



How to design scientific control

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Control group:

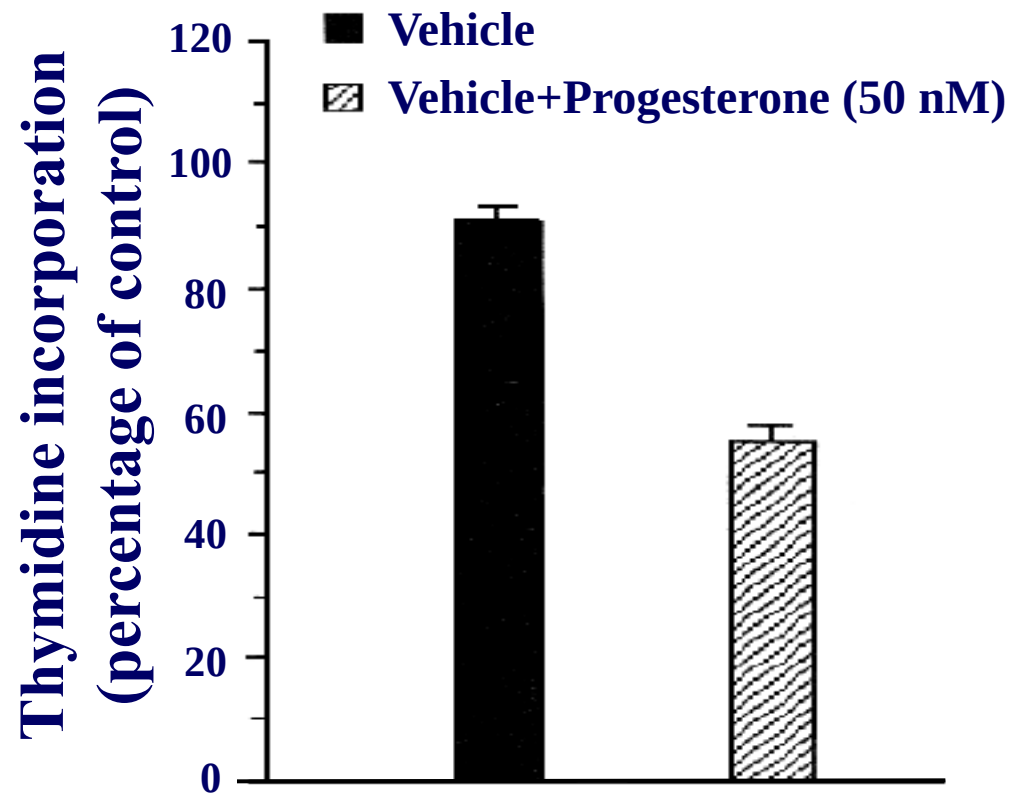
Control groups are the foundational point for which to compare the experimental group against. They are not manipulated.

Experimental group:

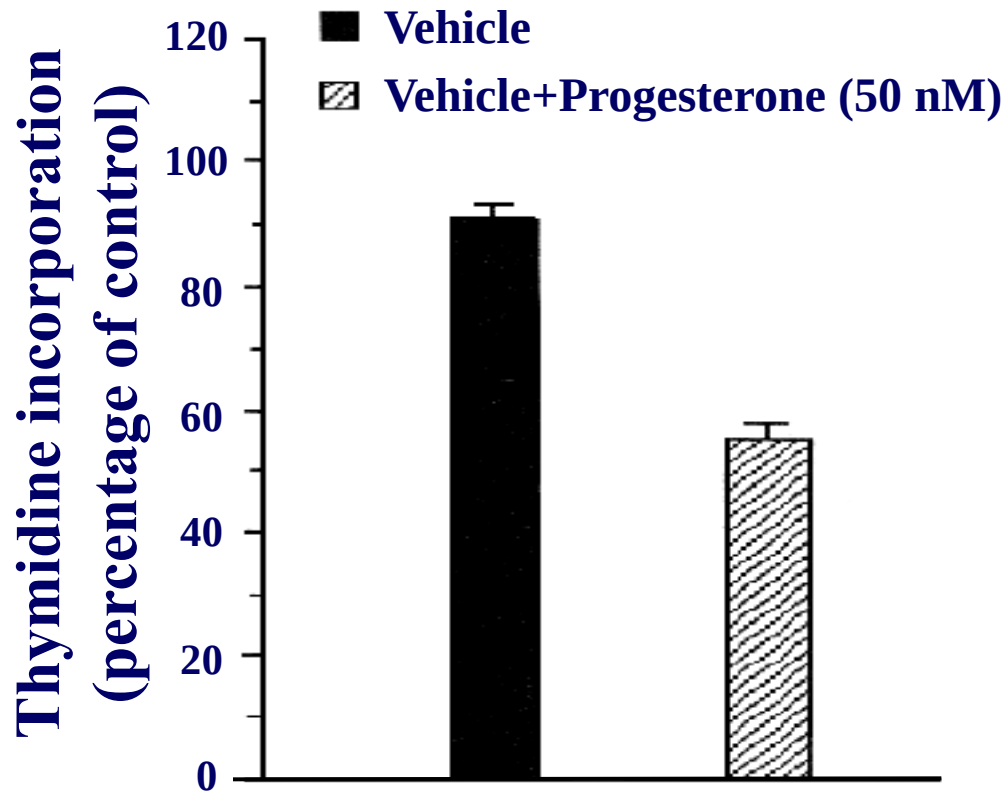
Experimental groups are the group being treated, or otherwise manipulated for the sake of the experiment. This group is identical to the control group except that it is manipulated.

**Can progesterone inhibit the growth
of vascular smooth muscle cells?**

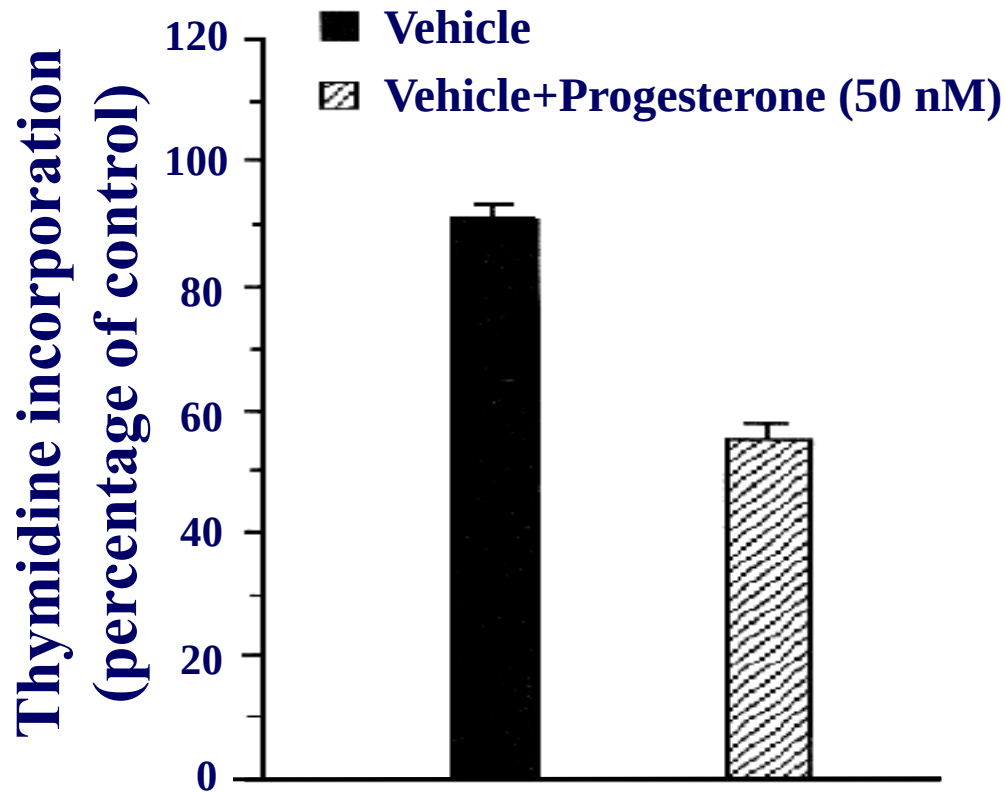
?



Progesterone reduces [3H]thymidine incorporation into RASMC



Vehicle does not affect the [3H]thymidine incorporation into RASMC

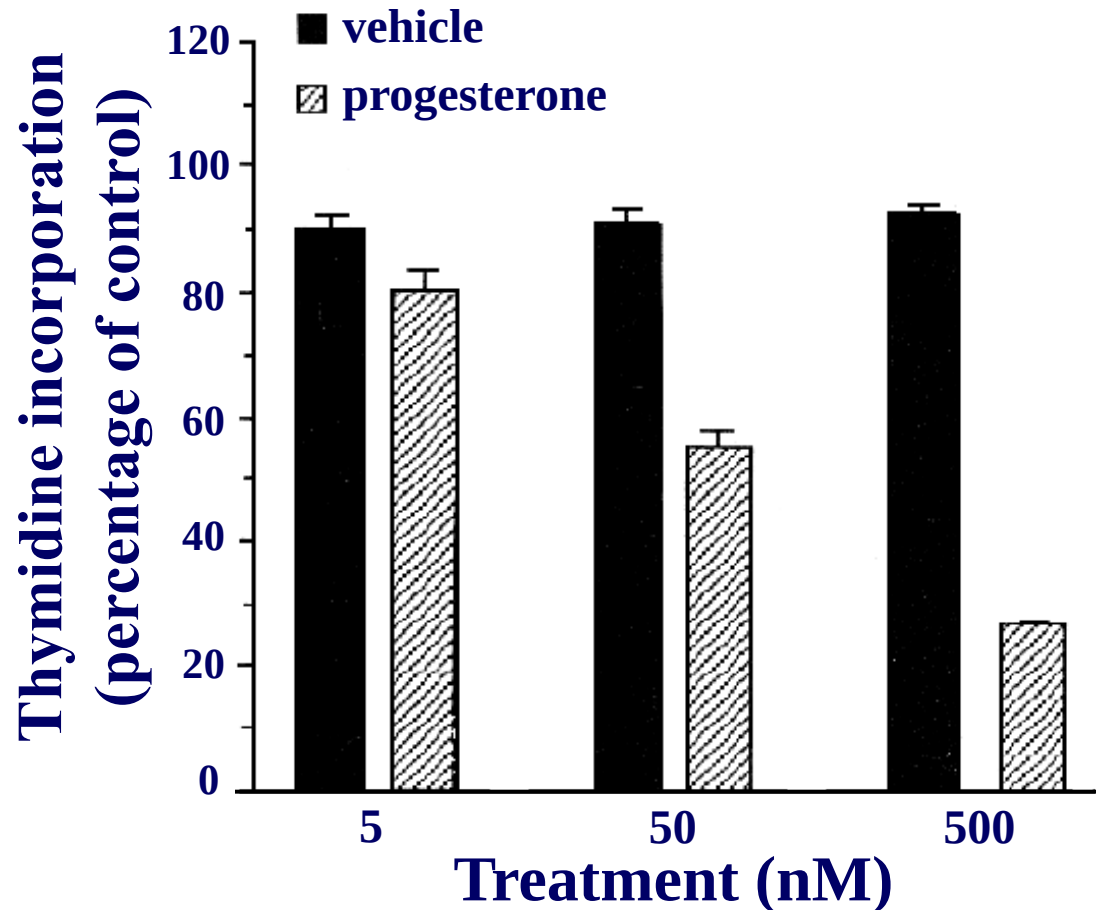


**Is this effect due to
osmolarity change?**

**How to prove that this effect is due
to progesterone treatment?**

**Whether this effect is
concentration-dependent?**

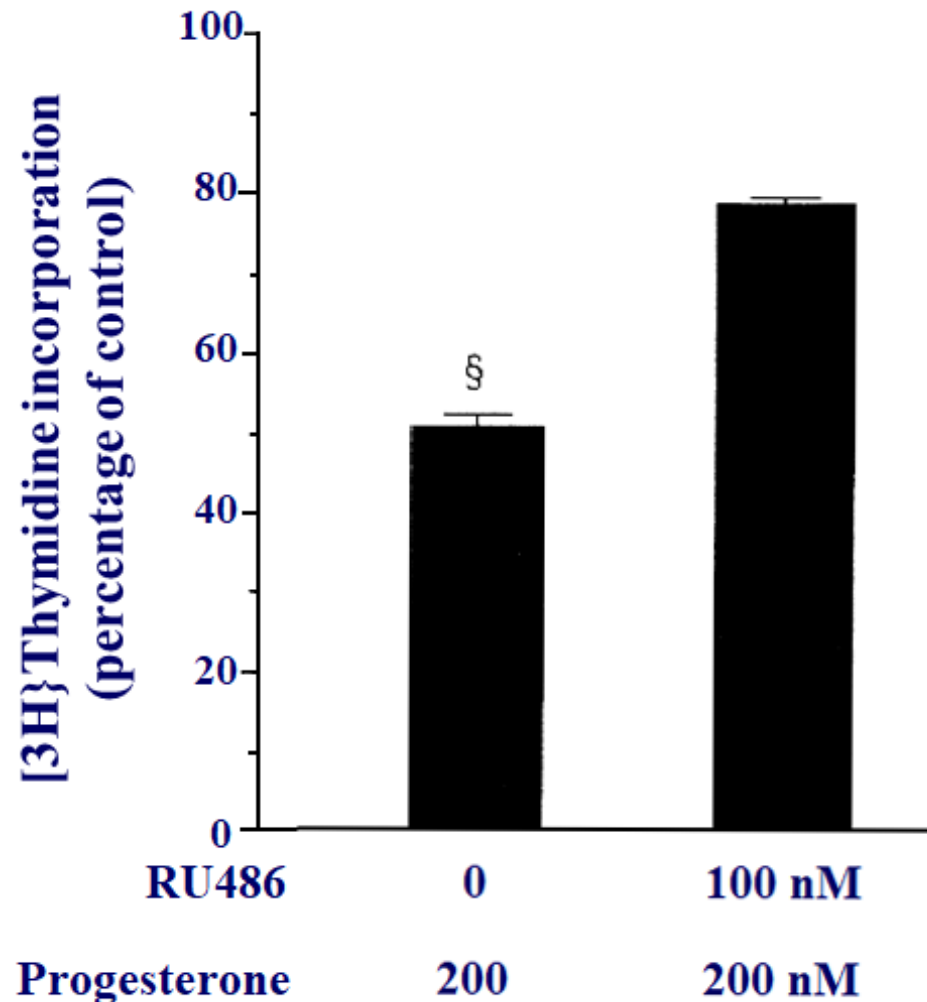
Concentration-dependent inhibition of [3H]thymidine incorporation into RASMC by progesterone



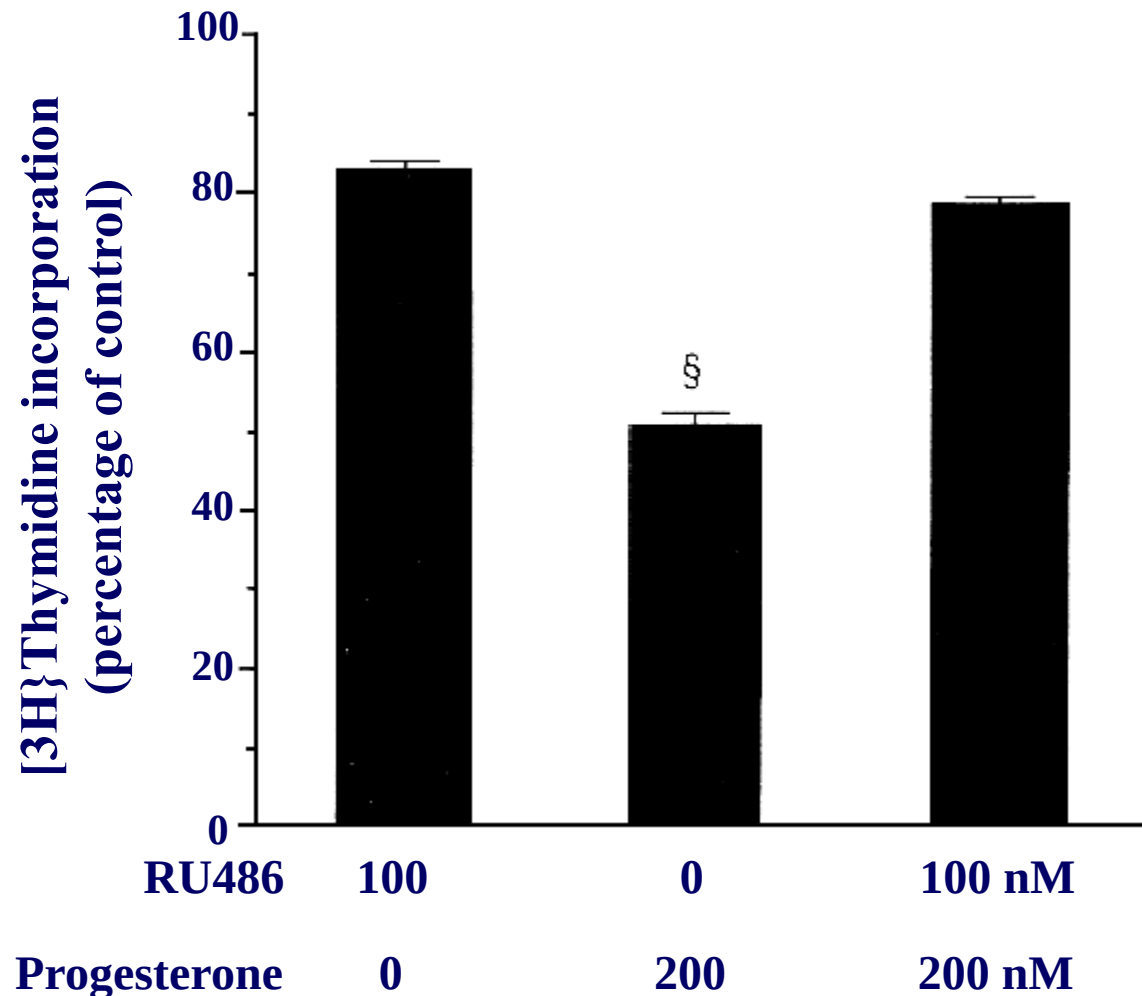
**How to prove the specificity of
the progesterone's effect?**

**Can progesterone receptor
antagonist block this effect?**

Progesterone-induced inhibition of [3H]thymidine incorporation into RASMC is blocked by the progesterone receptor antagonist RU486



Progesterone-induced inhibition of [3H]thymidine incorporation into RASMC is blocked by the progesterone receptor antagonist RU486



**What should be concerned for
using an antagonist ?**

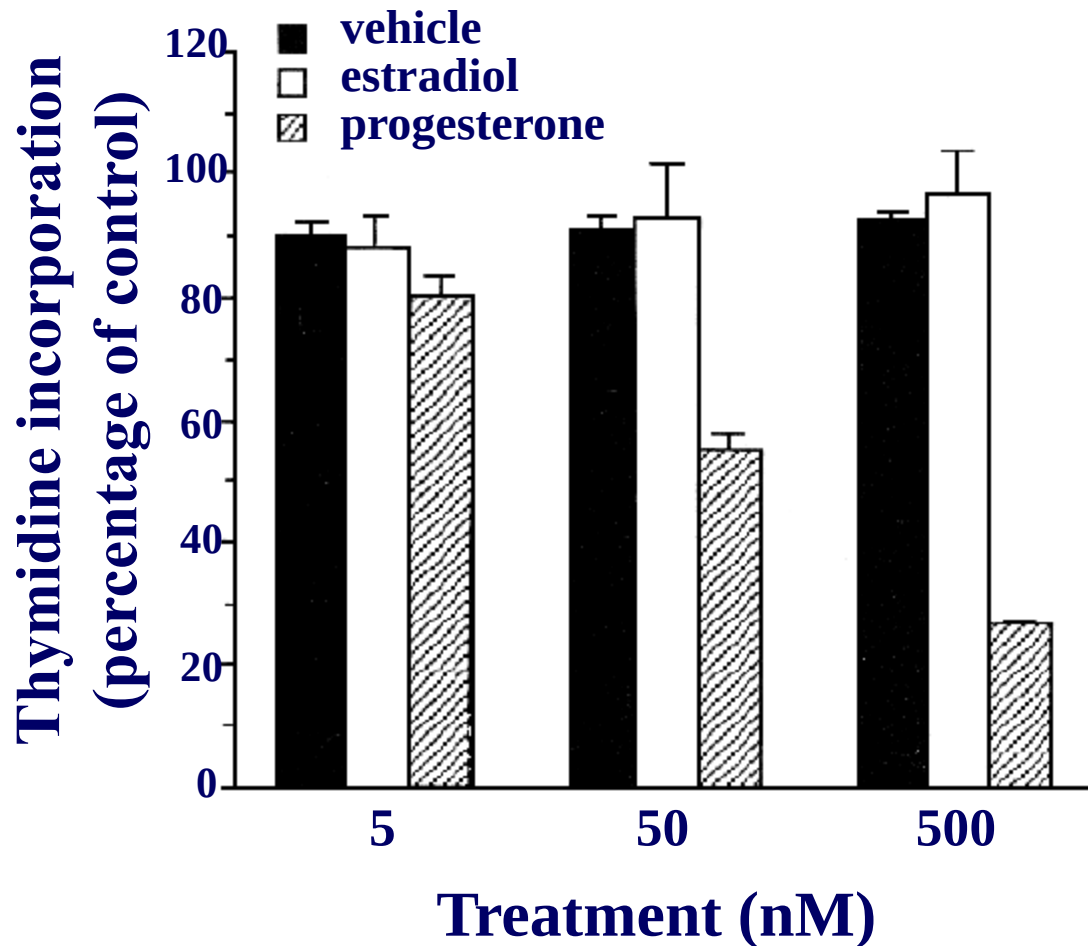
Cross reactivity

It has been demonstrated that
RU486 can also react with
glucocorticoid receptor

To address the issue of cross activity, molecular biology techniques to knock-down the candidate molecule using siRNA or antisense oligonucleotide has been developed.

If the receptor had not been identified, then how could you prove this effect is due to progesterone treatment?

Concentration-dependent inhibition of [3H]thymidine incorporation into RASMC by progesterone, but not estradiol

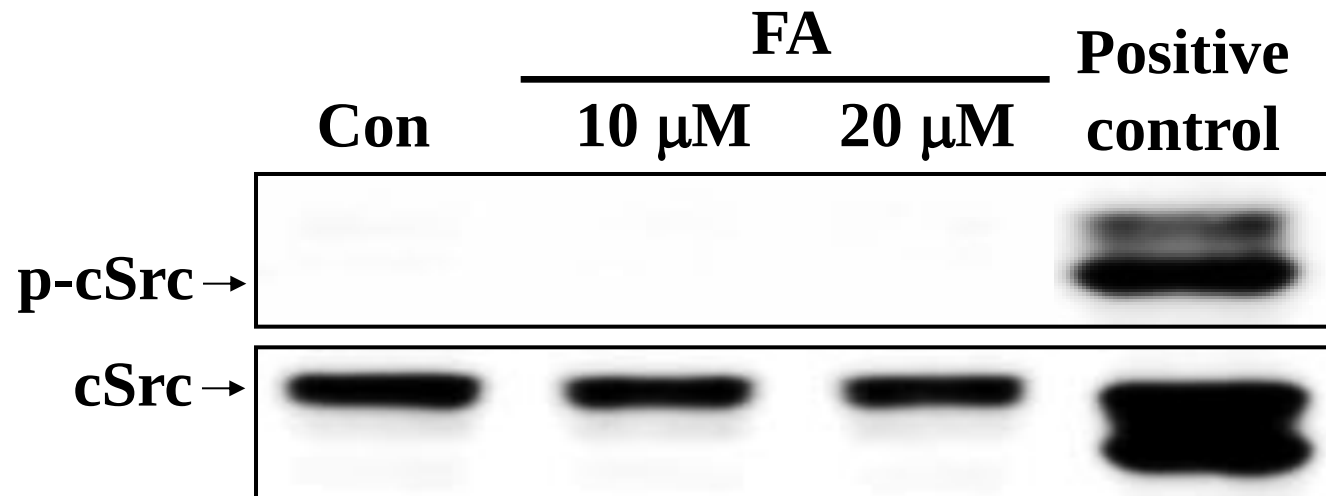


Positive control

Positive controls confirm that the procedure is effective in observing the effect (therefore minimizing false negatives).

**How to clarify that the
immunoreactivity negative is a
real negative or false negative**

HCT-116



Do smooth muscle cells in neointima express Thy-1?



What can cause false negative?

1. Experimental protocol

a. Fixation

b. Experimental procedure

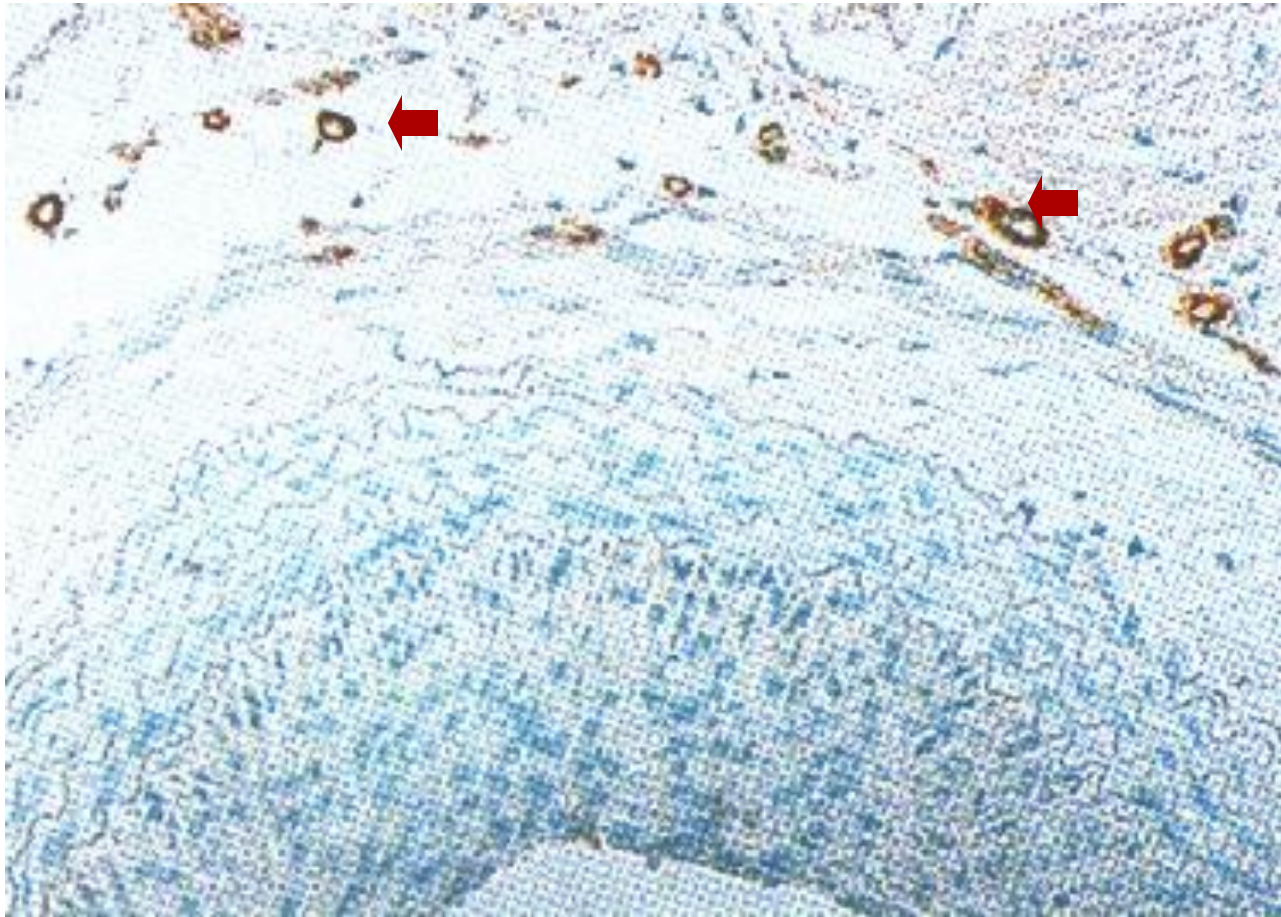
2. Chemicals

a. Antibody concentration

b. Expired date of chemicals

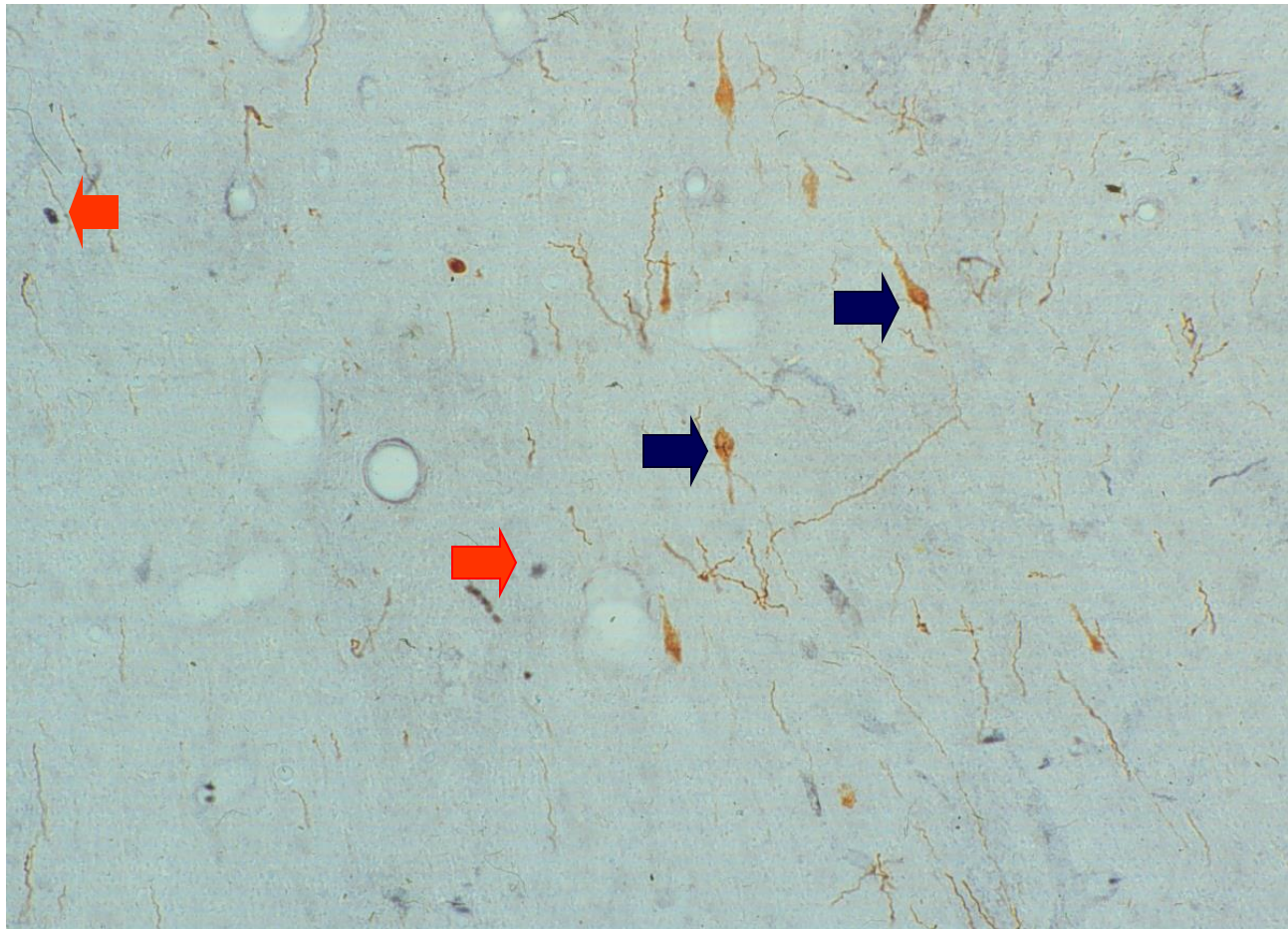
c. PH value of solutions

Thy-1 immunoreactivity was detected in the adventitia but not neointima

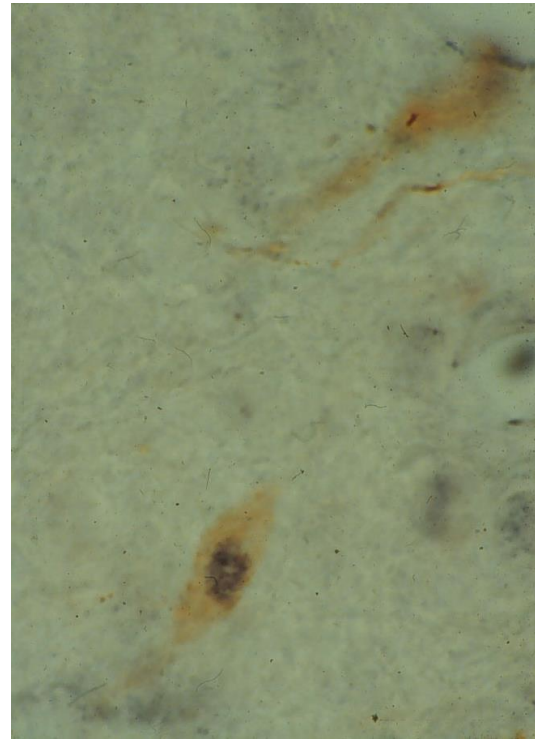
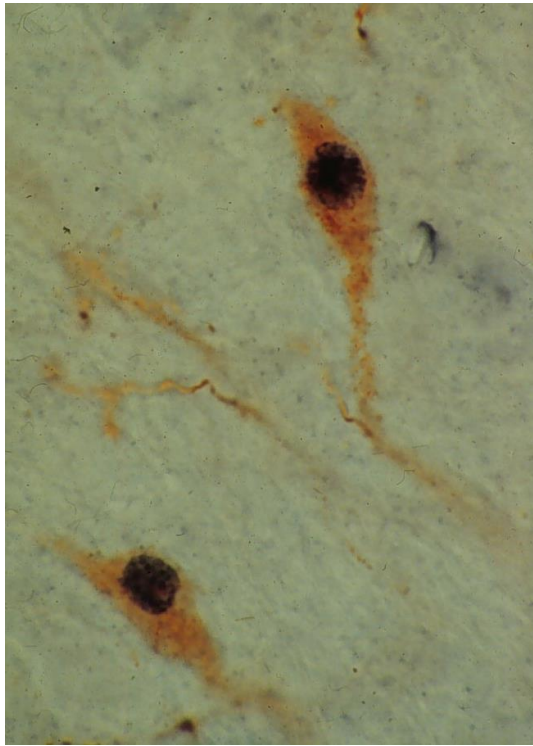


**Use of the same tissue for positive control
for immunocytochemical staining**

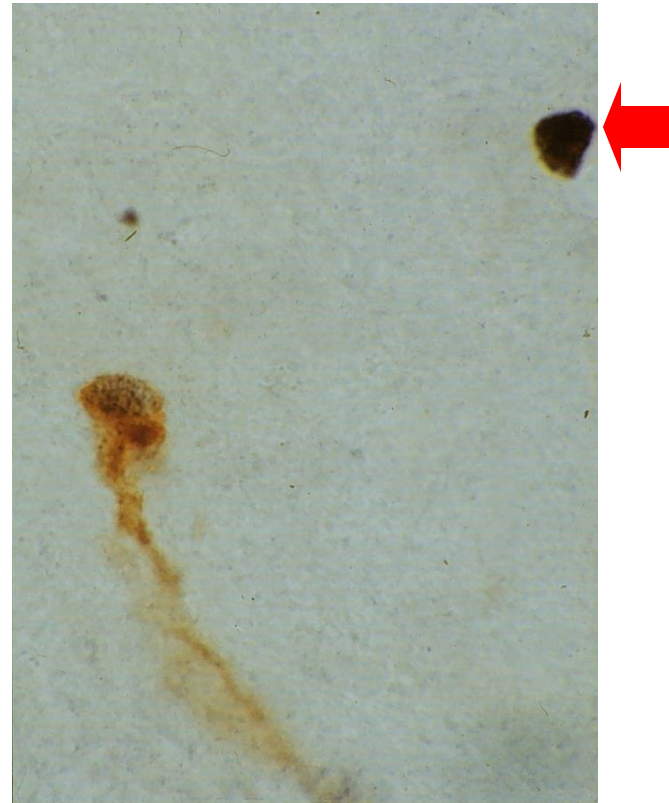
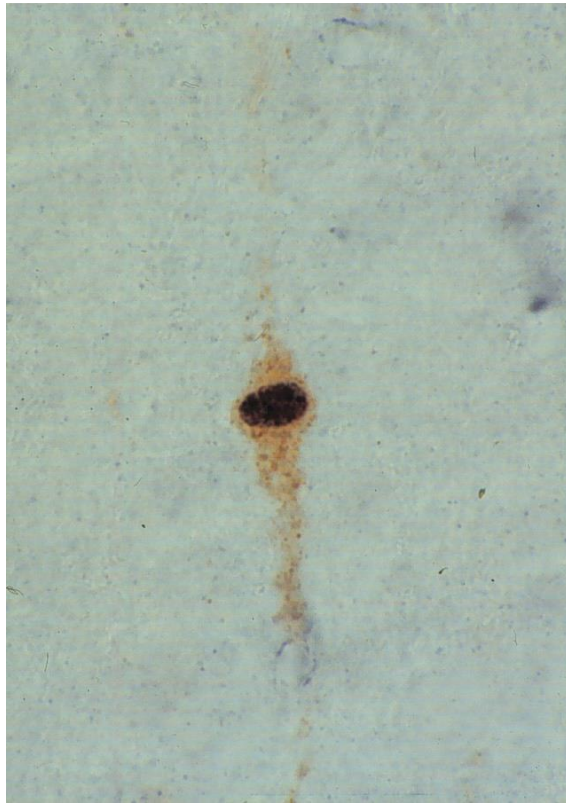
Pentobarbital blocks the expression of cFos in LHRH neurons on the afternoon of proestrus



Quantitation for immunocytochemical staining

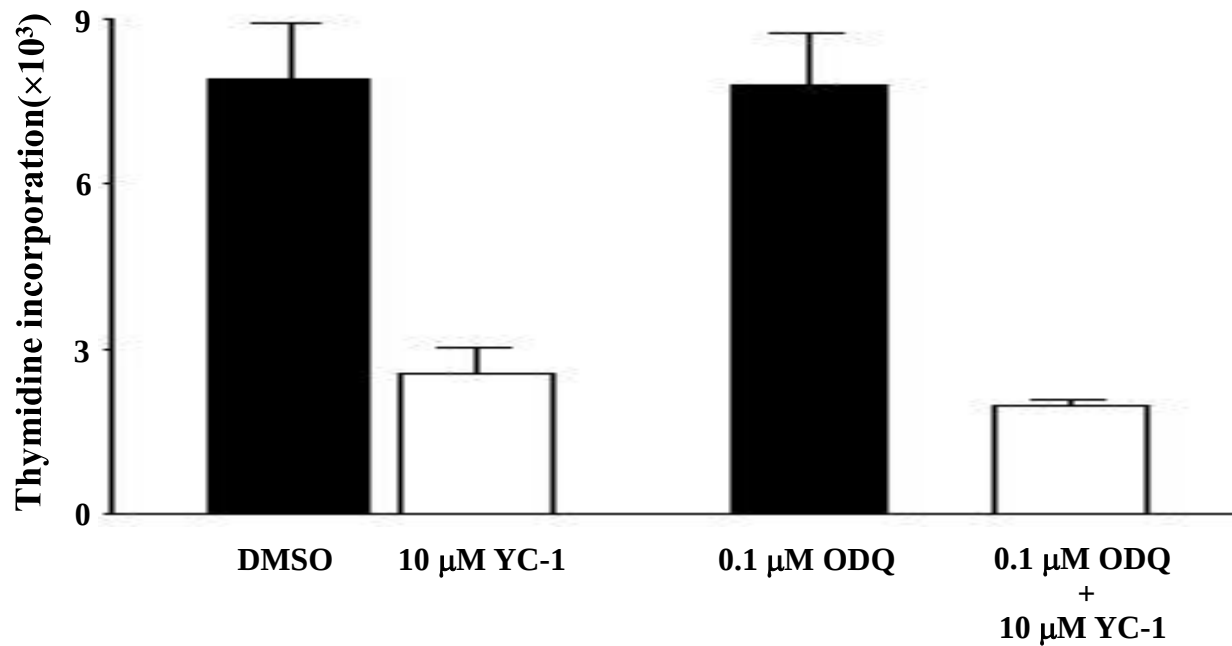


Positive control for immunocytochemical staining



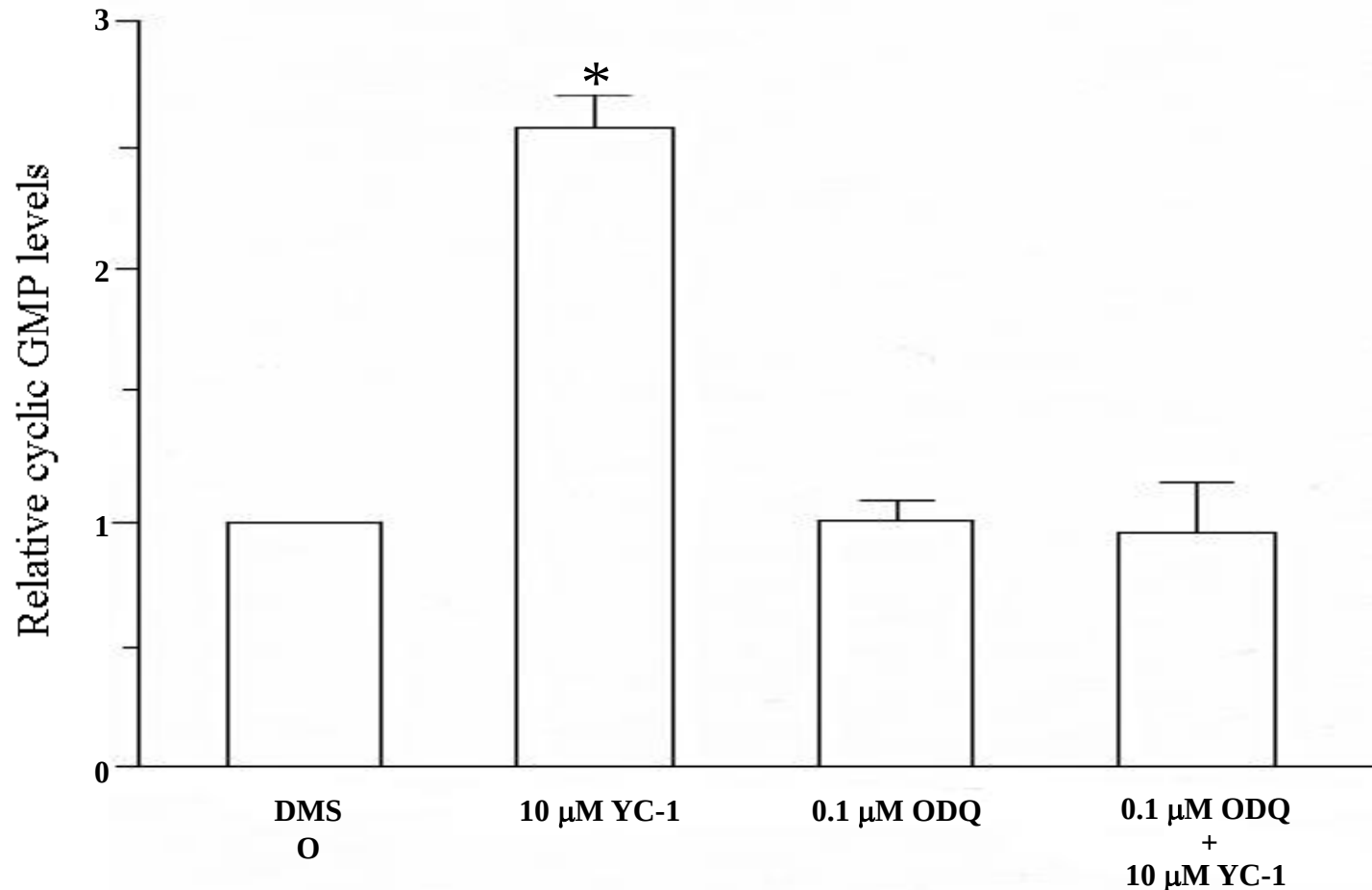
Use of the tissue from different organs or different animals for positive control for immunocytochemical staining

cGMP is not involved in the YC-induced [3H]thymidine incorporation inhibition in HUVEC



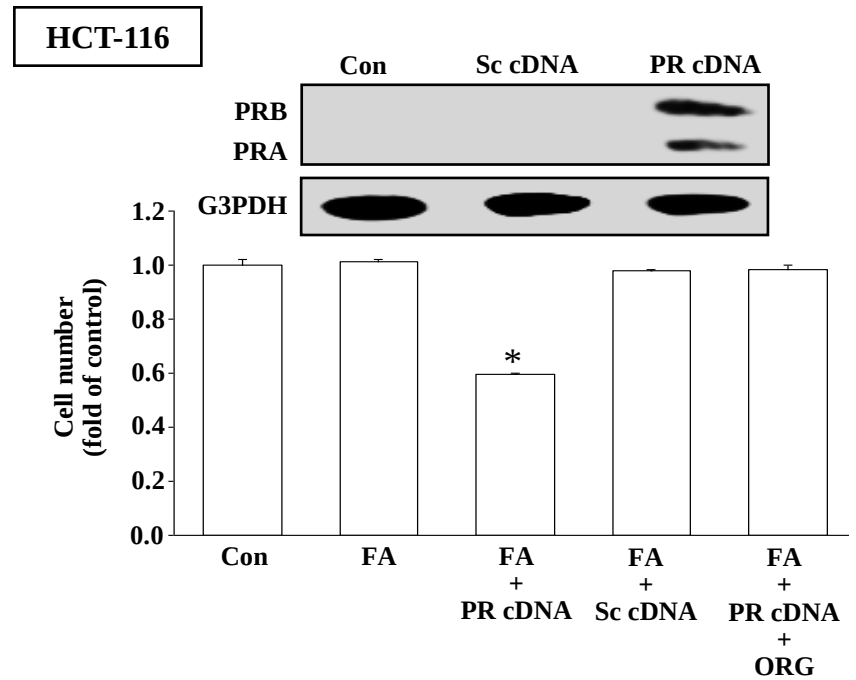
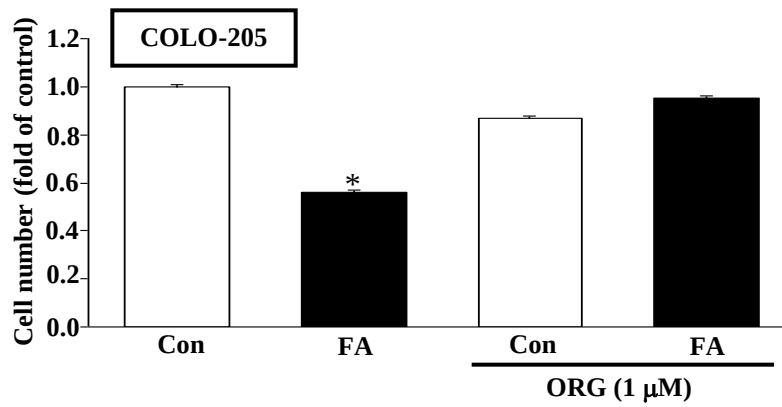
How to clarify that the cGMP inhibitor, ODQ, is effective?

ODQ inhibits the YC-1-induced increase of cGMP level

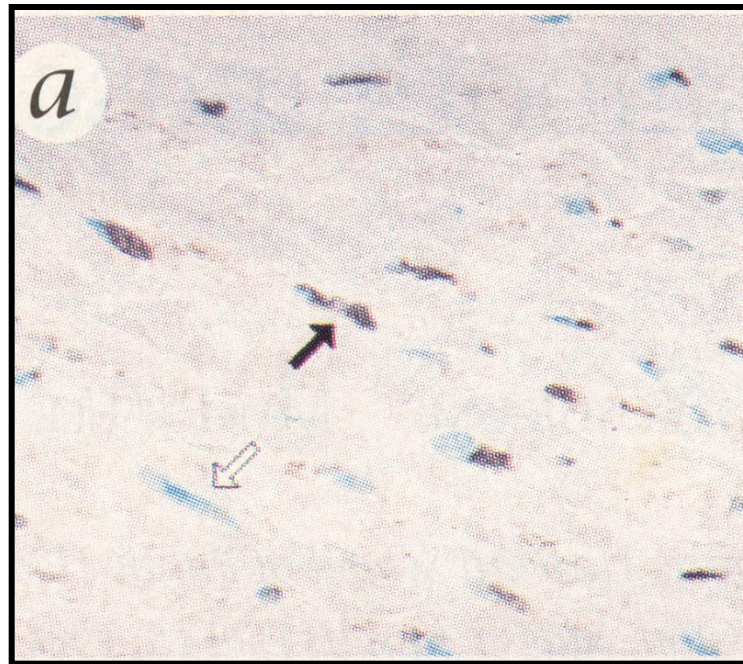


Negative control

Negative controls confirm that the procedure is not observing an unrelated effect (therefore minimizing false positives).



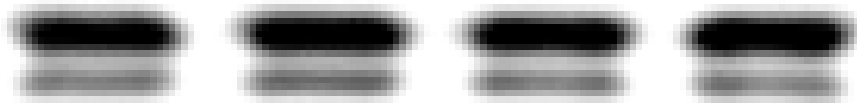
Immuno-localization of progesterone receptor on the aortic smooth muscle cells



anti-P4R Ab
DAB staining

Human aorta

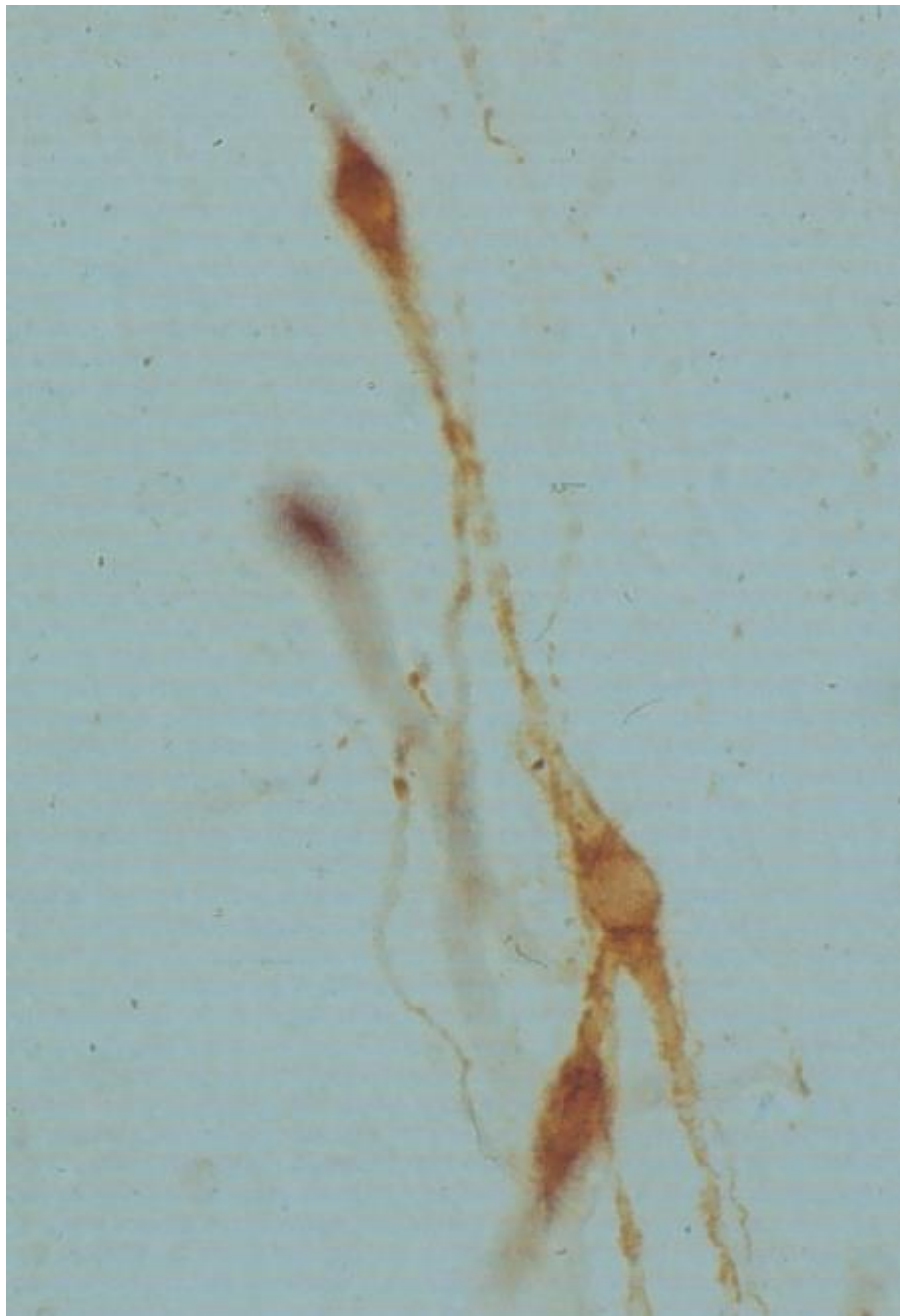
Western blot analysis



Which band is
real positive

Molecular weight

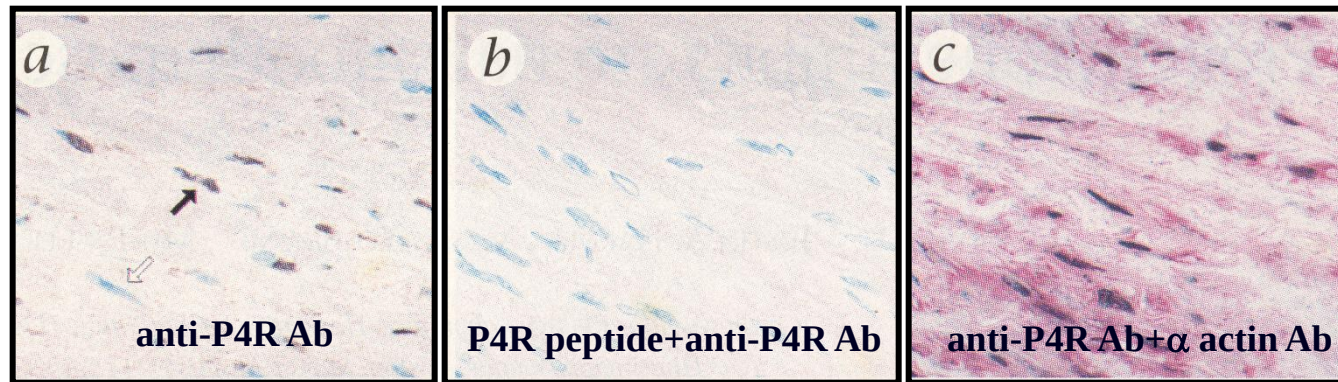
**How to clarify that the
immunoreactivity positive is a
real positive or false positive**



Current methods used for immunocytochemical staining negative control

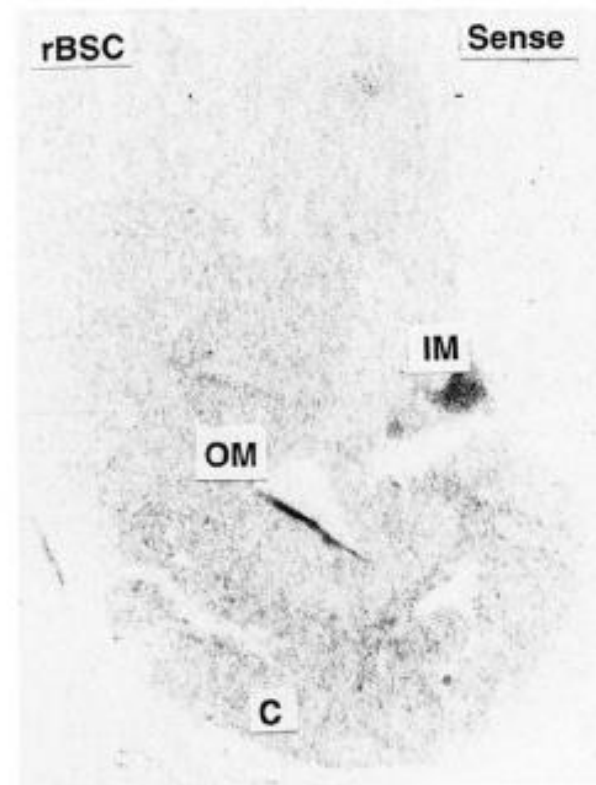
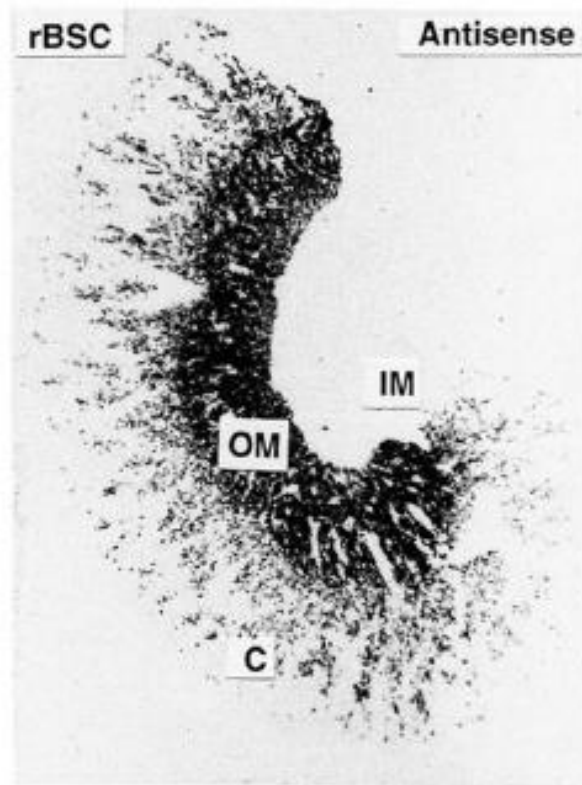
- 1. Use of preimmune serum to replace the primary antibody**
- 2. To omit the primary antibody**
- 3. Antigen preabsorption**

Immuno-localization of progesterone receptor on the aortic smooth muscle cells

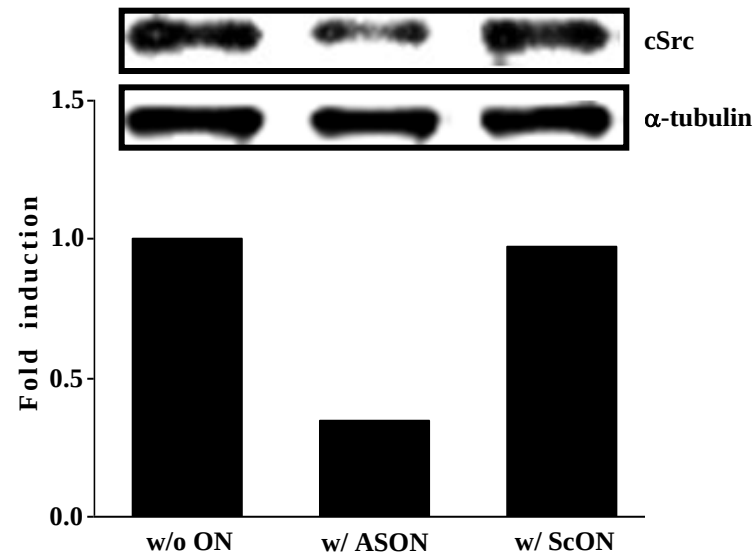


Human aorta

In situ hybridization of rat kidney with rBSC



Knock down cSrc expression with cSrc antisense oligonucleotide



sense vs. scramble antisense

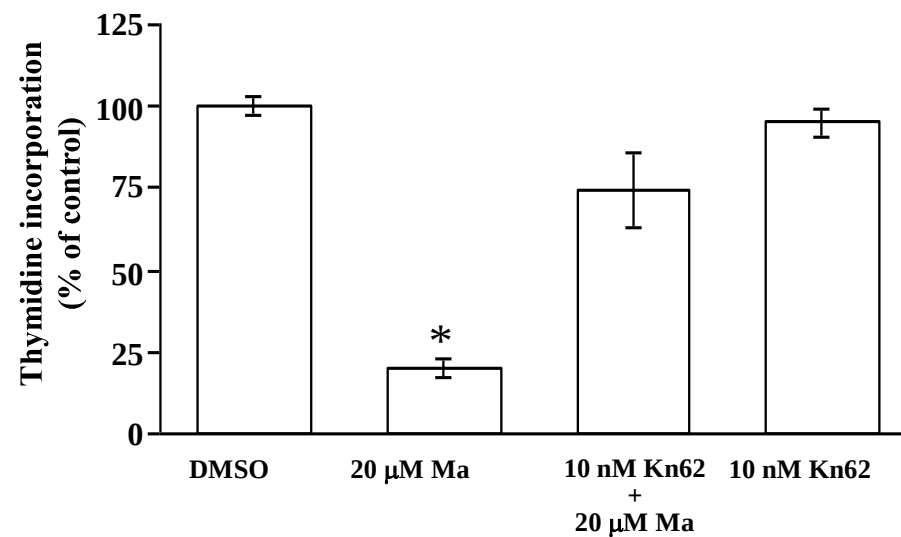
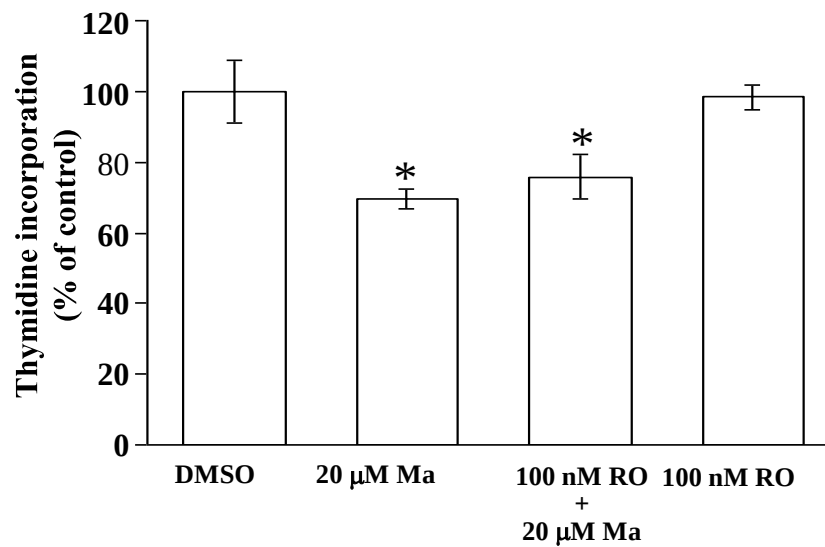
Antisense: ACCGATTTCG

Sense: TGGCTAAGC

Scramble: ACTGCATGC

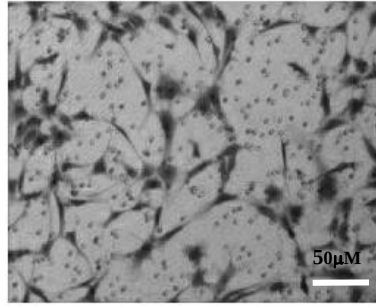
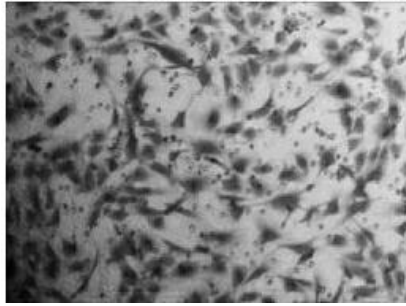
Shame operation vs. without operation

Intra-group vs. inter-group comparison



C

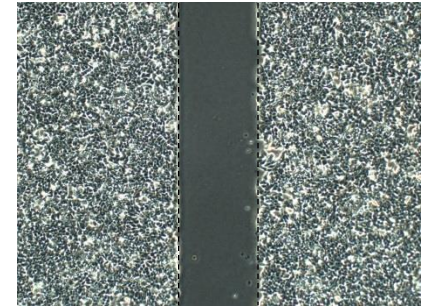
TB (60 μ M)



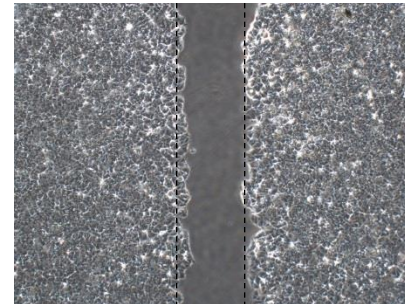
C

PMA (50 ng/mL)

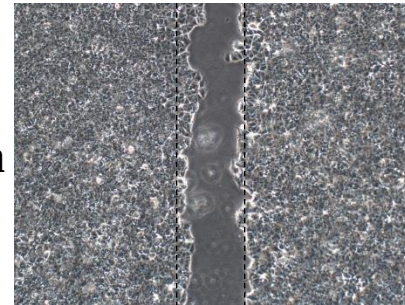
0 h



9 h



23 h



Double-blind study