INVESTIGATION OF ISOLATED GOAT ILEUM PREPARATION FOR THE SUITABILITY AS A TEACHING AID FOR UNDER GRADUATE AND POST GRADUATE PRACTICALS

Deepraj Paul, K.Nandakumar

DEPARTMENT OF PHARMACOLOGY, P.E.S COLLEGE OF PHARMACY
HANUMANTHANAGAR, BANGALORE-560050
E-mail: deeprajcology81@gmail.com

Summary

In the present experiment we tried to find the suitability of goat ileum as a teaching aid for under graduate (UG) and post graduate (PG) practicals so that it can be used as a substitute for guinea pig ileum. For this purpose first goat ileum was mounted in different physiological salt solutions (PSS) at various temperatures and the response to Ach was recorded. It was observed that goat ileum had shown maximum stability in Tyrode solution at 37^0C for a period of 9 hours. Where as the stability decreased for Kreb's and Dejalon solution to 6 and 3 hours respectively. Following selection of PSS and temperature concentration response curve (CRC) of Ach was constructed till ceiling height was achieved. Also CRCs of Ach were constructed in the presence of different concentration of antagonist (1, 3 and 10 nM). Four different antagonists viz. atropine, dicyclomine, valethamate and hyoscine were used. The same method was followed for guinea pig ileum. The EC_{50} value of Ach alone in goat ileum was found to be 1.71µM (EC_{50} range 1.63 ─ 1.79 µM) and pEC_{50} value is -5.76 ± 0.01. In guinea pig ileum preparation the EC_{50} value of Ach alone was found to be 1.06µmolar (EC_{50} range 1.01─ 1.10 µM) and pEC_{50} value is -5.97 ± 0.009 . All these antispasmodics had antagonized the effect of Ach. All these drugs had shown a right ward shift of the CRC of Ach in goat and guinea pig ileum preparation. The pA_2 values for atropine, dicyclomine, valethamate and hyoscine in goat ileum were found to be 9.59 ± 0.022, 8.92 ± 0.237, 9.04 ± 0.227 and 9.09 ± 0.022 respectively. For guinea pig ileum the pA_2 values for atropine, dicyclomine, valethamate and hyoscine were found to be 9.93 ± 0.04, 9.39 ± 0.12, 9.80 ± 0.12 and 9.46 ± 0.05 respectively. Atropine and hyoscine had shown competitive type of antagonism where as dicyclomine and valethamate had shown non competitive type of antagonism in both goat and guinea pig ileum preparation, concluded based on the slope value obtained from Schild plot.

The EC_{50} value of Ach confirmed that goat ileum is less sensitive compared to guinea pig ileum and also there is a significant difference in the pA_2 values for atropine, valethamate and hyoscine in both the isolated tissue preparation, concluded based on "un paired t test". How ever the nature of the antagonists did not change in the tissue preparations. Based on this we can predict that goat ileum can be used as a substitute for guinea pig ileum only for teaching purpose but not for research.

Key words: Acetyl choline, ileum, antispasmodic, atropine, dicyclomine, valethamate, hyoscine.

Introduction

Isolated tissue preparations are also used for bioassay of drugs, to characterize specific receptor or its subtypes, to determine concentration response curve of an agonist .These preparations are also used to study antagonism of drug and in new drug discovery.[1]

The commonly used preparations for teaching undergraduate and post graduate students are isolated heart and rectus abdominis of frog. How ever there is lack of licensed frog breeders in India, this has arisen a situation to catch the frogs from the environment for teaching purposes. In this regards committee for the purpose of control and supervision of experiments on animals (CPCSEA) has raised several issues for the usage of frogs as a teaching aid. Hence many institutions have shifted the frog experiments to mammalian isolated tissue preparation. Guinea pig ileum is one of the widely used tissue preparation to study various effect of drugs for teaching purpose. Various committee and agencies try to reduce the usage of animals for teaching purposes. This has driven us to conduct alternative isolated tissue experiments. Ileum consists of a number of receptors including muscarinic, histaminic, GABAergic, serotonergic and adreno receptors.[2]

To study the changes mediated by the receptors, guinea pig ileum has been used as reference intestinal in vitro preparation. Various receptors including histaminic, GABAergic and adrenoreceptors are characterized in these tissue preparations. Guinea pig ileum preparations are very sensitive to common spasmogens and anti spasmogens and respond well to these drugs. However it is expensive for routine practical purposes. Isolated ileum preparations from other species like mice and rats are being characterized for interactions between specific receptors and drugs.[3,4]

Isolated tissue preparations are also used for research to identify new receptors or to determine the variation in species produced by anti cholinergic drugs. How ever to use this preparation for teaching purposes, the laboratory animal species has to be sacrificed. But if goat ileum is used for teaching purposes, which is easily available from slaughter house then animals need not be killed just for teaching purposes. The present investigation sought to examine the suitability of the easily and economically available goat ileum as a teaching aid for pharmacological teachers. This will reduce, refine and replace the number of animals to be used for teaching purpose.The present study aims to study various antispasmodic agents and its interaction with Acetyl choline (Ach) on isolated goat ileum. The results of goat ileum are to be compared with guinea pig ileum to examine the suitability of this preparation as a teaching aid in pharmacological experiments.

Materials

Acetyl choline, atropine sulfate, dicyclomine hydrochloride, valethamate bromide and hyoscine butyl bromide.

Methods

1. Standardization of goat ileum: Ileum from male goat was collected from slaughter house and cut into 2-3 cm piece like that of guinea pig ileum[1] and was mounted in 30ml organ bath with different PSS viz. Tyrode, Kreb's, Ringer and Dejalon at 37^0c. The preparation was aerated and allowed to relax for a period of 45 minutes under a constant load of 1 gram. CRC to Ach was constructed with three different dose levels of Ach ($1.81x10^{-7}$, $5.49x10^{-7}$ and $1.81x10^{-6}$ M) in this tissue preparation. The height of this contraction was measured and the sensitivity of the tissue to these PSS was assessed. Similar experiment was conducted at various temperatures (room

temperature, 32^0c, 35^0c and 37^0c) using this tissue preparation in Tyrode solution. The stability of the preparation was also assessed by repeatedly constructing the CRC of Ach at three different dose levels ($5.49x10^{-7}$, $1.81x10^{-6}$ and $5.49x10^{-6}$ M) at an interval of 1 hour for a period of 10 hours.

2. Construction of the concentration response curve (CRC) of the agonist alone and in the presence of different concentrations of the antagonist: Following standardization of the goat ileum preparation CRC of agonist (Ach) was established after a 45 minutes stabilization period given to the tissue preparation. CRC was recorded in Tyrode solution at 37^0C in 30 ml tissue bath volume using different molar concentration of Ach ($1.65 \text{ X } 10^{-8}$ to $5.49 \text{ X}10^{-3}$). After recording the response with a particular concentration of the agonist the tissue was washed thrice with PSS at an interval of 1 minute time.

The time cycle for this preparation were as follows –

0 – 30 sec : Base line recording.

30 – 60 sec : Response of Ach (contact time).

60 – 120 sec : First wash.

120 – 180 sec: Second wash.

180 – 240 sec : Third wash.

The response of Ach were taken till the ceiling dose was achieved. From this EC_{50} value of Ach was calculated. Once ceiling effect was observed and confirmed with the next higher dose the tissue was washed with physiological salt solution and incubated with 1nM concentration of the antagonist for half an hour and again CRC was established in the presence of 1nM concentration of the antagonist being dissolved in the physiological salt solution. Like this contractions were recorded in the same manner for 3 and 10nM concentration of the antagonist. Thus from these

graphs height of contractions were measured and converted into percentage responses with respect to the maximum contraction of the agonist alone, being assigned cent percent. Following this mean, standard error of mean (SEM) of the percentage response and log molar concentrations of the dose of the Ach were obtained . Based on the sigmoid shaped graph pattern the type of antagonism were predicted but further confirmation regarding type of antagonism was obtained from Schild plot.

3. Determination of EC_{50} value of the agonist and pA_2 value of the antagonist: EC_{50} value was obtained from graph pad prism software and pA_2 value of the antagonist was obtained using the formula-

pA_2=log {(EC_{50} of agonist in the presence of antagonist/ EC_{50} of agonist in the absence of antagonist)-1}-log{molar concentration of antagonist}.

Results

Effect of Ach on isolated goat ileum preparation

To observe the effect of Ach on isolated tissue preparation different molar concentration of Ach was administered (1.65×10^{-8} to 5.49×10^{-3}). It was found that Ach had shown a dose dependent increase in contraction in goat ileum preparation with EC_{50} value 1.71 µM. (EC_{50} range 1.63 to 1.79 µM.) and pEC_{50} value -5.767 ± 0.01 [Figure (l)].

Effect of antispasmodics on the CRC of Ach on isolated goat ileum

Atropine (1, 3 and 10 nM) , dicyclomine (1, 3 and 10 nM) , valethamate (1, 3 and 10 nM) and hyoscine (1, 3 and 10 nM) shifted the CRC of Ach towards right with a change in EC_{50} and pEC_{50} value of Ach [Table.1 ; Figure 2,4, 6 and 8].Antagonism produced by atropine and

hyocine were found to be competitive in nature as shown by Schild plot [Figure 3 and 9] , where as antagonism produced by dicyclomine and valethamate were found to be non competitive in nature concluded based upon Schild plot [Figure 5 and 7].

Drugs	**EC_{50} (µM)**	**pEC_{50}**	**pA_2/ pK_B value**
Ach alone	1.71(1.63 to 1.79)	-5.767 ± 0.0103	--------
1nM Atropine	12.5 (8.42 to 18.4)	-4.9 ± 0.08	9.59±0.022
3nM Atropine	37.0 (18.4 to 74)	-4.43 ± 0.15	
10nM Atropine	102 (70.8 to 147)	-4.0 ± 0.07	
1nM Dicyclomine	7.35 (2.24 to 24)	-5.13 ± 0.25	8.92 ± 0.237
3nM Dicyclomine	6.25 (2.24 to 17.4)	-5.20 ± 0.22	
10nM Dicyclomine	10.5 (4.43 to 24.8)	-4.97 ± 0.18	
1nM Valethamate	0.99 (0.93 to 1.04)	-6 ± 0.01	9.04 ± 0.227
3nM Valethamate	2.34 (2.17 to 2.51)	-5.6 ± 0.01	
10nM Valethamate	20.5 (18.6 to 22.6)	-4.6 ± 0.02	
1nM Hyoscine	3.16 (2.73 to 3.64)	-5.5 ± 0.03	9.09 ± 0.022
3nM Hyoscine	6.33 (5.40 to 7.39)	-5.19 ± 0.03	
10nM Hyoscine	20.9 (17.8 to 24.3)	-4.6 ± 0.03	

Table:1 Effect of Ach alone and in the presence of atropine, dicyclomime, valethamate and hyoscine on isolated goat ileum preparation.The values in the parenthesis indicate EC_{50} range at 95% confidence interval. The values of pEC_{50} and pA_2/pK_B are expressed as mean ± s.e.m. (n=6)

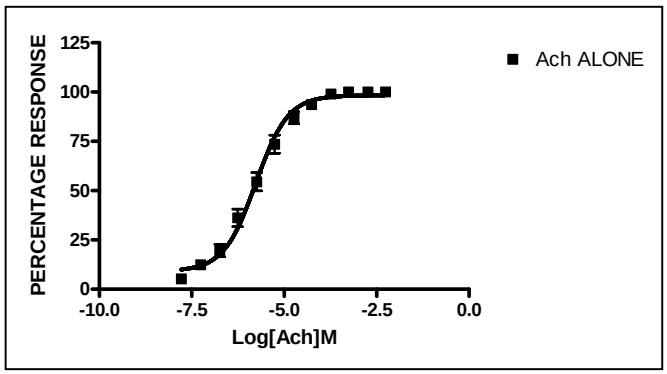
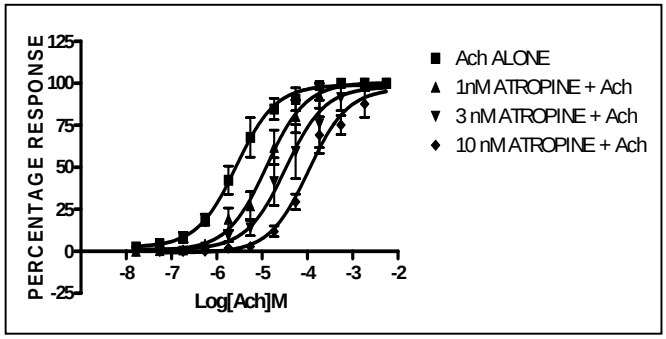


Figure : 1



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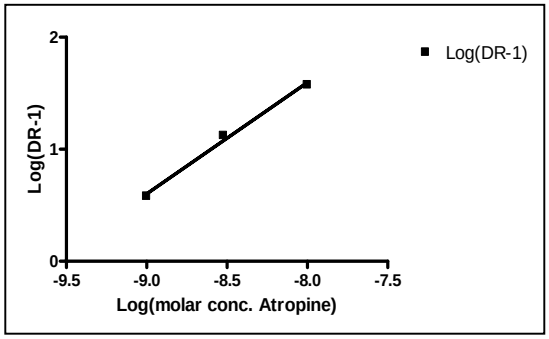

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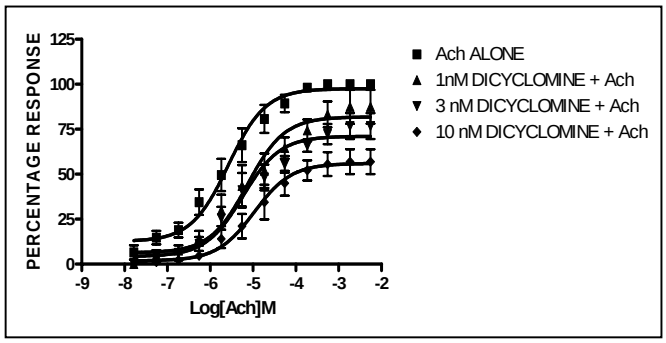

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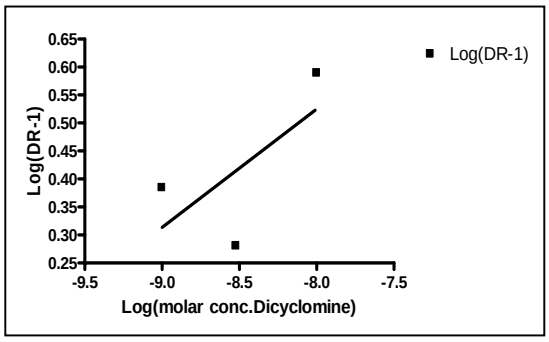

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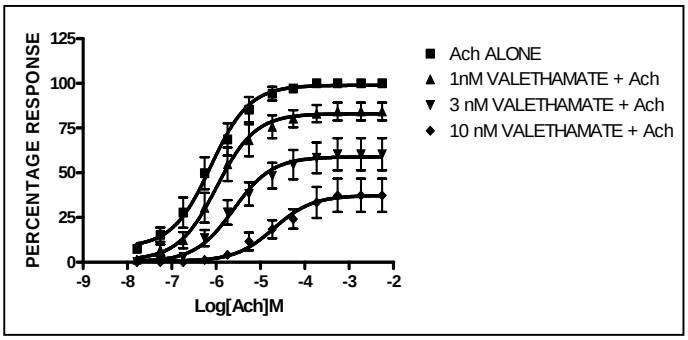

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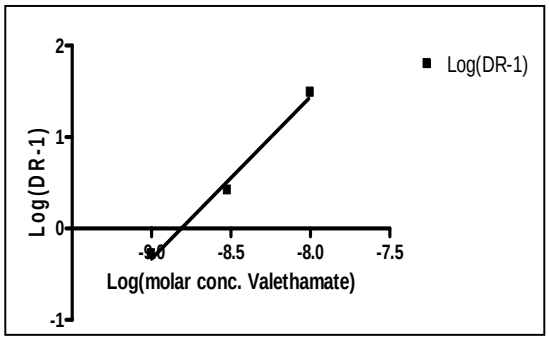

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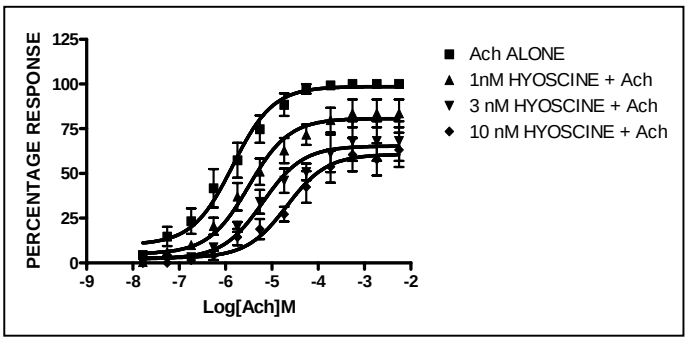

Figure:8

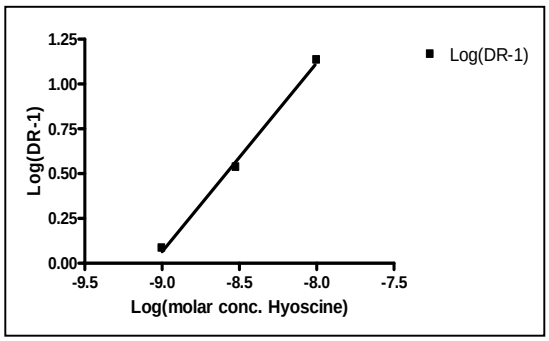

Figure:9

Figures : **(1)**: Effect of Ach alone on contraction shown by goat ileum

(2) Effect of Atropine on contractions induced by Ach in goat ileum. Results are expressed as percentage decrease in contractions induced by Ach. Values are mean ± S.E.M of 6 observations for each concentrations of Atropine. **(3)** Schild plot for Atropine against Ach with slope 0.9931 ± 0.0766, which is near to unity. **(4)** Effect of Dicyclomine on contractions induced by Ach in goat ileum. Results are expressed as percentage decrease in contractions induced by Ach. Values are mean ± S.E.M of 6 observations for each concentrations of Dicyclomine. **(5)** Schild plot for Dicyclomine against Ach with slope 0.2111 ± 0.2329, which is significantly different from unity. **(6)** Effect of Valethamate on contractions induced by Ach in goat ileum. Results are expressed as percentage decrease in contractions induced by Ach. Values are mean ± S.E.M of 6 observations

for each concentrations of Valethamate. **(7)** Schild plot for Valethamate against Ach with slope 1.771 ± 0.1704, which is significantly different unity. **(8)** Effect of Hyoscine on contractions induced by Ach in goat ileum. Results are expressed as percentage decrease in contractions induced by Ach. Values are mean ± S.E.M of 6 observations for each concentrations of Hyoscine. **(9)** Schild plot for Hyoscine against Ach with slope 0.9931 ± 0.0766, which is near to unity.

Comparison of pA_2 value of atropine, dicyclomine, valethamate and hyoscine obtained from goat ileum preparation with that of guinea pig ileum preparation

The pA_2 value of atropine, dicyclomine, valethamate and hyoscine are shown in table 2

Antagonist	pA_2 value in goat ileum	pA_2 value in g. pig ileum	P value	Significance
Atropine	**9.60 ± 0.02**	**9.93 ± 0.04*****	**0.0018**	**Highly significant**
Dicyclomine	**8.92 ± 0.23**	**9.39±0.12**	**0.1552**	**Not significant**
Valethamate	**9.05 ± 0.22**	**9.8±0.12***	**0.0443**	**significant**
Hyoscine	**9.09 ± 0.02**	**9.46±0.05*****	**0.0042**	**Highly significant**

Values are expressed as mean ± s.e.m (n = 3). ***P<0.001, **P<0.01 and *P<0.05
Table.2 Significance level in the difference between pA_2 values of antagonists.

Discussion

The present study was designed to determine the suitability of isolated goat ileum preparation as a teaching aid for UG and PG practicals.

Different PSS viz. Tyrode, Kreb's, Ringer and Dejalon were used to mount the isolated tissue preparation at 37^0C. It was found that Ach could not produce any contraction with Ringer solution indicating tissue may not be viable in this solution, while with Tyrode, Kreb's and

Dejalon solution different concentration of Ach produced dose dependent increase in contraction in these solution. The contraction produced in Tyrode solution was maximum compared to other two solutions. The stability of the tissue was assessed at 37^0C with various PSS. It was found that with Tyrode solution the tissue had produced consistent response up to 9 hours while with Kreb's and Dejalon consistent responses were observed up to 6 and 3 hours respectively. From the results it was found that tissue had produced maximum contraction in Tyrode solution with different doses of Ach and the tissue was stable for a longer period of time than the other PSS. Hence this solution was found suitable to mount the isolated tissue preparation.

Further the effect of temperature on Tyrode solution was also studied. At room temperature and at 32^0C the tissue did not show any contraction with Ach hence these temperatures were not suitable to study the response of Ach. At 35^0C and 37^0C the tissue responded to various concentration of Ach in a dose dependent manner and the tissue did not produce any inherent contraction at these two temperatures. Hence these two temperatures were found suitable for the experiment. However at 37^0C Ach had produced greater responses than observed at 35^0C for the same concentration and the tissue was stable at 37^0C for 9 hours. Hence we optimized the PSS to be Tyrode solution at a temperature of 37^0C.

CRC of Ach was constructed in goat ileum preparation. Ach produced concentration dependent increase in contractions of the goat ileum preparation. Ach produced the response at 5.88 $x10^{-8}$M ± 2.33 x 10^{-8}M and ceiling effect was observed at 1.36 $x10^{-4}$M ± 3.58 x 10^{-5}M. This study indicated that Ach produced the contractions of the tissue through muscarinic receptor. The EC_{50} value of Ach from the goat ileum preparation was found to be 1.71µM and EC_{50} range is 1.63 to 1.79 µM.

Effect of various antispasmodic drugs was observed by constructing the CRC of Ach in the presence of different concentrations of antagonists. It was found that all the concentration of antagonist (1, 3 and 10 nM) shifted the CRC of Ach towards right for goat ileum preparation. Atropine and hyoscine had shown competitive type of antagonism where as dicyclomine and valethamate had shown non competitive type of antagonism in both the sets of tissue preparation, concluded based on Schild plot. These results suggest that muscarinic blockers were able to block the Ach response confirming contraction produced by Ach was due to muscarinic receptors.

The EC_{50} values of Ach alone on goat ileum was found to be 1.71 µM and for guinea pig ileum the EC_{50} value was found to be 1.06 µM. This indicated that goat ileum is less sensitive than guinea pig ileum. Also the difference in pEC_{50} values of both the preparation was statistically significant ($P<0.0001$).

The pA_2 values for atropine, dicyclomine, valethamate and hyoscine in goat ileum were found to be 9.59 ± 0.022, 8.92 ± 0.237, 9.04 ± 0.227 and 9.09 ± 0.022 respectively where as the pA_2 values for atropine, dicyclomine, valethamate and hyoscine in guinea pig ileum were found to be 9.933 ± 0.0445, 9.391 ± 0.1205, 9.800 ± 0.1253 and 9.4610 ± 0.0585 respectively. The difference in pA_2 values for atropine, valethamate and hyoscine were found to be statistically significant concluded based on "un paired t test". How ever the pattern of response to Ach and nature of antagonism with the various antispasmodic drugs were similar to that of guinea pig ileum preparation. Hence goat ileum preparation can be used as a substitute for guinea pig ileum preparation only for teaching purpose not for research purpose.

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