Identifying the pharmacological properties of unknown drugs

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The experiment aimed to establish the pharmacological attributes of drug (B2) which is relatively unknown to many people. A more important thing to consider is the effect of Antagonistic in blocking the effects of B2. Another thing of utmost value is the question which tries to explain whether the effects and usefulness of B2 can actually be replicated in other drugs. It is paramount to understand that B2 is a drug which brings side effect as a result of interaction with the digestive substance from Pseudo-Cholinesterase. Another area of interest is the hindering of b2 effects by the introduction of an inhibitor known as cholinesterase; it is not clear whether it can actually hinder the potential effect of. The receptor factor of B2 is also considered important.

Introduction

There exist two categories of pharmaceutical drugs; agonist and antagonist. Agonist drugs acts on the principle that it binds itself to the receptor substance of the respective cell. Normally agonists exists in form of hormones or neurons a fact that makes them very popular in the human body. In this scenario the unknown B2 drug belongs to the agonist category. On the other hand antagonists operate on the reverse principle of the agonists in that they tend to block the receptors. In order to evaluate and asses the pharmacological properties of B2 it is vital to examine two unique properties; efficacy and potency. Efficacy refers to the overall capacity of a drug to produce the desired effects. Potency on its part refers to the level of response that is generated by a drug. The higher the potency the lower the response level of a particular drug. For instance in order to generate a 50%

response value, the dosage of the drug being administered needs to quite high.

The experiment is composed of two distinct phases; phase2 and phase3. Phase2 focused on establishing the effect of administering a selective antagonist dosage on the two substances; chlorphenamine and Atropine. As a result Atropine appeared to be blocked primarily because it is exhibit antagonistic attributes towards muscurinic and nicotinic receptors. On the other hand chorphenamine appeared to inhibit the effects of histamine more because it blocks autocoid histamine receptors remain blocked. In order to determine the blockage effect of B2 it was necessary to thoroughly test the selective dosage. This will allows for easier identification of the actual receptors which not works with B2 but those that blocks it affects. Another aspect examined in phase2 is related with how other agonists mimic the effect of B2. In this case it was vital to evaluate and compare the behavior of log-dose curves with the sole aim of deriving both the efficacy and the potency values.

Phase 3 involved the use of pseudocholiesterase from horse blood and an esterase inhibitor known as physostigmine. Cholinesterase action involves hydrolyzing the ester bond found in acetylcholine. Basically there exists two categories of cholinesterase; acetyl-cholinesterase and pseudo-cholinesterase. Another substance used in this phase is carbachol which is rather resistant to the effect of esterase digestion. This means that its presence is used to protect or inhibit the digestion of acetycholine, histamine and B2. In addition an interaction between an antagonist like physostigmine

and agonist substance will result in an increase in ED50. In some cases this can be attributed to the ever increasing potential of grugs by many people.

Methods

An organ bath is initially setup in presence of an ileum tissue from a guinea pig. Prior to using the tissue, a Petri dish is first filled with ringer solution and then subjected to oxygen supply. It is paramount to note that the tissue lacks any spontaneous patterns but rather it is characterized by contractions. The ileum tissue contains substances such as 5Ht, H1, nicotinic and muscurinic receptors. These substances are easily affected by contraction. In addition ileum tissue experience relaxation probably due to the fact that it lacks both beta and alpha receptors.

In order to produce good result the tissue required to be attached to a transducer using a threading string. Additionally this tissue was submerged in ringer’s solution at a room temperature of 37 degrees Celsius. In a normal scenario the addition of an agonist may cause the ileum to contract; this tension would be amplified by the transducer, which would then record the trace of response on a computer. Each test was preceded by a complete wash out of the drug. Oxygen supply needs to quite constant in order to sustain the life of the cell. Data from the races is used to plot the graph which shows the behavior of each agonist in response to the effect of log-dose.

Results

For B2 laced with Chlorphenamine 1 in 10000 dilutions Emax was 98/% while ED50 was 1. 0X10-6M.

For B2 laced with atropine 1 in 5000 dilutions, Emax and ED50 were 4. 4 x 10^-6 and 72% respectively.

It is evident that competitive antagonism was dominant. It is as a result of Atropine blocking specific masculine receptors. It means that B2 is a cholinergic agonist, which might be either acetylcholine or Carbachol.

Mimicry effect:

B2 Gave an Emax value of 90% and an ED50 value of 1. 44 x 10^-6M. The values for Acetylcholine were 60% and ED50 value of3. 1 x 10 ^-6 M. However. Carbachol gave a high efficacy 100% Emax value and an ED50 value of 3. 0 x 10^-6M. Additionally Histamine gave Emax and ED50 50% and4. 4 x 10 ^-6M respectively. The lowest point was recorded while using Serotonin which had an ED50 value of 7. 5 x 10^-6 and an Emax value of 21%.

Acetylcholine in the presence of physostigmine

Tthe Emax was 100% and the ED50 1. 3×10^-7 while Carbacho was 98. 4% Emax and ED501. 7×10^-6. However in the presence of physostigmine EMAX was 100% and the ED50 was 6. 0×10^-7.

Conclusion

Both the mimicry graphs of B2 and Carbachol exhibits similar characteristics hence the same ED50 values Despite this there is no enough evidence to establish what exactly what the receptors acts on. When antagonistic atropine was used the ED50 of the respective B2 was considerably reduced. This mainly occurred at both the selective and effective dosage levels. It can be concluded that B2 indeed acted on cholinergic receptors. This is given

more strength by the increase in ED50 reduction in potency level. To get even more definite results chlorphenamine was used as the sole histaminergic antagonist. Results indicated that both the effective and selective dose of Chlorphenamine had no antagonistic effect on B2. Additionally the potency did not reduce. It is evident that B@ does not in any way acts on histaminergic receptors. Thus it is prudent to argue that B2 does indeed acts upon cholinergic receptors

Experiment 2

Purpose: To demonstrate the pharmacological properties of unknown drug B16

Experimental phases (phase 1 and phase 2) are essential in determining these properties. Key attributes investigated include selective and effective dose of Atropine, Atropine and B2, effective and selective dose of Chlorphenamine, Chlophenamine and B2, Mimicry of B2 , digestion by pseudo-cholinesterase on B2, protection by Physostigmine of B2, and potentiation of B2.

Acetylcholine is regarded as an acetic acid such as ester of choline. It acts on cholinergic synapses to propagate nerve impulses. Acetylcholine has high and equal potency for muscurinic and nicotinic receptors. It is also highly susceptible to breakdown by cholinesterase. Carbachol which is is agonist of the muscurinic and nicotinic receptors is more potent on nicotinic receptors. In addition it is not broken down by Cholinesterase. Health applications of Acetylcholine includes but not limited to the treatment of Glaucoma. Its treatment remedy is based on the contraction principle; causes contraction

of circular muscle in the eye leading to an increase in output of aqueous humour.

Obtained from Atropa belladonna also known as deadly nightshade, Atropine which is alkaloid in nature serves to block the cholinergic receptors. Medical applications of Atropine involve dilation of the pupil which is most common during examinations of the eyes. Another substance Chlorphenamine is rather antihistamine in nature and thus it blocks histamine receptors. It clinical uses involve the treatment of allergic reactions such as itching. (Youngson, 1999) Physostigmine is regarded as being one of those substances that tends to bring reversible cholinesterase inhibition. Since Physostigmine normally interferes with the breakdown of Acetylcholine , its overall effects is significantly ppextended. Major medical use encompasses the boosting of the muscle tone of people with Myasthenia Gravis (Youngson, 1999).

Effective dose of atropine

The purpose of the first experiment was to identify the effective dose of Atropine. Three doses of atropine were added to Acetylcholine; Acetylcholine with atropine 1/1000, Acetylcholine with atropine 1/10000 and Acetylcholine with atropine 1/5000. The three concentrations of atropine (1. 4 x 10^-10M, 2. 88 x 10^-10M and 1. 4 x 10^-11M) were first carried out on both carbachol and acetylcholine. The three specimens showed a shift in the dose response curves to the right. This makes the drugs to appear to be below potent as they tend to increase their ED50 values. The results prove that both acetylcholine and carbachol are blocked by atropine. After observing results from graphs used in the experiment, it is evident that there is a

distinct shift in all the dose response curves to the right. This helps to lower the potency of the drug at all concentrations.

Selective dose Atropine

The aim of this section of experiment is to establish whether the effective dose of Atropine is also a selective dose. In this case histamine was titrated with the three concentrations of atropine to identify if histamine is actually blocked antagonist. In a normal scenario histamine ought not to be antagonized by atropine. Instead there should not be a significant shift in the dose response curve or reduction in potency. However at high dose concentration, atropine can indirectly block histamine.

To analyze the selective dose of atropine, three different concentrations of atropine were used on histamine. The 1/5000 and 1/1000 dilutions of atropine i. e. 1. 4 x 10^-10M and 1. 4 x 10^-9M became the histamine to shift to the right. This shows that high concentrations of atropine can cause an indirect antagonistic affect to histamine. Despite this, the 1. 4 x 10^-11M (1 in 10000 obtained was found to be 2. 3×10-6M. Actually dilution does not reduce the efficacy or the potency of histamine. Additionally the dose of 1. 4 x 10^-11M (1/10000 dilution of atropine) does not shift histamine to the right thus retaining its ED50 value. This dose of atropine is both effective and selective for cholinergic agonist such as acetylcholine and Carbachol. It effectively blocks acetylcholine and carbochol especially when the affect on histamine is not great. Consequently the effective and selective dose for Atropine was resolved to be 1. 4 x 10^-11M. It means Atropine did not affect Histamine activity.

Atropine and B16

Aim of the experiment was to find the impact of both the effective and selective dose of Atropine (1×10-7M) on the unknown drug B16. The effective and the selective dose were obtained by testing different concentrations of atropine on acetylcholine, carbachol and histamine. A distinct shift in the dose response curve to the right was found when administering the 1. 4 x 10^-11M of atropine to unknown drug B16 there was. Therefore this dose of atropine result in drug B2 appearing to be less potent by lowering its ED50 value. It is clear that drug B2 is capable of acting upon cholinergic receptors. This is primarily because the effective and selective dose of atropine that was determined previously blocked the actions of drug B2. In effect it makes it to appear to be less potent and reducing its ED50 value. This means that Atropine was blocking Cholinergic receptors, which B2 acts on. Hence it may be deduced that B2 is a cholinergic agonist, and it may be Acetylcholine, or Carbachol.

Effective dose of Chlorphenamine

Experiment aim was to find out an effective dose of Chlorphenamine-an antagonist of histamine receptors. An effective dose will decrease the potency of histamine; however the same dose should not affect the Emax of histamine. Histamine when free of antagonist Emax was 100% while ED50 was 3. 5×10-6M. However histamine when added to Chlorphenamine 1/ 10000 dilutions an Emax became 96% and an ED50 became 1. 0×10-5M. This means that with the lesser dose of the antagonist the efficacy will be increased, while the potency will be decreased. The remarkable shift to the right confirms a decrease in potency with only a 4% variance in Emax. This is

evidence that the effective and selective dose has shifted histamines Log-dose response curve to the right thus decreasing potency.

Selective dose of Chlorphenamine

Acetylcholine was in two different concentrations of Atropine. Chlorphenamine on its part does not result in the shift of the graph to the right. Additionaly this shift does not result in the reduction of potency. In addition, there was a small shift of the curves to the left with the addition of Chlorphenamine which may be due to re-sensitization. On the other hand as there is no shift to the right of the dose response curves with the addition of Chlorphenamine. It will be possible to realize that it is not antagonist towards Acetylcholine

Effective and selective dose of Chlorphenamine on B2

The aim of experiment was to determine whether the effective and selective dose for Chlorphenamine was able to competitively antagonise the unknown drug B2. Results indicate three combinations; B2 only, B2with Chlorphenamine 1 in 10000 dilution, and B2with Chlorphenamine 1 in 5000 dilution. Consequently the display of Emax`s is 98%, 92%, 100% and respectively. The respective ED50 values are 2. 2 x10-6M, 1. 0×10-5M, and 7. 0×10-6M respectively. From the results it can be demonstrated that the effective and selective dose of Chlorphenamine did not have any considerable effect on the drug B2. This shows that B2 is not acting on the autocoid receptor H1.

Mimicry of B2 with other muscurinic agonists

Aims to study the mimicry effects of muscurinic agonists as wellas study parallel efficacy and potency of unknown drub B2. The mimicry data appears

to express that B2 mimics Carbachol as it has a similar ED50 value. In essence, B2 gave ED50 90% and Emax 1. 44 x 10^-6M while Carbachol was ED50 100% and Emax 1. 00 x 10^-6M. That saids it is not enough proof to ascertain that B2 acts on receptors. The cholinergic antagonist Atropine was used because whenever it decreased the ED50 of B2 at the effective and selective dose then this would confirm the B2 acted upon cholinergic receptors. An affirmative result proved that B2 acted upon cholinergic receptors as the ED50 increased and the potency decreased. In order to confirm this, Chlorphenamine was used as a histaminergic antagonist. The effective and selective dose of Chlorphenamine had no antagonistic effect on B; it failed to reduce the potency. It means that B2 does not act upon histaminergic receptors. Thus it can be concluded that drug B2 acts upon cholinergic receptors

Digestion by pseudo-cholinesterase/protection by physostigmine

The effects of Acetylcholine explain that when presented alone a 100% response is guaranteed.. In another perspective, Acetylcholine by pseudo-esterase gave a 0% response. However with the addition of Acetycholine, esterase, and physostigmine 95% response was acquired. Basically it means that Acetycholine is prone to the digestion by Pseudo-Cholinesterase obtained from the horse’s blood. Additionally it is protected from being digested by physostigmine. When carbachol was treated with both blood esterase and physostigmine each every response was almost identical yielding only a 10% discrepancy. Evidently is not in any way capable of being digested into blood esterase. As a result, physostigmine is not suitable to be used to block the digestive effects of the esterase.

Other results (from graphs 1. 5, 1. 6 and 1. 7) indicate that B2 was indeed broken down by blood esterase suggesting that it is potentially digestible by the former. Indeed if blood esterase were to be added to drug B2 alone, esterase would immediately digest drug B2 reducing its reaction to approximately 0%. However when an indirect agonist such as physostigmine is used, drug B2 is capable will be able to produce a significant. One thing to note is that the blood esterase virtually broke down all of drug B2. Relevant indications appear to reveal that the concentration of drug B2 is slightly low. This explains the minimal response of drug B2 to blood esterase.

Another substance that was broken down and digested by blood esterase was acetylcholine. Additionally, physostigmine effectively inhibited the effects of the blood esterase on both acetylcholine and drug B2. This result helps to explain the mimicry phenomenon; drug B2 mimics the procedures of acetycholine as well as acting upon the cholinergic receptors.

By studying graph 1. 8, there is revelation of the effects of histamine when treated with both blood esterase and physostigmine. From the data available it is evident that all four responses appear to be quite identical with only a 5-10% discrepancy. Graph 1. 7, reveals that blood esterase does not digest histamine. This means that histamine would need physostigmine in order to block the digestive effects of the esterase.

Potentiation

From graph 1. 9 it appears that physostigmine is acting as an indirect agonist towards Acetylcholine. This is because there is an obvious potentiation; the Emax leaped from 86. 2% to 100% while at the same time

the ED50 increased slightly with a shift left from 1. 3×10^-7M to 3. 0×10^-7M

In graph 2. 0 there is no potentiation of Emax or ED50. This helps to explain the fact that physostigmine does not work as an indirect agonist towards Carbachol. In essence the Emax for both trails are almost identical the same as for ED50 which runs very close

Physostigmine raises thee Emax but fails to lower the ED50. It is manifested by the fact that physostigmine acts as an indirect agonistThere is increased level of Emax to B2 mainly due to re-sensitisation occuring through-out the experiment as well as biological variance of the tissue

Summary

The antagonist Atropine appears to act on the unknown B2 drug which is associated with bringing about competition for inhibition factors. The same case applies to B2 cholinergic agonist. In another analysis Chlorphenamine appears to lack proper antagonistic effect on B2. Again B2 fails to directly act on autocoid H1 receptors. From these findings it is evident that B2 is a Cholinergic Agonist. The fact that carabcol and histamine were not digested in blood esterase while acetylcholine got digested means that B2 is indded acetylcholine. The two attributes provides some of the unique agonistic properties of a pharmacological drugs.