The Mechanism of the Hypotensive Action of Veratrum Alkaloids.* (19279)

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The characteristic action of veratrum alkaloids in producing hypotension, bradycardia and depression of respiration has long been recognized(1,2). The importance of the motor fibers of the vagus in the production of the circulatory component of this so-called "Bezold reflex" has been equally well known (2). It has not been clear, however, whether this reflex action is the entire mechanism whereby veratrum alkaloids produce a persistent lowering of blood pressure in the intact animal. As a matter of fact, there are several reasons to believe that the vagal stimulation may be of secondary importance. For example, it has been shown that after section of the vagi or after the administration of atropine, veratrum alkaloids still produce a dramatic fall in systemic blood pressure(3,4). Furthermore, it has recently been shown that a wide variety of chemical compounds are capable of inducing the Bezold or Bezold-like effect, and yet none of these produce a hypotensive effect after vagotomy(5,6,7).

The availability of several new alkaloids (8,9) and the revival of clinical interest in the possible use of vertrum alkaloids in hypertension has prompted the studies reported here with the hope that they will contribute to a better understanding of the mechanism of action of this group of agents. Since the major part of the work reported in this paper was done with germitrine and neogermitrine,† it also seemed important to determine whether these two new alkaloids behave in a fashion similar to those which had been studied previously. The alkaloids in water or dilute acetic acid solutions (pH 6-7) were injected intravenously over a period of 5-30 seconds.

Action of germitrine in isolated and perfused head preparation. A series of dogs was prepared according to the method of Heymans and Heymans(10) in such a manner that the head of recipient dog B was connected to its trunk only by means of the vagus nerves. Appropriate doses of germitrine and neogermitrine were injected into: a) the carotid arteries of the isolated head, b) intravenously into the intact donor dog A, and c) into the isolated trunk of dog B. Fig. 1 illustrates results from a typical experiment. It is particularly to be noted that germitrine injected into donor dog A produced the reflex circulatory effect only in the intact animal with no response in the recipient dog. Furthermore, injection of germitrine into the isolated trunk induced a typical effect in the recipient dog. Heymans and de Vleeschhouwer(11) have reported with veratridine that bradycardia can be produced not only by stimulation of receptors in the isolated trunk but also by stimulation of the chemo-receptors of the carotid body and by direct stimulation of the vagal center of the isolated and perfused head. It should be pointed out, however, that in the experiments by Heymans and de Vleeschhouwer much larger doses of veratridine were required to induce the typical response from stimulation of the carotid sinus itself or the vagal center than was the case for stimulation of receptors in the trunk. It is possible that higher concentrations of germitrine and neogermitrine might also produce similar results. It seems fair, however, to conclude that with doses of veratrum alkaloids not too different from clinical doses in the intact animal the major part of the reflex circulatory effect originates from stimulation of receptors in the trunk and which travel centrally by way of the vagus.

Fig. 1 is also of interest in that it shows the short duration of the reflex circulatory effect which is produced in the recipient dog; whereas, in the intact animal there is a much slower recovery of blood pressure, suggesting that in the intact animal the persistent hypo-

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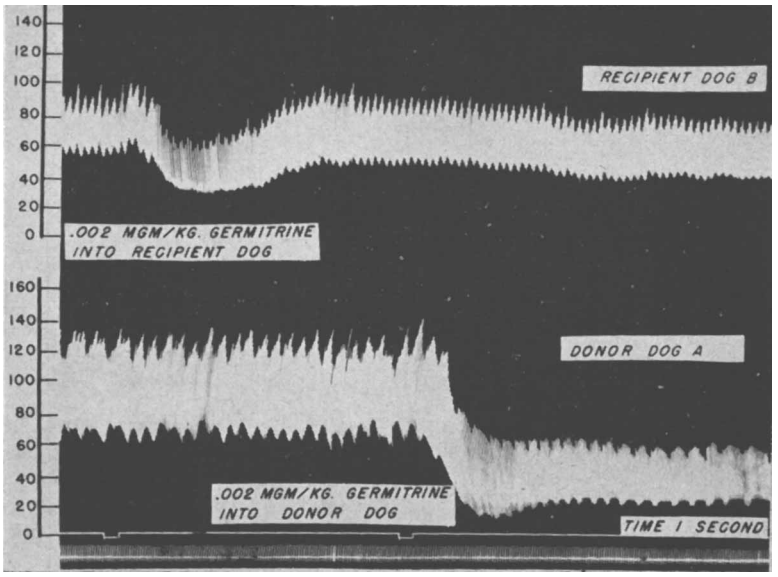

FIG. 1. Effect of germitrine on the vagal cardio-inhibitory mechanism of the dog. Animals prepared according to the technic of Heymans and Heymans. Note typical bradycardia induced in recipient dog when drug is injected into trunk but not when same dose is injected into donor dog.

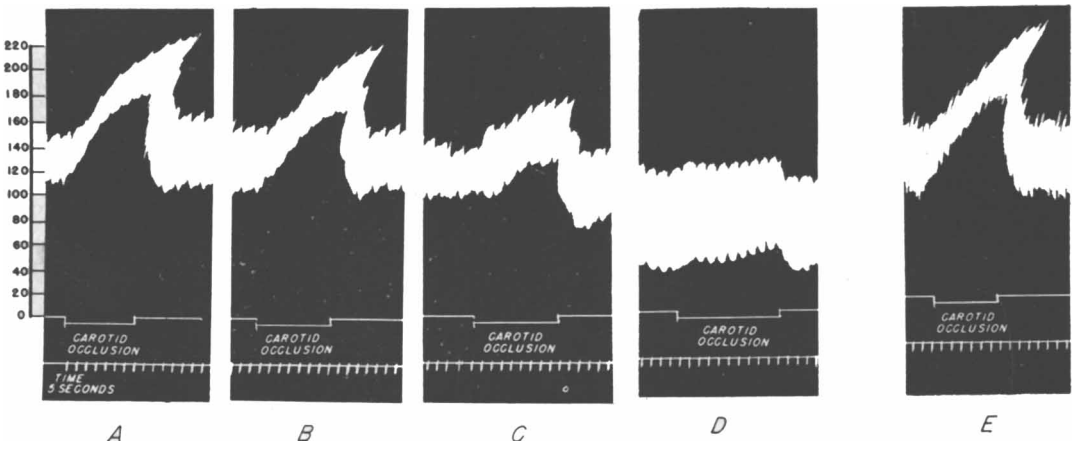

FIG. 2. Typical experiment illustrating effect of germitrine on the carotid sinus pressor reflex of a dog, animal anesthetized with chloralose and morphine, both vagi cut and animal given .5 mg/kg of atropine sulfate. A. Carotid response, B. After .0005 mg per kilo, C. After .001 mg per kilo, and D. After .0015 mg per kilo of germitrine, E. Repeat carotid occlusion 1.5 hr after D.

tension is in part due to some phenomenon other that the Bezold effect.

Effects on carotid sinus pressor reflex. In a routine study of the action of veratrum alkaloids, it was found that they possessed a marked ability of blocking the carotid sinus pressor reflex in dogs. Dogs were prepared with chloralose and morphine as described previously(12). After obtaining control observations on the carotid sinus pressor reflex, repeated doses of various veratrum alkaloids were injected intravenously at 5 minute intervals and the response to carotid sinus occlusion tested after each administration of drug. A typical record of such an experiment is shown in Fig. 2 in which germitrine produced a pro-

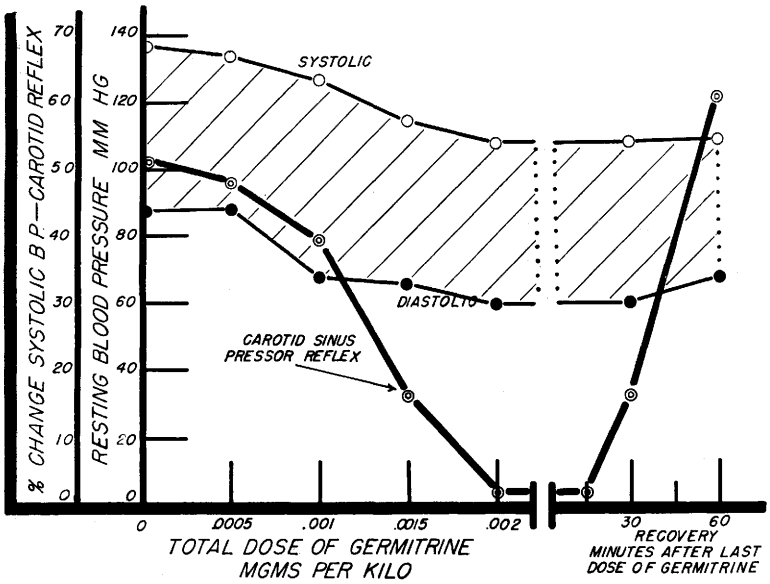

FIG. 3. Summary of effect of germitrine on the resting blood pressure and carotid sinus pressor reflex of dogs anesthetized with chloralose and morphine. Mean results of six separate experiments.

gressive and finally almost complete blockade of the carotid sinus pressor reflex. Following essentially complete blockade animals were tested at intervals until there was recovery of the carotid sinus pressor reflex. Five to 7 dogs were used to determine the activity of each active compound and the results were averaged together as shown in Fig. 3. The dose producing a 50% blockade of the carotid sinus pressor reflex was determined graphically from the dosage-response curve from each series of experiments.

Table I summarizes the results obtained with a series of veratrum alkaloids and semi-purified veratrum preparations. In general, the activity of all compounds tested by this method was similar to that previously reported for other methods of assay(13).

Previously, it had been demonstrated that blockade of the carotid sinus pressor reflex was produced by the following 3 methods: 1) Synaptic blocking agents(12), 2) by a decrease in resting blood pressure(14) or effective blood volume, *i.e.*, hemorrhage(15), and 3) adrenergic blocking agents(16). None of the veratrum preparations used in this study proved to possess either adrenergic blocking or synaptic blocking action. It is possible, however, that the depression of the carotid sinus pressor reflex might have been secondary to a general vasodilatation, either directly or indirectly, since a decrease in resting blood pressure was observed as shown in Table I and Fig. 3. That this seemed unlikely was demonstrated in experiments in which the carotid pressor reflex was blocked by an effective dose of germitrine and despite the infusion of 2-3 liters of 6% dextran solution into several animals, the reflex remained blocked. As additional evidence, it should be pointed out in Fig. 3 that there was practically complete recovery of the carotid sinus pressor reflex in one hour without significant changes in resting blood pressure. Thus, it appears that the blockade of the carotid sinus pressor reflex produced by the veratrum alkaloids can not be explained on the basis of the above-mentioned means of inducing such an effect. Subsequent experiments tend to suggest that an important action of veratrum alkaloids may be on the vasomotor center itself.

Effect on vasomotor center. A series of dogs was prepared according to the technic of Sollmann and Pilcher(17). Animals were anesthetized with chloralose and morphine. Blood pressure was recorded from the femoral artery by means of an Anderson glass manometer. The spleen was mobilized and a major artery cannulated. The corresponding vein

TABLE I. Relative Activity of Veratrum Alkaloids on Carotid Sinus Pressor Reflex.

Compound or preparation	No. dogs used	Dose for 50% blockade of carotid sinus pressor reflex,* mg/kilo	Ratio of activity, germitrine = 1	Time for recovery, min	Mean resting blood pressure, mm Hg systolic/diastolic	
					Before treatment	After carotid sinus blockade
Germitrine	6	1.3 ± .1	1	60	139/90	115/65
Neogermitrine	5	1.6 ± .2	.8	60	153/99	112/69
Protoveratrine	6	3 ± .5	.4	60	130/90	115/80
Germerine	5	4 ± .6	.3	60	152/99	110/65
Germidine	5	4 ± .7	.3	60	152/106	132/89
Veriloid	7	9 ± 1.5	.14	45	135/89	112/75
Veratramine	1	>505	<.0025	—	—	—
Jervine	3	>7000	<.0002	—	—	—
Germine	1	>9333	<.0001	—	—	—

* ± Standard deviation.

was also isolated and cannulated. Immediately following this procedure, infusion of Locke-Ringer's solution into the splenic artery was begun. All other vessels to the spleen were isolated and tied off, care being taken not to include any nerves in the various ligatures. Pressure in the perfusion fluid was obtained by placing the storage bottle of solution approximately three feet above the level of the dog. A Shipley rotameter was inserted into the system at a point just before the perfusing fluid entered the spleen. Flow of perfusing fluid was determined by means of a visual galvanometer which had previously been calibrated. In a properly prepared animal the outflow from the spleen became clear approximately one-half hour after the operation was completed. In order to test the effect of complete isolation of the splenic circulation, epinephrine was injected intravenously. Under proper conditions, this procedure caused no effect on the rate of flow of perfusing fluid in the spleen, although it caused a considerable rise in blood pressure. Both carotid arteries were isolated so that they could be occluded in the usual test for sensitivity of the carotid sinus pressor reflex.

Fig. 4 illustrates the type of results obtained with this preparation. Panel A represents the effects of control carotid occlusion and shows the typical rise in blood pressure and decrease in splenic flow as a result of this maneuver. Panel B illustrates the effects of injecting 0.002 mg per kilo of neogermitrine intravenously into the animal. The typical fall in blood pressure in the systemic circulation is illustrated. Significantly, however, this fall in pressure was accompanied by a vasodilatation of the vessels of the spleen and since the spleen was essentially isolated from the rest of the body except for its nerve supply, it is apparent that this effect must have been mediated by nervous pathways. Panel C represents a repetition of the carotid occlusion 5 minutes after the injection of neogermitrine, illustrating a complete blockade of the carotid sinus pressor reflex as well as no effect upon flow through the spleen. Panel D illustrates the effects of carotid occlusion one hour after the injection of neogermitrine in which instance there had been a partial recovery of the carotid sinus pressor reflex and of the concurrent reflex decrease in splenic flow. Results similar to these were obtained in three dogs each using neogermitrine and protoveratrine.

In order to further eliminate the possibility that the actions described above might have been due to direct effects of the veratrum alkaloids in spite of extreme care in isolating the splenic circulation, neogermitrine and protoveratrine were injected into the perfusate going directly into the spleen. Under such circumstances neogermitrine consistently caused a vaso-constriction rather than a dilatation (Fig. 5). This is of interest in connection with observations of Heymans and de Vleeschhouwer(11) that injection of veratridine into a completely isolated and surviving trunk produces a rise in blood pressure rather than a fall. We have also observed that doses of neogermitrine of the order of 0.01 mg per kilo of this alkaloid tend to produce a rise in blood pressure rather than a fall. Such effects were

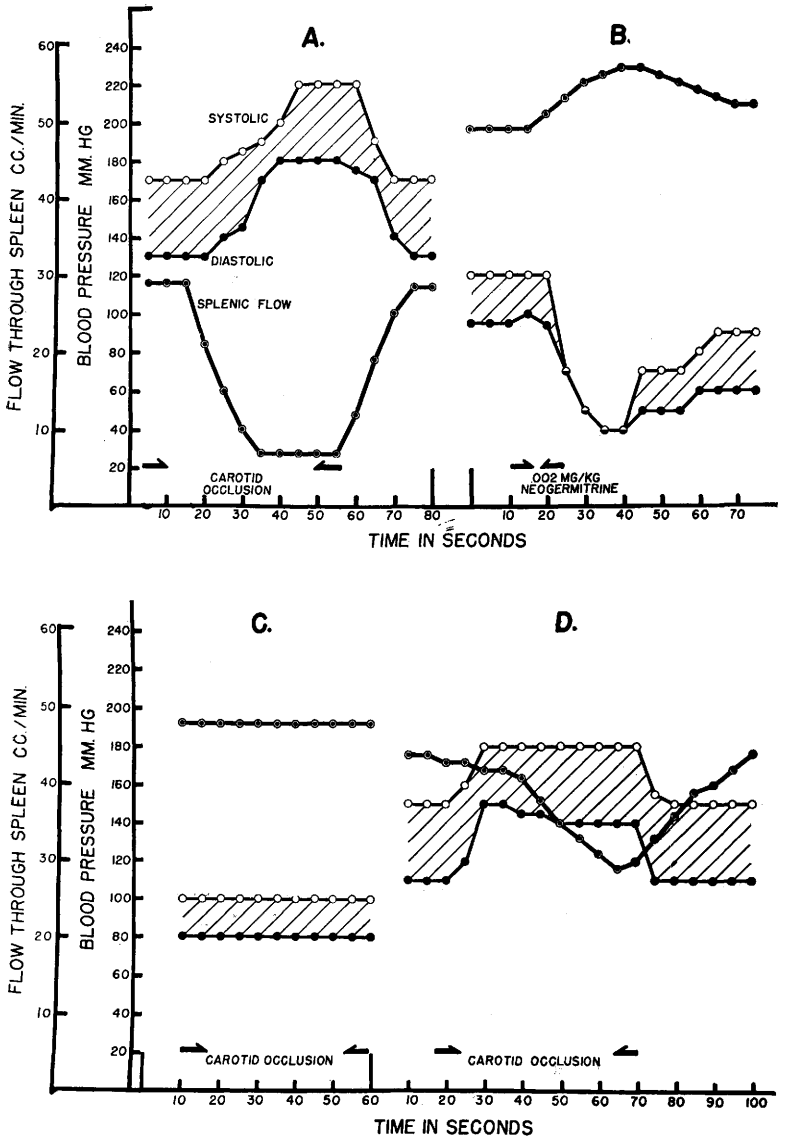

FIG. 4. The effect of neogermitrine on the circulation of the isolated and perfused spleen of the dog prepared according to the technic of Sollmann and Pilcher. Splenic flow determined with a Shipley rotameter placed in perfusion system just before entrance into the splenic artery. A. Effect of carotid sinus occlusion before treatment. B. Effect of intravenous injection of .002 mg per kilo of neogermitrine. C. Carotid occlusion 5 min after B. D. Carotid occlusion 1 hr after B.

not noted with protoveratrine and as shown in Fig. 5, protoveratrine injected directly into the splenic circulation produced little effect upon the rate of flow.

Discussion. The above observations clearly indicate that veratrum alkaloids which have thus far been studied, produce two distinct actions upon the circulation of the dog. One of these is reflex stimulation of the cardio-inhibitory vagal center by means of the Bezold reflex. The second is a blockade of the carotid sinus pressor reflex, which has as its function maintenance of blood pressure at normal levels. The present experiments do not furnish evidence as to the direct site of this blockade, but are suggestive of the point of view that

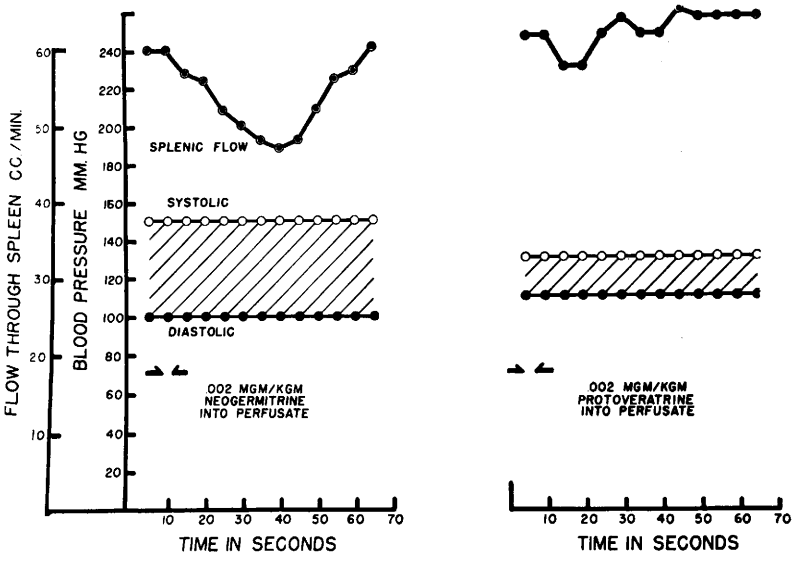

FIG. 5. Same preparation as described in Fig. 4. Injections made directly into the splenic arterial circulation. Note that under these conditions neogermitrine produces a marked vasoconstriction while protoveratrine is almost without effect.

this second action of veratrum alkaloids is directly upon the vasomotor center. Other evidence (18,19) also indicates that the site of the hypotensive action may be central.

The experiments reported here are of interest in another connection in that they suggest the possibility of developing a more precise method of assay of the activity of veratrum alkaloids than is now available. This possibility has been explored by Rubin and Burke (20) and preliminary observations indicate that the method based on the ability of these alkaloids to block the carotid sinus pressor reflex will, in fact, give a more sensitive and more reproducible bioassay result.

Summary. 1. A series of veratrum alkaloids including two new ones, germitrine and neogermitrine, which have recently become available have been studied for their effect upon the circulation of dogs. 2. Germitrine and neogermitrine, as well as other veratrum alkaloids, produce reflex respiratory and circulatory effects. In major part these responses are initiated by stimulation of afferent fibers which run in the vagi-aortic nerves. 3. All clinically active veratrum alkaloids produce a reversible blockade of the carotid sinus pressor reflex. Activity upon this particular reflex corresponds approximately to the reported therapeutic action of these agents in hypertension. It is suggested that this may be made the basis of bioassay procedures for this group of substances. 4. Results are suggestive that an important action of the veratrum alkaloids may be directly on the vasomotor center. 5. In excessive dosage, germitrine and neogermitrine produce a rise in blood pressure rather than a fall.

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Increase in B-Vitamin Concentrations in Digestive Tract Contents of Young Dairy Calves.* (19280)

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In a previous report(1) the authors presented data which indicated that levels of certain of the B vitamins in the contents of the digestive tract of calves were not related to the age of the animal. Concentrations were as high or higher in calves 2 and 4 weeks of age as in older calves up to 14 weeks, and these levels were greater generally than those in the feed consumed. It seemed desirable to make additional studies using the younger calf in an attempt to determine whether the indicated high values were a consistent phenomenon.

Experimental. Male Holstein calves were quartered and fed in individual pens in an artificially heated and ventilated barn. A medium grade of alfalfa hay and a calf starter were fed *ad libitum* to the animals from the time they entered the barn. Each calf had received colostrum for approximately 3 days, then was placed on a milk feeding schedule which would allow 270 lb of Holstein herd milk during the ensuing 36 days. Twenty calves were divided at random into age groups for slaughter, 5 being sacrificed at each of the following ages: 8, 16, 24 and 32 days. The final feeding of milk occurred 6 hours prior to slaughter; however, hay and grain were accessible to the calf until the time of slaughter. The contents of the rumen, abomasum, small intestine and large intestine were collected in sample jars, iced, taken to the laboratory and blended into a slurry. Chemical assays for thiamine, riboflavin, niacin and pteroylglutamic acid were performed on the slurried contents of the digestive tracts of these calves. Thiamine was determined by the thiochrome method(2), riboflavin by a modification of the fluorometric procedure as set forth by Loy(3), niacin by the cyanogen bromide-metol method(4) and pteroylglutamic acid (PGA) analyses were performed according to the fluorometric method as outlined by Alfrey, *et al.*(5). The milk, grain, and hay which were being fed were similarly assayed several times during the trial. Concentrations of all factors were calculated on a dry matter basis. Since records had been made of the amounts of milk, hay and grain which each calf had received, it was possible to calculate an average level of vitamin intake for each calf on a dry matter basis. This level of intake varied slightly among calves, depending upon the relative proportions of the three feedstuffs consumed.

Results and discussion. The results of the assays for the four vitamins studied are pre-

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