

COMPARISON OF THE EFFECT OF SYMPATHETIC AGONIST AND ANTAGONIST ON RABBITS PERFUSED HEART: A STUDY ON SIMPLE AND SUCCESSED DILUTIONS

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Background: The pharmacological effect of Adrenaline and Atenolol dilutions/succussion is still unexplainable for their reverse effect on tissues. This effect of potentization is observed in the recent study with differences in the effect of simple and succussed dilutions on heart rate. **Methods:** For this purpose, both simple and succussed dilutions of Adrenaline & Atenolol were prepared serially, ranging from 10^{-3} to 10^{-36} for testing on the isolated perfused Rabbit's heart. Langendorff heart assembly was used to perfuse the heart and its activity was recorded on Oscillograph through isotonic transducer. **Results:** The significant difference between the effects of simple and succussed dilutions of Adrenaline at 10^{-3} and 10^{-4} and for Atenolol 10^{-5} , 10^{-11} , 10^{-27} , 10^{-30} , 10^{-33} and 10^{-36} was observed on heart rate respectively. **Conclusion:** This study confirms that there are differences in the effects of simple & succussed dilutions. While, potentization or reverse effect observed than normal has been found in-consistently throughout the range of dilutions used. Thus in-consistency expresses the instability of change in parent drug molecule on succussion.

Key words: potentization, succussion, Adrenaline, Atenolol, heart rate, Langendorff, isolated heart.

INTRODUCTION

Physiological effects of sympathomimetic agents are well established and available through out the literature, even their mechanism of action with respect to direct & indirect effect¹ through membrane receptor and cytoplasmic substrate/messenger is also known. It has been observed that different dilutions of these substances have been used in Physiological & Pharmacological studies. In this connection method of dilution is also important. In the present study the method of serial dilution used in Homeopathy is employed to test Physiological response of isolated heart. The purpose of this study is to obtain a relation of various dilutions of Adrenaline with its effect on Heart Rate (HR) along with the use of its antagonist, Atenolol.

Adrenaline produces strong positive inotropic and chronotropic action on heart. It has also been reported that, adrenoceptors agonist increases the Ca^{++} transient amplitude to exert positive inotropic effect, as reported in rat myocytes.² It has been also reported that, the effects of β -Adrenergic stimulation are due to increased Na^+K^+ pump activity, the K^+ current³ & long lasting inward Ca^{++} current.⁴ This long lasting Ca^{++} current is thereby responsible for increased rate of diastolic depolarization & concomitant increase in the HR.^{4,5}

On the other hand, chronic blocker of stimulatory β -Adrenergic receptors, i.e, Atenolol leads to a coordinate trans-regulation of inhibitory receptors and Gi-proteins.⁶ It slows down the strength of heart's contractions & reduces its oxygen requirements.⁷

The use of drug dilution is not new and number of workers have tested various drug dilution for bio-assay on animal tissue, even beyond Avagadro's number, 6.24×10^{-24} .⁸⁻¹²

In addition omeopathic procedure of dilution not only responsible to potentize (succussed) the drug dilution but also responsible to produce reverse effect (opposite from the normal effect of the original drug). A number of scientists have reported potentized and reverse (opposite to normal) effects on biological tissues. These are, Isolated ears of Rabbits;¹¹ metamorphosis of tadpoles;⁹ Guinea Pig atria;¹³ frog heart;⁸ isolated Gastrocnemius;¹² plants growth;¹⁴ growth of Yeast;¹⁵ plant growth;¹⁶ isolated intestine;¹⁷⁻¹⁹ isolated heart.^{20,21}

In the light of the above mentioned facts the present study has been carried out on Rabbit's perfused heart to observe the difference in the effect of simple & succussed Adrenaline dilutions (10^{-3} to 10^{-36}) in the presence of its antagonist Atenolol including the potentized & reverse effects on heart rate.

MATERIAL AND METHODS

Rabbits (*Oryctolagus cuniculus*) of either sex ranging from 1.0-1.5 Kg were used for experiments. These experimental animals were killed by decapitation for immediate isolation of heart. The isolated heart was mounted and perfused through aorta by using Langendorff assembly. The perfusate was oxygenated Krebs's Henseleit Buffer having pH regulated at 37°C. The perfusion was carried out through the aorta at a constant pressure. The composition of oxygenated Krebs's Henseleit Buffer

was 118mM; NaCl, 4.70 mM; KCl, 1.25 mM; CaCl₂, 1.20 mM; MgSO₄, 25 mM; NaHCO₃ & 11 mM; Glucose.

In all the experiments, simple dilutions & succussed dilutions of Adrenaline & Atenolol were prepared by dissolving 0.01gm of drug in 10ml distilled/de-ionized water. This dilution (10⁻³) contains 5.5mM of original drug, from which further dilutions were prepared upto 10⁻³⁶ dilution; this last dilution may contains 5.5 * 10⁻³³ mM of Ad & Ate i.e., above Avagadro's number. For the preparation of SUD the diluted drugs from 10⁻³ to 10⁻³⁶ were shaken for two hours in a mechanical shaker.

The isolated heart was perfused through aorta for coronary perfusion and its ventricular activity was recorded through isotonic transducer on Oscillograph (Harvard apparatus Ltd. UK, Cat no. 50-8622). The records obtained before and after the infusion of 1 ml of the dilutions (simple and succussed) were later used to measure the Heart Rate.

All calculated values of various parameters were then calculated for their average and standard error. The statistical comparison was however made between the effect of simple and succussed dilutions by using students t-test at a significance level of 0.05.

RESULTS

EFFECT OF ADRENALINE ON HEART RATE:

Simple dilution of Adrenaline (10⁻³ to 10⁻³⁶) have been found to increase the heart rate at lower most dilutions, i.e., 10⁻³ to 10⁻⁶ only (Table 1). This increase was found maximum, i.e., 27% higher than control at 10⁻⁴. However, SD at 10⁻⁷ to 10⁻³⁶ have been found to decrease this rate that represents a reverse effect. This decrease (reverse effect) was found maximum, i.e., 8% lower than control at 10⁻³⁶. Similarly, the succussed of Adrenaline (10⁻³ to 10⁻³⁶) have also been found to increase the heart rate at 10⁻⁴ & 10⁻⁵ only. This increase was found maximum at 10⁻⁴, i.e., 11% higher than control (Table 1). However, Adrenaline dilutions at 10⁻³ & 10⁻⁶ to 10⁻³⁶ have demonstrated a reduction in the heart rate that was the reverse effect of Adrenaline on this parameter. This reverse effect was found maximum at 10⁻²⁷ Adrenaline dilution being 10% higher than control. Statistical comparison between the effects of simple & succussed dilution of Adrenaline on heart rate demonstrates significant difference at 10⁻³ (P<0.005) & 10⁻⁴ (P<0.01) only (Table 1).

Table 1: Statistical comparison between the effect of various simple & succussed dilutions of Adrenaline on the average values of Heart rate.

Dilutions	Simple Dilutions	Succussed Dilutions	Significance Level
10 ⁻³	114.18 + 4.42 (26)	*97.64 + 3.87 (18)	P<0.005
10 ⁻⁴	127.72 + 5.96 (30)	111.41 + 3.47 (18)	P<0.01
10 ⁻⁵	105.68 + 1.54 (13)	104.72 + 5.16 (15)	P>0.05
10 ⁻⁶	103.61 + 3.21 (14)	*99.29 + 1.32 (13)	P>0.05
10 ⁻⁷	*99.01 + 1.67 (8)	*99.00 + 1.92 (10)	P>0.05
10 ⁻⁸	*93.99 + 4.56 (9)	*93.76 + 2.58 (11)	P>0.05
10 ⁻⁹	*96.29 + 1.65 (18)	*96.77 + 0.82 (13)	P>0.05
10 ⁻¹⁰	*97.89 + 0.98 (11)	*98.27 + 1.48 (10)	P>0.05
10 ⁻¹¹	*96.68 + 1.97 (17)	*98.55 + 0.74 (19)	P>0.05
10 ⁻¹²	*96.65 + 1.83 (19)	*96.39 + 1.89 (13)	P>0.05
10 ⁻¹⁵	*96.51 + 0.98 (21)	*93.36 + 2.67 (19)	P>0.05
10 ⁻¹⁸	*94.27 + 2.63 (14)	*92.53 + 3.52 (13)	P>0.05
10 ⁻²¹	*95.83 + 2.62 (12)	*96.05 + 0.53 (13)	P>0.05
10 ⁻²⁴	*98.19 + 1.55 (17)	*97.64 + 1.08 (14)	P>0.05
10 ⁻²⁷	*97.48 + 2.05 (16)	*90.21 + 4.79 (18)	P>0.05
10 ⁻³⁰	*97.91 + 2.71 (14)	*93.56 + 3.30 (14)	P>0.05
10 ⁻³³	*96.52 + 3.19 (13)	*98.35 + 3.85 (12)	P>0.05
10 ⁻³⁶	*92.36 + 4.12 (16)	*94.42 + 1.78 (13)	P>0.05

* Reverse effect of Adr. **Significant difference**

All values are presented as mean + standard error.

While figure in parenthesis represents the number of observations

Table 2: Statistical comparison between the effect of various simple & succussed dilutions of Adrenaline in the presence of Atenolol on the average values of Heart rate.

Dilutions	Simple Dilutions	Succussed Dilutions	Significance Level
10^{-3}	*94.65 + 2.49 (28)	*99.20 + 4.62 (18)	P>0.05
10^{-4}	**104.27 + 3.24 (30)	**105.06 + 4.06 (13)	P>0.05
10^{-5}	**106.82 + 3.73 (30)	*90.83 + 3.13 (11)	P<0.005
10^{-6}	*99.03 + 1.27 (11)	*94.76 + 3.59 (14)	P>0.05
10^{-7}	*95.98 + 1.19 (18)	*98.95 + 2.67 (9)	P>0.05
10^{-8}	**100.84 + 3.03 (13)	**104.99 + 5.49 (10)	P>0.05
10^{-9}	**103.58 + 5.18 (16)	*94.79 + 1.67 (10)	P>0.05
10^{-10}	*97.62 + 1.23 (20)	*92.00 + 5.82 (10)	P>0.05
10^{-11}	**105.31 + 6.16 (15)	*91.54 + 1.92 (13)	P<0.025
10^{-12}	*96.95 + 3.10 (20)	*94.71 + 2.34 (16)	P>0.05
10^{-15}	*89.32 + 1.40 (10)	*92.22 + 1.41 (10)	P>0.05
10^{-18}	*99.46 + 1.34 (10)	*99.00 + 0.96 (10)	P>0.05
10^{-21}	*96.72 + 1.08 (10)	*95.55 + 2.69 (10)	P>0.05
10^{-24}	*94.51 + 2.03 (10)	*95.23 + 0.88 (12)	P>0.05
10^{-27}	*85.40 + 2.57 (10)	*67.31 + 8.21 (10)	P<0.025
10^{-30}	*89.16 + 1.47 (10)	*79.39 + 2.07 (12)	P<0.0005
10^{-33}	*83.74 + 3.25 (10)	*93.49 + 2.03 (19)	P<0.01
10^{-36}	*81.52 + 4.64 (17)	*93.17 + 4.48 (10)	P<0.05

* Reverse effect of Adr ** Reverse effect of Ate **Significant difference**

All values are presented as mean + standard error.

While figure in parenthesis represents the number of observations

EFFECT OF ADRENALINE IN THE PRESENCE OF ATENOLOL ON HEART RATE:

Simple dilution of Adrenaline (10^{-3} to 10^{-36}) used after the administration of their respective dilutions of the antagonist Atenolol (Ate) have been found to decrease the heart rate at most of the dilutions except at 10^{-4} , 10^{-5} , 10^{-8} , 10^{-9} & 10^{-11} . This decrease was found maximum, i.e., 19% lower than control at 10^{-36} being the reverse (opposite to the normal) effect of Adrenaline. While, at 10^{-4} , 10^{-5} , 10^{-8} , 10^{-9} & 10^{-11} simple dilution, the heart rate was increased, represents the reverse effect of Atenolol. Thus, indicating that Atenolol did not antagonize the effect of Adrenaline at these dilutions. This reverse effect of Ate was found maximum, i.e., 6% higher than control at 10^{-5} , as shown in (Table 2).

Succussed of Adrenaline (10^{-3} to 10^{-36}) after the administration of its antagonist Atenolol has also been found to decrease the rate at all dilutions except 10^{-4} & 10^{-8} . This decrease was found maximum i.e., 33% lower than control at 10^{-27} , thus representing the reverse effect of Adrenaline. However, dilutions of 10^{-4} & 10^{-8} were not antagonized by Atenolol and thus an increase in the heart rate was found, representing reverse or opposite effect of Atenolol. This reverse effect was found maximum by 5% higher than control at 10^{-4} (Table 2).

Statistical comparison between the effect of simple & succussed dilutions of Adrenaline after the administration of its antagonist Atenolol demonstrated the significant difference in the heart rate at 10^{-5} (P<0.005), 10^{-11} (P<0.025), 10^{-27} (P<0.005), 10^{-30} (P<0.0005), 10^{-33} (P<0.01) & 10^{-36} (P<0.05).

DISCUSSION

EFFECT OF ADRENALINE ON HEART RATE:

At lower simple dilutions (10^{-3} to 10^{-6}) & succussed dilutions (10^{-4} & 10^{-5}) of Adrenaline, positive chronotropic effect was observed, that was the normal effect of Adrenaline. It indicates that in these dilutions the original drug molecule is retained. However, at 10^{-3} (succussed dilution) an unexpected 3% lesser heart rate was observed than control. While, from 10^{-7} to 10^{-36} dilutions (simple & succussed) the negative chronotropic effect of Adrenaline was observed.

It is the reverse effect (opposite to normal) of Adrenaline. It is therefore clear that the serial dilution is also responsible for drug water interaction in addition to succussion that may cause molecular changes in drug.²¹⁻²³

The succussed dilutions of Adrenaline have shown specific succussed effects by decreasing them from normal effects than their respective simple dilutions. It demonstrates that the molecules in these

dilutions have undergone changes due to succussion. It has been reported that in high-energy collision, particles may be created or destroyed.²⁴ Similarly in the process of homeopathic potentization the frictional electricity or electrostatic charge has been reported to generate, when a liquid is flown over a solid surface.²⁵

EFFECT OF ADRENALINE IN THE PRESENCE OF ATENOLOL ON HEART RATE:

In the present study, at most of the simple & succussed dilutions of Adrenaline after the administration of Atenolol, lower than control values of heart rate were observed. In the presence of Atenolol, maximum decrease in heart rate was observed (i.e., 33% lower than control values) at 10⁻²⁷ (succussed dilution) and 19% lower than control values at 10⁻³⁶ (simple dilution). This decreasing effect of both dilutions on heart rate represents the reverse effect of Adrenaline. Maximum decrease in heart rate at higher simple & (beyond Avagadro's number) represents specific activity that can be produced by the dilutions even when the original molecule had been diluted until it no longer existed.²⁶ It has been reported that β₂-adrenergic agonist, Salbutamol causes increase in heart rate that is blocked by non-selective adrenoceptor antagonist (Propranolol).²⁷ On the other hand, higher than control values of heart rate observed in this series of experiments, at various lower simple & succussed dilutions (ranging from 10⁻⁴ to 10⁻¹¹), represents the reverse effect of Atenolol. This reverse effect of Atenolol is however, non-significant (not more than 6%) indicating that Atenolol instead of producing antagonistic effect, the β-adrenergic agonist Adrenaline was allowed to produce normal effect & thus increasing the heart rate.

Statistical comparison between the effects of simple & succussed dilutions of Adrenaline in the presence of Atenolol has been found significant mostly at higher dilutions, i.e., beyond Avagadro's number. It has been observed & discussed that variations in the antagonistic effect exerted by these dilutions might be responsible for the inconsistent effects produced by both, simple & succussed dilution.

The power or strength of succussion has been demonstrated by observing its dynamic properties at higher dilutions. Therefore, many researchers worked on higher potencies to find out the margin of the potent action of succussion. Madam Kolisko (1926) has demonstrated that plant growth occur up to 60th decimal (10⁻⁶⁰) potency and even beyond to the 200th decimal (10⁻²⁰⁰) potency.²⁸ Further, there is no any reported evidence about the

number of succussion required to complete the information transfer from one dilution to the next.

CONCLUSION

Both simple & succussed dilutions reported to have differences in terms of their physical nature & the present study confirms these differences in their normal, potentized & reverse effects on cardiac tissues. Thus, a possibility for the presence of reprints of original or parent drug molecule at high dilutions (simple & succussed), cannot be ignored. While the potentization & reverse effects of both simple & succussed dilutions are not consistently observed, indicates the instability of reprints of the parent drug molecule.

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