ELECTROPHYSIOLOGICAL EFFECTS OF VERATRAMINE ON CAT ATRIA

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of

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Harold A. Sures

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ABSTRACT

The purpose of this investigation was to study some of the electrophysiological correlates of the negative chronotropic action of veratramine, as well as those correlated with the production of "periodic rhythm" induced by veratramine treatment. The preparation used was the isolated spontaneously beating right atrium of the cat.

The effect of veratramine on the spontaneous rhythm of the isolated atrial preparation was dependent on the concentration of drug to which the atrium was exposed and the duration of exposure. Veratramine 10^{-7} to 10^{-5} g/ml routinely caused only slowing of the spontaneous rate. In the presence of 1-3 x 10^{-5} g/ml veratramine, spontaneous atrial rate decreased progressively prior to the development of periodic rhythm. The establishment of periodic rhythm in the presence of veratramine was marked by a period of asystole which usually lasted 15-20 seconds and was followed by recommencement of atrial rhythm. Thereafter alternating periods of inactivity and activity of the atria continued for up to 30 minutes. The active phases of the periodic rhythm were characterized by a gradually decreasing rate leading to a period of asystole.

In the presence of 1-3 x 10^{-5} g/ml veratramine, the periodic rhythm eventually resolved into either a regular but slower rhythm (as compared to control), or the atria

became quiescent.

When the atrial preparation became quiescent following periodic rhythm, it was found that regular electrical stimuli could still evoke responses if stimulus strength was increased over that necessary to drive untreated atria, but the response did not follow regularly on a one to one basis, indicating a possible blockade of conduction. When veratramine 1-3 x 10^{-5} g/ml was left in contact with the muscle for one hour and then washed out, electrical stimulation had no effect. However, although the atrium was quiescent, a small "pacemaker" area of about 1.5 - 2.5 sq. mm was active electrically and mechanically. This small area contracted regularly and was easily identified by these contractions.

When the isolated atrium was exposed to a larger dose of veratramine (7 x 10^{-5} g/ml), the preparation became entirely quiescent without passing through the stage of periodic rhythm. The process was complete in five to ten minutes and the drug was washed out. The discrete active "pacemaker" area became obvious shortly after the drug was washed out and remained active while the rest of the preparation remained quiescent.

Coincident with the decline in spontaneous rate in the presence of veratramine, the atrial transmembrane action potential was altered in a progressive continuous manner prior to and during the occurrence of periodic activity.

The effect of veratramine on the atrial action potential was manifest as a reduction of the action potential amplitude and overshoot, an increase in the rise time of the upstroke of the action potential, and a prolongation of the repolarization phase of the action potential. The resting membrane potential remained not significantly different from that of the untreated control. Qualitatively, the effects of veratramine on the atrial action potential during periodic activity were similar to those observed when spontaneous rate had only been reduced by veratramine. Quantitatively, however, the effects of veratramine, reflected in the values of the action potential parameters, were greater during the active phases of periodic rhythm than when spontaneous atrial rate had only been decreased in the presence of veratramine. The effects of veratramine on action potential parameters appeared to be consistent with an interference with the sodium and potassium processes that occur during the depolarization and repolarization phases of the action potential.

In the area of the discrete "pacemaker" which remained active following veratramine treatment and washout, cells displayed action potential parameters consistent with pacemaker or latent pacemaker action potential characteristics. Around the "pacemaker" area was found an area of tissue approximately 5 mm in diameter in which some cells were active; the record of intracellular transmembrane potential

showing abortive spikes, small summating depolarizations with irregular action potentials sometimes superimposed.

The microelectrode was positioned so that the cells impaled in sequence were located progressively closer to and finally within the pacemaker area. With proximity to the pacemaker area amplitude of transmembrane electrical activity increased. Abortive spikes, small depolarizations and irregular action potentials were less apparent in the records of transmembrane potential of cells located more proximal to the pacemaker area. Electrical activity appeared to become more regular and smooth diastolic depolarization was observed. Within the pacemaker area rhythm appeared to be regular and irregular electrical activity did not impinge on the recorded action potentials. Outside the area immediately surrounding the pacemaker area, the cells examined maintained the normal resting membrane potential, but no action potentials occurred despite the ongoing activity of the cells of the pacemaker area. Conduction of electrical impulses from the veratramine-resistant pacemaker area to the common atrial tissue appeared to be interrupted in a decremental fashion through a "transitional" area of tissue surrounding the pacemaker area.

The negative chronotropic effects of veratramine were transiently antagonized by catecholamines. In addition, the effects of veratramine on the atrial action potential were partially antagonized by catecholamines, which con-

currently restored a regular rhythm when periodicity had been established or when veratramine had caused cessation of atrial activity. Catecholamines did not completely restore the action potential to the configuration observed before veratramine treatment and eventually periodic activity replaced the regular atrial rhythm which had been produced by the addition of catecholamines.

Aminophylline, caffeine, dibutyryl cyclic AMP, valinomycin or modification of the ionic composition of the fluid bathing the atrial preparation did not prevent or reverse the negative chronotropic effects of veratramine, did not prevent the development of periodic activity, and did not restore a regular rhythm when periodicity had been established. These treatments were also ineffective in reversing the effects of veratramine on the atrial action potential.

Carbachol which in the untreated atrial preparation slowed spontaneous rhythm and increased the rate of repolarization of the atrial action potential, did not increase repolarization rate slowed in the presence of veratramine.

Pretreatment of the atrial preparation with tetrodotoxin enhanced the effects of a given dose of veratramine. In the presence of tetrodotoxin, veratramine, in a dose which routinely caused only slowing of the spontaneous atrial rhythm, now produced periodic activity. In the isolated atrial preparation variation of the concentrations of sodium and potassium in the bathing fluid had little

effect on the consequences of veratramine treatment. It is suggested that the mechanism of action of veratramine involves modification of specific sodium and potassium processes resistant to or not readily affected by alteration of the bathing fluid medium. Changes induced in the atrial preparation by veratramine appear to be irreversible.

To my Wife Susan

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INTRODUCTION

INTRODUCTION

Initial interest in the veratrum alkaloids was stimulated by their structural resemblance to the cardiac glycosides (Fig. 1). As earliest information on the physiological properties of the veratrum alkaloids was based on results obtained with extracted mixtures of alkaloids, it was proposed that study of the pure individual alkaloids was imperative for the development of these agents as significant pharmacological tools and in the development of a rational basis for the clinical use of these agents. Although reference is made to the use of crude preparations of veratrum alkaloids in obstetric clinics in the treatment of eclampsia, and single alkaloids in hypertensive crisis (Krayer and Acheson, 1946), the severe toxicity of these compounds has, to date, precluded their clinical use.

Veratramine, when given to anesthetized dogs and cats, can, via a stimulating action on the central nervous system, produce clonic convulsions despite the depression of the higher areas of the central nervous system produced by the anesthetic (Krayer, 1949a; 1949b; Krayer and Reiter, 1950). Only one instance of administration of veratramine in man appears in the literature (Marsh et al, 1951). After the administration of 250 μg of veratramine, orally in water to a 32 year old male, no convulsions were observed over a period of 2 hours. The following observations were recorded by Marsh et al., 1951, beginning 51 minutes after

administration of veratramine:

> "At this time the subject became unable to write, talked incoherently for a few minutes, slobbered copiously, and talked almost no more for two hours. During this two-hour period he would not sit up nor lie down but remained huddled in a semi-squatting position in a corner of the room. He would raise his head long enough to vomit but would not cooperate to have blood pressure readings taken. ..... Only a minor amount of muscular rigidity was observed and no convulsions."
>
> "After the two-hour period he slowly improved, asked to be taken home, slept restlessly for two hours, and some time after awakening ate a normal meal. Two hours later some dark urine was voided and three hours later more. There was some bronchoconstriction as evidenced by mild wheezing and a dry cough. He complained that he became weak and had palpitations on sudden exertion. He was essentially recovered the following day and commented only on the severe vertigo that preceeded the first vomiting attack and the general feeling of severe malaise during his uncooperative period." (Marsh et al., 1951).

The severe reactions that occurred in the subject were sufficient to discourage any further consideration of a therapeutic value of veratramine.

The potential of the veratrum alkaloids as important investigative pharmacological tools remains a viable possibility, especially in the light of modern findings in the field of excitable tissues. The present investigation is concerned specifically with the action of one alkaloid, Veratramine, and its action upon the electrophysiological properties of heart.

FIGURE I: The structure of veratramine.

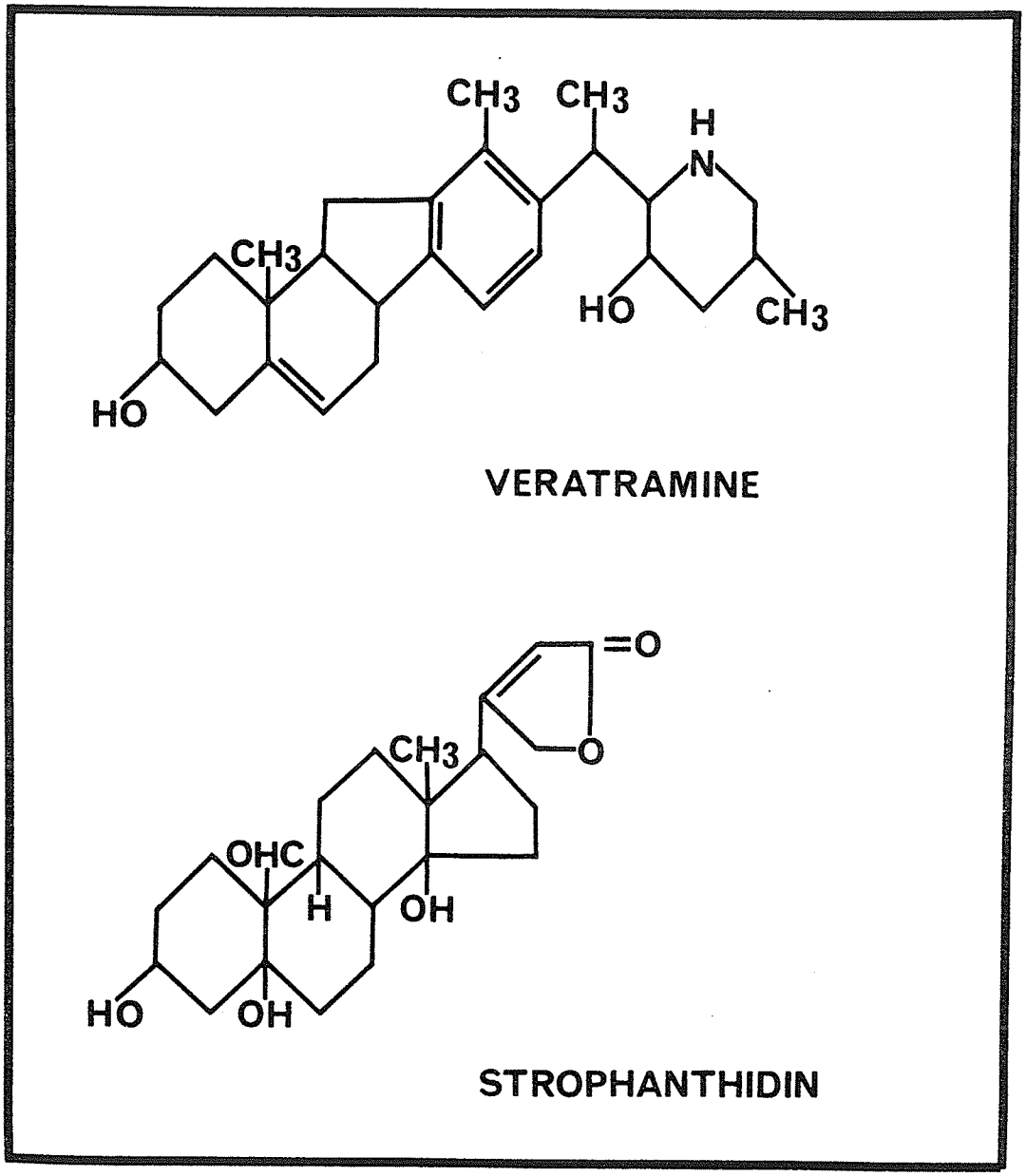
CH3
CH3
H
N
CH3
HO
CH3
HO
VERATRAMINE
=O
O
CH3
OHC
H
OH
HO
OH
STROPHANTHIDIN

FIGURE 1

Chemistry of the Veratrum Alkaloids

The veratrum alkaloids comprise a large number of compounds which are obtained from a group of liliaceous plants belonging to the suborder Melanthaceae. The plant species: Veratrum album, Linneaus; Veratrum viride, Aiton; and Veratrum sabadilla, Retz, yield a number of alkaloids that may be subdivided into three classes: the ester alkaloids, represented by veratridine and cevadine; the glycosidic alkaloids represented by veratrosine and pseudojervine; and the alkamines represented by veratramine and jervine. The ester alkaloids and the glycosidic alkaloids, on hydrolysis, yeild additional alkaloids. For example, the hydrolysis products of veratrosine are veratramine and d-glucose (Krayer and Acheson, 1946). Distinction between the classes of alkaloids becomes significant when mode of action is considered. The name veratrine is commonly used to denote the crude alkaloid mixture containing, veratridine, cevadine, cevine, cevadilline and sabadine (Merck Index, 7th edition). The proper use of the name veratrine is in reference to the pure alkaloid cevadine (Merck Index, 7th edition). Similarly, the name veratrum has been commonly used to describe an extract of Veratrum album, Linneaus, which contains large numbers of alkaloids. The use of such mixtures by earlier and contemporary investigators (Horakova and Vassort, 1973) should be noted, so as to avoid the possibility of ascribing the properties of mix-

tures of alkaloids to individual veratrum alkaloids, the actions of which vary markedly from alkaloid to alkaloid. The tertiary amine bases and their esters (veratridine, cevine) have a positive inotropic effect and elicit a reflex decrease in heart rate and blood pressure (Krayer and Acheson, 1946; Moe and Krayer, 1943), while the secondary amine bases and glycosides (veratramine, veratrosine) lack positive inotropic action and antagonize selectively the positive chronotropic action of epinephrine (Krayer, 1949a; 1949b; Krayer and Reiter, 1950).

Veratramine

Structure

The secondary amine veratramine, $C_{27}H_{39}O_2N$, molecular weight 409.59, was isolated from Veratrum grandiflorum, Loes. fil. by Saito in 1940 (Saito, 1940), and from Veratrum viride, Aiton by Jacobs and Craig in 1945 (Jacobs and Craig, 1945). The structure of veratramine is shown in Figure 1.

"Antiaccelerator" Effect of Veratramine

Krayer (1949) demonstrated the ability of veratramine to antagonize the positive chronotropic action of epinephrine and norepinephrine, in the isolated denervated heart of the dog (heart-lung preparation), as well as in the complete circulatory system of dogs and cats under anesthesia, or of spinal or pithed cats (Krayer, 1949). In as much as pretreatment of the preparations with atropine did not modify this veratramine effect, Krayer concluded

that the effect was not mediated by either the vagus nerves to the heart or the cardiac sympathetic nerves. Krayer concluded that the site of action of veratramine was in the pacemaker tissue of the heart. Krayer also reported that even though veratramine antagonized the chronotropic action of epinephrine on the heart, it did not abolish the positive inotropic, coronary vasodilator or circulatory pressor actions of epinephrine (Krayer, 1949a). Krayer maintained that as such, the action of veratramine was unique, and could be employed to study either the chronotropic or the inotropic actions of adrenaline separately (Krayer, 1949a).

In subsequent studies, the ability to inhibit the positive chronotropic action of epinephrine and norepinephrine in a manner similar to veratramine, was demonstrated with: veratrosine, the glycoside of veratramine; pseudojervine, the glycoside of jervine; and jervine (Krayer, 1949a; 1949b; Krayer and Van Mannen, 1949; Krayer and Reiter, 1950; Krayer, 1950). Veratridine and cevine were ineffective as antagonists of the chronotropic actions of epinephrine and norepinephrine (Krayer, 1949a).

While it was concluded that both veratramine and veratrosine reduced the sensitivity of the pacemaker of the heart to exogenous epinephrine and norepinephrine, certain differences in the actions of the two alkaloids were noted. The effect of veratrosine on heart rate was slow to develop as compared to that of veratramine, and

veratrosine did not possess the convulsant properties which had been observed with veratramine (Krayer, 1949a; 1949b). Moreover, the LD_{50} of veratrosine (intravenous injection in mice) was more than ten times larger than that of veratramine (Krayer 1949a; 1949b). These differences between the properties of veratramine and veratrosine were attributed to the glycosidic character of veratrosine (Krayer, 1949b).

The effects of veratramine on the heart were extended to include antagonism of the positive chronotropic effects of electrical stimulation of the cardiac sympathetic nerves (Krayer and Van Mannen, 1949; Krayer and Reiter, 1950), as well as, antagonism of the chronotropic actions of a wide variety of sympathomimetic agents (Krayer and Ourisson, 1954). Krayer, in 1950, introduced the term "antiaccelerator" to describe the atropine-resistant negative chronotropic action of veratramine in the presence of cardioacceleration produced by sympathomimetic amines or "electrical stimulation of the accelerans nerves" (Krayer, 1950). Krayer then suggested the possibility that this antagonism by veratramine and its related compounds was of a competitive nature in that it seemed to be specific and was surmountable.

Negative Chronotropic Action of Veratramine

Krayer and Ourisson (1954), using the dog heart-lung preparation, and Matallana et al.(1955), using cat

heart-lung and spinal cat preparations, demonstrated that veratramine could decrease the activity of the sino-atrial node independently of cardioacceleration produced by sympathomimetic amines. In order to exclude effects of peripheral vagal stimulation, atropine (1 mg/kg) was given to the spinal cat (Matallana et al., 1955). Atropine was also employed in several of the dog heart-lung preparations (Krayer and Ourisson, 1954). Treatment of the preparations with atropine did not affect the negative chronotropic action of veratramine (Krayer and Ourisson, 1954; Matallana et al., 1955).

In the spinal cat, heart rate was reduced by veratramine from 186 beats/min to 65 to 90 beats/min. Continuous infusion of 0.028 mg veratramine per kg per minute was needed in order to cause 50 per cent of the total decrease in heart rate (Matallana et al., 1955).

Innes et al. (1956), using the spinal cat preparation, confirmed the findings that both veratramine and veratrosine (0.1 to 0.3 mg/kg) could decrease basal heart rate, as well as, diminish the increase in heart rate due to stimulation of accelerator nerves. Chronic sympathetic denervation of cats (performed two weeks prior to exposure to veratramine) did not alter the effect of veratramine on basal heart rate or on the accelerator action of epinephrine (Innes et al., 1956). Innes and Krayer (1958) also showed that veratramine could produce a negative chronotropic

effect on the heart in the dog heart-lung preparation depleted of catecholamines by pretreatment with reserpine (5 mg/kg). A dual action of veratramine was suggested by Innes et al. (1956): a competitive antagonism responsible in part for the antiaccelerator action, and an independent negative chronotropic action.

Inhibition, by veratramine, of the oxidative phase of glucose metabolism, in both intact rat atrial tissue and rat ventricular homogenate was demonstrated by Reiter (1950). Veratramine, at a dose level comparable to that required to produce antiaccelerator effects in heart, inhibited the oxidation of lactic acid, pyruvic acid, and fumaric acid (Reiter, 1950). Succinic dehydrogenase and the cytochrome oxidase system were not influenced by veratramine (Reiter, 1950). Veratramine had no demonstrable effect on anaerobic glycolysis (Reiter, 1950).

It was suggested by Innes and Krayer (1958), that the negative chronotropic action of veratramine might be caused by interference in the "mechanism of cardiac impulse generation" at a "stage more fundamental than at the receptor site for sympathomimetic amines". This interference could result in an overall reduction of pacemaker activity and thus contribute to the antagonism of sympathomimetic agents (Innes and Krayer, 1958). It was further suggested that the inhibition of tissue respiration by veratramine, found by Reiter (1950), might be responsible for, or associated with

the proposed decrease in pacemaker activity (Innes and Krayer, 1958).

That the inhibitory effects of veratramine are not confined to the region of the sino-atrial node was demonstrated by Krayer et al. (1955), who showed in the heart-lung preparation of the dog that the heart rate was influenced by veratramine in qualitatively the same fashion in the presence of atrio-ventricular rhythm and of sino-atrial rhythm. These findings were confirmed in 1961 by Benforado and co-workers.

Veratramine-induced "Periodic Rhythm"

Veratramine-induced "periodical cessation of the heart beat" (periodic rhythm) in the spinal cat preparation was reported by Matallana et al. (1955) and Kosterlitz et al. (1955). In addition to the spinal cat preparation, periodic rhythm has been produced by veratramine treatment, in isolated right atrial preparations of guinea-pig and rabbit (Hawkins, 1962a; 1962b; Reuse-Blom, 1959).

While the characteristics of periodic rhythm produced by veratramine in cat, guinea-pig and rabbit are essentially similar, the most explicit description of periodic rhythm (spinal cat preparation) was given by Kosterlitz et al. (1955) as follows:

> "When veratramine was injected intravenously in a dose of 1 mg/kg, the heart rate rapidly decreased from 160 to 40-60

> beats/min. This was followed by a peculiar periodic rhythm characterized by phases of complete absence of electrical and mechanical activity alternating with phases of apparently normal sino-auricular activity."
>
> "The inactive phases usually lasted between five and twenty seconds, occasionally thirty, and once even sixty seconds. After an inactive phase, the frequency was higher than that of the regular rhythm immediately before the onset of the periodic rhythm, but declined more or less rapidly prior to the next inactive phase. This cycle of activity and inactivity recurred at regular intervals for ten to thirty minutes, the inactive phases becoming progressively shorter and the active phases longer. Eventually the periodic rhythm was replaced by a slow but regular rhythm of 60-80 beats/min" (Kosterlitz et al., 1955).

The slow but regular rhythm that replaced the periodic rhythm in the spinal cat preparation gradually increased in frequency, over a period of time but did not reach pre-

veratramine treatment levels (Kosterlitz et al., 1955). In addition, Kosterlitz et al. (1955) found that during the inactive phases of periodic rhythm in the spinal cat preparation, arterial blood pressure fell to about 10-15 mm Hg and rose immediately with resumption of cardiac activity.

Periodic cessation of heart beat, during sino-atrial rhythm, in the presence of veratramine had not been observed by Krayer in his experiments with dog heart, although "irregularities" in heart rate with high doses of veratramine had been observed (Krayer and Ourisson, 1954). Periodic rhythm was, however, produced by veratramine in the heart-lung preparation of the dog in the presence of atrio-ventricular rhythm (Krayer et al., 1955; Benforado, 1961).

Action of Sympathomimetics on Periodic Rhythm

Sympathomimetic agents antagonize the negative chronotropic effects of veratramine. With respect to periodic rhythm, the actions of sympathomimetics are interesting in that, while they are capable of antagonizing ongoing periodic rhythm, they can also precipitate periodic rhythm after periodic rhythm has given way to a regular rhythm. In the spinal cat preparation, Kosterlitz and co-workers (1955) found that infusion of 1.6 µg/kg/min of epinephrine during periodic rhythm caused an increase in the total number of beats/min from 50 to 90 beats/min. The inactive

phases of the periodic rhythm became less frequent and the active phases more prolonged. When the rate of epinephrine infusion was raised to 2.5 µg/kg/min, a regular rhythm of approximately 110 beats/min replaced the periodic rhythm. Cessation of epinephrine infusion was followed by a reduction in heart rate and resumption of periodic rhythm. If epinephrine was infused at a rate of 1.6 µg/kg/min at a time when periodic rhythm had given way spontaneously to a regular rhythm, periodic rhythm reappeared after about 3 minutes. Discontinuation of infusion at this point was followed by a marked periodic rhythm which lasted for 2 or 3 minutes after which a regular rhythm was resumed. Norepinephrine had essentially the same effects as epinephrine (Kosterlitz et al., 1955).

The effects of stimulation of the cardioaccelerator nerves during the various stages of periodic rhythm produced similar results as had been observed by Kosterlitz and co-workers with epinephrine and norepinephrine infusions.

In addition to the effects of epinephrine on periodic rhythm, Krayer et al. (1955) noted also that the increase in heart rate due to stimulation of the cardioaccelerator nerves was poorly maintained after veratramine treatment compared with the effect before veratramine.

In the isolated guinea-pig right atria, Hawkins (1962a) confirmed the findings of Kosterlitz et al. (1955)

with respect to the actions of epinephrine on periodic rhythm. Hawkins (1962a) also studied the action of veratramine and the influence of epinephrine on veratramine action at both 37°C and 32°C. The responses of the atria to veratramine and epinephrine, and the time course of the responses at 32°C were similar to those observed at 37°C (Hawkins, 1962a). At 32°C the resting rates of the preparation were lower and the response to veratramine correspondingly smaller. A most interesting observation was that periodic rhythms were more common at 37°C than 32°C (Hawkins, 1962a).

In a further study, Hawkins (1962b) observed that in the presence of veratramine, chronotropic responses to epinephrine are poorly maintained. This finding was in agreement with the findings of Kosterlitz et al. (1955) and Innes et al. (1956) who in the spinal cat preparation showed that the cardioaccelerator response to stimulation of the cardioaccelerator nerves is poorly maintained in the presence of veratramine. In addition, Hawkins (1962b) pointed out that the impairment by veratramine of the cardiac responses to epinephrine is more marked at 37°C than at 32°C.

Mode of Action of Veratramine

To account for the observed effects of veratramine on atrial tissue, Innes et al. (1956) and Innes and Krayer (1958) proposed a dual action of veratramine.

These investigators suggested that the antiaccelerator effect of veratramine resulted from a combination of competitive antagonism of the sympathomimetics by veratramine, as well as an independent negative chronotropic action of veratramine. Furthermore, it was proposed that the negative chronotropic action of veratramine might result from a reduction in pacemaker activity associated with an inhibition of tissue respiration, an effect of veratramine observed by Reiter (1950). To explain the slowing of heart rate after veratramine and the eventual cessation of the beat, Kosterlitz, Krayer, and Matallana (1955) suggested that an unknown substance, "X", was required to maintain normal activity of the sinoatrial node, and veratramine either interferes with the formation of this substance, or competes with it for a site at the pacemaker. The regularity of the alternating active and inactive phases of periodic rhythm suggested to Kosterlitz et al. (1955) that the resumption of cardiac activity appeared to be conditioned by a process which took place during the inactive phase of periodic rhythm.

> "A certain stage in this process must be reached before the sino-auricular node regains its ability to discharge rhythmically. The time required, and therefore the duration of the inactive phase, decreases slowly as the veratramine effect

wears off."

(Kosterlitz et al. 1955)

Kosterlitz and co-workers (1955) proposed that the sinoatrial node ceases to be active when the concentration of substance "X" falls below a certain level, and becomes active again with the accumulation of substance "X" during the inactive phase. To account for the ability of sympathomimetics to both antagonize or precipitate periodic rhythm, Kosterlitz et al. (1955) proposed that the increased rate of discharge of the sinoatrial node, induced by sympathomimetics, could lead to accelerated use of substance "X". In support of this concept, Kosterlitz et al. (1955) cited their observation of increased incidence of inactive phases and shortened active phases, which occurred after cessation of administration of epinephrine or norepinephrine.

Hawkins (1962a) disagreed with the suggestion that the antiaccelerator effect of veratramine was due to combined effects of competitive antagonism and a direct slowing action. Hawkins (1962a) pointed out that if it could be shown that the resting heart rate was maintained by endogenous epinephrine or norepinephrine, the effects of veratramine could be interpreted as due to competitive antagonism. Innes and Krayer (1958) had demonstrated that the sinus rate of hearts depleted of catecholomines by administration of reserpine is reduced to the same degree by the same doses of veratramine as that of untreated pre-

parations. In addition, it had been demonstrated (Hawkins, 1962a) that the amounts of veratramine required to slow the epinephrine-accelerated heart were the same as those required to decrease the resting rate and that veratramine was no less active in slowing the atrium accelerated by a $5^{o}C$ rise in temperature than it was in slowing the atrium accelerated by epinephrine. Hawkins (1962a) concluded veratramine acted in a direct fashion to slow sinus rate and was a physiological antagonist of the sympathomimetic agents rather than a competitive one. Langer and Trendelenburg (1964) lent support to the idea of veratramine as a physiological antagonist (of the responses of the isolated atrium to) of epinephrine. Langer and Trendelenburg (1964) maintained that, whereas the actions of a competitive agonist should be specific, the antiaccelerator action of veratramine on isolated atria was nonspecific in that norepinephrine and histamine (in equieffective concentrations) were antagonized to the same extent by veratramine (Langer and Trendelenburg, 1964).

The findings of Hawkins (1962a , 1962b) support the hypothesis of Kosterlitz et al. (1955) that periodic rhythm, in particular, the periods of asystole, could be due to exhaustion of a necessary metabolite. Hawkins found, that experimental conditions that favoured a higher rate of tissue activity, and hence, a greater metabolite utilization rate by the atrial tissue, also resulted in more

marked effects of veratramine. For example, Hawkins (1962a, 1962b) observed that, in the isolated guinea-pig atrial preparation, veratramine-induced periodic rhythms were more likely to occur at 37°C than at 32°C. Also, at 37°C, as opposed to 32°C, the impairment by veratramine of the accelerator effects of epinephrine were more marked (Hawkins, 1962a,1962b). Hawkins maintained, however, that the "necessary metabolite" hypothesis as an explanation of periodic rhythm lacked a degree of complexity, in that the hypothesis did not explain the observed spontaneous resolution of periodic rhythm in some isolated preparations continuously exposed to veratramine.

Statement of the Problem

The foregoing discussion has been concerned with the negative chronotropic actions of veratramine on the heart, and the apparently unique ability of veratramine to produce a "periodic rhythm" in the heart of various animal species. Despite the information provided by the investigations that have been considered in this text, knowledge of the mechanisms whereby veratramine exerts its effects on the heart remains incomplete.

It was considered that investigation of the actions of veratramine in terms of the electrophysiological parameters of the atrium could provide significant information concerning not only the electrophysiological mechanisms involved in the action of veratramine, but could also pro-

vide clarification of basic electrophysiological mechanisms concerned with some aspects of cardiac automaticity.

The objective of this study, therefore, was to determine, in the isolated spontaneously beating cat atria, some of the electrophysiological correlates of the negative chronotropic action of veratramine, as well as those correlated with the production of periodic rhythm by veratramine. This information would be considered in terms of the relationships and implications concerning current knowledge and theories of cardiac electrophysiology.

As a basis for this discussion, some of the pertinent current concepts of cardiac electrophysiology are considered in the following section of the introduction.

Resting Membrane Potential and Action Potential

It is generally accepted that in cardiac tissue the genesis of the resting membrane potential and the action potential can be interpreted according to the general concepts developed for nerve by Hodgkin, Huxley, Katz and Keynes (Hodgkin, 1951; 1957; Hodgkin and Huxley, 1952a, 1952b); Hodgkin and Katz, 1949; Hodgkin and Keynes, 1954, 1955; Katz, 1966). The theory of the ionic basis of resting and action potential in nerve must be modified in order to account for events taking place in cardiac tissues. The necessity for modification is indicated not only by the structural dissimilarities between nervous and cardiac tissues but also by the observed dissimilarities in the action potentials of these tissues. The most striking of these dissimilarities are the prolonged repolarization of the cardiac action potential and the occurence of a plateau phase during the repolarization.

Ionic Basis of Resting and Action Potential in Nerve

The ionic theory of membrane potential is ultimately based on the concept of the cell membrane as a semipermeable barrier to the free movement of charged particles. The electrical potential difference that exists across the cell membrane is thought to be the result of a sustained difference in the ionic compositions of the fluids on the interior and exterior of the cell. For example, the intracellular fluid contains a high concentration of potassium

and a low sodium concentration, while the extracellular fluid contains a relatively high concentration of sodium and a low concentration of potassium. The difference in the ionic concentrations across the cell membrane is thought to serve as the electrochemical source of membrane potential.

Due to the semipermeable properties of the cell membrane there is a continual exchange of ions between the intracellular and extracellular fluids. This process, if unopposed, would result in eventual decay of ionic concentration gradients and hence membrane potential. Several mechanisms have been proposed to account for the maintenance of the ionic concentration gradients in the face of these diffusional pressures.

Donnan Equilibrium

In a Donnan equilibrium, membrane potential is generated by the diffusion of permeable ions across the membrane, down their concentration gradients. The assymmetric distribution of ions, and hence the development of an electrostatic potential difference is caused by the presence of charged nondiffusible ions (i.e., cellular proteins). The distribution of diffusible ions is influenced as well by their relative permeabilities. If the membrane is permeable to several ions, sodium, potassium and chloride, then the membrane potential will be a function of the relative permeabilities and concentration differences for all the ion species. The Goldman equation is the mathematical expression

of this relationship.

Energy Dependent Ion Pumps

This mechanism involves a metabolically driven transport system which couples the movement of sodium and potassium across the cell membrane against their respective concentration gradients. The movement of sodium and potassium down their concentration gradients is governed by the selective permeability properties of the membrane. Concentration gradients created by a coupled transport system could, in part, generate and maintain membrane potential. Hodgkin and Keynes (1955b) demonstrated that the uphill movement of sodium from the nerve cytoplasm to the extracellular fluid could be stopped by metabolic inhibitors and restarted by intracellular injection of adenosine triphosphate. As well it was found that metabolic inhibition of sodium extrusion simultaneously reduced the inward movement of potassium (Hodgkin and Keynes, 1955b). Sodium inward and potassium outward movements (passive) were not affected.

Energy dependent ion pumps may be neutral, that is, result in no net movement of charge across the membrane. Alternately, the activity of ion pumps may cause a net transfer of charge across the membrane and contribute directly to generation of membrane potential. The two types of ion transport systems are referred to as "neutral" and "electrogenic" respectively. The existence

of electrogenic ionic transport systems in nerve and muscle (cardiac, striated, smooth) is supported by the work of several investigators (Keynes and Steinhardt, 1968; Marmor and Gorman, 1970; Taylor et al., 1970; McDonald and MacLeod, 1971; Thomas, 1972; Glitsch, 1972; 1973; McDonald and MacLeod, 1973).

Action Potential Generation in Nerve (Hodgkin-Huxley Theory)

At rest the membrane potassium permeability is relatively high in comparison with membrane permeabilities of other ionic species. As a result, the potassium equilibrium potential dominates the resting transmembrane potential and this potential is primarily modified by the potassium gradient across the cell. In both nerve axons and cardiac cells the resting potential has been shown to vary as a linear function of the extracellular potassium concentration (Hoffman and Cranefield, 1960).

Although the amplitude of the resting membrane potential can be modified by manipulation of external ion concentrations, the voltage changes observed during the action potential are not the result of changes in the equilibrium potentials of sodium and/or potassium. Changes in the potential difference across the membrane that occur during the action potential result from sequential changes in the relative membrane permeabilities of sodium and potassium, such that the equilibrium potentials of these ions alternately dominate the transmembrane potential.

Depolarization of the membrane increases the permeability of the membrane to sodium, thereby permitting the influx of sodium ions which in turn leads to further depolarization. The process is regenerative, and the transmembrane potential approaches the sodium equilibrium potential. Return of the transmembrane potential to resting value (repolarization) is proposed to take place as a result of further changes in the membrane permeabilities to both sodium and potassium. Hodgkin and Huxley (1952a) proposed that the initial increase in sodium permeability that leads to regenerative membrane depolarization is ultimately a self limiting and transitory process. As the transmembrane potential approaches the sodium equilibrium potential, "inactivation" takes place, and sodium permeability decreases. With the decrease in sodium permeability, the transmembrane potential moves toward the resting level, as there is a relative increase in domination of the transmembrane potential by the potassium equilibrium potential. In addition to the increase in sodium permeability, depolarization leads to a delayed increase in membrane potassium permeability, the delay being such that decreasing sodium permeability and increasing potassium permeability coincide. The transmembrane potential, as a result, approaches the potassium equilibrium potential (towards resting level) with a concomitant efflux of potassium from the cell.

Cardiac Action Potential

It is evident that modification of the Hodgkin-

Huxley hypothesis of action potential generation in nerve must be made for cardiac muscle in order to explain the prolonged phase of repolarization that is typical of the cardiac cell. Much of the research that has been done in an effort to explain the cardiac action potential involves a complex consideration and re-evaluation of the relative contributions of various ion currents to the configuration of the action potential.

Voltage Clamp Technique

The ionic basis of the cardiac action potential has been the subject of extensive recent review (Sonenblick and Stam, 1969; Johnson and Lieberman, 1971; Langer, 1973; Trautwein, 1973). Much of the recent work in delineating the various ion currents involved in the generation of the cardiac action potential has made extensive use of voltage clamp techniques. Basically, the voltage clamp technique involves the measurement of current, that is required to hold the potential across a prescribed area of cell membrane at a known value, that is uniform with respect to distance and time. Application of voltage clamp technique to cardiac muscle preparations becomes complex when one considers the multicellular geometry of the preparation. Johnson and Lieberman (1971) pointed out the difficulties involved in the separation of the capacitive from ionic current components of current measured following step changes in clamped potential. They

expressed a need for caution in the interpretation of current measurement in the presence of possible inadequate temporal and spatial control (due to tissue mass) of clamped membrane potential. Langer (1973) accepted the validity of Johnson and Lieberman's criticism, but pointed out the value of the at least qualitative contributions of the voltage-clamp investigations.

Initial Rapid Sodium Current

In cardiac muscle as in nerve, the upstroke of the action potential is considered to be secondary to an increase in sarcolemmal sodium permeability. This concept is supported by the finding that tetrodotoxin, which selectively decreases sodium permeability, is capable of abolishing the inward excitatory current in a variety of cardiac tissues (Hagiwara and Nakajima, 1965; Coraboeuf and Vassort, 1968; Rougier et al., 1969).

Secondary Slow Inward Current

In addition to the initial rapid sodium current of the action potential spike, a slower secondary inward current, which occurs during depolarization, has been described by investigators using the voltage-clamp technique in cardiac preparations (Rougier et al., 1969; Beeler and Reuter, 1970a, 1970b; Vassort, 1973; Kohlhardt et al., 1973). It has been suggested (McDonald and MacLeod, 1973) that this slow inward current contributes to the maintenance of cardiac action potential amplitude and duration, and is

influenced to some extent by metabolic processes.

Considerable discussion has taken place as to the ion species responsible for the slow inward current (sodium and/or calcium), and as to the extent of the contribution of this current to the maintenance of amplitude, duration, and plateau phase of the action potential.

Langer (1973) in reviewing the literature concerning the origin of the secondary slow current, indicated the concensus that the slow inward current is, at least partially, attributable to calcium flux.

Beeler and Reuter (1970a, 1970b) had observed that the slow inward current was not affected by removal of sodium from the perfusing fluid, or by treatment of the preparation with tetrodotoxin in a dose sufficient to reduce or abolish the initial fast sodium current. These investigators also found that the slow inward current was sensitive to alterations in the external calcium concentration. Rougier et al. (1968, 1969), using a double-sucrose gap frog atrial preparation, found that the slow inward current could only be abolished if both sodium and calcium were removed from the bathing fluid. Manganese, an inhibitor of calcium permeability, also reduced the slow inward current. Beeler and Reuter concluded that the slow inward current was carried by calcium ions, and Rougier et al. (1968, 1969) concluded that the current was carried by both sodium and calcium. While Langer (1973) proposed

that the most plausible explanation of the slow inward current was that it is carried, at least in part, by calcium ions. He cautioned that such may not be the case in all tissues. For example, in sheep ventricle (Mascher and Peper, 1969) and in guinea-pig ventricle (Ochi, 1970) evidence has been presented in favour of a slow sodium current.

A possible contribution of the slow inward current (sodium and/or calcium) to the transmembrane action potential is suggested by the findings of Coraboeuf and Vassort (1968) in rat ventricle, and McDonald (1972) in guinea-pig ventricle. These workers showed that manganese, which reduced the slow inward current, reduced the peak amplitude of the action potential, the amplitude and duration of the action potential plateau and the action potential duration. A contribution of the slow inward current to the maintenance of the peak amplitude, plateau amplitude and duration of the transmembrane action potential in heart is thereby implied.

Johnson and Lieberman (1971) suggest that the acceptance of a slow calcium current as contributing to the action potential is premature. They propose that "during a depolarizing step in membrane potential there is a transient inward sodium current, that rises to a peak within a time equal to the duration of the depolarizing phase of the action potential and that this inward current declines

in two phases, that is to say inactivates at two different rates." They suggest that the evidence for two components of inward current, one sodium and the other all, or in part calcium, is inconclusive and is likely the consequence of inadequate spatial and temporal control of the transmembrane potential with the voltage clamp technique.

Role of Chloride in Cardiac Action Potential Generation

Chloride has been implicated in generation of the transmembrane action potential (Sonenblick and Stam, 1969). An increased outward chloride current occurring at the junction of the action potential spike and plateau has been associated with both the mechanism of early repolarization, and as well as the inactivation of the initial rapid sodium current (Dudel et al., 1967; Reuter, 1968; Sonenblick and Stam, 1969; Fozzard and Gibbons, 1973; Fozzard and Hiraoka, 1973).

Role of Potassium in Cardiac Action Potential Generation

The role of potassium ions in cardiac action potential repolarization has been discussed by Langer (1968). To explain the plateau phase of the cardiac action potential, Weidman (1951) proposed that sodium conductance was not rapidly inactivated following an initial rapid increase (action potential spike), but rather sodium inward current was maintained at a relatively high level. At the same time, Weidman (1951) proposed that there was a delayed rise in potassium conductance ("delayed rectification"),

the overall result being an inward sodium current of the same order of magnitude as the outward potassium current and a stabilization of the membrane potential (plateau).

Evidence, not only for a delayed rise in potassium conductance but also for a reduction in potassium conductance following depolarization has been provided by several investigators (Brady and Woodbury, 1960; Noble, 1960, 1962; Hall and Noble, 1963; Johnson and Tille, 1960; Johnson and Wilson, 1962; Saito, 1971; Juncker et al., 1972).

Juncker et al. (1972), in amphibian atrium, observed an initial brief augmentation of potassium efflux followed by a marked suppression (of potassium efflux) which lasted for approximately the duration of the plateau of the action potential and then returned to resting level. Juncker et al. (1972) concluded that diminished membrane permeability to potassium plays an important part in the generation of the plateau of the cardiac action potential.

During the final phase of repolarization, according to Langer (1968), outward potassium current exceeds the rapidly falling inward sodium current and thus the membrane potential falls to diastolic levels. Noble and Tsien (1969) have identified a potassium channel that leads to a delayed increase in outward potassium current perhaps involved in the repolarization process (Fozzard and Gibbons, 1973).

Pacemaker Cells - Diastolic Depolarization

Normally, most of the atrial and ventricular cells

maintain a stable membrane potential throughout the diastolic period. Such cells are termed non-pacemaker cells. As discussed earlier, in the resting state, the membrane potassium permeability is relatively high (as compared to sodium permeability) and a resting potassium efflux occurs. It is thought that the stability of the diastolic membrane potential of the non-pacemaker cell is attributable largely to an unchanging potassium permeability (Langer, 1968).

The pacemaker cells of the myocardium (sinus node, latent atrial pacemakers, purkinje tissue) characteristically demonstrate a progressive diastolic depolarization, which can lead to the generation of an action potential. Mechanisms that have been suggested to account for the process of progressive diastolic depolarization include: an increasing sodium permeability (net sodium influx which is almost but not entirely balanced by potassium efflux and chloride efflux) (Hoffman and Cranefield, 1960); a decreasing potassium permeability, and a reduction in active sodium pumping. Any of these mechanisms theoretically could, if operative, lead to a reduction in diastolic membrane potential. Langer (1968) has concluded that the rate of active sodium pumping is too low to contribute significantly to the membrane potential, or to play a significant role in diastolic depolarization.

To date, experimental evidence would appear to support the concept that diastolic depolarization is the result of

a decreasing potassium conductance (decreasing potassium efflux) as opposed to an inward sodium influx.

Vassalle (1965) demonstrated that exposure of purkinje fibers to low potassium concentrations, resulted in a decrease in membrane potassium conductance, and an increase in the rate of diastolic depolarization. Alternatively, when extracellular potassium concentration was raised, potassium conductance increased and the rate of diastolic depolarization declined. In a subsequent study (1966) Vassalle, using a voltage clamp technique, demonstrated the occurrence of a time-dependent fall in potassium conductance during diastolic depolarization. Vassalle concluded that this decrease in potassium conductance was a major contributing factor in the production of diastolic depolarization.

Spontaneous activity can be induced in quiescent atrial and purkinje tissues by exposure of the preparations to solutions of low potassium content (Carmeliet, 1961; Vassalle, 1965; Brown et al., 1972; Vassalle et al., 1973). Correspondingly, spontaneous activity of sinoatrial and purkinje tissue can be reduced or abolished when exposed to solutions containing raised concentrations of potassium (Vassalle et al., 1973). Carmeliet (1961) observed pacemaker potentials in guinea-pig left atrial preparations, which had been exposed to low potassium tyrode solution. In frog atria, Brown et al. (1972) determined that the diastolic

depolarization (induced) of the atrial cell resulted from the decay of an outward current, rather than from the time-dependent onset of an inward current.

The theory of mechanisms underlying diastolic depolarization has been developed mainly from studies of purkinje fibers. On the basis of common responses to experimental manipulations, Vassalle et al. (1973) have inferred that similar mechanisms are operative in producing diastolic depolarization in both sino-atrial and purkinje tissues. However, Vassalle et al. (1973) also indicated that the differences in the responses of the two tissue types suggests a degree of differentiation in the basic function of these tissues.

Both purkinje fibers and sinoatrial cells respond to an increase in membrane potassium conductance (High extracellular potassium concentration or treatment with acetylcholine) with a decrease in automaticity (decreased rate of diastolic depolarization and decreased spontaneous rate) (Vassalle et al., 1973). When potassium concentration in the perfusing fluid is reduced from a high to a normal level, the maximum diastolic potential of the sinus nodal cells increases, but to a lesser extent than that observed in purkinje fibers (Vassalle et al., 1973). It is also of interest to note that the membrane potential of the sino-atrial cells is influenced to a lesser degree than that of atrial muscle cells by treatment of these tissues with

excess potassium or barium (Toda, 1971). Vassalle has suggested that the smaller increase, as compared to purkinje fibers, in the maximum diastolic potential in the sinus node may be due to a higher membrane sodium conductance at normal potassium levels.

The apparent resistance, to pharmacological manipulation, of the electrophysiological properties of the sinoatrial cells in comparison to both purkinje and common atrial cells is well documented (Tomlinson and James, 1968; Hashimoto and Chiba, 1969; Toda, 1971; 1973; Hashimoto and Moe, 1973).

Vassalle et al. (1973) have demonstrated that automaticity of the sinus node is more resistant than that of purkinje fibers to the depressant effects (negative chronotropic) of high potassium concentrations. This resistance is enhanced by catecholamines and by the presence of increased calcium concentrations (Vassalle et al., 1973). The cells of the sinoatrial node are more resistant to the suppressant action of tetrodotoxin (Tomlinson and James, 1968; Hashimoto and Chiba, 1969) than are either purkinje fibers (Vassalle et al., 1973), or atrial muscle cells (Sano et al., 1968; Huang, 1970, 1973). Yamagishi and Sano (1966) showed that tetrodotoxin, which is said to block sodium entry during excitation, decreased the maximum rate of rise and amplitude of the action potential in rabbit sinus node, but did not induce a significant change in the

slope of slow diastolic depolarization. This is understandable if the slope of diastolic depolarization is determined mainly by a changing potassium conductance. Hashimoto and Moe (1973) observed that the specialized atrial tissue appeared to be more resistant than purkinje or atrial cells to the action of acetylstrophanthidin to induce self sustained automatic activity.

The reasons for the different sensitivities of sino-atrial, atrial and purkinje cells are not well understood. It has been suggested (Vassalle et al., 1973) that part of the explanation may lie in the differences in the individual membrane characteristics, as well as in the differential extent of sympathetic innervation. Vassalle et al. (1973) have shown that the abundant sympathetic innervation of the sinus node, as compared to purkinje fiber, is in part responsible for the observed resistance of the sinus node to the depressant effect of potassium on automaticity.

Other factors which should be considered when examining the responses of atrial tissue are the specialized structure of the sinus node, and the atrial specialized conduction system.

Atrial Conducting System and Sino-Atrial Block

The anatomical, biochemical and electrophysiological evidence for the existence of specialized tissues and preferential conduction pathways in the atria of the heart has been reviewed by James and Sherf (1971). Evidence for the

existence of preferential conduction pathways running from the sinus node to the atrial tissue, and to the atrioventricular node has been found in human, rabbit, bird, dog, monkey, rat and horse (James and Sherf, 1971). James and Sherf (1971) found that, in addition to an interatrial pathway, there are three connecting pathways between the human sinus node and the atrioventricular node; the anterior, middle and posterior internodal pathways. Fibers from all three tracts intermingle by way of crossovers superior to the level of the atrioventricular node. Fibers of the anterior and middle tracts enter the superior part of the atrioventricular node, while fibers from the posterior internodal pathway pass along the convex right atrial surface and enter the inferior portion of the atrioventricular node (James and Sherf, 1971; Kawamura and James, 1971). Since the fibers of the posterior internodal tract anatomically circumvent most of the atrioventricular node they are referred to as "by-pass" fibers.

Using a brush electrode consisting of ten microelectrodes, van Cappelle et al. (1972) have confirmed, in rabbit, the existence of at least two distinct conducting pathways that enter the atrioventricular node from the atria. They also confirmed the finding of James and Sherf (1971) that the main input into the atrioventricular node during normal sinus rhythm occurs over the crista terminalis (posterior internodal tract). James and Sherf (1971) and James (1973)

discussed the function of intra-atrial conduction pathways to the atrioventricular node in terms of normal and disturbed cardiac rhythm.

Sano and Yamagishi (1965), using intracellular microelectrodes, also found three intra-atrial conduction pathways to the atrioventricular node from the sinus node in rabbit. Using multiple microelectrode technique, they mapped the conduction pathways of impulses originating in the sinus node, and measured conduction velocities along the pathways to the atrioventricular node. As described by Sano and Yamagishi (1965), the excitation starts from the sinus node, moving radially at first and then proceeding obliquely to the crista terminalis. Movement of the impulse is forced obliquely towards the crista terminalis by virtue of a zone of tissue with low conduction velocity posterior to the sinoatrial node, and by virtue of a zone of relatively faster conduction at the basal wall of the superior vena cava. Once the excitation impulse enters the crista terminalis, it travels rapidly in two opposite directions through two branches of a ring-like structure formed by the crista terminalis and its extension. The two ring-like branches encircle the inferior vena cava and superior vena cava respectively, and approach the atrioventricular node. The third and slowest conduction pathway is within the rings formed by the crista terminalis, and proceeds along the basal wall of the superior vena cava towards the atrioven-

tricular node. Sano and Yamagishi found that the main pathway of conduction from sinus node to atrioventricular node was along the branch of crista terminalis that encircled the inferior vena cava. This pathway corresponds to the posterior internodal pathway described by James and Sherf (1971) and James (1963).

Sano and Yamagishi (1965) and Sano and Iida (1968) outlined a functional preferred conduction pathway from the sinus node to the atrial tissue. The sinus node was found to be composed of a network of "very delicate, palely stained, primitive muscle fibers" (Sano and Iida, 1968) embedded in a connective tissue matrix (Bonke, 1972). From the sinus node, a bridge-like connection extends to the crista terminalis obliquely and cranially (Sano and Iida, 1968). This anatomical extension corresponded to the route of preferred conduction mapped by Sano and Yamagishi (1965). Histologically, the extension was found to be composed of parallel running muscle fibers and "corresponded to a kind of transitional tissue between the nodal tissue and the ordinary atrial tissue" (Sano and Iida, 1968).

The characteristics of the transmembrane action potentials and conduction velocities were examined along this route from the sinus node to the atrial tissue (Sano and Yamagishi, 1965). In the sinus node, the characteristic features of the transmembrane action potential included a

small action potential with a slow upstroke, little or no membrane potential reversal, and a low "resting" potential with a very prominent slow diastolic depolarization (phase 4) (Sano and Yamagishi, 1965). The characteristics of the action potential were found to change gradually along the conduction pathway towards the atria. Action potential amplitude, resting potential and rate of rise increased, and the rate of diastolic depolarization was reduced progressively until, distal to the sinus node at the end of the conduction route, the action potential resembled that of common atrial tissue (Sano and Yamagishi, 1965). Conduction velocity also varied along the sino-atrial pathway. Within the sinus node, and in the immediate area surrounding the node, conduction velocity was markedly slow (2 to 6 cm/sec). Beyond the sinus node the conduction velocity increased progressively through the crista terminalis (30 to 40 cm/sec) attaining a maximum velocity at and within the common atrial tissue (70 cm/sec) (Sano and Yamagishi, 1965; Bonke, 1972). Sano et al. (1966) and Sano and Iida (1968) examined the sino-atrial conduction pathway in terms of the possible location of a site of conduction blockade between the sinus node and the atrium. They produced and examined the progress of sino-atrial block in high potassium solution, and found that the sinus node, the sino-atrial connection and its immediate vicinity were resistant to the blockade. The atrial musculature lost electrical

activity long before the sino-atrial pathway was blocked (Sano et al., 1966; Sano and Iida, 1968; Sano, 1969).

Strauss and Bigger (1972) examined, in rabbit, the elctrophysiological properties of the transitional cells which surround the sinus node, lying between the sinus node and crista terminalis. These cells, which they termed "perinodal fibers", were proposed to constitute a junctional zone, which introduces a delay between the time of impulse formation in the sinus node and initial depolarization in the crista terminalis. Strauss and Bigger (1972) found that the transmembrane action potentials of the perinodal fibers were intermediate between those of the sinus node and those of the crista terminalis. Premature impulses either were not propagated, or were propagated with great delay through the region of perinodal fibers; perinodal fibers did not discharge spontaneously at induced heart rates as low as 60/min. Resistance of the perinodal fibers to blockade was intermediate between atrial and sinus node tissue. Strauss and Bigger suggested that the junctional zone of perinodal fibers might constitute a mechanism of sinoatrial block in the abnormal heart, damaged or drug-treated. Blockade of conduction between the sinus node and atrial tissue, termed sino-atrial block, has been documented in both man and experimental animal (Marshall and Vaughan Williams, 1956; Marshall, 1957; Torres and Angelakos, 1964; Sano et al., 1966; Angelakos et al., 1971; Grazel and Angeles, 1972; deAzeudeo et al., 1973).

SECTION II

METHODS

METHODS

GENERAL PROCEDURES

Isolated Atrial Preparation

Kittens weighing 0.7 to 1.5 kg of either sex were killed by a blow on the head. The heart was removed as quickly as possible and placed in Krebs-Henseleit solution at 4°C. The right atrium was cut from the heart with the sino-atrial node intact and suspended horizontally in a plexiglass organ bath containing Krebs-Henseleit solution maintained at 37°C and bubbled with 95% oxygen and 5% carbon dioxide. The same orientation of the tissue in the bath was maintained from experiment to experiment to facilitate the location of the sino-atrial node. The organ bath contained about 10 ml of bathing fluid, and was drained and filled through openings at the bottom of the chamber. Openings were also located in the upper section of the chamber to allow for overflow washouts. The atria were allowed to equilibrate for ½ hour before tests were started. During this time the bathing fluid was changed every 5 minutes.

Tension developed by the atrium was recorded isometrically with a force displacement transducer (Grass FT03) with a resting tension of 500 mg. Electrical activity of single atrial cells were measured with intracellular glass capillary microelectrodes filled with 3M potassium chloride.

The electrode resistance varied between 30-100 megohms. Microelectrodes were mounted on a silver/silver chloride electrode which was attached to a platinum-iridium wire of .004 inch diameter formed into a helix of 1 cm diameter and approximately 5 revolutions. The microelectrode and coil were suspended vertically above the organ bath from a Brinkman Micromanipulator (Brinkman Instruments, New York). Electrical signals of membrane potentials and tension were recorded on a Hewlett Packard 3960 tape recorder and monitored on a Hewlett Packard 141B storage oscilloscope. Electrical signals so displayed were photographed with a Shackman oscilloscope camera type no. A.C. 2/25 and measurements were made from projected photographic recordings. Electrical supramaximal stimuli, when required, were delivered by a Grass S8 stimulator through a stimulus isolation unit. Electrical stimulation was applied by way of two platinum electrodes, one contacting the tissue directly and the other immersed in the bathing fluid in close proximity to the tissue.

Spinal Preparation

Cat spinal preparations were obtained under ether anesthesia by a minor modification of the method of Barger and Dale (1910), the approach to the spinal cord being through the lamina of the third, instead of the second vertebra. Cats weighing 2.8 to 4.5 kg of either sex were used. The hemorrhage from the bony structure was negligible

in this procedure. The brain was pithed by an aluminum rod introduced through the foramen magnum. Femoral arterial pressure was measured through a polyethylene catheter filled with a solution containing 4% heparin and 0.9% NaCl connected to a Statham pressure transducer model P23AC and recorded with a Grass Model 5 polygraph.

The chest was opened and the pericardium cut and tied so as to form a supporting "basket" for the heart. Platinum stimulating electrodes were clipped to the right atrium. A plastic polyethylene film was used to prevent dehydration of the exposed heart. Stimulii were obtained from a Grass Model SD5 'square wave' stimulator. Veratramine was given as the hydrochloride and injected into the femoral vein over a period of fifteen seconds. At least one hour elapsed between the cessation of ether anesthesia and the injection of veratramine.

EXPERIMENTAL PROCEDURES

I. Bathing Media

The following bathing fluids were made with glass distilled deionized water.

A. Krebs-Henseleit Solution

NaCl 118 mM; KCl 4.7 mM; $CaCl_2$ 2.5 mM; KH_2PO_4 1.1 mM; Mg_2SO_4 1.2 mM; $NaHCO_3$ 25 mM; and glucose 11 mM. Calcium chloride and magnesium sulphate were added after the other compounds had been dissolved and the solution had been equilibrated with 95% oxygen, 5% carbon dioxide for 30

minutes.

B. Modified Krebs-Henseleit Solution

Alteration of individual ion concentrations of the Krebs-Henseleit solution, bathing the isolated atrial preparation, was performed by pipetting appropriate volumes of stock solutions of calcium chloride, potassium chloride, or sodium chloride into the organ bath. No substitution was made to compensate for changes in osmolarity of the bathing fluid due to these additions. The effect, on the course of veratramine action, of increasing individual ion concentrations was examined when the ion concentration was increased before administration of veratramine and when the ion concentration was increased after the effects of veratramine had been allowed to develop. The effects of the following solutions were examined:

1. High Calcium Solution

The composition was similar to that of Krebs-Henseleit solution except that the calcium concentration was increased from 2.5 mM to 5.0 mM.

2. High Potassium Solution

The potassium content of Krebs-Henseleit solution was increased from 5.8 mM to 11.6 mM.

3. High Calcium, High Potassium Solution

The potassium and calcium concentrations of Krebs-Henseleit solution were increased from 5.8 mM and 2.5 mM to 11.6 mM and 5.0 mM, respectively.

4. High Sodium Solution

The Krebs-Henseleit solution was modified to contain sodium chloride in a concentration of 238 mM instead of 118 mM.

II. Preparation of Microelectrodes

The glass used for preparing microelectrodes was a high quality pyrex glass tubing (Corning Glass Works, Corning, New York), outside diameter 1.2 - 1.5 mm, which was cut into 6 cm lengths and the ends flame polished. The glass tubing was thoroughly cleaned before use by soaking in acid solution, rinsed with double distilled water, soaked in an alcohol-acetone mixture and then dried in a vacuum dessicator. Electrodes were prepared with glass fibers according to the method of Tasaki et al. (1968), and pulled on a David Kopf 700 C vertical pipette puller. Electrodes were then stored dry, tip up, in a covered plexiglass holder. The microelectrodes were filled with 3 M potassium chloride immediately before use.

The potassium chloride solution was introduced into the micropipette with a 1 cc tuberculin syringe and a 2 inch 27 hypodermic needle. The 3 M potassium chloride solution was prepared by dissolving potassium chloride (Fisher Scientific Company Certified A.C.S.) in glass distilled water. This solution was then filtered under slight negative pressure through a 100 millimicron millipore filter.

The resistance and tip potential of the microelectrodes were measured using a Grass P16 microelectrode D.C. amplifier. Electrodes were discarded if the resistance was less than 30 megohms or if the tip potential exceeded 5 mV.

III. Measured Parameters of the Atrial Transmembrane Action Potential

The following parameters of the atrial transmembrane action potential were obtained from measurement of projected photographic recordings. These parameters are illustrated in figure 2.: (i) the resting membrane potential (mV); (ii) the amplitude of transmembrane action potential (mV); (iii) the overshoot of the transmembrane action potential (mV); (iv) the rise time of the transmembrane action potential (ms), i.e. the duration from the onset of the action potential to the peak of the upstroke; (v) the duration of the transmembrane action potential (ms), i.e. the duration from the onset of the transmembrane action potential to the 90% repolarization level; (vi) the 25, 50, and 90% repolarization times (ms), i.e. the duration from the peak of the upstroke of the transmembrane action potential to the point of 25, 50, and 90% level of repolarization.

FIGURE 2: Diagramatic representation of the measured parameters of the atrial transmembrane action potential.

i. resting membrane potential

ii. Amplitude of transmembrane action potential

iii. overshoot of transmembrane action potential

iv. rise time of transmembrane action potential

v. duration of transmembrane action potential

vi. % repolarization time (X= 25,50 or 90%)

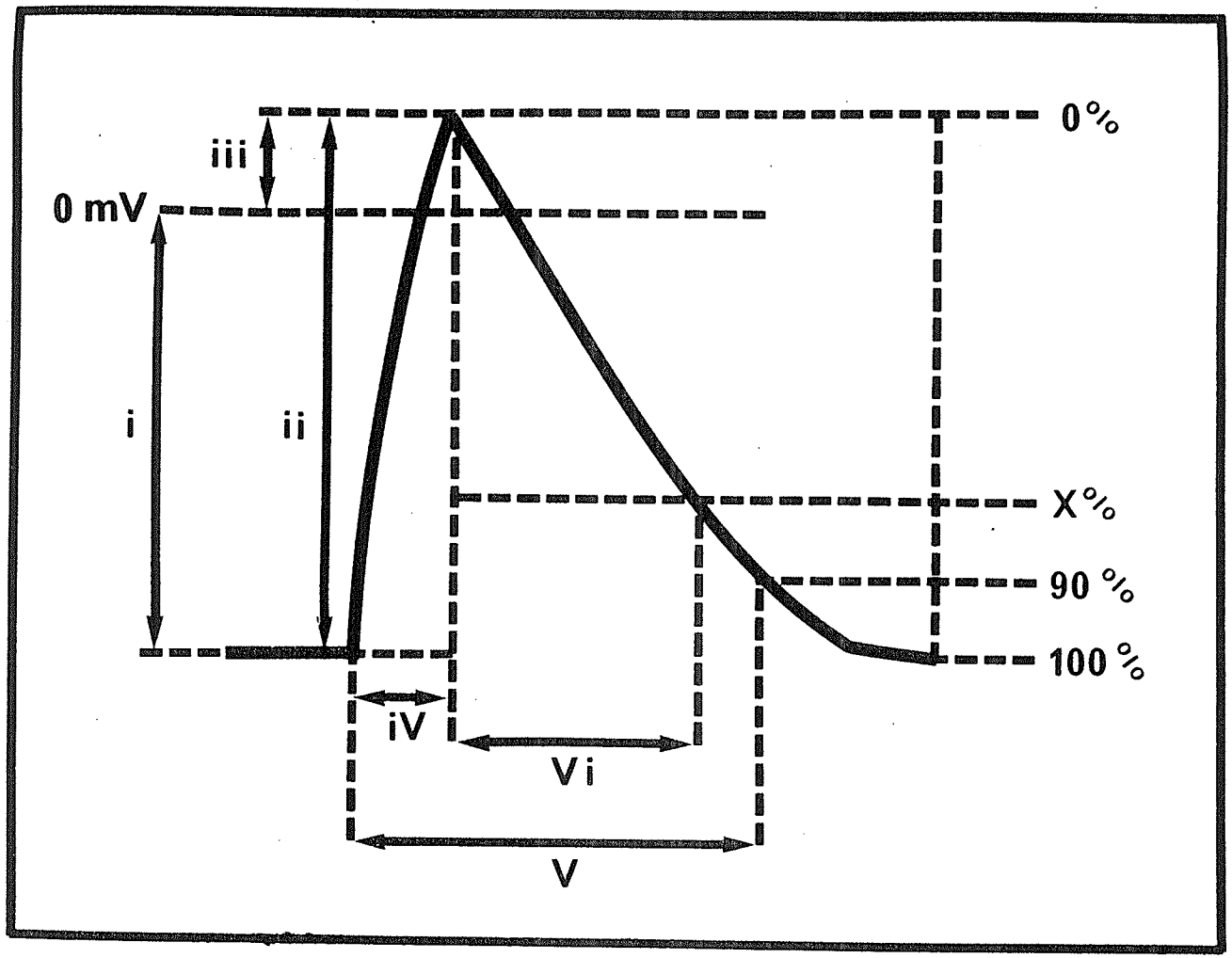

FIGURE 2

IV. Measurement of Heart Rate

Calculation of heart rate (beats per minute, B.P.M.) was obtained from projected photographic recordings of trains of transmembrane action potentials and isometric tension records. A calculation of heart rate was performed in conjunction with each individual transmembrane action potential analysed.

V. Statistical Analysis

Statistical analysis of the parameters measured in the isolated atrial preparation was performed with Student's t-test for unpaired observations (Steel and Torrie, 1960). Significance limits of the Student distribution (P values) were obtained from a two tailed t-table (Documenta Geigy Scientific Tables, sixth edition). The means of the measured parameters and their standard errors are presented in the Results section of this text.

VI. Experimental Protocol

Immediately after the equilibration period of the isolated preparation (see General Procedures), control values were established for the atrial action potential before the commencement of any experimental procedure. In general the experimental protocols were as follows:

A. Veratramine Treatment

Veratramine, in various concentrations, (range 10^{-7} to 10^{-4} g/ml) was added to the fluid bathing the tissue. Action potentials were monitored throughout

the course of development of resultant drug action.

B. Drug Treatment before Veratramine Treatment

Pharmacological agents (in varied concentrations) were added to the bathing fluid before veratramine. This was done to ascertain the effects of the agents on the course of development of veratramine effect, as well as to establish the effects of these agents themselves on the atrial preparation.

C. Drug Treatment Following Veratramine Treatment

In order to ascertain the ability of pharmacological agents to antagonize or enhance the effects of veratramine on the atrial preparation, the effects of veratramine on the preparation were developed prior to the addition of other agents to the bathing fluid.

D. Cold Treatment

In addition to the effects of pharmacological agents observed using the foregoing protocols, the effects of reduced temperatures on the untreated and veratramine treated preparation were examined. Progressive reduction in the temperature of the atrial preparation was achieved by switching off the circulation pump which normally was responsible for maintaining the organ bath at 37^{o}C. The temperature of the fluid bathing the preparation was monitored throughout this procedure.

Throughout all the experimental procedures transmembrane action potentials were continuously sampled.

The microelectrode was positioned and repositioned so as to sample atrial cells over a wide area of the preparation.

VII. Drugs

The chemical compounds used in this study and the suppliers of these compounds are listed in Table 1. All solutions were made weight/volume in terms of the base unless otherwise specified. Stock solutions were stored at $4^{o}C$ with the exceptions of caffeine and aminophylline which were kept at room temperature to prevent precipitation. Concentrations in the text refer to final concentration in the bath fluid in g/ml. In volume, there was never greater than 1 ml of any test solution added to the bathing fluid.

With the exception of specified agents, stock solutions of the biogenic amines were prepared in 0.01 M HCl and other drugs in glass-distilled water. On the day of the experiment, stock solutions were diluted as required with 0.9% sodium chloride solution.

Other Solutions

Veratramine stock solution (1 mg/ml) was prepared from the powder immediately before the experiment. 10 mg of veratramine powder was weighed and placed in a 10 ml volumetric cylinder. Immediately after the addition of approximately 2 drops of 6.0 M HCl to this cylinder, 6 ml of glass distilled water were added and the contents agitated. When the veratramine had dissolved, the volume of

the solution was made up to 10 ml. The addition of up to 1 ml of this solution to the organ bath did not alter the pH of the Krebs-Henseleit solution.

Valinomycin stock solution (1 mg/ml) was prepared by dissolving the powder in 95% alcohol.

Appropriate volumes of drug solvents were tested as to their effects on the atrial preparation.

VIII. Stimulus Threshold in the Spinal Cat

The minimum voltage required to electrically drive the heart in the spinal cat preparation (see Spinal Preparation pg 43) was determined as follows:

Duration of stimulus was set constant at 1 ms. The frequency of stimulation (usually 3 Hz) was set at a rate greater than the observed spontaneous rate. The stimulus strength (V) was gradually increased until the heart began to follow the applied stimulus. Stimulus threshold was calculated as the mean voltage of several trials. Stimulus threshold was determined before and after experimental manipulation of the preparation.

TABLE 1

Acetylcholine chloride	Calbiochem
l-Adrenaline bitartrate	Mann Research Laboratories
Aminophylline	British Drug Houses
Caffeine	Calbiochem
Carbachol	Calbiochem
Dibutyryl cyclic AMP (dibutyryladenosine 3'5' cyclic phosphate)	Calbiochem
dl-Isoprenaline bitartrate	Winthrop Laboratories
Ouabain	Nutritional Biochemical Corporation
Valinomycin	Calbiochem
Veratramine	S.B. Penick and Company

SECTION III

RESULTS

RESULTS

Effect of Veratramine on Spontaneous Atrial Rate and Rhythm

The effects of veratramine on the heart rate of the isolated spontaneously beating right atrial preparation of the cat are summarized in Table II. Control values of heart rate (beats per minute) were established in the untreated preparations after the initial equilibration period (see Materials and Methods; General Procedures) immediately before administration of veratramine. In addition, values of mean heart rate were established when spontaneous atrial rate had decreased in the presence of veratramine before development of periodic rhythm (Table II, Veratramine non-periodic), as well as when periodic rhythm occurred in the presence of veratramine (Table II, Veratramine-Periodic). The mean heart rate, when spontaneous atrial rate had decreased in the presence of veratramine before development of periodic rhythm, was significantly reduced as compared to control ($P < .01$). Furthermore, the mean atrial rate in the presence of veratramine during periodic rhythm was significantly reduced as compared to both control atrial rate ($P < .01$) and as compared to atrial rate in the presence of veratramine before the development of periodic rhythm ($P < .01$). Values of the atrial rate during periodic rhythm refer to atrial rate during the active phases of periodic rhythm. The duration of the inactive

TABLE II

EFFECTS OF VERATRAMINE ON THE SPONTANEOUS RIGHT ATRIAL HEART RATE

	Control	Veratramine	
		non-periodic	periodic
Mean atrial rate (beats per minute)	147.4	86.6*	64.8**
Standard error	±6.0	±5.5	±4.3
N	56	25	25

* significantly different from control $P < .01$

* * significantly different from veratramine non-periodic $P < .01$

N = number of determinations

phases was not included in calculation of atrial rate during periodic activity.

The effect of veratramine on the spontaneous rhythm of the isolated atrial preparation was dependent on the concentration of the drug to which the atrium was exposed and the duration of exposure of the atrium to veratramine. Exposure of the atria to veratramine, 10^{-7} - 10^{-5} g/ml, routinely caused only slowing of the spontaneous rate. In the presence of veratramine, 1-3 x 10^{-5} g/ml, the spontaneous atrial rate decreased progressively from 147.4 $\pm$ 6.0 to 86.6 $\pm$ 5.5 beats per minute before the development of periodic rhythm. Figure 3 shows a typical recording of the electrical and mechanical events occurring with the onset of periodic rhythm. The establishment of periodic rhythm in the presence of veratramine was marked by a period of complete asystole which usually lasted 15-20 seconds and was followed by recommencement of atrial rhythm. Thereafter, alternating periods of inactivity and activity of the atria continued for up to 30 minutes. The duration of the active phases was variable during periodic activity, usually increasing from approximately 5 seconds at the beginning of the periodic rhythm to 30 seconds at the end, when regular rhythm was resumed. A typical record of the tension developed by the atria during periodic activity is shown in Figure 4. After a period of asystole, the first atrial contraction of the

FIGURE 3: Periodic activity induced by veratramine, 3×10^{-5} g/ml, in the isolated right atrium preparation. Transmembrane potential is the upper trace, tension the lower trace.

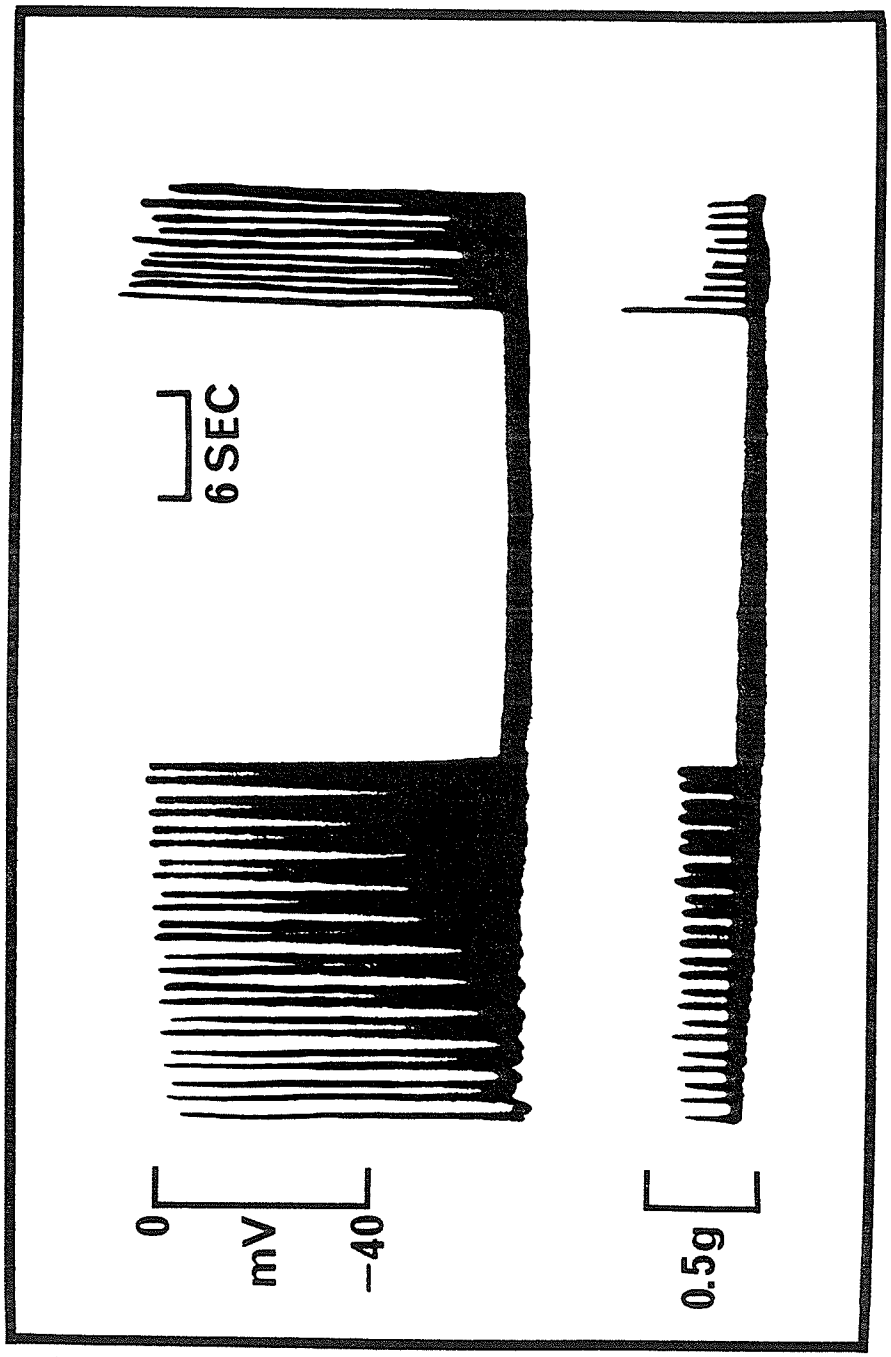

FIGURE 3

FIGURE 4: Record of atrial tension development during periodic activity induced by veratramine 3×10^{-5} g/ml.

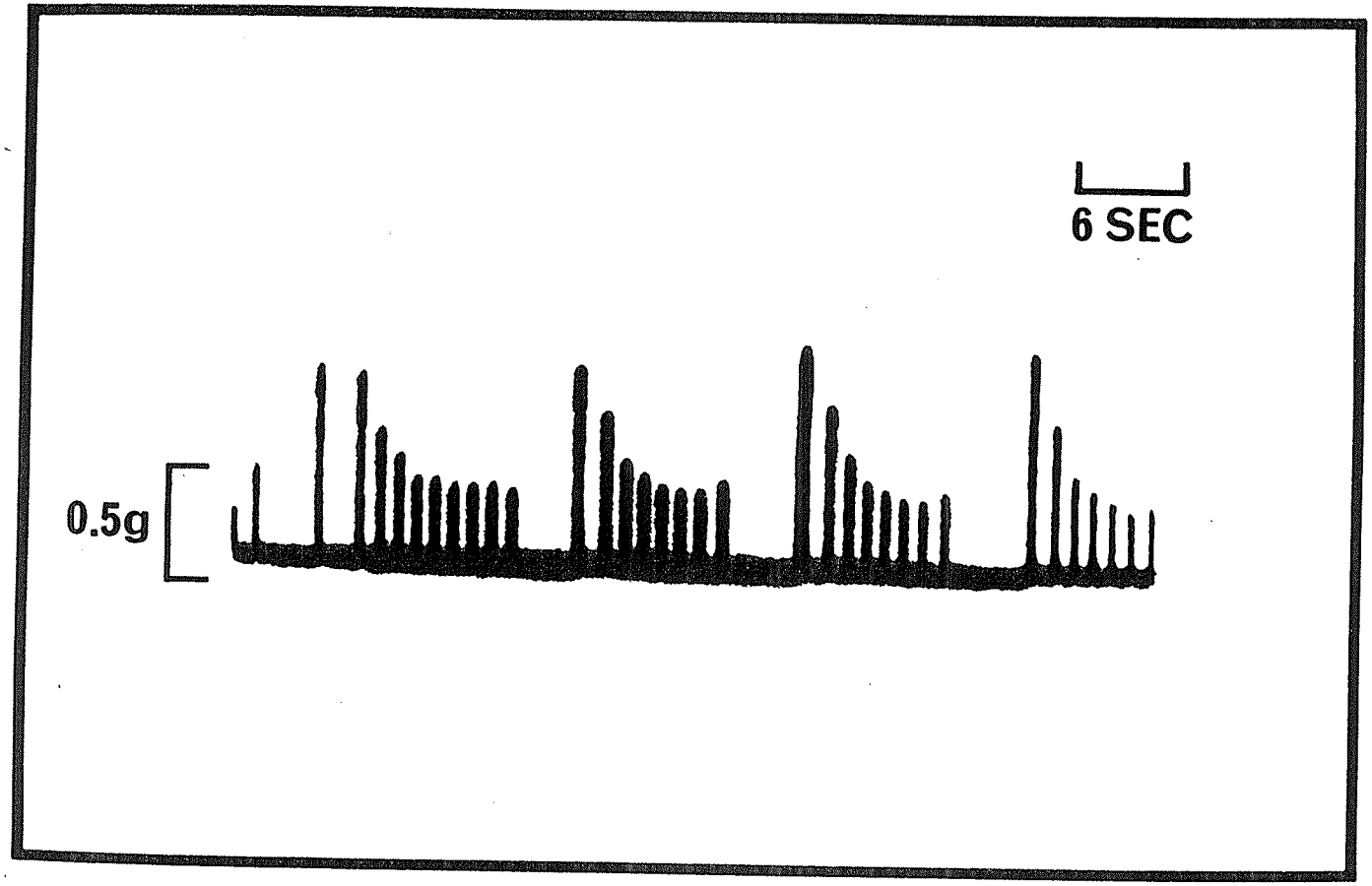

FIGURE 4

active period was considerably larger than the ensuing contractions. Subsequent contractions were progressively smaller reaching their lowest strength at the 5th or 6th contraction. The active phases of the periodic rhythm were characterized by a gradually decreasing rate leading to a period of asystole. The average atrial rate during the active phase of periodic rhythm was 64.8 $\pm$ 4.3 beats per minute (Table II).

In the presence of veratramine, 1-3 x 10^{-5} g/ml the periodic rhythm of the atrial preparation eventually ended. Either a regular rhythm ensued, slower than the control rate, or the atrium became completely quiescent. Typical resolution of periodic rhythm into a regular rhythm is shown in sequential atrial tension records in Figures 5-8. As the periodic rhythm evolved into a regular rhythm, the duration of the active phases increased progressively until a regular rhythm was established.

When in the presence of veratramine, 1-3 x 10^{-5} g/ml, the atrial preparation became completely quiescent following periodic rhythm, regular electrical stimulii could still evoke responses if stimulus strength was increased over that necessary to drive untreated atria, but the response did not follow regularly on a one to one basis, indicating a possible blockade of conduction. When veratramine, 1-3 x 10^{-5} g/ml, was left in contact with the muscle for one hour, and then washed out, electrical stimulation had no

FIGURE 5: Sequential record of atrial tension during resolution of periodic activity into a regular rhythm in the presence of veratramine, 3×10^{-5} g/ml (see also FIGURES 6,7, and 8). The corresponding chronological order of tension records is 5a, 5b, 6a, 6b, 7a, 7b, 8a, 8b.

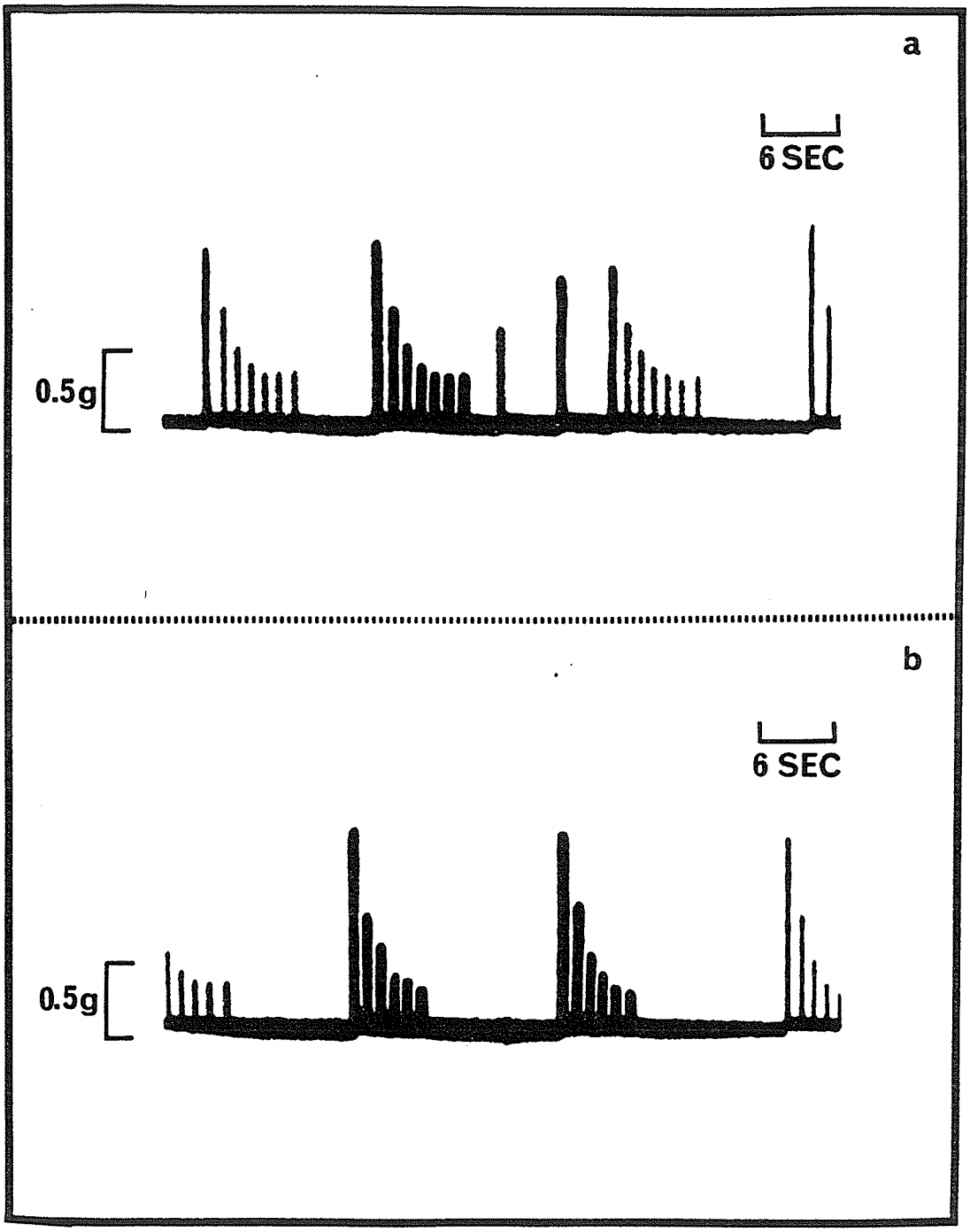

FIGURE 5

FIGURE 6: Sequential record of atrial tension during resolution of periodic activity into a regular rhythm in the presence of veratramine, 3×10^{-5} g/ml (see also FIGURES 5,7, and 8). The corresponding chronological order of tension records is 5a, 5b, 6a, 6b, 7a, 7b, 8a, 8b.

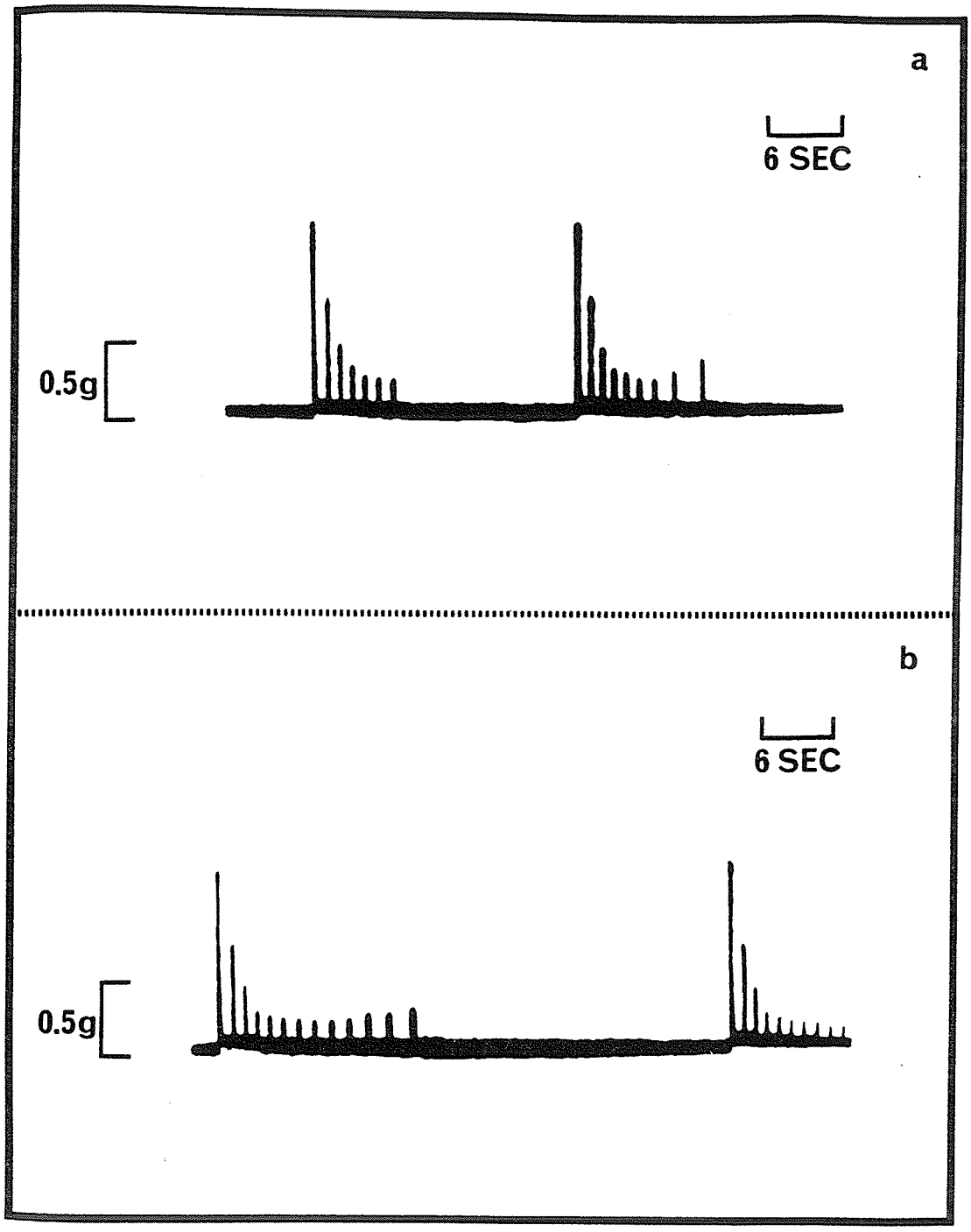

FIGURE 6

FIGURE 7: Sequential record of atrial tension during resolution of periodic activity into a regular rhythm in the presence of veratramine, 3×10^{-5} g/ml (see also FIGURES 5,6, and 8). The corresponding chronological order of tension records is 5a, 5b, 6a, 6b, 7a, 7b, 8a, 8b.

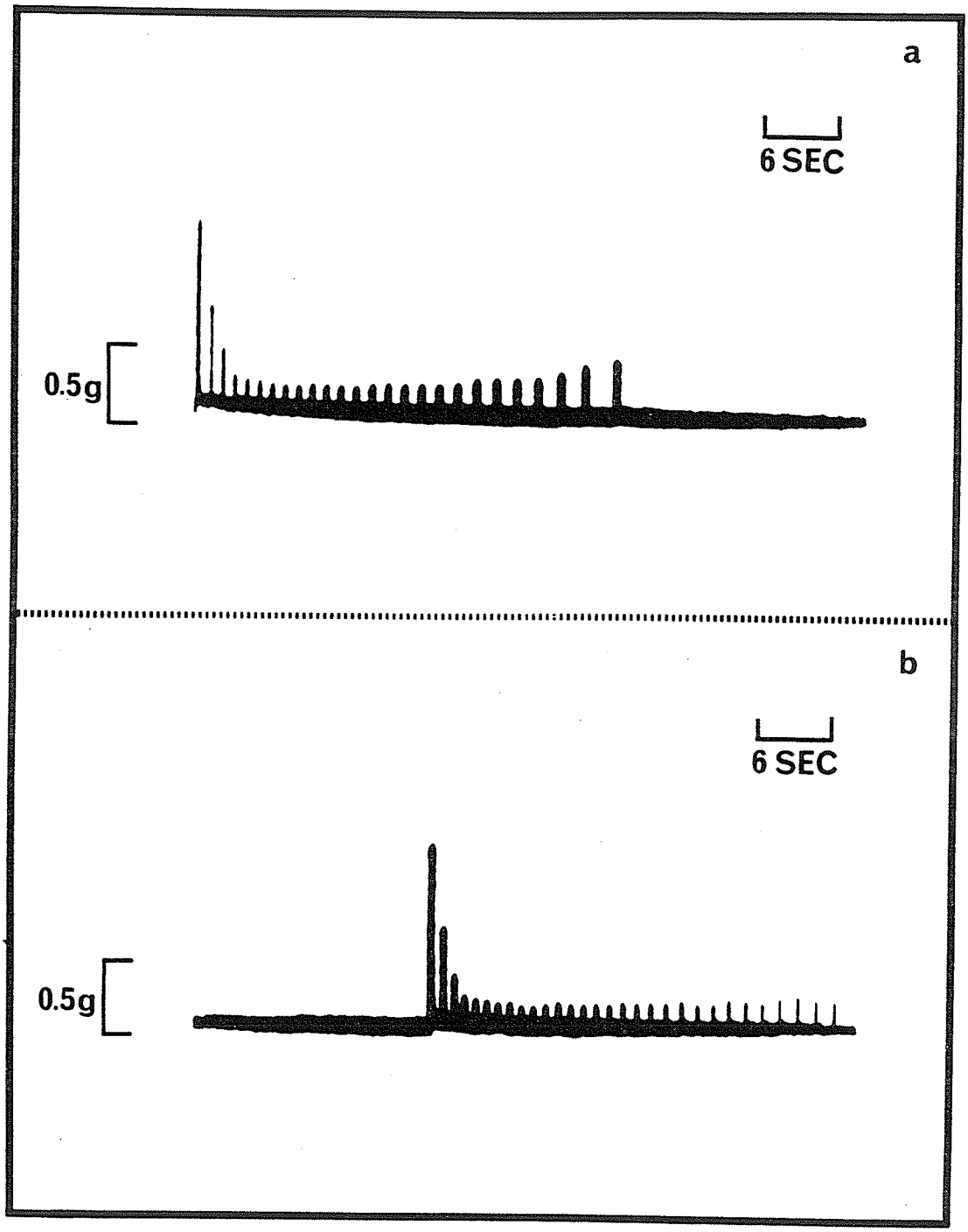

FIGURE 7

FIGURE 8: Sequential record of atrial tension during resolution of periodic activity into a regular rhythm in the presence of veratramine, 3×10^{-5} g/ml (see also FIGURES 5,6, and 7). The corresponding chronological order of tension records is 5a, 5b, 6a, 6b, 7a, 7b, 8a, 8b.

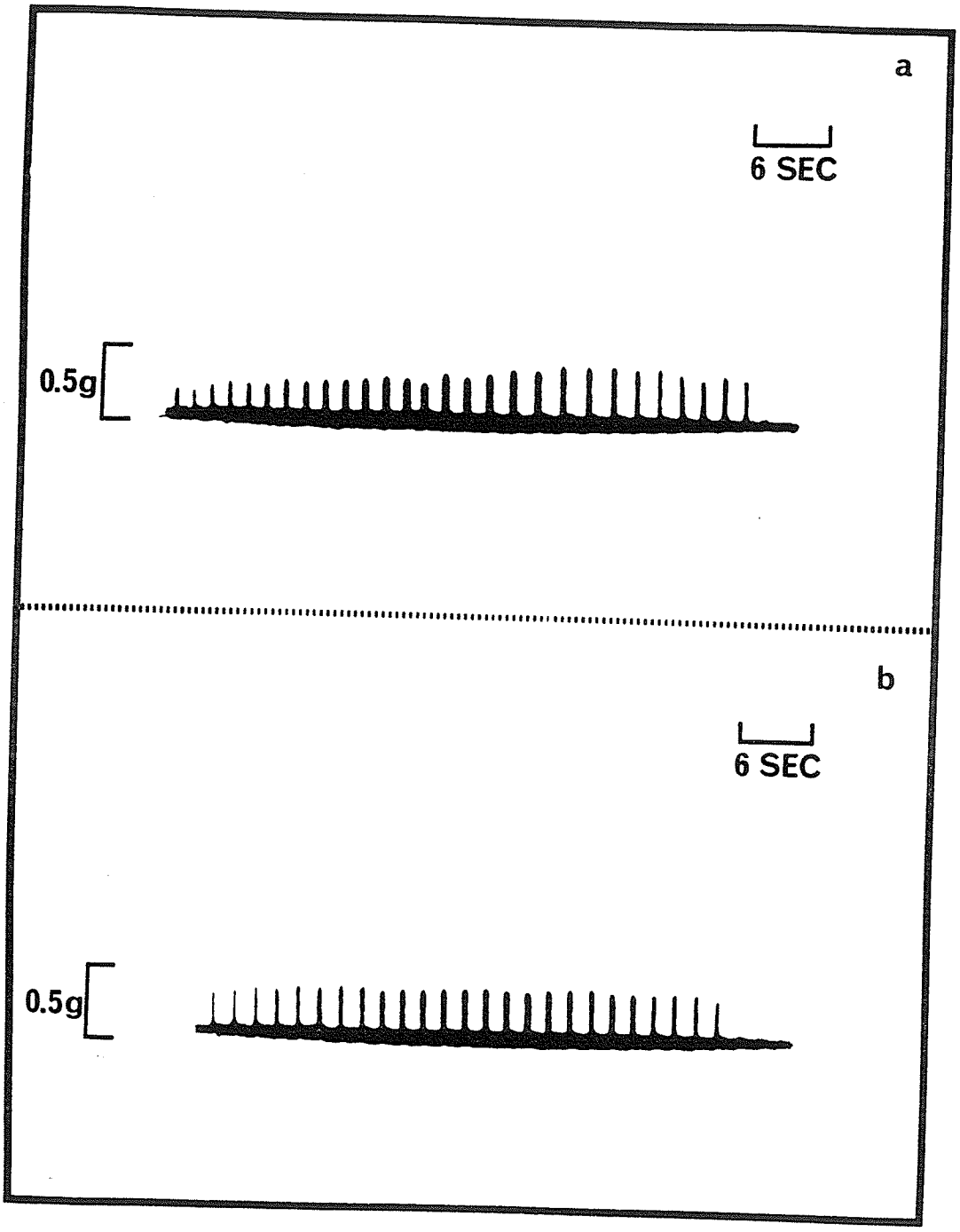

FIGURE 8

effect. However, although the atrium was quiescent, a small "pacemaker" area of about 1.5 - 2.5 sq. mm was active electrically and mechanically. This small area contracted regularly and was easily identified by these contractions. This phenomenon continued for up to two hours.

When the isolated atrium was exposed to a larger dose of veratramine, 7×10^{-5} g/ml, the preparation became quiescent without passing through the stage of periodic rhythm. Sequential segments of a continous record of the transmembrane potential and isometric tension of the atrial preparation in the presence of veratramine, 7×10^{-5} g/ml, are shown in Figures 9-12. These records of transmembrane potential are of a single cell held continously. Coincident with the gradual decline in atrial rate, the amplitude of atrial contractions and the amplitude of the atrial transmembrane action potential decreased. Throughout the process, the resting membrane potential remained unchanged. A dissociation of mechanical and electrical events in the atrial preparation is illustrated in Figure 11b, where contractile events are not consistently related to the electrical events occurring in the impaled recorded cell. The process whereby the atrial preparation became quiescent in the presence of veratramine, 7×10^{-5} g/ml, was complete in 5-10 minutes, and drug was washed out after 10 minutes exposure. The discrete active "pacemaker" area became obvious shortly after the drug was washed out

and remained active while the rest of the preparation remained quiescent.

FIGURE 9: Veratramine-induced quiescence in the isolated right atrial preparation. FIGURES 9,10,11 and 12 are sequential segments of a continuous record of the atrial preparation in the presence of veratramine, 7×10^{-5} g/ml. The chronological order of records is 9a, 9b, 10a, 10b, 11a, 11b, 12a, 12b. In each record segment, transmembrane potential is the upper trace, tension the lower trace.

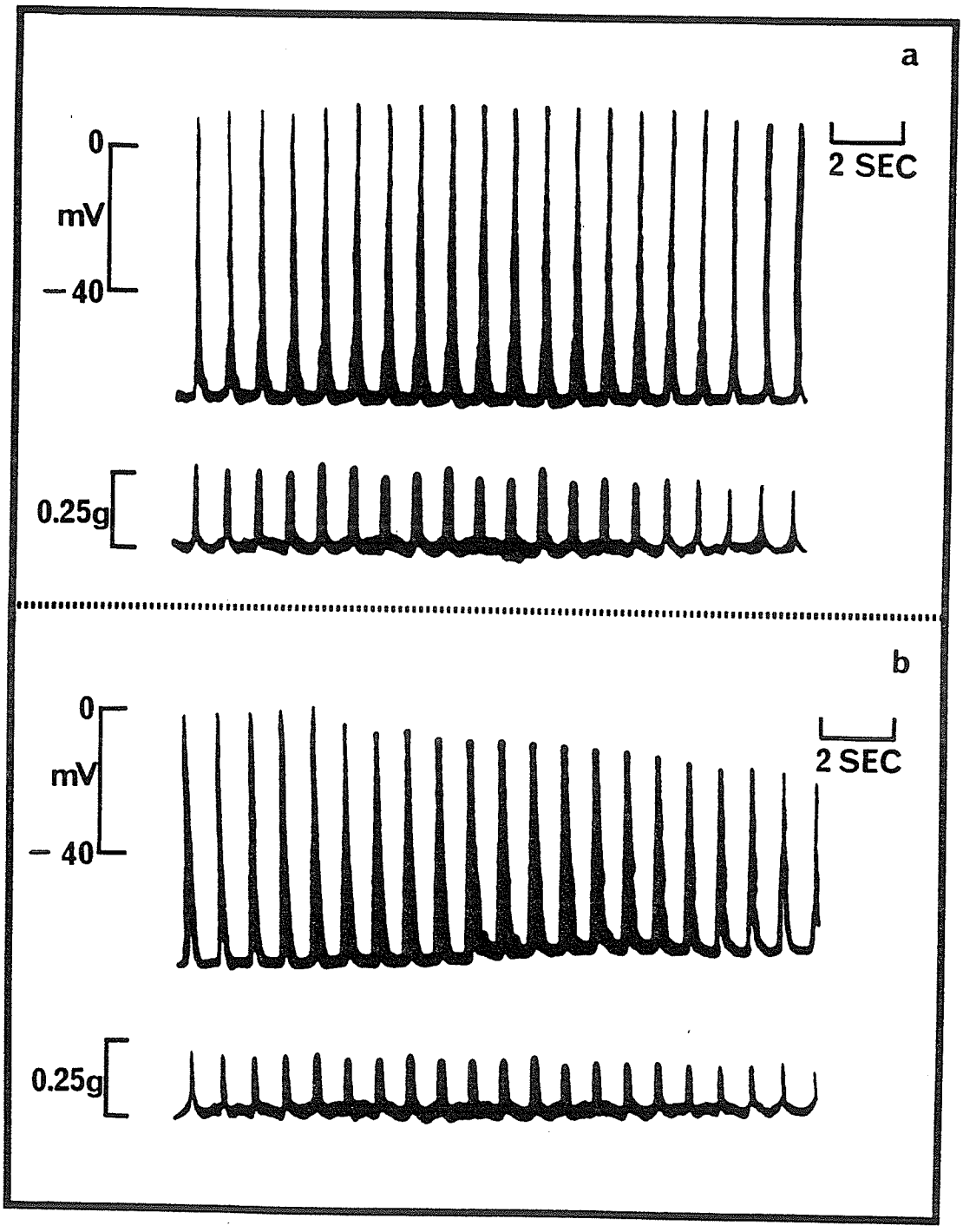

FIGURE 9

FIGURE 10: Veratramine-induced quiescence in the isolated right atrial preparation. FIGURES 9,10,11, and 12 are sequential segments of a continous record of the atrial preparation in the presence of veratramine, 7×10^{-5} g/ml. The chronological order of records is 9a, 9b, 10a, 10b, 11a, 11b, 12a, 12b. In each record segment, transmembrane potential is the upper trace, tension the lower trace.

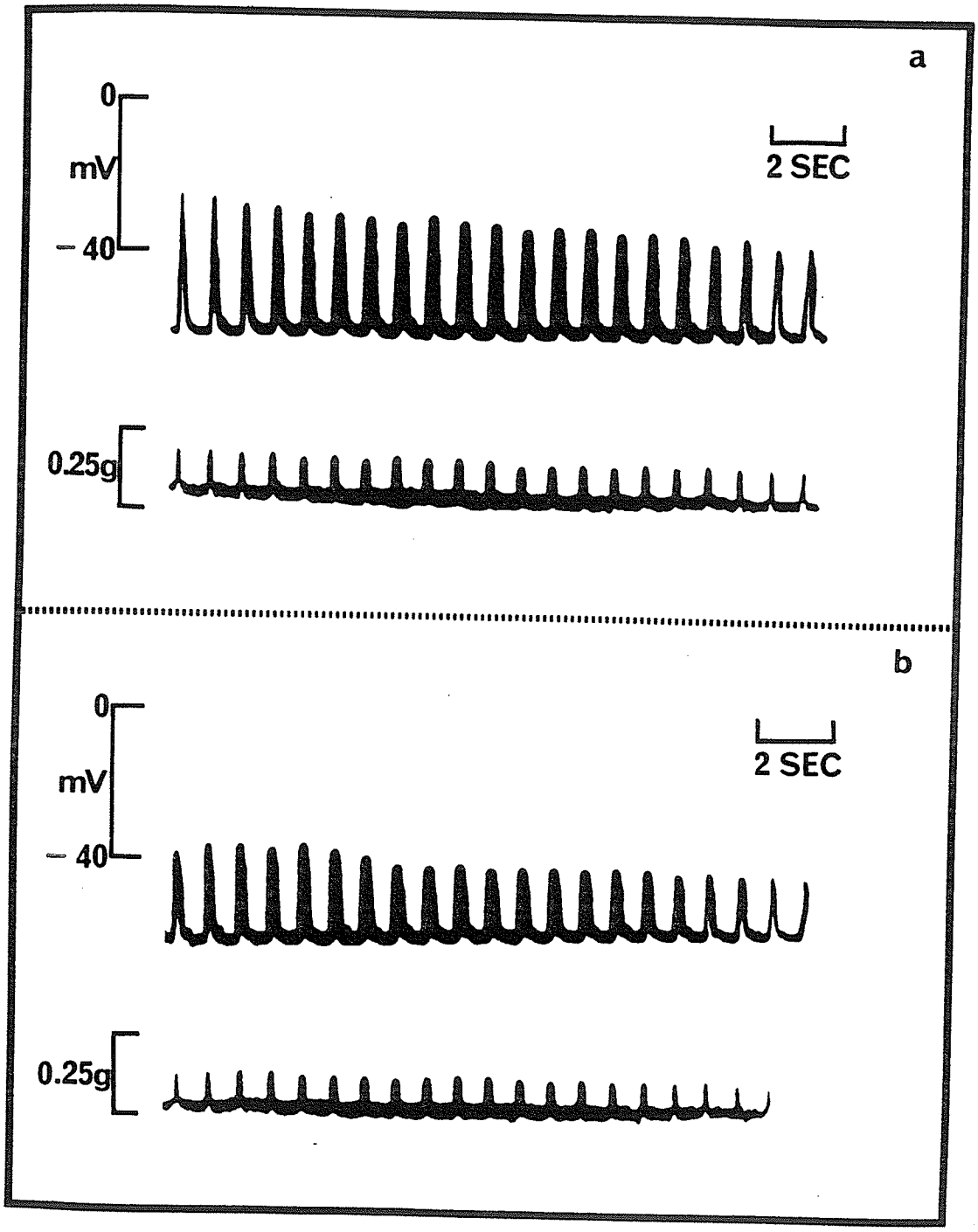

FIGURE 10

FIGURE 11: Veratramine-induced quiescence in the isolated right atrial preparation. FIGURES 9,10,11, and 12 are sequential segments of a continous record of the atrial preparation in the presence of veratramine, 7×10^{-5} g/ml. The chronological order of records is 9a, 9b, 10a, 10b, 11a, 11b, 12a, 12b. In each record segment, transmembrane potential is the upper trace, tension the lower trace.

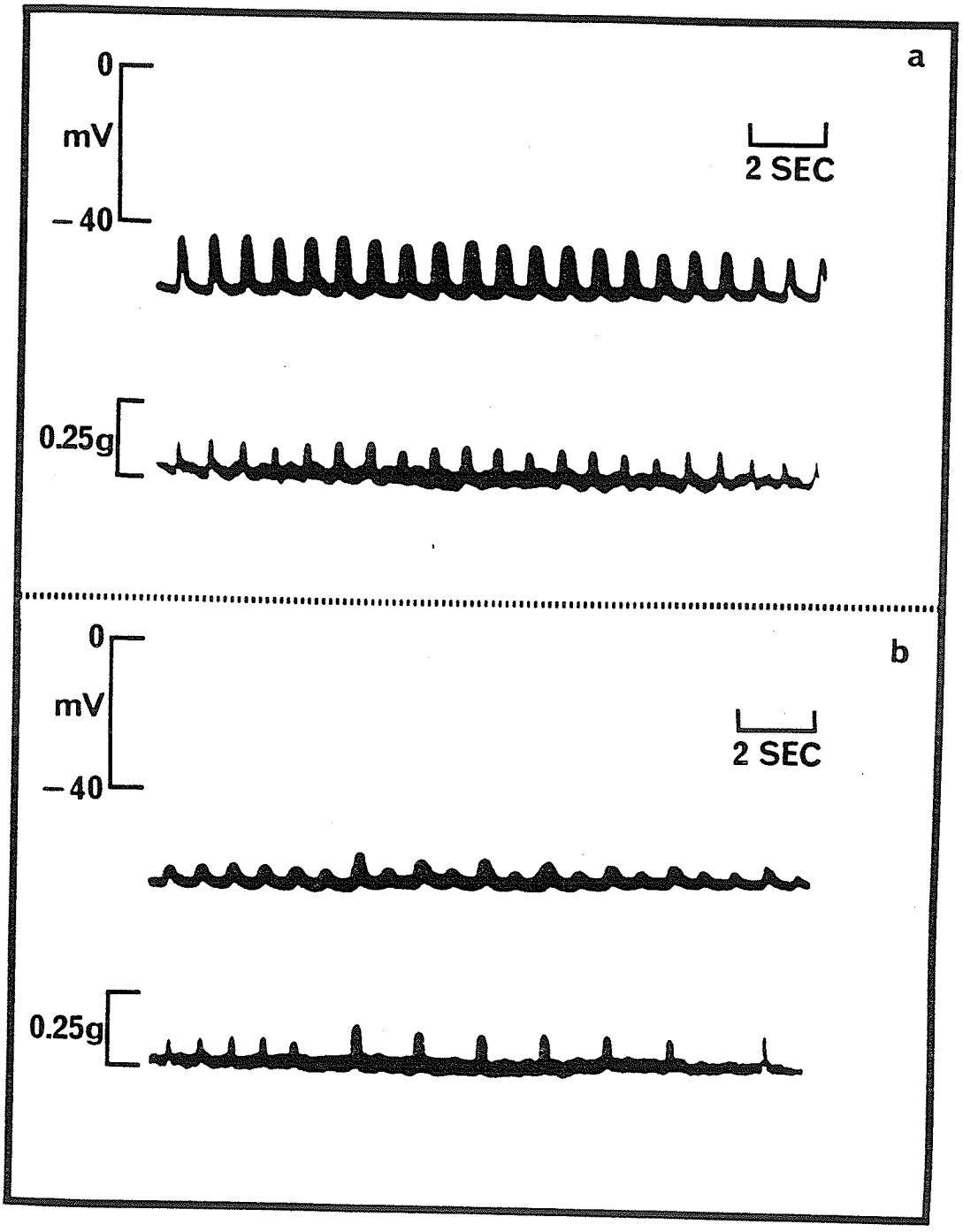

FIGURE 11

FIGURE 12: Veratramine-induced quiescence in the isolated right atrial preparation. FIGURES 9,10,11, and 12 are sequential segments of a continuous record of the atrial preparation in the presence of veratramine, 7×10^{-5} g/ml. The chronological order of records is 9a, 9b, 10a, 10b, 11a, 11b, 12a, 12b. In each record segment, transmembrane potential is the upper trace, tension the lower trace.

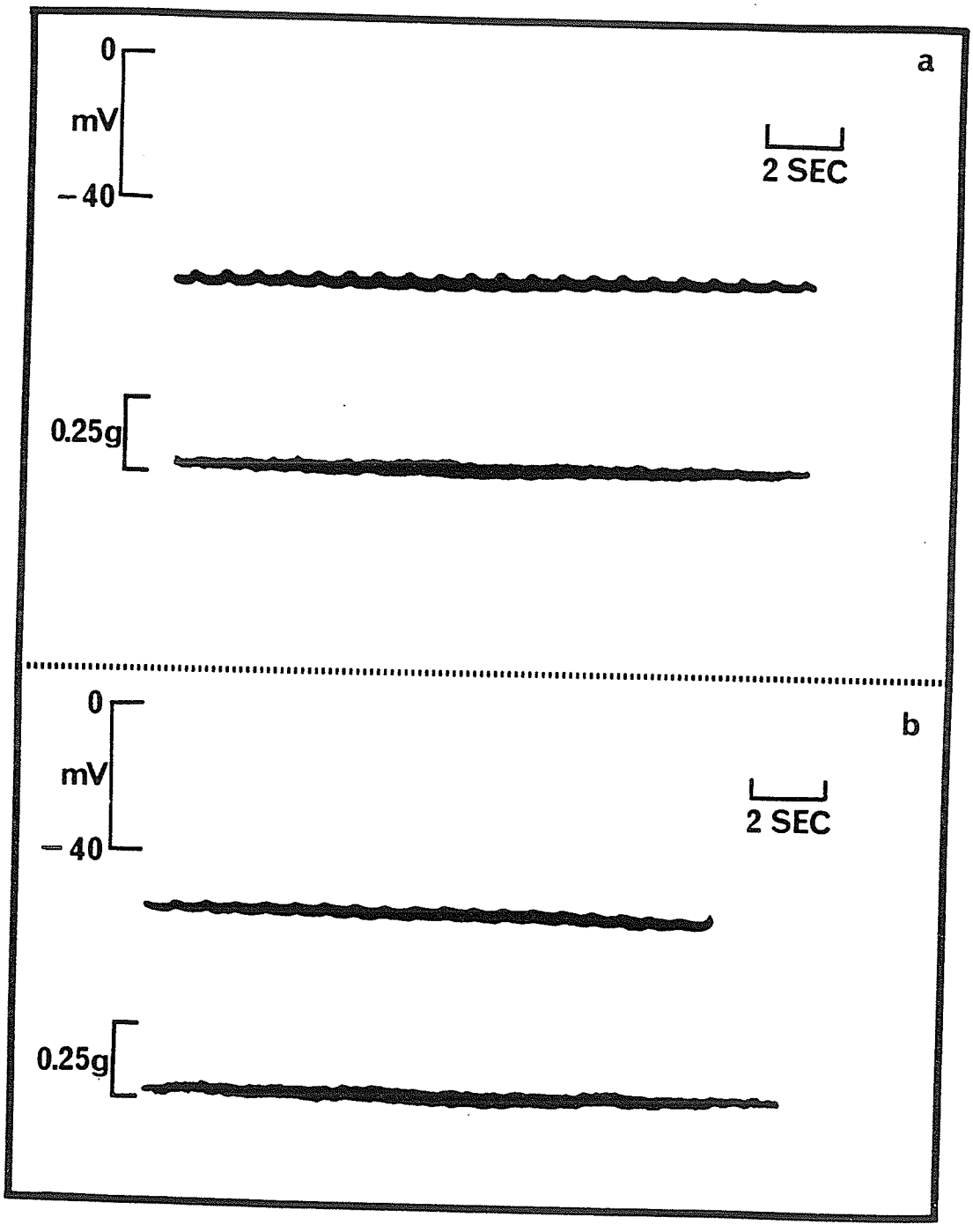

FIGURE 12

Stimulus Threshold During Periodic Rhythm

In the isolated atrial preparation it had been observed that in the presence of veratramine the atrium often did not respond to electrical stimulation on a one to one basis, and that the atrium, when it became quiescent due to veratramine did not respond to electrical stimulation. It was decided to determine, in the spinal cat preparation, the effect of veratramine on the stimulus threshold required to drive the heart electrically. Intravenous injection of veratramine, 1 mg/kg, produced periodic rhythm in the spinal cat. Five spinal preparations were used and it was determined that the stimulus threshold required to electrically drive the heart was greatest during periodic rhythm during the periods of asystole (approximately 0.74V). During the active phases of periodic rhythm, the stimulus threshold required to electrically drive the heart was approximately 0.70 V. Before the administration of veratramine, a stimulus of approximately 0.68 V was required to electrically drive the heart.

When the heart was electrically driven at 3 or 6 Hz during periodic rhythm for periods of time exceeding one minute, the heart did not always follow the stimuli on a one to one basis, and could in fact escape the driving stimulus altogether and beat on its own.

In the spinal cat preparation, as well as in the isolated atrial preparation, periodic rhythm eventually

was resolved into a regular rhythm. When the heart was driven by electrical stimulation after periodic rhythm had resolved into a regular rhythm, periodic rhythm occurred after the cessation of the stimulation. The heart was usually driven at a rate which did not exceed the spontaneous rate of the untreated preparation. Periodic rhythm did not occur during the period of electrical stimulation, however escape from the driving stimuli was observed, as well as an inability of the heart to follow stimuli on a one to one basis.

Effects of Veratramine on the Atrial Action Potential

The effects of exposure of the isolated atrial preparation to various concentrations of veratramine on the intracellular transmembrane action potential were quantitated in terms of measurements made from projected photographic recordings of individual action potentials. The parameters of the transmembrane action potential that were measured are defined in the Materials and Methods section of this text and include; resting membrane potential, action potential amplitude, action potential overshoot, rise time of the transmembrane action potential, action potential duration and the 25, 50, and 90% repolarization times. The results, with appropriate comparisons, are presented in Table III.

Coincident with the decline in spontaneous atrial

TABLE III

EFFECTS OF VERATRAMINE ON ATRIAL TRANSMEMBRANE ACTION POTENTIAL

Action Potential Parameters Measured when Atrial Rate is Slowed by Veratramine, 1-3 x 10^{-5} g/ml, Before Periodic Rhythm (Veratramine Non-Periodic) and During Periodic Rhythm Induced by Veratramine, 3-5 x 10^{-5} g/ml (Veratramine Periodic).

	Resting Potential (-mV)	Amplitude (mV)	Overshoot (mV)	Rise time (mSec)	90% Duration (mSec)	Repolarization time (mSec)		
						25%	50%	90%
Control								
Mean	-67.6	79.3	11.8	8.9	146.5	34.1	66.2	137.7
± SE	± 0.7	±0.8	±0.4	±0.2	±3.4	±0.9	±1.7	±3.4
N = 56								
Veratramine Non-Periodic								
Mean	-70.5	74.4*	3.8*	34.7*	219.1*	61.8*	99.1*	184.4*
± SE	± 1.6	±1.7	±0.8	±4.3	±7.7	±2.7	±3.1	±4.7
N = 25								
Veratramine -Periodic								
Mean	-67.4	66.4**	-1.0**	55.7**	270.0**	78.7**	123.4**	214.5**
± SE	± 1.2	±1.8	±1.0	±6.0	±9.3	±4.9	±5.0	±5.8
N = 25								

* Significantly different from control $P < .01$

** Significantly different from Veratramine Non-Periodic and Control

rate produced by veratramine, the action potential was altered in a progressive continous manner before and during periodic activity.

Action Potential During Veratramine - Induced Reduced Spontaneous Atrial Rate Without Periodic Rhythm

Figures 13 and 14 illustrate the typical appearance of individual atrial transmembrane action potentials when the spontaneous atrial rate had been reduced by veratramine. For purposes of comparison, atrial transmembrane action potentials recorded from the same atria before veratramine are included in Figures 13 and 14. Isometric tension records, recorded simultaneously with the respective transmembrane action potentials are also included.

Figures 13 and 14 show that reduction of the spontaneous atrial rate by veratramine was accompanied by reduction of the amplitude of the atrial transmembrane action potential . In addition, the overshoot of the action potential was reduced while the resting membrane potential remained not significantly different from that of the control. The time course of the action potential was also affected by veratramine. Depolarization and repolarization processes were slowed as compared to the controls.

The mean values of the measured action potential parameters of untreated atrial preparations (Control), and of atrial preparations exposed to veratramine at a dose level which slowed spontaneous atrial rate but did

FIGURE 13: The appearance of the atrial transmembrane action potential when spontaneous atrial rate is reduced by veratramine, 3×10^{-5} g/ml. (a) Control, (b) Veratramine. In both (a) and (b) transmembrane potential is the upper trace, tension is the lower trace.

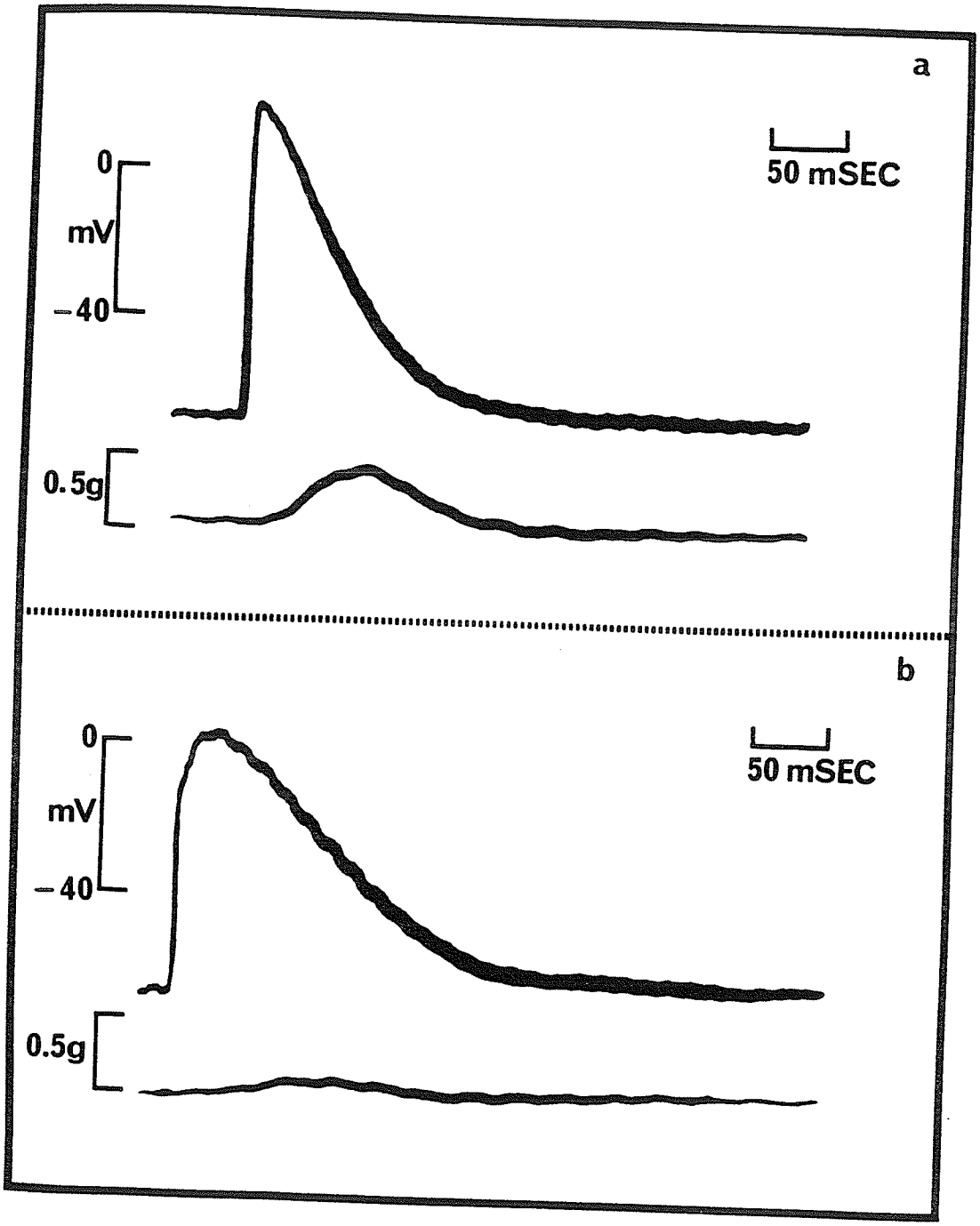

FIGURE 13

FIGURE 14: The appearance of the atrial transmembrane action potential when spontaneous atrial rate is reduced by veratramine, 3×10^{-5} g/ml. (a) Control, (b) Veratramine. In both (a) and (b) transmembrane potential is the upper trace, tension is the lower trace.

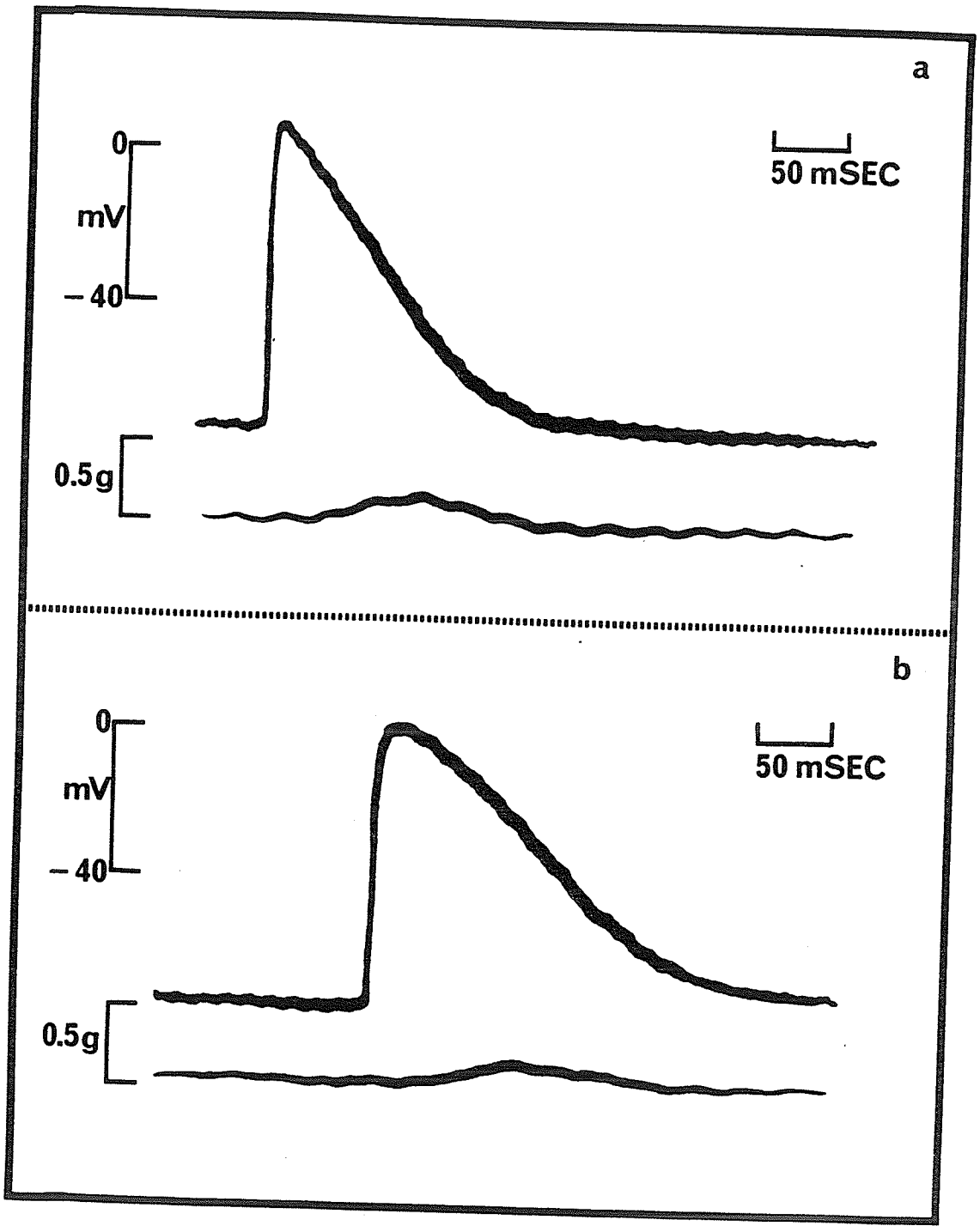

FIGURE 14

not precipitate periodic rhythm (Veratramine Non-Periodic) are presented in Table III.

Veratramine reduced spontaneous atrial rate from 147.4 ± 6.0 to 86.6 ± 5.5 beats/min (Table II). Coincident with this reduction in the spontaneous rate, the amplitude of the atrial transmembrane action potential was significantly reduced ($P < 0.01$) from mean value of 79.3 ± 0.8 mV to a mean value of 74.4 ± 1.7 mV (Table III). In addition, when spontaneous atrial rate had been reduced in the presence of veratramine, the overshoot of the action potential was significantly reduced ($P < .01$) from a control mean value of 11.8 ± 0.4 mV to a mean value of 3.8 ± 0.8 mV (Table III). The mean resting membrane potential in the presence of veratramine was not significantly different from that of control (Table III).

Exposure of the atrial preparation to veratramine also resulted in significant alterations in the time course of the depolarization and repolarization processes of the action potential. The rise time of the upstroke of the action potential was calculated to provide an evaluation of the function of processes governing the rate of upstroke of the action potential. As the spontaneous atrial rate declined in the presence of veratramine from 147.4 ± 6.0 to 86.6 ± 5.5 beats/min, the mean rise time of the action potential was increased from 8.9 ± 0.2 ms to 34.7 ± 4.3 ms (Table III). The mean rise time of the Veratramine Non-

Periodic action potential upstroke was significantly greater than the control mean rise time ($P < 0.01$), and represented an increase over control by a factor of approximately 4. The total duration of the action potential (measured to the 90 per cent repolarization level) was increased from a control mean value of 146.5 $\pm$ 3.4 ms to 219.1 $\pm$ 7.7 ms (Table III), when spontaneous rate had been reduced to 86.6 $\pm$ 5.5 beats/min in the presence of veratramine. Analysis of repolarization time indicated that, while the earlier phase of repolarization (25 per cent repolarization time) was affected to the greater degree by veratramine, The later phases of repolarization (50 and 90 per cent repolarization times) were also prolonged. When spontaneous atrial rate had been reduced by veratramine, the mean 25 per cent repolarization time was increased by approximately 81%, from a control mean value of 34.1 $\pm$ 0.9 ms to a mean value of 61.8 $\pm$ 2.7 ms. The mean 50 per cent repolarization time was increased by approximately 50%, from a control mean value of 66.2 $\pm$ 1.7 ms to 99.1 $\pm$ 3.1 ms when spontaneous rate was reduced by veratramine. The mean 90 per cent repolarization time was increased as compared to control by approximately 34%, from a mean value of 137.7 $\pm$ 3.4 ms to 184.4 $\pm$ 4.7 ms in the presence of veratramine. It is apparent in Figures 13 and 14 that while the duration of the action potential repolarization was increased by veratramine, the increased duration did not re-

sult in the appearance of a distinct plateau phase during repolarization. In addition, the "spike" quality of the action potential upstroke was less prominent in the presence of veratramine than in the control.

Resting Membrane Potential During the Quiescent Phases of Veratramine - Induced Periodic Activity

There was no sign of electrical activity in atrial cells during the quiescent phases of the veratramine-induced periodic activity and the resting membrane potential was not significantly different from that recorded in the atrial cells of the untreated preparation.

Action Potential During Veratramine - Induced Periodic Activity

The typical configuration of atrial transmembrane action potentials during veratramine induced periodic activity is shown in Figures 15 and 16 with accompanying atrial transmembrane action potential records from the same atria before veratramine. The simultaneously recorded isometric tension is also included in Figure 16.

When the atrial preparation had been exposed to veratramine at a dose level which caused periodic activity (3×10^{-5} g/ml), the observed effects on the atrial action potential were qualitatively similar to those observed when atrial rate had only been reduced in the presence of veratramine. During veratramine-induced periodic rhythm, the resting membrane potential was not significantly

different from that recorded in the atrial cells of the untreated preparation. This was also the case when spontaneous atrial rate had only been reduced by veratramine.

The effect of veratramine on the atrial action potential during periodic activity was manifest as a reduction of the action potential amplitude and overshoot, an increase in the rise time of the upstroke of the action potential, and a prolongation of the repolarization phase of the action potential. Qualitatively, these effects of veratramine were similar to those observed when spontaneous atrial rate had only been reduced by veratramine treatment. Quantitatively, however, the effects of veratramine, reflected in the values of the action potential parameters, were greater during the active phases of periodic rhythm than when spontaneous atrial rate had only been decreased by veratramine. The mean values of the measured action potential parameters during veratramine-induced periodic rhythm are shown in Table III. With the exception of resting membrane potential, which was not altered by veratramine treatment, the mean values of action potential parameters during veratramine-induced periodic activity were significantly different not only from those of the untreated preparation, but also from the respective mean values of action potential parameters when spontaneous atrial rate had only been reduced by veratramine treatment. The consistent trend indicated by the values of the trans-

FIGURE 15: Configuration of the atrial transmembrane action potential during veratramine-induced periodic activity. (a) Control, (b) Veratrmine, 3×10^{-5} g/ml.

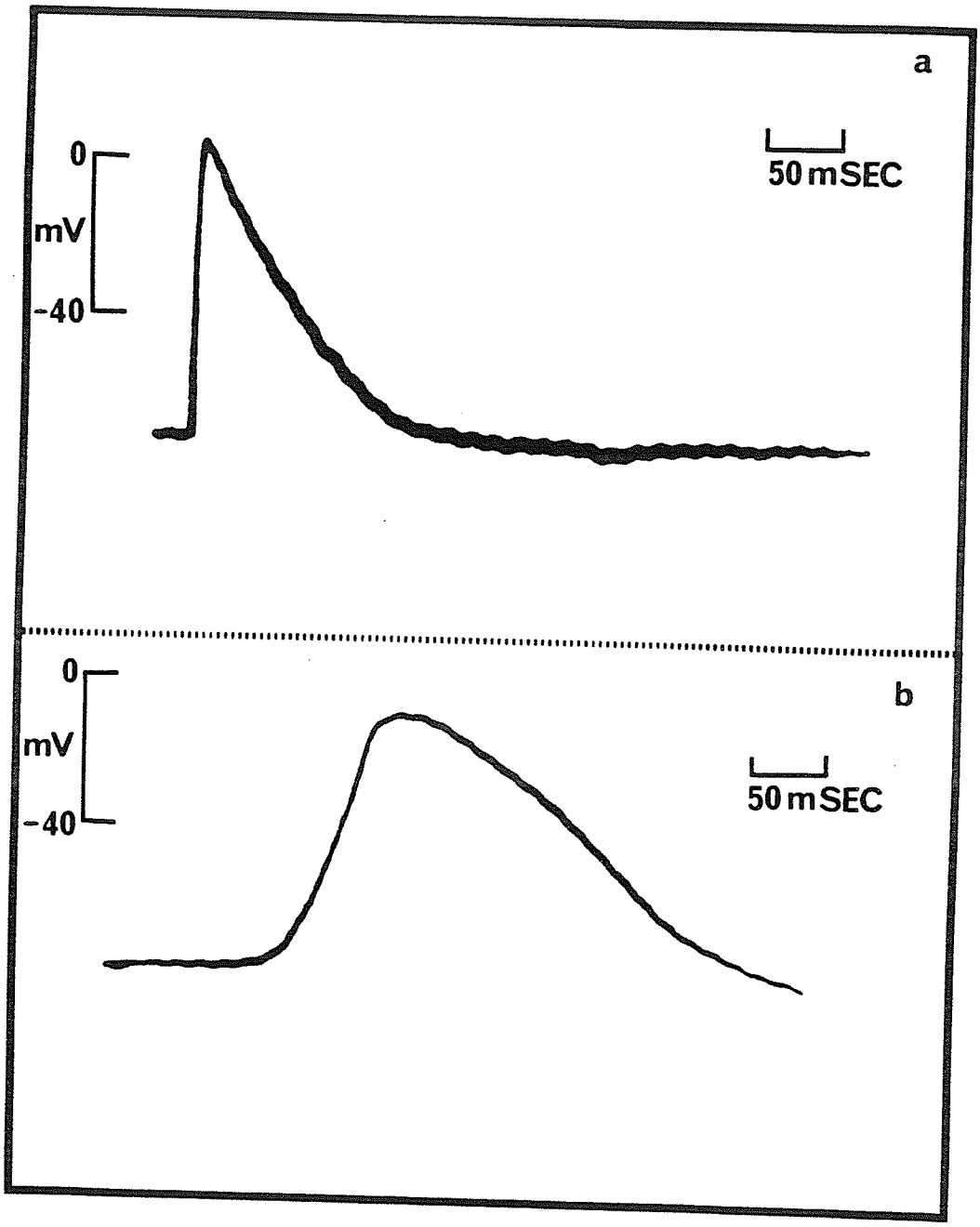

FIGURE 15

FIGURE 16: Configuration of the atrial transmembrane action potential during veratramine-induced periodic activity. (a) Control, (b) Veratramine, 3×10^{-5} g/ml. In both (a) and (b) transmembrane potential is the upper trace, tension is the lower trace.

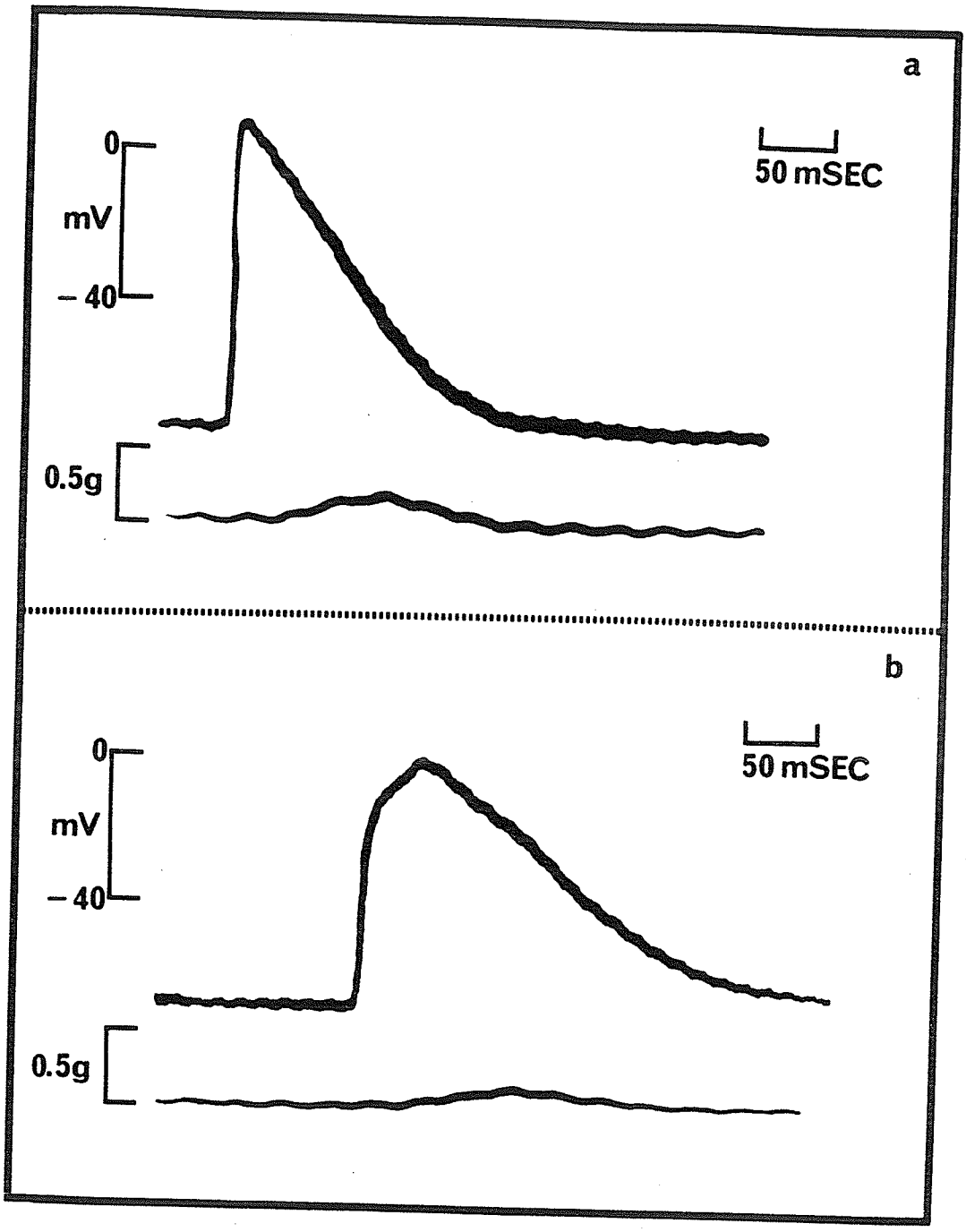

FIGURE 16

membrane action potential parameters at different dose levels of veratramine (Table III) would seem to reflect a development in degree of effect of veratramine on atrial cells at higher dose levels.

The mean atrial rate during the active phases of veratramine-induced periodic activity was 64.8 ± 4.3 beats/min in contrast to the control rate of 147.4 ± 6.0 beats/min. During periodic activity the amplitude of the atrial transmembrane action potential was reduced from a mean control value of 79.3 ± 0.8 mV to a mean value of 66.4 ± 1.8 mV. The value of the mean amplitude of the atrial action potential during periodic rhythm was less than, and significantly different from the value of mean action potential amplitude of the untreated atrial preparation (79.3 ± 0.8 mV) and the value of mean action potential amplitude when spontaneous atrial rate had only been reduced by veratramine (74.4 ± 1.7 mV).

The mean value of the overshoot of the atrial transmembrane action potential, which was reduced from a control mean value of 11.8 ± 0.4 mV to a mean value of 3.8 ± 0.8 mV when spontaneous atrial rate had been decreased in the presence of veratramine, was reduced to a greater extent during veratramine-induced periodic activity to a mean value of -1.0 ± 1.0 mV. During veratramine-induced periodic activity most action potentials showed no overshoot.

The alterations in the time course of the depolar-

ization processes of the atrial action potential that had been observed when spontaneous atrial rate had been reduced by veratramine were exaggerated during veratramine-induced periodic rhythm. The mean rise time of the upstroke of the atrial action potential during veratramine-induced periodic rhythm was 55.7 $\pm$ 6.0 ms. This value represented an increase over the control mean rise time of 8.9 $\pm$ 0.2 ms by a factor of 6, and an increase over the Veratramine Non-Periodic mean rise time of 34.7 $\pm$ 4.3 ms by a factor of approximately 2. Statistically, the mean rise time of the action potential, during veratramine-induced periodic activity, was significantly greater than the control mean rise time ($P < .01$) and the Veratramine Non-Periodic mean rise time ($P < .01$).

The total duration of the action potential (measured to the 90 per cent repolarization level) was increased from a control mean value of 146.5 $\pm$ 3.4 ms to a mean value of 270.00 $\pm$ 9.3 ms during veratramine-induced periodic activity. When spontaneous atrial rate had only been reduced by veratramine the mean total action potential duration was 219.1 $\pm$ 7.7 ms.

Analysis of action potential repolarization time indicated that, as had been the case when spontaneous atrial rate had only been reduced by veratramine treatment, during veratramine-induced periodic rhythm, the earlier phase of repolarization, represented by the mean 25 per

cent repolarization time was affected to the greatest degree, but the later phases of repolarization (50 and 90 per cent repolarization times) were also increased as compared to the control and Veratramine non-periodic parameters. The mean values of the 25, 50, and 90 per cent repolarization times (Table III), during periodic activity, were significantly greater than the respective mean values of both control ($P < .01$), and Veratramine non-periodic ($P < .01$) parameters. During veratramine-induced periodic activity, the mean 25 per cent repolarization time of the action potential was increased by approximately 130%, from a control mean value of 34.1 $\pm$ 0.9 ms to a mean value of 78.7 $\pm$ 4.9 ms. The mean 50 per cent repolarization time was increased over control by approximately 86%, from a control mean value of 66.2 $\pm$ 1.7 ms to 123.4 $\pm$ 5.0 ms during periodic activity. The mean 90 per cent repolarization time was increased as compared to control by approximately 55%, from a mean value of 137.7 $\pm$ 3.4 ms to 214.5 $\pm$ 5.8 ms during periodic activity.

Discrete Pacemaker-like Area

When atria continued to be exposed for 30-60 minutes to veratramine (3×10^{-5} g/ml) after the occurrance of periodic rhythm or when the atria were treated with a larger dose of veratramine (7×10^{-5} g/ml) the preparation became quiescent. Shortly after washout of the veratra-

mine a discrete active "pacemaker" area became obvious and remained active for as long as 2 hours while the rest of the preparation remained quiescent. This active area was consistently located by sight on the superior surface of the posterolateral aspect of the right atrium in an area consistent with the location of the Sino-atrial node. Recording of pacemaker cells was greatly simplified in this preparation; virtually every cell penetrated here with the microelectrode displayed action potentials consistent with pacemaker or latent pacemaker transmembrane action potential characteristics. This included spontaneous slow diastolic depolarization. Transmembrane action potentials, typical of those recorded from cells in the "pacemaker" area of the preparation, are shown in Figure 17. A slow diastolic depolarization which blends into the upstroke of the action potential is readily apparent. The lack of coincident mechanical activity in the whole atrial preparation is indicated by the simultaneously recorded atrial isometric tension record.

The "pacemaker" cells behaved normally with respect to drugs so far as tested. A small dose of adrenaline, 10^{-8} - 10^{-7} g/ml, increased the rate of diastolic depolarization without interfering with the quiescence of the rest of the atrium; a larger dose, 10^{-6} g/ml, transiently restored the atrium to activity. Acetylcholine, 10^{-7} g/ml, decreased the rate of diastolic depolarization and in

FIGURE 17: Intracellular transmembrane potential recorded from cells within a "discrete pacemaker-like area". The preparation was exposed for 10 minutes to veratramine, 7×10^{-5} g/ml. In both (a) and (b) transmembrane potential is the upper trace, tension the lower trace. The lack of coincident mechanical activity in the whole atrial preparation is indicated by the atrial isometric tension record.

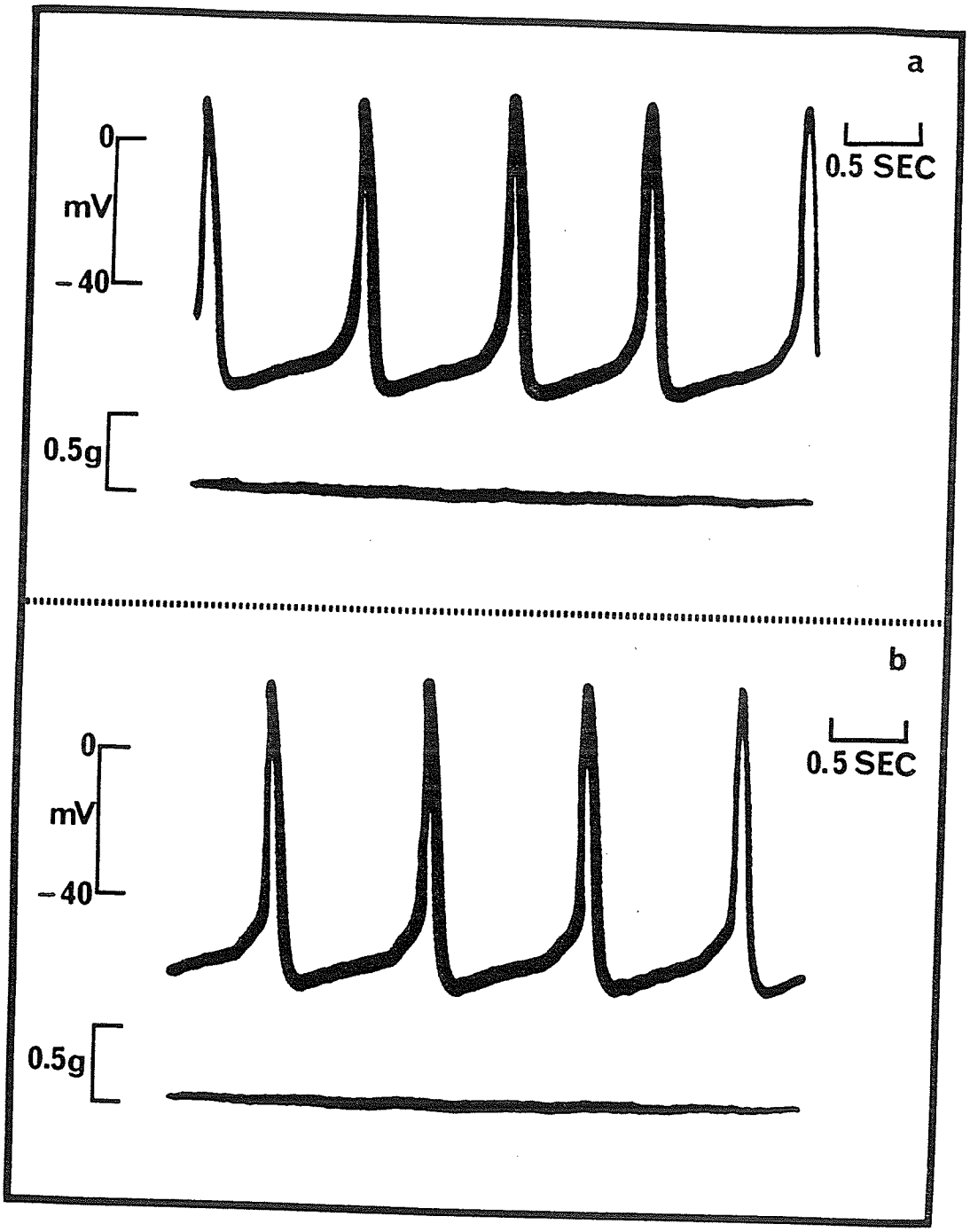

FIGURE 17

larger doses stopped the pacemaker from beating.

Around the "pacemaker" area, was found an area of tissue approximately 5 mm in diameter in which some cells were active; the record of intracellular transmembrane potential showing abortive spikes, small summating depolarizations, with irregular action potentials sometimes superimposed. The records of intracellular potential shown in Figure 18 illustrate this phenomenon.

With the use of the micromanipulator to position the microelectrode, transmembrane potentials of cells at varying distances from the pacemaker area were examined. The amplitude of the irregular electrical activity (see Figure 18) increased with the proximity of the cell examined to the pacemaker area, indicating possible decremental conduction in the area immediately surrounding the pacemaker area. Figures 19, 20, and 21 show a series of recorded transmembrane potentials from individual cells, when the microelectrode was positioned, so that the cells impaled in sequence were located progressively closer to, and finally within the pacemaker area. The atrial isometric tension, recorded simultaneously with the transmembrane potentials, is also shown in these figures, illustrating an absence of atrial contraction in the main body of the tissue, notwithstanding continued pacemaker activity. Conduction of electrical impulses from the pacemaker area to the common atrial tissue is interrupted.

FIGURE 18: Intracellular transmembrane potential recorded from cells within an area immediately surrounding the "discrete pacemaker-like area". The preparation was exposed for 10 minutes to veratramine, 7×10^{-5} g/ml. (a), (b), (c), and (d) are recordings from 4 individual cells located approximately 3 mm from the pacemaker area.

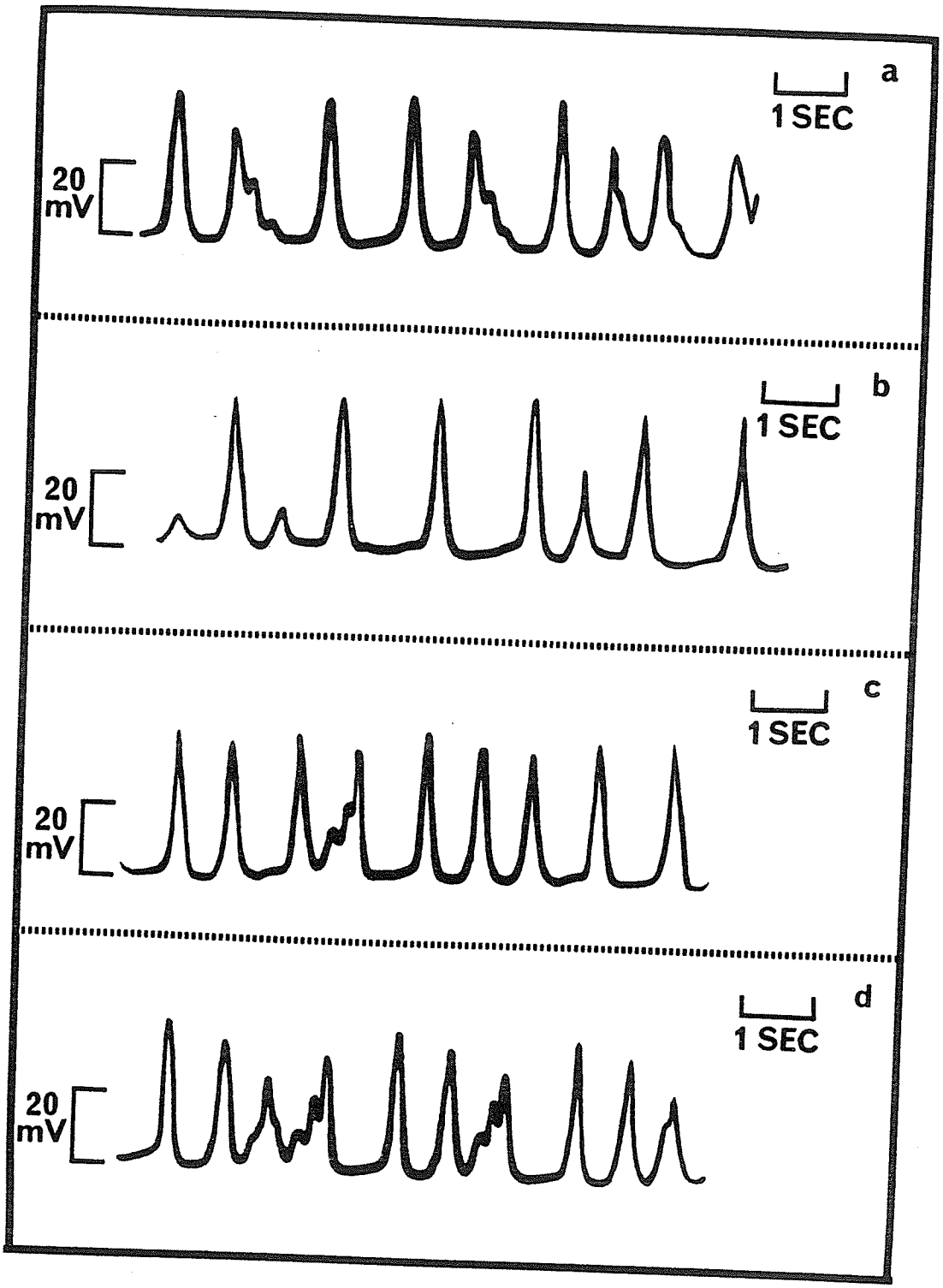

FIGURE 18

In addition to the increase in the amplitude of cellular transmembrane electrical activity with proximity to the pacemaker area, the irregular nature of cellular electrical activity was less prominent in cells closer to the pacemaker area. Abortive spikes, small depolarizations, and irregular action potentials were less apparent in the records of transmembrane potential of cells closer to the pacemaker area (Figure 19b, 20a). As well, with proximity of the cell to the pacemaker area, cellular electrical activity appeared to become more regular, and smooth diastolic depolarization was observed in the transmembrane action potentials (Figure 20). With respect to the electrical activity of cells contained within the pacemaker area, rhythm appeared to be regular, and irregular electrical activity did not impinge on the recorded action potentials (Figure 21).

Outside the area immediately surrounding the pacemaker area, approximately 4 mm from the pacemaker area, the cells examined maintained the normal resting membrane potential, but no action potentials occurred despite the ongoing activity of the cells of the pacemaker area. Conduction of electrical activity from cell to cell was clearly disrupted, and electrical excitability appeared to be reduced (no response to pacemaker activity and no response to electrical stimulation).

The untreated preparation typically gave maximum

inotropic responses to single and repetitive stimuli (1-3/second) of 20 volts and 1 ms duration. The normal parameters of applied stimuli were; threshold 6V; maximum 10V; supra-maximal 20V of 1 ms duration. During the quiescent phase of periodic activity electrical stimuli could still evoke electrical and mechanical responses but the responses did not follow regularly on a 1 to 1 basis. When the atria became quiescent after exposure to veratramine, 3×10^{-5} g/ml, for 30 to 60 minutes, or to veratramine, 7×10^{-5} g/ml, for 10 minutes, responses to stimulation were seldom observed even when stimulation parameters were increased to maximum acceptable experimental limits.

The implications of the apparently isolated veratramine-resistant pacemaker area, and the role of the apparent "transitional" area of tissue surrounding the pacemaker area, with respect to the development of periodic rhythmare considered in the discussion section.

FIGURE 19: Intracellular transmembrane potential of cells close to the "discrete pacemaker-like area". The preparation was exposed for 10 minutes to veratramine, 7×10^{-5} g/ml. In both (a) and (b) transmembrane potential is the upper trace, tension the lower trace. FIGURES 19, 20, and 21 are records of the transmembrane potential of cells located progressively closer to (19(a), 19(b), 20(a)) and finally within (20(b), 21) the "pacemaker" area. Figures 19(a), 19(b) and 20(a) represent records of cells located approximately 2.5, 2.0 and 1.0 mm respectively from the pacemaker area.

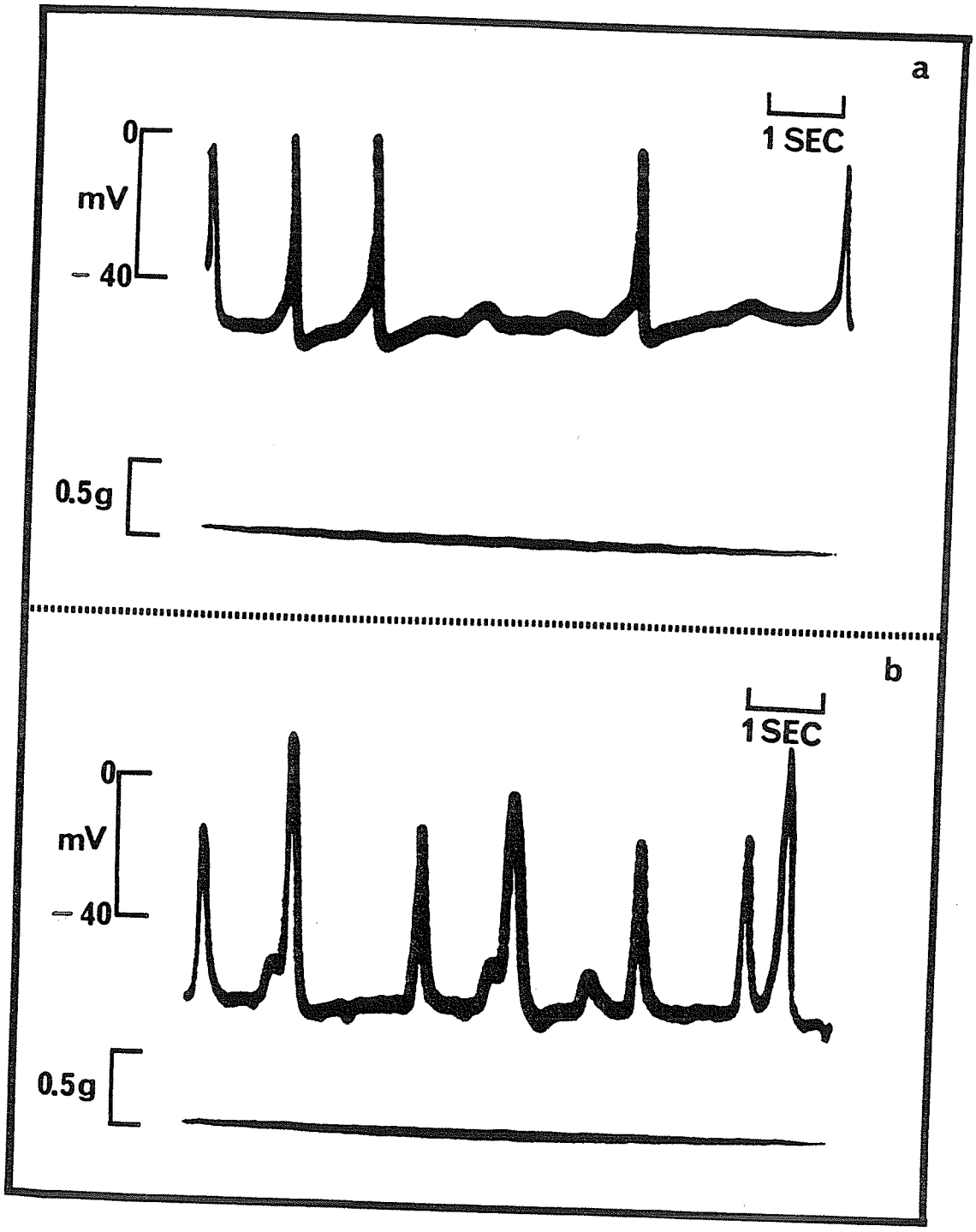

FIGURE 19

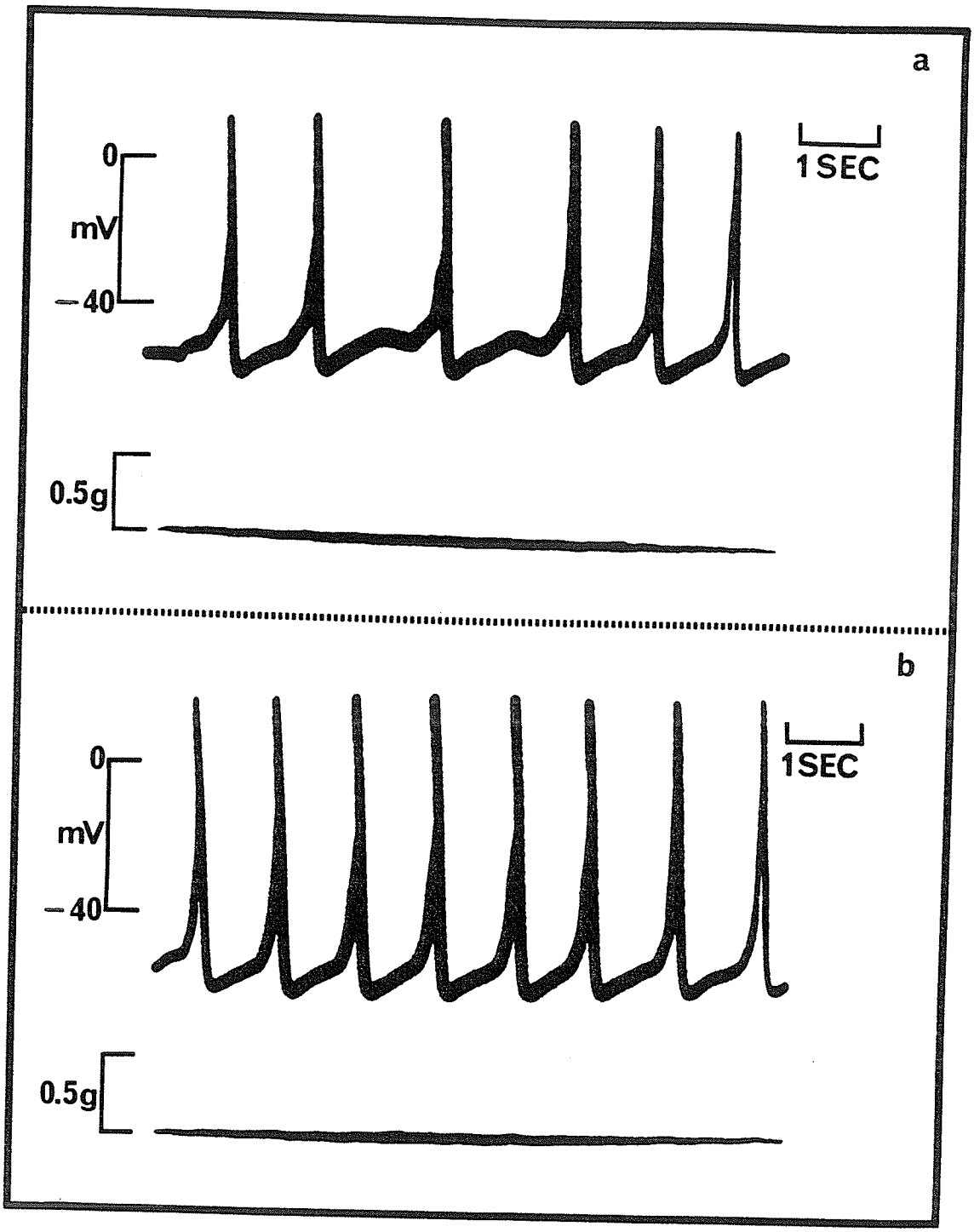

FIGURE 20

FIGURE 21: Intracellular transmembrane potential of cells close to the "discrete pacemaker-like area". The preparation was exposed for 10 minutes to veratramine, 7×10^{-5} g/ml. Transmembrane potential is the upper trace, tension the lower trace. FIGURES 19, 20, and 21 are records of the transmembrane potential of cells located progressively closer to (19(a), 19(b), 20(a)) and finally within (20(b), 21) the "pacemaker" area. Figures 19(a), 19(b) and 20(a) represent records of cells located approximately 2.5, 2.0 and 1.0 mm respectively from the pacemaker area.

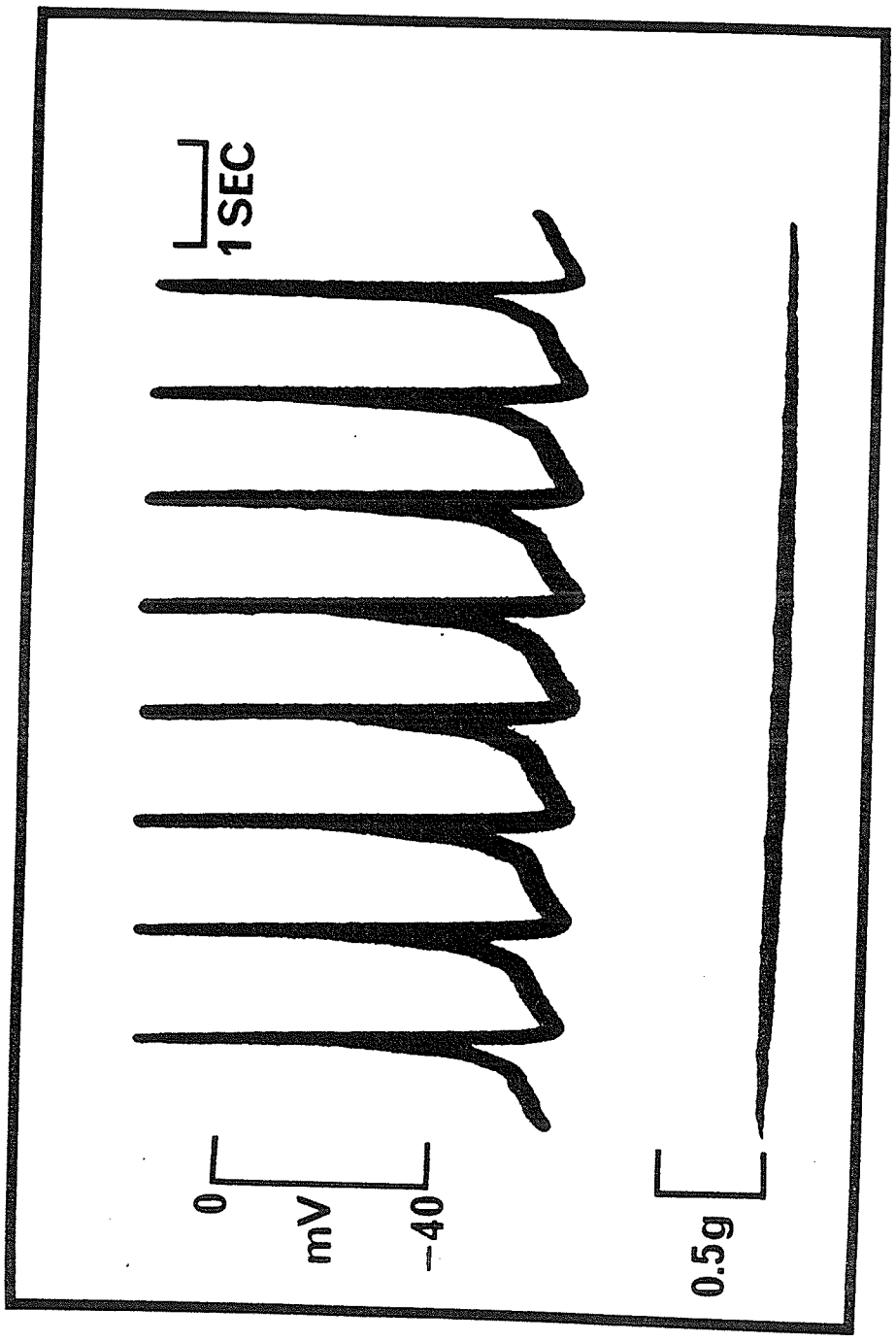

FIGURE 21

Modification of the Effects of Veratramine on Spontaneous Atrial Rate and Atrial Action Potential

Catecholamines

It was considered that manipulation of the veratramine-treated isolated atrial preparation by pharmacological agents and by modification of the ionic medium surrounding the preparation might provide some insight into the mechanisms of action of veratramine.

The negative chronotropic effects of veratramine on the isolated spontaneously beating right atrial preparation were transiently antagonized by adrenaline (10^{-6} g/ml) or isoprenaline (10^{-6} g/ml). In addition, when the atrial preparation was treated with veratramine at a dose which produced periodic activity or caused complete cessation of atrial activity the addition of catecholamines to the organ bath resulted in the restoration of a regular rhythm. These effects of catecholamines were transient, however, and within minutes periodic activity replaced the regular atrial rhythm which had been produced by the addition of catecholamines. Infrequently, after veratramine-induced periodic activity had spontaneously reverted to a slow regular rhythm, the addition of catecholamines caused the transient reappearance of periodic activity. Usually the periodic activity so produced lasted only a few minutes and then was replaced by a regular rhythm.

The effects of veratramine on the atrial transmem-

brane action potential were partially antagonized by catecholamines, which concurrently restored a regular rhythm when periodicity had been established. The antagonism by adrenaline (10^{-6} g/ml) of the effects of veratramine on the atrial transmembrane action potential is illustrated in Figure 22. The amplitude and overshoot of the atrial action potential, which had been reduced by veratramine (Figure 22b), were increased by adrenaline (Figure 22c) to the levels observed in the control action potential (Figure 22a). The duration of the repolarization phase of the action potential, which had been increased by veratramine (Figure 22b), was decreased by the addition of adrenaline (10^{-6} g/ml). However, as illustrated in Figure 22, the reversal of the effect of veratramine on the voltage-time relationship of the action potential was limited. Adrenaline did not completely restore the action potential to the configuration observed before veratramine.

As catecholamines had been found to antagonize the effects of veratramine on spontaneous atrial rhythm and on the atrial transmembrane action potential, it was decided to test the effects of aminophylline, caffeine and dibutyryl cyclic AMP on the veratramine-treated isolated right atrial preparation.

Xanthines

The effects of up to 4 mM caffeine (final bath concentration) on the rhythm and contractility of the untreated

FIGURE 22: Antagonism by adrenaline of the effects of veratramine on the atrial transmembrane action potential. (a) Control (b) Veratramine (3×10^{-5} g/ml) (c) Veratramine + adrenaline (10^{-6} g/ml). The records shown in (a), (b) and (c) were obtained from the same atrial preparation.

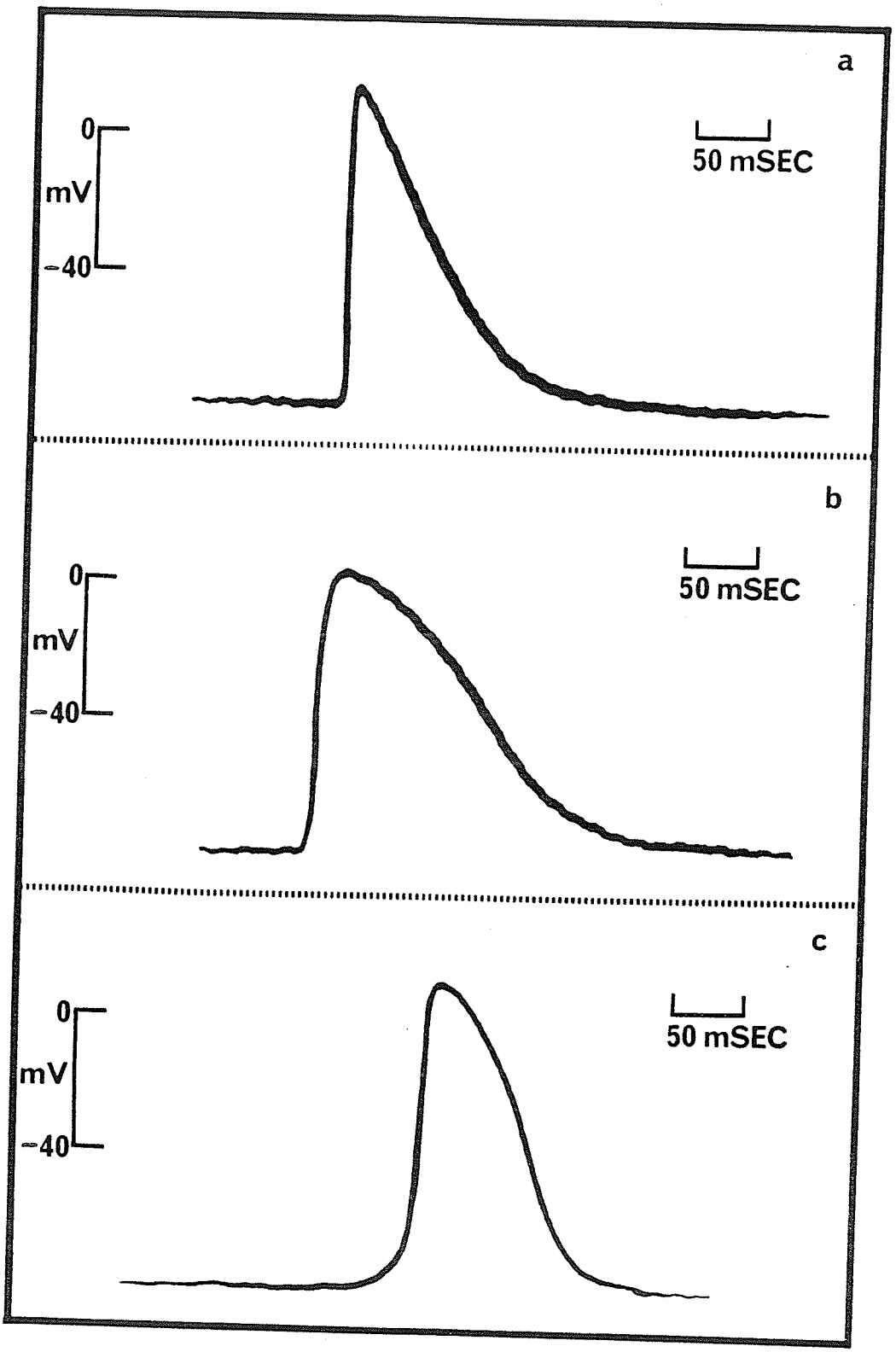

FIGURE 22

right atrial preparation were negligible. Occasionally, an initial transient positive chronotropic effect, lasting only seconds, could be discerned following the addition of caffeine to the organ bath. With respect to the atrial action potential, caffeine caused a slight reduction in the overshoot of the atrial action potential and increased the rate of repolarization of the atrial action potential (Figure 23).

Concentrations of aminophylline or caffeine from 1 to 6 mM did not prevent or reverse the negative chronotropic effects of veratramine (1 - 3 x 10^{-5} g/ml) on the isolated atrial preparation. Moreover, caffeine (1 - 6 mM) did not prevent the development of veratramine-induced periodic activity, or restore regular rhythm when periodicity had been established.

The appearance of the atrial action potential when the atrial preparation was exposed to veratramine and caffeine (Figure 24) differed markedly from that observed when the atrial preparation was exposed to caffeine alone (see Figure 23). When the atrial preparation was exposed to both veratramine and caffeine, a distinct "plateau" phase became apparent in the repolarization phase of the action potential (Figure 24). No such plateau occurred with caffeine alone (Figure 23). The amplitude of the action potential, which had been reduced by veratramine (Figure 24b), was increased when caffeine was also added (Figure 24c).

FIGURE 23: Effect of caffeine on the atrial transmembrane action potential. (a) Control (b) Caffeine (4 mM). In both (a) and (b) transmembrane potential is the upper trace, tension the lower trace. The records shown in (a) and (b) were obtained from the same atrial preparation.

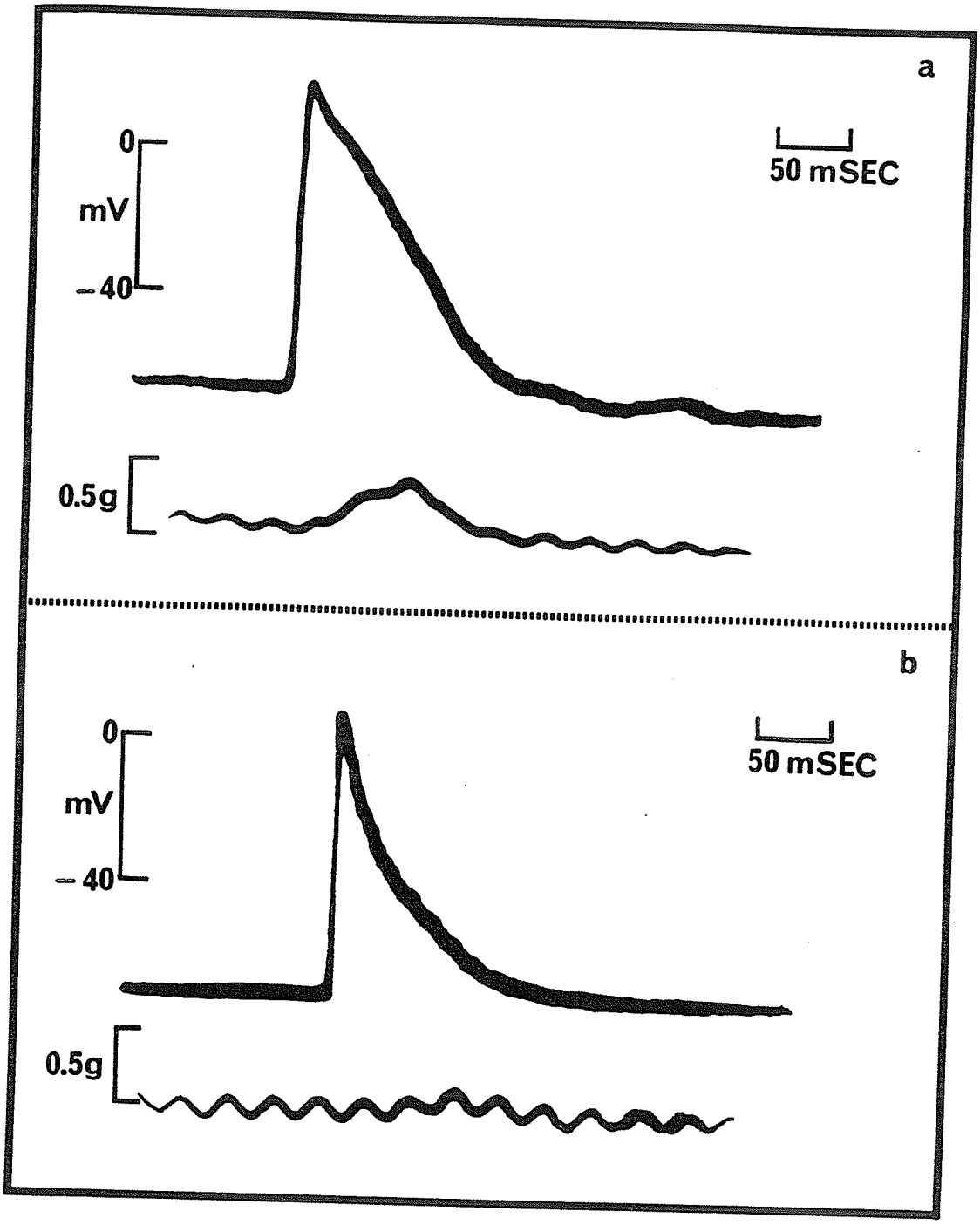

FIGURE 23

FIGURE 24: Effect of caffeine on the atrial transmembrane action potential in the presence of veratramine. (a) Control (b) Veratramine (3×10^{-5} g/ml) (c) Veratramine + caffeine (4mM). The records shown in (a), (b) and (c) were obtained from the same atrial preparation.

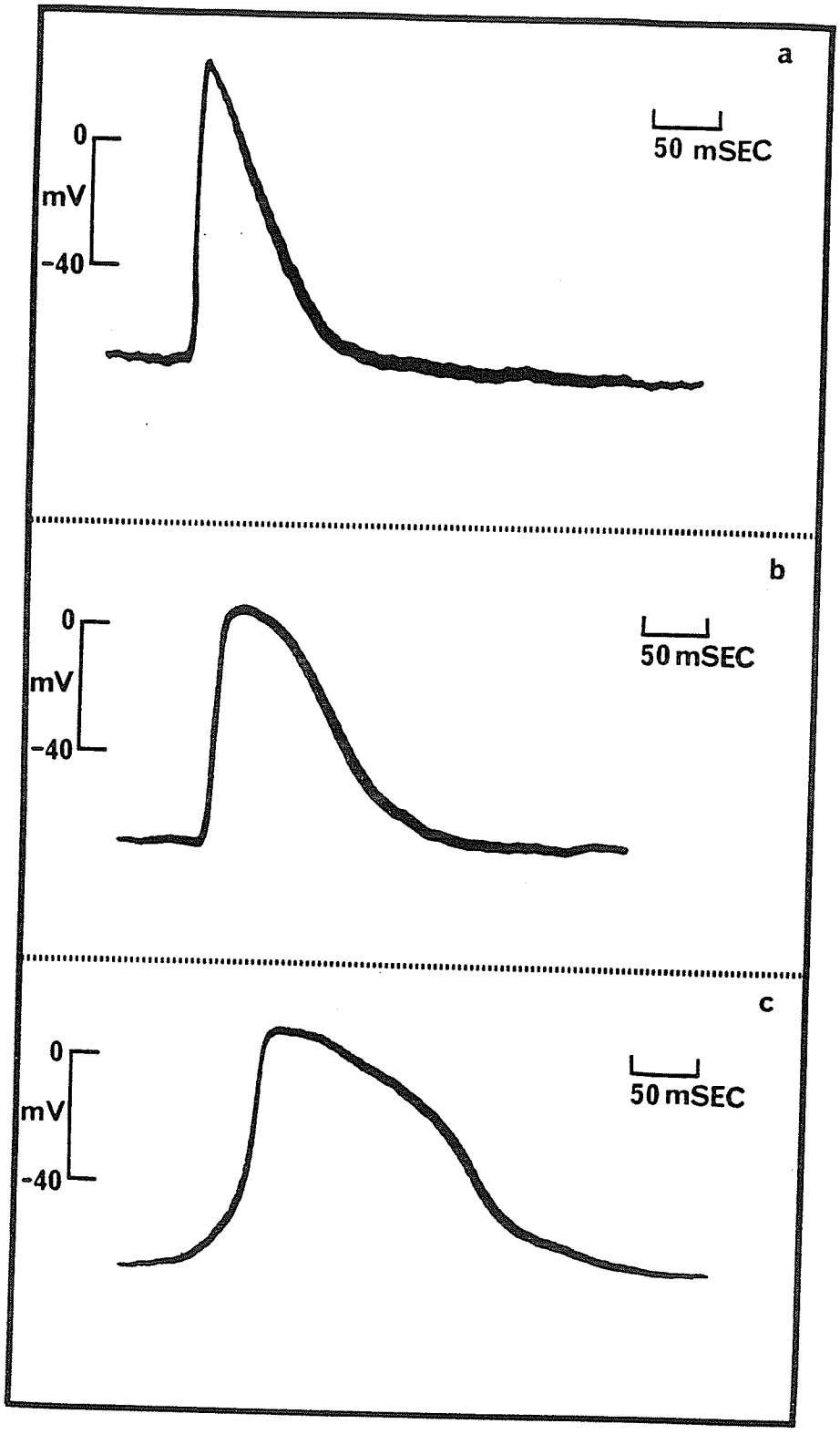

FIGURE 24

However, caffeine did not reduce the increased duration of the action potential due to veratramine.

Dibutyryl Cyclic AMP

Like caffeine, dibutyryl cyclic AMP (10^{-6} - 10^{-4} g/ml) failed to prevent or reverse the negative chronotropic effects of veratramine. Also, dibutyryl cyclic AMP (10^{-4} g/ml) did not prevent the development of veratramine-induced periodic activity, or restore a regular atrial rhythm when periodicity had been established.

The effect of dibutyryl cyclic AMP on the atrial action potential during veratramine treatment is shown in Figure 25. Dibutyryl cyclic AMP, like caffeine, did not reverse the effects of veratramine on the atrial action potential. Dibutyryl cyclic AMP, in the presence of veratramine, did not restore the action potential overshoot or shorten the duration of repolarization, but did result in the appearance of a more distinct "plateau" phase in the action potential (Figure 25c). In this latter respect the effects of dibutyryl cyclic AMP were similar to caffeine.

Consideration of the observed effects of veratramine on the configuration of the atrial action potential led to speculation that the mechanism of action of veratramine on atrial tissue involved at least some degree of modification of the normal cellular ionic processes responsible for the generation of the action potential. Alter-

FIGURE 25: Effect of dibutyryl cyclic AMP on the atrial transmembrane action potential in the presence of veratramine. (a) Control (b) Veratramine (3×10^{-5} g/ml) (c) Veratramine + dibutyryl cyclic AMP (10^{-4} g/ml). The records shown in (a), (b) and (c) were obtained from the same atrial preparation.

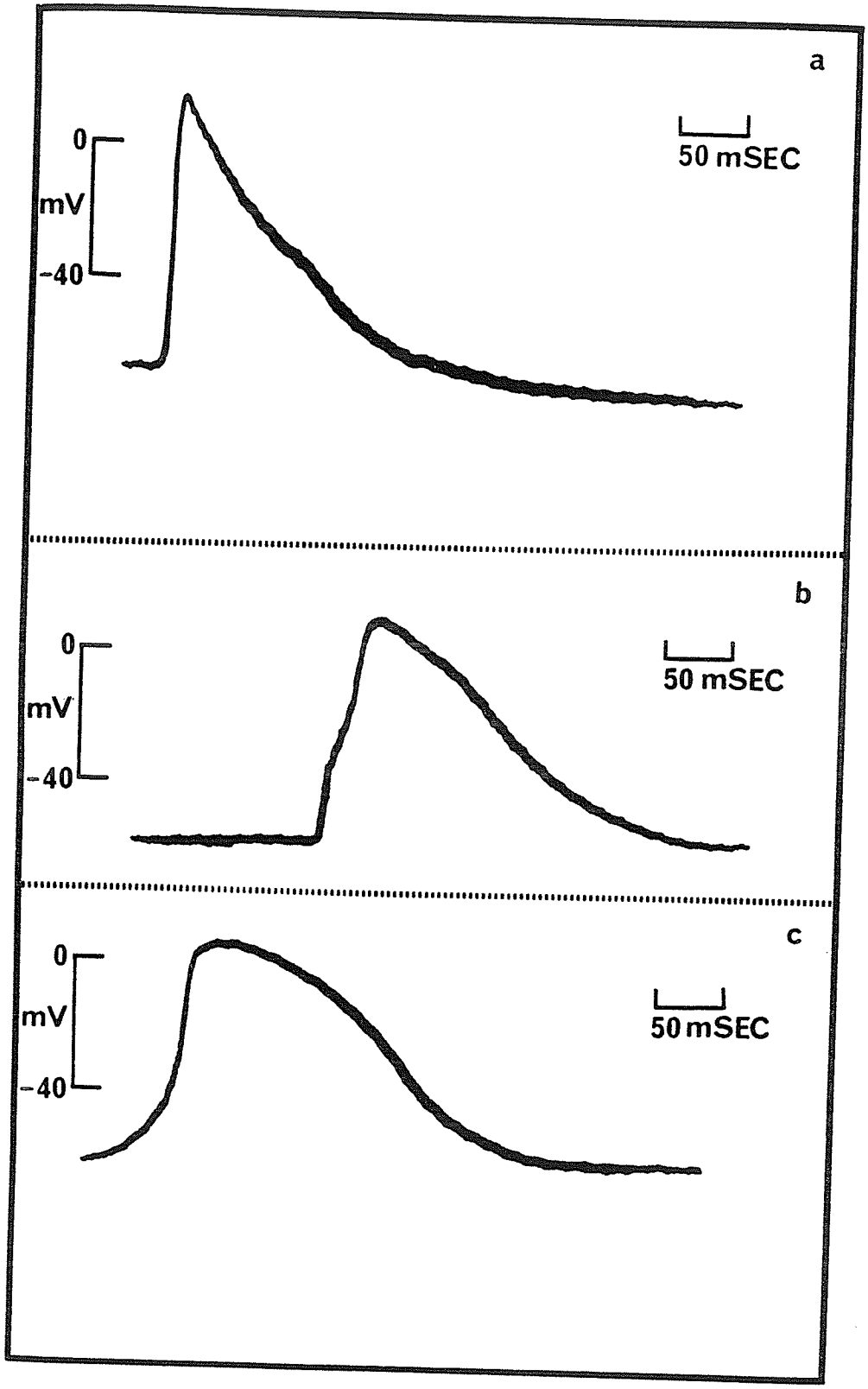

FIGURE 25

ation of the ionic composition of the fluid bathing the tissue was performed with the purpose of modifying the atrial cellular ionic environment, and hence, possibly the responses of the atrial preparation to veratramine. Towards this same end, pharmacological agents were also employed in an effort to modify atrial cellular ionic processes and the response of the atrial preparation to veratramine.

Bathing Fluid Modification

Modification of the ionic composition of the fluid bathing the atrial preparation to contain high concentrations of calcium (5mM), potassium (11.6 mM), calcium and potassium (5 and 11.6 mM respectively), or sodium (238 mM), failed to antagonize or reverse the effects of small or large doses of veratramine on the atrial preparation.

Valinomycin

Valinomycin, which has been reported to effect increases in potassium permeability (Moore and Pressman, 1964; Carafoli et al., 1969; Henderson et al., 1969) when added to the bathing fluid (10^{-6} to 10^{-4} g/ml), did not prevent slowing of the spontaneous atrial rhythm or the development of periodic activity due to veratramine. Furthermore, valinomycin (10^{-6} to 10^{-4} g/ml) had no apparent effect on the atrial action potential, when administered

before or after veratramine.

Carbachol

Carbachol (10^{-8} - 10^{-7} g/ml), which in the untreated atrial preparation slowed the spontaneous rhythm and increased the apparent rate of repolarization of the atrial action potential, did not increase the rate of repolarization of the atrial action potential when spontaneous atrial rate had been slowed in the presence of veratramine (Figure 26). Periodic rhythm was not precipitated in atrial preparations slowed by veratramine when carbachol was added to the bathing fluid.

Enhancement of the Action of Veratramine by Tetrodotoxin

Tetrodotoxin has been shown to block specifically the inward sodium current responsible for the upstroke of the action potential (Hagiwara and Nakajima, 1965; Coraboeuf and Vassort, 1968; Rougier et al., 1969; Vassort et al., 1969; Beeler and Reuter, 1970a, 1970b; Spero et al., 1973). The production of periodic activity by veratramine was facilitated when the atrial preparation was pretreated with tetrodotoxin (10^{-8} to 10^{-7} g/ml). Tetrodotoxin alone (10^{-8} to 10^{-7} g/ml) did not reduce spontaneous atrial rate. Pretreatment of the atrial preparation with tetrodotoxin enhanced the effects of a given dose of veratramine on the atrial action potential. In the presence of tetrodotoxin and veratramine, the increase in the rise time and

FIGURE 26: Effect of carbachol on the atrial transmembrane action potential in the presence of veratramine. (a) Control (b) Carbachol (10^{-8} g/ml) (c) Veratramine (3×10^{-5} g/ml) + carbachol. The records shown in (a), (b) and (c) were obtained from the same atrial preparation.

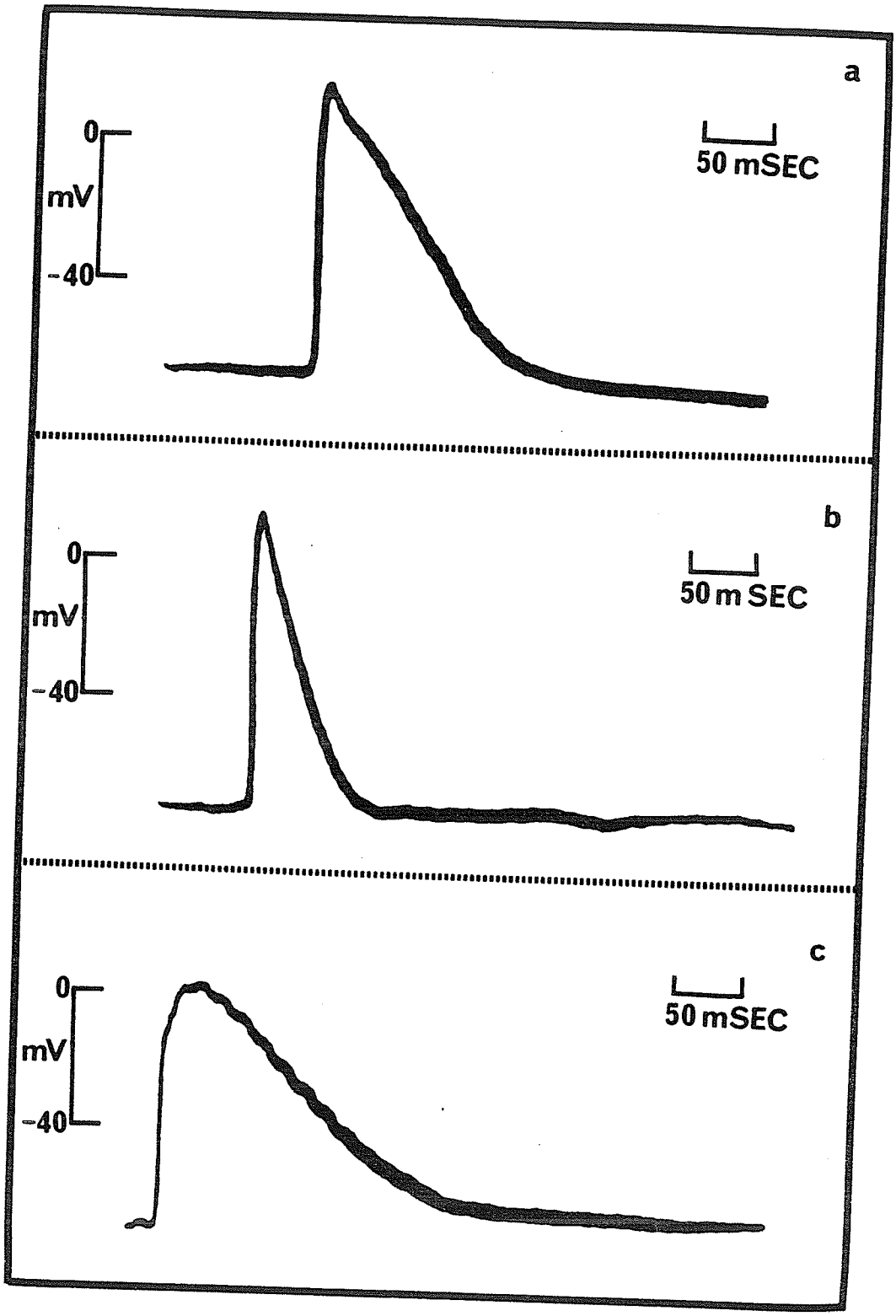

FIGURE 26

prolongation of the atrial action potential was more marked than that produced by the same dose of veratramine alone. In the presence of tetrodotoxin (10^{-8} to 10^{-7} g/ml), veratramine (10^{-5} g/ml) in a dose which routinely caused only slowing of the spontaneous atrial rhythm, now produced periodic activity. In the isolated atrial preparation, sodium and potassium (variable concentrations in the bathing fluid) were found to have little effect on the consequences of veratramine treatment, while tetrodotoxin, (a sodium-specific agent) enhanced the effects of veratramine on the atrial preparation. The involvement of more specific sources of sodium or potassium,or both (pools resistant to, or not readily affected by alteration of the bathing fluid medium) in the mechanism of action of veratramine is considered in DISCUSSION and CONCLUSIONS.

SECTION IV
DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

The current concepts concerned with processes which govern the generation of the transmembrane action potential in cardiac cells have been considered in the Introduction section of this text and serve as a basis for the discussion of the results of the present study wherein the effects of veratramine on the isolated right atrial preparation have been examined. Recently, the evidence regarding the ionic basis of membrane currents involved in the generation of the cardiac action potential has been the subject of extensive review (Langer, 1973; Trautwein, 1973). It is generally believed that the cardiac action potential is generated as a consequence of membrane currents which arise from the sequential transmembrane movement of various charged ion species. While much of the evidence regarding the ion currents that occur during the cardiac action potential requires confirmation, a tentative sequence of events may be summarized as follows: A rapid sodium inward current is responsible for the rapid depolarization of the heart cell from threshold to the crest of the spike. Subsequent to the initial rapid sodium inward current, a second slower transmembrane inward current occurs, and is proposed to contribute to the action potential amplitude and plateau. In addition, Ochi and Trautwein (1971) have demonstrated a dependent relationship between cardiac con-

traction and the slow inward current. Evidence indicated that the second transmembrane inward current is carried by calcium, or sodium or calcium and sodium ions, and is characterized by slow activation and inactivation as compared to the initial rapid sodium inward current (Reuter, 1967; Rougier et al., 1968, 1969; Mascher and Peper, 1969; Beeler and Reuter, 1970a, 1970b; Ochi, 1970; Kohlhardt et al., 1973a; Vassort, 1973). The initial rapid sodium inward current is reduced and abolished by tetrodotoxin. In contrast, the secondary slow inward current is not reduced by tetrodotoxin (Beeler and Reuter, 1970a, 1970b); it is reduced in the presence of lowered sodium and calcium concentrations (Rougier et al., 1968, 1969), and can be diminished selectively by antagonists of calcium transmembrane influx such as manganese or verapamil without significant change in the fast sodium current (Kohlhardt et al., 1972a, 1972b, 1973a). While the secondary slow inward current appears to be independent of the initial fast sodium current, Kohlhardt et al., (1973b) have demonstrated that the slow inward current can be carried non-specifically by calcium, barium, strontium or magnesium ions.

In addition to the initial fast sodium inward current and the secondary slow inward current (sodium or calcium or both), a transient outward current, ("positive dynamic current") carried by chloride ions and overlapping the secondary slow inward current in time, has been re-

ported to occur during action potential repolarization (Hiraoka and Fozzard, 1972; Fozzard and Hiraoka, 1973). The functional contribution to the cardiac action potential of this outward current has not been determined, but it has been proposed to modulate initial rapid repolarization (Dudel et al., 1967), as well as to contribute in concert with other ionic currents, to the formation of the action potential plateau (Hiraoka and Fozzard, 1972; Fozzard and Hiraoka, 1973).

The final phase of repolarization of the cardiac cell is considered to be produced by an outward potassium current which exceeds the rapidly falling inward sodium current and thus causes the membrane potential to fall to diastolic levels (Langer, 1968; Noble and Tsien, 1969; Fozzard and Gibbons, 1973).

Alteration of the Intracellular Atrial Action Potential by Veratramine

The results of the most recent investigations of the electrophysiological effects of the veratrium alkaloids on nerve and muscle are of considerable significance to the present consideration of the mechanisms of action of veratramine in atrial tissue.

On the basis of their effects on the membrane potential and conductance of squid and crayfish giant axons, eleven veratrium alkaloids have been classified into three groups (Ohta et al., 1973).

The first group, which includes the alkaloids veratridine, cevadine and protoveratrines A and B cause depolarization of the squid and crayfish giant axons. This depolarization, caused by veratridine has been shown to be due primarily to a selective increase in the resting sodium permeability of the nerve membrane and is antagonized by tetrodotoxin (Ohta et al., 1973).

The second group, which includes the alkaloids, veratramine, isorubijervine, muldamine and 5-veratranine-3β, 11α-diol are capable of blocking the action potential in the giant axon and cause little or no depolarization. Work done with 5-veratranine-3β, 11α-diol indicates that the alkaloids of this second group (which includes veratramine) block the axon action potential by inhibiting both sodium and potassium conductance increases, with an additional action that causes potassium inactivation to appear (Ohta et al., 1973).

The third group of alkaloids including cyclopamine, jervine, rubijervine and veratrosine were shown to have no effect on the resting and action potentials of the squid and crayfish giant axons (Ohta et al., 1973).

The work of Horackova and Vassort (1973), Ulbricht (1972a, 1972b) and Varga et al. (1972), on frog atrial muscle, myelinated nerve fibers and nodes of Ranvier of frog and frog skeletal muscle respectively, support the evidence that the alkaloids veratrine, cevadine and pro-

toveratrines A and B cause depolarization by increasing sodium inward current and that this effect may be blocked by tetrodotoxin. In addition Horackova and Vassort (1973) found that veratrine (a mixture of veratridine, cevadine, cevadilline and sabadine) did not substantially change the slow "calcium" inward current when the action of veratrine on sodium inward current was prevented with tetrodotoxin. Ulbricht (1972a) using myelinated nerve fibers of the frog studied the kinetics of the veratridine effect on sodium permeability and suggested that the increased sodium permeability produced by veratrine was the result of a modification of the existing sodium channels as opposed to the creation of new sodium channels. Further, Ulbricht (1972a) suggested that the modification of a sodium channel was an all or none event and that drug concentration determined the number of channels converted.

Ohta et al. (1973) discussed the possibility that the differences in activity between the three groups of veratrum alkaloids; the depolarizers (eg. veratridine); the nondepolarizing blockers (eg. veratramine) and the inactive alkaloids (eg. cyclopamine), might be accounted for by the differences between the groups in the basic veratrum alkaloid structure. "It was found that some of them exert a highly specific action on the resting sodium permeability and that modifications of the chemical bring about drastic changes in the mode of action" (Ohta et al., 1973).

Consideration of the results of the present study leads one to the conclusion that veratramine acts upon the cells of the spontaneously beating cat atria with actions similar to the group of veratrum alkaloids described by Ohta et al. (1973) as nondepolarizing blockers of squid and crayfish giant axon. In the cat right atrial preparation veratramine blocks the action potential of atrial cells with little or no depolarization of the resting membrane potential and appears to inhibit both sodium and potassium conductance increases. In the right atrial preparation treated with veratramine, disturbance of the sodium conduction system is indicated by the increased rise time of the action potential upstroke and by the reduced action potential overshoot. The increased duration of the repolarization phase of the action potential observed in the veratramine treated atrial cells is consistent with slowed inactivation of the sodium conductance system, interference with potassium conductance increases or both.

The actions of veratridine appear to be in direct opposition to the actions of veratramine in both nerve and muscle preparations. Veratridine has been shown to cause depolarization and to increase sodium conductance in both nerve and muscle preparations (Ohta et al., 1973; Horackova and Vassort, 1973; Honerjager, 1973; Ulbricht, 1972a; Varga et al., 1972). On the other hand, the present evi-

dence with atrial tissue, as well as the results of previous work with nervous tissue (Ohta et al., 1973) indicate that veratramine causes negligible depolarization and appears to decrease sodium conductance. In view of the fact that tetrodotoxin antagonizes the effects of veratridine, (Ohta et al., 1973; Horackova and Vassort, 1973; Honerjager, 1973; Ulbricht, 1972a; Varga et al., 1972) one might be led to suspect that tetrodotoxin would facilitate the effects of veratramine. This indeed was the case in the isolated atrial preparation. Ohta et al. (1973) successfully antagonized veratridine depolarization in their nerve preparation with tetrodotoxin or by reducing external sodium concentration. However, in the isolated atrial preparation, sodium and potassium (variable concentrations in the bathing fluid) had little effect on the electrophysiological consequences of veratramine treatment, while treatment of the atrial preparation with tetrodotoxin enhanced the effects of veratramine on the atrial action potential. Tetrodotoxin has been shown to block specifically the inward sodium current responsible for the upstroke of the action potential (Hagiwara and Nakajima, 1965; Coraboeuf and Vassort, 1968; Rougier et al., 1969; Vassort et al., 1969; Beeler and Reuter, 1970a, 1970b; Spero et al., 1973). Veratramine as well appears to reduce this fast inward sodium current. The degree to which veratramine affects the atrial action potential

parameters is dependent not only on the dose of veratramine to which the atrium is exposed but also on the duration of exposure of the atrial tissue to a given dose of veratramine. The effects of a given dose of veratramine are long-lasting and resistant to washout. Ulbricht (1972a) suggested that the action of veratridine results in a permanent modification (opening) of the existing sodium channels such that the passage of sodium ions across the membrane is facilitated and leads to depolarization. If in the atrial preparation veratramine irreversibly modifies membrane function so that the transmembrane passage of sodium ions is permanently impaired, then the enhancement of the effects of veratramine by tetrodotoxin as well as the inability of increased bathing media sodium concentration to reverse the veratramine effects can be understood. Whereas the action of tetrodotoxin on the cellular membrane would impair the passage of sodium ions across the membrane and add to the effects of veratramine, an increased sodium concentration in the bathing fluid would not be expected to affect a process that had been irreversibly impaired by the action of veratramine. In contrast, in the case of veratridine, where sodium channels are irreversibly locked in an "open position", decreasing extracellular sodium concentration would reverse the depolarization caused by veratridine-induced increased sodium permeability simply by reducing the sodium ions available for trans-

membrane passage.

Adrenaline partially reversed the effects of veratramine on the atrial action potential. Exposure of the atrial preparation to adrenaline subsequent to veratramine treatment resulted in a restoration towards control of action potential amplitude, overshoot, and to a limited extent rise time and action potential duration (See Figure 22). The mechanism mediating this action of adrenaline conceivably could involve the action of adrenaline on the transmembrane movement of calcium ions. In the INTRODUCTION the role of a slow inward current carried by calcium, or sodium, or calcium and sodium ions was discussed in terms of its contribution to the generation of the late part of the upstroke and early part of the plateau phase of the cardiac action potential (Reuter, 1967, 1968; Mascher and Peper, 1969; Rougier et al., 1969; Beeler and Reuter, 1970a, 1970b; Ochi, 1970). The action of adrenaline on the "slow inward current" was examined by Carmeliet and Vereecke (1969) and Vassort et al. (1969) who showed that adrenaline increased the slow calcium inward current but did not appreciably modify the "fast" sodium current occurring during the upstroke of the action potential. Coincident with the increased slow inward current, action potential amplitude was increased (Carmeliet and Vereecke, 1969; Vassort et al., 1969). Several investigators have described the development of calcium dependent conducted

action potentials in cardiac cells after the fast sodium-carried inward current had been inactivated and action potentials abolished by treatment of the tissue with tetrodotoxin; by bathing solutions containing an increased concentration of potassium; by sodium-free bathing media (Carmeliet and Vereecke, 1969; Scholz, 1971c, Verdonck et al., 1972; Busselen et al., 1972; Shigenobu and Sperelakis, 1972; Delahayes, 1972; Tritthart et al., 1973; Thyrum, 1974). Calcium-mediated action potentials, under the above described conditions, have been reported to occur in calf and cow ventricle (Carmeliet and Vereecke, 1969; Verdonck et al., 1972), cardiac muscle preparations of goldfish, trout, frog, rabbit, dog and cow (Busselen et al., 1972), chicken cardiac muscle preparation (Shigenobu and Sperelakis, 1972), frog atrial muscle (Delahayes, 1972), cat papillary muscle (Tritthart et al., 1973), guinea-pig atria (Thyrum, 1974) and sheep and calf ventricle (Scholz, 1971c). The propagated calcium-mediated action potentials are characterized by slow rates of rise, the presence of overshoots and increased action potential duration. In some preparations only local graded depolarizations were elicited by electrical stimulation after the fast sodium current had been inactivated; however, treatment of the preparations with adrenaline, isoproterenol, caffeine, theophylline, cyclic 3'5' AMP or dibutyryl cyclic AMP resulted in the appearance of the propagated

calcium-mediated action potentials described above (Carmeliet and Vereecke, 1969; Scholz, 1971c; Verdonck et al., 1972; Shigenobu and Sperelakis, 1972; Delahayes, 1972; Thyrum, 1974). Cyclic 3'5' AMP, dibutyryl cyclic AMP, theophylline and caffeine were less effective than the catecholamines in producing the calcium-mediated action potentials and the development of the response of the tissues to the former agents was much slower than the response to the catecholamines (Shigenobu and Sperelakis, 1972). Treatment of preparations with manganese ions and verapamil, specific antagonists of calcium transmembrane influx (Hagiwara and Nakajima, 1966; Rougier et al., 1969; Nayler and Szeto, 1972; Singh and Vaughan Williams, 1972), as well as lowered concentration of extra-cellular calcium abolished the ability of the sodium-inactivated tissues to respond to stimulation with electrical activity. However, subsequent exposure of the preparations to increased concentration of calcium ion, adrenaline, or isoproterenol overcame these inhibitory effects (Carmeliet and Vereecke, 1969; Shigenobu and Sperelakis, 1972; Tritthart et al., 1973; Thyrum, 1974).

In consideration of the foregoing discussion it would not seem unreasonable to conclude that the antagonism by adrenaline of the effects of veratramine on the atrial action potential is at least in part mediated by the ability of adrenaline to effect an increase in the

transmembrane movement of calcium ions in the presence of inactivated fast inward sodium current.

It remains an open question as to whether or not this action of adrenaline involves mediation by cyclic AMP. Methylxanthines (caffeine, theophylline, aminophylline), which have been reported to increase transmembrane influx of calcium both by direct membrane action as well as indirectly through increased levels of cyclic AMP induced by inhibition of the enzyme phosphodiesterase (Scholz, 1971a, 1971b, 1971c; Skelton et al., 1971; Kukovetz and Poch, 1972; Kimoto, 1972; Massingham and Nasmyth, 1972; Chiba et al., 1973; Hamakawa et al., 1973), failed to restore the amplitude of the atrial action potential which had been reduced by veratramine treatment. Similarly, dibutyryl cyclic AMP which is reported to penetrate cell membranes more readily and which is more resistant to degradation by phosphodiesterase than cyclic AMP (Posternak et al., 1962; Skelton et al., 1971; Drummond and Hemmings, 1972; Kukovetz and Poch, 1972), failed to reverse the effects of veratramine on the atrial action potential. In the veratramine treated atria, the occurrence in the atrial action potential of a more distinct "plateau" phase in the presence of caffeine or dibutyryl cyclic AMP (See Figures 24,25), could be interpreted in terms of the ability of these agents to effect an increase in the inward calcium current (Kobayashi et al., 1971; Skelton et al.,

1971; Drummond and Hemings, 1972; Kukovetz and Poch, 1972; Bertelli et al., 1972; Meinertz et al., 1973; Tuganowski et al., 1973; Sobel and Mayer, 1973).

Area of Transitional Tissue Between the SA Node and Common Atrial Tissue

When atria were exposed to veratramine (3×10^{-5} g/ml) for a prolonged period of time, or when the atria were treated with a larger dose of veratramine (7×10^{-5} g/ml), the preparation became quiescent. Shortly following washout of the veratramine, a discrete active "pacemaker" area became obvious, and remained active while the rest of the preparation remained quiescent. The area of tissue immediately surrounding the "pacemaker" area is of particular interest in that the response of the cells within this area to veratramine appears to be intermediate between that of the "pacemaker" and that of the common atrial cell. This tissue surrounding the "pacemaker" may represent an area of "transitional" cells lying between the sino-atrial node and the common atrial tissue.

Various investigators have proposed the existance of an area of tissue lying between the SA node and the common atrial tissue wherein cellular characteristics are intermediate between those of the pacemaker and those of the common atrial cell (Sano and Yamagishi, 1965; Sano and Iida, 1968; Sano, 1969; Strauss and Bigger, 1972). Sano and Yamagishi (1965) found that the characteristics of

the action potential underwent a gradual progressive change between the cells of the sinus node and the cells of common atrial tissue. When cells were examined in steps progressively more distant from the sinus node, it was found that action potential amplitude, resting potential and rate of rise increased, and rate of diastolic depolarization was reduced progressively until the action potential resembled that of common atrial tissue. In addition, Sano and Yamagishi (1965) as well as Strauss and Bigger (1972) found that resistance of cells to blockade of conduction by solutions containing high concentrations of potassium increased progressively with proximity of the cells to the sinus node. Other workers have confirmed the increased resistance of the cells in the region of the sinus node to blockade of conduction by high potassium concentration (Vassalle et al., 1973) and tetrodotoxin (Tomlinson and James, 1968; Sano et al., 1968; Hashimoto and Chiba, 1969; Huange, 1970, 1973). This resistance is enhanced by catecholamines and by the presence of increased calcium concentrations (Vassalle et al., 1973). Treatment of cardiac tissue with solutions containing high concentrations of potassium or with tetrodotoxin are experimental manipulations that result in the inactivation of the fast inward sodium current responsible for the upstroke of the action potential (Carmeliet and Vereecke, 1969; Scholz, 1971c; Verdonck et al.,

1972; Busselen et al., 1972; Shigenobu and Sperelakis, 1972; Delahayes, 1972; Tritthart et al., 1973; Thyrum, 1974). Within the area of cells comprising the "transitional" cells, the increasing resistance of the cells to this blockade of the action potential with increasing proximity of the cells to the sinus node implies a progressive reduction in the dependence of the action potential on sodium ion flux. In addition, the fact that resistance to action potential blockade by potassium or tetrodotoxin is enhanced in the presence of high calcium solution or adrenaline (Vassalle et al., 1973) suggests an increasing importance of calcium ion flux in the generation of the action potential in the cells of the sinus area.

In the present study, after exposure of the isolated atria to veratramine for a prolonged period of time, or after exposure of the atria to larger doses of veratramine, it was possible to observe a discrete pacemaker area which remained active while the main body of the atrial preparation was quiescent. Around the "pacemaker" area, was found an area of tissue in which some cells were active; the record of transmembrane potential showing abortive spikes, small summating depolarizations, with irregular action potentials sometimes superimposed. When the microelectrode was positioned so that the cells impaled in sequence were located progressively closer to

and finally within the "pacemaker" area various changes in electrical activity were observed. The amplitude of the irregular electrical activity was found to increase with the proximity of the cell to the "pacemaker" area. Abortive spikes, small depolarizations, and irregular action potentials were less apparent in the records of the transmembrane potential of cells located closer to the "pacemaker" area. As well, with increasing proximity of the cell to the "pacemaker" area the rhythm of electrical activity appeared to become more regular and smooth diastolic depolarization was observed. Within the "pacemaker" area rhythm was regular, and irregular electrical activity did not impinge on the recorded action potentials. Outside the area immediately surrounding the "pacemaker" area, the cells examined maintained the normal resting membrane potential, but no action potentials were observed despite the ongoing activity of the cells of the "pacemaker" area.

The effects of veratramine appear therefore to decrease progressively when the atrial cells examined are located progressively closer to the "pacemaker". This apparent gradation of veratramine effect in the cells lying between the quiescent common atrial cells and the active "pacemaker" could be interpreted as a consequence of the existance of "transitional" atrial cells whose elecrophysiological characteristics are in-

termediate between common atrial cells and pacemaker cells. In the common atrial cell, the action of veratramine appears to be manifested as an inhibition of the inward sodium current responsible for the upstroke of the action potential. With respect to the electrical activity recorded in the cells of the "transitional" area, the increase in the amplitude of electrical activity, with increasing proximity of the cell to the "pacemaker" area, could represent an inability of veratramine to produce action potential blockade by virtue of a progressive reduction in cellular dependence on sodium ion flux for generation of the action potential. Disturbance of conductance and electrical activity by veratramine is most apparent in cells distal to the "pacemaker" area and decreases progressively as cells are examined closer to the "pacemaker" area. Finally, within the pacemaker area the effects of veratramine on cellular electrical activity appear to be minimal.

The foregoing observations support the concept of the existence of "transitional" atrial cells which are located between the cells of the sino-atrial node and common atrial tissue and whose cellular characteristics appear to be intermediate between those of pacemaker and those of common atrial cell.

Veratramine-Induced Periodic Activity

It has been proposed that the periodic rhythm produced by veratramine in the cat heart is a consequence of properties of the sino-atrial node (Kosterlitz et al., 1955). Kosterlitz et al. (1955) proposed the hypothesis that the periods of asystole of periodic rhythm may be due to the exhaustion of a necessary metabolite. Kosterlitz et al. (1955) suggested that veratramine, which has been demonstrated to have an inhibitory effect on oxidative metabolism (Reiter, 1950), might interfere with the availability of a metabolite necessary for the activity of the pacemaker. The alternating periods of asystole and activity, according to Kosterlitz et al. (1955), represent a decrease in concentration of the limited "substance X" below a critical level causing inactivity of the sino-atrial tissue (asystole), and during the period of inactivity, accumulation of "substance X" occurs and leads to the subsequent active phase. The ability of adrenaline to either antagonize or precipitate periodic activity is consistent with the hypothesis of Kosterlitz et al., (1955) in view of the capability of adrenaline to increase both the production and utilization of metabolites in cardiac muscle. The findings of Hawkins (1962a, 1962b) support the hypothesis of Kosterlitz et al. (1955) that periodic rhythm, in particular, the periods

of asystole, could be due to exhaustion of a necessary metabolite. Hawkins found, that experimental conditions that favoured a higher rate of tissue activity, and hence a greater metabolite utilization rate by the atrial tissue, also resulted in more marked effects of veratramine. For example, in the isolated guinea-pig atrial preparation, veratramine-induced periodic rhythms were more likely to occur at 37°C than at 32°C. Also at 37°C, as opposed to 32°C, the impairment by veratramine of the accelerator effects of epinephrine were more marked (Hawkins, 1962a, 1962b). Hawkins (1962), however, proposed that a more complex explanation of periodic rhythm was necessitated by the fact that infrequently, the rhythm resolved spontaneously in isolated preparations in the presence of veratramine. To date, while no direct evidence has been found to link the metabolic action of veratramine (Reiter, 1955) to the production of periodic rhythm, this does not preclude an involvement, at least in part, of the metabolic action of veratramine in the production of periodic rhythm.

The differential effects of veratramine on atrial tissue as opposed to the sino-atrial tissue, observed in the present investigation may provide some insight into the mechanism of production of periodic rhythm by veratramine. Exposure of the atrium to veratramine produces progressively, an increased threshold for electrical stimulation during the quiescent phase of periodic act-

ivity, followed by an inability of the tissue to follow electrical stimuli on a one to one basis, and eventually, total blockade of conduction occurs in the atrial tissue in the presence of continued activity of pacemaker-like cells. Interpretation of these findings lead one to conclude that while the atrial pacemaker cells are subject to a negative chronotropic action of veratramine, they are resistant to the action of veratramine that leads to a blockade of the action potential and conduction in the cells which compose the main body of the atrium. This conclusion is supported by the findings of Reuse-Blom (1959) who, working with isolated spontaneous beating rabbit atria, reported that veratramine decreased conduction velocity and eventually produced a blockade of conduction in her preparation. In addition, she reported a resistance of the cells of the sino-atrial node to the effects of veratramine in that, even in the presence of concentrations of veratramine which reduced the amplitude of contraction to practically zero, the decrease in the rate of the sino-atrial node did not exceed 25%. Reuse-Blom (1959) also reported that adrenaline was capable of reversing the effects of veratramine on conduction, conduction velocity and sino-atrial rate.

The possibility exists therefore, that periodic rhythm produced in the presence of veratramine could result from a progressive development of conduction

blockade between the cells composing the main body of the atrium (due to the inhibitory action of veratramine on inward sodium movement) in the presence of continuous or resumed activity of the sino-atrial node (the cells of which are resistant to action potential blockade by veratramine). In this context, antagonism of the effects of veratramine by adrenaline could be the result of not only a positive chronotropic action but also an ability of adrenaline to restore atrial action potential and conduction by increasing the transmembrane flux of calcium. The spontaneous resolution of periodic rhythm could represent a recovery in degree, of the sino-atrial node from the negative chronotropic effects of veratramine at a time when blockade of conduction in the main body of the atrium was incomplete. Support for such a recovery process could be indicated by the work of Kosterlitz et al. (1955) who, observing transient veratramine-induced periodic rhythm, noted that as periodic rhythm progressed towards resolution into a regular rhythm, the inactive phases became progressively shorter and the active phases longer. Eventually the periodic rhythm was replaced by a slow but regular rhythm the frequency of which continued to gradually increase with time but never attained control levels.

Resolution of the mechanisms of action of veratramine on the heart requires further investigation, including simultaneous multiple microelectrode recording

of activities of both atrial and sino-atrial cells throughout the complete course of action of veratramine. Certainly the metabolic effects of veratramine cannot be excluded from possible involvement in the cardiac effects of veratramine. Investigation of the action of veratramine on the sino-atrial node appears to offer most promise if only from the standpoint that this drug permits the development of a unique preparation in which study of the cells composing the sino-atrial node is greatly facilitated.

SECTION V

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BIBLIOGRAPHY

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