Influence of Pentobarbital on Some Cardiovascular Effects of Cryptenamine in Dogs

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Abstract □ In a series of 32 conscious and anesthetized paired dogs, pentobarbital anesthesia significantly lowered basal systolic/diastolic arterial pressures, raised basal heart rate, and altered their responses to selected autonomimetic treatments. Pentobarbital anesthesia also significantly increased the effective hypotensive and/or bilateral carotid occlusion pressor response inhibiting dose of cryptenamine. Further examination of the data revealed that the conscious dog systolic depressor response to cryptenamine is the most sensitive and accurate bioassay criterion for the evaluation of cryptenamine potency.

Keyphrases □ Pentobarbital–cryptenamine—cardiovascular effects, dogs □ Cryptenamine–pentobarbital—cardiovascular effects, dogs □ Anesthesia, pentobarbital—influence on cryptenamine cardiovascular effects, dogs

In an earlier study (1) the carotid sinus pressor reflex bioassay procedure for the hypotensive principles of *Veratrum viride* was examined. The results obtained indicated that the date on which the experiment was performed significantly influenced the mean basal arterial pressure level and the magnitude of the pressor response to bilateral carotid occlusion, before as well as after drug treatment, but did not significantly alter the relationship between the drug dose and its blood pressure lowering response. It was speculated that seasonal influences are possibly the results of rhythmic changes in disposition and deposition of adipose tissue and other physiological changes, as reported by Fisher *et al.* (2) and Hilditch (3).

The assays presently employed (4, 5) to determine the ratio of emetic to effective hypotensive potency of *V. viride* samples obtains these measures separately in conscious (emetic assay) and anesthetized (hypotensive assay) dogs. Conceivably the reported (4) superior ratio of emetic to hypotensive dose effects of cryptenamine is due to the physiological state of the animal as used in each assay procedure. Ideally, both assays should be performed simultaneously in the same preparation so that a more meaningful ratio can be obtained.

It is well known that the physiological state of the experimental preparation can profoundly influence both the quality as well as the amplitude of a fixed treatment response. Schneider and Rinehart (6) reported that, in the anesthetized dog, the alkaloid ibogaine produced a marked hypotension, while the conscious dog was devoid of this effect. The alkaloid yohimbine was reported by Gershon and Lang (7) to produce a hypertensive response in the conscious dog while evoking a depressor response when the dog was anesthetized with pentobarbital.

A series of experiments was performed in chronically prepared dogs in the conscious and anesthetized states in an attempt to elucidate the influence of anesthesia on the nature and intensity of some cardiovascular effects of cryptenamine. This report is concerned with the findings obtained in this study.

Table I—Effects of Pentobarbital Anesthesia on the Systolic/Diastolic Arterial Pressures and Heart Rates and Its Influence on the Responses by Cryptenamine, Bilateral Carotid Occlusion (BCO), Tyramine (Tyr), Noradrenaline (Nad), and Isoproterenol (Isup) in Dogs

	Conscious			Anesthetized		
	Systolic, mm. Hg	Diastolic, mm. Hg	Heart Rate, beats/min.	Systolic, mm. Hg	Diastolic, mm. Hg	Heart Rate, beats/min.
Control:						
Basal	132.6[a]	60.7[a]	112.1[a]	110.6[a]	73.1[a]	164.8[a]
BCO	+32.5[a]	+30.6[a]	+3.8[a]	+25.9[a]	+25.6[a]	+16.8[a]
Tyr	+27.9	+18.5	−35.0	+36.5	+20.6	−40.8
Nad	+107.1[a]	+62.7[a]	−64.5	+155.0[a]	+92.9[a]	−56.2
Isup	−39.0	−39.0[a]	+136.7[a]	−41.6	−48.4[a]	+105.8[a]
After 1 mcg./kg. i.v. cryptenamine (total dose):						
Basal	−5.0[a, b]	−2.9	−6.9	−0.8[b]	−0.3	+0.3
BCO	+27.1	+27.4	+1.61[b]	+23.6	+24.4	+15.3[b]
After 3 mcg./kg. i.v. cryptenamine (total dose: 4 mcg./kg.):						
Basal	−22.1[a, b]	−18.4[a, b]	−20.0[a]	−6.3[b]	−7.3[b]	−16.0[a]
BCO	+17.7[a]	+20.2[a]	+0.3[b]	+16.2[a]	+16.7[a]	+11.8[b]
Tyr	+30.0	+23.0	−14.0[a]	+36.4	+24.1	−23.6[a]
Nad	+91.0[b]	+61.0	−19.9[a, b]	+147.7[b]	+98.6[b]	−47.7[b]
Isup	—	—	—	−31.4	−51.4	+115.0
After 10 mcg./kg. i.v. cryptenamine (total dose: 14 mcg./kg.):						
Basal	−48.8[a, b]	−44.8[a, b]	−49.8[a]	−23.7[a],	−27.4[a, b]	−42.4[a]
BCO	+4.8[a]	+5.0[a]	+2.5	+3.6[a]	+3.1[a]	0[a]
Tyr	+28.8[b]	+20[b]	−29.3[b]	+46.7[b]	+34.7[a, b]	−17.0[a, b]
Nad	+95.7[b]	+72.2[b]	−37.6[a]	+165.9[b]	+105.7[b]	−29.4[a]
Isup	−26.2[a]	−30.4[a, b]	+96.4[a]	−33.1[a]	−45.3[b]	+107.8

[a] $p = 0.05$ for differences between groups, conscious *versus* anesthetized. [b] $p = 0.05$ for differences between groups, control and after cryptenamine.

Table II—Variance, Difference in Variance, and Power of the Test Analysis on the Influence of Pentobarbital Anesthesia on Basal Systolic Level and the Systolic Depressor Response to Doses of 1, 3, and 10 mcg./kg. Cryptenamine

Treatment	Conscious		Anesthetized		F	p
	Variance	N	Variance	N		
Basal	156.6	8.09(80) 15.88(95)	479.52	8.09(80) 15.99(95)	14.79	$\ll 0.005$
1 mcg./kg.	82.5	21.1(80) 41.3(95)	22.5	11,000(80) 22,000(95)	6.66	<0.005
3 mcg./kg.	898.9	5.03(80)	158.3	187.6(80) 367.6(95)	15.38	$\ll 0.005$
10 mcg./kg.	2700	1.03(80) 2.02(95)	733.3	13.3(80) 26.2(95)	32.32	$\ll 0.005$

MATERIALS AND METHODS

Eight male and female beagle dogs were surgically prepared with bilaterally externalized carotid artery loops by a modification (8) of the Van Leersum method (9). From this preparation, it is quite convenient to measure systolic and diastolic arterial pressure directly, to induce a reflex pressor response to bilateral carotid occlusion, and to measure concomitantly other autonomic functions such as electrocardiogram (EKG), heart rate, and respiration while the animal is fully conscious and only slightly restrained.

Direct systolic and diastolic arterial pressure (lateral) was measured from a Medicon cannula (model AR-3218) inserted and fixed into an externalized carotid artery. The cannula was connected to a Statham pressure transducer (model P23AA), which supplied input into a Beckman strain gauge coupler (model 9872) of the recorder. The cannula was maintained patent by the slow retrograde infusion of saline through the pressure transducer, the rate of infusion not altering the arterial pressure measurements.

Heart rate was measured by the conversion of the R-R wave interval by a Beckman cardiotachometer coupler (model 9857) from a bipolar electrocardiograph input obtained through Beckman biopotential electrodes positioned and affixed to the skin over the area of the sternum. A homemade electrode paste composed of NaCl (0.07 mole/454 g.) was used with these electrodes to facilitate conduction.

The indirect rate and amplitude of respiration were measured through a pneumograph belt positioned around the thoracico-abdominal junction and connected to the pressure transducer connected, in turn, to the strain gauge coupler.

All recording was made on a Beckman Type R dynograph at a paper speed of 5 mm./sec.

To control for order effect, the eight animals were treated in a random order as follows:

Dog No.	Order Code
43	1, 2, 1, 2
44	2, 1, 2, 1
50	1, 2, 2, 1
51	2, 1, 1, 2
52	1, 1, 2, 2
53	2, 2, 1, 1
19	1, 2, 1, 2
1110	2, 1, 2, 1

with 1 or 2 indicating that the dog was to be used in the conscious or anesthetized state, respectively. Each dog was used in accordance with the schedule shown on a frequency of twice per week. After this schedule was completed ($N = 32$), a 1-week recovery period was allowed; then the procedure was repeated, but each dog was moved down one step in this schedule. In this manner, a total of 64 tests was performed, 32 each in conscious and anesthetized pairs. Thus, each dog served as its own control and received a series of eight tests, four in the conscious state and four while anesthetized.

All dogs received an indwelling venocatheter (PE-50) in the brachialcephalic or saphenous vein for all drug solution administration. When the dogs were to be anesthetized, sodium pentobarbital, 30–35 mg./kg., was administered through the indwelling venous catheter. Care was taken to position the pressure transducer at the appropriate position (heart-horizontal) for each dog, particularly when anesthesia was employed.

A 10–15-min. equilibration period was allowed following the preparation of the animal into its experimental condition. After the equilibration period, the animal received a 30-sec. (A) bilateral carotid occlusion (BCO); noradrenaline bitartrate (NAd), 2 mcg./kg.; tyramine hydrochloride (Tyr), 200 mcg./kg.; isoproterenol hydrochloride (Isup), 2 mcg./kg.; and a second 30-sec. BCO episode (B). Intravenous doses of 1, 3, 10, and 30 mcg./kg. of cryptenamine[1] were administered, with a 30-sec. BCO episode after each dose. The maximum dose of cryptenamine administered was determined by the degree of inhibition of the BCO-induced pressor response; *i.e.*, the dose was not increased when the BCO pressor response was maximally inhibited. At this time, the fixed-dose treatments with noradrenaline, tyramine, and isoproterenol were repeated.

The data were analyzed for statistical significance by one of two standard methods. When applicable, the matched-pair t test (10) was used. Otherwise, Student's t test was used (11).

RESULTS AND DISCUSSION

The data from this study on the influence of pentobarbital anesthesia in dogs on basal systolic/diastolic arterial pressures and heart rate and on their response to selected autonomimetic treatments, before and after graded doses on cryptenamine, are summarized in Table I.

It was reported (12) that the intravenous administration of barbiturate (thiopental) in man resulted only in a transient lowering of arterial pressure shortly after drug treatment. In the study reported here, the intravenous administration of sodium pentobarbital, 30–35 mg./kg., produced a statistically significant change of basal systolic/diastolic arterial pressure and an associated increase in heart rate. Moreover, pentobarbital anesthesia resulted in a significant lowering of the arterial pressure pressor response to a 30-sec. BCO episode while increasing the tachycardia produced by this treatment. The noradrenaline-induced pressor response, on the other hand, was significantly enhanced by the induced anesthesia without apparent change in heart rate. The tyramine pressor response was also greater during anesthesia, but the difference did not achieve statistical significance. The isoproterenol-induced diastolic depressor response was also significantly enhanced by the anesthetic, although the positive chronotropic response was considerably less after anesthesia.

These divergent results can be explained on the basis of pentobarbital-evoked partial inhibition of buffer mechanisms. Since the carotid occlusion pressor response is largely dependent upon a functional buffer mechanism, inhibition of this buffer mechanism would result in a diminished pressor response to carotid occlusion. Likewise, removal of this homeostatic control would also result in an increased pressor response to noradrenaline, which acts primarily at the effector site rather than through an established "pressor reflex."

It is also possible to explain the apparent influence of pentobarbital anesthesia on these responses by applying the Law of Initial Values (13). Prochnik *et al.* (14) reported that the carotid occlusion response is basal level dependent, with increasing response amplitude associated with increased basal level. The authors previously reported (15) that the amplitude of the pressor response to tyramine is inversely related to basal level, *i.e.*, increasing response amplitude with diminished basal level, while the pressor response to noradrenaline is independent of basal level. Therefore, it is

[1] Active component of Unitensen Aquenous, supplied by Dr. Arvid Zuber, Neisler Laboratories, Decatur, Ill.

conceivable that the observed difference in response amplitude, at least to tyramine, appearing in the conscious and anesthetized physiological states may have been reflections of the differences in basal level between the two preparations.

The intravenous dose of 1 mcg./kg. cryptenamine produced a significant hypotensive response in the conscious dog; the response was also significantly different from the almost complete lack of arterial pressure response this dose of cryptenamine produced in the pentobarbital-anesthetized matched pairs. After the higher doses of 3 and 10 mcg./kg. i.v. cryptenamine, the systolic/diastolic hypotensive responses of the conscious dogs were significant when compared with their respective pretreat levels. Only the hypotensive response produced by the 10 mcg./kg. cryptenamine differed significantly from the control in the anesthetized preparation. Most interesting was the finding that the arterial pressure hypotensive response of the conscious dog continued to demonstrate greater sensitivity than that exhibited by the anesthetized dog. The systolic/diastolic hypotension was statistically significantly greater in the conscious dog than in the anesthetized pair after both these higher doses of cryptenamine.

The pressor response to BCO was inhibited to the same degree in the conscious and anesthetized dog pairs following high dose treatment with cryptenamine. Although the inhibition of the BCO response significantly differed from their respective controls, there was insignificant difference between the conscious and anesthetized pairs. However, the apparent inhibition of the BCO response could have been secondary to the hypotension, which would profoundly diminish the amplitude of the pressor response (14).

After treatment with the 3- and 10-mcg./kg. doses of cryptenamine, the tyramine, noradrenaline, and isoproterenol response relationships between the conscious and anesthetized pairs remained consistent with their respective differences obtained during the control evaluation. These findings are somewhat inconsistent with the findings of Jandhyala and Buckley (16) who concluded that, based on a variety of organ system functions, cryptenamine appeared to potentiate the response of isoproterenol on β-adrenergic receptors. Findings in the present study do not indicate any consistent effect of treatment with cryptenamine on the systolic/diastolic and heart rate responses to the β-adrenergic stimulant isoproterenol in either the conscious or anesthetized dog.

The data were subsequently analyzed for difference in variance of the basal systolic level and the systolic depressor response to cryptenamine between the conscious and anesthetized matched pair dogs by the analysis of variance *F*-test (17). Calculation was also made from the available data for the minimum number (N) of animal tests for basal level systolic and systolic depressor response at the 80 or 95% Power of the Test (18). These two sets of calculated data are presented in Table II.

The variance of the conscious dog basal systolic level was significantly lower ($p \ll 0.005$) than that calculated for the anesthetized population. However, after receiving treatment with cryptenamine (1, 3, and 10 mcg./kg.), the dose-dependent increasing variance was significantly greater ($p < 0.005$) in the conscious dogs compared to the matched paired anesthetized preparation. It should be emphasized here that the cryptenamine mean systolic depressor responses were significantly more pronounced in the conscious dog, which may account for some of the apparent increased variance.

It is speculated that cryptenamine alters homeostatic mechanisms operating in the conscious state more profoundly than those operating in the anesthetized state, thus resulting in the increased variance. The reciprocal variance relationship obtained for the basal systolic levels can be explained as due to a partial inhibition of homeostatic mechanisms by the anesthetic, while in the conscious dog these mechanisms are maximally homeostatic.

The 80 and 90% Power of the Test analysis revealed that the minimum number (N) of matched paired tests necessary to demonstrate significant difference between conscious and anesthetized dog basal systolic levels was 8.09 and 15.88, respectively. The minimum N necessary to exhibit a significant systolic depressor response to cryptenamine, at the three doses employed, was considerably larger in the anesthetized dog than in the conscious dog.

It is concluded, therefore, from the Power of the Test analysis that the conscious dog systolic depressor response is the most sensitive, accurate, and efficient bioassay criterion for the evaluation of cryptenamine potency.

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