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Keyphrases

Phenobarbital effects—motor activity, mice
Stimulant effects—phenobarbital
Motor activity—phenobarbital administration
Environment effect—phenobarbital activity
Fasting, satiation effects—phenobarbital activity

Mechanisms of Action of Cryptenamine

By MARIE L. JANDHYALA and JOSEPH P. BUCKLEY

The hypotensive effect of cryptenamine has been reported to be markedly attenuated by adrenalectomy or by treatment with *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286) in atropinized animals. Cryptenamine also appeared to potentiate the response of isoproterenol on β-adrenergic receptors. This present study was conducted to further investigate these two phenomena. Cryptenamine potentiated the response of epinephrine on the β-adrenergic receptors in the intact dog heart, partially blocked the effect of isoproterenol on the isolated guinea pig auricle, and had no effect on the isoproterenol-induced relaxation of the isolated guinea pig tracheal chain. Cryptenamine produced positive inotropic and chronotropic effects on the isolated guinea pig heart and potentiated the inotropic effect of epinephrine and inhibited its chronotropic effects in this preparation. These data suggest that the effects of cryptenamine on β-adrenergic receptors are variable and that either potentiation or inhibition may be observed depending on the effector organ and on the species of animal studied. Cryptenamine also significantly decreased the epinephrine content of the adrenal venous blood while increasing the norepinephrine content suggesting that the drug may inhibit the methylation of norepinephrine in the adrenal medulla.

CRYPTENAMINE, an alkaloidal mixture, prepared from *Veratrum viride* by a nonaqueous benzene triethylamine extraction procedure, has been reported to have a ratio of emetic to effective hypotensive dose superior to that of other veratrum preparations (1, 2). Finnerty (2) reported that in humans the divergence between the hypotensive and emetic doses of cryptenamine was apparent on intravenous administration. McCall and his colleagues (3) studied the effects of cryptenamine on cerebral circulation and cerebral oxygen consumption in patients with toxemia of pregnancy. They observed that on intravenous administration of cryptenamine cerebral blood flow and cerebral oxygen metabolic rate were increased significantly while the respiration quotient of the brain remained normal. In these experiments, comparison with other veratrum preparations indicated fewer side effects. Although a satisfactory ambulatory treatment of hypertension by the oral administration of cryptenamine has been reported (4), Abreu (5) failed to demonstrate any superiority of cryptenamine over protoveratrine *A* in dogs as to the ratio of emetic to hypotensive doses.

Recently, Jandhyala and Buckley (6) reported that cryptenamine sensitized β-adrenergic receptors and that it might possibly stimulate the release of epinephrine from the adrenal medulla and that these factors might contribute to the overall hypotension. They reported that the hypotensive effects of cryptenamine were inhibited or abolished by pretreatment with *N,N*-diisopropyl-*N'*-isoamyl-*N'*-diethylaminoethylurea (P-286), bretylium, pronethalol, reserpine, α-methyl-dopa, and by adrenalectomy in atropinized animals. Cryptenamine also potentiated the isoproterenol-induced relaxation of the vasculature in the denervated perfused hind limb of the dog. Cryptenamine potentiated the

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depressor response induced by minute doses of epinephrine, suggesting a sensitization of β-adrenergic receptors. In addition, these investigators reported that cryptenamine acted on the baro- and chemoreceptors in the carotid sinus complex inducing a reflexogenic hypotensive response. Most of the evidence for the increased release of epinephrine from the adrenal medulla, as reported by Jandhyala and Buckley (6) was indirect.

The purpose of this investigation was to further study the mechanism of action of cryptenamine, especially as related to the release of catecholamines from the adrenal medulla and to the effects of cryptenamine on β-adrenergic receptors in selected tissues in different species.

METHODS

Effects of Cryptenamine and Protoveratrine A on the Epinephrine Content of Adrenal Venous Blood—Mongrel dogs of either sex were anesthetized with sodium pentobarbital, 35 mg./kg., i.v. The left adrenal vein was catheterized with PE 205 tubing, and 500 units/kg. of heparin administered i.v. Blood was permitted to flow freely from the catheter and collected continuously. Blood flow was measured by collection of 1-min. samples into a 10-ml. graduated cylinder from which an 8-ml. sample was withdrawn for catecholamine analysis prior to, and 5, 15, 30, 60, and 120 min. after the administration of cryptenamine, 5 mcg./kg., i.v. or protoveratrine-*A*, 4 mcg./kg., i.v. Samples to be analyzed were collected under ice, centrifuged at 2000×*g* for 10 min., and the plasma frozen and maintained at −10° until analyzed. Blood not used for catecholamine analysis was injected *via* a femoral vein in 20–30 ml. aliquots. Blood volume was maintained by addition of saline containing red blood cells from the centrifuged blood in amounts equal to plasma removed.

After allowing the frozen plasma samples to thaw at room temperature, an equal volume of 1.6 *N* perchloric acid was added and the samples agitated on a Vortex mixer to precipitate the proteins. Assays of catecholamines were performed according to the method of Brodie *et al.* (7), as modified by Bennett *et al.* (8).

Effects of Cryptenamine, Protoveratrine A and Protoveratrine B on Isoproterenol-Induced Relaxation of Vasculature in the Denervated Perfused Hind Limb of the Dog—Mongrel dogs of either sex were anesthetized with sodium pentobarbital, 35 mg./kg., i.v., and a femoral artery was catheterized to record arterial blood pressure. One hind limb was denervated by severing the femoral and sciatic nerve trunks and vascularly isolated by ligating the muscle with a 21-gauge stainless steel wire placed under the femoral artery and vein and tightened with a Schiffrin wire tightener. The distal segment of the femoral artery of the isolated limb was catheterized with PE 205 tubing and perfused with blood drawn from the central segment of the same artery. A constant flow of blood was maintained utilizing a Sigmamotor pump and perfusion pressure determined from a T tube placed between the pump and the isolated limb *via* a Statham pressure transducer.

In order to study the effects of cryptenamine, protoveratrine *A*, and protoveratrine *B* on β-adrenergic receptors, four different doses of isoproterenol, 1, 2, 5, and 10 mcg., at 10-min. intervals, were administered intraarterially into the limb followed by the i.v. administration of one of the following doses: cryptenamine, 5 mcg./kg.; protoveratrine *A*, 1 mcg./kg., 2 mcg./kg., or 5 mcg./kg.; or protoveratrine *B*, 1 mcg./kg. or 2 mcg./kg. After a 30-min. stabilization period, the doses of isoproterenol were repeated.

Effect of Cryptenamine on the Cardiac Responses of Epinephrine in Atropinized Dogs—Mongrel dogs of either sex were anesthetized with sodium pentobarbital, 35 mg./kg., i.v. and a femoral artery cannulated for the recording of blood pressure. The trachea was cannulated and the animal placed on positive pressure respiration (Mine Safety Appliance Co.), utilizing a mixture of 95% oxygen and 5% carbon dioxide. The thoracic cavity was entered at the fourth intercostal space utilizing electrocautery to minimize bleeding, and the pericardium incised and sutured to the chest wall so as to gain easy access to the heart. A Walton-Brodie strain gauge arch was sutured to the right ventricle to determine the force of contraction and the physiological responses recorded on a Grass polygraph. The animals were pretreated with atropine sulfate, 1 mg./kg., i.v., to block the parasympathetic receptors involved in the reflexogenic mechanism, thus making it possible to study the nature of the efferent pathways.

Effect of Cryptenamine on the Epinephrine Response in Isolated Guinea Pig Heart—Guinea pigs were sacrificed by cervical dislocation and the lungs, heart, and ascending aorta quickly excised. The pericardium was removed, the pulmonary vessels ligated, and the lungs separated from the heart and saline perfused into the aorta to clear the coronary arteries of blood. A cannula was inserted into the aorta, the heart suspended in a Langendorf unit, and perfused with oxygenated Locke's solution (NaCl, 9.0 g.; KCl, 0.42 g.; $CaCl_2$, 0.24 g.; $NaHCO_3$, 0.5 g.; dextrose, 2.0 g., glass-distilled water q.s. 1,000 ml., warmed to 38°). The reservoir was adjusted to produce an infusion pressure of 40–50 mm. Hg which closed the semilunar valves thus forcing the fluid through the coronary vessels. Contractions of the heart were recorded on a Grass polygraph utilizing a force-displacement transducer.

Drugs were administered directly into the cannula, *via* a three-way stopcock. Epinephrine HCl (0.5 or 1.0 mcg. of the base) was administered initially and the perfusion continued for approximately 5 min., at which time cardiac activity had returned to basal level. This procedure was repeated until the response was reproducible. Cryptenamine, 10 mcg., was then injected; and after cardiac activity reached a constant level, the same dose of epinephrine was repeated.

Effect of Cryptenamine on Isoproterenol Response in Isolated Guinea Pig Atria—Guinea pigs were sacrificed by cervical dislocation, the thoracic cavity opened, and the pericardium removed. The ventricles were removed by cutting through the atria-ventricular junction, and the remaining parts of the aorta and pulmonary artery freed and gently drawn apart, producing an atrial strip approxi-

mately 3 cm. in length. Silk thread was tied to each end, and the preparation suspended in oxygenated Locke's solution which was maintained at a constant temperature at 29–30° in a 50-ml. muscle bath. The contractions were recorded on a Beckman dynograph utilizing a Grass force-displacement transducer.

Drugs were administered into the isolated organ bath in volumes less than 5% of the total bath volume after the preparation had been permitted to stabilize for 15–30 min. Between each administration of isoproterenol (2–8 ng./ml.), the preparation was washed at least 3 times at 5-min. intervals. When the response was reproducible, cryptenamine was added (50 ng./ml.), and 30 min. later, the same dose of isoproterenol repeated. Preliminary data indicated that a minimum period of 30 min. was necessary to alter the responses of isoproterenol.

Effect of Cryptenamine on Isoproterenol Responses in Guinea Pig Tracheal Smooth Muscle—Guinea pigs were sacrificed by cervical dislocation, the trachea removed, and sectioned into 12 equal rings. These were connected in series by short loops of silk thread so that the dorsal smooth muscle bands were vertical. The chain was set up in a 10-ml. organ bath containing oxygenated (95% O_2 + 5% CO_2) Krebs-Henseleit solution (NaCl, 6.90 g.; KCl, 0.35 g.; $CaCl_2$, 0.28 g.; $NaHCO_3$, 2.10 g.; KH_2PO_4, 0.16 g.; $MgSO_4$, $7H_2O$, 0.29 g.; dextrose, 1.0 g.; glass-distilled water q.s. 1,000 ml. maintained at 38°). An isotonic lever was utilized to record the contractions on a slowly moving kymograph. The effect of isoproterenol (0.2–0.8 ng./ml.) prior to and following cryptenamine, 50 ng./10 ml. bath were investigated. Isoproterenol (0.2–0.8 ng./ml.) was administered, the maximal effect recorded, and the preparation washed five times at 5-min. intervals. This was repeated until reproducible responses were obtained. Cryptenamine was added to the bath following the last injection and isoproterenol (concentration equal to that administered prior to cryptenamine) administered 5 min. later.

Statistical significance was determined using Student's *t* test throughout this study.

RESULTS

Effects of Cryptenamine and Protoveratrine A on the Epinephrine Content of Adrenal Venous Blood—Cryptenamine and protoveratrine *A* decreased the rate of secretion of epinephrine from the adrenal medulla and increased the rate of norepinephrine secretion (Tables I–IV and Figs. 1 and 2). These results suggest that cryptenamine and protoveratrine *A* may block the enzymes which methylate norepinephrine into epinephrine. It is also probable that the increase in the pressor response that Krayer (9) and Krayer and Mendez (10) obtained when they administered blood plasma from dogs which received veratridine or protoveratrine to pithed rats or cats may not have been due to an increase in epinephrine content but in the amount of norepinephrine.

Effects of Cryptenamine, Protoveratrine A and Protoveratrine B on Isoproterenol-Induced Relaxation of Vasculature in the Denervated Perfused Hind Limb of the Dog—The results are summarized in Tables V–X. Neither protoveratrine *A* nor protoveratrine *B* potentiated the effects of iso-

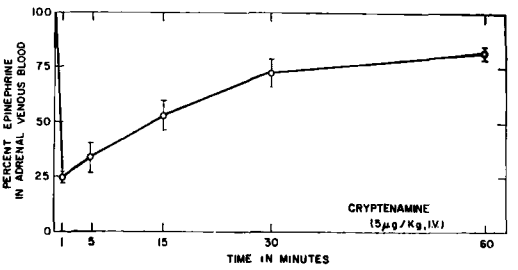

Fig. 1—Percent of control epinephrine content in adrenal venous blood.

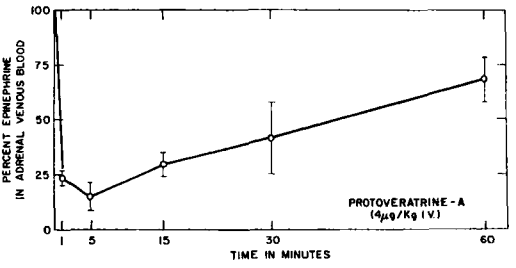

Fig. 2—Percent of control epinephrine content in adrenal venous blood.

TABLE I—EFFECT OF CRYPTENAMINE (5 MCG./KG., I.V.) ON THE ADRENAL VENOUS BLOOD EPINEPHRINE CONTENT (MCG./MIN.)

Dog	Control	*I*[a]	*II*[b]	*III*[c]	*IV*[d]	*V*[e]	*VI*[f]
1	0.899	0.203	0.298	0.487	0.756	0.813	—
2	0.894	0.204	0.596	0.659	0.840	—	0.831
3	2.467	0.941	1.150	1.277	1.468	2.058	2.139
4	0.510	0.160	0.192	0.403	0.438	0.456	0.593
5	0.334	0.104	0.031	0.096	0.229	0.289	0.310
6	2.701	0.608	1.094	1.154	1.849	2.181	2.139
7	1.602	0.139	0.192	0.468	0.486	1.082	1.165
8	1.105	0.236	0.182	0.711	0.885	0.953	1.033
Mean	1.314	0.324	0.466	0.657	0.869	1.109	1.193
± *SE*	0.308	0.104	0.202	0.138	0.192	0.279	0.283
p		<0.01	<0.05	<0.01	<0.05	<0.10	<0.10

[a] Blood collected immediately after administration of cryptenamine. [b] Blood collected 5 min. after administration of cryptenamine. [c] Blood collected 15 min. after administration of cryptenamine. [d] Blood collected 30 min. after administration of cryptenamine. [e] Blood collected 60 min. after administration of cryptenamine. [f] Blood collected 120 min. after administration of cryptenamine.

TABLE II—EFFECT OF CRYPTENAMINE (5 MCG./KG., I.V.) ON THE ADRENAL VENOUS BLOOD NOREPINEPHRINE CONTENT (MCG./MIN.)

Dog	Control	I[a]	II[b]	III[c]	IV[d]	V[e]	VI[f]
1	0.053	0.185	0.195	0.469	0.402	0.116	—
2	0.053	0.127	0.187	0.235	0.141	—	0.138
3	0.035	0.153	0.215	0.211	0.079	0.076	0.073
4	0.015	0.047	0.084	0.206	0.200	0.184	0.129
5	0.021	0.037	0.043	0.034	0.021	0.021	0.022
6	0.176	0.239	0.166	0.159	0.122	0.117	0.117
7	0.238	0.495	0.546	0.699	0.562	0.449	0.335
8	0.248	0.236	0.286	0.318	0.220	0.295	0.295
Mean	0.090	0.190	0.215	0.278	0.217	0.180	0.157
± *SE*	0.034	0.051	0.062	0.069	0.064	0.055	0.043
p		<0.05	<0.05	<0.05	<0.10	<0.10	<0.10

[a] Blood collected immediately after administration of cryptenamine. [b] Blood collected 5 min. after administration of cryptenamine. [c] Blood collected 15 min. after administration of cryptenamine. [d] Blood collected 30 min. after administration of cryptenamine. [e] Blood collected 60 min. after administration of cryptenamine. [f] Blood collected 120 min. after administration of cryptenamine.

TABLE III—EFFECT OF PROTOVERATRINE *A* (4 MCG./KG., I.V.) ON THE ADRENAL VENOUS BLOOD EPINEPHRINE CONTENT (MCG./MIN.)

Dog	Control	I[a]	II[b]	III[c]	IV[d]	V[e]	VI[f]
1	0.462	0.097	0.028	0.154	0.152	0.358	0.615
2	1.806	0.343	0.226	0.343	0.349	0.874	1.658
3	0.265	0.079	0.068	0.097	0.194	0.217	0.247
Mean	0.844	0.173	0.107	0.198	0.232	0.483	0.840
± *SE*	0.484	0.042	0.086	0.057	0.060	0.199	0.422

[a] Blood collected immediately after administration of protoveratrine *A*. [b] Blood collected 5 min. after administration of protoveratrine *A*. [c] Blood collected 15 min. after administration of protoveratrine. *A* [d] Blood collected 30 min. after administration of protoveratrine *A*. [e] Blood collected 60 min. after administration of protoveratrine *A*. [f] Blood collected 120 min. after administration of protoveratrine *A*.

TABLE IV—EFFECT OF PROTOVERATRINE *A* (4 MCG./KG. I.V.) ON THE ADRENAL VENOUS BLOOD NOREPINEPHRINE CONTENT (MCG./MIN.)

Dog	Control	I[a]	II[b]	III[c]	IV[d]	V[e]	VI[f]
1	0.015	0.117	0.151	0.220	0.225	0.196	0.048
2	0.349	0.428	0.443	0.528	0.526	0.481	0.300
3	0.011	0.142	0.158	0.274	0.273	0.091	0.016
Mean	0.125	0.229	0.251	0.341	0.341	0.256	0.122
± *SE*	0.112	0.099	0.096	0.095	0.093	0.138	0.089

[a] Blood collected immediately after administration of protoveratrine *A*. [b] Blood collected 5 min. after administration of protoveratrine *A*. [c] Blood collected 15 min. after administration of protoveratrine *A*. [d] Blood collected 30 min. after administration of protoveratrine *A*. [e] Blood collected 60 min. after administration of protoveratrine *A*. [f] Blood collected 120 min. after administration of protoveratrine *A*.

proterenol on the β-adrenergic receptors, but a slight blocking effect was observed with some of the doses, 90–120 min. after administration of the alkaloids. Cryptenamine did potentiate the response to isoproterenol in the denervated perfused hind limb (Table V).

Effects of Cryptenamine on the Cardiac Responses of Epinephrine in Atropinized Dogs—Pretreatment of the animals with atropine sulfate partially blocked the hypotension and bradycardia, and slightly decreased the duration of activity of cryptenamine as has previously been reported (6). Three doses of epinephrine, 50 ng./kg., 100 ng./kg., and 200 ng./kg. were administered i.v. to the animals before and after administration of cryptenamine, 5 mcg./kg., i.v. Cryptenamine potentiated the effect of epinephrine on the myocardial contractile force (Table XI) but did not alter the effect of epinephrine on the blood pressure.

Effect of Cryptenamine on the Epinephrine Response in Isolated Guinea Pig Heart—Cryptenamine, 10 mcg., produced positive inotropic effects on the heart and also appeared to potentiate the inotropic effect of epinephrine; however, the chronotropic activity was inhibited (Table XII).

Effect of Cryptenamine on Isoproterenol Response in Isolated Guinea Pig Atria—Cryptenamine, 50 ng./ml., markedly reduced the positive chronotropic and inotropic effects of isoproterenol (Table XIII). If cryptenamine remained in contact with the isolated atria for more than 1 hr., it produced a negative chronotropic effect, which as reported by Hawkins (11) was irreversible and did not disappear when the bathing fluid was replaced with fresh Locke's solution.

Effect of Cryptenamine on Isoproterenol Response in Guinea Pig Tracheal Chain—The data, summarized in Table XIV, indicate that cryptenamine, 5 ng./ml., did not affect the isoproterenol-induced relaxation of the guinea pig tracheal chain.

DISCUSSION

It has been reported that cryptenamine appeared to sensitize β-adrenergic receptors in the vasculature

TABLE V—EFFECT OF CRYPTENAMINE (5 MCG./KG., I.V.) ON THE ISOPROTERENOL-INDUCED RELAXATION IN THE DENERVATED PERFUSED HIND LIMB OF THE DOG

Sex and Weight, kg.	Before Cryptenamine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg				After Cryptenamine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg			
	1 mcg.	2 mcg.	5 mcg.	10 mcg.	1 mcg.	2 mcg.	5 mcg.	10 mcg.
F 10.4	16	18	23	28	20	26	30	39
M 12.0	25	35	40	45	30	45	47	53
M 10.8	20	25	33	35	25	33	40	42
F 9.4	26	30	38	40	32	38	45	48
Mean	21.8	27.0	33.5	37.0	26.8	33.5	40.5	45.5
± *SE*	2.32	3.63	3.80	3.63	2.69	4.01	3.80	3.12
p					<0.01	<0.01	<0.01	<0.01

TABLE VI—EFFECT OF PROTOVERATRINE *A* (1 MCG./KG., I.V.) ON THE ISOPROTERENOL-INDUCED RELAXATION IN THE DENERVATED PERFUSED HIND LIMB OF THE DOG

Sex and Weight, kg.	Before Protoveratrine: Decrease in Perfusion Pressure after Isoproterenol, mm. Hg				After Protoveratrine: Decrease in Perfusion Pressure after Isoproterenol, mm. Hg			
	1 mcg.	2 mcg.	5 mcg.	10 mcg.	1 mcg.	2 mcg.	5 mcg.	10 mcg.
F 11.8	42	47	62	65	37	46	60	62
M 9.5	10	16	24	32	10	17	25	33
F 11.0	20	25	33	35	20	25	30	30
M 12.0	23	32	35	38	25	33	38	40
Mean	23.8	30.0	38.5	42.5	23.0	30.3	38.3	41.3
± *SE*	6.69	6.54	8.19	7.60	5.61	6.18	7.73	7.23
p					>0.95	>0.95	>0.95	>0.95

TABLE VII—EFFECT OF PROTOVERATRINE *A* (2 MCG./KG., I.V.) ON THE ISOPROTERENOL-INDUCED RELAXATION IN THE DENERVATED PERFUSED HIND LIMB OF THE DOG

Sex and Weight, kg.	Before Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg				After Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg			
	1 mcg.	2 mcg.	5 mcg.	10 mcg.	1 mcg.	2 mcg.	5 mcg.	10 mcg.
M 10.7	23	43	46	51	23	25	38	49
M 12.9	35	38	40	44	15	20	25	34
M 10.4	23	25	35	42	22	20	25	30
M 12.0	25	32	35	38	20	27	35	38
Mean	26.5	34.5	29.0	43.8	20.0	23.0	30.8	37.8
± *SE*	2.87	3.88	2.16	2.71	1.78	1.80	3.38	4.09
p		—			>0.3	>0.1	>0.1	>0.2

TABLE VIII—EFFECT OF PROTOVERATRINE *A* (5 MCG./KG., I.V.) ON THE ISOPROTERENOL-INDUCED RELAXATION IN THE DENERVATED HIND LIMB OF THE DOG

Sex and Weight, kg.	Before Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg				After Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg			
	1 mcg.	2 mcg.	5 mcg.	10 mcg.	1 mcg.	2 mcg.	5 mcg.	10 mcg.
F 10.0	17	20	34	36	13	15	20	30
F 7.6	25	32	35	37	35	33	40	43
F 8.5	25	28	40	45	25	25	36	40
M 8.5	18	20	24	29	15	12	14	20
Mean	21.5	25.0	33.3	36.8	22.0	21.2	27.5	33.3
± *SE*	2.09	3.0	3.35	3.28	5.07	4.80	6.24	5.21
p					>0.9	>0.2	>0.3	>0.4

of dogs and nictitating membrane of cats (6). Cryptenamine and several other veratrum alkaloids were investigated for their effect on β-adrenergic receptors in various organs. The data indicated that cryptenamine did not affect isoproterenol-induced relaxation of guinea pig tracheal chain, potentiated the inotropic effect and inhibited the chronotropic action of epinephrine on the isolated guinea pig heart, potentiated the positive chronotropic effect of epinephrine in the intact heart of atropinized dogs, and inhibited the characteristic isoproterenol response on the isolated guinea pig atria. These results are consistent with the published work by Lands *et al.* (12) which indicated that there was a certain degree of variation in affinity and intrinsic activity of β-adrenergic receptors in various organs. This and other published work (13) suggest that the epinephrine-sensitive β-adrenergic receptors may be activated to a greater or lesser degree in various organs and species. Although the emphasis in this discussion has been placed on the action of cryptenamine on receptor sites, it is

TABLE IX—EFFECT OF PROTOVERATRINE *B* (1 MCG./KG., I.V.) ON THE ISOPROTERENOL-INDUCED RELAXATION IN THE DENERVATED PERFUSED HIND LIMB OF THE DOG

Sex and Weight, kg.	Before Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg				After Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg			
	1 mcg.	2 mcg.	5 mcg.	10 mcg.	1 mcg.	2 mcg.	5 mcg.	10 mcg.
M 13.6	30	45	48	50	25	37	50	50
M 11.4	40	45	50	55	40	50	55	60
F 9.7	8	15	20	25	10	15	20	25
M 10.7	40	45	48	52	32	32	42	50
Mean	29.5	37.5	41.5	45.5	26.8	33.5	41.8	46.3
± *SE*	7.54	7.50	7.18	6.91	6.37	7.24	7.73	7.47
p					>0.4	>0.4	>0.4	>0.7

TABLE X—EFFECT OF PROTOVERATRINE *B* (2 MCG./KG., I.V.) ON THE ISOPROTERENOL-INDUCED RELAXATION IN THE DENERVATED PERFUSED HIND LIMB OF THE DOG

Sex and Weight, kg.	Before Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg				After Protoveratrine: Decrease in Perfusion Pressure to Isoproterenol, mm. Hg			
	1 mcg.	2 mcg.	5 mcg.	10 mcg.	1 mcg.	2 mcg.	5 mcg.	10 mcg.
M 12.3	30	45	47	50	30	37	40	50
F 8.4	40	45	55	55	35	35	50	55
F 13.1	25	35	43	48	25	30	35	38
M 8.4	23	25	28	30	20	22	25	27
Mean	29.5	37.5	43.3	45.8	27.5	31.0	37.5	42.5
± *SE*	3.80	4.79	5.66	5.45	3.23	3.34	5.20	6.28
p					>0.1	>0.05	>0.05	>0.3

possible that these alkaloids are affecting one or more enzyme systems within the cell (possibly adenylcyclase or phosphordiesterase) or acting directly on the vascular smooth muscle contractile elements. Since protoveratrine *A* and *B* failed to enhance the vasodilator effect of isoproterenol in the denervated dog hind limb, this activity must be attributed to other constitutents in cryptenamine.

Krayer (14) and others (15) have reported that at the peak blood pressure rise following intravenous injection of 1 mg. of protoveratrine to vagotomized dogs, a concentration of epinephrine in the order of 1:100,000 may be reached in the suprarenal venous blood suggesting that the release of epinephrine was a direct effect of veratrum alkaloids on the adrenal medulla. Mendez (15) and Krayer and Acheson (16) reported that an increase in pressor responses was obtained when they administered blood plasma from dogs which received protoveratrine or veratrine to pithed cats and rats and suggested that the rise in blood pressure was due to an increase of epinephrine levels. Jandhyala and Buckley (6) reported that the release of epinephrine from the adrenal medulla may contribute to the hypotensive activity of cryptenamine *via* its action on β-adrenergic receptors. This conclusion was made from indirect evidence in adrenalectomized dogs and dogs pretreated with P-286, reserpine, guanethidine, and α-methyldopa, which suggested the possible release of catecholamines from the adrenal medulla by cryptenamine. However, the actual content of catecholamines in the adrenal venous blood was not determined in their studies.

TABLE XI—EFFECT OF CRYPTENAMINE (5 mcg./kg.) ON INOTROPIC RESPONSE TO EPINEPHRINE IN ATROPINIZED DOGS (1 mg./kg.)

Epi Dose, mcg./kg.	*N*	Before Cryptenamine Average % Increase in Contractile Force ± *SE*	After Cryptenamine Average % Increase in Contractile Force ± *SE*
0.05	3	9.33 ± 2.46	15.63 ± 3.66
0.10	4	15.72 ± 3.91	22.0 ± 3.47
0.20	4	20.43 ± 3.53	32.50 ± 8.85

The results obtained in this present investigation did not agree with the above conclusions, in that the total adrenal venous catecholamine content was slightly decreased following the administration of cryptenamine and protoveratrine *A*. The epinephrine content of adrenal venous blood was decreased while the norepinephrine content markedly increased, suggesting that cryptenamine and protoveratrine *A* may be interfering with the conversion of norepinephrine into epinephrine. Epinephrine is synthesized in the adrenal medulla from norepinephrine by the enzyme, phenylethanolamine-*N*-methyl transferase (PNMT) (17), in a reaction in-

TABLE XII—EFFECT OF CRYPTENAMINE ON EPINEPHRINE RESPONSE IN THE ISOLATED GUINEA PIG HEART

Epi Dose, mcg./ml.	*N*	Before Cryptenamine[a]: Average % Increase in Amplitude ± *SE*	Before Cryptenamine[a]: Average % Increase in Rate ± *SE*	After Cryptenamine: Average % Increase in Amplitude ± *SE*	After Cryptenamine: Average % Increase in Rate ± *SE*
0.5	4	32.88 ± 7.03	40.78 ± 10.10	81.83 ± 17.34[b]	17.13 ± 4.55
1.0	6	68.25 ± 8.69	35.40 ± 9.67	116.87 ± 17.44[b]	20.80 ± 3.09

[a] 10 mcg. [b] Significant at $p < 0.05$ when compared to response prior to cryptenamine.

TABLE XIII—EFFECT OF CRYPTENAMINE (50 NG./ML.) ON ISOPROTERENOL-INDUCED RESPONSES ON GUINEA PIG ATRIA

Iso Dose, mcg./ml.	N	Before Cryptenamine: Average % Increase in Amplitude ± SE	Before Cryptenamine: Average % Increase in Rate ± SE	After Cryptenamine: Average % Increase in Amplitude ± SE	After Cryptenamine: Average % Increase in Rate ± SE
2	4	54.76 ± 5.44	22.40 ± 4.76	15.26 ± 6.94[a]	15.82 ± 3.71
4	4	120.18 ± 16.80	39.15 ± 5.06	42.53 ± 4.83[a]	31.88 ± 3.32
8	4	110.31 ± 18.58	27.98 ± 0.32	45.88 ± 4.58[a]	26.05 ± 6.45

[a] $p < 0.05$ when compared to response prior to cryptenamine.

TABLE XIV—EFFECT OF CRYPTENAMINE[a] ON ISOPROTERENOL-INDUCED RELAXATION IN GUINEA PIG TRACHEAL CHAIN

Iso Dose ng./ml.	N	Before Cryptenamine Average (mm.) Relaxation ± SE	After Cryptenamine Average (mm.) Relaxation ± SE
0.2	4	12.5 ± 0.87	12.5 ± 0.87
0.4	4	16.4 ± 1.10	16.4 ± 0.10
0.8	4	22.4 ± 0.93	22.6 ± 0.93

[a] 5 ng./ml.

volving *S*-adenosyl methionine as a methyl donor (18). One control mechanism for the synthesis of catecholamines, which applies to the synthesis of epinephrine in the adrenal medulla has recently been described (19). It was suggested (19) that the synthesis of PNMT, responsible for the formation of epinephrine, is controlled by adrenal glucocorticoids. When adrenal steroid secretion was depressed by hypophysectomy, the activity of the epinephrine-synthesizing enzyme falls, but can be restored by pituitary adrenocorticotropic hormone (ACTH) or adrenal glucocorticoid administration. It is possible that cryptenamine and protoveratrine *A* affect the PNMT enzyme *via* this endocrine system and prevent the formation of epinephrine from norepinephrine and thus increase the norepinephrine concentration in the adrenal venous blood.

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Keyphrases

Cryptenamine
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Epinephrine, norepinephrine activity—cryptenamine effect
Protoveratrine *A*, *B* activity—cryptenamine effect
Atropine activity—cryptenamine effect