Hydrogen-Rich Saline Attenuates Remifentanil Induced Hyperalgesia via Regulation of NMDA Receptor Trafficking in Rats

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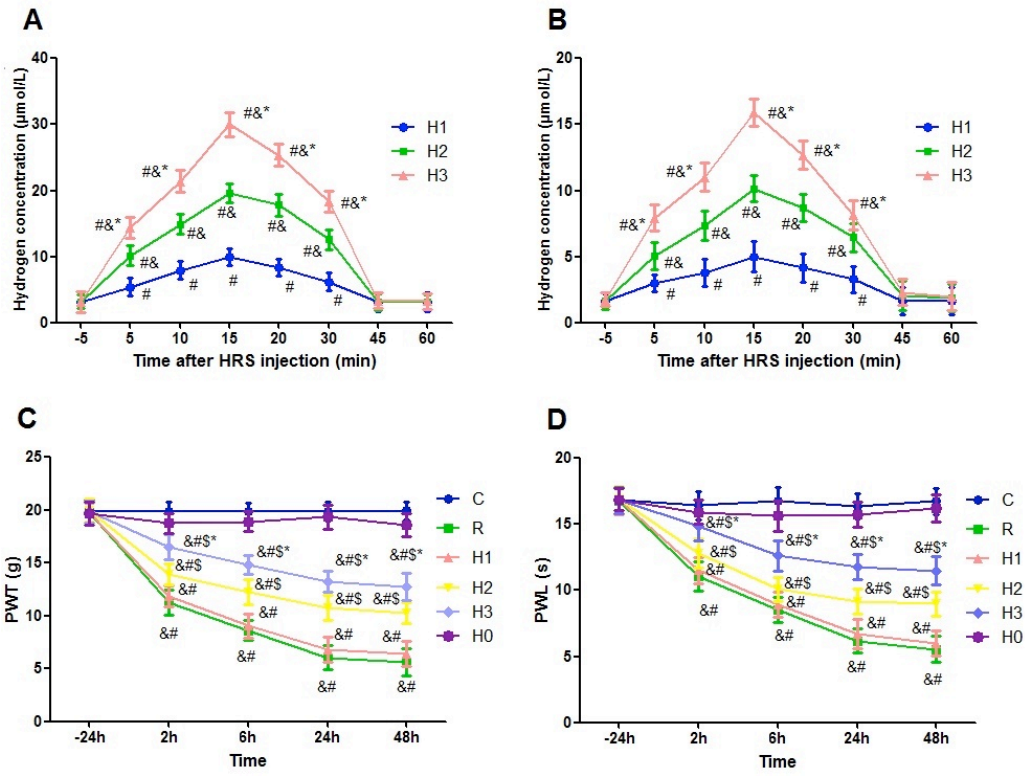
Background: Opioid administration may subsequently cause paradoxical and neuropathic hyperalgesia, but mechanisms remain unclear. MnSOD nitration caused by generation of superoxide and activation of NMDAR is involved in the induction and maintenance of central neuropathic pain. Hydrogen which selectively removes superoxide has gained much attention in recent years. In this study, we investigated antinociception of hydrogen-rich saline (HRS) on remifentanil-induced postsurgical hyperalgesia in a rat model of incisional pain.

Methods: HRS with various doses was injected intraperitoneally after remifentanil infusion. A noncompetitive NMDAR antagonist MK801 and a selective NR2B antagonist Ro25-6981 with subthreshold doses were used to investigate whether antihyperalgesic effect of HRS is associated with NMDAR. Nociception was evaluated by mechanical and thermal tests. We examined time course of hydrogen concentration in blood after HRS injection. RT-qPCR、IHC and Western blot were applied to analyze MnSOD expression and nitration, and NR2A and NR2B expression and trafficking in the L_4-L_6 segments of the right dorsal horn.

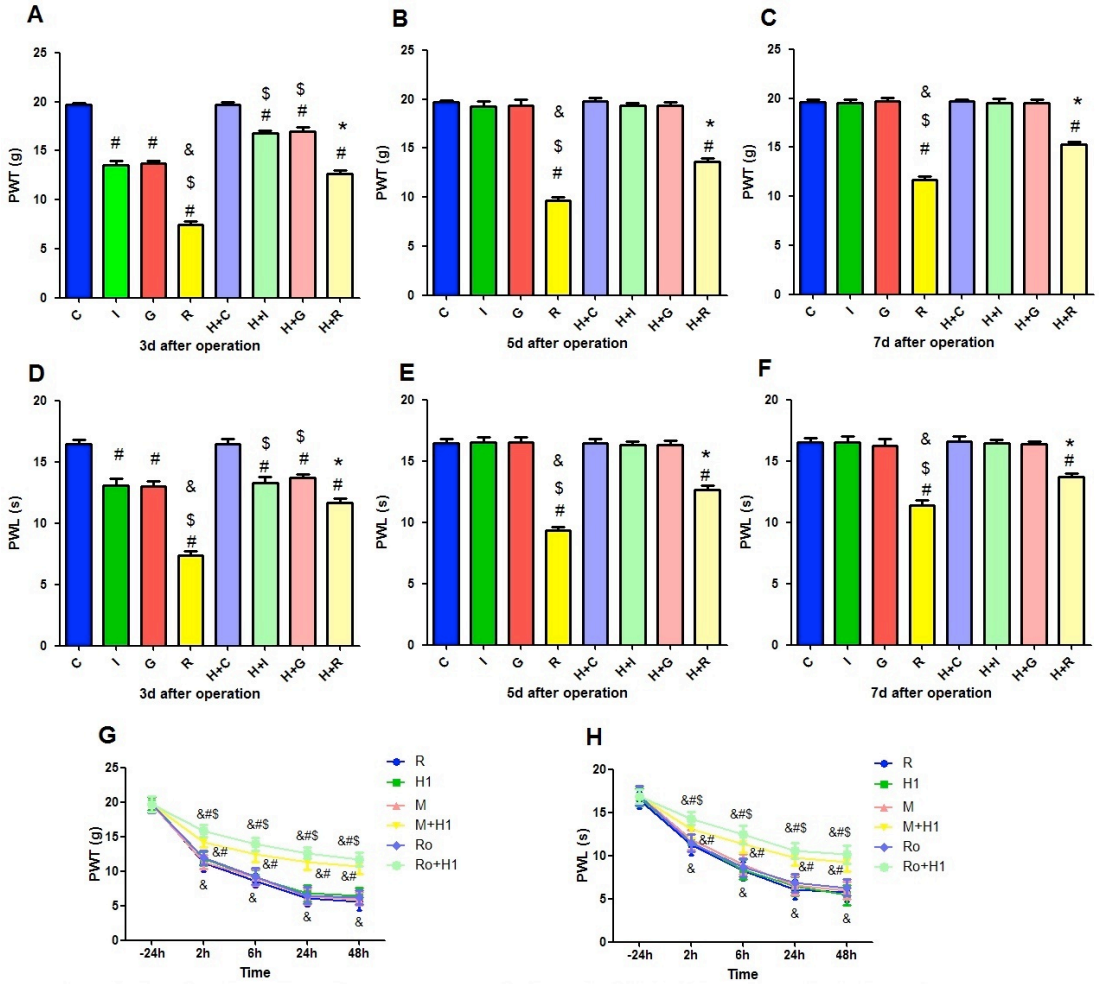
Results: The analgesic effect of remifentanil was followed by long-term hyperalgesia lasting at least postoperative 7 days, which was accompanied with increase in NR2B trafficking and MnSOD nitration in dorsal horn ($P<0.01$), however, there are no significant changes in NR2A and MnSOD level in all groups ($P>0.05$). Hydrogen concentration dose-independently increased 5min, peaked 15min, and returned to basal level 45min after HRS administration ($P<0.01$); HRS not 2.5 but 5 and 10 ml/kg dose-dependently attenuated mechanical and thermal hyperalgesia, and minimal effective concentration was observed to be higher than 10 μmol/L ($P<0.01$); HRS (10 ml/kg) blocked NR2B trafficking and decreased MnSOD nitration in dorsal horn after remifentanil infusion ($P<0.01$), hyperalgesia and MnSOD nitration were also further attenuated after the combination of HRS (2.5ml/kg) and Ro25-6981 than that observed after HRS (2.5ml/kg) and MK801 injection ($p < 0.01$).

Conclusions: HRS dose-dependently plays a preventive role in remifentanil-induced hyperalgesia, furthermore, the dose of 10ml/kg exerts a best result. The underlying mechanism is that HRS could inhibit expression and trafficking of NR2B-containing NMDAR to enhance MnSOD activity.

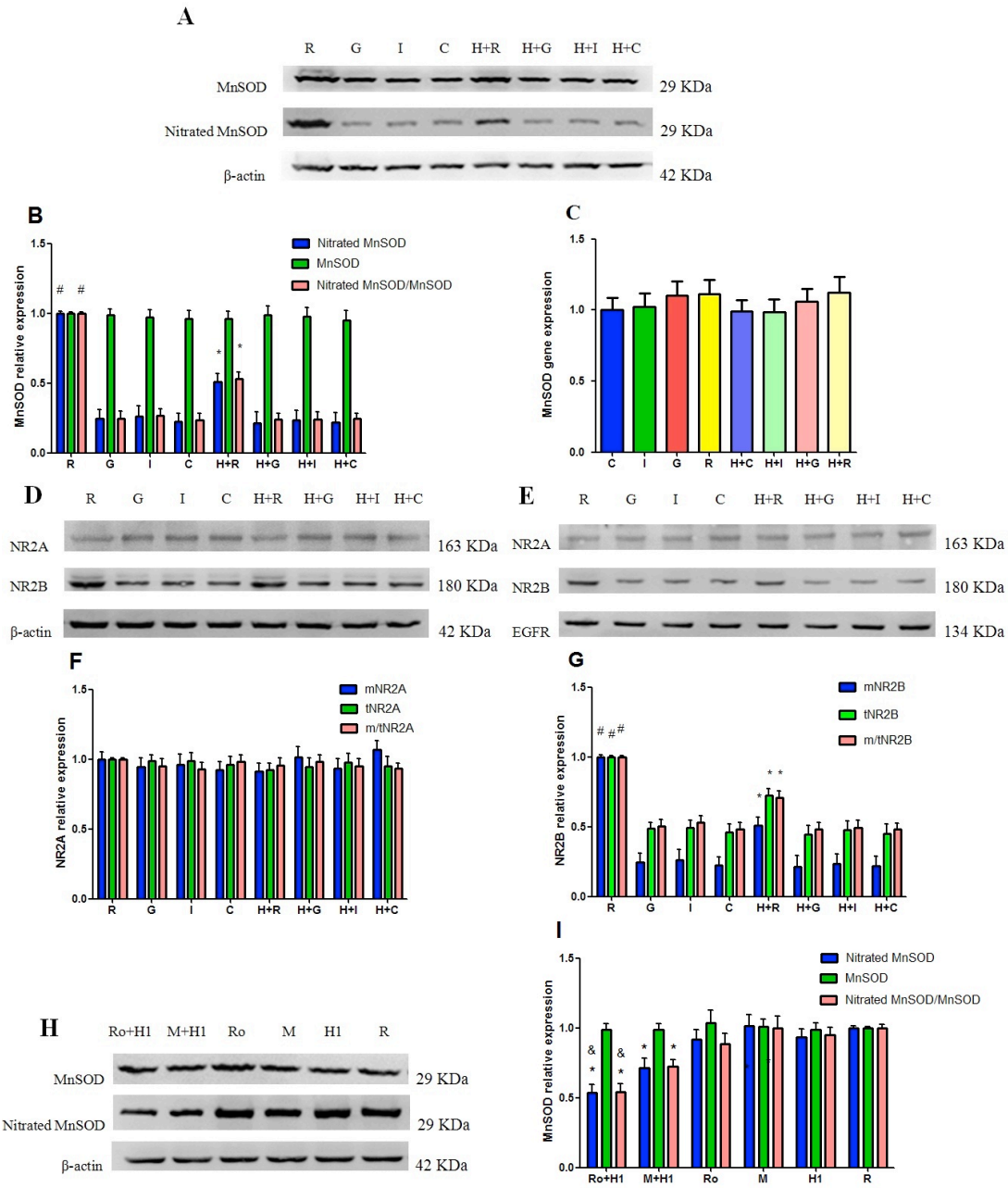
Summary: Our study shows that Pretreatment with hydrogen-rich saline could attenuate mechanical and thermal hyperalgesia induced by remifentanil via regulation of NR2B-containing NMDAR trafficking and MnSOD nitration in a dose-dependent manner.



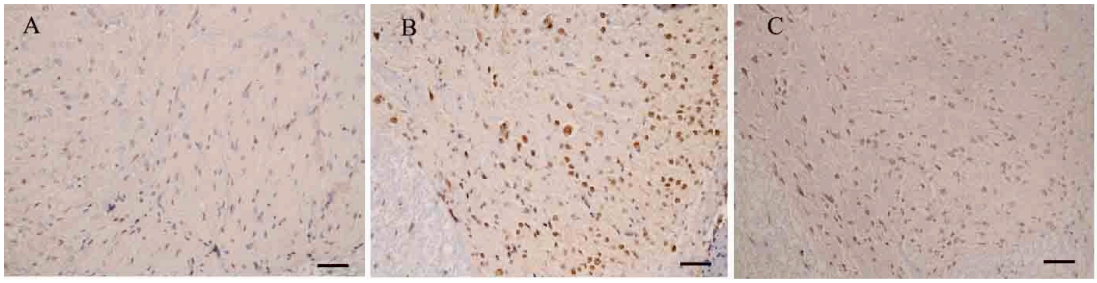
Variation of hydrogen concentration in blood after HRS administration and dose-dependently antinociception of HRS on hyperalgesia induced by remifentanil. group H1, H2, H3 (administration of HRS 2.5ml/kg, 5ml/kg, 10ml/kg) Hydrogen concentration (μmol/L) in arterial (A) and venous (B) blood was monitored and recorded at 5min before and 5, 10, 15, 20, 30, 45 and 60min after administration of HRS. Data were expressed as mean ± SD (n=8). $^{\#}P<0.01$ vs baseline, $^{\&}P<0.01$ vs group H1, $^{*}P<0.01$ vs group H2. PWT (C) and PWL (D) were evaluated at 24h before (baseline) and 2, 6, 24 and 48h after remifentanil administration. Data were expressed as mean ± SD (n=8). $^{\&}P<0.01$ vs baseline, $^{\#}P<0.01$ vs group C, $^{\$}P<0.01$ vs group R, $^{*}P<0.01$ vs group H2.



The antinociceptive effect of HRS on long-term postoperative hyperalgesia induced by remifentanil and effects of NMDAR antagonist on antinociception of HRS. Pretreatment with HRS (10ml/kg) was injected intraperitoneally. PWT(A,B,C) and PWL(D,E,F) were measured at 3, 5 and 7d after operation. Data were expressed as mean ± SD (n=8). $^{\#}P<0.01$ vs group C, $^{\$}P<0.01$ vs group I, $^{\&}P<0.01$ vs group G, $^{*}P<0.01$ vs group R. MK801 and Ro25-6981 were given intrathecally before remifentanil infusion. PWT(G) and PWL(H) were recorded. Data were expressed as mean ± SD (n=8). $^{\&}P<0.01$ vs baseline, $^{\#}P<0.01$ vs group R, $^{\$}P<0.01$ vs group M+H1.



Effects of HRS on MnSOD nitration and NR2B-containing NMDAR trafficking, and NMDAR was involved in inhibition of HRS on MnSOD nitration in OIH. (A, H) Bands of Western blot for the expression and nitration of MnSOD protein. β-actin was the internal standard. (B, I) Values for the ratios of MnSOD/β-actin, nitrated MnSOD/β-actin and nitrated MnSOD/MnSOD are normalized to R group. (C) Values for MnSOD mRNA expression were presented as fold increase over group C and normalized to the expression of GAPDH.(D) and (E) Bands of Western blot for the expression of total(t) and membrane(m) NR2A and NR2B. β-actin and EGFR were the internal standard. (F) Values for the ratios of tNR2A/β-actin, mNR2A/EGFR and mNR2A/tNR2A are normalized to R group. (G) Values for the ratios of tNR2B/β-actin, mNR2B/EGFR and mNR2B/tNR2B are normalized to R group. Data were expressed as mean ± SD (n=4). $^{\#}P<0.01$ vs group C, $^{*}P<0.01$ vs group R, $^{\&}P<0.01$ vs group M+H1.



Effect of HRS on NR2B expression in OIH. Representative immunohistochemistry micrographs of dorsal horn of L4-L6 spinal cord showed that NR2B presents the brown staining. When compared with vehicle (A), the expression of NR2B dramatically increased in incision-remifentanil rats (B). Intraperitoneal delivery of HRS (10ml/kg) blocked the increasing NR2B expression (C). Scale bar=50 um.