Author's response to reviews

Title: Organ culture: a new model for vascular endothelium dysfunction

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PDF covering letter
Dear Editor,

Thank You for the letter regarding the manuscript 1502131161160893 "Organ culture: a new model for vascular endothelium dysfunction", by Alm et al. Below is a list with the changes made in the manuscript that has been performed following the referees suggestions. All the queries have been taken care of as follows:

**Reviewer 1:**

Specific comments:

1. **Methods**

   We agree that it might be confusing for the reader to term the control artery segments (kept in 8°C for 20h) “fresh”. This has been changed to “control” throughout the manuscript. In the methods section (page 6, 1st paragraph) it now reads: “The vessels were then cut into 1 mm long cylindrical segments and divided into two groups; one that was kept in a 8 °C refrigerator (control segments) and the other in organ culture for 20 h (cultured segments).”

   Standard buffer solutions were used for the *in vitro* pharmacology (bicarbonate based buffer solution containing 1 g/L glucose) and for the organ culture (DMEM containing 4.5 g/L glucose). These buffer solutions have been used throughout previous work involving organ culture and *in vitro* pharmacology (Adner et al., 1996; Hoel et al., 2001). A glucose concentration within this range is physiological. In the absence of glucose during culture, a phenotypic change of artery smooth muscle cells does not occur (Adner et al., 1998).

2. **Statistics**
“n=6” means 6 animals in 6 different experiments. We agree that this is not clearly explained. This has been changed in the statistics section on page 8, 1st paragraph; “n denotes the number of experiments that were performed, each in a different animal” and in the tables legend; “n denotes the number of experiments (animals)”.

3. Preconstrictors

The results of U46619 are given on page 9, 2nd paragraph. Here n=6. We can therefore not see the necessity of including a table.

Single-dose experiments with U46619 were performed and the maximum contraction (E\text{max}) was calculated. Consequently, the potency cannot be calculated from the performed experiments. We believe that only the maximum contraction of U46619 is of interest. It has been shown that the amount of preconstriction affects the following dilatory experiment. EDHF-mediated dilatation is more pronounced at a low preconstriction, while NO dominate at a higher preconstriction (Plane et al., 1996; Zygmunt et al., 1994).

We agree. The variability was larger in the cultured segments as compared to the controls. The reason for this is not known. Still, the results from the cultured and controls did not differ significantly.

The level of preconstriction was not significantly different in the presence of L-NOARG and indomethacin as compared to the controls. The basal release of NO, EDHF and prostaglandins from isolated artery segments is not large enough to affect the preconstriction, and the presence of the different mediator inhibitors is therefore not an issue (Malmsjo et al., 1998). Conversely, in methods where artery perfusion systems are used, an effect of a basal endothelium-derived mediator release is of importance.

4. Discussion

First we need to clarify. The relaxant effect of acetylcholine at 0.1 mM, in the cultured artery segments, was:

80 % for EDHF (not 25 % as the reviewer states)
84 % for NO (not 15 % as the reviewer states)
16 % for PG (not 60 % as the reviewer states)

These results support our hypothesis on page 12. In order to avoid confusion, we have clarified this further in the discussion on page 12, 3rd paragraph: “After organ culture, the EDHF-mediated dilatation amounted to 80 %, NO to 84 % and prostaglandins to 16 % of preconstriction. The additive NO, prostaglandin and EDHF responses thus exceeded the total ACh-dilatation by far.”
The suggestion to test substance P or bradykinin in order to confirm our results is excellent. We will consider performing these experiments in the future.

Reviewer 2:

Mandatory corrections:

1. The fact that a K⁺ channel opener was not used in the present experiments has been pointed out to the reader in the discussion on page 12, 2nd paragraph: “EDHF induced a maximum dilatation of the artery even after organ culture (80% before and 83% after organ culture). It can therefore be expected that the smooth muscle cell response to EDHF is functioning normally after culture, although there is no proof of this since a K⁺ channel opener was not used in the present experiments.”

2. We agree with the reviewer that such a statement would not hold true unless binding studies had been performed. In the discussion, the sentence, “the total ACh-dilatation was not reduced, indicating that the muscarinic receptor expression on the endothelium remained intact”, was therefore removed.

Minor points:

1. “Vasodilatation was expressed as % of preconstriction with U46619” has been added to the methods section of the abstract.

2. Page 4, paragraph 3: Reference 13 has been cited earlier in the sentence and references (Lobato et al., 1980; Svendgaard et al., 1977) have been added to substantiate the statement on smooth muscle cell alterations in disease, as suggested by the reviewer.

3. Page 12, paragraph 3: CHF has been replaced by “congestive heart failure”.

4. The discussion has been shortened as suggested.

References


We hope that the manuscript can now be accepted for publication in BioMed Central.

 Yours Sincerely,

Malin Malmsjö, MD, PhD