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Formaldehyde regulates vascular tensions through nitric oxide-cGMP signaling pathway and ion channels

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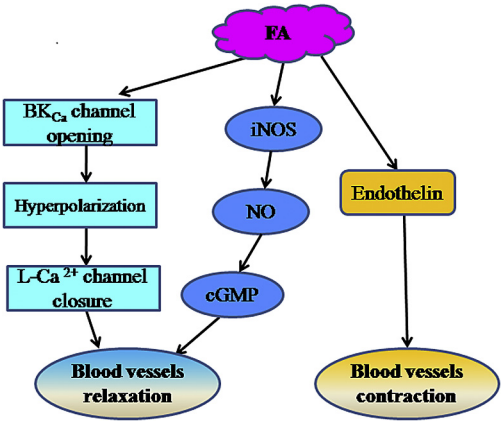
HIGHLIGHTS

- Vascular effects of FA are biphasic depending on its concentration.
- Effects of FA on the isolated rat aortas are endothelium-dependent.
- Vasoconstriction of FA at low concentrations might be related to endothelin.
- NO-cGMP pathway and ion channels are involved in the FA-induced vasodilation.
- Vasodilation of FA linked to expression changes of NOS, BK_{Ca}, and L-Ca^{2+} channel.

GRAPHICAL ABSTRACT



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ABSTRACT

Formaldehyde (FA) has been linked to the detrimental cardiovascular effects. Here, we explored the effects and mechanisms of FA on rat aortas both *in vivo* and *in vitro*. The results presented that FA evidently lowered the blood pressures of rats. The expression levels of BK_{Ca} subunits α and β1 and iNOS of the aortas were up-regulated by FA *in vivo*. However, FA markedly reduced the levels of $Ca_v1.2$ and $Ca_v1.3$, which are the subunits of L-Ca^{2+} channel. Furthermore, the contents of NO, cGMP and iNOS in the aortas were augmented by FA. To further confirm these findings, the mechanisms accredited to these effects were examined *in vitro*. The data showed that FA contracted the isolated aortic rings at low concentrations (<300 μM), while it relaxed the rings at high concentrations (>500 μM). The FA-induced vasoconstriction at low concentrations was blocked partly by an inhibitor of ACE. The relaxation caused by FA at high concentrations was attenuated by the inhibitors of NO-cGMP pathway, L-Ca^{2+} channel and

Abbreviations: 4-AP, 4-aminopyridine; ACh, acetylcholine; L-NAME, NG-nitro-L-arginine methyl ester; ACE, angiotensin-converting enzyme; VSMCs, vascular smooth muscle cells; BK_{Ca}, big-conductance Ca^{2+}-activated K^+ channel; FA, formaldehyde; IbTx, iberiotoxin; iNOS, inducible nitric oxide synthase; K_{ATP}, ATP-sensitive K^+ channel; eNOS, endothelial nitric oxide synthase; Kv, voltage-dependent K^+ channel; sGC, soluble guanylate cyclase; NE, norepinephrine; nNOS, neuronal nitric oxide synthase; NS-2028, 4H-8-bromo-1,2,4-oxadiazolo(3,4-*d*)benz(b)(1,4)oxazin-1-one; PGI_2, prostacyclin; PKC, protein kinase C; cGMP, cyclic guanosine monophosphate; TEA, tetraethylammonium.

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Thoracic aorta ring
Vascular tension
Ion channels
Signal pathway

BK_{Ca} channel, respectively. Similarly, the expression of iNOS was strongly enhanced by FA *in vitro*. The effects of FA on the aortic rings with endothelium were significantly greater than those on the rings without endothelium. Our results indicate that the vasoconstriction of FA at low concentrations might be partially pertinent to endothelin, and the FA-caused vasorelaxation at high concentrations is possibly associated with the NO-cGMP pathway, L-Ca^{2+} channel and BK_{Ca} channel. This study will improve our understanding of the pathogenic mechanisms for FA-related cardiovascular diseases.

1. Introduction

Formaldehyde (FA) is a ubiquitous pollutant (NTP, 2011; IARC, 2012). It is widely used in the manufacturing processes of building materials, wood, fertilizer, plastics, cement, resins, and paper products. Moreover, FA is served as a fixative in histology and anatomy laboratories, and as a disinfectant in medicine. Due to broad and versatile range of use, millions of people worldwide are exposed to FA (Rager et al., 2014). After ingestion, FA metabolized into formic acid and methanol inside human body and is excreted through respiration, feces, and urine (Conklin et al., 2004). In addition to exogenous ingestion and exposure, FA is formed endogenously through normal cellular metabolism. FA has been extensively studied for the common nature of both exogenous and endogenous FA exposures since the 1980s (NTP, 2011; IARC, 2012; Rager et al., 2014). Hundreds of reports revealed that FA causes damages to a lot of tissues, impairs energy metabolism regulation, and decreases the antioxidant capacity (Bakar et al., 2014; Bono et al., 2010; Aydin et al., 2015). Moreover, some international organizations have identified FA as a definite human carcinogen (NTP, 2011; IARC, 2012).

FA inhalation has been related to adverse cardiovascular effects. It has been reported that the FA toxicity reduced heart rate and arterial pressure and caused Sinus bradycardia and AV-arrhythmia in the Electro-Chemical Grinding (Strubelt et al., 1990). Treatment of both hypertrophic and normal hearts with FA caused cardiac failure modulated likely by damaging the intracellular Ca^{2+} regulation (Takeshita et al., 2009). Güleç et al. depicted that the continuous treatment of rats with FA for 4 or 13 weeks evidently elevated the lipid peroxidation products and diminished the antioxidant enzyme activities in the hearts (Güleç et al., 2006). It has been discovered that methylamine could relax the isolated human blood vessels but it could be metabolized to FA by semicarbazide sensitive amine oxidase, which suggested that FA produced by methylamine might contribute to the methylamine-induced vascular effects (Conklin et al., 2004). Nevertheless, the effects and mechanisms of FA on vascular tensions are unknown. In the blood vessels, ion channels and signaling pathways are essential for vascular activities. We hypothesized that FA may mediate the vascular activities by regulating some ion channels and signaling pathways. Hence, the roles of different ion channels and signaling pathways in line with the effects of FA on the vascular tensions were explored both *in vivo* and *in vitro*. The current findings may improve our knowledge of the pathogenic mechanisms for the cardiovascular diseases induced by FA and would be a significant contribution in the existing facts of FA toxicology.

2. Materials and methods

2.1. Rat treatment protocols and blood pressure measurement in vivo

Male Wistar rats (210–230 g) were purchased from Hebei Medical University and handled following Animal Use Committee of Shanxi University guide lines. Twenty-four rats were housed under 50% ± 5% humidity and 24 ± 2 °C and equally divided to 4 groups. Three groups were treated with 0.5 ± 0.04, 3 ± 0.24, 18 ± 0.37 mg m^{-3} (0.42 ± 0.03, 2.50 ± 0.20, 15 ± 0.31 ppm) FA in treatment chambers for 7 d, 4 h d^{-1}, respectively. Animals in the control group were exposed to clear air for the same period of time (Zhang et al., 2016; Ye et al., 2013). The blood pressures of rats were determined following the procedure reported previously by us (Meng et al., 2003). Using a rat blood pressure meter from the China–Japan Friendship Institute of Clinical Sciences (Beijing, China), the systolic pressures were obtained by monitoring the blood flow through rats' tails. In order to measure the blood pressures of rats in FA inhalation and its control groups, the blood pressures were examined every day, 20 min after FA exposure. The final value of blood pressure for each rat was the mean of 6 continuous measurement values. To investigate the involvement of the three types of NOS in the FA-evoked effects, blood pressures were recorded in the rats pretreated with L-NAME (30 mg kg^{-1}) before FA exposure in another series of experiments (Talbot et al., 2012). Twenty-four hours after the last treatment, rats were killed *via* anesthetic overdose. The rat aortic tissues were harvested rapidly, and placed into liquid nitrogen for freezing and subsequently transferred to storage at −80 °C.

2.2. RT-PCR

The experiments were carried out as reported previously (Zhang et al., 2015; Li et al., 2014). In brief, TRIzol Reagent was used to extract total RNA of the above aortic tissues and reverse transcription kit (TaKaRa, Dalian, China) was used to synthesize cDNA following manufacturer's instructions. An iQ iCycler test system (Bio-Rad, CA, USA) was applied to measure mRNA levels. β-actin was used as an internal control and relative expression levels of the interest target were examined. The primer sequences of interest genes were depicted below: BK_{Ca} α (NM 031828), sense: 5′-TTACAGCACACTCCGCAGAC-3′, antisense: 5′-CACCAAACAACC ACCATCC-3′, BK_{Ca} β1 (NM 019273), sense: 5′-ATCCTCCTCTTCT CCTTCTTCTG-3′, antisense: 5′-GCCGTTCCTGGTGACTCC-3′, $Ca_v1.2$ (NM 012517), sense: 5′-CATCATCAT CATTGCCTTCTTC-3′, antisense: 5′-ACTGGTGCTGGTTCTTGG-3′, $Ca_v1.3$ (NM 012517), sense: 5′-CTTCTTCTTCATCATCATCTTC-3′, antisense: 5′-TCATACAT CACCGCATTCC-3′, neuronal nitric oxide synthase (nNOS) (NM 052799), sense: 5′-CTGCAAAGCCCTAAGTC CAG-3′, antisense: 5′-AGTGTTCCTCTCCTCCAGCA-3′, inducible nitric oxide synthase (iNOS) (NM 012611), sense: 5′-CTCTCAGCAGCATCCACG-3′, antisense: 5′-GCGGCTGGAC TTCTCACT-3′, endothelial nitric oxide synthase (eNOS) (NM 021838), sense: 5′-CCGAGGCAATCTTCGTTC-3′, antisense: 5′-GCTGGCTGTTCCAGATCC-3′, β-actin (NM 017008), sense: 5′-CCTATGCCAACACAGTGCTG TCT-3′, antisense: 5′-GCTCAGGAGGAG CAATGA TCTTGA-3'.