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Effects of Training on Atrial Rate and Sensitivity of Isolated Rat Atria to Catecholamines and Acetylcholine

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EFFECTS OF TRAINING ON ATRIAL RATE AND SENSITIVITY OF ISOLATED RAT ATRIA TO CATECHOLAMINES AND ACETYLCHOLINE

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and Acetylcholine

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TABLE OF CONTENTS

Abstract ii

Acknowledgement iv

Introduction and Literature Review . . . 1

Materials and Methods 10

Results 15

Discussion 66

Literature Cited 77

ABSTRACT

Thirty five male rats were subjected to a treadmill running program and body weight, heart weight and effects of neurotransmitters were measured. Rats engaged in training programs show a lower body weight, a lower heart rate and lower intrinsic heart rate. The response of all isolated rat atria to different drugs were observed. Epinephrine in concentrations of $1x10^{-5}M$, $1x10^{-6}M$ and $1x10^{-7}M$ increased the atrial rate in trained rats by averages of 41.3%, 20.04% and 15.3% respectively, and in control animals by averages of 18.6%, 11.1% and 8.6% respectively. Norepinephrine in concentrations of $1x10^{-6}M$ and $1x10^{-7}M$ increased the atrial rates of trained animals by averages of 13.3% and 8.3% respectively.

Acetylcholine of $1x10^{-6}M$ decreased the atrial rate in trained rats averages of 48.4% and 28.2% in control animals. Atrophine in concentrations of $1x10^{-5}M$, $1x10^{-6}M$ and $1x10^{-7}M$ increased the atrial rates in all preparations, but the percent change was higher in trained rats. $1x10^{-5}M$ atropine added to isolated rat atria of trained rats increased the

atrial rate to a rate almost identical to the basal atrial rate of control rats. A biphasic response of atrial rate was observed when equimolar concentrations of acetylcholine and norepinephrine were added to isolated rat atria. It is concluded that trained rats have a lower resting heart rate and a lower intrinsic heart rate than control rats. The isolated rat atria of trained animals were more sensitive to catecholamines, acetylcholine and atropine. Increased stores of acetylcholine in the region of the pacemaker may account for the lowered heart rate. The negative chronotropic action of acetylcholine was blocked and the heart rate was brought to the basal rate of control animals in the presence of 1×10^{-5}M atropine.

ACKNOWLEDGMENT

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INTRODUCTION AND LITERATURE REVIEW

It is a common observation that exercise leads to an improved performance. The most significant effect of exercise is an increase in the efficiency of the cardiovascular system. For this reason, exercise has been recommended as a means of modifying the risk factors contributing to heart disease (Herrlich et al., 1960 and Fox et al., 1964). It also protects against the development of myocardial infarctions in humans (Milles et al., 1954). Similar types of vascular changes have been observed in various animal species subjected to physical exercise (Tepperman et al., 1963).

It is well known that repeated exercise involving vigorous muscular activity, in competitive sports such as swimming and running, produce a characteristic functional change in the cardiovascular system of trained individuals (Knehr et al., 1942 and Coton, 1932). In the athlete this amounts to an improvement in performance. The rate of improvement depends on how rigorous a regime is followed in order to achieve the most striking results.

The outstanding change in cardiac performance that takes place during repeated exercise is a decrease in the resting heart rate (Herrlich et al. 1960). Astrad (1964) and Saltin et al. (Suppl. VII) for example have shown that heart rate is a sensitive indicator of physiological conditions at various levels of exercise. Intensity of exercise is thought to have a greater influence on cardiovascular conditioning than duration and frequency of exercise in causing the maximum training effect on heart rate.

Many explanations have been proposed to account for this bradycardia, but the mechanism of its origin is not yet well understood. Of the many explanations proposed the concept of enhanced vagal tone and/or sympathetic inhibition has been the most commonly favored (Herrlich et al. 1960; Hall 1963).

There have been attempts to determine whether the relationship between physical activity and heart rate is a true indication of a cholinergic mechanism (Mellerowicz 1956).

The heart can be pharmacologically isolated from the natural sympathetic and parasympathetic activities by simultaneous administration of the adrenergic beta-receptor blocker propranolol and the cholinergic antagonist atropine. The alkaloid derivative atropine bears some distinct chemical similarities to acetylcholine; for example, the distance

between the nitrogen (N) and the carbonyl group (C=O) is roughly 7A° as shown in figure 1B. It is known that atropine does not interfere with acetylcholine formations, but compete with acetylcholine for receptor sites.

Jose and Stitt (1967) measured the heart rate following combined injections of atropine and propranolol. This method has been used in dogs and should be applicable to rats with appropriate adjustment of dosages. Under such a state, which closely resembles the cardiac isolation in intact animals and humans, the measurement obtained was termed intrinsic heart rate (IHR).

Experiments in mammals including man indicate that atropine accelerates the heart rate (Raab et al. 1960), but produces slight or no effect on ventricular contraction (Herxheimer 1921). This suggests that a difference exists in the sympathetic regulation of chronotropic functions on the one hand and inotropic functions on the other. Raab et al. (1965) noted a slight difference between the cardioacceleration in trained and untrained men. Tipton et al. (1965) showed that the resting heart rate of trained rats was lower than untrained rats and that atropine caused a greater cardiac acceleration in control rats than in trained rats. These results were interpreted based on the assumption that trained animals have more stores of acetyl-

choline in the pacemaker to compete with atropine for the receptor sites. The heart rate depends on both sympathetic and vagal activities in the pacemaker. According to Herxheimer atropine produces no effect on ventricular contraction in man. It is then thought that the contractility is chiefly adrenergic, this may not be entirely true since the bound acetylcholine has been found in ventricular tissue (Rothschuh et al. 1955).

Studies have shown that in a spontaneous heart beat acetylcholine is liberated from cardiac pro-cholinergic stores and it is rapidly resynthesized such that there in no pronounced alteration in the total acetylcholine content of the heart (Briscoe 1954; Day 1956 and Rothschuh et al. 1955).

The effects of acetylcholine and epinephrine on atrial muscle cells were demonstrated by many investigators. In all species studied acetylcholine was found to produce a decrease in the repolarization phase, and epinephrine produced a prolongation of the repolarization phase (Burgen et al. 1953; Hoffman et al. 1953; Johnson 1956 and Webb et al. 1956). The marked decrease in the repolarization phase caused by acetylcholine is thought to be the cause for the decrease in contractile strength of atrial muscle (Burgen 1953). Furchgott et al. (1960) showed that with guinea pig atrial preparations, acetylcholine at minimal concentration causes

a reduction in contractile strength. A subsequent addition of epinephrine enhances the restoration of the strength of contraction by exerting a complete counteracting effect on acetylcholine. Ginzel et al. 1953 and Giotti 1954 previously reported that in the presence of atropine at concentrations of 10^{-6}M and 10^{-5}M in guinea pig and other mammal atria, the addition of high concentration acetylcholine induces the release of catecholamines and counteracts completely the negative inotropic action of acetylcholine. The cholinergic neurotransmitter is known to play a role in myocardial carbohydrate metabolism through its inhibition of the glycogenolytic effect of epinephrine in isolated perfused heart (Vincent 1959 and Ellis 1963).

Allotey et al. (1969) reported that acetylcholine inhibits the positive inotropic effect of epinephrine in isolated rabbit heart. This finding is in agreement with those of Meester et al. (1967). Murad et al. (1962) indicated that in the presence of both acetylcholine and catecholamines the rate of synthesis of cyclic, 3', 5' adenylic acid was reduced.

In 1965 Sutherland and Robison postulated that epinephrine exerts its inotropic action by acting on the membrane adenyl cyclase to accelerate the rate of synthesis of cyclic AMP which in turn enhances the chronotropic and the inotropic

responses. Similarly stimulation of beta-receptors by catecholamines enhances the production of cyclic AMP which in turn acts as a second messenger to initiate a positive inotropic and chronotropic response (Chamales et al. 1971).

Roberts et al. (1969) in their study suggested that differences exist between the cholinergic receptors on the pacemaker and the contractile cells of the rat atrium. They also noted that pacemaker cell receptors appears to be highly selective in their agonist requirements and are more effectively blocked by atropine than contractile cell receptors.

The pacemaker is known to receive its nerve supply from both divisions of the autonomic nervous system. Since both the adrenergic and the cholinergic nerve systems act synergistcally to maintain a heart rate, one may conclude that simultaneous activity of both nerves must account for autonomic control (Levy et al. 1969).

According to Samnan (1935) and Warner et al. (1969) the autonomic control of cardiac pacemaker activity could not be expressed as an algebriac sum of the response to seperate stimulation. During simultaneous stimulation of both sets of fibers the resulting heart rate cannot be expressed mathematically as an arithmetic mean elucidating the effect of combined parasympathetic and sympathetic stimulation of the pacemaker (Warner et al. 1969). The response of spontaneously

contracting rat atria is a possible interaction between neurotransmitters. The parasympathetic neurotransmitter acetylcholine is capable of suppressing the response of the sinoatrial node to the sympathetic transmitter, norepinephrine. The latter is much less likely to inhibit the effect of acetylcholine (Grodner et al. 1970). If this evidence holds true the results are similar to the findings of Samnan (1935); Warner et al. (1962) and Levy et al. (1969) who showed in intact animals that an increase in vagal tonic activity will initiate a progressively less pronounced sympathetic activity on heart rate. Herrlich et al. (1960) reported that the atria of trained rats have a higher acetylcholine content than control rats. Gordon et al. (1966) indicated that exercise enhanced an increase in the incorporation of ^{14}C-tyrosine into catecholamines in rat heart. This is in agreement with the most recent review by Sheldon et al. (1975) who showed that forced running caused an increase in the incorporation of ^{14}C-tyrosine into catecholamines in the rat heart on the assumption that running may increase the specific activity of tyrosine which results in an increase in catecholamines synthesis. De Shryver et al. (1967) reported that the catecholamines content of the myocardium is lower in hearts of trained rats.

Grodner et al. (1970) indicated that there exists a dominancy of the cholinergic system on the pacemaker frequency caused by interaction of the neurotransmitters, and that the presence of acetylcholine seems to prevent the action of the adrenergic transmitters. He also studied the response of isolated perfused rat atria to atropine in the presence of acetylcholine and noted an increase in the atrial rate. Carrier et al. (1971) indicated in their study that the cholinergic transmitter, acetylcholine has a greater influence in altering the chronotropic response to rabbit atria even when both neurotransmitters were present in equimolar concentrations. Also in an effective concentration the change in heart rate could not be expressed as an algebriac sum of the two effects. This is similar to and in agreement with results obtained in isolated rat atria preparations (Grodner et al. 1970).

Bolter et al. (1973) presented evidence that the intrinsic heart rate of trained rats was significantly lower than control rats and that the atria of trained rats had a marked subsensitivity to acetylcholine. Similar observations in humans established that regular exercise may also lower the intrinsic rate in proportion to an increase in aerobic capacity (Sutton et al. 1967 and Frick et al. 1967). From all these studies it appears that the control of heart rate may

be a function of the interaction between the neurotransmitters; catecholamines and acetylcholine. An increase in tonic parasympathetic activity may be a possible mechanism for the bradycardia by causing a change in atrial stretch which might thereby modify the rhythm of the sinoatrial node (Hall 1963). Or alternatively a change in sensitivity of the pacemaker to acetycholine in physically trained rats (Bolter et al. 1973) might be responsible.

The continous change in intrinsic heart rate in trained animals has not yet been studied, nor the possibility that the degree of change in sensitivity to catecholamines may be correlated with exercise induced intrinsic heart rate depression.

The overall objective of this investigation was to initiate a program to study these possibilities and to establish a more precise quantitative relationship between the neurotransmitters and intrinsic heart rate in rats.

MATERIALS AND METHODS

Forty seven male Sprague-Dawley albino rats (Madison, Wisconsin) initially weighing 120-200 grams were randomly assigned to control or exercised groups. All rats were kept in an environmentally controlled animal suite and allowed rat chow and water ad libitum. Experimental animals were exercised on a staggered schedule so that the isolated atrial preparation could be done on an approximate two per day basis as the rats finished the training program.

Exercised rats were trained twice daily for one hour six days per week. The training period lasted for seven to nine weeks. The rats were always exercised at the same time of the day. The rate of exercise was on the order of 12 meters per minute, (10.5 cycles/min.). Control rats were quartered with experimentals and were periodically handled and placed in the motorized chamber for 15 minutes twice a week, but were not exercised. The motorized chamber is a special six compartment motor driven activity wheel made by Wahmann Manufacturing Company. Each rat ran in a four inch wide wire space, fourteen inches in diameter.

At first the rats did not run readily in the treadmill, as some rats learned to ride the axle to avoid running. Therefore the axle was removed and the drum was modified so that it could be driven from the ends.

Preliminary experiments showed that there was no significant difference in heart rate between lightly anesthetized and unanesthetized rats. In order to obtain as constant results as possible, all animals received an identical anesthetic dose (sodium pentobarbital 60 mg/kg) intraperitoneally before heart rates were recorded. Resting and intrinsic heart rates were obtained on alternate weeks in both control and experimental rats by means of an electrocardiogram. The intrinsic heart rate was measured in intact rats by recording the EKG following a combined injection with appropriate dosages of atropine and propranolol (Jose and Stitt, Cir. Res. 25; 53, 1969). At the end of the training period animals were sacrificed. In this procedure they were stunned by a blow to the back of the neck and the hearts were rapidly removed into a dish containing Kreb's-Henseleit bicarbonate solution. The atria were then carefully dissected, ensuring that the sinoatrial node was intact. For the continous perfusion of the heart a modified Kreb's-Henseleit solution pH 7.2$\pm$.1 was made up of the following; NaCl (154mM),

KCl (5.4mM), $CaCl_2$ (2.4mM), $NaCO_3$ (6mM), and dextrose (11mM) to one liter of distilled water. The constant 34°C organ bath was oxygenated continously with 95% oxygen, 5% carbon dioxide during the experiment. The modified Kreb's-Henseleit solution was not recirculated.

Immediately upon placing the spontaneously beating atria in the bath, the atrial tension was recorded by means of a thread tied to an S-shaped pin hooked into the tip of the atrium. One end of the thread passed to a strain gauge transducer. The other tip of the atria was attached to a metal wire fastened to the bottom of the organ chamber. After the initial equilibration period, the atrial rate was maintained and the influence of various concentrations of epinephrine (10^{-5}M, 10^{-6}M and 10^{-7}M), norepinephrine (10^{-6}M and 10^{-7}M), acetylcholine 10^{-6}M, combined equimolar concentrations of acetylcholine and norepinephrine 10^{-6}M, and atropine (10^{-5}M, 10^{-6}M and 10^{-7}M) were tested on the response of the pacemaker of the isolated rat atria. Recording was made with a polygraph (Physiograph).

Dose response curves were obtained, each representing a different group of rat atria. After each exposure of the atria to a certain concentration of the drug or drugs, the preparations were washed twice with Kreb's-Henseleit solution and allowed to re-equilibrate to base line before

they were subjected to an additional exposure of the drug or drugs. Once the maximum responses of the atria to epinephrine, noreponephrine, acetylcholine, combined acetylcholine and norepinephrine or atropine had been determined, the atria were discarded. The following dose-response relationships were obtained:

1. The chronotropic effects of various concentrations (10^{-5}M, 10^{-6}M and 10^{-7}M) of epinephrine and norepinephrine were tested on the isolated perfused atrial rate.
2. The effects of 10^{-6}M of acetylcholine was tested on atrial rates. The atrial rate was determined over a period of 30 seconds between 1-2 min after addition of acetylcholine.
3. The interaction of acetylcholine and norepinephrine in combined equimolar concentrations of 10^{-6}M was tested on the chronotropic response of spontaneously beating rat atria.
4. The influence of various concentrations (10^{-5}M, 10^{-6}M and 10^{-7}M) of atropine on atrial rate.

Statistical analysis

All measures of variation of means used in this thesis are standard error of the mean. Students' t-test was used to assess the significance of difference between mean

values. Statistical tests were done according to methods described by Freeze (1967).

Drugs

Drugs used in this study were propranolol hydrochloride (Ayerst Laboratories Incorporated, N.Y. 10017), atropine sulfate (Nutritional Biochemicals Corporation, Cleveland, Ohio 44128), adrenaline chloride solution (Park, Davis & Company, Detroit, Michigan 48232), acetylcholine bromide (Eastman Organic Chemicals, Rochester N.Y.), levarterenol bitartrate (Winthrop Laboratories Division of Sterling Drug Inc., New York, N.Y. 10016), sodium pentobarbital 60mg/kg (Abbott Laboratories, North Chicago, Illinois 60064). All drugs were dissolved in distilled deionized water and the concentrations are expressed as molarities.

RESULTS

I. Body weight and heart weight

Forty seven rats with an initial average weight of 218 grams were divided into sedentary and exercise groups. The mean body weight of the eleven sedentary controls increased from 225 to 358 grams over the seven weeks for an average gain of 123 grams, while that of the thirty six trained rats increased from 212 to 297 grams, a gain of 85 grams (Table II). Thus, the body weight gain was higher in untrained rats. Trained rats consumed less food and gained less weight than untrained animals. This observation is in agreement with those obtained by Thomas et al. (1958) and Ahrens et al. (1972). Possibly this difference is due to an "anorexic" effect or some kind of a stress imposed on the rats. Or it may be in part related to the increase of body metabolic demands during the training period. The ventricles and the atria at the end of the experiment were blotted dry and weighed on an analytical balance. The total heart, ventricular and atrial weight of trained rats and their ratios to body weight at the end of the seven to nine weeks of exercise were similar to those of the control

Table I

Heart weights, ventricular weights, atrial weights and their ratios to body weight of male rats (Experiment #1).

	Controls	Experimentals
Heart weight (gm)	1.195 ± .0547 (6)	1.092 ± .023 (17)
Ventricular weight (gm)	1.056 ± .0563 (6)	0.915 ± .0247 (17)
Atrial weight (gm)	0.139 ± .0098 (6)	0.161 ± .02136 (12)
Heart weight/ body weight (mg/gm)	3.0 (6)	3.3 (17)
Ventricular weight/ body weight (mg/gm)	2.7 (6)	2.8 (12)
Atrial weight/ body weight (mg/gm)	0.3 (6)	0.5 (12)

Values are mean ± standard error with number of observations in parentheses. No significant difference between controls and experimentals.

Table II

Average body weights

Number of weeks	Controls (12)	Exercised (35)
Pre-exercise	225 ± 11.44	211.8 ± 10.70
1	268.6 ± 9.62	238.5 ± 35.47
2	292 ± 7.07	261.2 ± 5.48
3	308.8 ± 7.17	268.5 ± 4.49
4	326 ± 7.06	283.6 ± 3.87
5	335.9 ± 6.43	283.6 ± 4.33
6	344.8 ± 6.44	288.4 ± 4.47
7	358.1 ± 6.62	296.9 ± 3.66

Values are mean ± standard error with number of observations in parentheses.

group. No significant differences between control and trained rats were observed in heart, ventricular, and atrial weights nor in the heart, ventricular or atrial weight/body weight ratios (Table I).

II. Resting heart rate and heart rate during pharmacologic autonomic blockade

The groups of animals summarized in Figures 1, 2, 3 and in Table III and IV show that trained rats had a significantly lower resting heart rate than control rats during the sixth, seventh and eighth week of exercise in the first experiment ($P<.01$, Table III). No significant difference was seen prior to the sixth week of exercise in this experiment ($P<.05$, Table III). In the second experiment trained rats had a significantly lower resting heart rate than control rats by the third and the fourth week of exercise ($P<.05$, $P<.01$, Table IV).

The pharmacologically blocked heart rate in trained animals showed lower intrinsic rates than hearts of control rats in the first and second experiment ($P<.05$, $P<.02$, $P<.01$, Figures 4, 5 and 6).

III. Isolated atrial rate

In isolated rat atria the intrinsic atrial rate was lower in trained rats than in controls with averages of

Table III

Resting heart rates and intrinsic (pharmacologically blocked) atrial rates of control and trained rats (Experiment #1). Values for controls are means ± SEM from 6 animals. Values for experimentals are means from 18 animals (through 4th week) or 17 animals (week 5 through 9).

	RHR (b/min)		IHR (b/min)	
Weeks	Controls	Experimentals	Controls	Experimentals
Pre-ex.	429.3 ± 14.37	445.8 ± 8.35	345 ± 13.13	347.6 ± 7.66
1	414 ± 18.24	425.9 ± 6.92	355 ± 11.32	320.2 ± 10.74
2	456.6 ± 13.73	453.6 9.39		
3	437.5 ± 10.20	423.3 ± 15.02	387 ± 11.25	359.3* ± 6.15
4	436.5 ± 11.17	434 ± 7.55		
5	439.2 ± 6.62	419.2 ± 6.86	374 ± 15.38	321.8* ± 11.9
6	458.2 ± 13.8	413.9*** ± 7.69		
7	457.7 ± 10.92	410.6*** ± 6.62	377 ± 8.40	343.8** ± 7.12
8	450.2 ± 13.65	407.1*** ± 6.49		
9	442.7 ± 12.3	416.7 ± 6.36	372 ± 8.34	340.2* ± 7.86

* $P < .05$

** $P < .02$

*** $P < .01$

Table IV

Resting heart rates and intrinsic (pharmacologically blocked) atrial rates of control and trained rats (Experiment #2). Values for controls are means ± SEM from 6 animals. Values for experimentals are means ± SEM from 18 animals.

	RHR (b/min)		IHR (b/min)	
Weeks	Controls	Experimentals	Controls	Experimentals
Pre-ex.	438.7 ± 5.58	426.7 ± 3.04	376 ± 4.00	379.6 ± 5.53
1	418.8 ± 11.92	429.1 ± 3.73	384 ± 9.98	357*** ± 3.49
2	440 ± 8.78	423.8 ± 4.03		
3	438.3 ± 17.56	413.4* ± 3.33	382 ± 7.69	356.3*** ± 4.20
4	439 ± 4.87	412.5*** ± 4.65		
5	423.8 ± 7.19	413.9 ± 3.33	380 ± 5.06	357*** ± 3.7
6	436.5 ± 4.17	427.2 ± 3.47		
7	428 ± 9.72	421.1 ± 3.56	382 ± 5.72	362*** ± 3.59

* $P<.05$

** $P<.02$

*** $P<.01$

Figure 1

Resting heart rates of 12 controls and 35 trained rats over a period of seven weeks. •——•, control rats; o- - - o, trained rats.

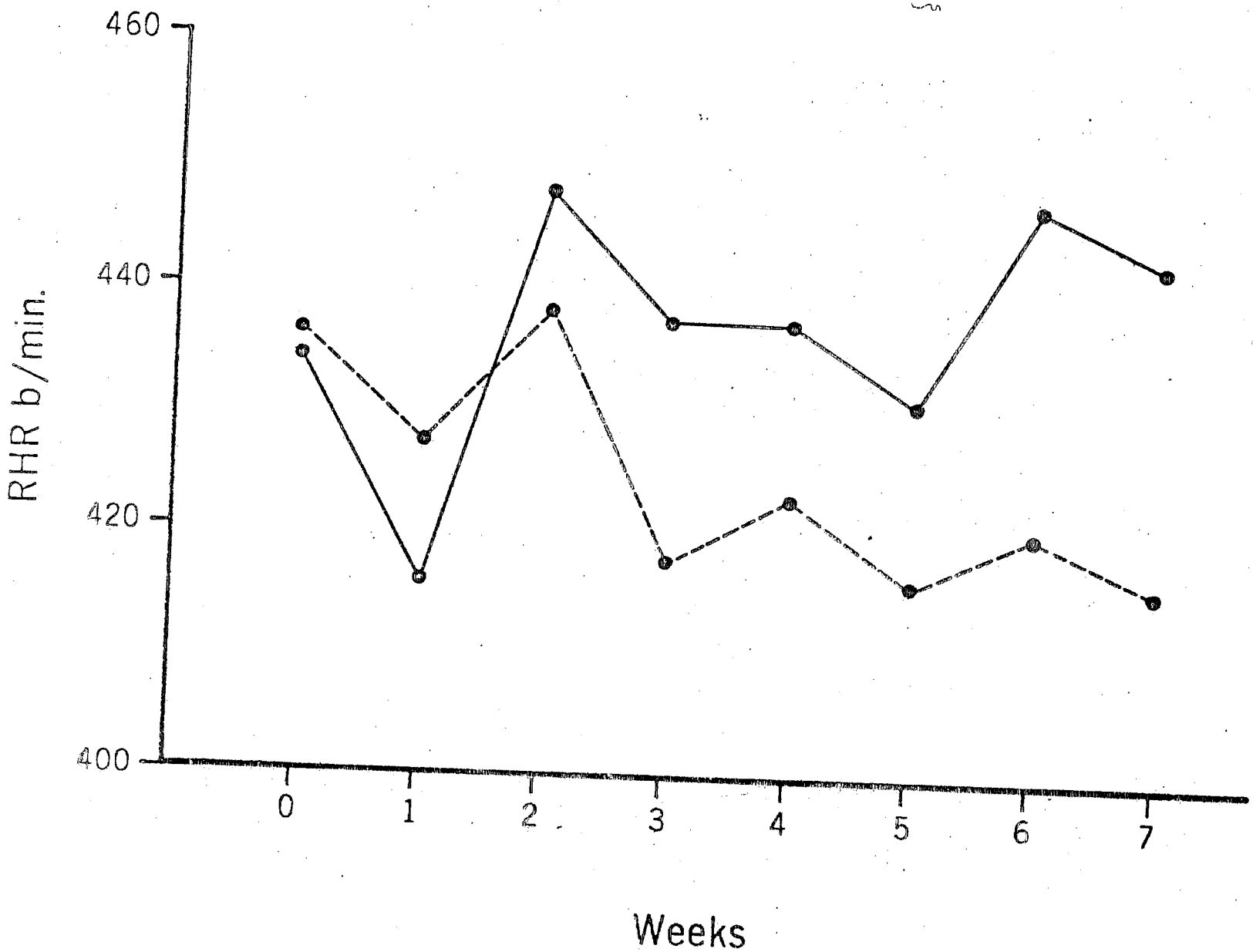

460
440
420
400
RHR b/min.
0
1
2
3
4
5
6
7
Weeks

Figure 2

Experiment #1. Resting heart rates of 6 controls and 17 trained rats over a period of nine weeks. o——o control rats; o- - -o , trained rats. Points represent mean from 23 animals ± SEM.

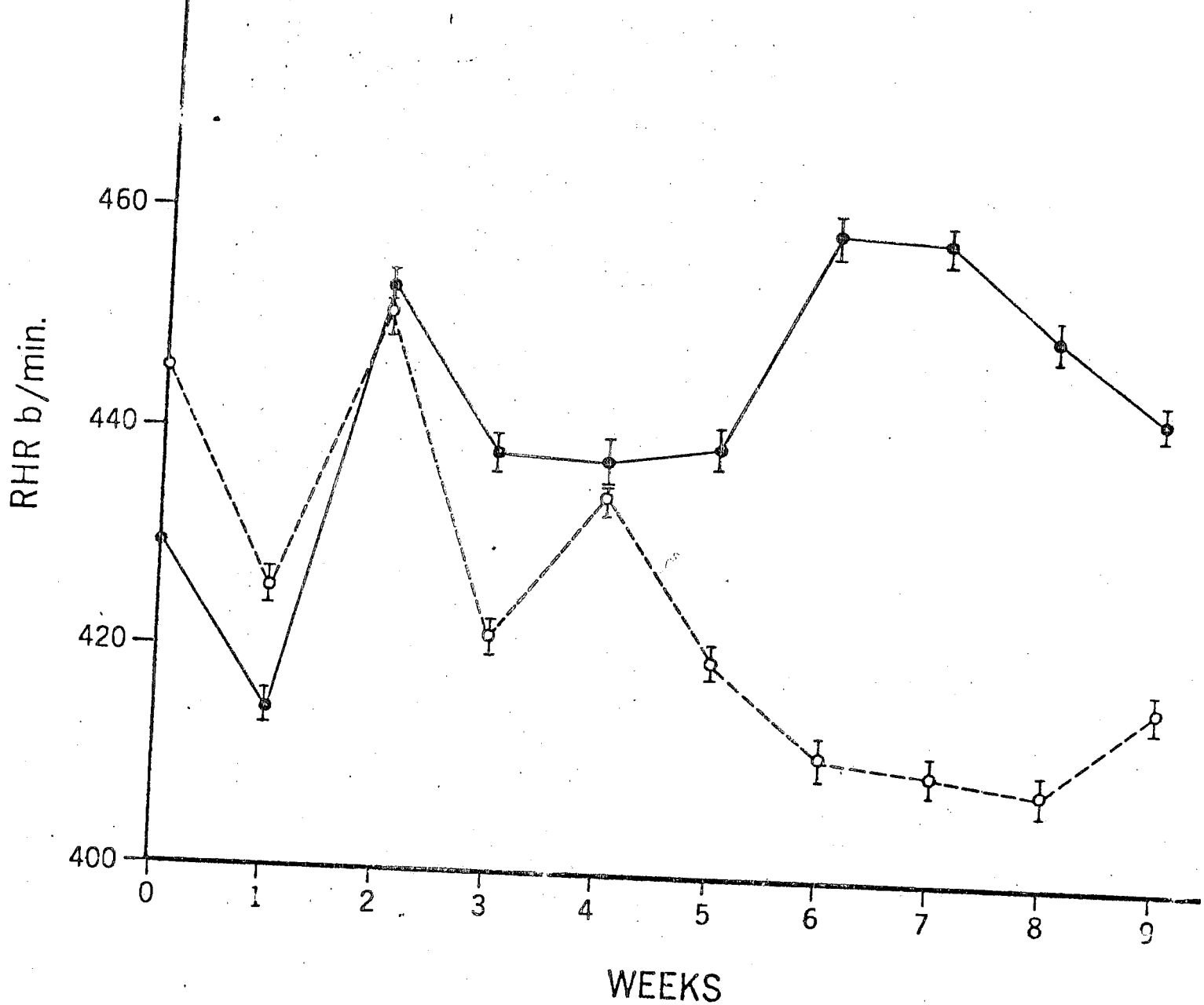
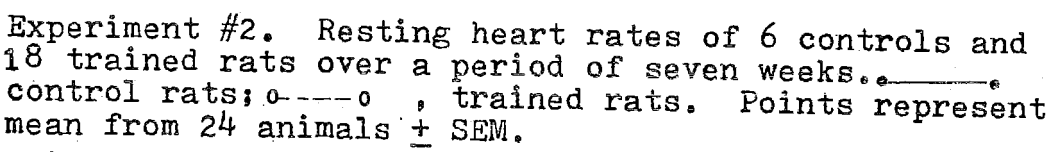

460
440
420
400
RHR b/min.
0
1
2
3
4
5
6
7
8
9
WEEKS

Figure 3



Experiment #2. Resting heart rates of 6 controls and 18 trained rats over a period of seven weeks. •——• control rats; o---o , trained rats. Points represent mean from 24 animals ± SEM.

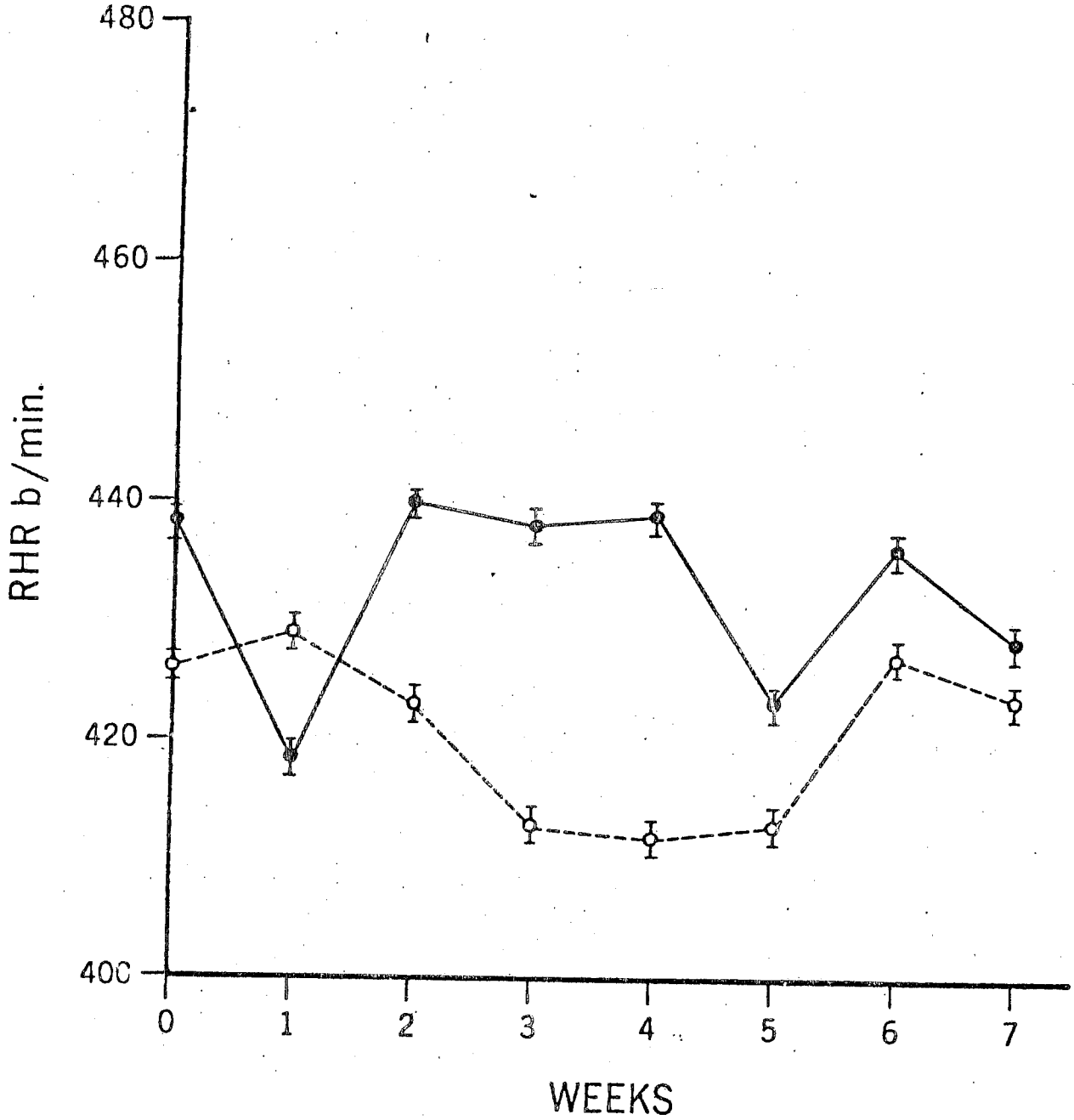

480
460
440
420
400
RHR b/min.
0
1
2
3
4
5
6
7
WEEKS

Figure 4

Intrinsic (pharmacologically blocked) heart rates of 12 controls and 35 trained rats over a period of seven weeks. •——• control rats; •- - -• , trained rats.

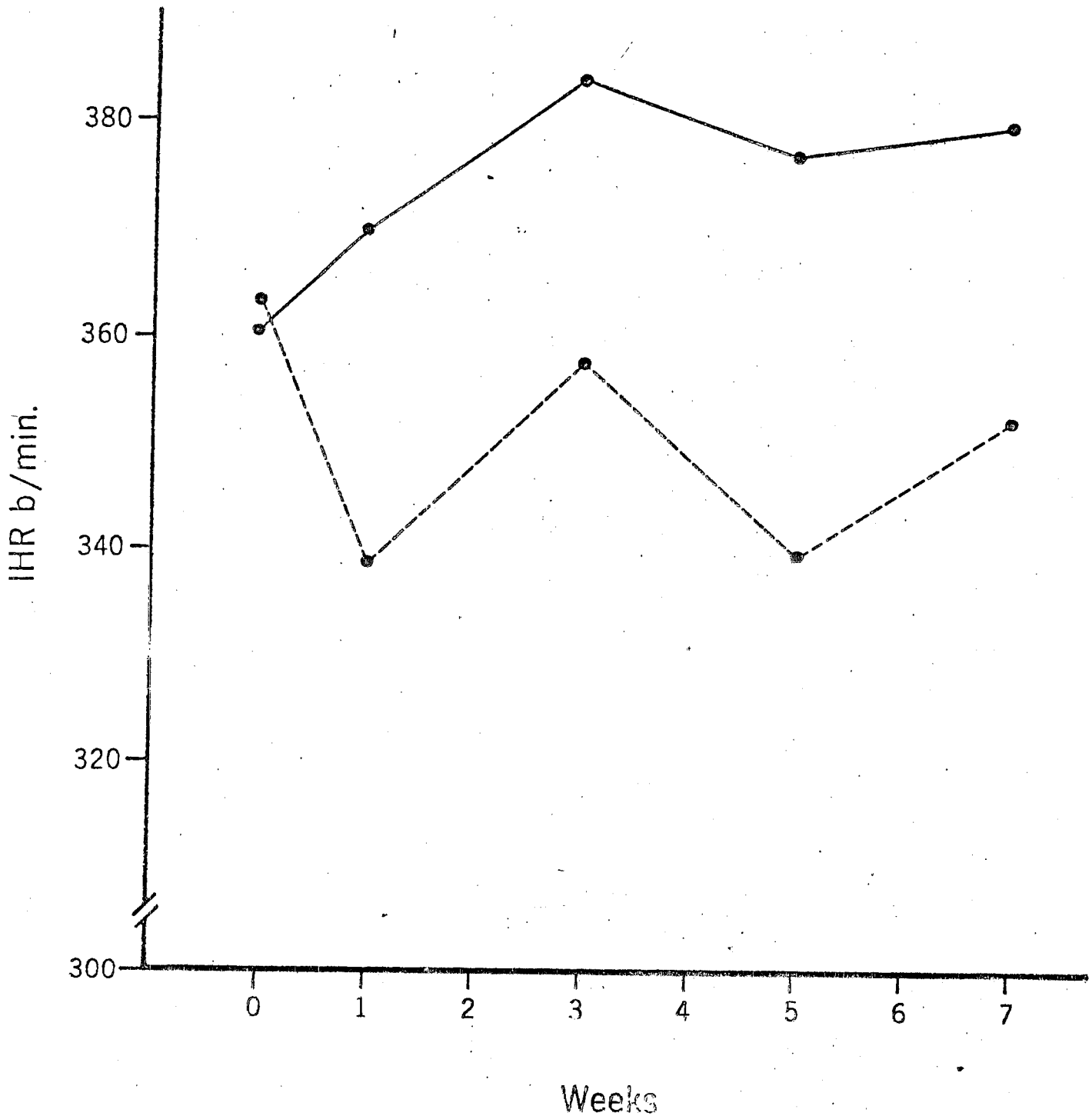
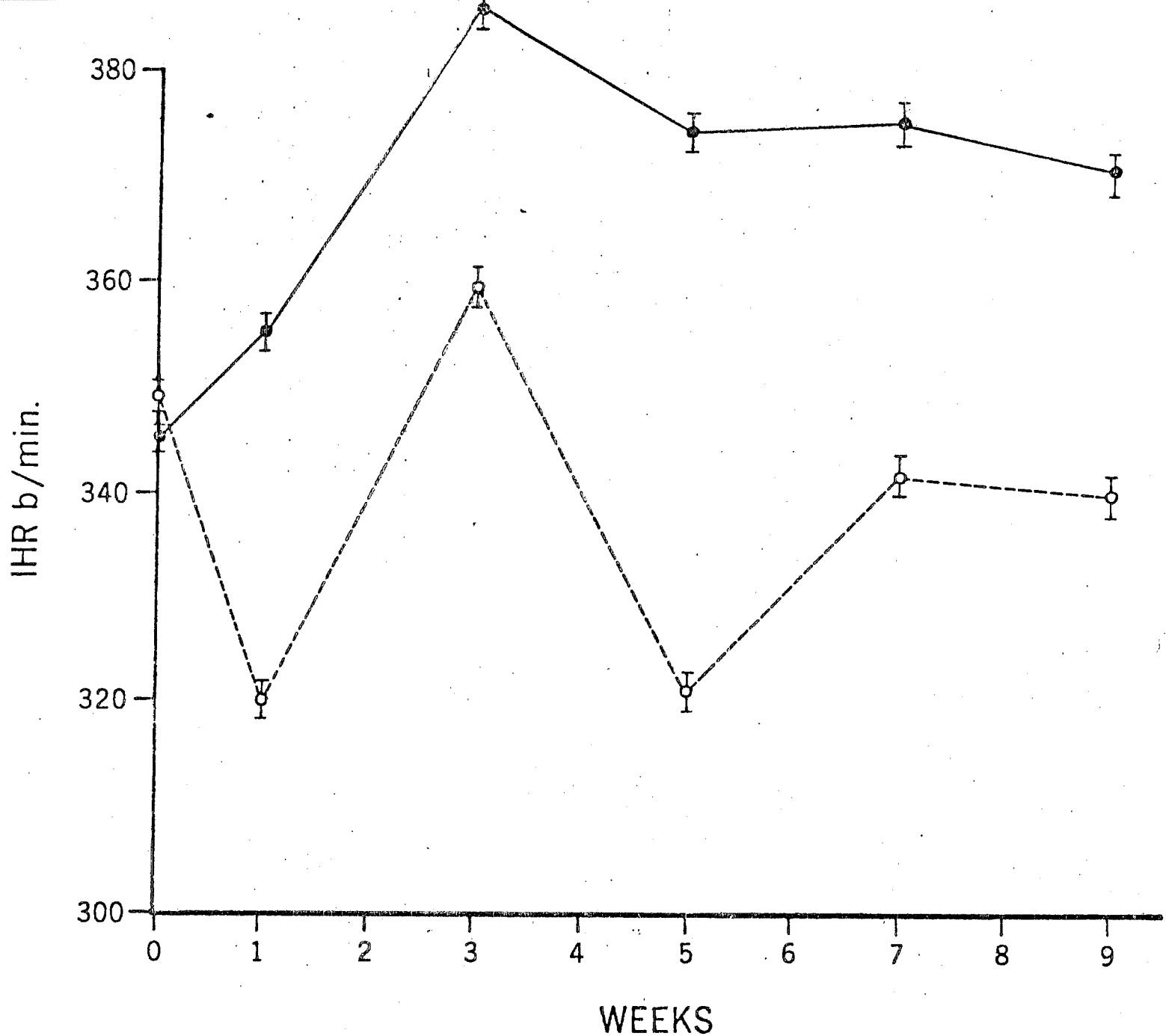

380
360
340
320
300
IHR b/min.
0
1
2
3
4
5
6
7
Weeks

Figure 5

Experiment #1. Intrinsic (pharmacologically blocked) heart rates of 6 controls and 17 trained rats over a period of nine weeks. •———•, control rats; o----o, trained rats. Points represent mean from 23 animals ± SEM.


380
360
340
320
300
IHR b/min.
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1
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3
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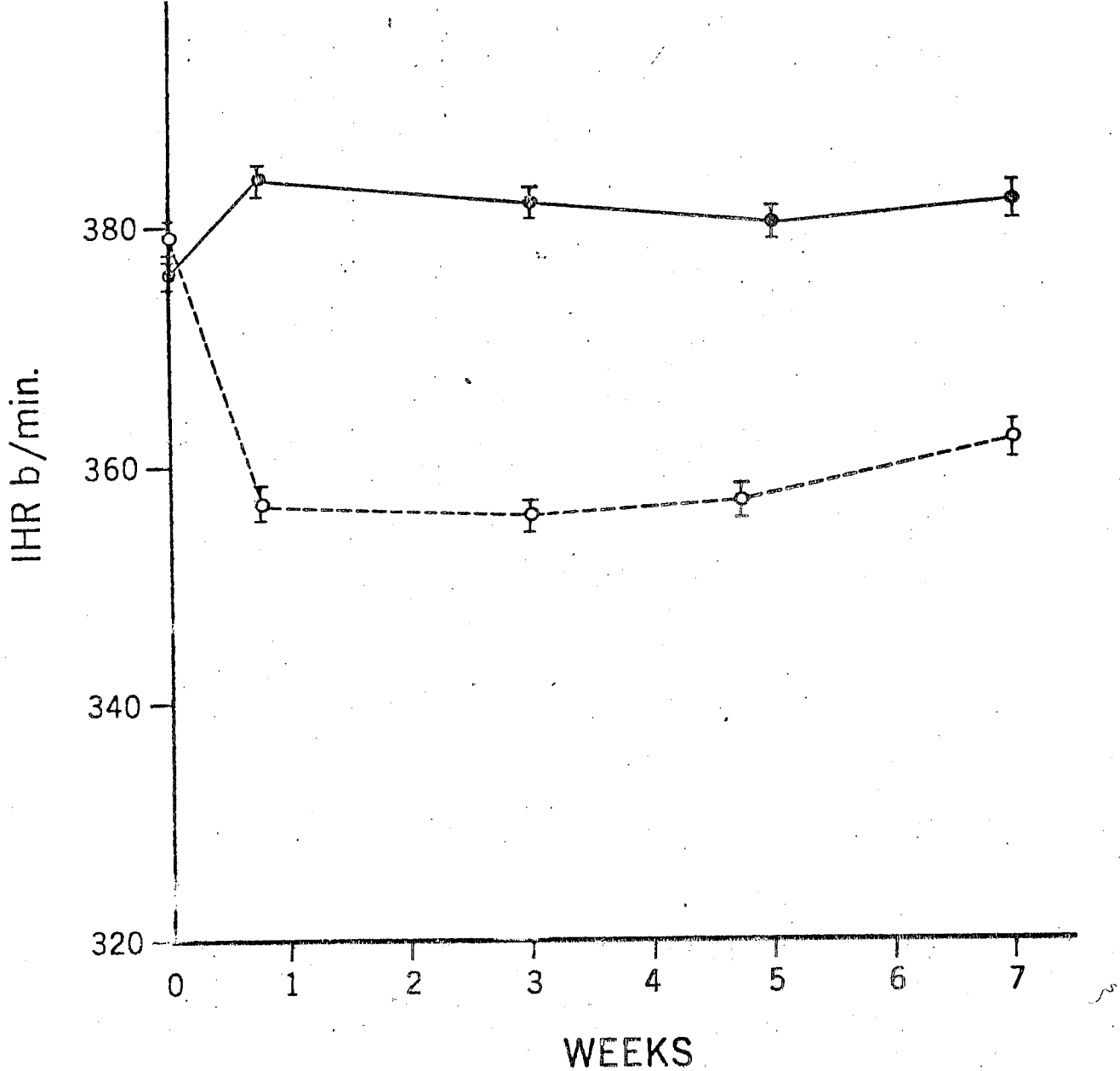
6
7
8
9
WEEKS

Figure 6

Experiment #2. Intrinsic (pharmacologically blocked) heart rates of 6 control and 18 trained rats over a period of seven weeks. •——•, control rats; o— — o, trained rats. Points represent mean from 24 animals ± SEM.


380
360
340
320
IHR b/min.
0
1
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4
5
6
7
WEEKS

191±11.7 beats/min and 252±12.01 beats/min in the first experiment ($P<.01$, Table VII, Figure 8). In the second experiment the rate difference was significant at 5% level of confidence ($P<.05$, Table VI). This is in close agreement with the results of Bolter et al. (1973), who reported a lower intrinsic heart rate in trained animals than controls with averages of 128±3 beats/min and 146±5 beats/min respectively.

IV. Atrial rate response to epinephrine

Isolated rat atria of control and experimental animals were treated with various concentrations of epinephrine. The basal atrial rate was first determined, than rate changes caused by various concentrations were measured. A microliter syringe was used to withdraw an amount of epinephrine from a vial which was then added to the organ bath in order to make the final concentration of epinephrine 10^{-5}M, 10^{-6}M and 10^{-7}M. After each exposure of the atria to epinephrine, the preparations were washed twice with Kreb's-Henseleit solutions and allowed to re-equilibrate to base line before they were subjected to a different concentration. As shown in Figures 10, 11 and Table V the addition of epinephrine in concentrations of $1x10^{-5}$M, $1x10^{-6}$M and $1x10^{-7}$M caused an increase in atrial rate in both control and experimental rats. Results are given as

Figure 7

Isolated atrial rates of 12 controls and 35 trained rats. Cont. = control, exp = experimental (trained) rats. Extension bar ± SEM..

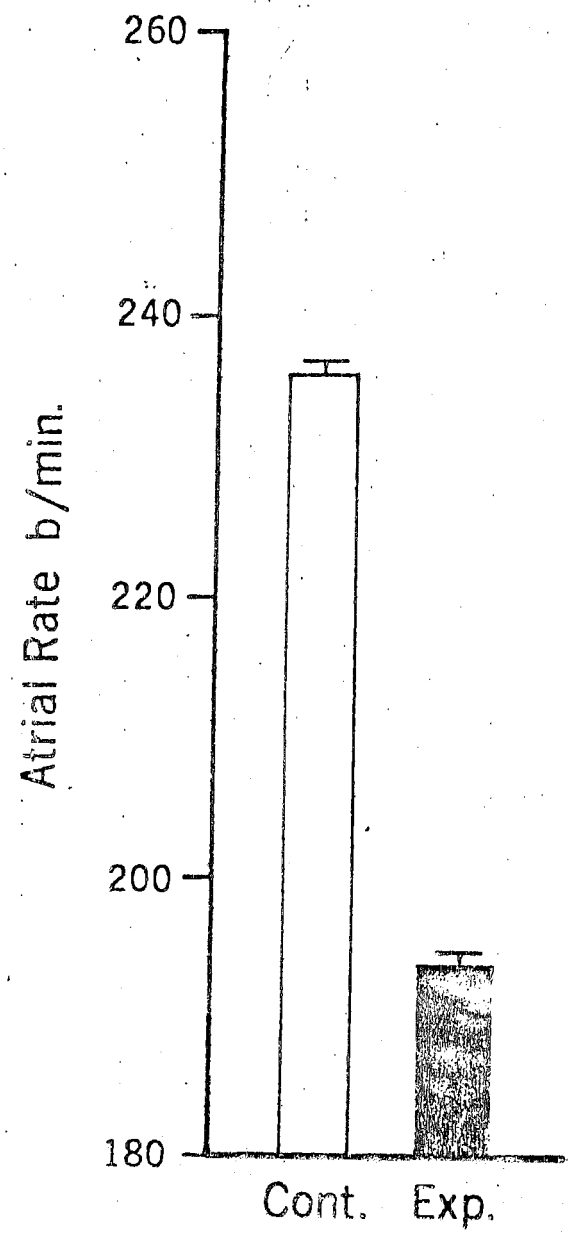

260
240
220
200
180
Atrial Rate b/min.
Cont.
Exp.

Figure 8

Experiment #1. Isolated atrial rates of 6 controls and 17 trained rats. Cont. = control, exp. = experimental (trained) rats. Extension bar = ± SEM.

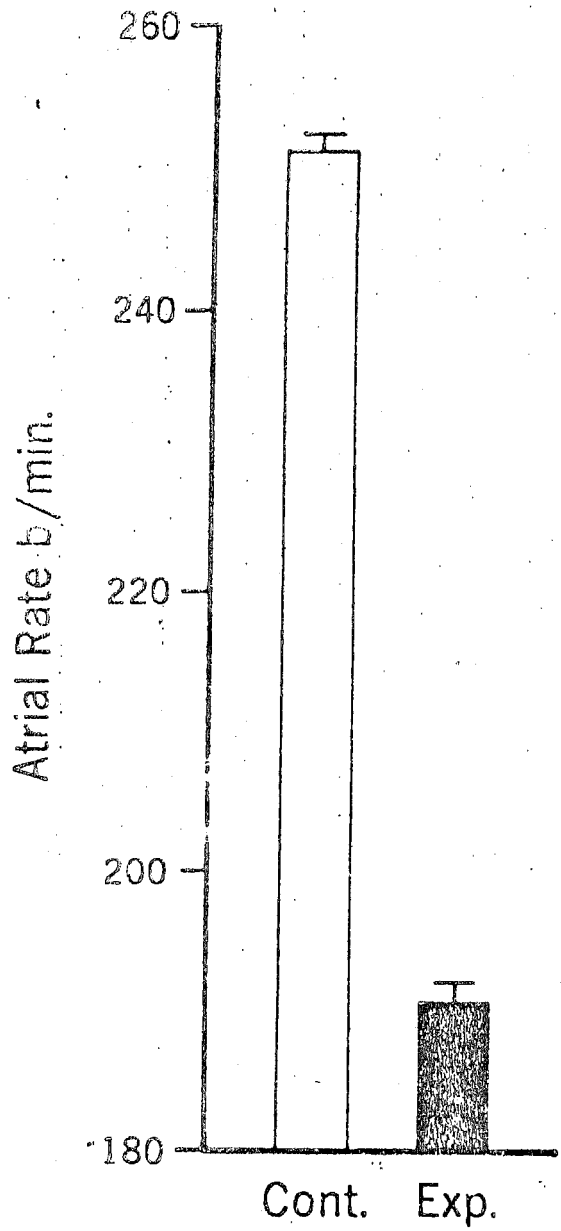

260
240
220
200
180
Atrial Rate b/min.
Cont.
Exp.

Figure 9

Experiment #2. Isolated atrial rates of 6 controls and 18 trained rats. Cont. = control, exp. = experimental (trained) rats. Extension bar = $\pm$ SEM.

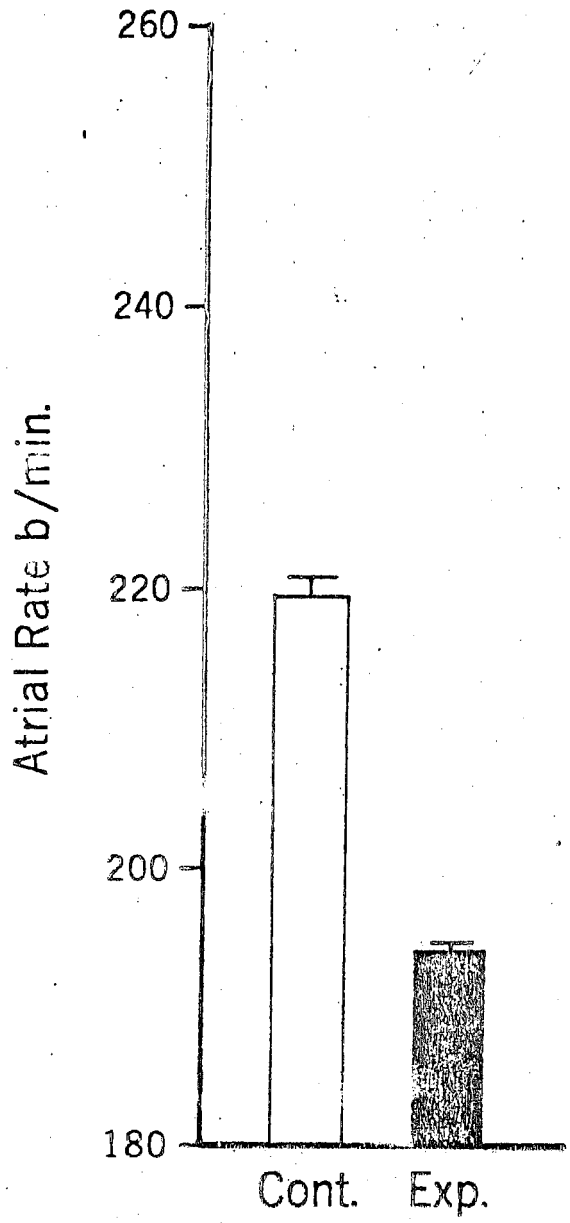
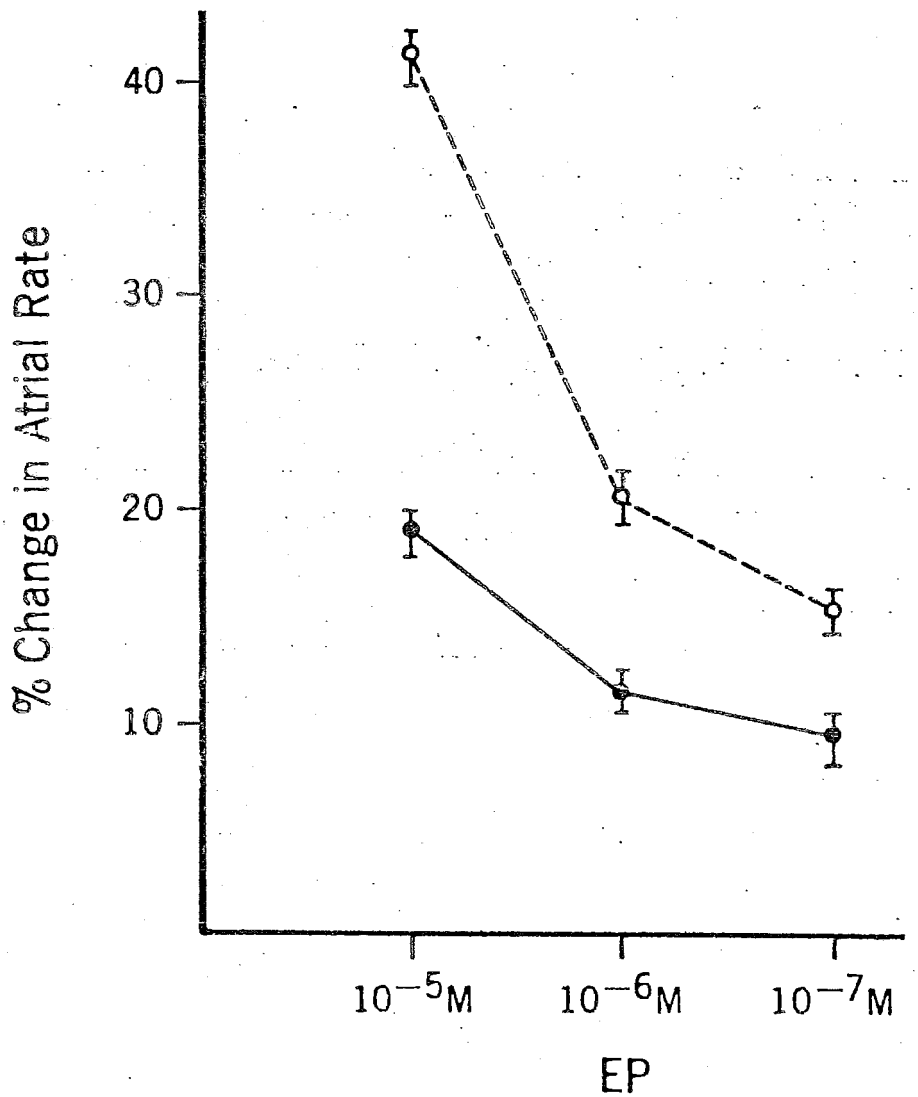

260
240
220
200
180
Atrial Rate b/min.
Cont.
Exp.

Figure 10

Effect of epinephrine on rate of isolated atria of control and trained rats in Experiment #1. •——• control rats; o---o trained rats. Points represent mean from 23 animals ± SEM.


40
30
20
10
% Change in Atrial Rate
10^{-5}M
10^{-6}M
10^{-7}M
EP

the percent change in atrial rate. In all preparations tested, trained rats show a greater increase in atrial rate, that is to say they were more sensitive to epinephrine. In trained rats epinephrine in the following concentrations of $1x10^{-5}M$, $1x10^{-6}M$ and $1x10^{-7}M$ increased the atrial rates by averages of $41.36 \pm 4.95\%$, $20.04 \pm 2.61\%$ and $15.32 \pm 2.82\%$ respectively. While the same order of concentrations of epinephrine added to isolated atria of control rats increased the atrial rates by averages of $18.61 \pm 2.54\%$, $11.1 \pm 1.42\%$ and $8.58 \pm 2.21\%$ respectively (Table V, Figure 11). The percent change in atrial rate after the addition of $10^{-5}M$ epinephrine in trained rats was significantly different from untrained rats ($P<.02$, Table V).

V. Atrial rate response to norepinephrine

Preparations of isolated atria of both groups were tested in the presence of norepinephrine in concentrations of $1x10^{-6}M$ and $1x10^{-7}M$. These amounts of norepinephrine caused a greater increase in the rate of atria from trained animals than from control animals. Average increases were $25.9 \pm 2.04\%$ and $19.7 \pm 4.06\%$ respectively. Norepinephrine $10^{-6}M$ caused a significantly greater rate change in trained animals than controls ($P<.01$, Table VI, Figure 12). From these results it is apparent that trained

Table V

Rate of isolated atrial rates of control and exercised rats (Experiment #1). Response to epinephrine.

	Experimentals (16)	Controls (6)	Probability
Atrial rate	190.8 ± 11.77***	252.5 ± 12.01	P<.01
10^{-5}M epi	262.2 ± 10.17	298 ± 10.79	
% change	41.26 ± 4.95**	18.61 ± 2.54	P<.02
Atrial rate	199.8 ± 10.69***	257.5 ± 12.18	P<.01
10^{-6}M epi	238.8 ± 12.62	286 ± 12.65	
% change	20.04 ± 2.61	11.10 ± 1.42	
Atrial rate	179.1 ± 10.16***	243 ± 19.44	P<.01
10^{-7}M epi	204.4 ± 15.5	262 ± 5.93	
% change	15.32 ± 2.82	8.58 ± 2.21	

Values are mean ± standard error with number of observations in parentheses.

** P<.02

*** P<.01

Table VI

Rate of isolated atrial rates of controls and exercised rats (Experiment #2). Response to norepinephrine.

	Experimentals (18)	Controls (6)	Probability
Atrial rate	193.5 ± 5.48*	218.2 ± 9.05	$P < .05$
10^{-6}M nor	242.6 ± 3.69	247 ± 9.74	
% change	25.9 ± 2.04***	13.3 ± 2.12	$P < .01$
Atrial rate	187.2 ± 7.92**	227.2 ± 13.76	$P < .02$
10^{-7}M nor	220.7 ± 7.28	245.0 ± 11.22	
% change	19.78 ± 4.06	8.26 ± 1.97	

Values are mean ± standard error with number of observations in parentheses.

* $P < .05$

** $P < .02$

*** $P < .01$

Figure 11

Effect of epinephrine on rate of isolated atria of control and trained rats in experiment #1.

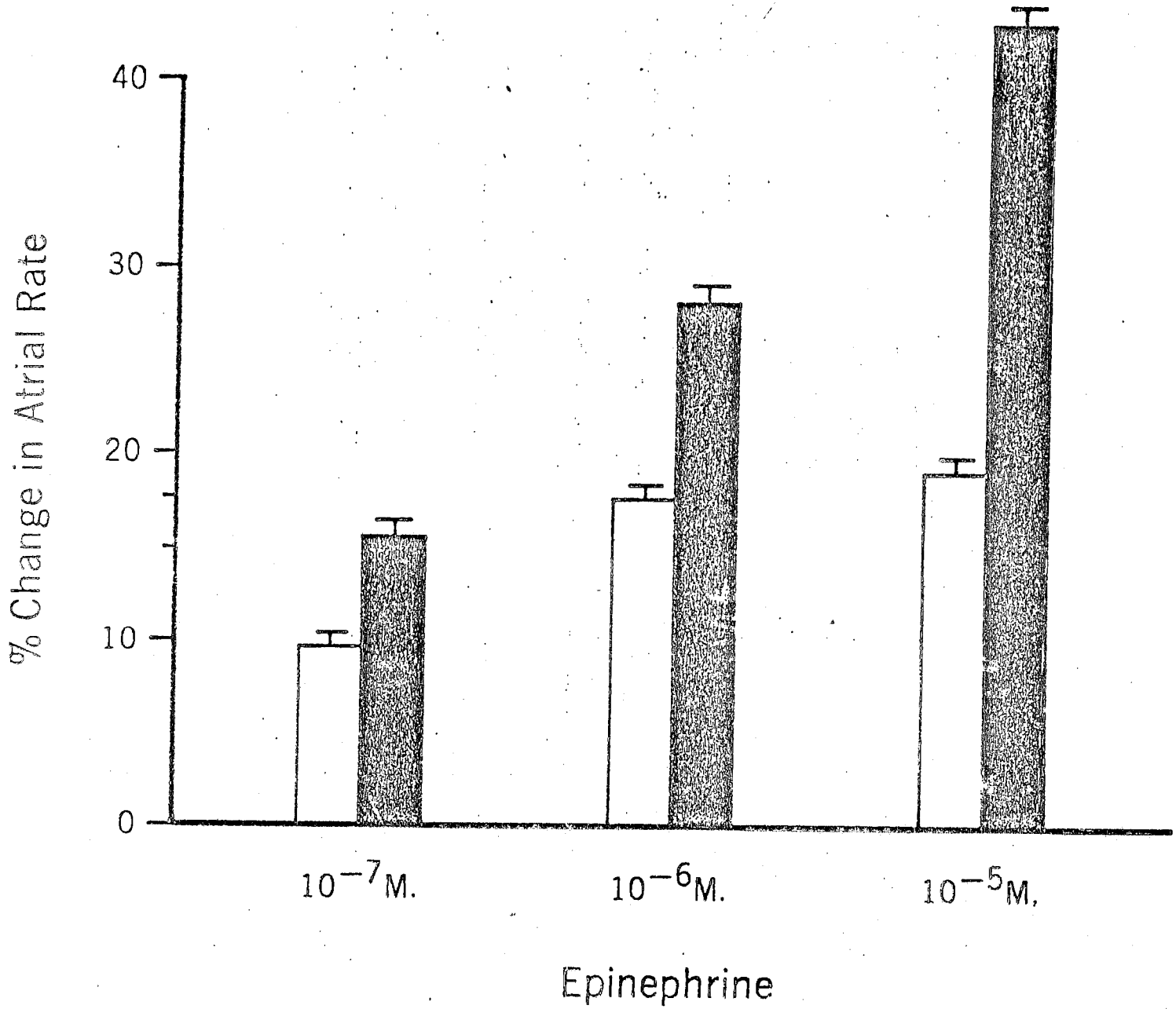
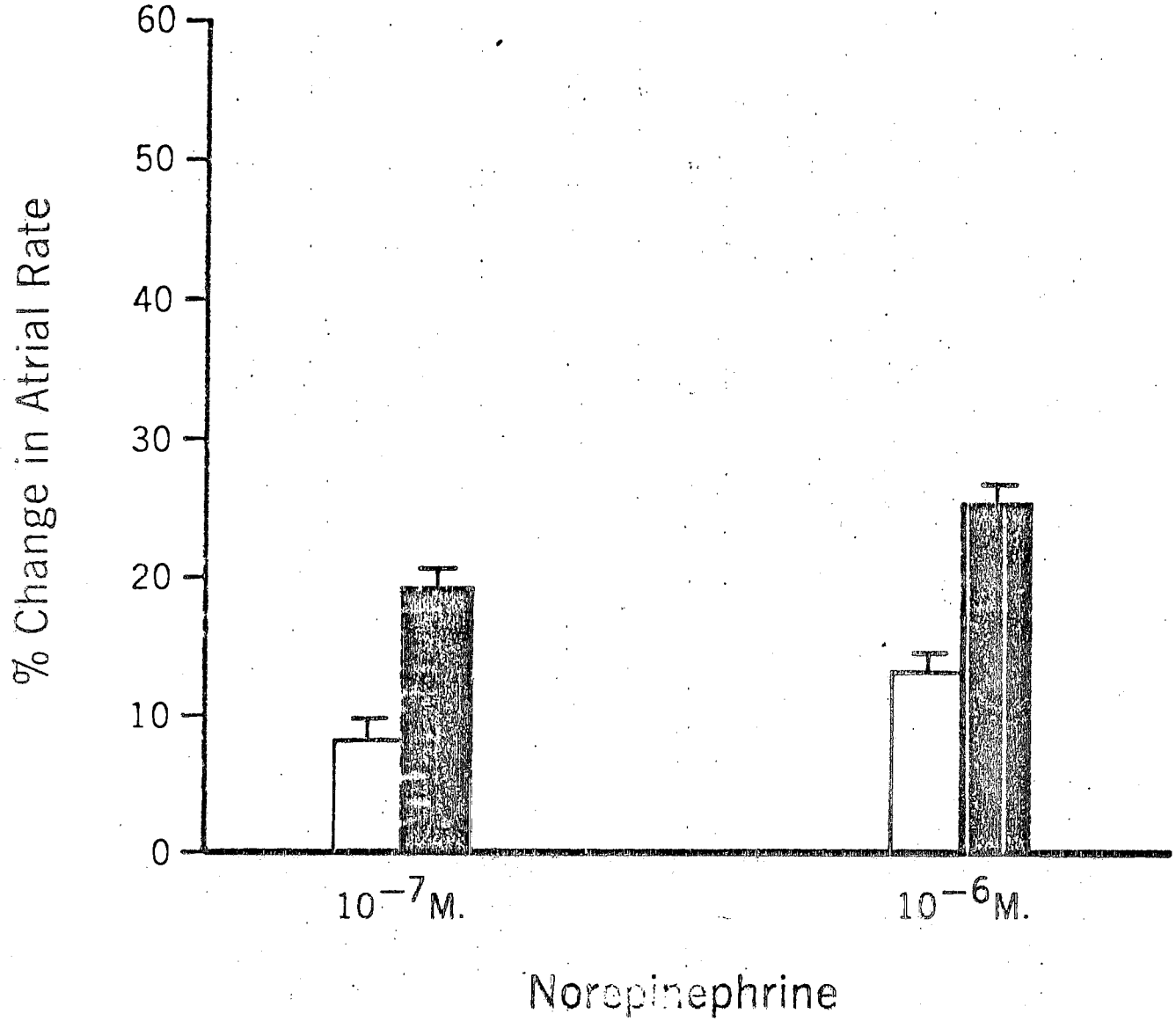

40
30
20
10
0
% Change in Atrial Rate
10^{-7}M.
10^{-6}M.
10^{-5}M.
Epinephrine

Figure 12

Effect of norepinephrine on rate of isolated atria of control and trained rats in experiment #2. Extension = ± SEM.


60
50
40
30
20
10
0
% Change in Atrial Rate
10^{-7}M.
10^{-6}M.
Norepinephrine

animals were more sensitive to both of these catecholamines than untrained animals.

VI. Atrial rate response to atropine

Atropine sulfate in concentrations of $1x10^{-5}M$, $1x10^{-6}M$ and $1x10^{-7}M$ added to isolated atria of control rats increased the atrial rates by averages $3.99 \pm 0.85\%$, $3.93 \pm 1.31\%$ and $1.88 \pm 1.21\%$ respectively (Table VII, Figures 13 and 14). When the same order of atropine concentrations were added to isolated atria from trained animals a greater increase in atrial rate was observed with averages of $24.1 \pm 4.5\%$, $11.19 \pm 4.35\%$ and $7.9 \pm 5.12\%$ respectively. Table VII shows that there is a significant difference between trained and untrained rats in the percent change in atrial rate after the addition of $10^{-5}M$ atropine ($P < .01$). The $1x10^{-5}M$ atropine added to the organ bath containing the isolated atria of trained rats caused an increase in atrial rate of trained rats to 242 beats/min, a rate almost identical to the basal atrial rates of control rats (250 beats/min). Student's t test indicated that there was no significant difference between the basal atrial rate of controls and that of experimental rats after the addition of $1x10^{-5}M$ atropine. While comparing both basal atrial rates before the addition of $1x10^{-5}M$ atropine there was a significant difference between

Table VII

Rate of isolated atrial rates of control and exercised rats (Experiment #1). Response to atropine.

	Experimentals (16)	Controls (6)	Probability
Atrial rate	201.1 ± 12.86*	250.2 ± 8.61	P<.05
10^{-5}M atropine	241.5 ± 12.33	260 ± 9.38	
% change	24.07 ± 4.50***	3.99 ± 0.850	P<.01
Atrial rate	203.8 ± 10.41***	260.5 ± 9.93	P<.01
10^{-6}M atropine	223.1 ± 9.14***	271 ± 11.32	P<.01
% change	11.19 ± 4.35	3.93 ± 1.31	
Atrial rate	203 ± 11.35*	253.5 ± 12.65	P<.05
10^{-7}M atropine	216.4 ± 12.28	258 ± 11.89	
% change	7.91 ± 5.12	1.88 ± 1.21	

Values and mean ± standard error with number of observations in parentheses.

* P<.05

** P<.02

*** P<.01

Figure 13

Effect of atropine on rate of isolated atria of control and trained rats in experiment #1. Extension bar = ± SEM..

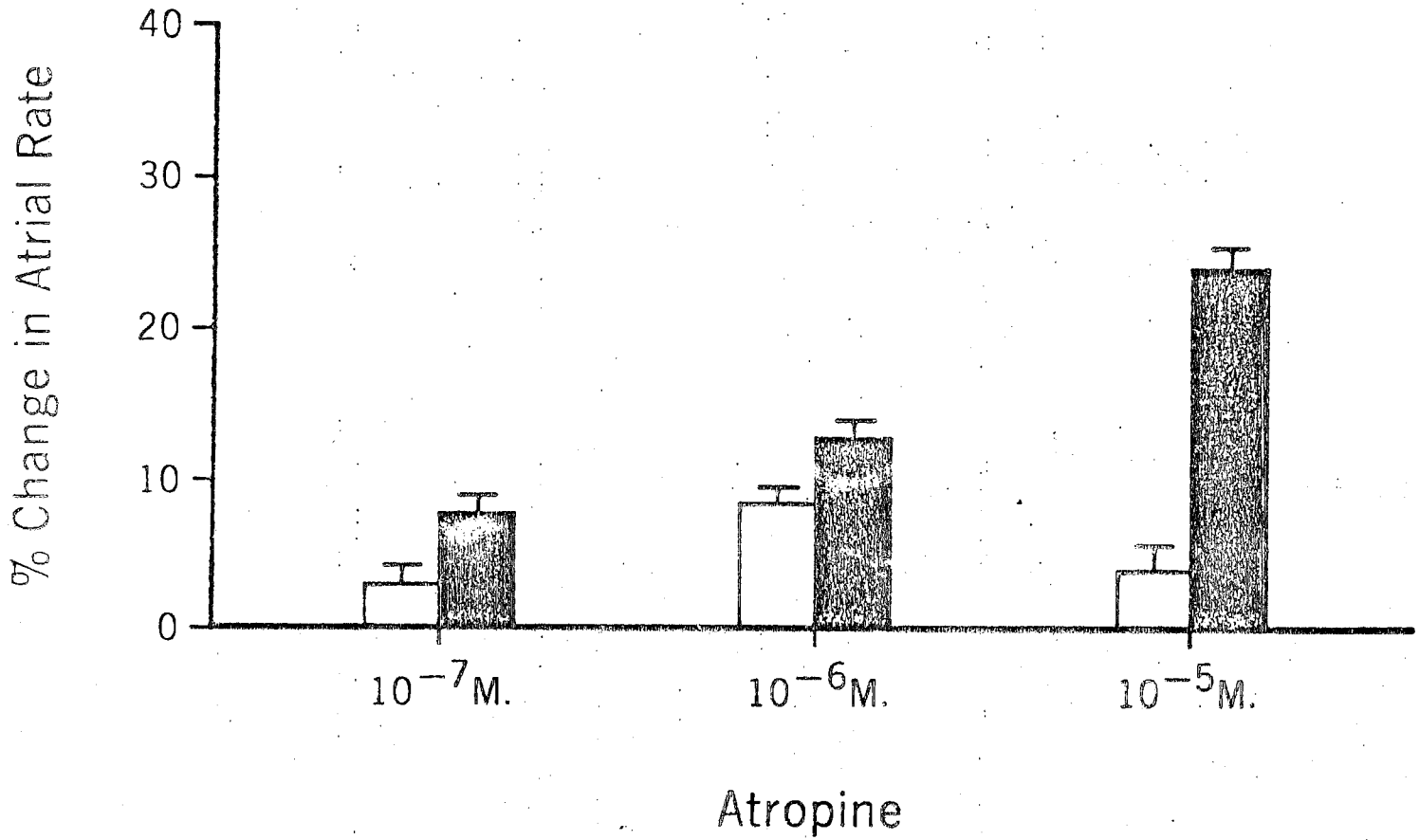
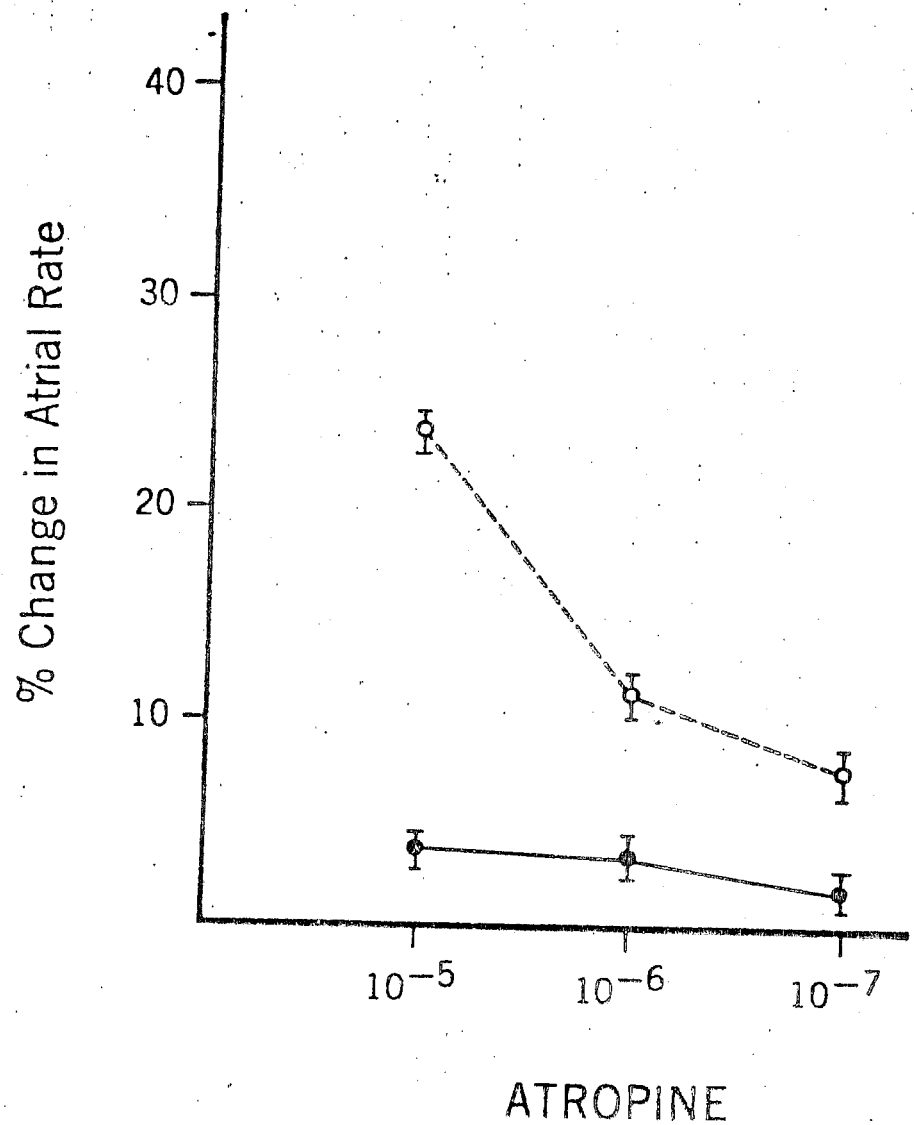

% Change in Atrial Rate
40
30
20
10
0
10^{-7}M.
10^{-6}M.
10^{-5}M.
Atropine

Figure 14

Effects of atropine on rate of isolated atria of control and trained rats in Experiment #1. ●——● control rats, o——o trained rats. Points represent mean from 23 animals ± SEM.


40
30
20
10
% Change in Atrial Rate
10^{-5}
10^{-6}
10^{-7}
ATROPINE

control and trained rats ($P < .05$, Table VII).

VII. Atrial rate response to acetylcholine and combined equimolar concentrations of acetylcholine and norepinephrine

When acetylcholine in concentration of 1×10^{-6}M was added alone to isolated atria of trained rats, there was a cessation (complete arrest) of atrial rate an average of 30 seconds immediately after administration. The atrial rate was counted following 1 to 1.5 minutes after the addition of acetylcholine. All atria resumed beating at their pre-acetylcholine rates after acetylcholine effective rate was measured. In control rats, however, no cessation of atrial rate was observed after the addition of acetylcholine (Figure 16). The percent change in atrial rate of trained rats was significantly less than controls with averages of $-28.2 \pm 6.40\%$ and $-48.4 \pm 4.3\%$ for control and trained rats respectively (Table VIII, Figure 15).

Since the cholinergic and adrenergic neurotransmitters individually produce opposite effects on heart, a procedure to determine whether one agent could counteract the effect of the other using equimolar concentrations of acetylcholine and norepinephrine would be of interest.

When equimolar concentrations of both acetylcholine and norepinephrine were present, a biphasic response in

Table VIII

Rate of isolated atrial rates of control and exercised rats (Experiment #2). Response to acetylcholine, norepinephrine and combined equimolar concentration of acetylcholine and norepinephrine.

	Experimentals (18)	Controls (6)	Probability
Atrial rate	178.2 ± 7.31*	213.5 ± 17.8	P<.05
10^{-6}M ACH	92.0 ± 8.34***	157.3 ± 24.07	P<.01
% change	-48.4 ± 4.3**	-28.2 ± 6.40	P<.02
Atrial rate	193.5 ± 5.48	218.2 ± 9.05	
10^{-6}M nor	242.6 ± 3.69	247 ± 9.74	
% change	25.9 ± 2.04***	13.3 ± 2.12	P<.01
Atrial rate	161.7 ± 10.54	203.0 ± 15.75	
Nor + ACH 10^{-6}M	187.7 ± 12.07*	243.0 ± 12.17	P<.05
% change	23.2 ± 11.7	22.6 ± 16.7	

Values are mean ± standard error with number of observations in parentheses.

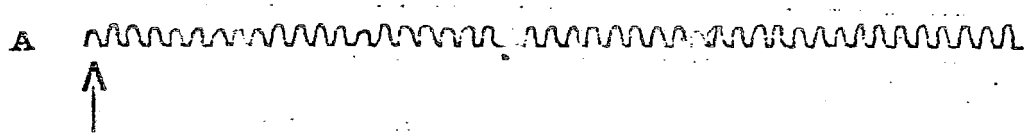
* P<.05

** P<.02

*** P<.01

Figure 16

Effect of acetylcholine on rate of isolated atria of control and trained rats in Experiment #2. A = control rats, B = trained rats.


A

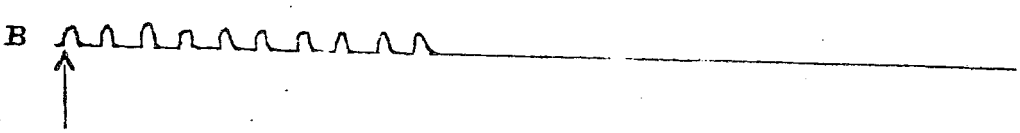
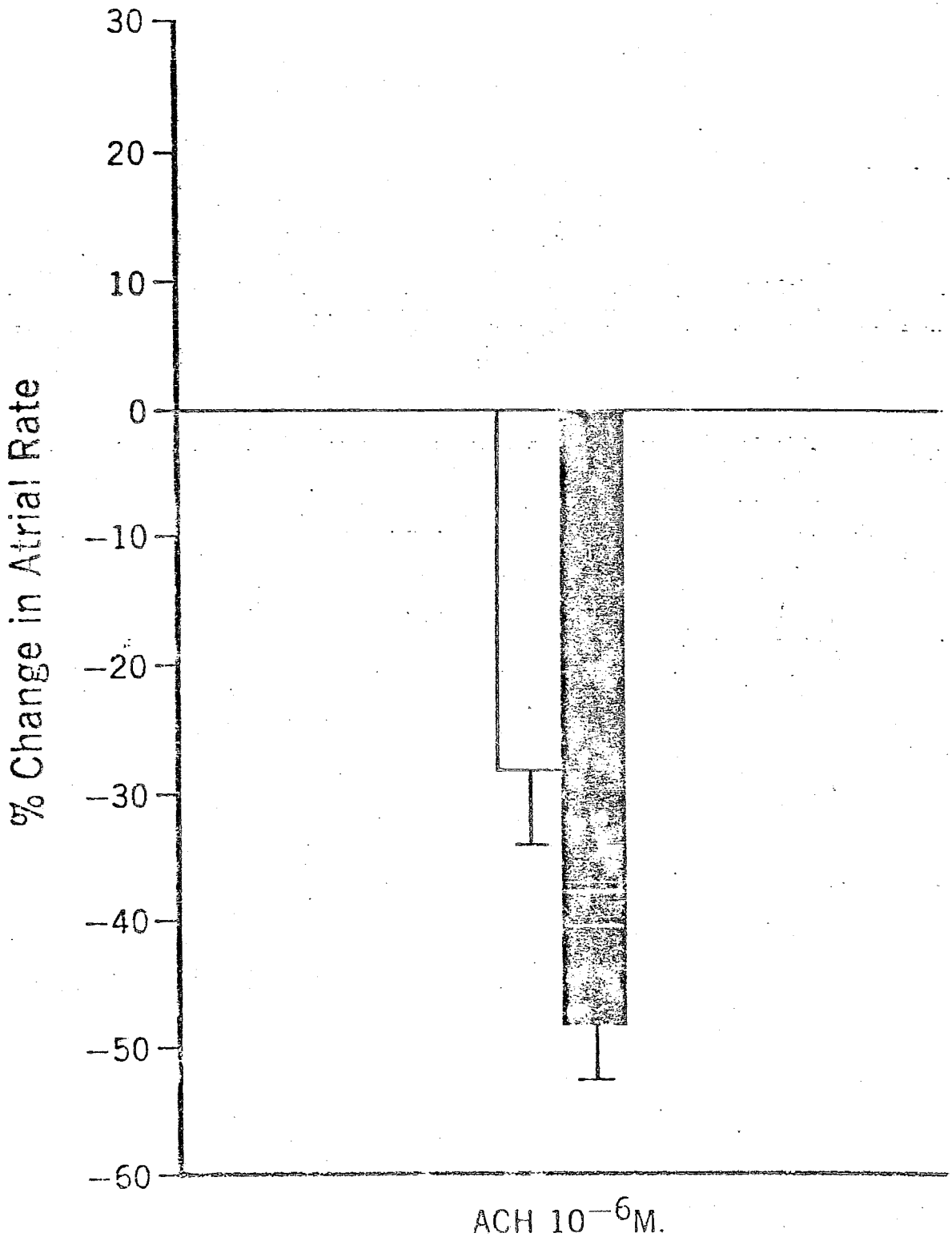

B

Figure 15

Effect of acetylcholine on rate of isolated of control and trained rats in Experiment #2. Extension bar = ± SEM.


30
20
10
0
−10
−20
−30
−40
−50
−60
% Change in Atrial Rate
ACH 10^{-6}M.

atrial rate was observed in atria from eight of the trained rats. At first a reduction in the atrial rate was observed which was similar to the cessation seen when acetylcholine was present alone in isolated atria of trained rats. The cessation was then followed by atrial acceleration. In the rest of the trained animals the presence of both neurotransmitters produced a simple decrease in atrial rate, but the beat was not arrested (Figure 17). One may conclude that acetylcholine somehow interfered with the chronotropic effects of norepinephrine (Table VIII).

Figure 17

Effect of combined equimolar concentrations of acetylcholine and norepinephrine. Extension bar = ± SEM.

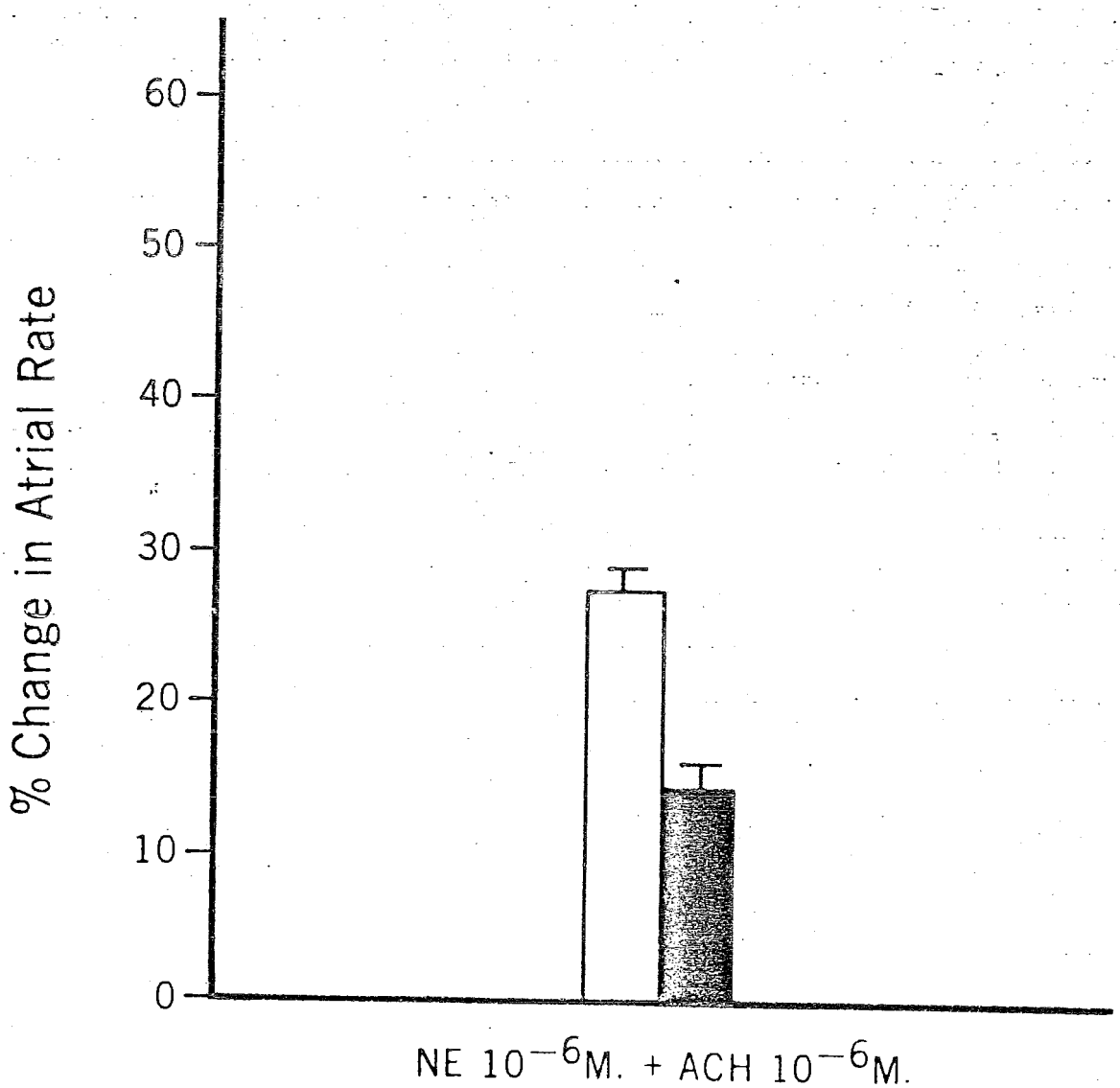
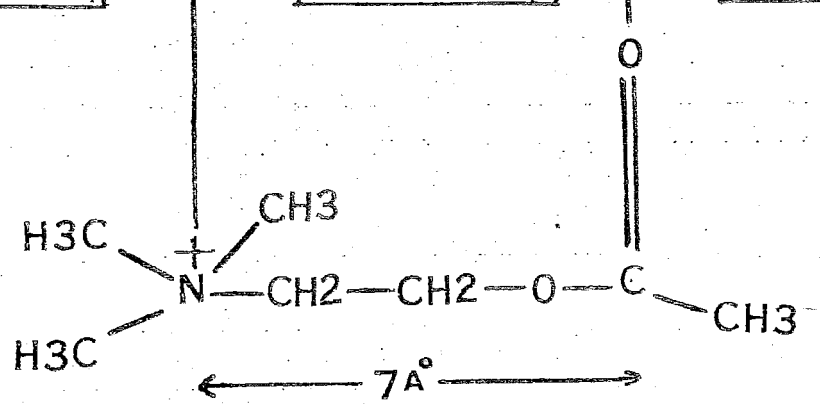

% Change in Atrial Rate
60
50
40
30
20
10
0
NE 10^{-6}M. + ACH 10^{-6}M.

Figure 18

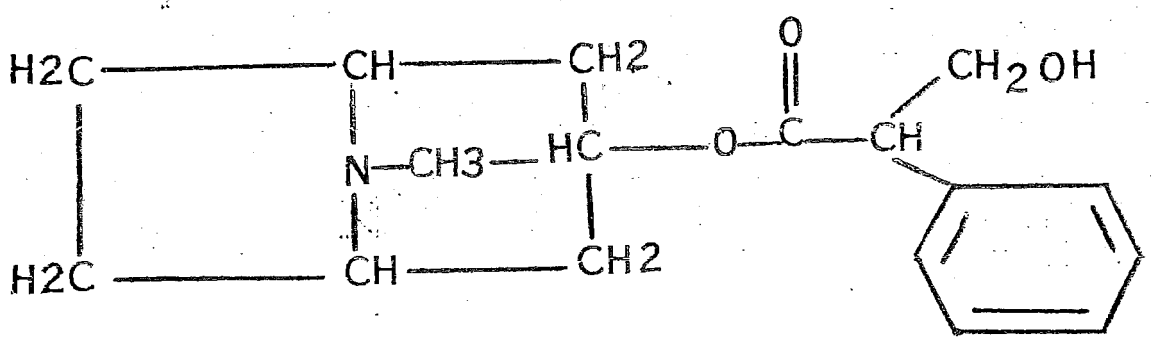
Possible interaction between acetylcholine, the antagonist (atropine) and the hypothetical receptor site.
A -- Hypothetical muscarinic receptor
B - Acetylcholine
C - Atropine

A

B


H3C
CH3
N
CH2
CH2
O
C
CH3
H3C
7A°

C


H2C
CH
CH2
O
CH2OH
N
CH3
HC
O
C
CH
H2C
CH
CH2

DISCUSSION

Training induces physical stress which leads to a characteristic change in body physiology, and more specifically a change in the cardiovascular system (Fox et al., 1964 and Saltin et al., 1968).

The body weight was lower in trained animals than in controls (Table II). This observation could be accounted for by an "anorexic" effect or an increase in the metabolic demands of the body (Thomas et al., 1958 and Stevenson et al., 1966). The lack of a significant change in heart weight, ventricular weight, atrial weight and their ratios to body weight between trained and non-trained male rats in the first experiment indicate no cardiac hypertrophy as manifested by heart weight during the course of this training period (Table I). A change in cardiac function resulting from the training program is evidenced by the resting and intrinsic heart rates. Trained animals showed lower resting and intrinsic heart rates than control animals (Tables III and IV). The term "intrinsic heart rate"

has been used in a number of ways in the literature. Ideally the term would indicate the rate of a heart which is under no neural, humoral or mechanical influence. Since such a condition is not possible under most experimental conditions, the term has been used to indicate a heart rate during pharmacologic autonomic blockade (Jose and Stitt, 1966) or the rate of an isolated atrium (Bolter et al., 1973). The term is used here to indicate a pharmacologically blocked heart. The heart can be pharmacologically isolated from the natural sympathetic and parasympathetic activities when the beta receptor adrenergic blocker, propranolol, and the cholinergic muscarinic receptor blocker, atropine, are given simultaneously (Jose and Stitt, 1966). The change in cardiovascular system associated with repeated exercise is a decrease in heart rate (Coton, 1932 and Knehr et al., 1942). This characteristic is pronounced when athletes are compared with non-athletes. Many explanations have been posited to account for this. Hall (1963), for example, associated the bradycardia with an increase vagal cholinergic activity or sympathetic inhibition. Herxheimer (1921) suggested that training period augments metabolic demands of the heart and this may develop vascular changes which will cause an increase in heart volume and eventually leads to bradycardia.

Some of the responses evoked by training could be accounted for by the activity of multiple muscarinic cholinergic receptors. These are associated with post-synaptic vagal nerve endings and are specifically blocked by atropine. If the decrease in heart rate was due to increased parasympathetic activity it would have been eliminated by atropine and the pre- and post-training heart rate would be identical.

Previous studies have shown the administration of atropine to intact animals will cause an increase in the resting heart rate by competing with acetylcholine for the receptor sites (Tipton et al., 1965, Figure 18). Tipton et al., (1965) reported that in intact animals, trained rats show less cardiac acceleration to atropine than non-trained rats. He attributed this to the increased stores of acetylcholine which compete with atropine for receptor sites. Grodner et al., (1970) confirmed the latter findings in non-trained isolated rat atria. Herrlich et al., (1960) found a considerable increase in the aceylcholine content in the atria of trained animals. In my study the isolated atrial rate data (atropine) from non-trained rats are in agreement with Grodner's finding. Tipton's results do indicate that animals with more available acetylcholine in the heart will exhibit a lesser cardiac response to cholin-

ergic inhibitors. It is known that the response to cholinergic inhibitors is related to dosage level.(Cullumbine, et al., 1955).

In the present study, the results of the isolated atria from trained rats showed significantly more acceleration in response to atropine than did atria from non-trained rats (Table VII). The source of difference in response between my results and those of Tipton's could be that they used intact rats and did not include higher atropine dosage levels that could compete with increased stores of acetylcholine. Thus, the lowered atrial rate observed in trained animals may be accounted for by increased stores of acetylcholine in the atria. The inhibitory effect of this acetylcholine could be blocked by atropine and the rate of beating of isolated atria from trained rats should be identical to the basal atrial rate of control rats. I found this to be the case in the present study. The $1x10^{-5}M$ atropine concentration employed in my experiments caused an increase in isolated atrial rates of trained rats to a rate almost identical to the basal atrial rate of atria from non-trained rats (Table VII). The acetylcholine-atropine antagonism in isolated atria appears to be large enough to offset the acetylcholine induced lower rates, although no attempts were made to determine the exact amount of atropine that

would prevent the decrease of heart rate produced by acetylcholine. The dynamic changes which enhance the synthesis release, and degradation of acetylcholine by cholinesterase could account for the bradycardia exhibited in trained animals.

Vik et al., (1962) reported that the atria from non-trained rats possess high cholinesterase activity. The fact that addition of 1×10^{-6}M acetylcholine to isolated atria of trained animals in the present study caused a complete arrest of 30 seconds, an observation not noted in non-trained animals, led me to wonder whether the cholinesterase activity is perhaps lower than that in non-trained rats at the pace-maker cholinergic receptor. It is also possible that the addition of acetylcholine diffused more homogeneously to the isolated atria than when it is released at the nerve endings.

One explanation for the bradycardia evoked by training is caused by increased stores of acetylcholine. This effect of increased stores of acetylcholine could then rule out the increase in vagal cholinergic activity as the major cause of exercise bradycardia. This is supported by the fact that the addition of atropine to isolated atria of trained rats caused the atrial rate to increase to a rate identical to the basal rate of isolated atria of non-trained rats (Table VII). Atropine does not interfere with acetylcholine formation

but competes with acetylcholine for receptor sites.

Another conceivable explanation for the bradycardia is a change in receptors. This could be a change in sensitivity of the receptors, for example, a change in adenyl cyclase activity or an increase in the number of receptor sites. Additionally a decrease in the cholinesterase activity in trained animals would permit increased store of acetylcholine.

Catecholamines are known to accelerate heart rate. Myocardial cells are considered to contain two distinct receptors, the alpha and the beta receptors. Gordon et al., (1966) and Sheldon et al., (1975) indicated in their studies that forced running caused an increase in the incorporation of ^{14}C-tyrosine into catecholamines in the heart. However, the increased synthesis of catecholamines did not increase the catecholamines content. This could be accounted for by an augmented turnover in catecholamines. The catecholamine content of the myocardium is lower in hearts of trained rats (De Shryver et al., 1969).

Russek et al., and Vendsalu (1960) associated the "appetite-suppressing" effect induced by exercise with increased levels of catecholamines. This is not in agreement with the most recent findings of Sheldon et al., (1975). In the present study the isolated atria of trained rats were

compared with control animal atria. In all preparations tested, trained rats show a greater increase in atrial rate in response to either epinephrine or norepinephrine; that is to say trained rats were more sensitive to catecholamines. The greater sensitivity of isolated atria of trained animals to catecholamines observed in this study favors and supports the most recent findings of Sheldon et al., (1975).

Sutherland et al., (1965) have shown that epinephrine added to broken cell preparations leads to enhanced cyclic AMP. There are indications that acetylcholine stimulates cyclic GMP production in rat hearts (George et al., 1970). Acetylcholine also acts by reducing the elevated cyclic AMP induced by catecholamines administration in guinea pig isolated hearts (Murad et al., 1962 and Chamales et al., 1971). Chamales et al., (1971) reported that only concentrations of acetylcholine that lead to a bradycardia can antagonize the chronotropic response of epinephrine. The interference of acetylcholine with the chronotropic response to norepinephrine and the blockade by atropine may suggest a possible role of cyclic AMP and/or cyclic GMP.

The change in heart rate observed in trained animals could be accounted for partly by a reduction of cyclic AMP formations caused by increased stores of acetylcholine and/or receptors. It has been suggested that the positive

effect of catecholamines on the inotropic and metabolic action is related to the activation of adenyl cyclase on the membrane and that the secondary messenger is somehow involved in differential effects of acetylcholine and norepinephrine. Krause et al., (1970) showed that N^6-$O^{2'}$-dibutyryl cAMP a derivative of cyclic AMP increased the contraction frequency in isolated cultured rat cells indicating that the positive chronotropic action of catecholamines is mediated by the formation of cyclic AMP.

Warner et al., (1969) have developed a model for heart rate control in which the heart rate is maintained by synergistic action of the adrenergic and the cholinergic systems. Rosenblaeth et al., (1934) reported that the influence of acetylcholine on the change in atrial rate is independent of the presence of norepinephrine and vice versa. This is in agreement with the recent findings of Grodner et al., (1970) and Carrier et al., (1971).

Acetylcholine blocks the chronotropic and inotropic action of catecholamines (Meester et al., 1969). Furchgott et al., (1969) reported that acetylcholine is capable of counteracting the effect of catecholamines in guinea pig atria, and that the ability of catecholamines to enhance glycogenolysis may be inhibited by acetylcholine (Allotey et al., 1969). In the presence of equimolar concentrations of both

norepinephrine and acetylcholine a pure cholinergic response was obtained (Carrier et al., 1971). In the present study a clear suppressing effect was seen when the isolated atria were subjected to equimolar concentrations of combined acetylcholine and norepinephrine.

The results obtained in this experiment on eight trained rats show that acetylcholine is capable of possessing a suppressor effect on the chronotropic response to norepinephrine. This is in agreement with the findings of Samann et al., (1935), Warner et al., (1969) and Carrier et al., (1971) who showed that an increase in vagal activity causes a reduction in sympathetic effects. In contrast the norepinephrine effect dominates in the other ten experimental rats. The 1×10^{-5}M atropine concentration blocked acetylcholine for the receptor sites. If propranolol was added to isolated atria to prevent norepinephrine from influencing acetylcholine, one may conclude that the interference of acetylcholine with the chronotropic effect of norepinephrine could not occur at the receptor as was suggested by Carrier et al., (1971).

The response observed in isolated rat atria in the presence of equimolar concentrations of acetylcholine and norepinephrine produces a biphasic atrial change. The term biphasic has been used to indicate the early decrease in

isolated atrial rates followed by a subsequent increase in atrial rate seen on eight trained rats in the second experiment. This biphasic response may be accounted for by the ability of acetylcholine to block the beta receptors, thus inhibiting the ability of norepinephrine to increase the atrial rates. The cessation seen in the isolated atrial rate of trained rats after the addition of acetylcholine seperately, and in combination is perhaps an indication of low cholinesterase activity in the pacemaker. This would permit an increase in the concentration of acetylcholine which would in turn induce a low rate.

In conclusion my results suggest that the bradycardia evoked by training is explainable in terms of increased stores of acetylcholine at the sinoatrial node or atrial myocardium which in turn accounts for the decrease in intrinsic atrial rate in trained animals. The rates measured in these experiments are in close agreement with the results of Bolter's et al., (1973). The higher atrial rates obtained in my experiment as compared to Bolter's observation could be accounted for by the difference in temperature at which the experiments were conducted. The difference in response to acetylcholine could be accounted for by the difference in time at which the atrial rate was measured. According to Jose and Stitt (1966) the intrinsic

rate depends on the electrolyte distribution at the myocardial membrane. It would be of interest to investigate the possibility that changes in electrolyte distribution in trained animal hearts may account for the bradycardia of exercise.

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