



Effect of Ceftriaxone on Isolated Smooth, Cardiac Muscles and Neuromuscular Junctions

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Abstract

The pharmacodynamic effect of ceftriaxone on smooth muscles was investigated in isolated organs. Maximum stimulation of isolated guinea pig's ileum, rabbit's duodenum and rat's fundic strip was achieved by addition of 1024 µg of ceftriaxone/ml bath. While in isolated rat's colon, it was achieved by 512 µg of ceftriaxone/ml bath. The effect of graded increased concentrations of ceftriaxone on isolated rat's uterine muscles was examined during various stages of sex cycle. Ceftriaxone in the tested concentrations produced a dose-dependant negative inotropic effect on isolated rabbit's heart and guinea pig's auricles. Ceftriaxone in all tested concentrations did not induce any effects on the resting tonus of isolated guinea pig's tracheal chain and rabbit's aortic strip. Neuromuscular blockade effect was investigated on isolated frog's gastrocnemius muscle and frog's rectus abdominis muscle preparation. It was concluded that, ceftriaxone directly stimulates the smooth muscles of gastrointestinal tract and depresses those of uterus as well as cardiac muscles. These findings indicated that ceftriaxone had scarcely any pharmacological properties which might be leading to severe adverse reactions in clinical use.

Key words: Ceftriaxone, Smooth muscles, Cardiac muscles, Neuromuscular junctions.

Introduction

Ceftriaxone is a broad spectrum cephalosporin resistant to various types of beta-lactamases, with potent activity against gram-positive and gram-negative bacteria, including *Enterobacteriaceae*, *Haemophilus influenzae*, *Streptococcus pneumoniae* and other



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nonenterococcal streptococci, *Methicillin-resistant staphylococci*, *Enterococci*, *Pseudomonas aeruginosa* and *Bacteroides fragilis* were typically resistant ¹. The drug acts through inhibition of transpeptidase enzymes responsible for the final step in bacterial cell wall synthesis and has broad stability against beta-hydrolysis ². In human medicine, ceftriaxone is widely used, because of its prolonged terminal half-life (5.4– 8.2 h) that allows its prescription on a single administration per day basis ^{3,4}. Expanded informations concerning the pharmacodynamic effects of ceftriaxone will be of benefits to physicians and their patients. Therefore, the purpose of this study was to investigate the pharmacodynamic effects of ceftriaxone on smooth, cardiac and skeletal muscles.

Material and Methods

2.1. Materials:

2.1.1 Drug

Ceftriaxone is a sterile, semisynthetic, broad-spectrum third generation cephalosporin antibiotic for intravenous or intramuscular administration. Ceftriaxone is a white to yellowish-orange crystalline powder which is readily soluble in water, sparingly soluble in methanol and very slightly soluble in ethanol. It was produced by Smithkline Beecham for Novartis Pharma Company (Egypt) and has the commercial name Ceftriaxone®.

2.1.2. Laboratory animals

Guinea pigs of both sexes and different weights (300-450 gm) were used for investigating the effect of ceftriaxone on the isolated ileum, auricles and tracheal strips. Rabbits of both sexes and different weights (1500-2000 kg) were used for studying the effect of ceftriaxone on isolated duodenum, heart and aortic strip. Rats of both sexes and different weights (150-220 gm) were used for studying the effects of ceftriaxone on isolated colon, fundic strip and uterine muscles in different stages of sex cycle. Egyptian toads were used for studying the effect of ceftriaxone on isolated rectus abdominis muscle and sciatic nerve gastrocnemius muscle preparations.

2.1.3. Devices

2.1.3.1. Glass jar bath

A glass water bath of about 750 ml capacity fitted into a metal stand in which a movable electric heater was located to maintain the temperature as required. An inner glass tube (organ bath) of 40 ml capacity passed through the bottom of the stand and was connected by a T-shaped glass tube.



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2.1.3.2. *Harvard universal oscillographe and transducers*

Two channels curvilinear oscillograph (HARVARD U.K) with an isotonic transducer (HARVARD APP LTD) which was employed for recording the effect of ceftriaxone on isolated tissues.

2.2. Methods:

The method explained by ⁵ was used for studying the effect of ceftriaxone on the isolated ileum of guinea pigs. The method described by ⁶ was used for studying the effect of ceftriaxone on isolated rabbit's duodenum, rat's colon and uterine muscle of rats at various stages of sex cycle. The effect of ceftriaxone on isolated rat's fundic strip was investigated according to the method described by ⁷. The method described by ⁸ was used for studying the effect of ceftriaxone on isolated guinea pig tracheal smooth muscle using the glass jar bath apparatus. The glass jar bath was used as described by ⁹ for studying the effect of ceftriaxone on isolated guinea pig's auricles. The method explained by ¹⁰ using Gunn's apparatus (heart infusion assembly) was used for studying the effect of ceftriaxone on rabbit's heart. The method explained by ¹¹ was used for studying the effect of ceftriaxone on rabbit's aortic strip. The method described by ¹² was used for investigating the effect of ceftriaxone on frog's gastrocnemius muscle-sciatic nerve preparation. The effect of ceftriaxone on the isolated frog's rectus abdominis muscle was investigated by using the method described by the ⁶.

Results

The effect of ceftriaxone on isolated guinea pig's ileum, rabbit's duodenum, rat's colon and rat's fundic strip and guinea pig's tracheal chain was recorded in **table (1)**. The effect of ceftriaxone on uterine motility of female rats at various stages of sex cycle was recorded in **table (2)**. Trials were performed to locate the site of action of ceftriaxone on the gastrointestinal motility and the results showed that, ceftriaxone had a direct intestinal smooth muscles stimulant effect and had a serotonin like effect on rat's fundic strip (**Figure 1**). Ceftriaxone depressed the uterine motility at various stages of sex cycle and these effects might be attributed to the direct effect of ceftriaxone as shown in **figure (2A & 2B)**. The effect of gradual increased concentrations of ceftriaxone on isolated guinea pig's auricles, rabbit's heart and aortic strip were demonstrated in **table (3)**. Ceftriaxone depressed the isolated guinea pig's auricles and rabbit's heart and this negative inotropic effect of ceftriaxone was not referred to either β_1 adrenergic blocking effect or cholinergic stimulant effect due to, in presence of ceftriaxone (1024 $\mu\text{g/ml}$ canula), adrenaline was able to produce its cardiac stimulatory effect and in presence of atropine sulphate, ceftriaxone (1024 $\mu\text{g/ml}$



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canula) was able to produce its inhibitory effect (**Figure 2C & 2D**). The effect of ceftriaxone on skeletal muscle preparations was summarized in **table (4)**. The neuromuscular blockade effect of ceftriaxone on isolated frog's gastrocnemius muscle was shown in **figure (2E)**.

Discussion

1- Effect of ceftriaxone on isolated gastrointestinal, tracheal and uterine smooth muscles:

The present investigation showed that, ceftriaxone *in vitro* stimulated the contractility of guinea pig's ileum, rat's colon and rabbit's duodenum. The stimulatory effect of ceftriaxone was proportional to the graded tested concentrations. These results proved that, ceftriaxone might directly stimulate the intestinal smooth muscles of rabbit's duodenum, guinea pig's ileum and rat's colon. These obtained results were similar to those obtained by ¹³ who found that; cefepiraxone *in vitro* enhanced slightly the motility of isolated rabbit's gastrointestinal tract at 0.001 g/ml. Also spontaneous motility of smooth muscle was temporarily increased with 800 mg/kg cefminox when administered intravenously and in upper doses ¹⁴. In contrast, cefamandole at concentrations of 512 and 1024 micrograms/ml bath caused complete relaxation in isolated guinea pig's ileum and rabbit's duodenum, respectively ¹⁵. Ceftriaxone stimulated contractility of the rat's fundic strip. Ceftriaxone in a high concentration produce a serotonin like effect on rat's fundic strip (a sensitive preparation for detection of serotonin). These results might be attributed to the ability of ceftriaxone to release serotonin from its stores. The serotonin stimulating effect of ceftriaxone overcame its direct effect on the smooth muscle of rat's fundic strip. The obtained results came in harmony with those obtained by ¹⁶ who recorded that, cefotaxime, ceftriaxone and ceftazidime produced concentration-dependent tonic contractions of rat's fundus. Also, cefamandole produced stimulation of rat's fundic strip ¹⁵.

Ceftriaxone *in vitro* inhibited the contractility of rat's uterus during non pregnant stages (estrus and non estrus) and during pregnant stages (early and late pregnancy). The effect was dose dependant and these obtained results were consistent with those recorded by ¹³ who found that, cefepiraxone depressed the uterine motility in two of six experiments while during pregnancy they found that, cefoperaxone might not be affected or depressed and / or stimulated the uterine motility. In other observation, cefepime had no effect on the delivery status of the offspring rats ¹⁷. The obtained results were not consistent with those obtained by ¹⁵ who recorded that, concentrations of 2048 and 4096 micrograms cefamandole/ml bath caused marked stimulation in force and frequency of rat uterine muscle in all stages of sex cycle. These differences were explained by ¹⁶ who proved that, effects of beta-lactam



antibiotics on smooth muscle isolated preparations were tissue and species dependent, indicating selectivity of their action.

The guinea pig's tracheal smooth muscles seemed to be insensitive to the tested concentrations of ceftriaxone. In presence of ceftriaxone, histamine was not able to produce its stimulatory effect. The obtained results in this study was similar with those obtained by ^{18,15} they recorded that, cefprozil and cefamandole respectively in different graded concentrations had no effect on the tracheal smooth muscles. On the other hand, ceftizoxime and cefminox relaxed the resting tonus of the isolated guinea pig's tracheal chain preparation ^{19,14}. Cefoperazone and cefteteram pivoxil respectively caused slight stimulation of the isolated guinea pig's tracheal smooth muscles ^{13,20}.

2- Effect of ceftriaxone on isolated cardiovascular muscles: Ceftriaxone had a negative inotropic effect on the isolated guinea pig's auricles and rabbit's heart. Ceftriaxone produced a direct and dose dependant depression of the myocardial contractility. This negative inotropic effect of ceftriaxone was not referred to either β_1 adrenergic blocking effect or cholinergic stimulant effect, as adrenaline was able to produce its cardiac stimulatory effect in presence of ceftriaxone and after addition of atropine sulphate, ceftriaxone was able to produce its inhibitory effect.

Contraction of the cardiac cells is believed to be dependant upon the intracellular concentration of available calcium ions in the vicinity of the contractile apparatus ²¹ so the direct myocardial depressant effect of ceftriaxone in the present work might be attributed to a modification of calcium function.

The negative inotropic effect of ceftriaxone on guinea pig's auricles and rabbit's heart in the present work was similar to that result obtained by ¹⁵ who recorded that, cefamandole directly depressed the contractility of isolated guinea pig's auricles and rabbit's heart in a dose dependant manner. This negative inotropic effect of ceftriaxone on guinea pig's auricles in the present work was not consistent with that result obtained by ^{22,19} they stated that, cefadroxil and ceftizoxime sodium respectively had no effects on the isolated hearts of rabbits and the spontaneous movement in isolated guinea pig's atrium. The obtained results were also inconsistent with those of ²³ who showed that, cefminox did not affect the spontaneous contraction of isolated guinea pig's atria and the blood vessels in perfused rabbit's ears.

It was observed that, ceftriaxone had no effect on the smooth muscle of aorta. In the presence of ceftriaxone, nor adrenaline was not able to produce its stimulatory effect, thus ceftriaxone appeared to cause an alpha adrenergic blocking effect on isolated rabbit's aortic strip. This result was consistent with that reported by ^{24,20,15} who stated that, cefbuperazone, cefteteram pivoxil and cefamandole respectively did not affect the rabbit's descending aorta and



the nor adrenaline and adrenaline fail to produce its stimulatory effect in the presence of these antibiotics. This was inconsistent with that recorded by ¹³ who found that; cefoperazone potentiated the pressor response to adrenaline in dogs.

3- Effect of ceftriaxone on the skeletal muscle preparations: The effect of ceftriaxone on skeletal muscle preparations (frog's gastrocnemius muscle sciatic nerve and frog's rectus abdominis muscle) was investigated. The ceftriaxone elicited a marked neuromuscular blocking activity in response to indirect muscle twitches; also ceftriaxone exhibited a local anaesthetic like activity on frog's gastrocnemius sciatic nerve preparation. The neuromuscular blocking activity of ceftriaxone on skeletal muscle preparations in the present work was similar to those obtained by ^{14,20} who found that, the twitch tension of gastrocnemius muscle evoked by electrical stimulation of sciatic nerve was slightly reduced following administration of cefminox and cefteram pivoxil respectively. Cefamandole had a neuromuscular blocking effect on isolated frog's gastrocnemius muscle and frog's rectus abdominis muscle ¹⁵. The obtained results were inconsistent with that reported by ¹³ who recorded that, cefoperazone enhanced slightly the twitch tension of musculus gastrocnemius induced by electrical stimulation in rats at 500 mg/kg. b.wt. Also, cefbuperazone had no effect on the neuromuscular junction ²⁴.

Conclusion: From the present study it could be concluded that, ceftriaxone directly stimulates the smooth muscles of gastrointestinal tract and depresses those of uterus as well as cardiac muscles. Ceftriaxone in all tested concentrations did not induce any effects on the resting tonus of isolated guinea pig's tracheal chain and rabbit's aortic strip. Ceftriaxone had a neuromuscular blocking activity on the skeletal muscle preparations.

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TABLES:-

Table (1): The effect of ceftriaxone on isolated guinea pig's ileum, rabbit's duodenum, rat's colon, rat's fundic strip and guinea pig's tracheal chain.



(-----) Not done

Concentrations ($\mu\text{g/ml}$ bath)	Responses of				
	Guinea pig's ileum	Rabbit's duodenum	Rat's colon	Rat's fundic strip	G. pig's Tracheal chain
8	No effect	No effect	No effect	No effect	No effect
16	No effect	Slight stimulation in the force	Slight stimulation in the force	No effect	No effect
32	Slight stimulation in the force	Slight stimulation in the force	Slight stimulation in the force	No effect	No effect
64	Slight stimulation in the force	Slight stimulation in the force	Slight stimulation in the force	Slight stimulation in the force	No effect
128	Slight stimulation in the force	Slight stimulation in the force	Marked inhibition in the force and rate of contraction	Slight stimulation in the force	No effect
256	Marked inhibition in the force and rate of contraction	Marked inhibition in the force and rate of contraction	Marked inhibition in the force and rate of contraction	Marked inhibition in the force and rate of contraction	No effect
512	Marked inhibition in the force and rate of contraction	Marked inhibition in the force and rate of contraction	Maximum stimulation	Marked inhibition in the force and rate of contraction	No effect
1024	Maximum stimulation	Maximum stimulation	-----	Maximum stimulation	No effect



Table (2): Effect of ceftriaxone on uterine motility of rats at various stages of sex cycle.

Concentrations ($\mu\text{g/ml}$ bath)	Response of uterine motility			
	Non estrus	Estrus	Early pregnant	Late pregnant
32	No effect	No effect	No effect	No effect
64	Slight inhibition in the force and frequency	Slight inhibition in the force and frequency	Slight inhibition in the force and frequency	No effect
128	Marked inhibition in the force and frequency	Marked inhibition in the force and frequency	Marked inhibition in the force and frequency	Moderate inhibition in the force and frequency
256	Complete relaxation	Complete relaxation	Complete relaxation	Complete relaxation

Table (3): The effect of ceftriaxone on isolated guinea pig's auricles, rabbit's heart and rabbit's aortic strip.

Concentrations ($\mu\text{g/ml}$ bath)	Responses of		
	Guinea pig's auricles	Rabbit's heart	Rabbit's aortic strip
128	No effect	No effect	No effect
256	Slight negative inotropic effect.	Slight negative inotropic effect.	No effect
512	Slight negative inotropic effect.	Slight negative inotropic effect.	No effect
1024	Marked negative inotropic effect.	Marked negative inotropic effect.	No effect
2048	Marked negative inotropic effect	Very marked negative inotropic effect	No effect



Table (4): The effect of ceftriaxone on skeletal muscle preparations

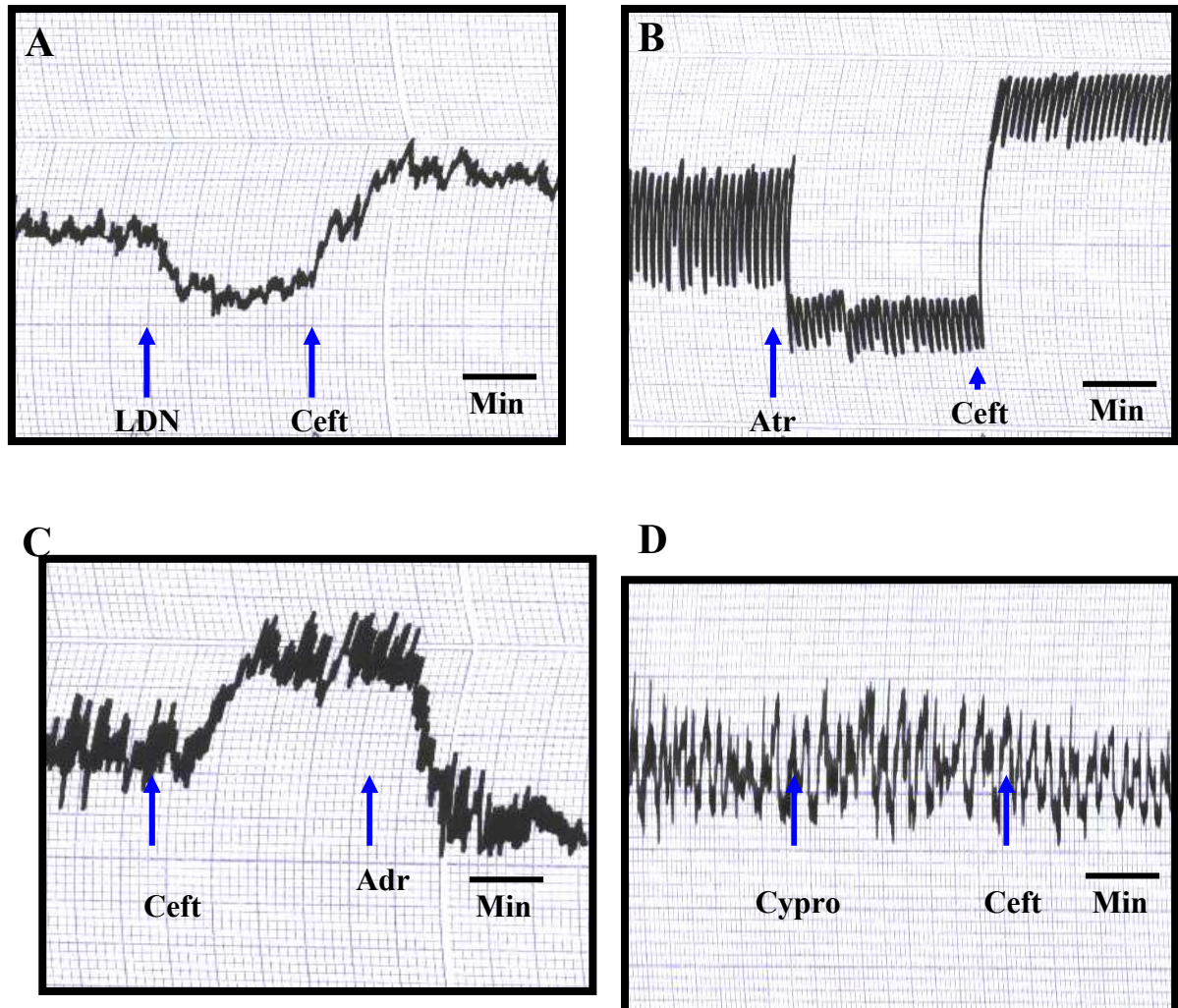
Concentrations ($\mu\text{g/ml}$ bath)	Responses of	
	Frog's gastrocnemius musc	Frog's rectus abdominis muscle
4	No effect	No effect
8	No effect	Slight neuromuscular blockade
16	No effect	Slight neuromuscular blockade
32	Slight neuromuscular blockade	Marked neuromuscular blockade
64	Slight neuromuscular blockade	Marked neuromuscular blockade
128	Marked neuromuscular blockade	Complete neuromuscular blockade
256	Marked neuromuscular blockade	-----

(-----) Not done



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Figures:





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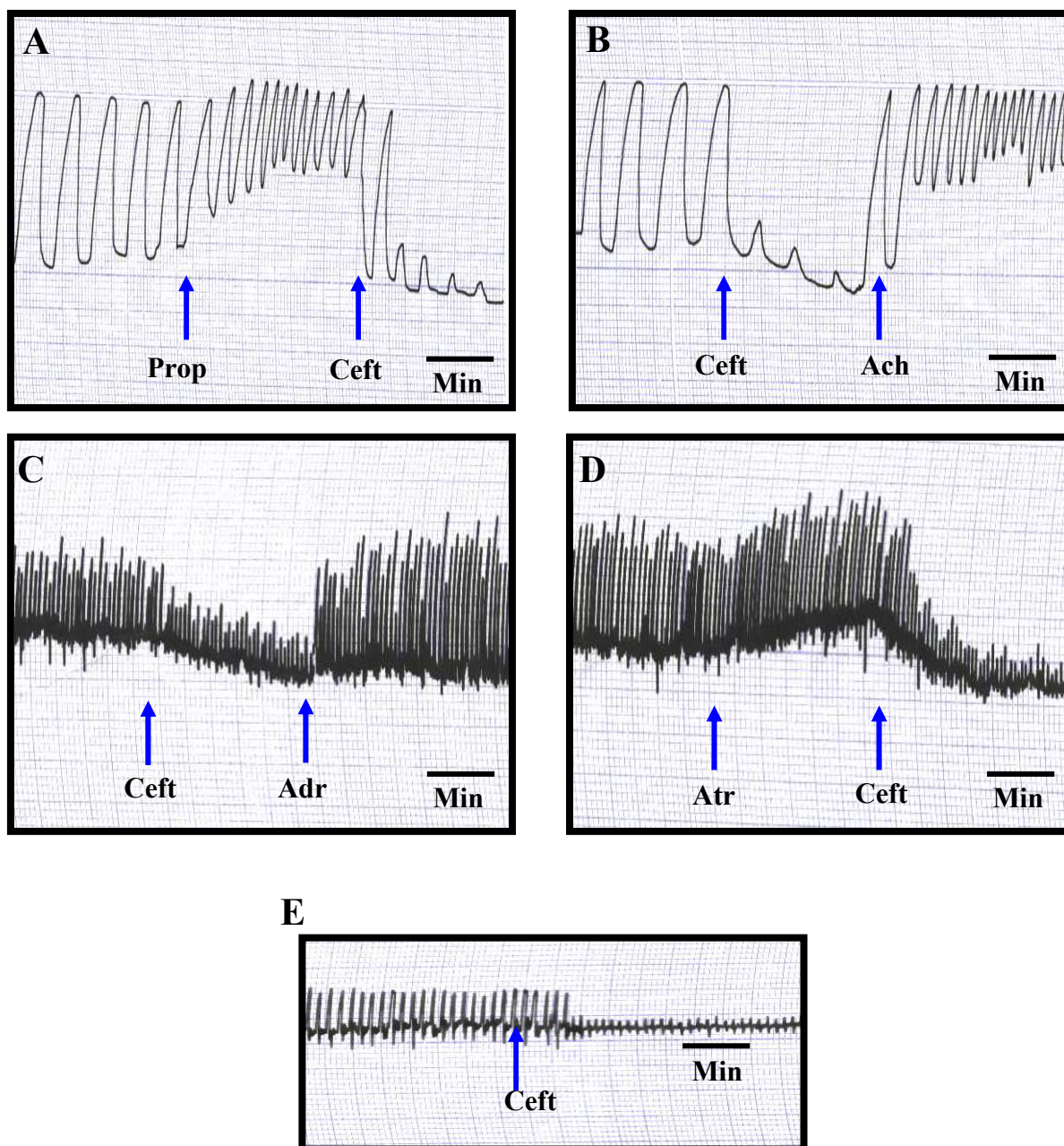


Figure (2): Site of action of ceftriaxone (Ceft.) on isolated rat's uterus during estrus stage and guinea pig's auricle.

- (A) 1 $\mu\text{g/ml}$ bath propranolol (Prop) followed by 256 $\mu\text{g/ml}$ bath ceftriaxone (Ceft) on isolated rat's uterus during estrus stage.
- (B) 256 $\mu\text{g/ml}$ bath ceftriaxone (Ceft) followed by 0.25 $\mu\text{g/ml}$ bath acetylcholine (Ach) on isolated rat's uterus during estrus stage.



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- (C) 1024 $\mu\text{g/ml}$ bath ceftriaxone (Ceft) followed by 1 $\mu\text{g/ml}$ bath adrenaline (Adr) on isolated guinea pig's auricle.
- (D) 0.1 $\mu\text{g/ml}$ bath atropine sulphate (Atr) followed by 1024 $\mu\text{g/ml}$ bath ceftriaxone (Ceft) on isolated guinea pig's auricle.
- (E) 256 $\mu\text{g/ml}$ bath ceftriaxone (Ceft) on isolated frog's gastrocnemius muscle.