*Physiol. Res. 44: 31–37, 1995*

# Role of Ion Fluxes in Hydrogen Peroxide Pulmonary Vasoconstriction

J. WILHELM, J. HERGET[1]

*Department of Medical Chemistry and Biochemistry and [1]Department of Physiology, Second Medical School, Charles University, Prague, Czech Republic*

*Received November 10, 1994*
*Accepted December 1, 1994*

## Summary

Hydrogen peroxide injected into the inflow cannula of isolated ventilated rat lungs produced a dose-dependent vasoconstriction in the range 0.25–10 mM, with maximum response between 2–5 mM. The effects of $H_2O_2$ can be influenced by ionophores or specific inhibitors of ionic channels or pumps. A key role is played by sodium ions which govern the subsequent inflow or outflow of calcium, an ion mediating the vasoconstriction. A physiological role for $H_2O_2$ generated by NADPH oxidase is postulated.

**Key words**
Hydrogen peroxide – Calcium – Ionophores – Sodium – Lung – Vasoconstriction

## Introduction

Hydrogen peroxide probably plays an important physiological role in the regulation of vascular tone. The hypothesis concerning the regulatory role of $H_2O_2$ is supported by the observations that vascular endothelial cells in culture release $H_2O_2$ into the extracellular space (Kinnula *et al.* 1992, Sundquist 1991). This production has a constant rate under oxygen concentrations between 100–10 % and decreases when oxygen concentration is lowered below 10 %, reaching one third of the original activity at 0 % oxygen (Kinnula *et al.* 1993). $H_2O_2$ is freely diffusible across cell membranes. It can therefore be expected that vascular smooth muscles might be influenced by the hydrogen peroxide produced by various sources such as alveolar macrophages or adhered neutrophils (Perry and Taylor 1988).

Hydrogen peroxide induces contractions in preparations of isolated pulmonary arteries (Sheehan *et al.* 1993) as well as in isolated lungs (Tate *et al.* 1982). It is noteworthy that some studies documented its vasodilator effect, e.g. in intrapulmonary arterial rings (Burke and Wolin 1987).

## Methods

Forty Wistar male rats with average body weight of 250–350 g were used for the experiments. Measurements were performed on the preparation of isolated ventilated lungs perfused by a physiological saline solution with 4 g/100 ml bovine albumin (fraction V, Sigma) and 0.016 mM sodium meclofenamate (Herget and McMurtry, 1985). The lungs were obtained from heparinized rats under pentobarbital anaesthesia (10 mg/100 g b.w.). The pulmonary artery and left ventricle were cannulated and then the lungs were suspended on the lever of a strain gauge (for the relative lung weight measurements) in a thermostated humid chamber. Isolated lungs were perfused by recirculation at constant flow of 0.04 ml/g b.w. They were ventilated with a humid and warmed normoxic gas mixture (21 % $O_2$+5 % $CO_2$ + 74 % $N_2$) by positive pressure (60 breath/min, peak inspiratory pressure 9 cm $H_2O$, end-exspiratory pressure 2.5 cm $H_2O$). After a 15 min stabilization period the lungs were challenged with two arterial injections of angiotensin II (0.2 $\mu$g) to establish the vasoreactivity of the preparation.

Hypoxia was induced by a mixture of 3 % $O_2$+5 % $CO_2$, balanced by $N_2$.

Hydrogen peroxide was measured in the medium taken from the outflow cannula by a luminometric method based on the reaction with luminol catalyzed by horseradish peroxidase.

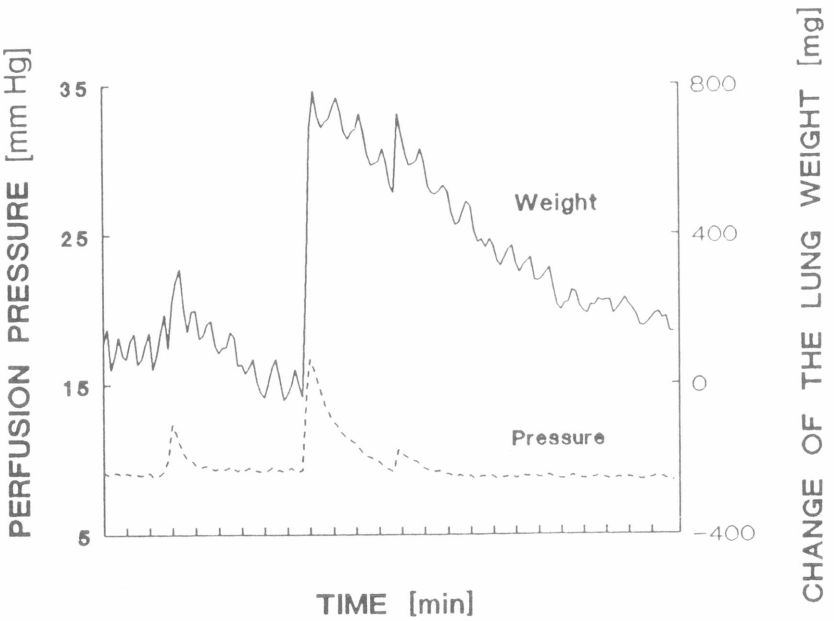


**Fig. 1**
Typical changes of perfusion pressure and lung weight after injection of hydrogen peroxide (0.7 mM). The increase of perfusion pressure reflects the vasoconstriction because the lungs are perfused at constant flow.

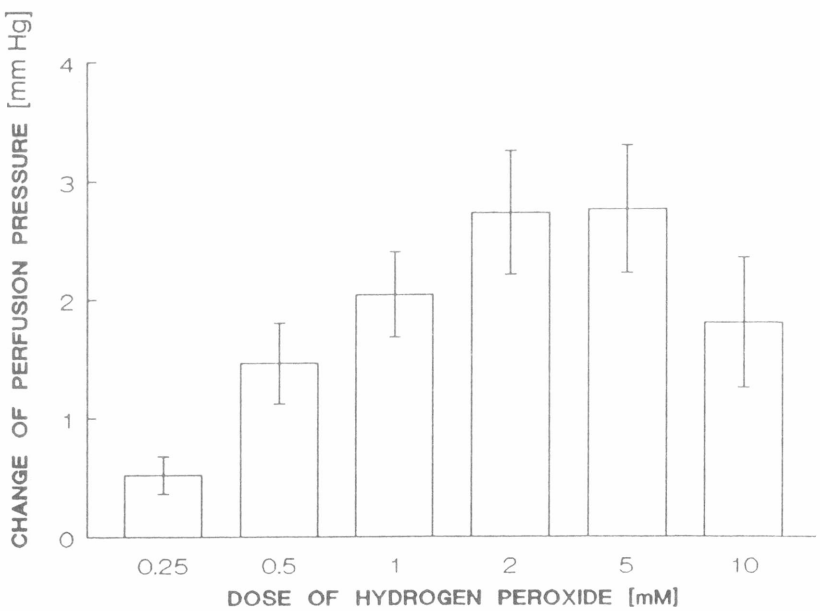


**Fig. 2**
Change in perfusion pressure in isolated rat lungs induced by injection of different doses of hydrogen peroxide in the inflow cannula (7 isolated lungs from male rats 195–240 g). Vertical bars represents S.E.M.

## Results and Discussion

In the present study we observed a transient vasoconstriction elicited by injection of hydrogen peroxide into the inflow cannula. The increase in vascular pressure was mirrored in a transient gain of lung weight (Fig. 1). The effect of hydrogen peroxide was dose-dependent, producing maximal vasoconstriction between 2–5 mM. A further increase in $H_2O_2$ concentration decreased the pressure (Fig. 2). The transient character of this pressure increase was caused by the rapid decomposition of hydrogen peroxide in the system, as we found no $H_2O_2$ in the outflow from the lung. However, if we induced sustained production of hydrogen peroxide by adding glucose plus glucose oxidase into the recirculating perfusate, persistent vasoconstriction was observed (Fig. 3).

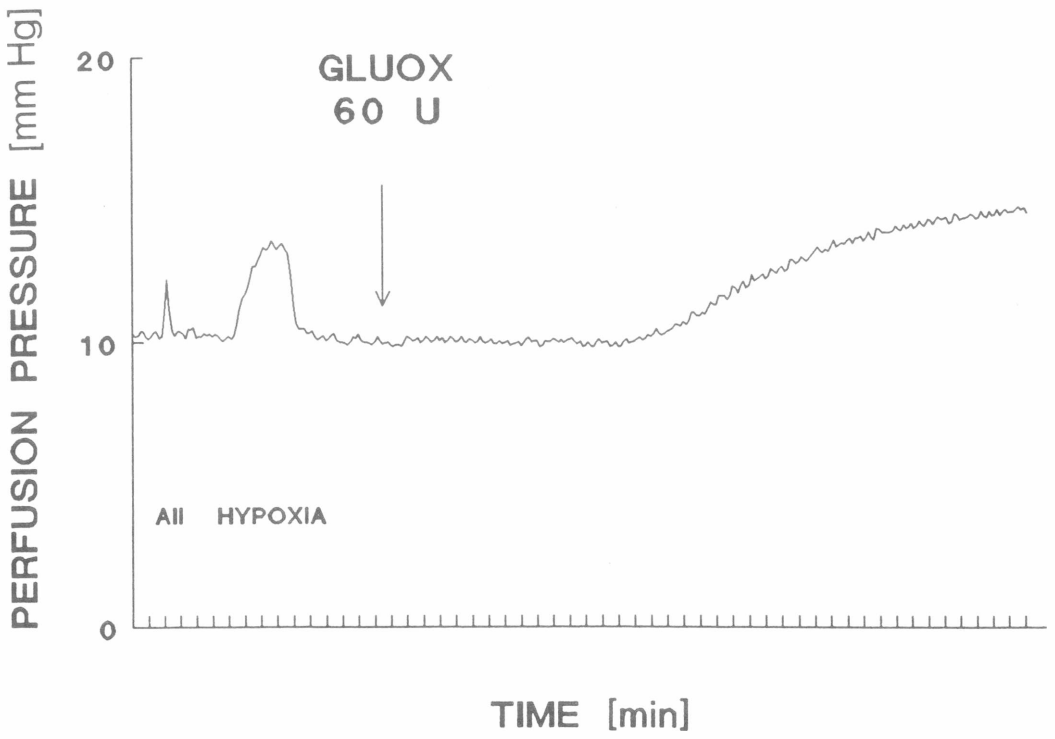


Fig. 3
Increase of perfusion pressure induced by 2 U/ml of glucose oxidase (GLUOX) added into the venous reservoir of isolated lung preparation. A II – angiotension II. Typical example from 3 experiments.

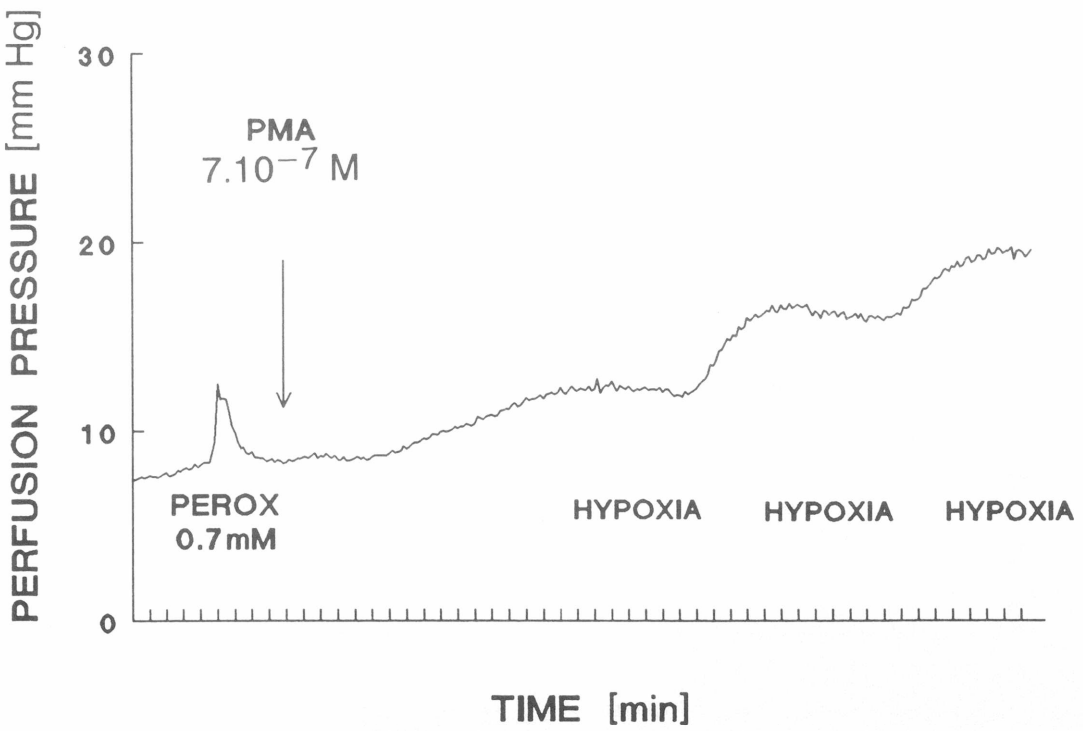


Fig. 4
Effect of phorbol myristate acetate (PMA) into the venous reservoir of isolated lungs. Hydrogen peroxide (PEROX) was injected into the inflow cannula. Typical example from 5 experiments.

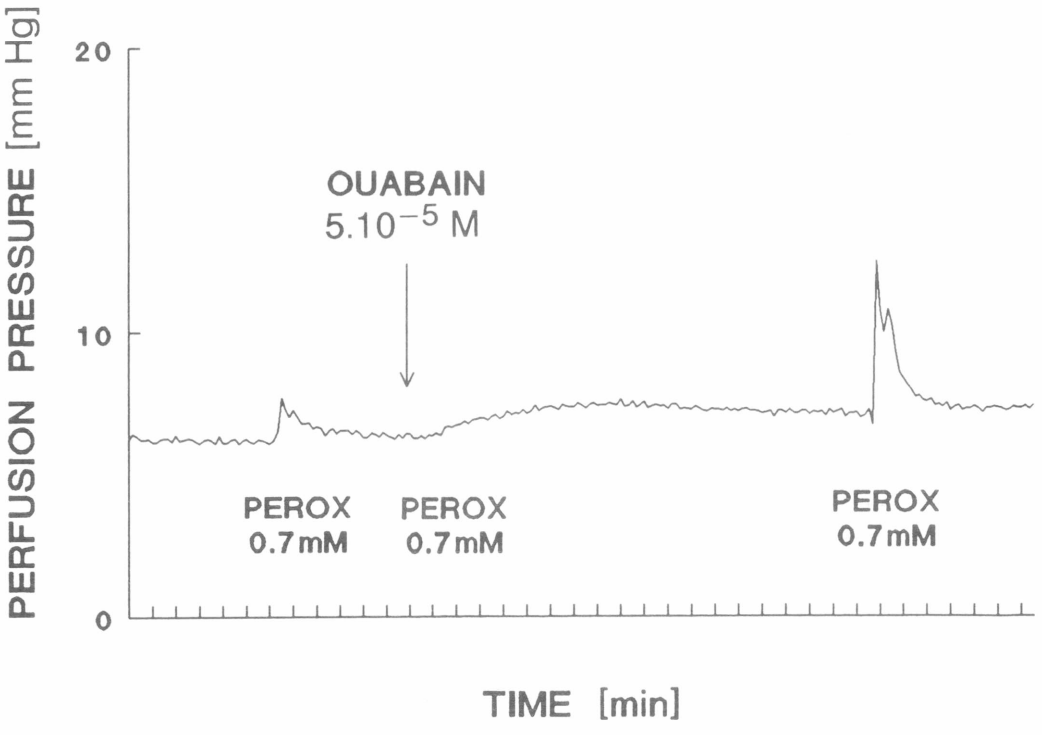


Fig. 5
Effect of ouabain (added into the venous reservoir) on the vasoconstriction induced by injection of hydrogen peroxide (PEROX) into the inflow cannula. Typical example from 9 experiments.

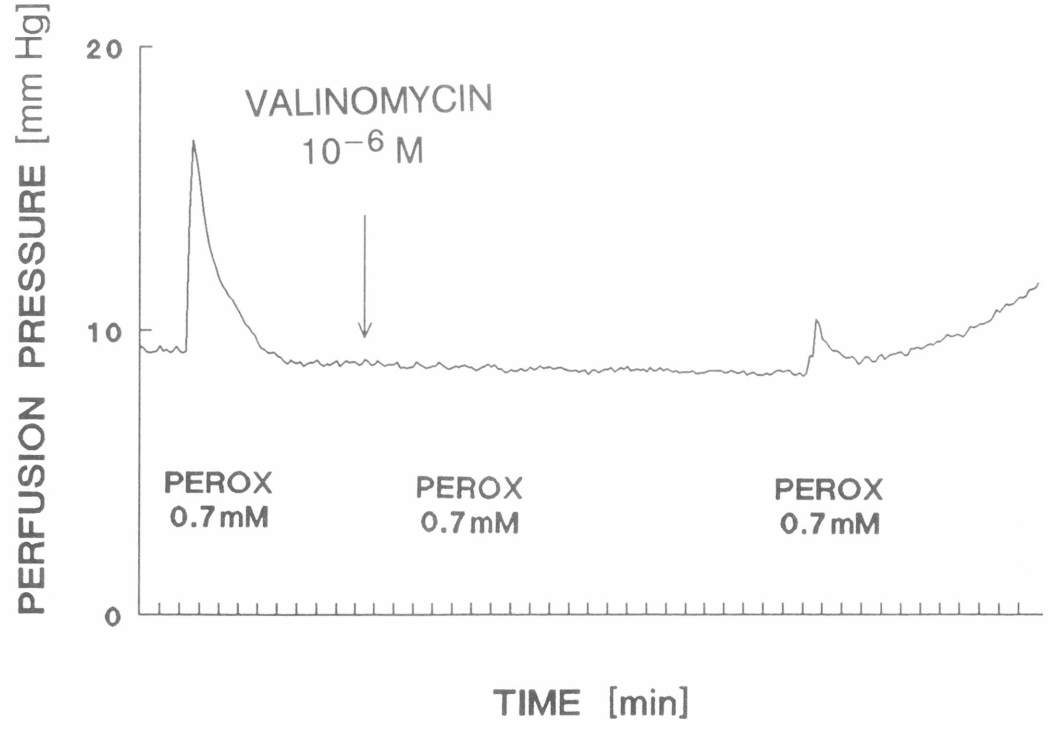


Fig. 6
Effect of valinomycin ($K^+$ ionophore) on vasoconstriction induced by hydrogen peroxide (PEROX). Typical example from 7 experiments.

We also attempted to induce endogenous production of hydrogen peroxide by phorbol myristate acetate (PMA) which triggers $H_2O_2$ production in neutrophils and macrophages (Perry and Taylor 1988). Though it was shown that PMA also influences other cellular activities by affecting ion fluxes (Owen 1985), our experiments (Fig. 4) pointed to the activation of $H_2O_2$-producing NADPH oxidase by PMA. This oxidase requires oxygen as a substrate for $H_2O_2$ generation. We have observed a continuous increase of perfusion pressure after incubation with PMA which could have been switched off by withdrawing oxygen from the ventilation gas. Reintroduction of oxygen in the ventilation apparently enabled further continuation of hydrogen peroxide production as perfusion pressure continued to rise again. The cycles of switching-off/switching-on could have been repeated several times. We believe that NADPH oxidase is the key participant, because otherwise hypoxia induces vasoconstriction instead of arresting it.

The effect of hydrogen peroxide could have been modulated by manipulation with cellular membrane polarization. Depolarization of the membrane by inhibition of Na,K-ATPase by ouabain, or by increased extracellular potassium led to slight vasoconstriction, and the subsequent effect of hydrogen peroxide was highly potentiated (Fig. 5, Table 1). On the other hand, when cells were hyperpolarized in the presence of valinomycin (a potassium ionophore) the following effect of hydrogen peroxide was strongly inhibited (Fig. 6).

**Table 1**
Effect of extracellular potassium on the relative vasoconstriction induced by injection of 0.7 mM $H_2O_2$

| $[K^+]_o$ | % of perfusion pressure increase |
|---|---|
| 5 mM | 100 |
| 20 mM | 160 |
| 25 mM | 330 |
| 30 mM | 200 |

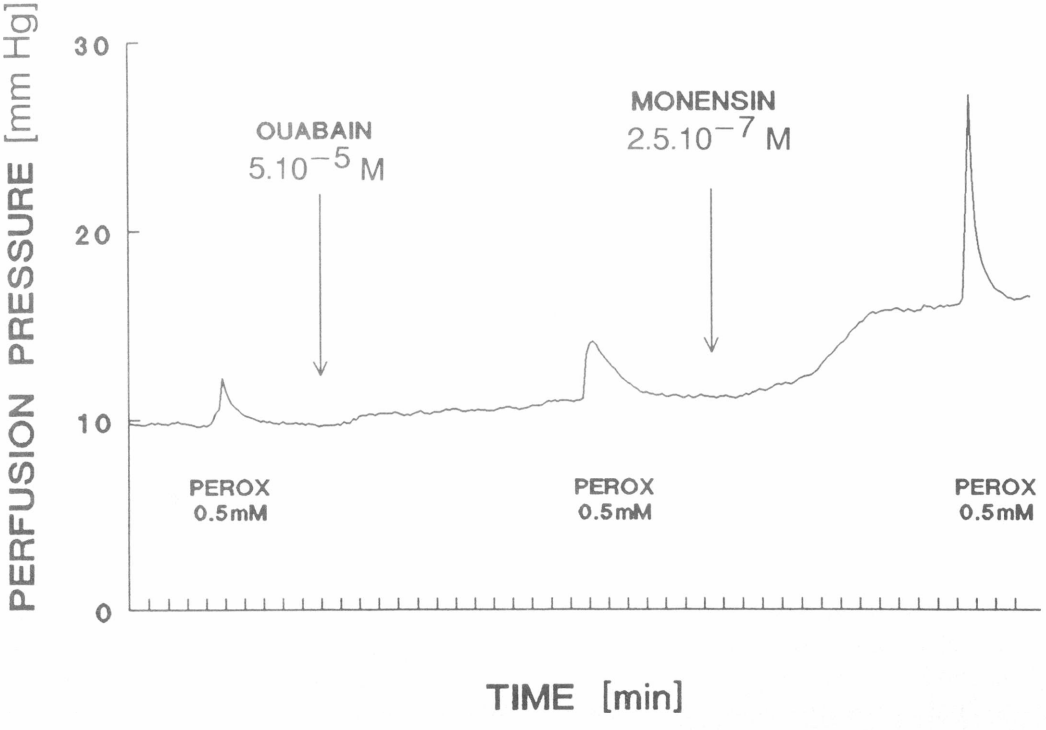


**Fig. 7**
Effect of monensin ($Na^+$ ionophore) on the vasoconstriction induced by hydrogen peroxide (PEROX). Typical example from 3 experiments.

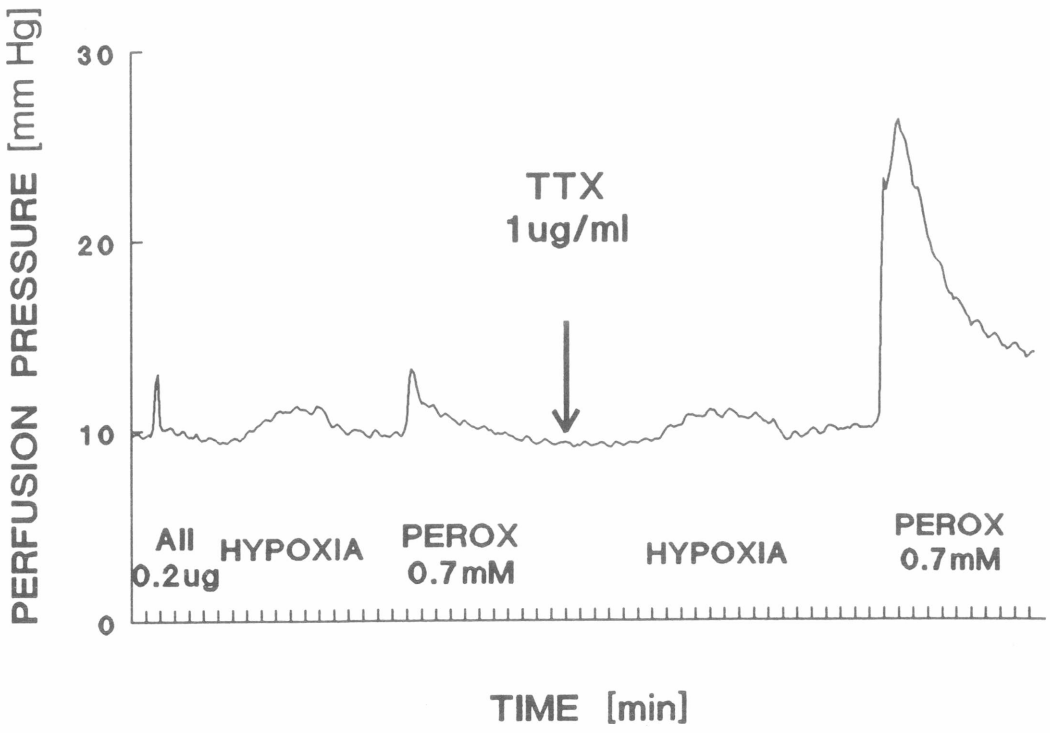


**Fig 8**
Effect of tetrodotoxin (TTX), blocker of voltage-dependent $Na^+$ channels, (added to the venous reservoir) on the vasoconstriction induced by hydrogen peroxide (PEROX) injected into the inflow cannula. Typical example from 3 experiments.

As the vasoconstriction is essentially dependent on the rise of cytosolic calcium, the report of Doan *et al.* (1994) is of special interest in that hydrogen peroxide increased cytosolic free calcium by both mobilization from endogenous stores and by activating the influx pathway in canine venous endothelial cells. If a similar process also takes place in vascular smooth muscle cells, it could explain our data. Another possibility is that the signal from endothelial cells is transferred to smooth muscle cells.

An important effector of $H_2O_2$ action will also probably be the $Na^+$-$Ca^{2+}$ exchanger which can move calcium ions in opposite directions, depending on the polarization of the membrane. If the cells are depolarized, the exchanger may mediate calcium influx. However, as the cells become repolarized, the exchanger will mediate calcium efflux. The direction of the calcium flux is dictated by orientation of the $Na^+$ gradient (Ashida and Blaustein 1987). Thus the rise in intracellular sodium concentration can elevate intracellular $Ca^{2+}$ and enhance contraction (Hermsmeyer 1982). The importance of Na,K-ATPase, which maintains the low intracellular sodium concentrations is apparent. So we might explain therefore the accentuation of the $H_2O_2$ effect after ouabain inhibition of Na,K-ATPase by the contribution of $Ca^{2+}$ influx due to increased intracellular $Na^+$.

The effect is even more apparent in the presence of the sodium ionophore, monensin. In the case of intact Na,K-ATPase, monensin does not produce vasoconstriction and subsequent addition of $H_2O_2$ does not produce greater constriction than in the absence of monensin. However, after addition of ouabain the vasoconstriction due to $Na^+$ loading caused by monensin starts to rise and subsequent addition of hydrogen peroxide produces a vigorous increase of perfusion pressure (Fig. 7).

Another aspect of $H_2O_2$ effects is revealed in the presence of tetrodotoxin (TTX). TTX is a specific blocker of voltage-gated sodium channels. Normally, voltage-gated channels are opened upon depolarization of the cell and rapid sodium influx discharges the membrane potential. This may serve as a signal for $Na^+$-$Ca^{2+}$ exchanger to pump calcium out of the cell to end vasoconstriction. As we have observed an increased effect of hydrogen peroxide after the addition of TTX, we can speculate that TTX locks the cell in the depolarized state for a longer time which results in higher calcium accumulation and greater vasoconstriction (Fig. 8).

We do not know the exact mechanism by which hydrogen peroxide induces cell depolarization, but the interpretation of our data can put certain limits to various theories. First, the mechanism of $H_2O_2$-induced vasoconstriction is completely different from that induced by hypoxia as is seen from the effect of PMA. Further, our data are in contradiction with the redox theory of hypoxic pulmonary vasoconstriction (Post *et al.* 1993) which presumes that oxidants block the influx of calcium. Finally, our study highlights the importance of sodium ion membrane fluxes in the mechanism of vasoconstriction.

**Acknowledgement**
This study was supported by the research grants of the Grant Agency of Charles University (No. 214) and the Grant Agency of the Czech Republic (No. 305).

## References

ASHIDA T., BLAUSTEIN M.P.: Control of cell calcium and contractility in mammalian arterial smooth muscle: the role of sodium-calcium exchange. *J. Physiol. Lond.* **392:** 617–635, 1987.

BURKE T.M., WOLIN M.S.: Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. *Am. J. Physiol.* **252:** H721–H732, 1987.

DOAN T.N., GENTRY D.L., TAYLOR A.A., ELLIOTT S.J.: Hydrogen peroxide activates agonist-sensitive $Ca^{2+}$-flux pathway in canine venous endothelial cells. *Biochem. J.* **297:** 209–215, 1994.

HERGET J., MCMURTRY I.M.: Effects of ouabain, low $K^+$, and aldosterone on hypoxic pressor reactivity of rat lungs. *Am. J. Physiol.* **248:** H55–H60, 1965.

HERMSMEYER K.: Electrogenic ion pumps and other determinants of membrane potential in vascular muscle. *Physiologist* **25:** 454–465, 1982..

KINNULA V.L., WHORTON A.R., CHANG L., CRAPO J.D.: Regulation of hydrogen peroxide generation by cultured endothelial cells. *Am. J. Respir. Cell Mol. Biol.* **6:** 175–182, 1992.

KINNULA V.L., MIRZA Z., CRAPO J.D., WHORTON A.R.: Modulation of hydrogen peroxide release from vascular endothelial cells by oxygen. *Am. J. Respir. Cell. Mol. Biol.* **9:** 603–609, 1993.

OWEN N.E.: Effect of TPA on ion fluxes and DNA synthesis in vascular smooth muscle cells. *J. Cell. Biol.* **101:** 454–459, 1985.

PERRY M., TAYLOR A.E.: Phorbol myristate acetate-induced injury of isolated perfused rat lungs: neutrophil dependence. *J. Appl. Physiol.* **65:** 2164–2169, 1988.

POST J.M., WEIR E.K., ARCHER S.L. HUME J.R.: Redox regulation of $K^+$ channels and hypoxic pulmonary vasoconstriction. In: *Ion Flux in Pulmonary Vascular Control*, E.K. WEIR (ed.), Plenum Press, New York, 1993, pp. 189–204.

SHEEHAN D.W., GIESE E.C., GUGINO S.F., RUSSELL J.A.: Characterization and mechanism of $H_2O_2$-induced contractions of pulmonary arteries. *Am. J. Physiol.* **264:** H1542–H1547, 1993.

SUNDQUIST T.: Bovine aortic endothelial cells release hydrogen peroxide. *J. Cell. Physiol.* **148:** 152–156, 1991.

TATE R.M., VAN BENTHUYSEN K.M., SHASBY D.M., MCMURTRY I.F., REPINE J.E.: Oxygen-radical-mediated permeability edema and vasoconstriction in isolated perfused rabbit lungs. *Am. Rev. Respir. Dis.* **126:** 802–806, 1982.

---

**Reprint Requests**
J. Wilhelm, Department of Medical Chemistry and Biochemistry, Second Medical School, Charles University, 150 00 Prague 5, Plzeňská 221, Czech Republic.