The Effects of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) on the Spontaneously Beating Isolated Auricles of the Rabbit Heart

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Abstract

The effects of NSAIDs (aspirin and indomethacin) on the isolated auricles of the rabbit heart were studied by observing the effects of these drugs on automaticity, excitability and contractility of the auricles. Aspirin (25,50,100 uM) produced concentration dependent depression in the automaticity of the SA node while indomethacin (10 uM, 25 uM) has stimulant effects and 50 uM produced depressant action. Only low concentration of indomethacin (10 uM) produced significant effect. Aspirin (25,50,100 uM) and indomethacin (50 uM) have significant depressant effects on the excitability of the auricles. Aspirin (100 uM) has suppressant effects ($p<0.05$) on the normal contractility while the adrenaline stimulated contractility is not suppressed by aspirin and indomethacin significantly. The results obtained in this study with aspirin and Indomethacin on chronotropicity and inotropicity of the rabbit atria have been discussed in the light of experimental work done by the workers regarding conventional antiarrhythmic drugs (JPMA 44:216,1994).

Introduction

Cardiac muscle has the properties of automaticity, excitability and contractility. Sinoatrial node (SA node) is the pacemaker of heart. Adrenaline has stimulant effects on the SA node due to its action onadrenergic receptors. Sympathetic stimulation causes positive chmnotropic and inotropic effects due to ncreased calcium influx. Like conventional antiarrhythmic drugs salicylates have been shown to depress SA node[1]. The surface electrical potential of excitable tissues is affected by the salicylates[2,3]. This study was done to see the effects of acetylsalicylic acid (aspirin) and any one of the others nonsteroidal anti-inflammatory like indomethacin on the SA node.

Material and Methods

In this study rabbit auricle preparation was used, as described by “Pharmacological experiments on the isolated preparations by the staff of the department of Phannacology, University of Edinburg[4]. Rabbits of either sex weighing 1.5-2 kg were sacrificed by cutting the throat and chest opened and heart removed as quickly as possible and placed in Ringer-locke solution at room temperature. All tissue was cut away until only auricle was left. Thread was tied, one to the tip of each auricle and preparation was mounted inRinger4ocke solution at 37±1°C, through which a brisk stream of pure oxygen was blown. One thread was attached to a fixed pin in the bath and other to the lever of isotonic transducer of “Harvard Oscillograph”. The tracing was recorded on a graph paper moving with the speed of 10 mm/sec. Ten seconds recordings were taken for each event and calculated later on by multiplying this with six to get value per minute. Volume of organ bath was 50 mls. After the equilibration period of 15-20 minutes, cumulative concentration response curve was determined by the stepwise increase of concentration of adrenaline[5]. Solutions were prepared daily with distilled water. Adrenaline was diluted in distilled water. The solution used in organ bath was having the following composition (Gram/Litre),

NaCI 9; KCI 0.42; CaCl 0.24; NaHCO3 0.15; Glucose 1.0.
The parameters selected were chronotropic activity (beats/mm.) and inotropic effect (contractility). Following steps were followed for observation.
1. Initial tension of 0.5-1 grain for 5-10 minutes was applied.
2. Increased heart rate or arrhythmias were produced by adrenaline concentration.
3. After each cumulative concentration response, the tissue was washed consecutively three times. Adrenaline was appliedfor2 minutes and contractions recorded for 10 seconds on the graph paper moving at 10 mm/second and rate calculated/minute.
4. For the antagonistic effect of experimental drugs, a period contact time of 30 minutes was given following the 15 minutes period of rest.
5. Agonist (adrenaline) was added and cumulative dose response obtained in the presence of a constant concentration of antagonist.
6. For the inotropic effect (contractility), we measured the height of each contraction, one smallest square of the graph paper wasequalto 1mm. Aspirin and indomethacin were dissolved in 2% NaHCO3 solution and then used in concentrations of 25,50,100 and 10,25,50 uM respectively. Effect of vehicle was also recorded. In preliminary experiments, the doses of the nonsteroidal anti- inflammatory drugs were calculated and six constant observations were recorded for each drug.

Statistical Analysis

The results were estimated and expressed as X- ± S.E(X-= mean:S.E = Standard error). Treatments were estimated at 5% level of significance. Wherever necessary paired or unpaired student's 't' test was used.

Results

In the preliminary experiments the doses of drugs under study were found which caused maximum suppression of the adrenaline stimulated activity of the isolated auricles of the rabbit heart. All those animals which did not respond to aspirin (25-100 uM) and indomethacin (10-50 uM) were discarded from the study. Six experiments were done for each drug.

Effects on Automaticity

The control value of atrial rate was 130±4beats/min. for aspirin and 132±6 beats/min. for indomethacin in six experiments for each drug. Treatment with aspirin caused a concentration dependent depressant effect while indomethacin produced stimulator)' effect at lower conceniration(10 and 25 uM) and depressant effect at higher concentrations (Table Ia).

Effects of Drugs on Chronotropic Activity of the Spontaneously Beating Isolated Auricles of the Rabbit Heart

Table I(a). Effects of Drugs on the Normal Activity (automaticity).

	Control	Test	%Change	p Value
Aspirin				
25uM	130±4	60±8	-53.84	<0.001
50uM	130±4	70±14	-46.15	<0.01
100uM	130±4	96±6.1	-26.15	<0.001
Indomethacin				
10uM	132±6	154±15.53	+16.66	<0.001
25uM	132±6	159±15.47	+20.45	>0.05
50uM	132±6	127±18.82	-3.78	>0.05

Values shown are Mean±SEM (beats/minute) of six observations.

The percent inhibition produced by aspirin at different concentrations (25,50,100 uM) was 53.8%, 46.1% and 26.1% respectively. Indomethacin produced 16.6% and 20.4% increase in heart rate at the concentrations of 10 and 25 uM respectively while next higher concentration (50 uM) exhibited 3.7% depressant action.

Effects on Excitability

The control value of the a trial rate was 207±15.91 beats/lmin. for aspirin and 239±9.68 beats/mm. for indomethacin in six experiments for each drug. Treatment with concentrations of aspirin (25,50,100 uM) caused depressant effects on the atrial rate while indomethacin produced slight stimulant effect at low concentration (10 uM) and depressant action at higher concentrations (25,50 uM) (Table Ib).

Table I(b). Effects of Drugs on the Maximal Activity Produced with Adrenaline (excitability).

	Control	Test	%Change	p Value
Aspirin				
25uM	207±15.91	130±9.41	-37.19	<0.01
50uM	207±15.91	135±22.41	-34.78	<0.001
100uM	207±15.91	135±37.63	-34.78	<0.05
Indomethacin				
10uM	239±9.68	241±22.10	+0.83	>0.05
25uM	239±9.68	212±4.66	-11.29	>0.05
50uM	239±9.68	114±20.66	-52.30	<0.001

Values shown are Mean±SEM (beats/minute) of six observations

The percent inhibition produced with aspirin at three different concentrations (25,50,100 uM) was 37.1%, 34.7% and 34.7% respectively. Indomethacin produced 0.8% increase in the atnal rate with 10

uM concentration while next higher concentrations (25 and 50 uM) produced 11.2% and 52.3% inhibition respectively.
Effects on Contractility (Table II a and b).

Table II(a). Effects of Drugs on the Normal Activity (Contracitility).

	Control	Test	%Change	p Value
Aspirin				
25uM	1.75	1.75	0	-
50uM	1.75	1.08±0.22	-38.28	>0.05
100uM	1.75	1±0.50	-42.88	<0.01
Indomethacin				
10uM	3	2.33±0.59	-22.33	>0.05
25uM	3	2.16±0.42	-28.00	>0.05
50uM	3	2.75±0.56	-8.33	>0.05

Values shown are Mean±SEM (mms) of six observations.

Table II(b). Effects of Drugs on the Adrenaline-Stimulated Maximal Activity (Contractility).

	Control	Test	%Change	p Value
Aspirin				
25uM	5.04±1.36	2.62±0.42	-48.01	>0.05
50uM	5.04±1.36	2.58±0.45	-48.80	>0.05
100uM	5.04±1.36	2.27±0.63	-54.96	>0.05
Indomethacin				
10uM	3.58±0.70	3.58±0.70	-	-
25uM	3.58±0.70	4.12±0.75	+15.08	>0.05
50uM	3.58±0.70	2.93±0.65	-18.15	>0.05

Values shown are Mean±SEM (mms) of six observations.

The control values for the normal contractility of spontaneously beating isolated auricles were 1.75 mms and 3 mms for aspirin and indomethacin respectively in six experiments for each drug. Treatment with aspirin caused a concentration dependent depression and all three concentrations of indomethacin also showed depressant effects. Lowest concentration of aspirin (25 uM) produced no change while percent inhibition caused by 50 and 100 uM aspirin were 38.2% and 42.8% respectively. Indomethacin produced
22.3%, 28%and 8.3% inhibition at the concentration of 10,25 and 50 uM respectively. The control values for the adrenaline stimulated maximal contractility of the isolated auricles were 5.04±1.36 and 3.58±0.70 mms for aspirin and indomethacin respectively in six experiments for each drug. Treatment

with aspirin produced concentration dependent depressant effects with all three concentrations (25,50,100 uM). Indomethacin <10 uM) produced no effect, 25 uM produced stimulant effect and 50 uM showed depressant action. The percent inhibition produced with aspirin with different concentrations (25,50,100 uM) was 84.0%, 84.8% and 54.9% respectively. Indomethacin produced 15.0% increase and 18.1% inhibition with 25 uM and 50 uM respectively. Effects of Aspirin and Indomethacin on the Log Dose Response Curves of Adrenaline (Figure 1,2). The dose response curves of adrenaline in the presence of aspirin are shifted towards right but maximum effect is not achieved which indicates that antagonism is insurmountable (Figure Ia and b).

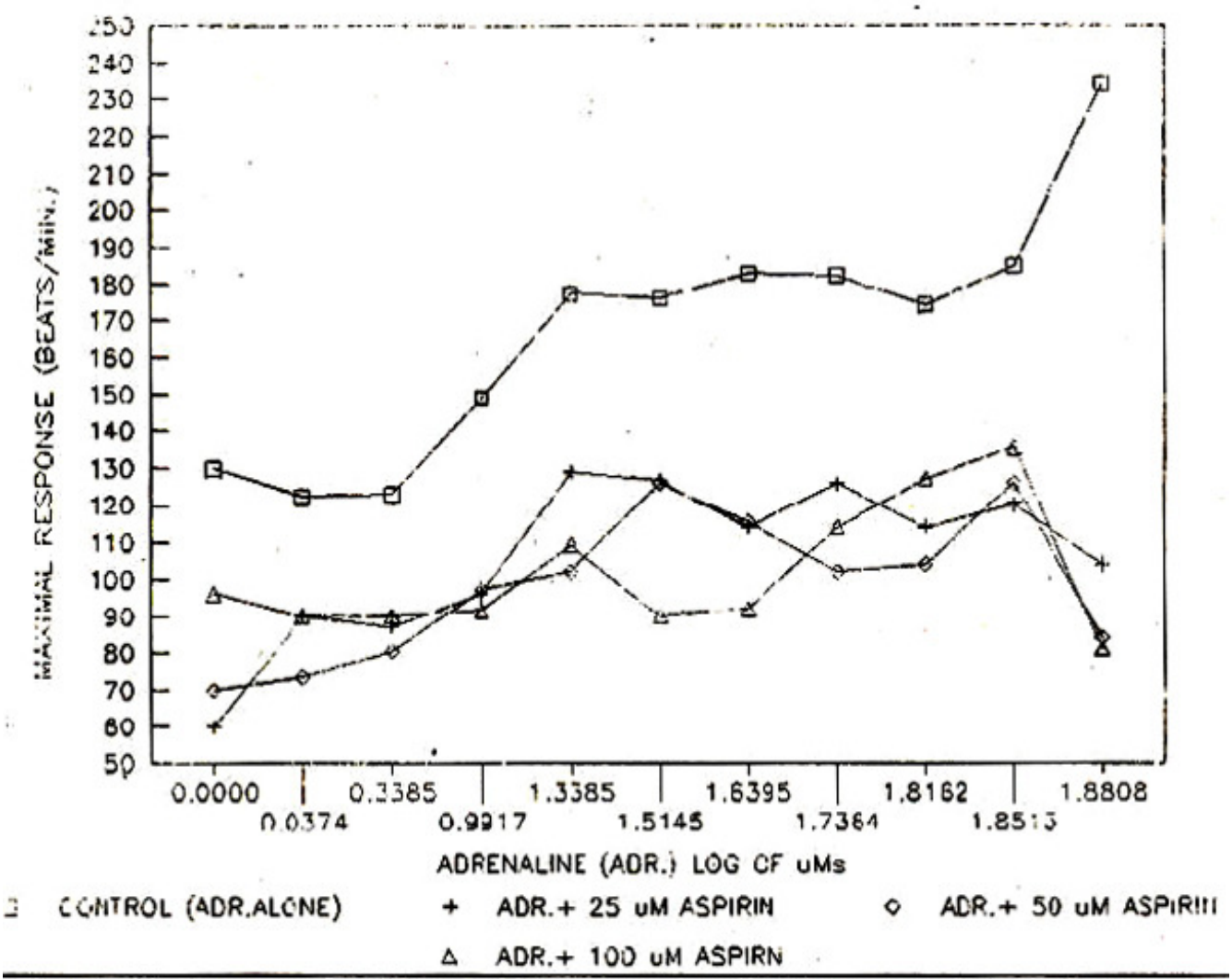

Figure 1 (a). Response of auricles of rabbit heart to adrenaline alone (control) and in the presence of aspirin.

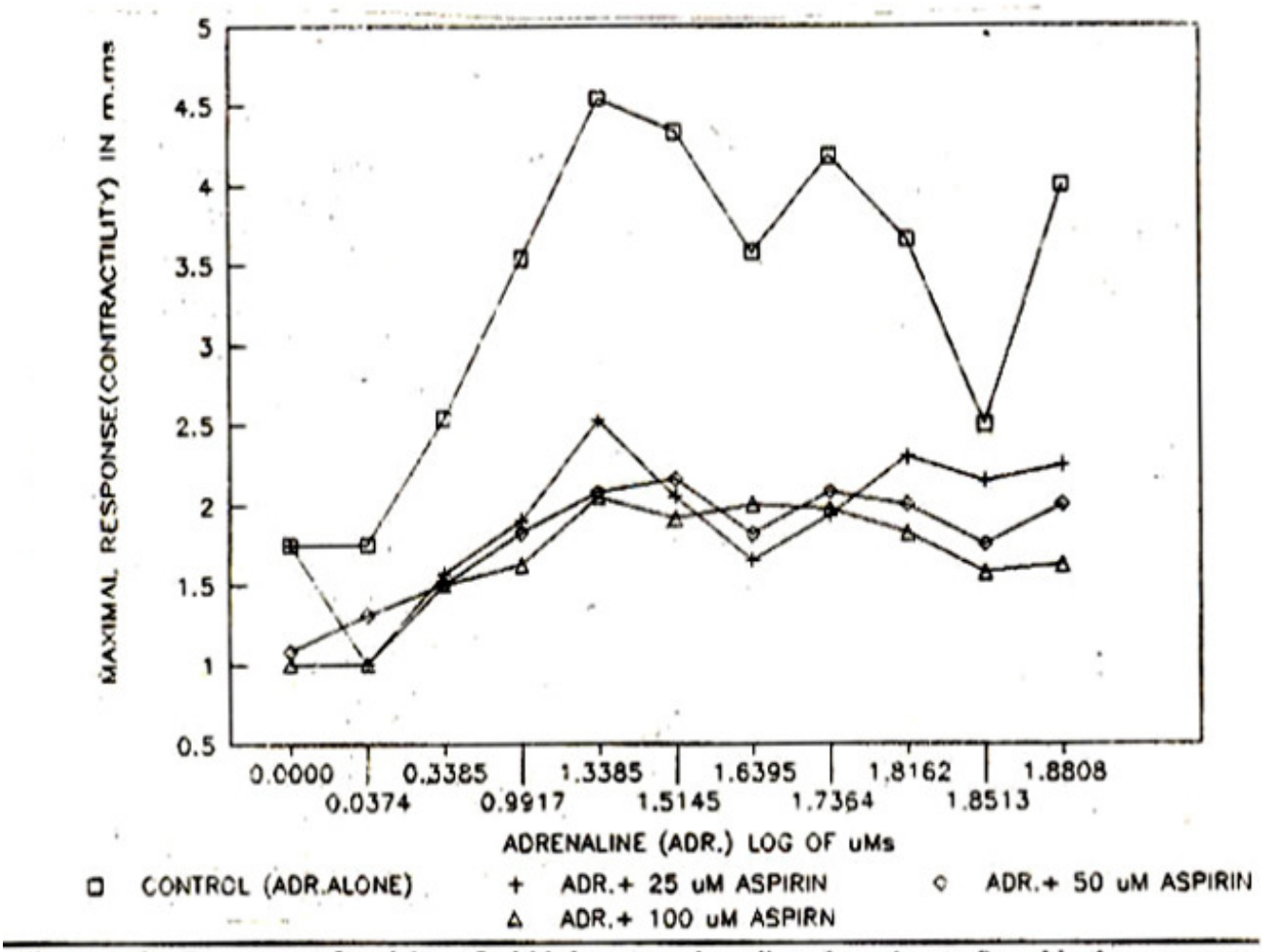

Figure 1 (b). Response of auricles of rabbit heart to adrenaline alone (control) and in the presence of aspirin.

Indomethacinin small doses has no effect on the adrenaline dose response curve while large doses (50 uM) led to marked shift towards right (Figure 2a).

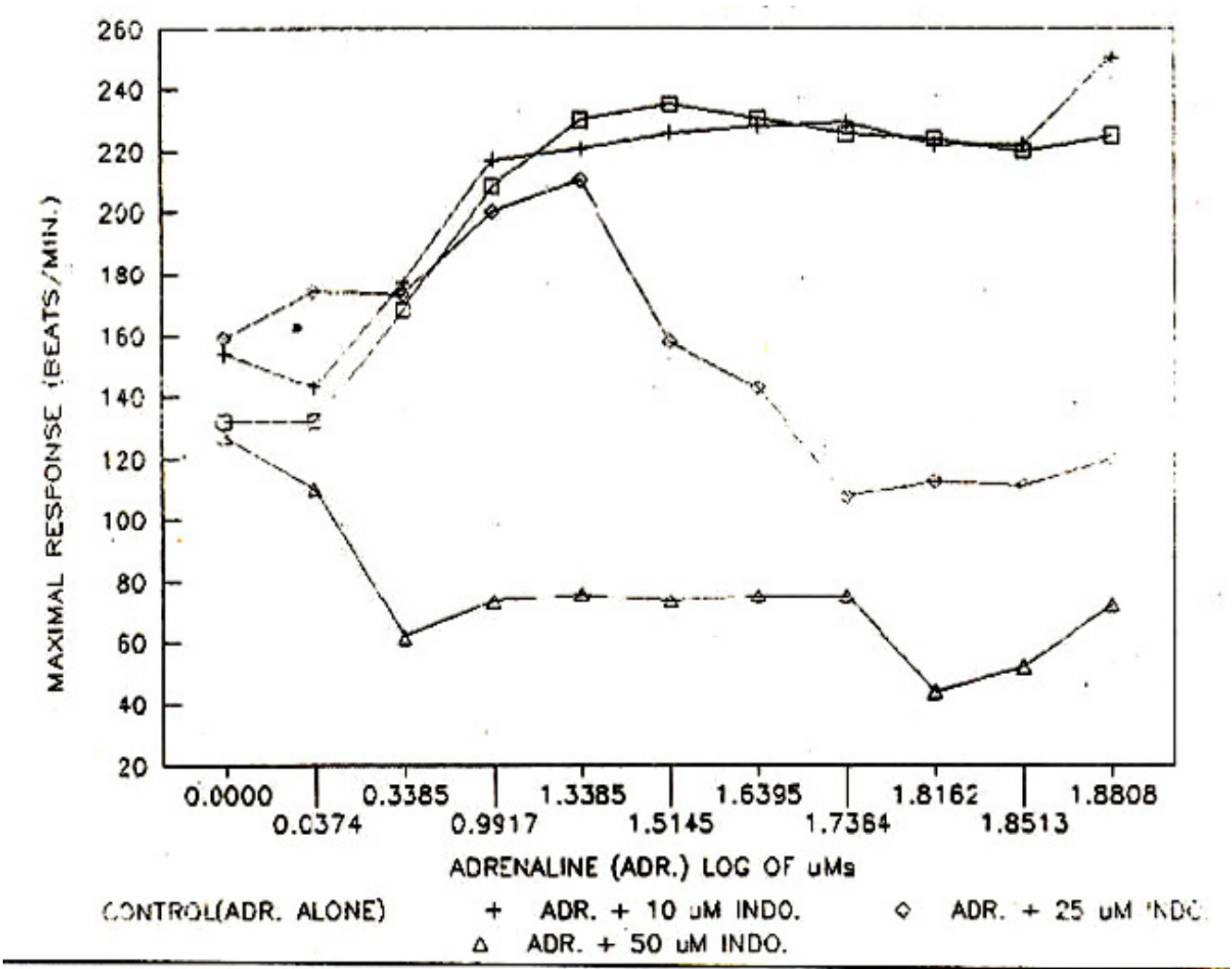

Figure 2 (a). Response of auricles of rabbit heart to adrenaline alone (control) and in the presence of indomethacin.

The dose response curve of contractility induced with adrenaline is little effected (Figure 2b).

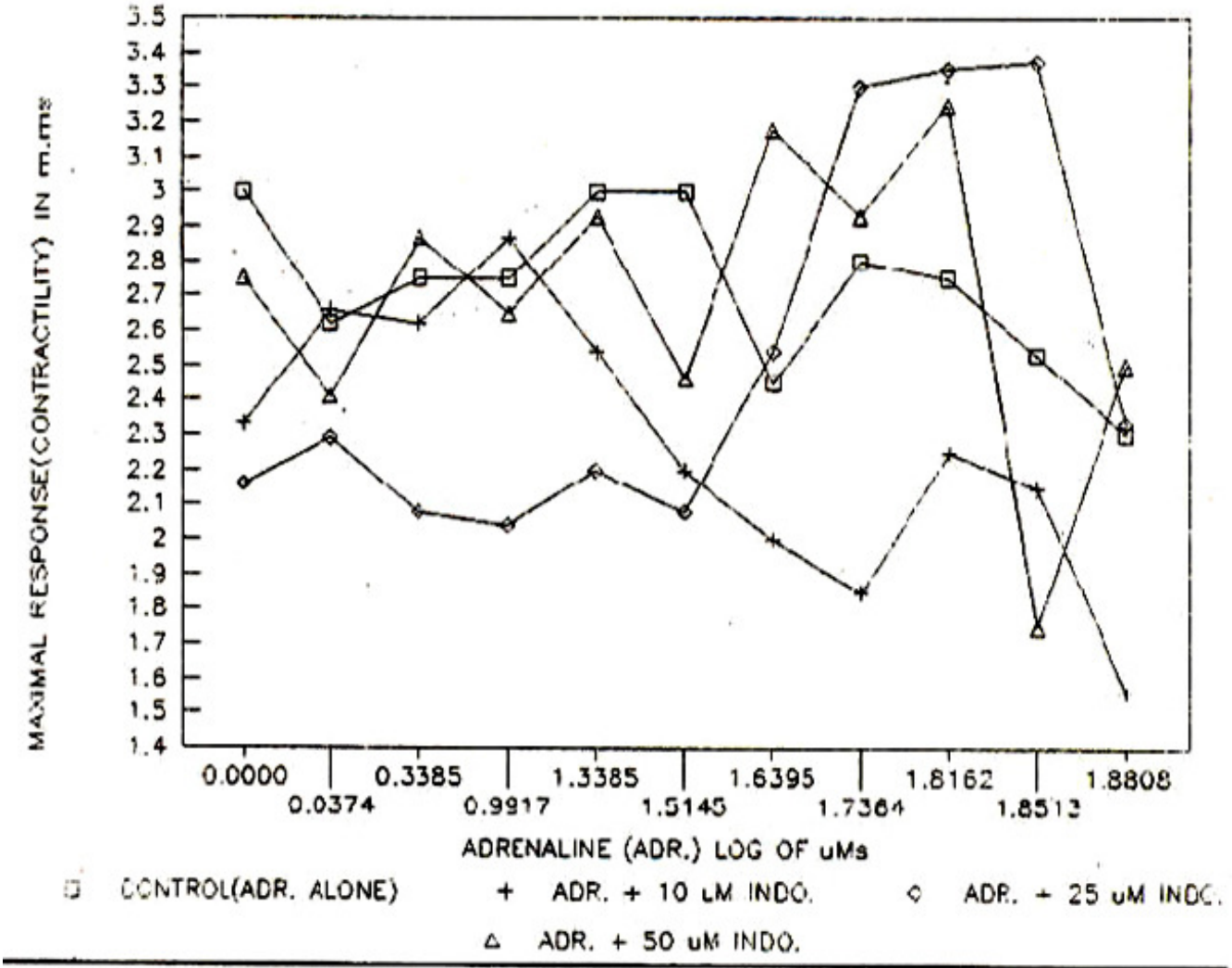

Figure 2 (b). Response of auricles of rabbit heart to adrenaline alone (control) and in the presence of indomethacin.

Discussion

Aspirin depressed the normal activity of the isolated anricles. Adrenaline stimulated activity is also depressed in the dose range of 25-100 uM. Dose response curves are shifted towards right. Our work is consistent with that performed by Neto[1], according to which 5-bromo-salicylates (30-100 uM) and sodium salicylate (100-300 uM) cause a dose dependent decrease in frequency of discharge of SA node. Salicylates possibly depress the slow inward current in both SA node and atrial muscle fibers of the rabbi heart[1]. However adrenaline did not restore the rhythm of heart stopped by greater concentration of salicylates (100-300 uM)[1]. Only higher concentrations of aspirin (100 uM) produced significant effect on the inotropic activity of the isolated auricles but adrenaline stimulated activity was not effected significantly by any of the concentrations of aspirin. Cat heart papillary muscles treated with indomethacin 10^{-4},10^{-3} M, acetylsalicylic acid did not exert significant positive or negative inotropic effect in the isolated papillary muscle fibers[6]. But in this study 100 uM aspirin produced significant depressant effect and this may be due to species difference. The effect of indomethacin(50 uM) is possibly due to forceful binding and slow dissociation from the receptor site. Carboxylic acid

NSAIDs, indomethacin, meclofenamate and neproxen inhibit calcium accumulation by mitochondria and microsomes while plasma membrane calcium transport is not inhibited. The mechanism of this inhibition is unknown[7]. Aspirin acts like agents such as verapamil which inhibits slow inward current directly and hence effects SA node discharge[8]. Moreover, verapamil markedly inhibits force of contraction in guinea pig atnal preparation. The degree of inhibition is greater when preparation is stimulated at higher frequencies[9]. It is also said that verapamil blocks conductance of slow Ca channels at high but not at low stimulation of frequencies[10]. These works are in consistent with our work of aspirin i.e., effect of aspirin on normal contractility and adrenaline stimulated contractility of isolated auricles. Calcium blocking activity has been shown by Neto[1] who has found that most important effects induced by the increase in the Calcium concentration on the action potential configuraion of the a trial muscle fibers were an elevation of the plateau height, a shortening of action potential duration and mean fall in sinus discharge. He also noticed the influence of increased potassium concentration on the effects of 5-bromo-salicylate and found that action potential duration is significantly reduced by increasing the potassium concentration.

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