# Pharmacology of Adrenoceptors WRAP UP

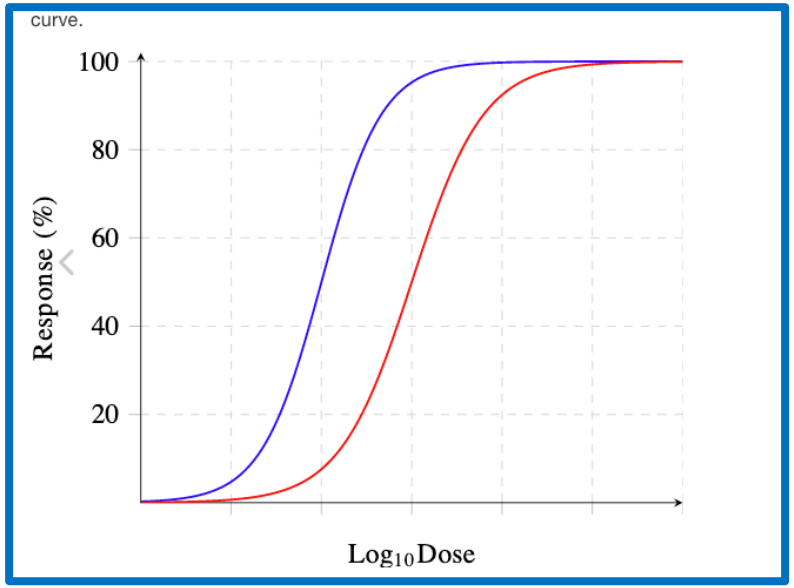
VB: Cardiovascular System

# Aims

- To characterize the adrenergic receptors on the sino-atrial node and on blood vessels

- ie **are they alpha (α) receptors or beta ( β) receptors?**

# How do we do that?

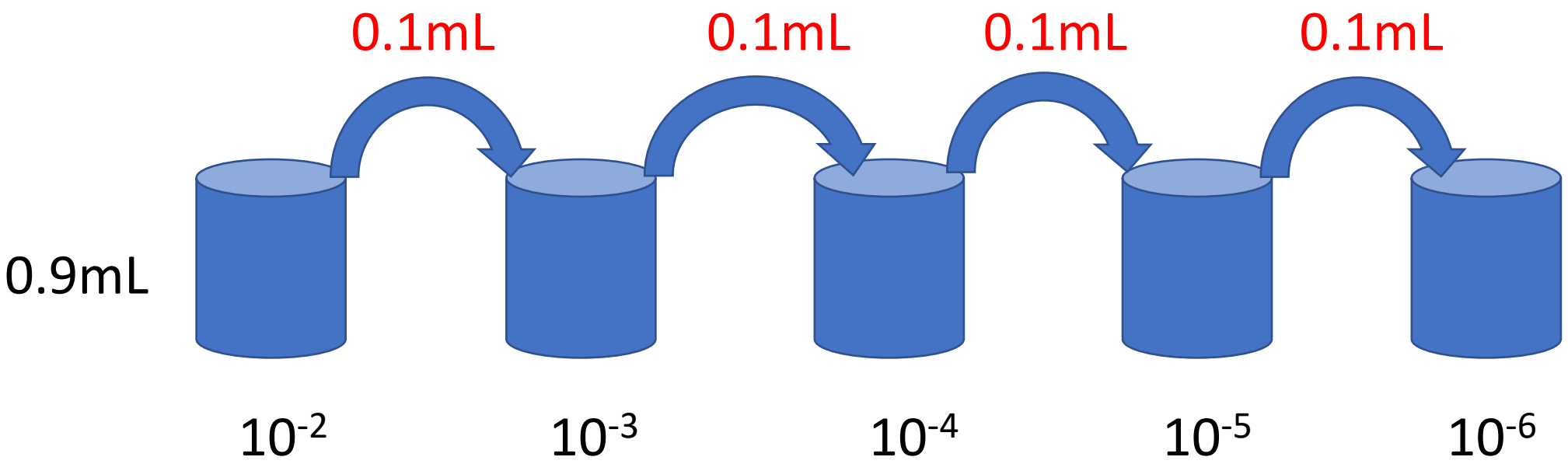



- Stimulate the tissue with a **known adrenergic agonist (Noradrenaline)**
- Repeat the stimulation in the presence of known antagonists at the **alpha** and **beta** receptor
- **Phentolamine**- an alpha adrenergic antagonist
- **Propranolol**- a beta adrenergic antagonist
- Record any changes in the response in the presence of the antagonist

# Dilutions…. Why do we do them???

- To “titrate” the volume of drug that we are administering
- In a concentration response curve we are typically administering doses across a dose range of **100,000 times :** ie from $10^{-8}$ – $10^{-3}$
- If the concentration is higher, and the dose is very low we can’t measure a small enough volume
- If we use a more dilute “stock” solution, the volume for higher doses becomes quite large and will distort the volume and hence concentration in the organ bath

# So… we performed serial dilutions to dilute our stock solution



# And then we made one:one thousand dilutions of this stock into our organ bath

- **Stock** 1 X $10^{-5}$
- Add 25 uL of this stock to a 25 mL organ bath (25,000uL organ bath)
- Yields an **organ bath concentration** of 1 X $10^{-8}$

# So.. If we wanted an organ bath concentration of $3 \times 10^{-8}$ ….

- We would add three times as much stock
- ie 3 X 25uL of stock.
- Or 75uL of $1 \times 10^{-5}$

# Or using the equation approach…

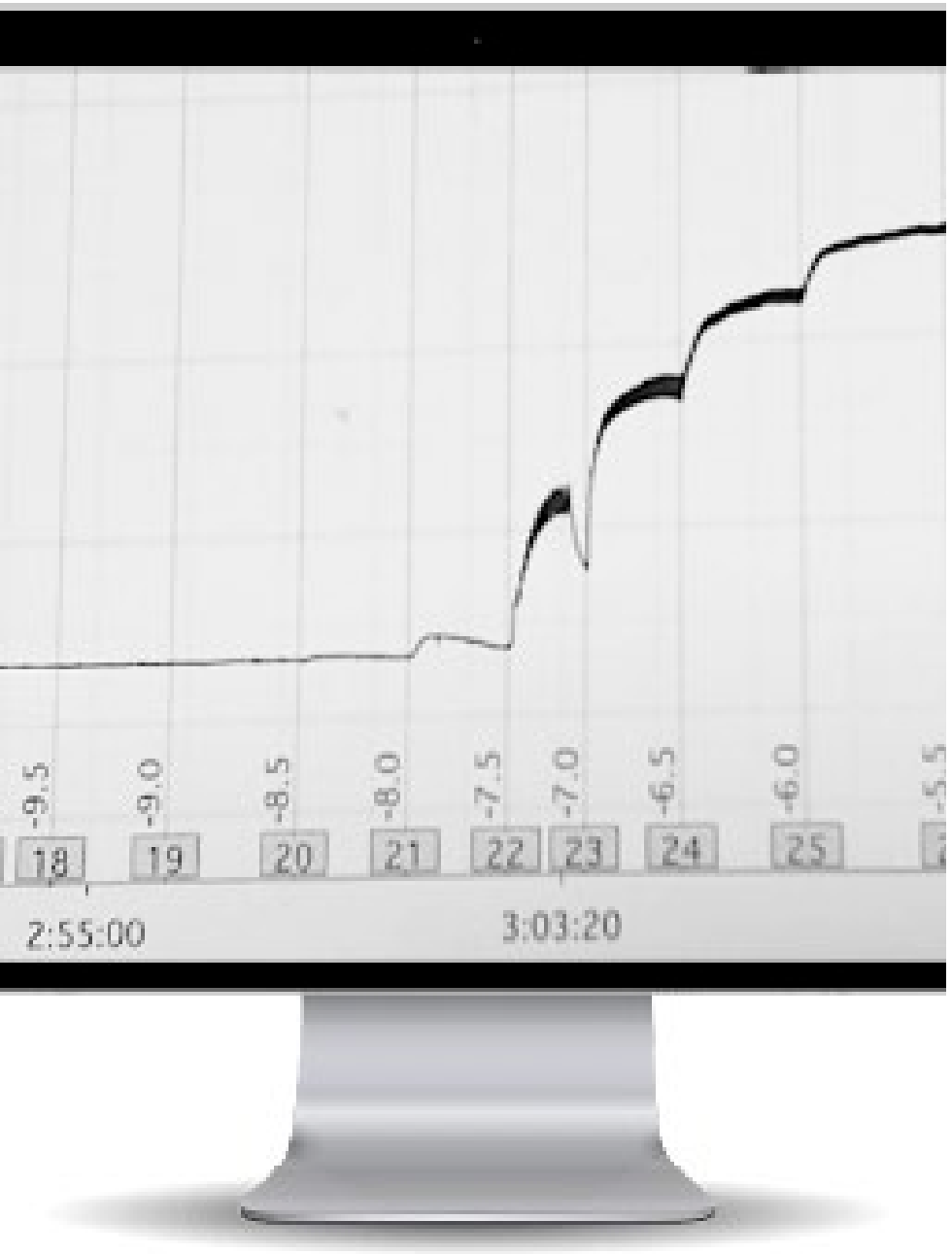
- $C_1V_1 = C_2V_2$
  (Stock) (Bath)

- $V_1 = C_2V_2/C_1$

  $= 1X\ 10^{-8}\ X\ 25000/\ 1X\ 10^{-5}$

  $=25000\ X\ 1\ X\ 10^{-3}$

  = 25 uL




# How do we create a concentration (dose) response curve?

- By cumulative additions of noradrenaline to the organ bath
- So that we go progressively from a very small concentration of NA in the organ bath (1 X $10^{-10}$ M) to a **one hundred thousand times higher concentration** (1 X $10^{-5}$ M)
- To achieve these different organ bath concentrations we need to add drug “doses” at different concentrations
- So….. we need to understand **dilutions**

# Creating a concentration response curve: what do cumulative additions look like?

| Required concentrations | | | | |
|---|---|---|---|---|
| log M* | M | conc. of NA stock solution | Volume of NA added | |
| -8.0 | 1 x $10^{-8}$ M (10 nM) | 1 X$10^{-5}$ | 25 ul | |
| -7.5 | 3.2 x $10^{-8}$ M (32 nM) | 1 X$10^{-5}$ | 55 ul | |
| -7.0 | 1 x $10^{-7}$ M (100 nM) | 1 X $10^{-4}$ | 17 ul | |
| -6.5 | 3.2 x $10^{-7}$ M (320 nM) | 1 X $10^{-4}$ | 55 ul | |
| -6.0 | 1 x $10^{-6}$ M (1 µM) | 1 X $10^{-3}$ | 17 ul | |
| -5.5 | 3.2 x $10^{-6}$ M (3.2 µM) | 1 X $10^{-3}$ | 55 ul | |
| -5.0 | 1 x $10^{-5}$ M (10 µM) | 1 X $10^{-2}$ | 17 ul | |
| -4.5 | 3.2 x $10^{-5}$ M | 1 X $10^{-2}$ | 55 ul | |

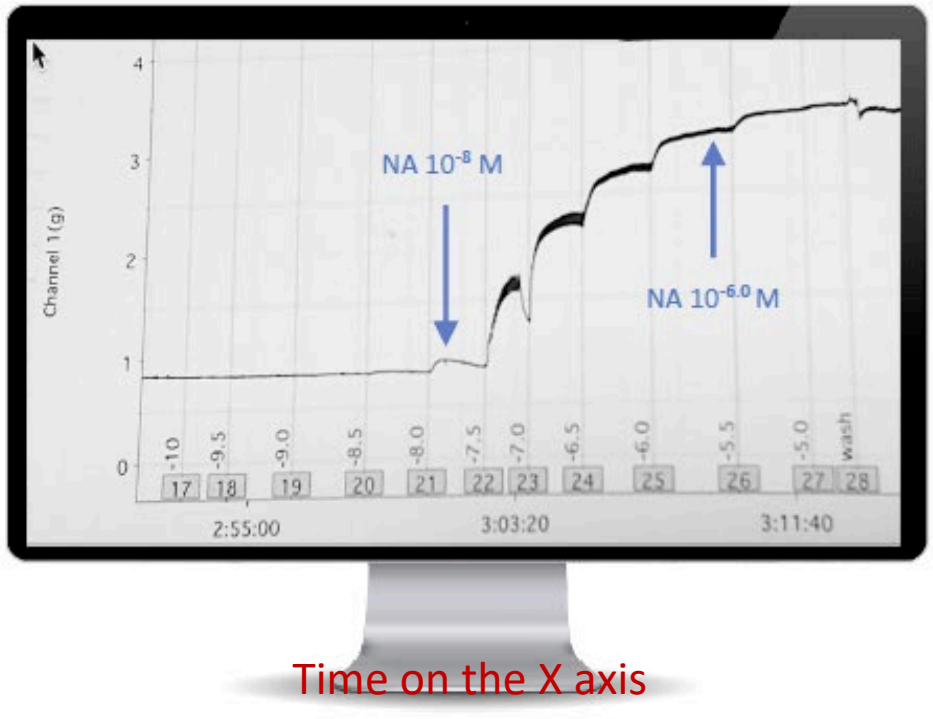
3.2 X 25ul= 80ul but already have 25ul therefore need to add 80-25= **55ul**

$1 X 10^{-7} = 10X 10^{-8}$
This would require 10X25 ul = 250ul
But we already have 80 ul, therefore we need to add 250-80= 170 of $1X 10^{-5}$
OR
**17ul of 1 X $10^{-4}$**

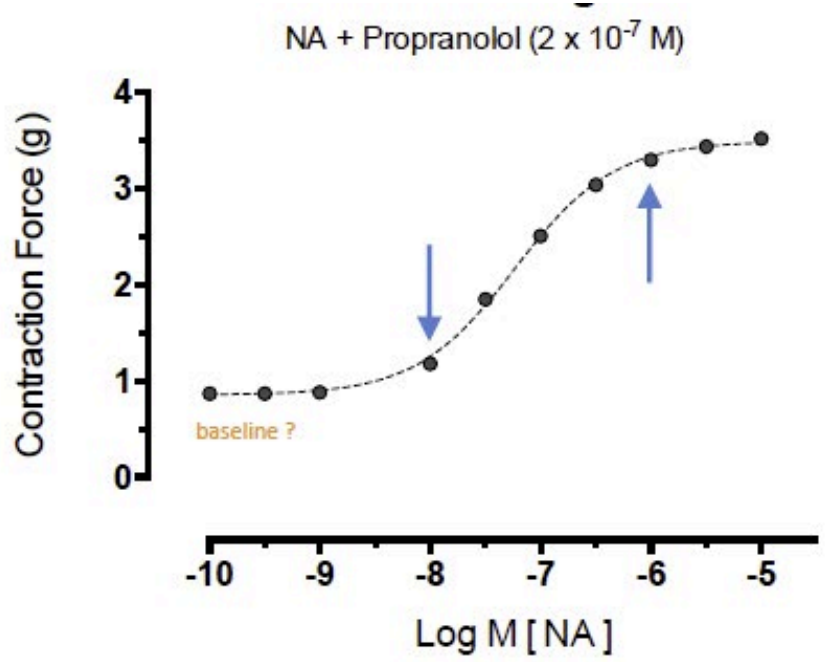
# Results- what did our charts look like?

Three charts for each tissue:

- Noradrenaline (control)
- Noradrenaline in presence of alpha antagonist
- Noradrenaline in presence of beta antagonist

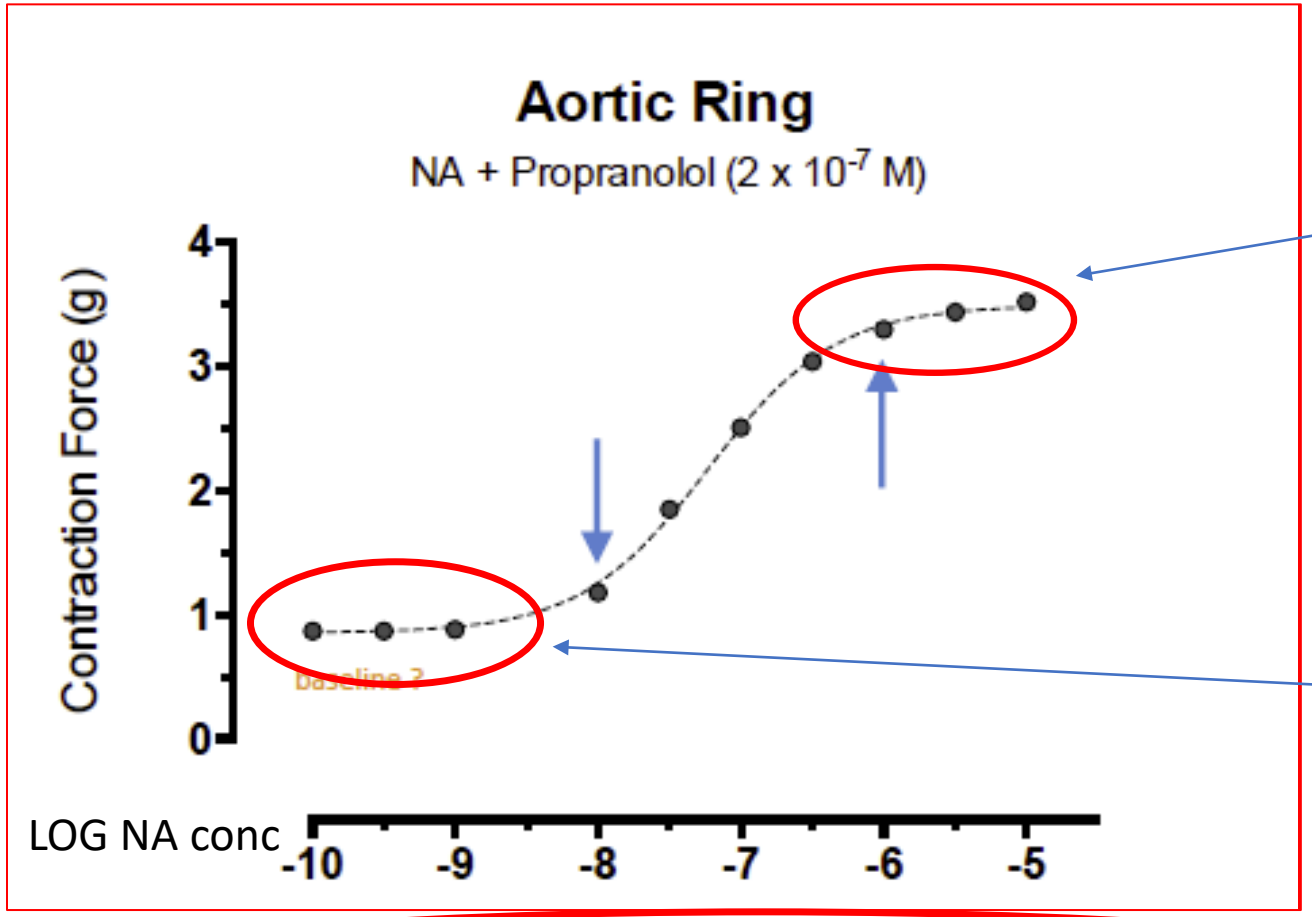



Time on the X axis




Concentration on the X axis

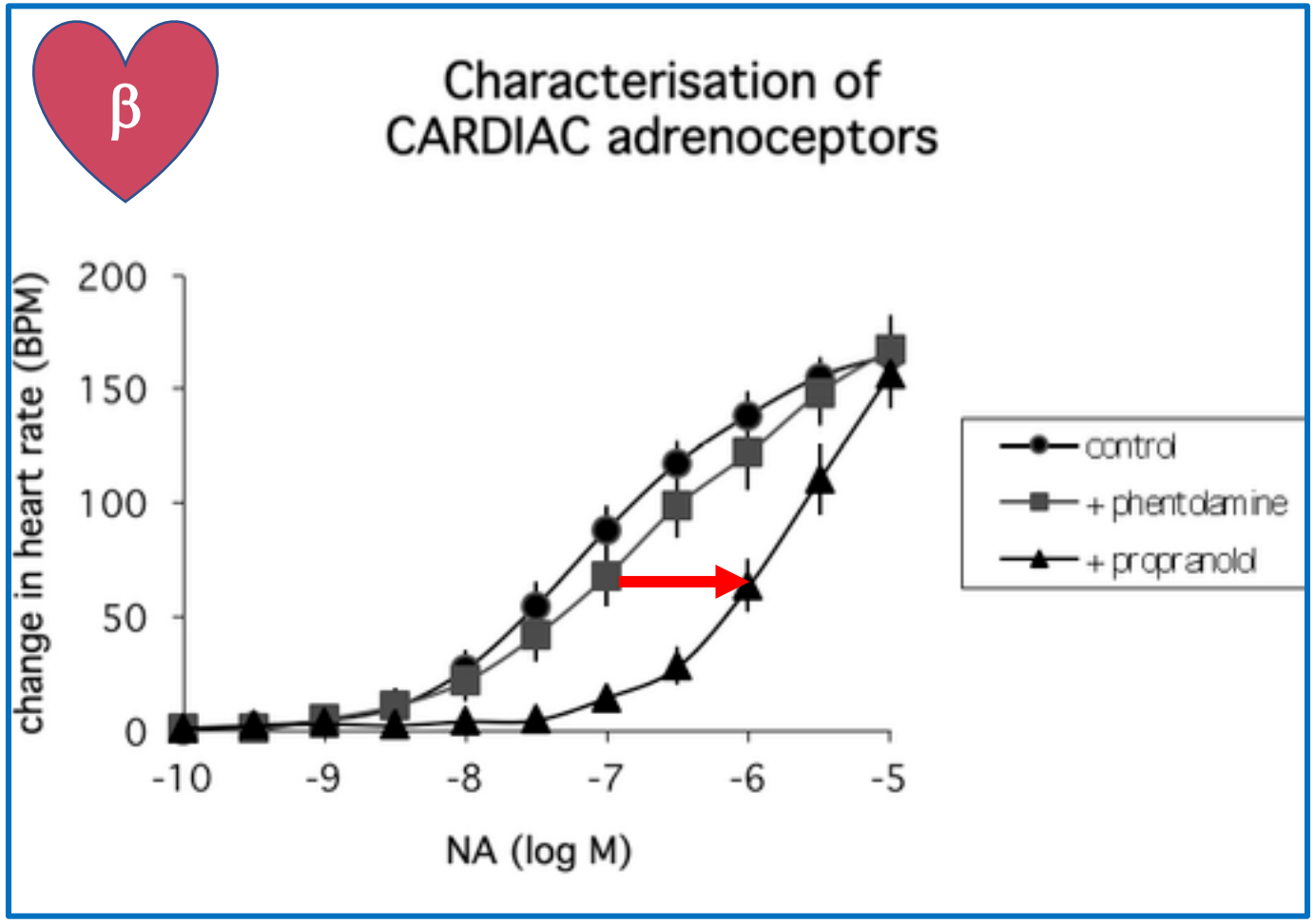
# What can we learn from a dose response curve?




- Sigmoid “S” shaped concentration response curve
- *Why is there no increase in response here with further addition of NA?*
- *Why does addition of NA elicit no contractile response here?*

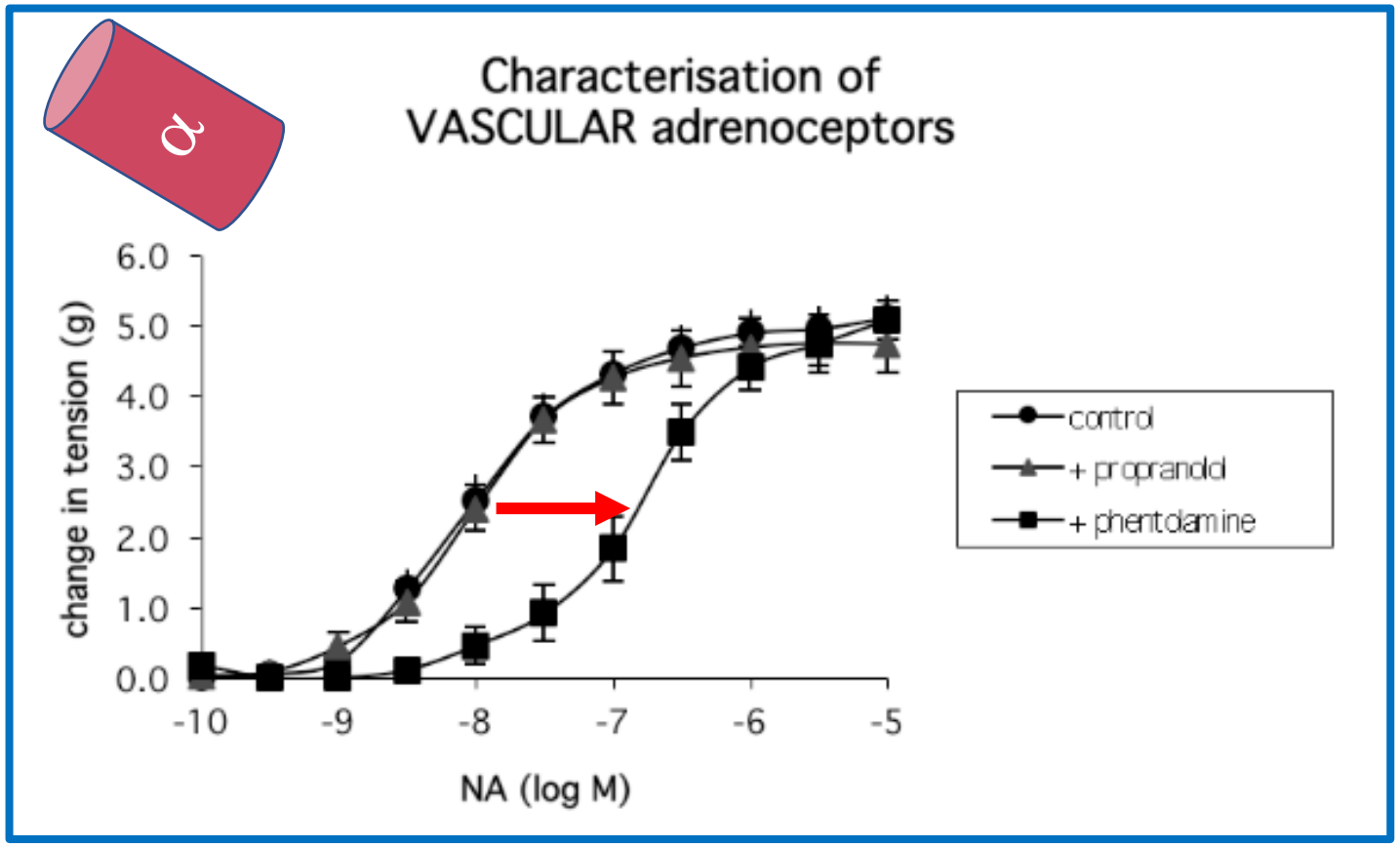
How many fold difference is there between the first and the last dose administered?

# Concentration response curves (CRC) for the Atrial Preparation: Are the adrenoceptors on the Sinoatrial node alpha or beta receptors?



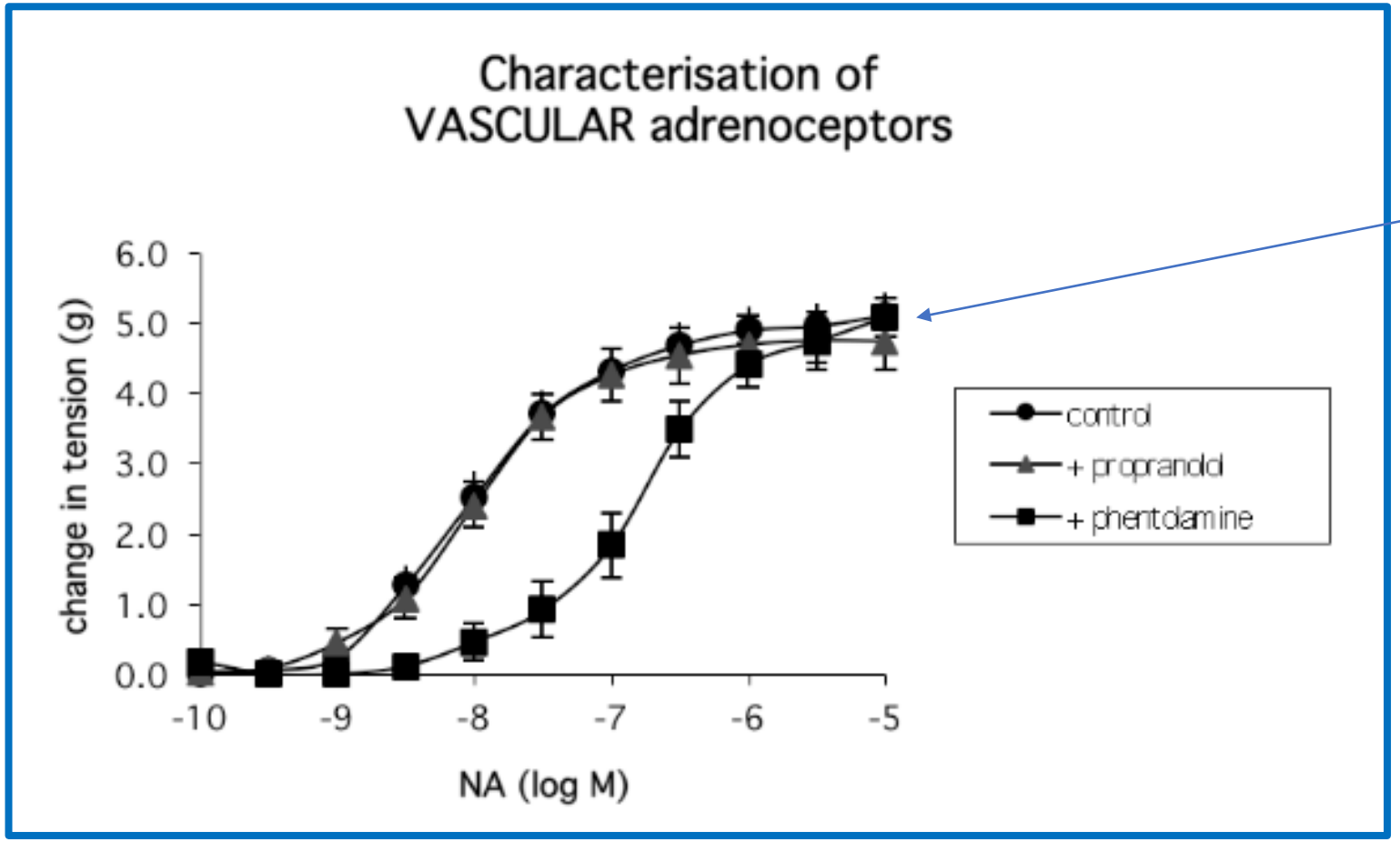

- In presence of phentolamine- no change to CRC
- In presence of propranolol parallel rightward shift in curve (→)
- **ie a beta blocker changed the response**
- **This is evidence that the adrenergic receptors on the SA node are beta receptors**

# Concentration response curves for the Aortic ring Preparation:
# Are the adrenoceptors responsible for constriction in the Aorta α or β receptors?



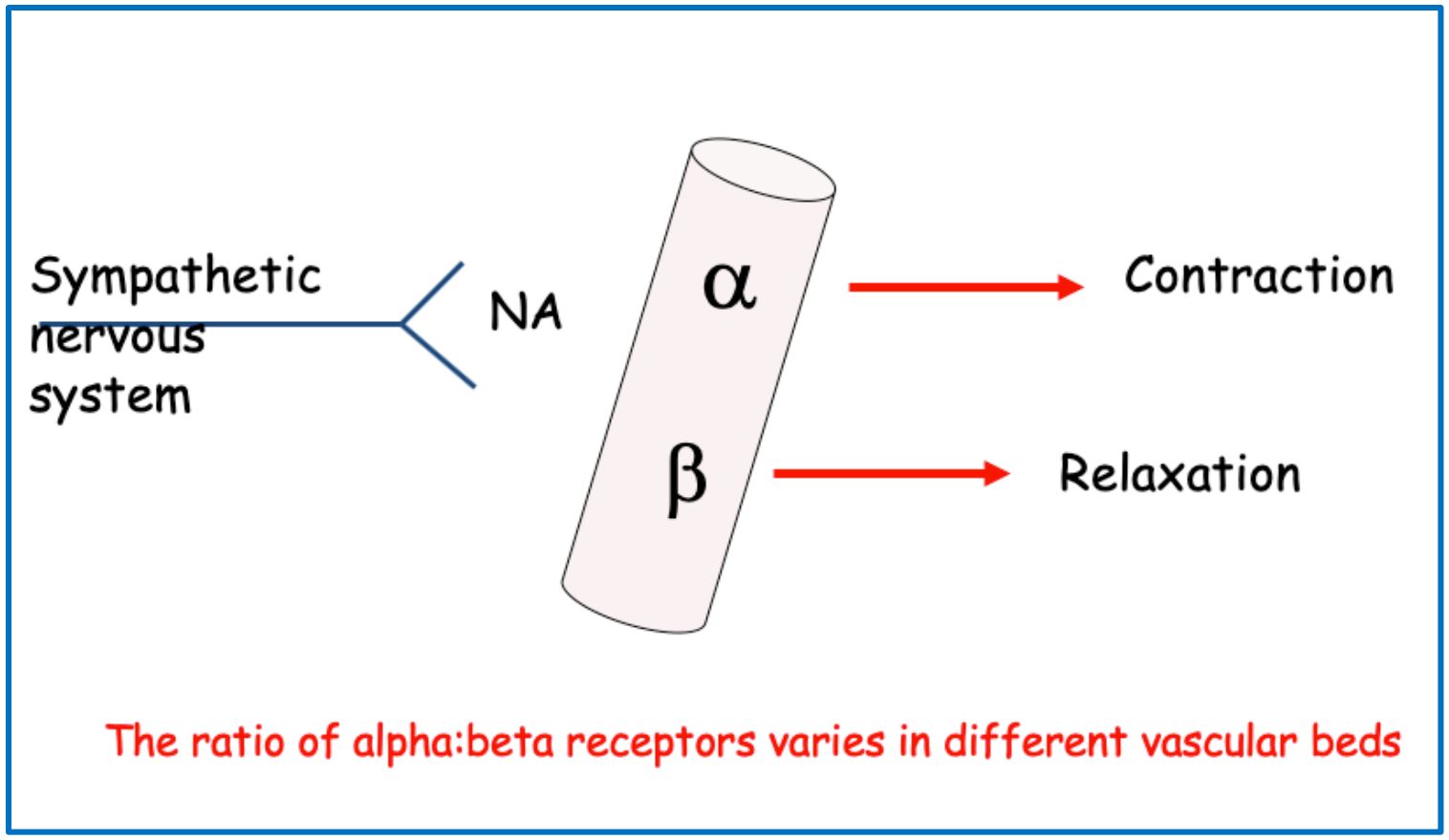

- In presence of propranolol- no change to CRC
- In presence of phentolamine **parallel rightward shift in curve.** ( → )
- **ie an alpha blocker changed the response**
- **This is evidence that the adrenergic receptors responsible for constriction in the aorta are alpha receptors**

Concentration response curves for the Aortic Ring Preparation:
NA induced vasoconstriction in the aorta is mediated  through alpha receptors

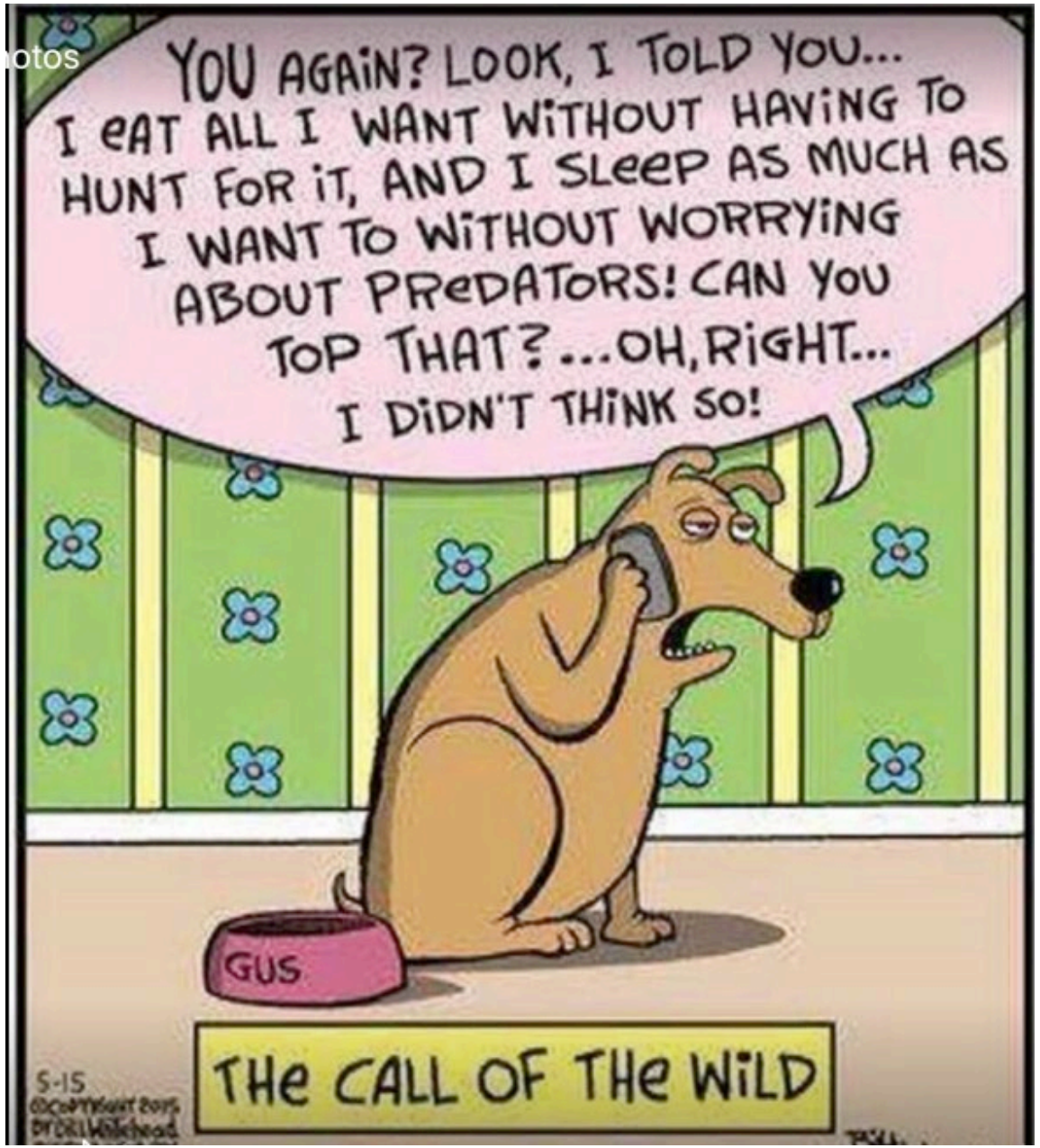



*Why is the response to NA in the presence of an alpha blocker restored at higher NA concentrations?*

# But are there beta receptors in blood vessels?




Yes, there are, but this setup does not allow us to observe relaxation




# Resisting the “Call of the Wild”

Thankyou…