Aim: Determination of pA_2 Value of Prazosin Using Rat Anococcygeus Muscle (Schild Plot Method)

References

1. Arunlakshana, O., & Schild, H.O. (1959). Some quantitative uses of drug antagonists. British Journal of Pharmacology and Chemotherapy, 14(1), 48-58.

2. Ghosh, M.N. (2008). Fundamentals of Experimental Pharmacology (6th ed.). Hilton & Company.

3. Rang, H.P., Dale, M.M., Ritter, J.M., & Flower, R.J. (2015). Rang & Dale's Pharmacology (8th ed.). Churchill Livingstone.

Introduction

The pA_2 value is a measure of the potency of a competitive antagonist. The rat anococcygeus muscle, which responds to adrenergic agonists like noradrenaline, is often used to determine the pA_2 value of adrenergic antagonists like prazosin. The Schild plot method involves creating a dose-response curve in the presence and absence of the antagonist and then using the Schild equation to calculate the pA_2 value.

Objective

To determine the pA_2 value of prazosin using the rat anococcygeus muscle by the Schild plot method.

Materials and Equipment

Male rats (to isolate the anococcygeus muscle)

Dissection tools (scissors, forceps, etc.)

Physiological saline (Tyrode's solution or Krebs solution)

Noradrenaline standard solutions (known concentrations)

Prazosin solutions (known concentrations)

Tissue bath setup

Aeration system (Oxygen supply)

Isometric transducer or force transducer

Recording device (kymograph or digital recorder)

Micropipettes and tips

Data analysis software (optional)

Procedure

1. Preparation of Rat Anococcygeus Muscle:

- Euthanize the rat using an appropriate method (e.g., CO2 inhalation or anesthetic overdose).
- Quickly dissect the perineal region to isolate the anococcygeus muscle.
- Mount the muscle in a tissue bath filled with Tyrode's solution or Krebs solution, maintained at 37°C and continuously aerated with oxygen.

2. Equilibration:

- Allow the muscle to equilibrate in the tissue bath for about 30 minutes, with constant aeration.
- Apply a resting tension of 1 gram to the muscle.

3. Baseline Recording: Record the baseline muscle tension to ensure stability and viability of the preparation.

4. Noradrenaline Dose-Response Curve:

- Prepare a range of noradrenaline concentrations (e.g., 1, 3, 10, 30, 100 nM).
- Add the lowest concentration of noradrenaline to the tissue bath and allow the muscle to contract.
- Record the maximum contraction.
- Wash the muscle with fresh Tyrode's solution or Krebs solution and allow it to return to baseline tension.
- Repeat the process for each concentration and plot the dose-response curve.

5. Noradrenaline Dose-Response Curve in Presence of Prazosin:

- Add a known concentration of prazosin to the tissue bath and allow the muscle to equilibrate.
- Repeat the dose-response curve for noradrenaline in the presence of prazosin.

- Record the maximum contractions.

6. Schild Plot Method:

- Calculate the dose ratio (DR) for each concentration of noradrenaline in the presence of prazosin.
- Plot the log(DR 1) against the log concentration of prazosin.
- Determine the slope and x-intercept of the Schild plot to calculate the pA_2 value.

Data Analysis

1. Plotting Dose-Response Curves: Plot the maximum contraction (response) on the y-axis against the concentration of noradrenaline on the x-axis for the control and prazosin-treated groups.

2. Schild Plot: Calculate the dose ratios (DR) using the formula:

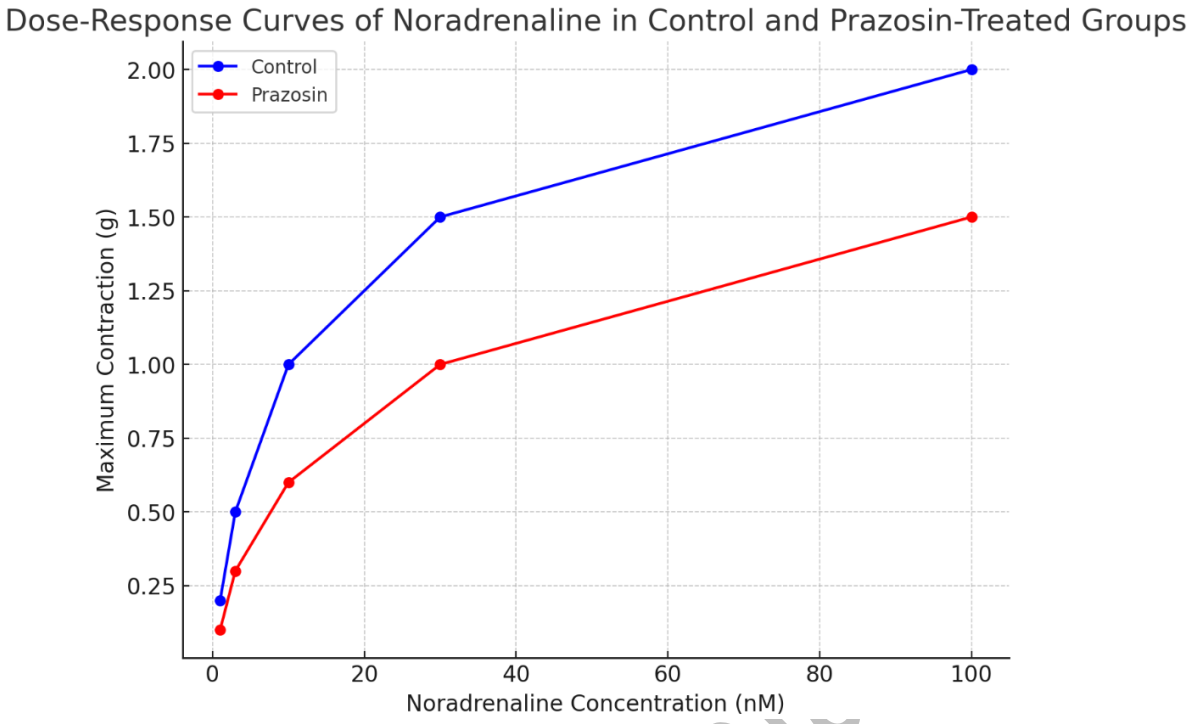
$$DR = \frac{EC50\ in\ presence\ of\ antagonist}{EC50\ in\ absence\ of\ antagonist}$$

- Plot the log(DR 1) on the y-axis against the log concentration of prazosin on the x-axis.
- The x-intercept of the Schild plot gives the pA_2 value.

Sample Data Table for Dose-Response Curves

Noradrenaline Concentration (nM)	Maximum Contraction (g) (Control)	Maximum Contraction (g) (Prazosin)
1	0.2	0.1
3	0.5	0.3
10	1.0	0.6
30	1.5	1.0
100	2.0	1.5

Sample Plot for Dose-Response Curves



Here's the plot showing the dose-response curves for noradrenaline in both control and prazosin-treated groups. The blue line represents the control group, while the red line represents the prazosin-treated group.

Next, let's calculate the dose ratios (DR) and plot the Schild plot to determine the pA_2 value of prazosin.

Sample Data for Dose Ratios and Schild Plot

Using the sample data from the dose-response curves:

$$\text{DR} = \frac{EC50\ in\ presence\ of\ Prazosin}{EC50\ in\ absence\ of\ Prazosin}$$

Given the data:

EC50 (Control): 10 nM (approximately)

EC50 (Prazosin): 30 nM (approximately)

$$\text{DR} = \frac{30}{10} = 3$$

For a range of prazosin concentrations, we would calculate the dose ratios and then plot log (DR 1) against log [prazosin concentration].

Sample Data Table for Schild Plot

Prazosin Concentration (nM)	Dose Ratio (DR)	log(DR 1)	log[Prazosin Concentration]
1	1.5	0.1761	0
3	2.0	0.3010	0.4771
10	3.0	0.4771	1.0
30	4.5	0.6532	1.4771
100	7.0	0.8451	2.0

Let's generate the Schild plot using this sample data.