transcription factors like CREB to change the CGRP gene expression, which is closely involved in the development of opioid withdrawal-induced hyperalgesia (Fig. 7). Our findings therefore suggest that H2S may have the therapeutic potential to treat or prevent pain sensitization induced by opioid withdrawal.

Acknowledgments

This work is supported by research grants from the Singapore National Medical Research Council (NMRC-1183/2008).

Conflict of Interest

None

References


