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BRIEF COMMUNICATION

EFFECTS OF EXTRACELLULAR pH ON PGI_2 AND TxA_2 RELEASE FROM PERFUSED CANINE VEINS

Jan S Brunkwall*, James C Stanley **, William E Burkel *, David Bergqvist***
Department of Surgery, University of Lund, Malmö General Hospital, Sweden*,
Section of Vascular Surgery, Department of Surgery **,
Department of Anatomy and Cell Biology***,
University of Michigan Medical School, Ann Arbor, Michigan, USA

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INTRODUCTION

The production and release of prostacyclin and thromboxane from the vessel wall are influenced by mechanical, radiological, immunological and chemical factors, as well as temperature and oxygen tension. In most reported studies, where buffered salt solution has been used as incubate or perfusate, the extracellular pH has been kept at the physiologic level of 7.4. Study of the effect of various pH values in rat aortic rings has shown that PGI_2 release was pH-dependent. On the contrary, pH did not determine the release of PGI_2 from perfused rabbit hearts (1). The release of prostacyclin in perfused vessel segments is not known.
The aim of this investigation was to study whether changes in extracellular pH would influence the release of PGI_2 and TxA_2 in perfused mongrel jugular veins.

MATERIAL AND METHODS

In anaesthetized mongrel dogs (Pentobarbital 20 mg/kg bw), the external jugular veins were carefully dissected free and all branches ligated. After flushing with calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS) each vessel segment was divided into two approximately 3 cm long segments and mounted in an open recirculating perfusion system that has been previously described (2,3). The segments were perfused for five times 15 minutes with Hank's balanced salt solution, HBSS, (temperature 37 °C, 290 mOsm) with the exchange of perfusate after each period and to the last period, arachidonic acid (AA) 4 µg/ml was added. The perfusate was stored at -20 °C until later determination for 6-keto-$PGF_{1\alpha}$ and TxB_2 by radioimmunoassay. Perfusion pressure was kept at 7 mm Hg and the nonpulsatile mean flow at 90 ml/min. The four vessel segments from each animal were divided into four groups with pH at either 6.0, 7.0, 7.4 or 7.8. The HBSS stock solution with a pH of 6.3 was titrated with NaOH or HCl to reach the desired pH. The vessel segments were perfused in parallel two by two and stratified so that each pH group was perfused equal times as number one and number two. In these experiments, pH of the perfusate was measured after each time period and did not vary more than ± 0.05. Accordingly the pH in the immersion bath did not vary more than ± 0.05.

Key words: Extracellular pH, PGI_2, prostacyclin, thromboxane, TxA_2

Prostanoid assay: Prostanoid extraction from the perfusates was performed according to the method described by Simmons (4). Prostacyclin was measured as its stable degradation product 6-keto-$PGF_{1\alpha}$ using a commercial kit (New England Nuclear). The percentage cross reactivity towards other prostaglandins is: PGE_2 2.5, $PGF_{1\alpha}$ 0.3, PGE_1 0.2, $PGF_{2\alpha}$ 0.05 and TxB_2 < 0.3 (manual New England Nuclear).
Thromboxane was measured as its stable degradation product TxB_2 according to a radioimmunoassay similar to that above also using ^3H-labelled tracer. The crossreactivity towards other prostaglandins is: PGD_2 1.0, PGD_1 0.32, $PGF_{1\alpha}$ 0.04 and $PGF_{2\alpha}$ 0.04. All samples were run in duplicate.
Specimens were prepared after perfusion for scanning electron microscopy (SEM) by pressure fixation at 7 mm Hg in 2.5 % glutaraldehyde in 0.1 M cacodylatebuffer. They were then dehydrated in ethanol, incubated in hexamethydisilazane for 10 minutes (5), air-dried, and sputtercoated with gold-palladium.
Data are expressed as pg/mm^2 luminal area/15 min (X ± SEM). Statistical analysis was assessed with test of variance and the Wilcoxon signed rank test and a p value < 0.05 was considered significant.

RESULTS

Control segments being perfused at a pH of 7.4 had an initial 6-keto-$PGF_{1\alpha}$ release of 57.1 ± 16.2 pg/mm^2/15 min declining to 10.2 ± 2.3 after 60 min ($p < 0.005$ vs initial value). When AA was added there was an increase to 117.9 ± 25.8 pg/mm^2/15 min ($p < 0.005$ vs 60 min value; Fig 1).

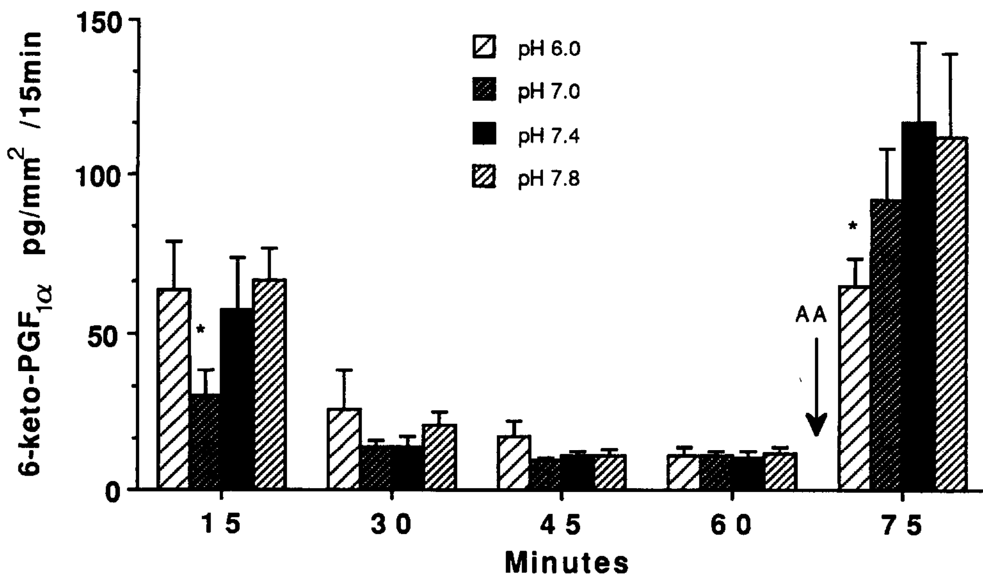

FIG 1 The effect of various pH of perfusate (Hank's balanced salt solution, HBSS) on the release of 6-keto-$PGF_{1\alpha}$ from mongrel veins. N = 7 in each group. AA = addition of arachidonic acid 4 μg/ml. Perfusion pressure 7 mm Hg and mean non-pulsatile flow 90 ml/min. * = $p < 0.05$ vs other groups (Test of Variance and Wilcoxon signed rank test at same measure point).

A pH of 6.0 exhibited the same release of 6-keto-$PGF_{1\alpha}$ as control segments for the first 60 minutes, but when AA was added, the response was only half the release compared to a pH of 7 or higher ($p < 0.05$).

A pH of 7.0 significantly decreased the initial 6-keto-PGF$_{1\alpha}$ release compared to higher or lower pH ($p < 0.05$), a difference that was abolished after the second perfusion and no difference versus controls was seen when AA was added.
A pH of 7.8 did not significantly alter the release of 6-keto-PGF $_{1\alpha}$ compared to control segments.
The release of TxB_2 from control segments was initially 3.8 ± 1.3 pg/mm^2/15 min declining to 0.7 ± 0.1 pg/mm^2/15 min after 60 min ($p < 0.05$) and when AA was given, the release increased to 3.2 ± 1.0 pg/mm^2/15 min ($p < 0.05$; Fig 2). A change in pH to 6.0, 7.0 or 7.8 did not significantly alter the TxB_2 release.

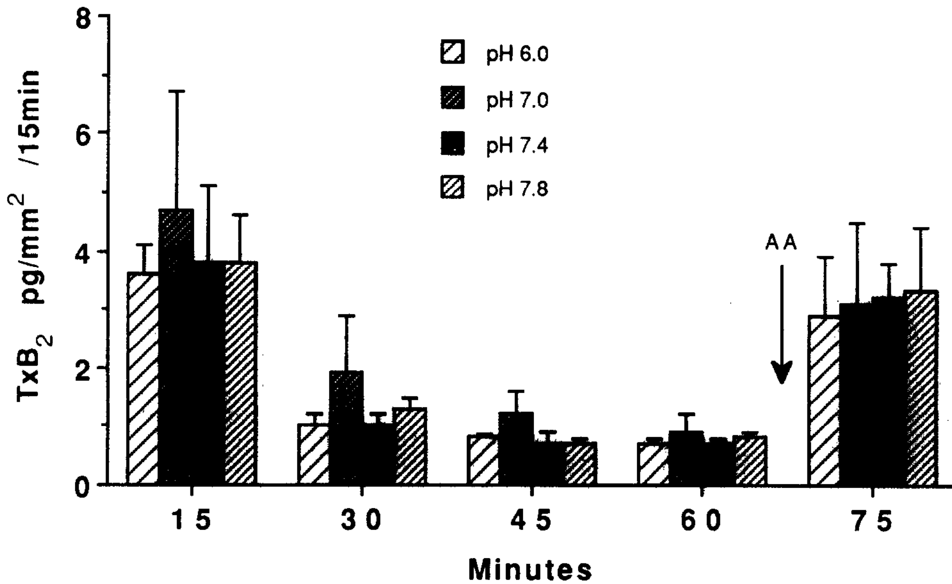

FIG 2 The effect of various pH of the perfusate (Hank's balanced salt solution, HBSS) on the release of TxB_2 from mongrel veins. N = 7 in each group. AA addition of arachidonic acid 4 µg/ml. Perfusion pressure 7 mm Hg and mean nonpulsatile flow 90 ml/min. No statistically significant difference at any point (Test of Variance and Wilcoxon signed rank test).

Scanning electron microscopy after perfusion revealed 75-90 % cover of the luminal surface by endothelial cells and without any difference between the various group studied.

DISCUSSION

The decreasing release of prostacyclin and thromboxane with time in this study as well as the increased release after addition of AA showed a similar pattern as in previous reports when mongrel arteries and veins (2,3) or human umbilical veins were perfused (6). The release is an active process and not an emptying of already produced and stored prostacyclin as indomethacin almost totally inhibited the release (2,6). Thrombin, bradykinin and AA all increased the release of prostacyclin when given either to each perfusate or only to the last, why the decline more probably is the result of accomodation, maybe by feedback mechanism (7). The release of thromboxane in the present study was low and the response to AA was low as in previous studies (2,3). Therefore, the release of TxA_2 from the vessel wall is probably of minor importance for the regulation of haemostasis under conditions with intact endothelium.

There are two interesting findings regarding the effect of pH on the release of prostacyclin and thromboxane from the vessel wall in this study which serve comments.
1. There was a lower initial release when perfused at a pH of 7 than at a higher or lower pH. A possible explanation is that the pH-sensitive calcium mobilization was reduced, with less phospholipase activity (8). The importance of calcium for the activation of phospholipase A_2 has been described in different ways. First, the calcium ionophore A23187 mobilizes intracellular calcium and increases PGI_2 synthesis (8,9) by activating phospholipase A_2 (10), phospholipase C (11) and diacylglycerol lipase (12). The action of A23187 though is independent of pH (8). Second, if TMB-8, which is an inhibitor of intracellular calcium mobilization (13) was administered to the incubate, there was a lower release of PGI_2 both in calcium-free and calcium-rich extracellular incubates (8). Third, extracellular calcium increases the release of PGI_2 whereas the release of TxB_2, PgE_2 and PGF_2 is uninfluenced (4,8,15). The effect of pH, seen in the present study, though, was rapidly abolished so that in the second 15 minute period, there was no difference. Another support for the theory that it was more likely the phospholipase activity that was lowered by a pH of 7.0 and not the cyclooxygenase or prostacyclin synthetase activity, is that when AA was added, and the phospholipase thereby bypassed, the release of prostacyclin was as high from segments perfused at a pH of 7 as from segments perfused at a higher pH.
2. A pH of 6.0 on the other hand, did not reduce the initial release of PGI_2 compared to a pH of 7.4 and 7.8. A possible explanation is that other phospholipases than phospholipase A_2, i e diacyylglycerol lipase and phospholipase C, could have higher activity at pH 6.0 than phospholipase A_2. On the contrary, an extracellular pH of 6.0 significantly reduced the release of prostacyclin when AA was added. This effect seems likely to be an effect on either the incorporation of AA through the cell membrane, or on the pool of cyclooxygenase that converts externally provided AA, and less likely on prostacyclin synthetase, as no effect was seen of a pH of 6.0 when perfused without AA. As the release of prostacyclin is to be seen as a result upon stimulation, a third explanation for the effect of a pH of 6.0 is that this pH "overstimulates" in the beginning of perfusion and thereby destroys the enzyme system. Thus it seems as an extracellular pH of 7.4 or 7.8 will allow the vessels to respond with a higher release of prostacyclin on various stimuli. We did not measure the intracellular pH in this experiment but Ritter (8) found that an extracellular pH of 6.4 corresponded to an intracellular pH of 5.9, 7.0 to 6,6, 7.4 to 6.9 and 8.0 to 7.4 in rat aortic rings. Thus the intracellular pH is lower than the extracellular under laboratory conditions.
It therefore seems as if experimental conditions influence the results, and under laboratory conditions, however, an extracellular pH of 7.4-7.8 seems optimal for studying the release of PGI_2 from the vessel wall.

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