plexity on passing from Compound II to III, one observes the beginning of kinetic selectivity by the enzyme.

It is noteworthy that if one sets $k_2 = k_{OH}[OH^-]$ for ACh, to achieve the enzymatic acylation rate one would have to use a hydroxide-ion concentration of 6×10^4 *M*.

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Carotid Sinus Pressure Reflex Bioassay for *Veratrum viride*

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Abstract □ From a total of 238 anesthetized dogs, a series of regression analyses was performed examining the relationship between the variables (sex of the animal, date on which the experiment was performed, effect of the three veratrum alkaloidal drug preparations studied, and the number of days the dogs were stored in the animal house) on the arterial pressure pressor amplitudes of the predrug and postdrug carotid occlusion response and on the blood pressure-lowering effects of the drug treatments. The seasonal date of the experiment and the drug treatments significantly influenced the arterial pressure responses, while sex of the animal or days stored prior to use did not consistently alter the slope constants or arterial pressure responses for the drug subgroups.

Keyphrases □ *Veratrum viride* alkaloids—bioassay □ Carotid sinus pressure reflex—*V. viride* bioassay □ Sex, time of year effects—blood pressure lowering, drugs □ Regression analysis—factors affecting blood pressure lowering

The method presently used for the quantitative assessment of extracts of *Veratrum viride* is based on the progressive loss of the pressor response induced by bilateral occlusion of the common carotid arteries in the anesthetized dogs as reported by O'Dell (1). The potency of the test material is expressed in terms of carotid sinus reflex (CSR) units defined as follows: "One CSR unit represents the amount of intravenously administered hypotensive agent per kilogram of body weight which just abolishes the pressor response to the carotid sinus reflex in dogs" (1).

In a study on anesthetized dogs, Prochnik *et al.* (2) concluded that minimal pressor response to bilateral carotid occlusion is to be expected when the mean arterial pressure is below 88 mm. Hg. Since the regression line approaches zero response at the basal mean arterial pressure level of 60 mm. Hg, it was suggested that minimum variability would be obtained if the carotid occlusion pressor responses were expressed as: (mm. Hg rise due to occlusion × 100)/(mean arterial pressure [mm. Hg] − 60).

Rubin and Burke (3) reported that since the changes in carotid pressor reflex response and in the existent mean arterial pressure are highly correlated, the steeper dose–response curve exhibited by the carotid pressor reflex response would be a more sensitive measure. They also concluded that the *V. viride* hypotensive principles examined did not act *via* adrenergic, sympathetic, or ganglionic blocking actions. However, more recently, Jandhyala and Buckley (4) reported that the effects of cryptenamine, an alkaloidal preparation from *V. viride*, were inhibited by reserpine, α-methyldopa, and adrenalectomy and therefore did act through a mechanism of action involving catecholamines and the adrenal medulla.

The authors have used the method as described by O'Dell (1) for the bioassay of several alkaloidal preparations from *V. viride*. The studies referred to previously alluded to the high correlation between the basal arterial pressure and the pressor response amplitude to bilateral carotid occlusion. The authors have also made unpublished observations on other possible influencing variables, such as time of the year the study was performed and length of time the dog was maintained in the animal quarter. To understand more fully the relationships between these variables, a series of regression analyses was performed on the data obtained over the last few years. This report is concerned with the findings obtained in this study.

METHODS

A total of 238 male and female mongrel dogs, weighing between 7.0 and 14.0 kg. each and anesthetized with pentobarbital sodium, 35 mg./kg. i.v., was used throughout.

Following the induction of anesthesia, both common carotid arteries were exposed and isolated by conventional surgical procedures. End arterial pressure was measured from either common carotid artery through a polyethylene tube inserted and fixed in the artery and connected to an E&M Physiograph pressure transducer which was coupled to an E&M Physiograph recorder. The cannula and pressure transducer were filled with a physiological saline solution containing 0.05% heparin sodium. A femoral vein was surgically exposed and used for the administration of all test drug solutions.

The experimental procedure was as follows: after a 5-min. equilibration period, a control pressor response was obtained by the unilateral occlusion of the remaining (either left or right) unused common carotid artery for a period of 45 sec. The test solution was then administered i.v.; 4 min. later, carotid occlusion again was performed. The test drug solution administration and challenging carotid occlusions were made at the time intervals mentioned previously until there was completely diminished carotid occlusion pressor response or until subsequent injections failed to alter the carotid occlusion induced pressor response.

The relationship between the variables, expressed by the regression equation $Y = a + bX$ (5) where the slope constant $b = xy/x^2$ and the intercept a is calculated from this formula by using the mean values of $x(X)$ and $y(Y)$, was examined with the use of a oneway analysis of variance. Using an IBM 360/50 computer, the following regression relationships were made:

A. Influence of sex (male or female) on the relationship between the basal arterial pressure and:

1. The amplitude of the predrug carotid occlusion response.
2. The amplitude of the postdrug carotid occlusion response.
3. The blood pressure-lowering effects by total drug treatment.

B. Influence of date (months October–March—Group 1, and April–September—Group 2) of experiment on the relationships between basal arterial pressure and 1, 2, and 3 of A.

C. Influence of drug: (*a*) cryptenamine alkaloids, (*b*) cryptenamine tannate, and (*c*) crude alkaloids on the relationship between basal arterial pressure and 1, 2, and 3 of A.

D. Influence of date of experiment on the relationship between basal arterial pressure and amplitude of predrug and postdrug carotid occlusion response clustered by drug treatments.

E. Relationship between days stored of dogs in animal house on basal arterial pressure and on amplitude of predrug carotid occlusion response clustered by drugs (*a*), (*b*), and (*c*).

RESULTS

The regression analysis of the relationships between the variables are summarized and presented in Tables I–V.

A. Influence of sex (male or female) on the relationship between the basal arterial pressure and predrug and postdrug carotid occlusion pressor response and on the blood pressure-lowering effects by total drug treatment (Table I).

It can be seen from Table I that the male dog population before drug treatment had a mean basal arterial pressure (BAP), Column Y, of 130.3 mm. Hg; a mean peaked blood pressure response to bilateral carotid artery occlusion (BCO), Column X, of 190.09 mm. Hg; with a regression slope constant, Column b, of 0.7319. The female dogs before drugs did not significantly differ from the male group and had calculated mean values of 132.55 and 195.05 mm. Hg for BAP and BCO response, respectively, and a regression slope of 0.6560.

Table I—Influence of Sex (Male or Female) on the Relationship between Basal Arterial Pressure and Response

Factors[a]	b	Y	X	a
BAP:BCO (B) M	0.7319	130.3	190.09	−8.83
BAP:BCO (B) F	0.6560	132.55	195.05	4.60
BAP:BCO (A) M	0.7424	75.07	101.12	0
BAP:BCO (A) F	0.6961	75.60	104.81	2.64
DD:BP M	−0.0531	10.339	55.091	13.264
DD:BP F	0.0014	9.949	56.780	9.149

[a] BAP = mean basal arterial pressure in mm. Hg; BCO = peak blood pressure (mm. Hg) during carotid occlusion episode; (B) = before drug; (A) = after drug; M = male; F = female; DD = drug dose in mcg./kg.; and BP = blood pressure-lowering response.

After treatment with total drugs, the male and female populations were not significantly different and demonstrated, in the case of the males, BAP, BCO, and slope values of 75.07 mm. Hg, 101.12 mm. Hg, and 0.7424, respectively, while the female population had values of 75.60 mm. Hg, 104.81 mm. Hg, and 0.6961, respectively.

Total drug treatment (DD) in the two sexes was not associated with a difference in: mean blood pressure-lowering activity (BP), Column X; mean dose in mcg./kg., i.v., Column Y; or slope constant, Column b.

B. Influence of time of the year experiment performed on the relationship between the basal arterial pressure and response.

As shown in Table II, the date when the experiment was performed significantly influenced the mean basal arterial pressure levels (Column Y) before as well as after total drug treatment. The experiments performed in the fall–winter (Group 1) showed a lower mean basal arterial pressure value of 129.97 mm. Hg compared to the mean value of 131.90 mm. Hg obtained from the experiments performed in the spring–summer (Group 2). Similarly, after total drugs the mean basal arterial pressure was significantly lower in Group 1 (73.49 mm. Hg) than in Group 2 (75.51 mm. Hg).

Table II—Influence of Date (Fall, Winter—Spring, Summer) of Experiment on the Relationship between Basal Arterial Pressure and Response

Factors[a]	b	Y	X	a
BAP:BCO (B) 1	0.6447	129.97[b]	185.54[c]	10.35
BAP:BCO (B) 2	0.7313	131.90[b]	195.11[c]	−10.78
BAP:BCO (A) 1	0.7437	73.49[d]	95.15[e]	2.73
BAP:BCO (A) 2	0.7162	75.51[d]	105.22[e]	0.15
DD:BP 1	0.158	10.91	56.11	11.80
DD:BP 2	0.0379	9.87	56.32	12.0

[a] BAP = mean basal arterial pressure in mm. Hg; BCO = peak blood pressure (mm. Hg) during carotid occlusion episode; (B) = before drug; (A) = after drug; 1 = experiments performed October through March; 2 = experiments performed April through September; DD = drug dose in mcg./kg.; and BP = blood pressure-lowering response. [b] $p < 0.036$. [c] $p < 0.024$. [d] $p < 0.048$. [e] $p < 0.016$.

The mean peaked blood pressure responses (Column X) also demonstrated significantly different amplitude of response when comparison was made between Group 1 (185.54 mm. Hg) and Group 2 (195.11 mm. Hg) before drug treatment, and Group 1 (95.15 mm. Hg) and Group 2 (105.22 mm. Hg) after total drug treatment.

The date of the experiment did not influence the mean blood pressure-lowering response of total drugs nor the mean total drug dose.

C. Influence of date of experiment performed on the relationship between dose of drug and amplitude of blood pressure-lowering response.

Summarized in Table III are the regression analyses of the relationship between the dose of the drug treatment and the blood pressure-lowering response. The drugs used were: (*a*) cryptenamine alkaloids, (*b*) cryptenamine tannate, and (*c*) an alkaloidal fraction of *V. viride*. It can be seen that the slope constants (Column b) of the relationship between the drug-dose treatment and the blood pressure-lowering response were significantly different, exhibiting negative slopes of −0.0035 and −0.109 for cryptenamine alkaloids and alkaloidal fraction, respectively, while a positive slope constant of 0.214 was obtained for the cryptenamine tannate. In the total population there was no difference in the three drug groups in their mean drug dose or in the mean blood pressure-lowering response.

When the data were analyzed according to the date when the experiment was performed, a similar finding of significantly different slope constants was observed in the October–March (Group 1) experiments.

Here again, cryptenamine alkaloids and alkaloidal fraction demonstrated negative slopes of −0.0404 and −0.622, respectively, and cryptenamine tannate showed a positive slope constant of 0.1638.

Table III—Influence of Date on the Relationship between Blood Pressure-Lowering Response and Dose of Drug Clustered by Drug Groups

Factors[a]	b	Y	X	a
DD:BP *a*	−0.0035[b]	9.27	51.71	9.29
DD:BP *b*	0.214[b]	11.06	52.73	0.22
DD:BP *c*	−0.109[b]	10.40	56.67	16.58
DD:BP *a* 1	−0.0464[c]	9.63[d]	53.25[e]	2.48
DD:BP *b* 1	0.1638[c]	14.0[d,f]	48.95[e,g]	−8.00
DD:BP *c* 1	−0.0622[c]	9.1[d]	63.05[e,g]	3.93
DD:BP *a* 2	0.0091	9.14	59.24	−0.53
DD:BP *b* 2	0.0246	9.80[f]	54.35[g]	1.35
DD:BP *c* 2	−0.1183	10.90	54.35[g]	6.44

[a] DD = drug dose in mcg./kg.; BP = blood pressure-lowering response; *a* = cryptenamine alkaloids; *b* = cryptenamine tannate; *c* = alkaloidal; 1 = experiments performed October through March; and 2 = experiments performed April through September. [b] $p < 0.0567$. [c] $p < 0.06$. [d] $p < 0.03$. [e] $p < 0.045$. [f] $p < 0.031$. [g] $p < 0.024$.

In these experiments there were also significant differences in the mean drug dose employed (Column Y) of 9.63, 14.0, and 9.1 mcg./kg. and between the amplitude of the mean blood pressure-lowering response (Column X) of 53.25, 48.95, and 63.05 mm. Hg for cryptenamine alkaloids, cryptenamine tannate, and alkaloidal fraction, respectively.

When the experiments were performed during April–September, Group 2, there were no significant differences between the slope constants obtained for the three experimental drugs. Also, the mean drug dose as well as the mean arterial pressure-lowering response did not significantly differ between the three subgroups.

When comparison was made of the Date 1 to Date 2 results, it was observed that a cryptenamine tannate dose of 14.0 mcg./kg. (Date 1) was significantly greater than the mean dose of 9.8 mcg./kg. used in the Date 2 experiments. Also, the mean blood pressure-lowering responses of cryptenamine and of alkaloidal fraction of 48.95 and 63.05 mm. Hg, respectively, for Date 1 were significantly different from the Date 2 values of 54.35 mm. Hg for these drug groups.

D. Influence of date experiment performed on the relationship between arterial pressure, drug doses, and the amplitude of the arterial pressure response to bilateral carotid occlusion.

As shown in Table IV, within the Date 1 group there was no significant difference between the three drug subgroups before treatment (B), in slope constant (b), basal mean arterial pressure (Y), or in peaked mean arterial pressure response (X) to carotid occlusion. Similarly, within the Date 2 group there were insignificant differences between the three drug subgroups before treatment (B), as far as slope constant (b) and peaked mean pressor response (X) to carotid occlusion are concerned, although the initial mean arterial pressure (Y) levels between the three subgroups were statistically different. When comparison was made of the regression analysis of each drug subgroup [(*a*), (*b*), or (*c*)] between Dates 1 and 2, before treatment (B), it is observed that there was a statistically significant difference in slope constants of 0.6244 to 0.7256 for cryptenamine alkaloids, 0.6475 to 0.7222 for cryptenamine tannate, and 0.6423 to 0.7182 for alkaloidal fraction. No significant difference in the drug subgroup values was observed between Dates 1 and 2 for the mean basal arterial pressure (Y). There was significant difference in pressor response to bilateral carotid occlusion between Dates 1 and 2 for cryptenamine alkaloids, 185.21 to 193.57 mm. Hg; and for cryptenamine tannate, 180.81 to 199.35 mm. Hg; whereas the difference on the mean response of the alkaloidal fraction was not significantly influenced by the date the experiment was performed.

Table IV—Influence of Date on the Relationship between Basal Arterial Pressure, Drug Treatment, and BCO Response Clustered by Drug Groups

Factors[a]	b	Y	X	a
BAP:BCO (B) *a* 1	0.6244[b]	128.63	185.21[c]	12.98
BAP:BCO (B) *b* 1	0.6475[b]	124.71	180.81[c]	7.64
BAP:BCO (B) *c* 1	0.6423[b]	137.5	191.3	14.6
BAP:BCO (B) *a* 2	0.7256[b]	126.53[d]	193.57[c]	−13.92
BAP:BCO (B) *b* 2	0.7222[b]	139.88[d]	199.35[c]	−4.09
BAP:BCO (B) *c* 2	0.7182[b]	131.62[d]	193.29	−7.20
DD:BCO (A) *a* 1	0.187[e,f]	9.6[g]	17.125[h]	3.193
DD:BCO (A) *b* 1	0.1598[e,f]	14.0[g,i]	23.667[h]	−3.781
DD:BCO (A) *c* 1	0.0361[e,f]	9.368[g]	26.316	−0.941
DD:BCO (A) *a* 2	0.0176[j,f]	9.143	29.6[h]	−0.512
DD:BCO (A) *b* 2	0.0303[j,f]	9.796[i]	31.796[h]	−0.954
DD:BCO (A) *c* 2	0.4620[j,f]	10.873	27.945	−12.90

[a] BAP = mean basal arterial pressure in mm. Hg; BCO = peak blood pressure (mm. Hg) during carotid occlusion episode; (A) = after drug; (B) = before drug; *a* = cryptenamine alkaloids; *b* = cryptenamine tannate; *c* = alkaloidal; 1 = experiments performed October through March; 2 = experiments performed April through September; and DD = drug dose in mcg./kg. [b] $p < 0.03$. [c] $p < 0.05$. [d] $p < 0.005$. [e] $p < 0.05$. [f] $p < 0.01$. [g] $p < 0.03$. [h] $p < 0.03$. [i] $p < 0.05$. [j] $p < 0.001$.

During Date 2, only the slope constants for drug (*a*), 0.0176; drug (*b*), 0.0303; and drug (*c*), 0.4620 were significantly different for the three drug subgroups. The mean drug doses (Y) and the peak mean pressor response to the bilateral carotid occlusion (X) did not vary significantly between the three drug subgroups.

The slope constants for the Date 1 cryptenamine alkaloids, 0.187; cryptenamine tannate, 0.1598; and alkaloidal fraction, 0.0361; significantly differed from their Date 2 values of 0.0176, 0.0303, and 0.4020, respectively. The mean drug dose of cryptenamine tannate of 14.0 mcg./kg. for Date 1 and 9.796 mcg./kg. for the Date 2 experiments were significantly different. The BCO responses (X) also differed significantly for cryptenamine alkaloids and cryptenamine tannate between Date 1 (17.125 and 23.667 mm. Hg) and Date 2 (29.6 and 31.796 mm. Hg), respectively.

E. Relationship of days stored in the animal house of the dogs on the mean basal arterial pressure and on the pressor response to BCO, before drug treatment, separated into drug subgroups.

The data, summarized in Table V, reveal that the duration of days the dogs were stored (dH) in the animal house was not significantly different for the three drug subgroups; however, there was a significant difference for the BAP (Column X) and for the slope constants for the three drug subgroups. The animals which subsequently received cryptenamine alkaloids had a mean BAP of 127.07 mm. Hg and slope constant of 0.0032; for the cryptenamine tannate group, a mean BAP of 135.33 mm. Hg and slope constant of −0.1669; and, in the case of the alkaloid fraction group, a mean BAP of 133.19 mm. Hg with a slope constant of 0.1176.

The influence of the duration of days held on the peaked blood pressure response to BCO did not significantly vary between the three drug subgroups.

DISCUSSION

Examination of the regression analysis presented in the *Results* section leads to the following conclusions:

The sex of the animal does not influence the mean BAP and the magnitude of the pressor response to BCO both before as well as after total drug treatment, nor influence the relationship between total drug-dose treatment and the intensity of the arterial pressure depression.

The date of the experiment, that is, whether the experiment was performed in October through March or in April through September, did have a profound influence on the mean BAP and on the magnitude of the pressor response to BCO both before and after total drug treatment, but did not significantly influence the relationship between total drug-dose treatment and blood pressure-lowering response.

The calculated mean doses of cryptenamine alkaloids, cryptenamine tannate, and the alkaloidal fraction were significantly different.

Table V—Influence of Days Stored in Animal House in the Basal Arterial Pressure and BCO Responses Clustered by Drug Groups

Factors[a]	b	Y	X	a
dH:BAP *a*	0.0032[b]	13.61	127.07[c]	13.20
dH:BAP *b*	−0.1669[b]	14.59	135.33[c]	37.18
dH:BAP *c*	0.1176[b]	13.73	133.19[c]	−1.93
dH:BCO (B) *a*	−0.0569	13.61	191.44	14.706
dH:BCO (B) *b*	−0.1023	14.59	193.79	34.41
dH:BCO (B) *c*	0.0271	13.73	192.76	8.16

[a] dH = days held in storage; BAP = mean basal arterial pressure in mm. Hg; BCO = peak blood pressure (mm. Hg) during carotid occlusion episode; (B) = before drug; *a* = cryptenamine alkaloids, *b* = cryptenamine tannate; and *c* = alkaloidal. [b] $p < 0.0148$. [c] $p < 0.03$.

There was a statistical difference in slope constant, mean drug dose, and in the amplitude of the blood pressure-lowering response for the experiments performed in October through March. When examination was made of the data from experiments performed April through September, there were no calculated significant differences in these relationships. There were no significant differences between the slope constants, mean drug dose, and blood pressure-lowering responses for the three drug subgroups when the data collected from October through March were compared to those obtained in April through September.

There were no differences in the slope constants, mean BAP, and in the amplitude of the pressor response to BCO before drug treatment between the three drug groups for the experiments performed during the October through March period. There was only a significant difference in mean BAP between the three drug groups when the experiments were performed during April through September. However, there were significant differences for the three drug subgroups in slope constant and amplitude of the pressor response to BCO when the two 6-month experimental periods were compared.

After drug treatment, there were significant differences between the three drug groups in slope constant and in mean drug dose for the experiments performed during October–March. Only the slope constants between the three drugs differed significantly when the experiments were performed during April–September. When comparison was made between the two 6-month periods, there was significant difference in slope constants, in mean drug dose (cryptenamine tannate), and in mean peak arterial pressure response to BCO for the cryptenamine alkaloids and cryptenamine groups.

It was observed that although there was no difference between the three drug subgroups in the mean number of days the dogs were stored in the animal house prior to use, there was a significant difference in slope constants and in mean BAP for the three drug subgroups.

Examination of the results reveals that although the mean BAP is markedly lowered by total drug treatment, the slope constants for the relationships between the mean BAP and the pressor response to BCO did not significantly differ. These findings thus support the conclusions of Prochnik *et al.* (2) who suggested that when employing the bilateral carotid occlusion pressor bioassay, correction be made for drug-induced changes in mean BAP and also for the findings that the pressor response was physiologically absent when the arterial pressure reached 60 mm. Hg.

Although there was no difference in mean total drug dose and blood pressure-lowering response, when these factors were separated by drug groups there was significant difference in slope constants. Here, cryptenamine tannate produced the most desirable slope constant indicative of an increase in blood pressure-lowering response with increasing dose. Cryptenamine alkaloids and the alkaloid fraction exhibited negative slopes, thus indicating that increasing drug dose decreases the degree the blood pressure falls. Further separation of these results into time of the year the experiment was performed showed that during October through March, cryptenamine tannate, although administered at a significantly higher dose, again demonstrated a positive slope while the other drugs exhibited negative slope constants in the relationship between drug dose and blood pressure-lowering effects. When the experiments were performed during April through September, cryptenamine alkaloids and cryptenamine tannate showed very low but positive slope constants, while the alkaloid fraction still demonstrated a negative slope constant for the relationship between the drug dose and the intensity of the blood pressure depression. It was of interest to note that for all three drug subgroups there was a significant increase in slope constants, while the peak BCO response (before drugs) only significantly increased for cryptenamine alkaloids and cryptenamine tannate, and no differences were observed in the mean BAP when comparison was made between the October–March and April–September groups. Similar findings were observed when examination of the relationship between drug dose and BCO (after drug) was made.

The days the animals were stored in the animal house prior to use did not consistently affect the slope constants nor the BAP on the BCO response amplitude for the three drug subgroups.

Examination of these results thus indicates that the date at which the experiment was performed did significantly influence most of the examined relationships and if this bioassay procedure continued to be employed, necessary corrections must be applied to adjust for these influences.

These seasonal influences are possibly the result of rhythmic changes in disposition and deposition of adipose tissue and other changes during different seasons as reported by Fisher *et al.* (6) and Hilditch (7). Since in all these experiments pentobarbital was used as the anesthetic, it is quite conceivable that the observed seasonal difference in mean BAP, in pressor response amplitude to BCO, and the blood pressure-lowering effects of the three drugs were the result of either a different level of anesthesia occurring during the different seasons or, more generally, a seasonally induced changed physiological state resulting in differential responding.

It is well known that the physiological state of the preparation can profoundly influence both the nature as well as the intensity of a fixed treatment response. This is particularly in evidence with the alkaloids ibogaine and yohimbine. It was reported (8) that in the anesthetized dog, ibogaine produced a marked lowering of arterial pressure while in the conscious preparation an equal dose of ibogaine appeared to be devoid of hypotensive activity. Likewise, yohimbine is reported (9) to produce a hypertensive response in the conscious animal while evoking an arterial pressure depressor response when the animal is anesthetized with pentobarbital sodium.

In order to examine the possibility that use of the anesthetic is a factor in influencing the responses as described, a series of experiments will be performed in chronically prepared dogs while conscious and in the anesthetized state. With each dog serving as its own control, it will be possible to assess the influence of the anesthetic on many of the relationships examined in this study.

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