ELECTROPHYSIOLOGICAL EFFECTS OF VERATRAMINE ON CAT ATRIA

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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

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.

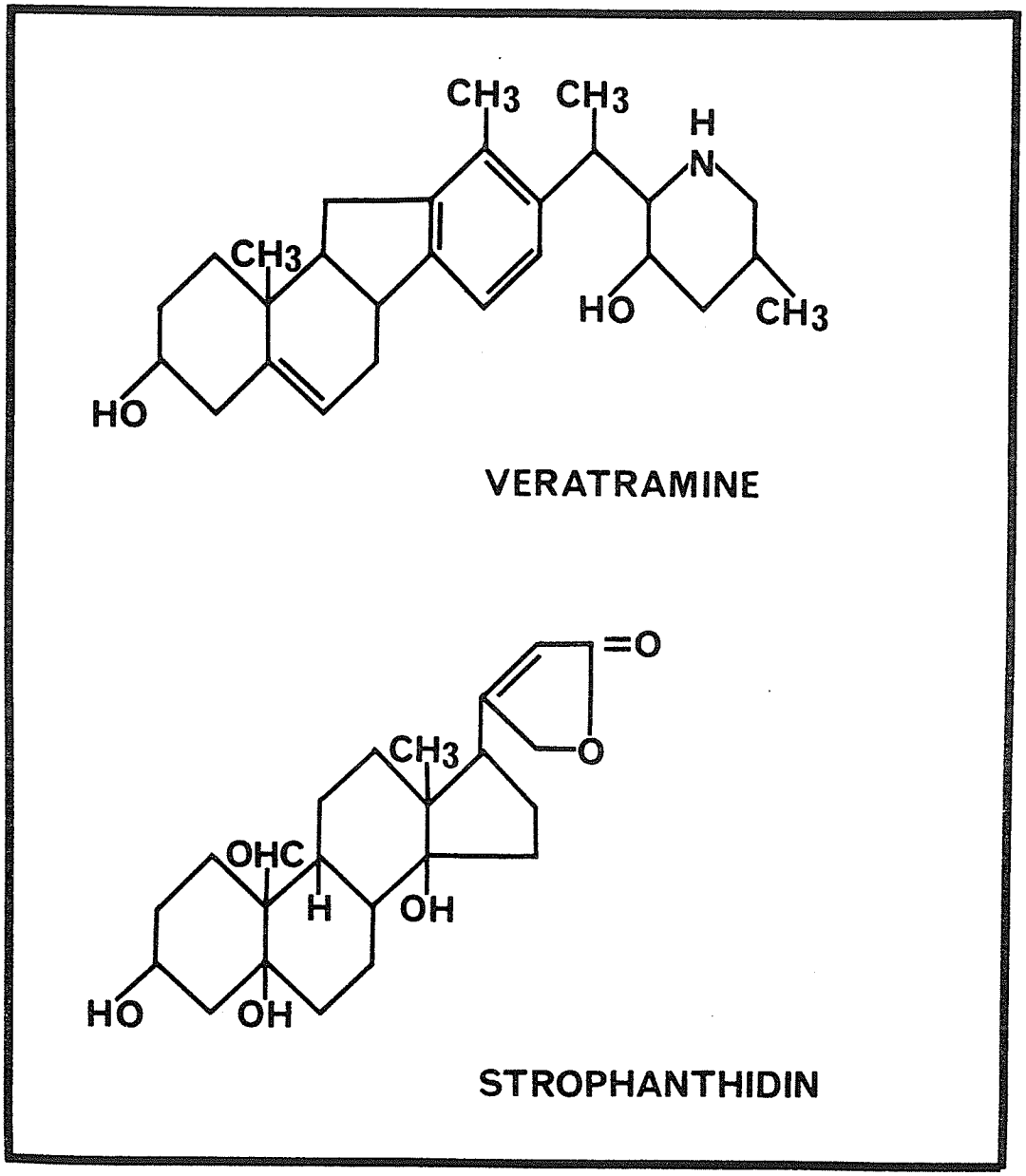
CH3
CH3
H
N
CH3
HO
CH3
HO
VERATRAMINE
=O
O
CH3
OHC
H
OH
HO
OH
STROPHANTHIDIN

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