

DOCTORAL THESIS

Analgesic effect of paeoniflorin in rats with visceral hyperalgesia induced by neonatal maternal separation

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**Analgesic Effect of Paeoniflorin in Rats with Visceral
Hyperalgesia Induced by Neonatal Maternal Separation**

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**A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy**

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Abstract

Paeoniflorin (PF) is one of the principle active ingredients of the root of *Paeonia lactiflora* Pall. (family Ranunculaceae), a Chinese medicinal herb traditionally used to relieve pain, especially visceral pain. Functional visceral pain is the cardinal feature of functional gastrointestinal disorders, such as irritable bowel syndrome (IBS), for which etiology remains elusive and effective therapeutic regime is deficient. A few recent clinical studies have suggested the effect of the root of *P. lactiflora* on visceral pain of IBS, but the experimental evidence is scarce. The present study aimed i) to explore the mechanism of visceral hyperalgesia in rats induced by neonatal maternal separation (NMS); ii) to investigate the analgesic effect of PF on colorectal distension (CRD)-evoked visceral pain in NMS rats; and iii) to study the possible mechanisms of PF analgesia.

In the present study, both electromyographic recording (EMG) and abdominal withdrawal reflex (AWR) test revealed that NMS rats, compared with non-handled (NH) rats, exhibited increased visceromotor response (VMR) and decreased pain threshold pressure to CRD, indicating the establishment of visceral hyperalgesia in adult NMS rats. Further studies on correlation between extracellular signal-regulated protein kinase (ERK) mitogen-activated protein kinase (MAPK) signaling transduction pathway and NMS-induced visceral hyperalgesia demonstrated that: i) ERK cascade is involved in CRD-evoked VMR in NMS rats, since significant CRD effect on increasing p-ERK and *c-fos* expression was observed in the dorsal root ganglia (DRG), laminae I-II of lumbosacral dorsal horn, as well as the supraspinal centers involving central medial thalamic nucleus (CM), paraventricular thalamic nucleus (PV), and anterior cingulate cortex (ACC); ii) ERK cascade is implicated in the NMS-induced long-term changes in CNS, as significant NMS effect on increasing p-ERK and *c-fos* expression was observed in the DRG as well as laminae I-II, III-IV and X of the lumbosacral dorsal horn; and NMS rats showed denser *c-fos* expression in supraspinal centers involving the

CM and ventroposterolateral nucleus of the thalamus (VPL) in the basal state than NH rats; iii) a significant interactive effect of NMS and CRD on p-ERK expression was found in laminae III-IV of the lumbosacral dorsal horn, indicating that elevated p-ERK expression in this region is probably associated with the visceral hyperalgesia of NMS rats; iv) p-ERK was expressed in neurons and colocalized with the downstream immediate early gene *c-fos*, and peptides SP and CGRP; correlation analysis revealed the positive association between *c-fos* and p-ERK immunoreactive (IR) nuclei numbers in almost all of the investigated CNS structures; v) the involvement of ERK cascade in CRD-induced VMR was confirmed by the analgesic effect of MEK inhibitor U0126, which not only elevated the pain threshold pressure of NMS rats but also decreased CRD-evoked p-ERK and p-CREB expression in the thalamus and cingulate cortex; vi) the CRD-evoked ERK activation in NMS rats was demonstrated to be NMDA receptor-dependent, as the NMDA receptor antagonist MK-801 produced analgesic effect in AWR test and markedly suppressed the CRD-evoked p-ERK and p-CREB expression in the lumbosacral dorsal horn, thalamus and cingulate cortex. Dynamic analysis of the extracellular excitatory neurotransmitters glutamate and aspartate by microdialysis sampling and capillary electrophoresis coupled with laser-induced fluorescence detection (CE-LIFD) revealed that noxious CRD evoked a rapid and transient increase of the extracellular glutamate but not aspartate in the ACC of NMS rats. These results provide substantial evidence for the participation of NMDA receptor-dependent ERK cascade in the visceral pain process of NMS rats, and suggest the possible involvement of ERK MAPK pathway in NMS-induced neuronal activation in the CNS.

This study showed that a dose-dependent analgesic effect was produced by PF (45, 90, 180, and 360 mg/kg, i.p.). Centrally administered PF (4.5 mg/kg, i.c.v) also produced a significant analgesic effect. The analgesic effect of PF (45 mg/kg, i.p.) was maximal at 30 min after administration. Time-course determination of PF in cerebral nuclei showed that the maximal concentration was observed also at 30 min after PF (180 mg/kg, i.p.) in several cerebral nuclei, including the amygdala, hypothalamus, thalamus and cortex. Furthermore, the analgesic effect of PF on CRD-evoked visceral pain was mediated by

multiple receptors and neurotransmitters. AWR test showed that the analgesic effect of PF could be significantly blocked by naloxone, nor-binaltorphimine and naltrindole (the selective μ -, κ - and δ -opioid receptor antagonists, respectively), as well as by reserpine (monoamine depletor), DL- α -Methyltyrosine (catecholamines synthesis inhibitor) and yohimbine (α_2 -adrenoceptor antagonist). It also could be significantly attenuated by pCPA (5-HT depletor). Moreover, the analgesic effect of PF was significantly blocked by DPCPX, an antagonist that could selectively block the adenosine A₁ receptors that are ubiquitous and abundant throughout the CNS and intimately associated with the opioidergic, noradrenergic and serotonergic pain modulatory pathways. Western blot and immunohistochemistry (IHC) analysis revealed that PF inhibited the CRD-evoked p-ERK and *c-fos* expression in the laminae I-II of lumbosacral dorsal horn and ACC, as well as CRD-evoked *c-fos* expression in the CM, PV and ventromedial hypothalamus (VMH). These results indicate that adenosine A₁ receptor is involved in the PF's analgesic effect, which was confirmed by the fact that DPCPX markedly reversed PF's inhibition on CRD-evoked ERK cascade in the thalamus and cingulate cortex. In addition, CE-LIFD analysis of the neurotransmitters in ACC microdialysate showed that PF significantly decreased CRD-evoked increase of extracellular glutamate. Thus, the present results clearly show that PF has a dose- and time-dependent analgesic effect on visceral pain of NMS rats. The analgesic effect of PF may be produced in the CNS. PF's inhibitory effect on CRD-evoked p-ERK is probably mediated via adenosine A₁ receptor through reducing the glutamate release in the ACC.

In summary, the present results extend our understanding of the intracellular mechanism of functional visceral pain and visceral hyperalgesia by revealing the participation of NMDA receptor-ERK-CREB-*c-fos* chain in visceral pain process as well as in NMS-induced neuronal activation in the CNS, which appears responsible for behavioral hyperalgesia in adult NMS rats. Furthermore, these findings demonstrate that PF has an analgesic effect on visceral pain in rats with visceral hyperalgesia induced by NMS. This result provides experimental basis for the traditional clinical use of the root of *P. lactiflora*, and indicates that PF is potentially useful in clinical therapy for visceral pain

associated with functional gastrointestinal disease, such as IBS.

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