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THE CHRONOTROPIC ACTION OF VERATRIDINE
STUDIED BY DIRECT PERFUSION OF THE
SINUS NODE THROUGH ITS ARTERY

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Veratridine and related veratum alkaloids have so many dramatic effects on the cardiovascular system that it is difficult to separate those which are primary from those which are secondary. With an experimental preparation permitting direct perfusion of the sinus node through its own artery we have studied the chronotropic action of veratridine and compared it to that of veratramine. Because nodal perfusion was direct, sufficiently small quantities of test substances could be employed so that on re-circulation they had no discernible effect on ventricular myocardium or extracardiac neuroreceptors.

METHOD

Nine dogs were anesthetized with intraperitoneal pentobarbital (30 mg/kg) and the trachea intubated for mechanical ventilation. Through a midline sternal-splitting incision the heart was exposed and cradled in the pericardial sac. The right coronary artery was dissected free between the atrial appendage and the margo acutus, and a small polyethylene cannula inserted into an opening cut in the artery and passed up its sinus node branch1,2. Collateral arterial circulation to the canine sinus node is so extensive that ligation of its primary arterial supply during cannulation has no significant effect on either rate or rhythm of the sinus node2. Details of the method have been published previously4,5.

For nerve stimulation the right vagus was isolated in the mid-cervical region and the right stellate ganglion within the thorax. Stimuli were delivered in rectangular waves, 30 cps, for 6 second trains of 1 millisecond impulses. Cannulae were routinely placed in the right atrium via the jugular vein and central aorta via the femoral artery. Throughout the experiments central aortic and central venous pressures were measured with transducers and monitored simultaneously with an electrocardiogram and tachogram on an oscilloscope at 50 mm/second. These phenomena were constantly recorded on a master recorder at 0.25 mm/second, with a slave circuit to a one-channel direct writing elec-

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trocardiograph permitting a separate permanent record (at 25 mm/second) from which the electrocardiographic complexes could later be studied. The tachogram was derived with an analog computer from successive R waves of the electrocardiogram and reflected instantaneously any change in heart rate. Final records of heart rate change were additionally plotted and graphed from each experiment as the average rate in successive 6-second intervals of the 25 mm/second electrocardiogram, where any changes in rhythm could be determined.

Veratridine hydrochloride* for injection into the sinus node artery was prepared by dilution with Ringer's solution in logarithmically increased concentrations from 0.001 to 1.0 |g/ml; similar concentrations were prepared in fresh autogenous arterial blood. Veratramine hydrochloride* was also prepared both in blood and Ringer's solution, in the same concentrations. Veratridine was studied in all nine dogs, and compared to veratramine in four. The injections into the sinus node artery were delivered from a hand syringe directly into the polyethylene cannula in 1.0 ml. volume in 30 seconds. Second and subsequent injections were made only after the heart rate had returned to control. When more than one series of increasing concentrations of test substance was given the same dog, at least one hour was allowed to elapse between series. Serial injections of veratridine or veratramine at concentrations of 1.0 |g/ml were administered as soon as heart rate returned to control.

RESULTS

In this experimental preparation any injection directly into the sinus node artery causes an initial slowing of sinus rate, followed regularly by a slight post-injection acceleration from Ringer's or Krebs-Henseleit solution, but rarely when injections are in fresh autogenous arterial blood. Neither veratridine nor veratramine at concentrations of 0.001 |g/ml had any effect different from control injections of Ringer's solution or blood. With concentrations of 0.01 |g/ml and greater there was no significant difference between the chronotropic actions of veratridine and veratramine. Both substances produced sinus acceleration when administered in Ringer's solution (Figures 1, 2), while both caused sinus bradycardia if prepared in fresh autogenous arterial blood (Figures 3, 4). None of the injections produced a significant change in right atrial or central aortic pressure.

Sinus acceleration from the preparations in Ringer's solution was consistent at 1.0 |g/ml but variable at lesser concentrations. In three of the nine dogs the degree of acceleration increased with each effective concentration (0.01, 0.1 and 1.0 |g/ml), but in the others the acceleration at the two lower concentrations was either transiently mixed with an associated period of slowing, or produced no more acceleration than the control injection of Ringer's solution. The acceleration produced by 1.0 |g/ml of either veratridine or veratramine began during the period of injection (Figure 2), and was in all respects identical to the type of acceleration observed in this same experimental model from norepinephrine or isoproterenol4. This similarity includes the onset, peak

*Kindly supplied by Dr. Otto Krayer, Department of Pharmacology, Harvard Medical School, Boston, Massachusetts.
effect and duration of action as well as the concave slope in heart rate curve during return to control. Acceleration in our experiments was observed without prior atropinization or vagotomy, conditions which have been reported to alter the chronotropic response to veratridine.

Sinus bradycardia from the preparations in blood consistently increased with each increasing concentration of either veratridine or veratramine (Figure 3). Atropinization (0.2 mg/kg atropine sulfate) did not prevent this bradycardia. At concentrations of

Veratridine in Ringer's solution injected into the sinus node artery produced sinus tachycardia, particularly evident with 1.0 μg/ml. The variable responses at lower concentrations are discussed in the text. The vertical bars indicate the beats per minute above and below control rate (C). A diphasic response in heart rate is characteristic of injections of Ringer's solution into the sinus node artery, and the average responses plotted here for 0.01 and 0.1 μg/ml of veratridine are not significantly different from responses to Ringer's solution alone. Veratramine in Ringer's solution had the same effect as veratridine.
0.01 and 0.1 μg/ml the slowing was entirely sinus bradycardia, while at 1.0 μg/ml there was sometimes transient sinus arrest with A-V nodal escape rhythm. Sinus bradycardia from either substance in blood was sometimes interrupted by transient periods of acceleration, just as the acceleration of sinus rhythm from preparations in Ringer's was sometimes interrupted by transient periods of bradycardia. However, the initial injection of 1.0 μg of veratridine or veratramine in Ringer's usually produced a pure sinus tachycardia, while that in blood usually produced a pure sinus bradycardia.

A characteristic response of heart rate to intranodal veratramine 1.0 μg in Ringer's solution is shown at the top of this graph; the same type of response was obtained with veratridine. This was an uninterrupted sinus tachycardia which was in all features similar to the response obtained from intranodal isoproterenol (middle graph) or norepinephrine (lower graph). The two lower graphs are from the same dog and are typical of hundreds of similar injections, while the upper graph is a different dog, but again typical of the veratridine-veratramine responses when administered in Ringer's solution. Heart rate is plotted as the average in successive 6-second intervals of a 25 mm/second electrocardiograph in which the P waves remained unchanged.
CHRONOTROPIC ACTION OF VERATRIDINE

For both veratridine and veratramine in Ringer's solution the time of onset and duration of chronotropic action changed with increasing concentration of the test substance. With both substances in concentrations of 0.01 and 0.1 μg/ml in Ringer's solution, the period of acceleration usually began near the end of the injection, or just after its completion, and was therefore similar to the period of post-injection acceleration from Ringer's solution alone, except of slightly longer duration. However, the sinus acceleration from 1.0 μg of either veratridine or veratramine in Ringer's solution began early during the period of injection, and was usually maximal by the end of the injection or shortly after it. Duration of this acceleration (averaging about 3 minutes before return to control rate) was always longer than the post-injection acceleration seen with control injections.

AVERAGE MAXIMAL AND MINIMAL HEART RATES AFTER INJECTION OF VERATRIDINE (IN BLOOD) INTO SINUS NODE ARTERY

Veratridine prepared in fresh autogenous femoral artery blood produced increasing degrees of sinus bradycardia in direct relation to the concentration. Compare in particular the difference in response to 1.0 μg here with that in Ringer's solution (Figure 1). Although some acceleration was apparent with increasing concentrations in blood, the predominant effect is clearly one of slowing. The same responses were obtained with veratramine in blood. The vertical bars indicate beats per minute above and below control rate (C).
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CHRONOTROPIC EFFECT OF INCREASING CONCENTRATIONS OF VERATRIDINE (IN BLOOD) INJECTED INTO SINUS NODE ARTERY

The increasing negative chronotropic effect of veratridine (or veratramine) in blood is demonstrated in this graph of responses in one dog. Heart rate is plotted in the same manner as in Figure 2.

Figure 4

A comparison in one dog of the initial and second injections of veratramine 1.0 μg in Ringer's solution; the same effects were produced by veratridine in Ringer's solution. The initial accelerative response shown on the left, which is an uninterrupted sinus tachycardia (same graph as top of Figure 2), is replaced by an alternate acceleration and slowing following the second injection.

Figure 5
CHRONOTROPIC ACTION OF VERATRIDINE

When given in blood, both veratridine and veratramine at concentrations of 0.01 and 0.1 μg/ml again seemed only to exaggerate the normal response, i.e. they were associated with more sinus bradycardia during the injection than was observed during injections of blood alone. As with the injections in Ringer’s solution at those concentrations, the duration of change corresponded generally to that observed from blood alone, although again there was slight prolongation of this effect. The slowing effect produced by 1.0 μg of an initial injection of either test substance in blood was not only much more profound during injection, but it always persisted after completion of the injection, lasting three to 12 minutes before returning to control rate.

With serial injections of 1.0 μg of veratridine (3 dogs) or veratramine (2 dogs) in blood the following pattern was usually observed. The first injection produced sinus bradycardia alone, the next injection (after return to control rate) produced sinus bradycardia with periodic rhythm and the slowing was usually more prolonged, while the third injection usually produced sustained sinus arrest with escape A-V nodal rhythm. Serial injection of 1.0 μg of either substance in Ringer's solution characteristically had the following effect (Figures 5, 6). The initial injection in Ringer’s produced sinus tachycardia instead of bradycardia; however, results of the second and third injections were similar to the results of the second and third injections in blood, namely, predominant slowing plus periodic acceleration with the second injection, and usually sinus arrest with A-V nodal escape rhythm following the third injection.

Because of the reported antiaccelerator effect of veratrum alkaloids as tested with various adrenergic stimuli 6,11 the response to supramaximal stimulation of the stellate ganglion was tested following intranodal veratridine in 2 dogs. In each of them veratridine 1.0 μg abolished the acceleration from stellate stimulation with the response gradually recovering to control levels (Figure 7). In the same dogs the response to submaximal stimulation of the right vagus nerve was enhanced by veratridine, with gradual recovery to the control response (Figure 8). For either stellate or vagal stimulation the effect of veratridine was unrelated to and considerably outlasted its primary chronotropic action.

DISCUSSION

On direct perfusion of the sinus node of the dog both veratridine and veratramine have distinct chronotropic effects which are the same for both substances. The observation that both veratridine and veratramine caused sinus tachycardia when administered in Ringer’s solution but sinus bradycardia when administered in blood is of potential significance in interpreting the results obtained by previous investigators utilizing isolated tissue or heart-lung preparations, some of which have been perfused with blood and others by various “physiologic” solutions.

The absence of reflex aortic hypotension following injection of veratridine or veratramine into the sinus node artery supplements the observations of Dawes12 on receptor sites for the coronary chemoreflex. He demonstrated that the reflex could be elicited by injections into the coronary arteries supplying the left ventricle, but not
Figure 6

Periodic rhythm observed with second injections of either veratridine or veratramine 1.0 μg is illustrated in this continuous ECG (lead a VR); the letters serve only to identify the lines. Periodic rhythm seen when utilizing the preparation employed in these studies differs from periodic rhythm seen after systemic administration of veratridine in that the intervals during sinus arrest are usually filled by an escape A-V nodal rhythm, since the A-V nodal pacemaker is unaffected during direct perfusion of the sinus node. Injection was begun at the arrow in line A and completed in line D. The initial sinus acceleration, lines A to D, terminates in abrupt bradycardia in line E, followed by an escape A-V nodal rhythm alternating with brief resumptions of sinus rhythm until recovery in line L.
by injections into those supplying the left atrium or right ventricle. In view of the abundant innervation of the sinus node it is of some interest that there do not appear to be receptor sites for the reflex in the area supplied by the sinus node artery, which perfuses not only the sinus node but several centimeters of adjacent right atrial myocardium.

Since an antiaccelerator action of veratrum alkaloids has been demonstrated to exist against numerous sympathomimetic amines as well as against accelerator nerve stimulation, it may be presumed that this action cannot occur exclusively at the nerve

**RIGHT STELLATE STIMULATION AFTER INTRANODAL VERATRIDINE**

![Graph showing heart rate over time after intranodal veratridine](image)

**MINUTES**

Figure 7

The antiaccelerator action of 1.0 µg intranodal veratridine (in Ringer's) is demonstrated in this experiment. Each vertical line indicates the response to supramaximal stellate ganglion stimulation and extends from the prestimulus rate indicated by a bar to the peak response indicated by a dot. The first stimulus after veratridine evoked no acceleration, while the response at 27 minutes was virtually back to control (C). The minutes on the horizontal axis refer to time after veratridine.
ending (although it may act there also) but must include the effector site. On the other hand a prominent feature of the histopathology in poisoning by veratrum alkaloids derived from sabadilla seeds is reported to be structural damage of nerves, with disintegration of ganglion cells and demyelination of peripheral nerve fibers. From these and related observations it seems likely that veratrum alkaloids act both on sinus node fibers and local nerve endings, although the enhancement of response to vagal stimulation in our experiments suggests that the local parasympathetic ganglia are not initially damaged.

**RIGHT VAGAL STIMULATION BEFORE AND AFTER INTRANODAL VERATRIDINE**

Intranodal veratridine (in Ringer's) enhanced the response to submaximal vagal stimulation. The responses to stimulation in this dog are plotted in the same manner as Figure 7. After 0.1 and 0.5 µg of veratridine there was slightly increased response to vagal stimulation, but the response to an identical stimulus following 1.0 µg was much increased over control (C). The response then gradually returned to control at 43 minutes and remained consistent for 20 more minutes. The minutes indicate time after the control response.
Concerning the accelerator action of veratridine or veratramine in dogs with intact vagi, there is considerable evidence that these alkaloids can release catecholamines. In our experiments serial injections of veratridine or veratramine in Ringer’s solution did not continue producing sinus tachycardia, whereas serial injections of norepinephrine at the same intervals in this preparation do. Similarly, repeated stimulations of the right stellate ganglion at these intervals continue to evoke maximum accelerative responses. The continued maximum responses to norepinephrine indicate the normal sinus node does not lose its ability to respond to local injection of this neurohormone, while the responses to stellate ganglion stimulation suggest the normal nodal adrenergic nerve endings do not lose their ability to liberate norepinephrine. Serial injections of veratridine and veratramine in Ringer’s solution must, therefore, do more than simply liberate local norepinephrine.

If the acceleration from these two substances when administered in Ringer’s solution is due to local release of norepinephrine, as all features of the graphed curves of heart rate response suggest (Figure 2), and as might be anticipated from other studies demonstrating the ability of certain veratrum alkaloids to release catecholamines, then the following considerations may be made. First injections of Ringer’s solution directly into the sinus node artery are regularly followed by a modest post-injection acceleration which is seldom seen after injections of blood. This post-injection acceleration is abolished by either the dichloro or naphthyl analog of isoproterenol, suggesting it may be due to norepinephrine locally released in the node by the calcium in Ringer’s solution. Thus injections in Ringer’s solution may cause local release of some norepinephrine while those in blood may not. Second, in Ringer’s solution all the calcium is in ionized form whereas only half that in whole blood is ionizable, and Dawes has demonstrated that the calcium ion opposes several cardiovascular effects of veratridine. It is possible then that veratridine or veratramine exert a direct negative chronotropic effect when given in blood, but that this effect is opposed by the presence of ionizable calcium in comparatively large amount in Ringer’s solution, leaving apparent only the effect of norepinephrine released locally by both Ringer’s solution itself and the veratridine or veratramine. Further studies will be required to establish whether these or still other factors are in fact responsible for the chronotropic responses observed in our experiments.

**SUMMARY**

Veratridine and veratramine have similar chronotropic effects when studied by direct perfusion of the canine sinus node through its own artery, but the effects of either substance differ according to whether administered in Ringer’s solution or blood. Both veratridine and veratramine in Ringer’s solution have a sinus accelerating effect, but in blood have a sinus slowing effect. Serial injections of either substance in either Ringer’s solution or blood lead first to periodic rhythm and then sinus arrest. Veratridine administered by direct perfusion of the sinus node suppresses the response to stellate ganglion stimulation and enhances the response to vagal stimulation. The absence of reflex hypotension following injection of veratridine or veratramine into the sinus node artery indicates the area perfused by that vessel probably does not contain receptors for the coronary chemoreflex of Von Bezold and Jarisch.
REFERENCES