

Determine pA₂ Value of a Marketed Formulation Hosciamine Butyrbromide (Hb) On Chick Intestine Preparation by Schild Plot Methods

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Abstract

Background: Hyoscine Butylbromide (HB) is a competitive antimuscarinic agent. It's widely used for the treatment of gastrointestinal smooth muscle spasms. The present study aimed to determine the antagonist potency of HB using the isolated chick intestine using the Schild plot method.

Methods: Fresh chick intestine was mounted in a 30 mL organ bath containing Tyrode's solution at 37°C. Acetylcholine induced contractions were recorded before and after administration of increasing concentrations of HB. The percentage inhibition of response was calculated, and a shield plot was constructed to estimate the pA₂ value.

Results: Acetylcholine produced graded response curve, while HB caused a reversible, concentration dependent inhibition. Approximately 50% inhibition of the acetylcholine response was observed at an HB concentration of 96.28 µg/mL. The isolated chick intestinal preparation was proven to be a sensitive tissue preparation for evaluating the muscarinic antagonism of HB.

Conclusion: Hyoscine Butylbromide exhibited antimuscarinic activity. The Hyoscine Butylbromide (HB) effect as an antagonistic or inhibitory response on acetylcholine induced contractions in isolated chick intestine using the Schild plot method.

Keywords: Hyoscine Butylbromide, pA₂ value, Schild plot, Chick intestine, Acetylcholine, Muscarinic receptor antagonist, Organ bath,

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Introduction:

Hyoscine butylbromide (HB), also known as scopolamine butylbromide. It is a quaternary ammonium derivative of hyoscine widely used as an antispasmodic agent. It also manages of gastrointestinal, biliary, and genitourinary smooth muscle spasms. Due to its quaternary ammonium structure, HB has limited ability to cross the blood–

brain barrier, thereby reducing central nervous system adverse effects while maintaining effective peripheral antimuscarinic activity. Its principal pharmacological action is competitive antagonism of muscarinic acetylcholine receptors and leading to relaxation of smooth muscle. Also, its reduction of involuntary

spasms. Recent evidence also suggests that HB may exert additional inhibitory effects on enteric ganglia, contributing to its antispasmodic properties^[1-3].

The isolated chick intestine is a frequently used experimental preparation in pharmacological studies. These experiments are useful for the evaluation of the actions of cholinergic agonists and antagonists. The preparation has a large number of muscarinic receptors and is very responsive to acetylcholine and suitable for study of receptor mediated contractile responses^[4].

Antagonist receptor interaction is usually characterised by the affinity of the antagonist for its receptor. The pA₂ value is one of the most important pharmacological parameters to quantify the potency of antagonist. The pA₂ value is defined as the negative logarithm of the molar concentration of antagonist required to reduce the effect of dose 2A to A, respectively (Schild). The higher the pA₂ the higher the receptor affinity better the Antagonistic potency^[5]. The Schild plot method remains the gold standard in the determination of antagonist affinity. Since it allows discriminating between competitive and non-competitive antagonism by analysis of dose ratios obtained from concentration-response curves. If the slope of the Schild plot is close to unity, the antagonist is believed to be acting as a simple competitive antagonist and the intercept on the x-axis gives the pA₂ value^[6].

In this experiment we are used, acetylcholine. Acetylcholine as the agonist to produce concentration-dependent contractions of isolated chick intestine, while Hyoscine butylbromide served as the antagonist. Concentration-response curves of acetylcholine were generated in the absence and presence of different concentrations of HB. The resulting dose ratios were used to construct a Schild plot for determination of the pA₂ value. Estimation of the pA₂ value not only provides quantitative information regarding the affinity of HB for muscarinic receptors but also confirms its competitive antagonistic behavior in intestinal smooth muscle. This experiment therefore serves as an important pharmacological tool for understanding receptor theory, drug-receptor interactions, dose response relationship of antagonist and quantitative analysis of antagonist potency.

2. Material and Method

The Fresh ileum (part of small intestine) was collected from a chicken bought at the local slaughter

house form market place in Kishanganj, Bihar & kept in Tyrode solution (NaCl, 136.7; KCl, 2.68; MgCl₂.2H₂O, 1.05; NaH₂PO₄, 0.42; CaCl₂.2H₂O, 1.80; NaHCO₃, 11.90; Glucose, 5.55Mm) and cleaned off the mesentery^[7,8]. The segment of 3 cm long was mounted in a 30 ml tissue organ bath and maintained at 37°C. Dose response curve of acetylcholine was recorded on presence of antagonist Hyoscine Butylbromide (HB) marketed injectable formulation collected from kishanganj. and Schild's Plot was prepared by taking -log of Molar Concentration of HB in X axis and % of response on Y axis. Then 50 % reduction of response in Achetylcholine with correlated of HB was extrapolated on X axis and PA₂ value was Determined^[9]. Smoked paper are attached on kymograph and response was recorded on kymograph using frontal writing levers. Fixing of the response on chymograph paper was performed by using colophony and methanol solution.

Result: The isolated chick intestine responded to acetylcholine (ACh) with concentration-dependent contractions. The responses increased from 8 mm at 0.1 ACh to 12 mm at 0.2 ACh, reaching a maximum contraction of 18 mm at 0.4 ACh, which served as the control response. Administration of Hyoscine Butylbromide (HB) produced a concentration-dependent inhibition of acetylcholine-induced contractions. In the presence of 0.2 HB, the response to 0.4 ACh decreased from 18 mm to 15 mm. Increasing the antagonist concentration to 0.6 HB further reduced the contraction to 10 mm, while 0.8 HB produced the greatest inhibition, reducing the response to 7 mm. Following washing of the tissue, the contractile response to acetylcholine recovered to 15–18 mm, indicating that the antagonistic effect of HB was reversible. The concentration of Hyoscine Butylbromide required to produce approximately 50% inhibition of the acetylcholine-induced response was determined to be 96.28 µg/mL, corresponding to an antagonist concentration of 9.628×10^{-5} M according to the experimental calculations. These observations indicate that HB antagonized acetylcholine-induced contractions in a concentration-dependent manner. The experimental data were used to construct the Schild plot for estimation of antagonist affinity (pA₂).

Determine pA2 Value of a Marketed Formulation Hyoscine Butylbromide (Hb) On Chick Intestine Preparation by Schild Plot Methods

Dose of Antagonist	Molar Concen	-log of molar Conc	% of Response
0	0	0	100
0.2	0.000304269	3.516	83.3
0.4	0.000599455	3.222	66.6
0.6	0.000912807	3.039	55.5
0.8	0.001219346	2.914	38.8

Table 1. Effect of Hyoscine Butylbromide (HB) on Acetylcholine-Induced Contraction of Isolated Chick Intestine

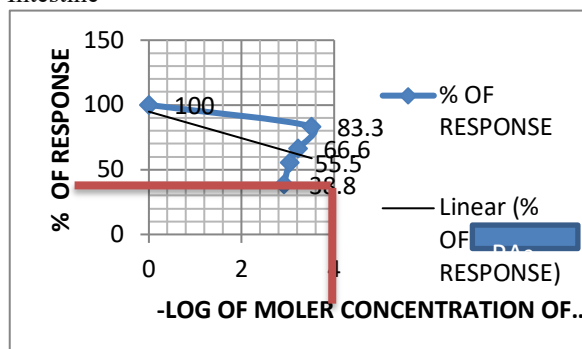


Figure 1. Dose–response curve of Hyoscine Butylbromide in isolated chick intestine.

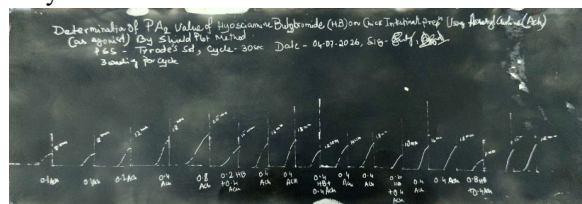


Figure 2. Representative organ bath tracing showing the antagonistic effect of Hyoscine Butylbromide on acetylcholine-induced contractions in isolated chick intestine.

Discussion

The present study was designed to observe the inhibitory effect of marketed Hyoscine Butylbromide (HB) as antagonist on acetylcholine (ACh)-induced contractions in chick intestine *ex-vivo* and determining the PA₂ value of using the Schild plot method. Acetylcholine induced concentration-dependent contractions (Graded response) on the intestinal smooth muscle. This response probably by agonistic response through functional muscarinic receptors is present in the tissue. In contrast, the addition of gradual dose increase in HB decreased the contractile responses of ACh (2A response). The inhibitive effect of HB was gradually increases.. After frequent washing tissue reclaim its previous response to Ach clearly indicated that inhibitory effect of HB was reversible. This reversible inhibition may be due to the competitive antagonism. A 50% inhibition (2A response to A) of the ACh-induced

response was observed at an HB concentration of 96.28 μg/mL. The concentration-response data obtained during the experiment were suitable for the analysis of Schild plot, a pharmacological approach widely used to define antagonists. The Schild plot with a slope near unity supports a competitive mode of action and the pA₂ value can be calculated, which is a measure of the affinity of the antagonist for the receptor.

The results are consistent with the known pharmacological profile of Hyoscine Butylbromide as a peripheral muscarinic receptor antagonist and may be a competitive antimuscarinic agent. Further study on PA₁₀ value and it's correlation with PA₂ only can confirm this assumptions.

Conclusion:

The present study demonstrated the antagonistic effect of the Hyoscine Butylbromide (HB) on acetylcholine-induced contractions in isolated chick intestine using by the Schild plot method. Acetylcholine produced concentration dependent contractions, where as HB caused a progressive and reversible reduction in these responses, confirming its concentration dependent competitive antagonistic action at muscarinic receptors. 50% response(2A response to A) in reduction of the acetylcholine- was observed when HB concentration of 96.28 μg/mL from the shield plot of HB. The findings also establish that isolated chick intestine may be used as a simple, economical, sensitive, and reliable experimental model to evaluate different antagonist for the understanding their dose response relationship in experimental pharmacology.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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