King Saud University 313 PHL

College of Pharmacy Lab # 1

Pharmacology Department

***Dose response curve of acetylcholine on isolated rabbit intestine***

***Points to be discussed:***

1. Pharmacology experiments can be largely divided in to:
   1. Qualitative
   2. Quantitative
2. Type of experiments:
   1. Invitro
   2. Invivo
3. Experimental conditions:

a- Physiological solution ingredients

b- Type of physiological solution.

***Lab work:***

1. Material:

* Physiological solution: Tyrode’s solution.
* Apparatus: Physiograph & thermostatically controlled organ bath.
* Preparation: isolated rabbit intestine.
* Temp.:37° C.

1. Method:
   * A piece of intestine is suspended in organ bath.
   * The aeration is adjusted by means of the screw clamp to give a steady stream of air bubbles
   * Adjust the tension on the muscle so that it’s kept stretched to moderate extent
   * Record the normal myogenic contraction of the muscle for 1 min.
   * Test the effect of different drugs on the muscle, wash twice and record normal contraction between the drugs (total cycle = 60sec. CT + 60sec.NT + washing twice each 30 sec. = 3 min.)

N.B. the intestine should be totally covered with Tyrode’s soln. & the level of solution should be kept constant during the experiment.

***Drugs:***

|  |  |
| --- | --- |
| 1) 0.1ml acetylycholine → | To test the efficiency of M3 |
| 2) 0.2ml acetylycholine → | To test the efficiency of M3 |
| 3) 0.4ml acetylycholine → | To test the efficiency of M3 |

***Result:***

1. The curve should be properly cut & pasted on the lab book with proper title & key.
2. Tabulate your result.

King Saud University 313 PHL

College of Pharmacy Lab # 2

Pharmacology Department

***Effect & site of action of different agonists on isolated rabbit intestine***

***Points to be discussed:***

1. Autonomic nervous system control of intestine
   1. Sympathetic
   2. Parasympathetic
2. Drugs act on intestine
   1. Stimulants "Spasomgenic"
   2. Relaxants "Spasmolytic"

***Lab work:***

1. Material:

* Physiological solution: Tyrode’s solution.
* Apparatus: Physiograph & thermostatically controlled organ bath.
* Preparation: isolated rabbit intestine.
* Temp.:37° C.

1. Method:
   * A piece of intestine is suspended in organ bath.
   * The aeration is adjusted by means of the screw clamp to give a steady stream of air bubbles
   * Adjust the tension on the muscle so that it’s kept stretched to moderate extent
   * Record the normal myogenic contraction of the muscle for 1 min.
   * Test the effect of different drugs on the muscle, wash twice and record normal contraction between the drugs (total cycle = 60sec. CT + 6osec.NT + washing twice each 30 sec. = 3 min.)

N.B. the intestine should be totally covered with Tyrode’s soln. & the level of solution should be kept constant during the experiment.

***Drugs:***

|  |  |
| --- | --- |
| 1) 0.1ml dil. nicotine (0.01%) → | To test the efficiency of Nn |
| 2) 0.1ml Ach (0.01%) → | To test the efficiency of M3 |
| 3) 0.1ml Bacl2 (6%) → | Acts directly on muscle fibers |
| 4) 0.1ml adrenaline (0.1%) → | To test the efficiency of α1 & β2 |
| 5) 0.1ml Mgcl2 (6%) → | Acts directly on muscle fibers |

***Result:***

1. The curve should be properly cut & pasted on the lab book with proper title & key.
2. Tabulate your result.

|  |  |  |
| --- | --- | --- |
| Drug / dose | Observation | Comment |
|  |  |  |

King Saud University 313 PHL

College of Pharmacy Lab # 3

Pharmacology Department

***Effect of certain agonists & their antagonists on the isolated rabbit intestine***

***Points to be discussed:***

1. Agonists & their specific antagonists acting on isolated rabbit intestine.
2. Determine the site of action of unknown drug.

***Lab work:***

1. Material:

* Physiological solution: Tyrode’s solution.
* Apparatus: Physiograph & thermostatically controlled organ bath.
* Preparation: isolated rabbit intestine.
* Temp.:37° C.

1. Method:
   * A piece of intestine is suspended in organ bath under appropriate conditions.
   * Record the normal myogenic contraction of the muscle for 1 min.then start to test the effect of agonists & their antagonists.
   * The agonist or antagonist is allowed to act for 60 sec.

N.B. No washing is done after addition of any antagonist & before the addition of its specific agonist

***Drugs:***

|  |  |
| --- | --- |
| 0.1ml dil. Nicotine (0.01%) | To test the efficiency of Nn-R |
| 0.1ml conc. Nicotine (0.1%) | To block the central Nn-R |
| 0.1ml dil. Nicotine (0.01%) | Testing for complete blocking of Nn-R |
| 0.1ml Ach (0.01%) | To test the efficiency of M3-R |
| 0.1ml Atropine | To block peripheral M3-R |
| 0.1ml Ach (0.01%) | Testing for complete blocking of M3-R |
| 0.1ml Bacl2 (6%) | Acts directly on muscle fibers |

***Design for Determination of site of action of unknown:***

**Blocker**

**+**

**Agonist**

**+**

**Unknown**

**Unknown**

**Agonist**

***Result:***

1. The curve should be properly cut & pasted on the lab book with proper title & key.
2. Tabulate your result.

King Saud University 313 PHL

College of Pharmacy Lab # 4

Pharmacology Department

**Effect of certain agonists & their antagonists on the isolated guinea-pig ileum**

**Points to be discussed:**

1. Ileum innervation.
2. Types of receptors in guinea-pig ileum.
3. Types of drugs act on guinea-pig ileum (spasmogens & spasmolytics)

**Lab work:**

1. Material:

* Physiological solution: Tyrode’s solution.
* Apparatus: Physiograph & thermostatically controlled organ bath.
* Preparation: isolated guinea-pig ileum.
* Temp.:32° C.

1. Method:
   * A piece of ileum is suspended in organ bath under appropriate conditions.
   * A normal record is taken for about 4 squares before starting drugs additions. In general:

1- Spasmogens (Ach, Histamine & Barium chloride) are allowed to act for 30 sec. except Bacl2 (1min. or longer).

2- Antagonists or spasmolytics (atropine, mepyramine & papaverine) are allowed to act for 60 sec.

**Identification of unk agonist on isolated GPI:**

|  |  |
| --- | --- |
| 0.1ml unk | It a stimulant it could be act through M3-R or H1-R or direct spasmogen. |
| 0.1ml Ach (0.01%) | To test the efficiency of M3-R |
| 0.1ml Atropine (for 1 min.) | To block peripheral M3-R |
| 0.1ml Ach (0.01%) | Testing for complete blocking of M3-R |
| 0.1ml unk | Either NO EFFECT→ the unk act through M3-R,or Produce stimulation→ the unk doesn’t act through M3-R , it could be…………..etc. |
| 0.1ml Histamine (0.1%) | To test the efficiency of H1-R |
| 0.2ml Mepyramine | To block H1-R receptors |
| 0.1ml Histamine (0.1%) | Testing for complete blocking of H1-R |
| 0.1ml unk | Either NO EFFECT→ the unk act through H1-R or Produce stimulation→ the unk doesn’t act through H1-R , it could be…………..etc. |

**Result:**

1. The curve should be properly cut & pasted on the lab book with proper title & key.
2. Tabulate your result.

King Saud University 313 PHL

College of Pharmacy Lab # 5

Pharmacology Department

**Dose response curve of Ach & drugs acting on the frog rectus abdominis**

**Points to be discussed:**

1– Neuromuscular transmission.

2– Drugs that affect neuromuscular transmission

**Lab work:**

**A- Material:**

* *Physiological solution*: Ringer’s solution.
* *Apparatus*: Physiograph & thermostatically controlled organ bath.
* *Preparation*: Isolated frog rectus abdominis.
* *Temp*.: Room temperature.

**B- Method:**

1-The frog is decapitated & pithed, the skin on ventral surface is removed to expose the two rectii.

2-The muscle should be kept moistened with Ringer’s solution during dissection. The muscle is properly isolated in away that one end is attached to aeration tube & the other one attached to the transducer.

**Dose response curve of Ach**

1-Recored the normal tone for 5cm.(No myogenic contraction i.e. straight line)

2-Study the effect of graded doses of Ach (start with 0.05ml). Plot the DRC of Ach using semi-log paper.

**Effect of drugs act on neuromuscular transmission**

5-A submaximal dose of Ach is chosen.

6-Study the effect of anticholinesterase (physiostigmine) on the action of Ach.

7-Study the effect of NM blocker (curare) on the action of Ach.

**Result:**

1-The curve should be properly cut & pasted on the lab book with proper title & key.

2-Tabulate your result.

King Saud University 313 PHL

College of Pharmacy Lab # 6

Pharmacology Department

***Effect & site of action of different agents on isolated rabbit heart (langendorff preparation)***

***Outlines:***

1. Autonomic nervous system control of the heart:
   1. Sympathetic
   2. Parasympathetic
2. Drugs act on the heart, type of receptors & transmitters in the heart.

**Lab work:**

1. Material:

* Physiological solution: Ringer Locke solution.
* Apparatus: Physiograph & thermostatically controlled organ bath.
* Preparation: isolated rabbit heart.
* Temp.:37° C.

1. Method:
   * The heart is carefully isolated from a scarified rabbit (the chest is opened & the heart is dissected with at least 1 cm of aorta).
   * The heart is then placed in a dish containing Ringer Locke solution and squeezed gently several times to remove the blood.
   * The aorta is tied to a cannula attached to the bottom of perfusion tube.
   * A hook is fixed to the apex of the heart & attached to the myograph by a thread.
   * The heart is perfused with oxygenated Ringer Locke at constant temp. of 37° C.
   * The drugs are injected through the rubber cap on top of cannula.

**Drugs:**

|  |  |
| --- | --- |
| 0.1ml dil. Nicotine (0.01%) | To test the efficiency of NN R |
| 0.1ml conc. Nicotine (0.3%) | To block the central NN R |
| 0.1ml dil. Nicotine (0.01%) | Testing for complete blocking of NN R |
| 0.1ml Ach (10g/ml) | To test the efficiency of M3 |
| 0.1ml Atropine | To block peripheral M3 R |
| 0.1ml Ach(10g/ml) | Testing for complete blocking of M3 R |
| 0.1ml Isoprenaline (2g/ml) | Testing the efficiency of 1 R |
| 0.1ml Propranolol (100g/ml) | To block R |
| 0.1ml Isoprenaline (2g/ml) | Testing for complete blocking of 1 R |
| 0.1ml Cacl2 (20mg/ml) | Direct acting myocardial stimulant |
| 0.1ml Kcl (10mg/ml) | Direct acting myocardial depressant |

**Result:**

1. The curve should be properly cut & pasted on the lab book with proper title & key.
2. Tabulate your result.

**Procedures:**

1. Tie the two ends of tissue with thread
2. Hang the tissue in the organ bath
3. One end should be attached to the transducer while the other end is attached to aerator
4. Adjust the tone of thread “Not too loose Not too tight”
5. Switch the physiograph on
6. Make interface on
7. Adjust the pen (Avoid the upper and lower limits)
8. Record normal contraction for 4 squares and immediately add the first drug
9. Record two squares after the drug addition then stop the wheal
10. Wash the tissue three times or till the pen return to normal
11. Record normal contraction for 4 squares then immediately add the second drug
12. Record two squares after the drug addition then stop the wheal and so on

King Saud University Practical Pharmacology

College of Pharmacy PHL 313

Pharmacology Department Lab No. ( )

**Title:**

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**Aim of the work:**

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**Material:**

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**Method:**

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**Results:**

**Title**

………………………………………………………………………………………………………………………………………………………………………………………………

**Curve:**

**Key:**

………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………….........………………………………………………………………………………………………………………………………………………………………………………………………

**Tabulate the results & record the observation**

|  |  |  |
| --- | --- | --- |
| Drug / volume | Observation | Comment |
|  |  | ……………………………………………….  ……………………………………………….  ……………………………………………….  ……………………………………………….  ……………………………………………….  ………………………………………………. |
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| --- | --- | --- |
| Drug / volume | Observation | Comment |
|  |  | ……………………………………………….  ……………………………………………….  ……………………………………………….  ……………………………………………….  ……………………………………………….  ………………………………………………. |
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**1st Tutorial Topics:**

**Different pharmaceutical dosage forms and routes of administration:** this will include

* Why we use different dosage forms and routes of administration.
* Different types of dosage forms.
* Routes of administration and their advantages and disadvantages .

**Receptors:** this will include

* -different type of receptors
* -Examples of drugs target each receptor
* -Receptor structure & Function

**Ion channels:** this will include

* -Different types of ion channels
* -Examples of drugs target ion channels
* -Structure and Function of ion channels

**Adrenergic receptors:** this will include

* -Classification of adrenergic receptors
* -Expression of adrenergic receptors
* -Pharmacological action
* -Examples of adrenergic agonists & antagonists

**G-protein:** this will include

* -Type of G-protein
* -Function of G-protein
* -Intracellular second messengers.

**2nd Tutorial Topics:**

* Cotransmissin in autonomic nervous system
* Muscarinic receptors
* Nicotinic receptors
* Treatment of glaucoma
* Skeletal muscle relaxants

Each group is asked to conduct an 8-12 min. - long slide presentation about one of the above topics in addition to oral discussion about the corresponding topic.

Each group is asked to submit an electronic copy of their presentation.

**PHL 313 lab tutorials**

**In Vitro Experiment**

Changes in Dose Response Curve

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ***Both*** | ***Tone*** | ***Amplitude*** |  |
| Contraction |  |  |  | ***Increase*** |
| Relaxation |  |  |  | ***Decrease*** |