

# Platelet Activating Factor affects Sigmoid Smooth Muscle Contraction in UC

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## Abstract

### **Platelet Activating Factor (PAF) affects Sigmoid Smooth Muscle contraction In Ulcerative Colitis (UC)**

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We have previously shown that sigmoid circular muscle cells from UC patients exhibit reduced contraction and  $\text{Ca}^{2+}$  signaling in response to the endogenous neurotransmitter neurokinin A (NKA) and that  $\text{H}_2\text{O}_2$  is elevated in the UC circular muscle layer. The  $\text{H}_2\text{O}_2$  scavenger catalase restored the decreased  $\text{Ca}^{2+}$  signal and cell shortening to normal levels, suggesting that  $\text{H}_2\text{O}_2$  contributes to the motor dysfunction of UC. It is known that PAF levels are elevated in colonic mucosa from patients with ulcerative colitis. In the present study we examined the role of PAF in motor dysfunction and  $\text{H}_2\text{O}_2$  production in UC.

PAF levels were significantly elevated in sigmoid circular muscle from patients with ulcerative colitis, when compared to normal controls. Treatment of normal sigmoid circular smooth muscle cells with PAF ( $10^{-6}\text{M}$ , 2 hours) significantly decreased NKA-induced contraction. Treatment of UC sigmoid muscle strips with the PAF antagonist CV3988 ( $10^{-6}\text{M}$ ) for 2 hours, however, did not improve the motor dysfunction, suggesting that other inflammatory mediators e.g.  $\text{H}_2\text{O}_2$  and  $\text{IL-1}\beta$  may be present and affect muscle contraction, even after blockade of PAF. PAF  $10^{-8}\text{M}$  significantly increased hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) production in sigmoid smooth muscle cells in primary culture, and  $\text{H}_2\text{O}_2$  production was abolished by the NADPH oxidase inhibitor apocynin, suggesting that NADPH oxidases mediate PAF-induced production of  $\text{H}_2\text{O}_2$ . PAF-induced  $\text{H}_2\text{O}_2$  was significantly reduced by the MEK1 inhibitor PD98059, the P38 MAP kinase inhibitor SB203580 and the cytosolic phospholipase A2 inhibitor AACOCF3, suggesting that PAF-induced  $\text{H}_2\text{O}_2$  production depends on activation of MAP kinases (ERK1/2, p38) and cytosolic phospholipase A<sub>2</sub>.

We conclude that PAF may contribute to motor dysfunction of sigmoid colon in UC and that PAF-induced  $\text{H}_2\text{O}_2$  production depends on activation of MAP kinases (ERK1/2, p38), cytosolic phospholipase A<sub>2</sub> and NADPH oxidases.

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# Background

- In animal models platelet activating factor (PAF) can induce mucosal inflammation and ulceration.
- PAF levels are increased in animal models of enterocolitis.
- Levels of PAF are elevated in mucosal tissues from patients with ulcerative colitis (UC).
- $H_2O_2$  levels are increased in sigmoid mucosa and circular muscle from patients with UC and enzymatically isolated UC muscle cells contain much higher levels of  $H_2O_2$  than normal muscle cells.
- NKA-induced contraction and calcium increase are significantly decreased in UC sigmoid muscle and this decrease is partly recovered by  $H_2O_2$  scavenger catalase. These data suggest that  $H_2O_2$  may contribute to motor dysfunction in ulcerative colitis.

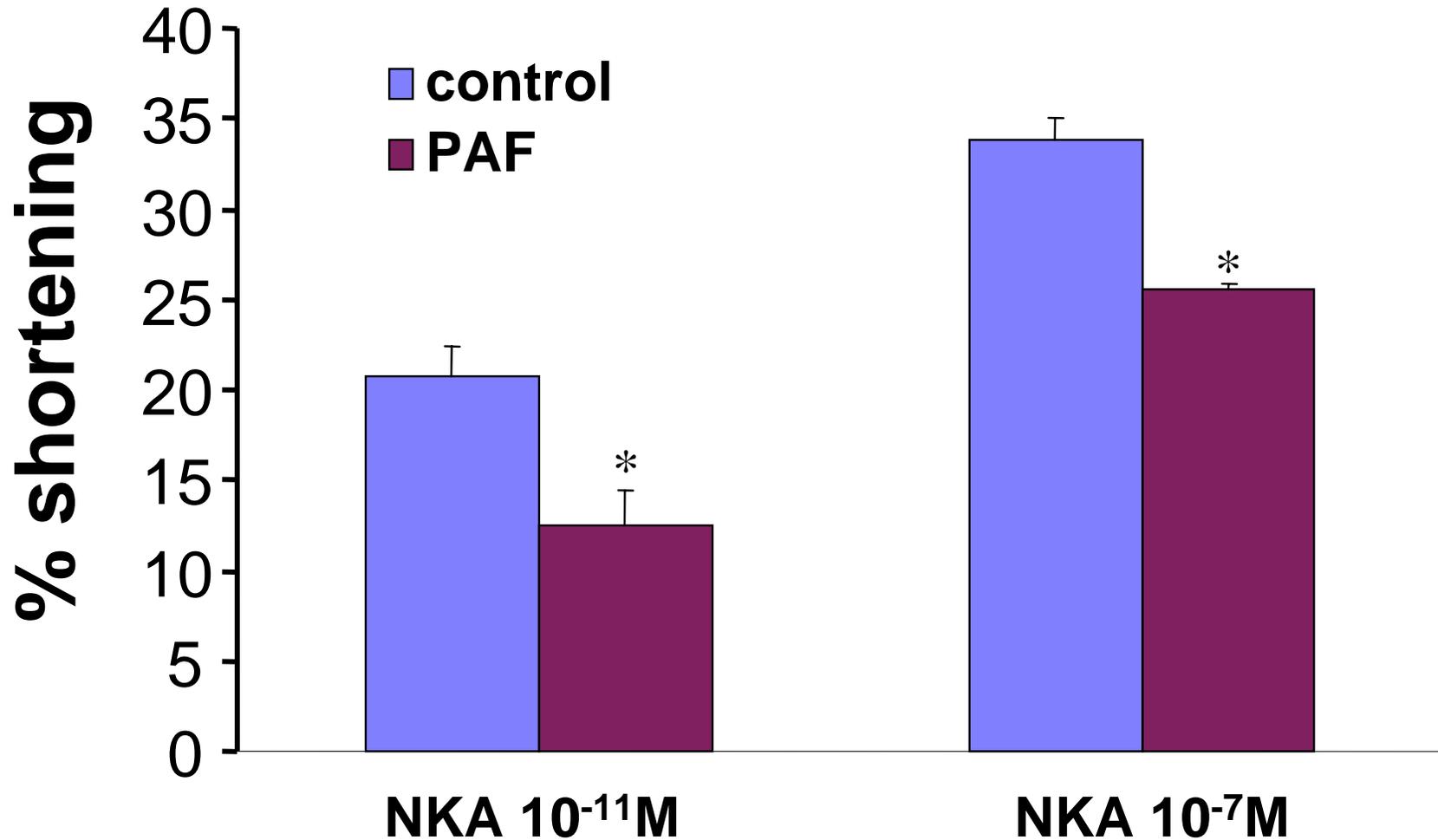
## **Aim**

**We propose to examine the role of PAF in causing motor dysfunction and in H<sub>2</sub>O<sub>2</sub> production in UC.**

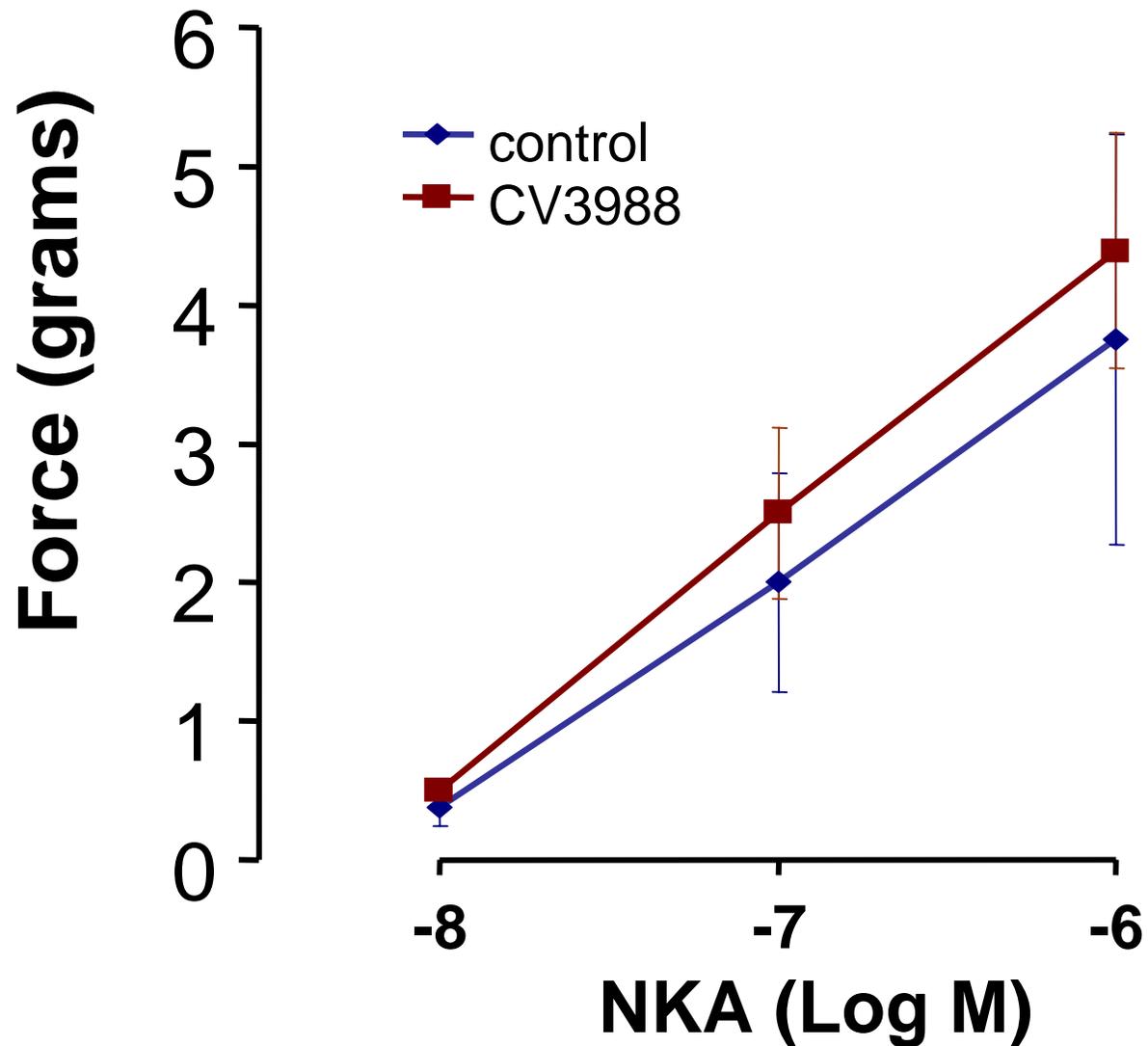
## Methods

- Sigmoid circular muscle was cut into thin strips and digested in HEPES buffered solution to obtain smooth muscle cells.
- After preincubation with PAF ( $10^{-6}\text{M}$ ) for 2 hours, normal sigmoid smooth muscle cells were treated with neurokinin A ( $10^{-6}\text{M}$  to  $10^{-12}\text{M}$ ) for 30 seconds. Cell length was measured with phase contrast microscope and NIH image software.
- In sigmoid circular muscle strips, cumulative dose response to neurokinin A was obtained before and after muscle strips were incubated with CV3988 (PAF antagonist) for 2 hours.

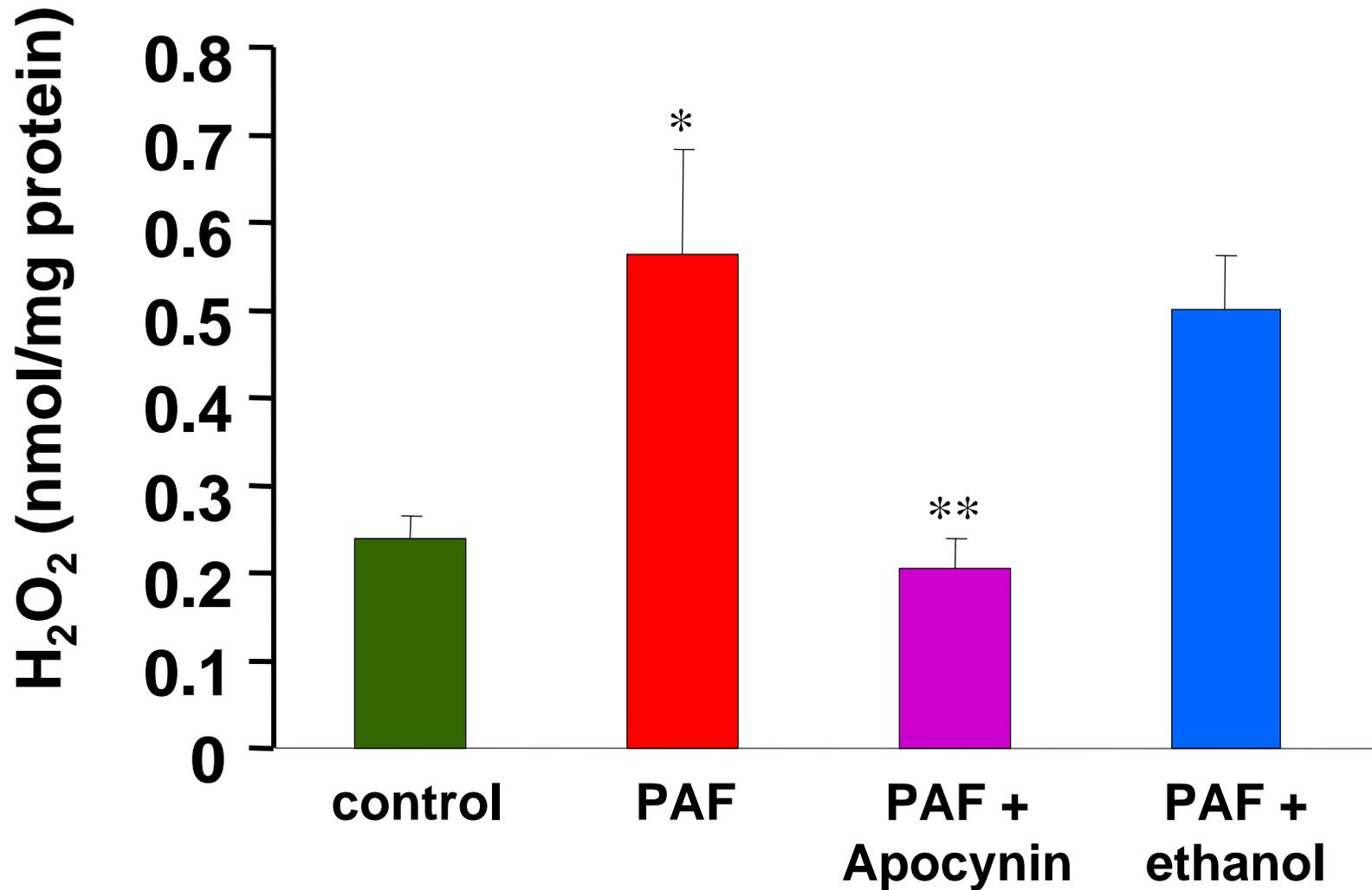
- **Sigmoid smooth muscle cells were kept in primary culture and treated with PAF  $10^{-8}$ M in the absence (control) or presence of apocynin ( NADPH oxidase inhibitor), PD98059 ( MEK1 kinase inhibitor), SB203580 (P38 MAP kinase inhibitor), and AACOCF3 ( cPLA2 inhibitor) for 24 hours. The culture medium was collected for measurement.**
- **H<sub>2</sub>O<sub>2</sub> was measured by using Amplex® Red H<sub>2</sub>O<sub>2</sub> Assay Kit (Molecular Probes, Inc., Eugene, OR).**



**Treatment of normal sigmoid circular smooth muscle cells with PAF (10<sup>-6</sup>M, 2 hours) significantly decreased NKA-induced contraction, suggesting that PAF may impair the smooth muscle contractility.**



**Treatment of UC sigmoid muscle strips with the PAF antagonist CV3988 ( $10^{-6}$ M) for 2 hours did not improve the motor dysfunction, suggesting that other inflammatory mediators e.g.  $H_2O_2$  and  $IL-1\beta$  may be present and affect muscle contraction, even after blockade of PAF.**

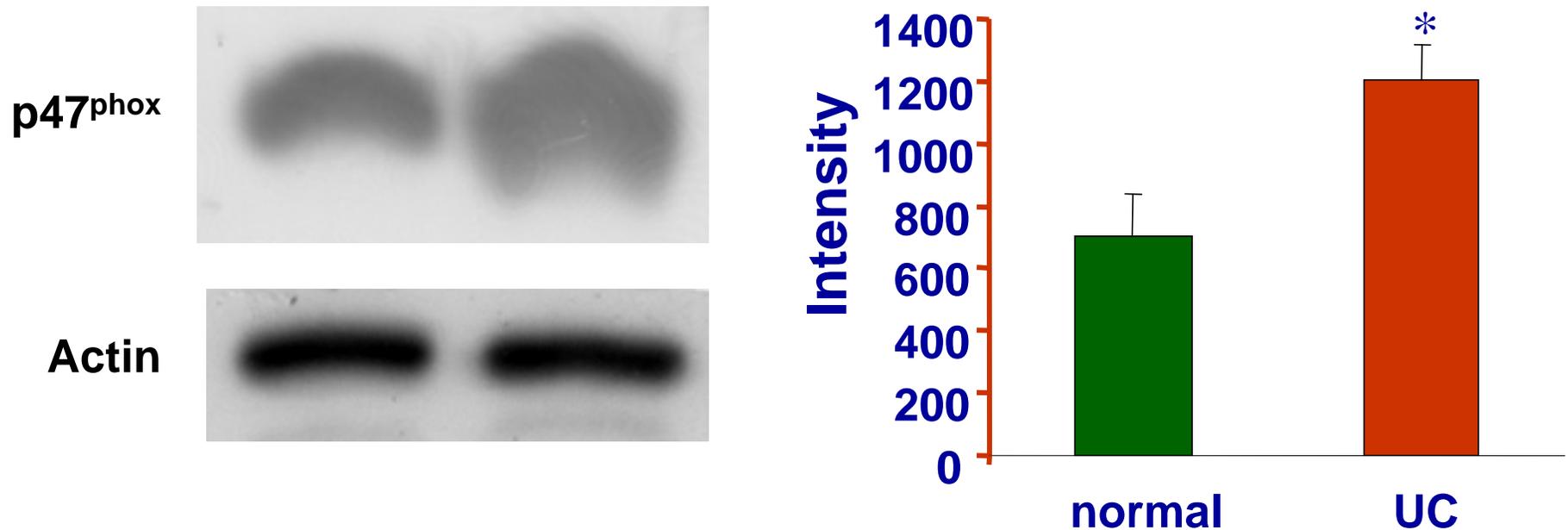


**PAF 10<sup>-8</sup>M significantly increased hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) production in sigmoid smooth muscle cells in primary culture, and H<sub>2</sub>O<sub>2</sub> production was abolished by the NADPH oxidase inhibitor apocynin, suggesting that NADPH oxidases mediate PAF-induced production of H<sub>2</sub>O<sub>2</sub>.**

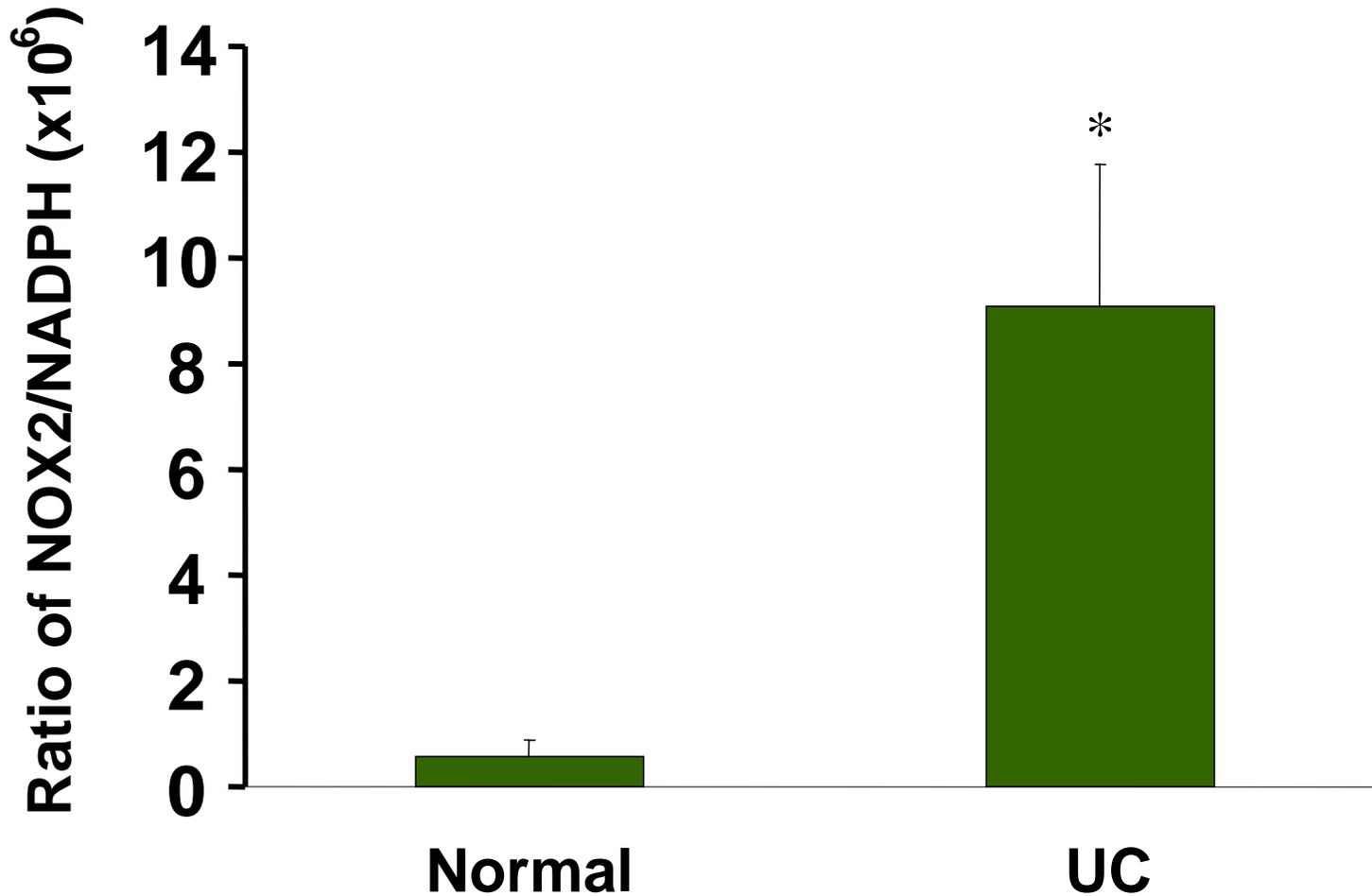


NOX1 NOX2 NOX3 NOX4 NOX5S NOX5L DOUX1 DOUX2

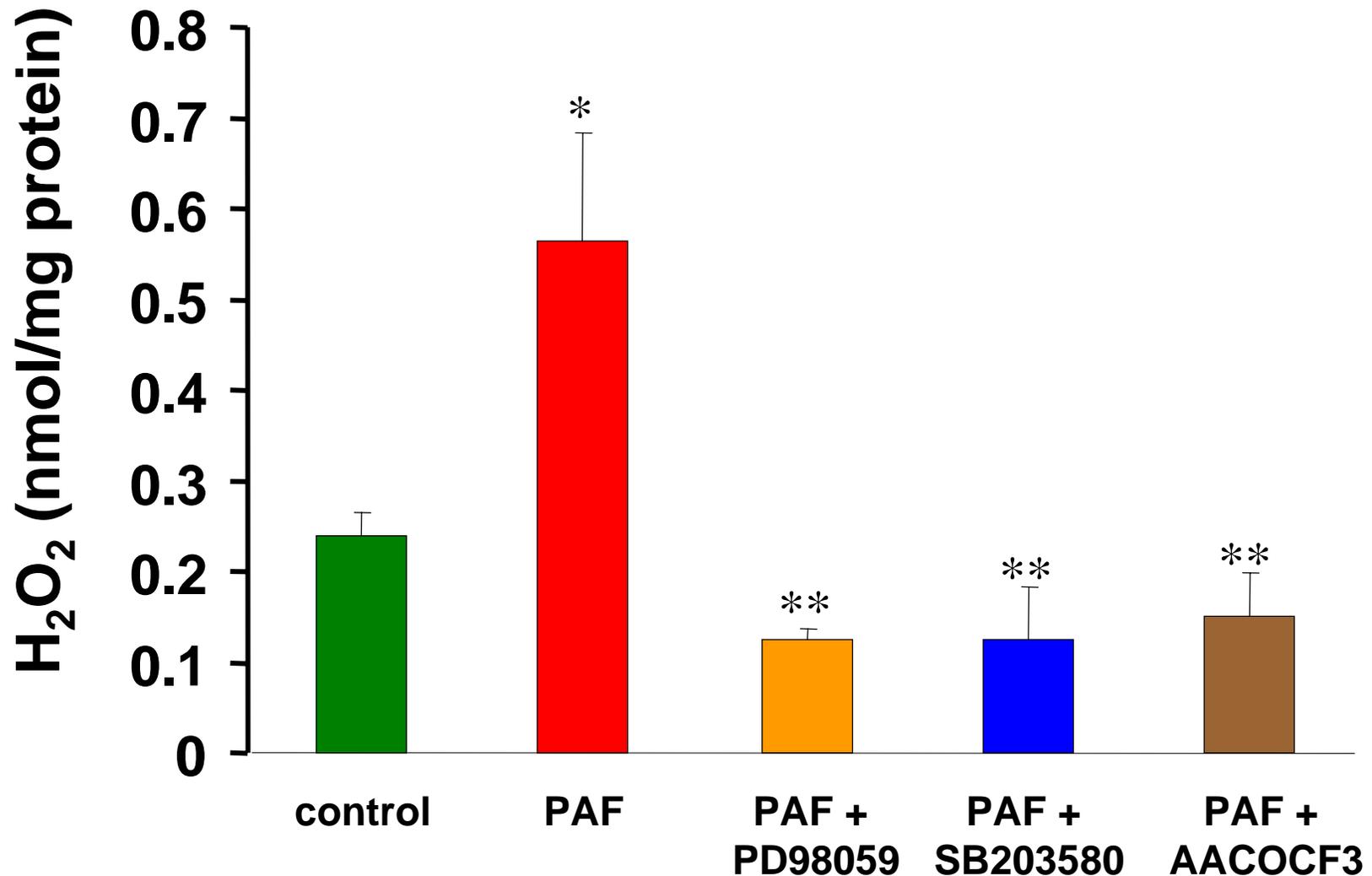
**RT-PCR showed that NOX2, DOUX1 and DOUX2 were present in human sigmoid circular smooth muscle cells.**



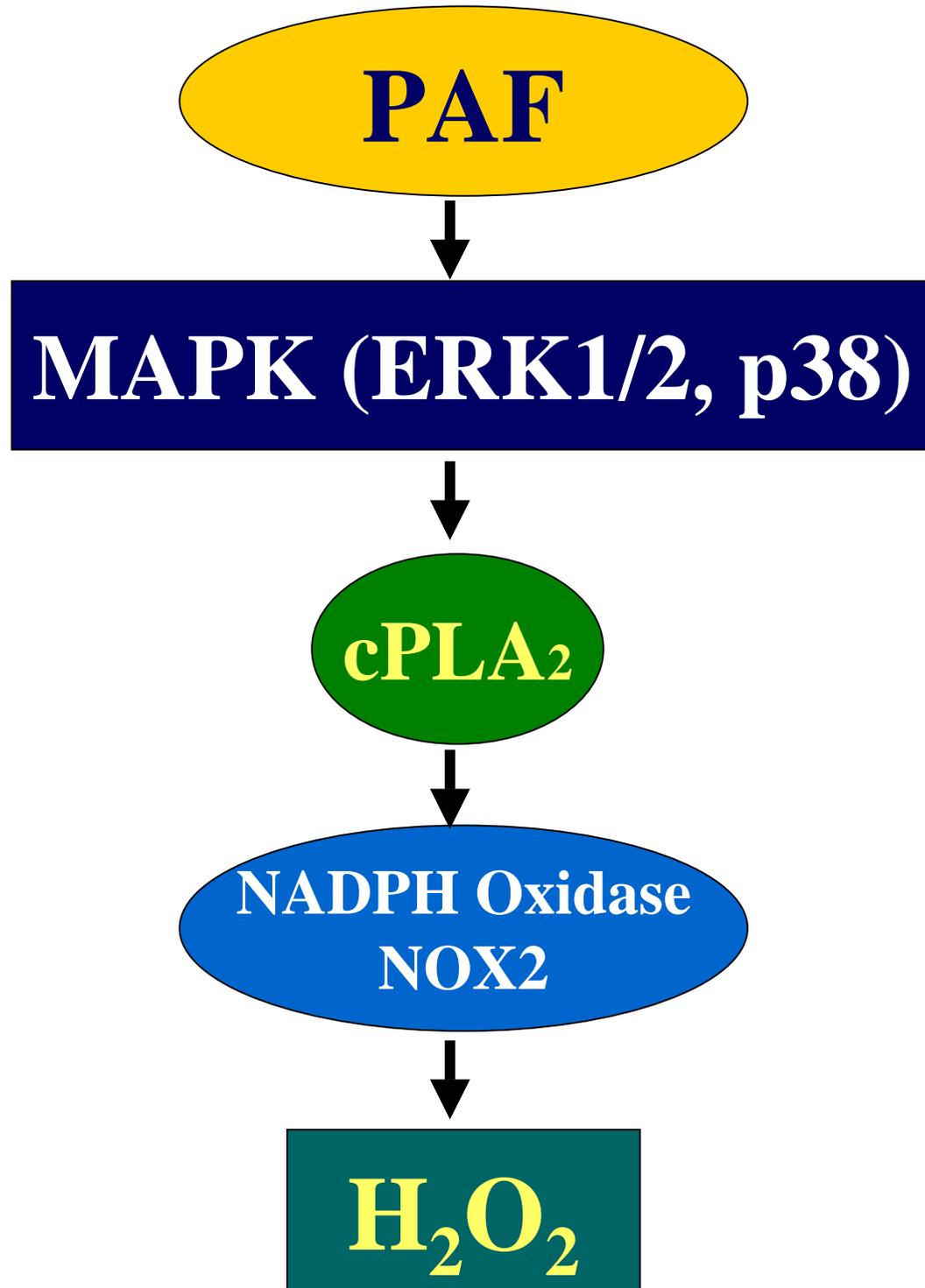
**Western blot by using an antibody against NADPH oxidase cytosolic subunit p47<sup>phox</sup> showed that NADPH oxidase was increased in UC sigmoid circular muscle, when compared with normal sigmoid muscle. Each lane was loaded with equal amount of protein (0.3mg). Intensity was calculated from the equation: OD\*area (pixels). N=3, \* P<0.05, unpaired t test.**



**NOX2 mRNA, as measured by real time PCR, was increased about 9.3 times in UC sigmoid muscle when compared with normal sigmoid muscle, indicating a possible role of NADPH oxidase NOX2 in the production of H<sub>2</sub>O<sub>2</sub> in UC.**



**In cultured smooth muscle cells PAF-induced H<sub>2</sub>O<sub>2</sub> production was significantly reduced by MEK1 inhibitor PD98059, the P38 MAP kinase inhibitor SB203580 and by the cytosolic phospholipase A2 inhibitor AACOCF3, suggesting that PAF-induced H<sub>2</sub>O<sub>2</sub> production depends on activation of MAP kinases (ERK1/2, p38) and cytosolic phospholipase A2. \* P<0.01, compared with control, \*\* P<0.01, compared with PAF group.**



## **Conclusion**

**PAF may contribute to motor dysfunction of sigmoid colon in UC and that PAF-induced H<sub>2</sub>O<sub>2</sub> production depends on activation of MAP kinases (ERK1/2, p38), cytosolic phospholipase A2 and NADPH oxidases.**