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Actions of Mescaline on Isolated Rat Atria

PETER K. S. SIEGL and RAYMOND F. ORZECHOWSKI ˣ

Abstract □ Mescaline, in concentrations of 5×10^{-4} and 1×10^{-3} *M*, produced negative chronotropic and positive inotropic responses in isolated, spontaneously beating rat atria. In tissues driven at a constant rate, the inotropic response was diminished greatly, indicating that the increment in the force of contraction was secondary to the reduction in rate. These chronotropic and inotropic responses were not altered consistently by pretreatment with the histamine antagonists chlorpheniramine and metiamide.

Keyphrases □ Mescaline—chronotropic and inotropic effects on isolated rat atria □ Chronotropic effects—mescaline on isolated rat atria □ Inotropic effects—mescaline on isolated rat atria □ Phenethylamines—mescaline, chronotropic and inotropic effects on isolated rat atria □ Cardiovascular effects—mescaline on isolated rat atria □ Psychotomimetics—mescaline, chronotropic and inotropic effects on isolated rat atria

In this study, the effects of mescaline (3,4,5-trimethoxyphenethylamine) on isolated rat atria were examined to reveal its possible actions directly on the pacemaker and atrial tissue. By the use of histamine antagonists, the involvement of histamine receptors (both H_1 and H_2) was examined as a possible mechanism of the *in vitro* cardiac action of mescaline.

BACKGROUND

The cardiovascular actions of the psychotomimetic amine mescaline were reported previously (1–8). Mescaline consistently evoked bradycardia in several species of animals. Mescaline-induced slowing of the frog heart was first observed by Dixon (1); others confirmed this action in the dog (2), rabbit, and cat (3). The bradycardia was inhibited by vagotomy or pretreatment with atropine (3); however, other published data (1, 2, 4) challenged these findings. Most evidence favors the conclusion that mescaline does not produce an effect on the heart by a vagal reflex or cholinergic stimulation.

Competition for epinephrine receptors by mescaline was proposed (5) because both mescaline-induced bradycardia and hypoglycemia in rats were reduced upon pretreatment with epinephrine. In another study (6), mescaline showed no propranolol-like antagonism of isoproterenol-induced relaxation of spontaneously contracting rat uterus. Mescaline failed to antagonize isoproterenol-induced positive chronotropism and hypotension (4) or to reverse an ethylnorepinephrine depressor response (6) in anesthetized dogs. From these data, β-adrenergic receptor blockade by mescaline or one of its metabolites was an unlikely explanation.

Histamine release or direct stimulation of histamine receptors by mescaline might account for the observed bradycardia. Potentiation of the hypotensive response to histamine in rats (7) was attributed to inhibition of the histaminolytic action of diamine oxidase by mescaline. Dogs receiving an intravenous infusion of mescaline showed (4) increased plasma histamine levels and a hypotensive response similar to that produced by compound 48/80, a known histamine-liberating substance (9). Alteration of respiratory dynamics and elevated right ventricular pressure, resembling responses to histamine, were observed in the guinea pig (8). Attempts to block these effects of mescaline with the antihistamine diphenhydramine were unsuccessful (4, 8).

More recent evidence suggested that histamine produced bradycardia by a stimulation of histamine receptor subtypes (H_2-receptors), which were not blocked by conventional antihistaminic agents such as diphenhydramine (10). The possible interaction of mescaline or mescaline-released histamine with H_2-receptors in the myocardium could be evaluated with an H_2-blocker, such as metiamide, in an attempt to confirm or refute this hypothesis.

EXPERIMENTAL

Sprague–Dawley rats of both sexes, 250–400 g, were sacrificed by cervical fracture and the hearts were rapidly removed. Paired atria were sectioned from the ventricles with a bridge of tissue joining the two atria. For examination of inotropic responses, left atria were electrically driven by paired platinum electrodes. Monophasic square wave pulses of 1-msec duration were delivered by a stimulator[1]. Tissues were suspended in a 10-ml glass tissue bath filled with Krebs–Henseleit solution (11) at a constant temperature of 35° and equilibrated with 95% O_2–5% CO_2. The solution pH was adjusted to 7.3.

A constant resting tension of 1.0 g was maintained throughout each experiment. All preparations were allowed to equilibrate for a minimum of 50 min with washes at 10-min intervals prior to drug challenge. Isometric contractions were measured with a force-displacement transducer[2] and recorded on a polygraph[3]. A linear tachometer[4] was used to record changes in heart rate. All drug solutions were prepared daily in Krebs–Henseleit solution and expressed as molar concentration of base.

Dose–Response Curves with Mescaline—To observe chronotropic and inotropic responses to mescaline, concentrations of 1×10^{-4}, 5×10^{-4}, and 1×10^{-3} *M* mescaline base were generated in a tissue bath containing spontaneously beating, paired atria. Heart rate and developed tension were recorded as maximum responses within 5 min. To minimize possible tachyphylaxis, only two drug challenges per preparation were employed. The challenges were separated by three washes and a 15-min reequilibration period. Sixteen atrial preparations were tested in this manner. All data were reported as the percent change from the pretreatment period and analyzed by Duncan's New Multiple Range Test (12).

The effects of mescaline were studied on separated left and right atria of the same heart to determine differences in inotropic responses in both the presence and absence of chronotropic responses. Right atria were allowed to contract spontaneously, while left atria were electrically driven (150% threshold voltage). After a 1-hr equilibration period, the rate of electrical stimulation was adjusted to equal the heart rate of the spontaneously contracting right atrium. At 5 min after the addition of mescaline to the tissue bath, changes in the developed tension for both atria

[1] Grass model S48.
[2] Grass model FT-03.
[3] Grass model 7B.
[4] Grass model 7P4-D.

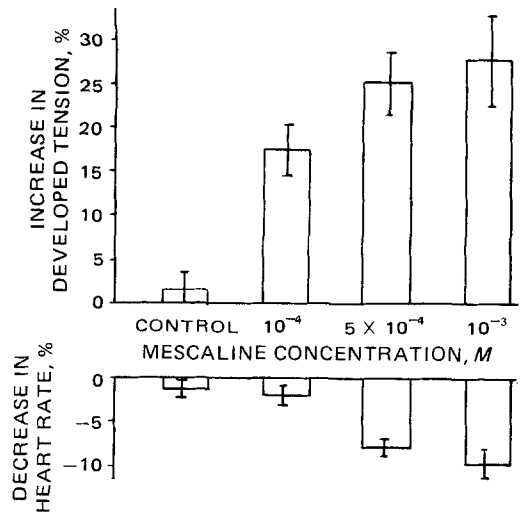

Figure 1—*Responses to mescaline challenge in spontaneously contracting paired rat atria. All bars indicate mean* ± SEM; n = *8.*

and the heart rate for the right atria were recorded. Twelve preparations were studied (*i.e.*, six hearts), each challenged with 1×10^{-4}, 5×10^{-4}, and 1×10^{-3} *M* mescaline base. Inotropic responses of left *versus* right atria were treated as paired data and tested by the Student *t* test (13).

To establish the relationship between rate and tension for rat atria and to allow interpretation of the effects obtained with mescaline, a Bowditch or rate–tension curve was constructed. Four left atria were electrically driven at 150% of threshold voltage and at various rates of stimulation. The maximum developed tension was recorded 5 min after the rate was altered. Results were graphed as the average developed tension (grams) *versus* the rate of stimulation (Hertz and beats per minute).

Histaminic Mechanism Study: Pretreatment with Antihistamines—Two concentrations of an H_1-antagonist (chlorpheniramine maleate, 1×10^{-6} and 1×10^{-5} *M*) and an H_2-antagonist (metiamide, 3×10^{-6} and 3×10^{-5} *M*) were each employed to pretreat six spontaneously contracting, paired atria 8 min prior to challenge with mescaline. Twelve control preparations were pretreated with 0.1 ml of Krebs–Henseleit solution. Pretreatments were randomized by the use of a table of random numbers.

Concentrations of mescaline were log spaced, and each challenge was separated by three washes and 8 min of incubation with the antagonist. Chronotropic and inotropic responses were measured before and 5 min after the addition of mescaline. Results were reported as the percent change from pretreatment values. Tukey's multiple range procedure for

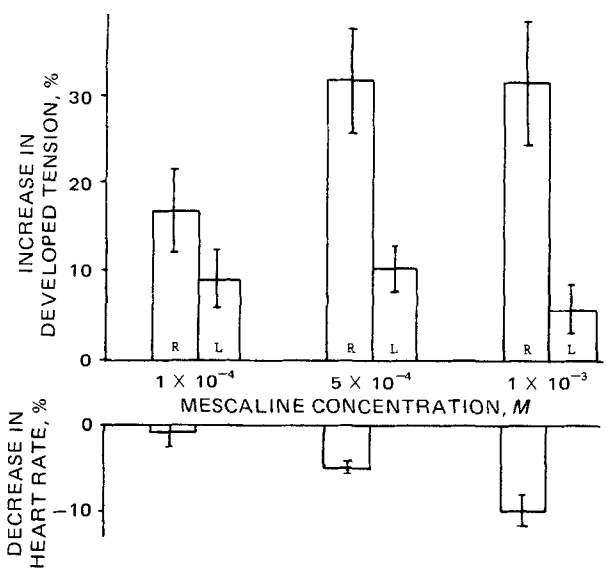

Figure 2—*Comparison of inotropic responses of electrically driven left atrium (L) and spontaneously contracting right atrium (R) to challenge with mescaline. All bars indicate mean* ± SEM; n = *6.*

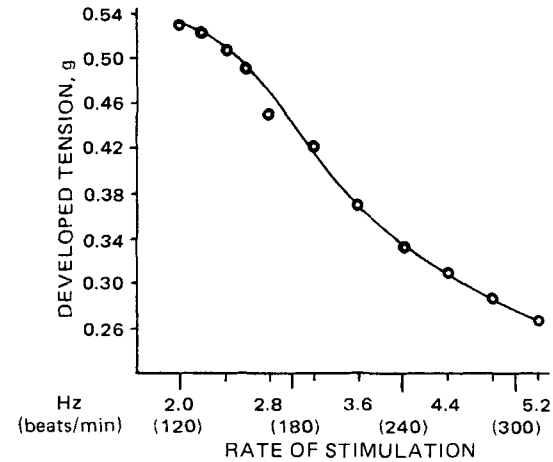

Figure 3—*Bowditch (rate–tension) curve for electrically stimulated left rat atrium.*

unequal subclass numbers[5] was used to test for statistical significance.

RESULTS

Dose–Response Curves with Mescaline—Dose-related positive inotropic and negative chronotropic responses were observed when spontaneously beating, paired rat atria were challenged with 1×10^{-4}, 5×10^{-4}, and 1×10^{-3} *M* mescaline (Fig. 1). All inotropic responses were significantly different from the control; however, only the two highest concentrations of mescaline produced a chronotropic response significantly different ($p < 0.05$) from the control.

Positive inotropic responses in electrically driven left atria challenged with 5×10^{-4} and 1×10^{-3} *M* mescaline were significantly less in the absence of a negative chronotropic response (Fig. 2). Since 10^{-4} *M* mescaline produced no significant change in heart rate, a difference in inotropic responses between spontaneously beating and electrically driven preparations was neither anticipated nor seen. Responses of spontaneously beating right atria were similar to those of spontaneously beating paired atria.

Since data from electrically driven atria suggested that a major component of the positive inotropic response was related to the negative chronotropic effect of mescaline, it was important to examine the effects of heart rate upon the developed tension in the control atria. A Bowditch or rate–tension curve for the electrically stimulated left rat atrium (Fig. 3) indicated that a positive inotropic response accompanied a decrease in heart rate. This relationship between rate and tension also was reported for rat papillary muscle (14, 15) and rat arterially perfused septal (16) preparations.

Histaminic Mechanism Study: Effects of Pretreatment with Antihistaminic Agents—Negative chronotropic responses to mescaline

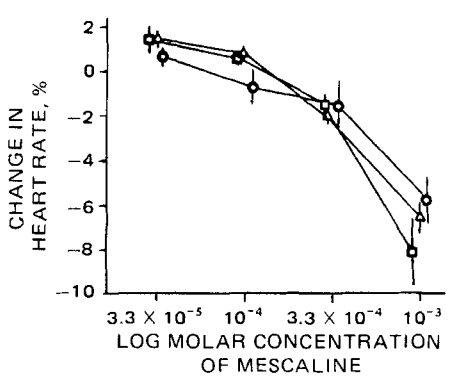

Figure 4—*Effect of pretreatment with chlorpheniramine upon the negative chronotropic response to mescaline challenge. Key: △, control,* n = *12;* □, *1* × *10^{-6}* M *chlorpheniramine,* n = *6; and* ○, *1* × *10^{-5}* M *chlorpheniramine,* n = *6.*

[5] J. L. Ciminera, Merck Institute for Therapeutic Research, West Point, Pa., personal communication.

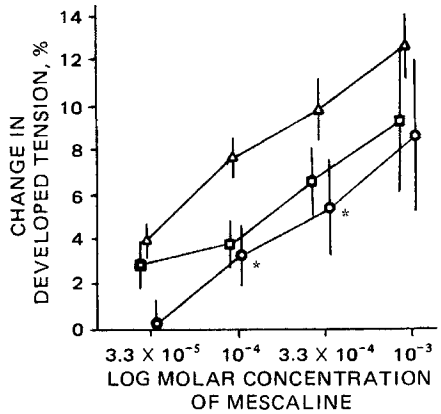

Figure 5—*Effect of pretreatment with chlorpheniramine upon the positive inotropic response to mescaline challenge. Key: same as Fig. 4; and *, p < 0.05.*

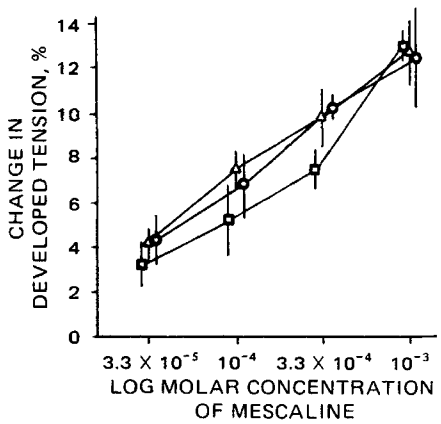

Figure 7—*Effect of pretreatment with metiamide upon the positive inotropic response to mescaline challenge. Key: same as Fig. 6.*

were unaltered by pretreatment with 1×10^{-5} or 1×10^{-6} *M* chlorpheniramine (Fig. 4). Both concentrations of chlorpheniramine produced a dose-related shift of the percent change in developed tension *versus* the log concentration of mescaline (Fig. 5); however, only responses to 1×10^{-4} and 3.3×10^{-4} *M* mescaline were reduced significantly by 1×10^{-5} *M* chlorpheniramine.

Metiamide, 3×10^{-5} and 3×10^{-6} *M*, failed to show a significant blockade of the negative chronotropic response, except at 1×10^{-3} *M* mescaline with 3×10^{-5} *M* metiamide (Fig. 6). Positive inotropic responses also were unchanged following pretreatment with metiamide (Fig. 7).

DISCUSSION

A significant role of endogenous histamine in the regulation of cardiac dynamics was proposed previously (17–19). The presence of histamine in rat atrial tissue (17) and evidence of the histamine-releasing activity of mescaline in vascular tissue (4) suggested that the cardiac actions of mescaline might involve activation of a histaminergic mechanism. Recent studies (10, 20, 21) indicated that separate receptors may mediate the chronotropic and inotropic responses to histamine in guinea pig atria. Positive chronotropic responses blocked by metiamide or burimamide, but not by promethazine or tripelennamine, suggested involvement of histamine H_2-receptor subtypes, whereas positive inotropic responses were altered only by promethazine and tripelennamine, histamine H_1-receptor blocking agents.

Exogenous histamine effects a negative chronotropic response in the isolated rat heart (17, 22). Based on the situation in the guinea pig heart, stimulation of H_2-receptors in rat atria by mescaline, either directly or *via* a release of endogenous histamine, could be responsible for the observed bradycardia (10, 20). That direct stimulation of histamine receptors could not account for the cardiac effects of mescaline was indicated by failure of histamine receptor blockers of the H_1 and H_2 type to obtund the response to mescaline. This explanation of mescaline-induced bradycardia in species other than the rat is untenable, since exogenous histamine evokes positive chronotropic responses in most species (22–27).

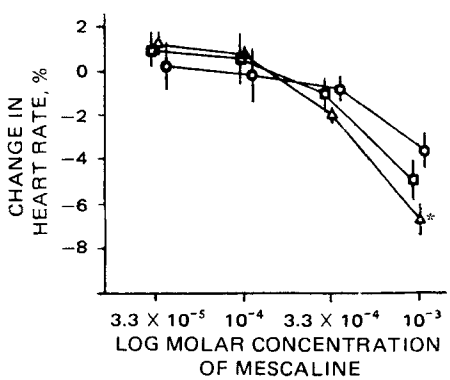

Figure 6—*Effect of pretreatment with metiamide upon the negative chronotropic response to mescaline challenge. Key: △, control,* n = *12;* □, *3 × 10⁻⁶* M *metiamide,* n = *6; and* ○, *3 × 10⁻⁵* M *metiamide,* n = *6.*

An inverse relationship existed between atrial rate and peak muscle tension in control rat atria; *i.e.*, the Bowditch curve showed that a decrease in atrial rate was associated with an increase in peak tension development (Fig. 3). Thus, it was of interest to determine whether the positive inotropic effect of mescaline would persist in atrial preparations electrically driven at a constant rate. The data indicated that a major component of the observed increase in tension development following mescaline was directly attributable to the slowing effect of the drug on atrial rate, since only slight increases in contractile force were seen in electrically driven tissues when the rate was held constant (Fig. 2).

A central action of mescaline might account for the bradycardia reported to occur in *in vivo* preparations. α-Adrenergic agonists injected into certain brain sites produced bradycardia and hypotension, due apparently to a reduction in sympathetic nervous tone (28). Mescaline penetrated into the central nervous system (29) and also evidenced α-adrenergic agonist properties in *in vitro* systems (30). Bradycardia was observed 1 min following injections of mescaline into the lateral brain ventricle of the mouse (31). Further investigation into the central mechanism appears to be warranted.

Finally, the concentrations of mescaline required to evoke alterations in myocardial rate in this study were relatively high (3.3×10^{-5}–1×10^{-3} *M*). Such concentrations, if extrapolated to the *in vivo* situation, would probably represent toxic concentrations of the drug (5).

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Comparative Pharmacokinetics of Coumarin Anticoagulants XXIV: Effect of Treatment with Phenobarbital on Serum Protein Binding of Warfarin and Dicumarol in Rats

AVRAHAM YACOBI, JOHN T. SLATTERY, and GERHARD LEVY [x]

Abstract □ Rats were treated with the enzyme inducer phenobarbital to determine if it would affect the serum protein binding of warfarin and dicumarol, possibly by changing the rate of formation or elimination of endogenous inhibitor(s). Daily administration of phenobarbital, 75 mg/kg, for 4 days increased relative liver size (a concomitant of enzyme induction) but had no apparent effect on the serum protein binding of warfarin and dicumarol.

Keyphrases □ Phenobarbital—effects on serum protein binding of warfarin and dicumarol, rats □ Protein binding, serum—warfarin and dicumarol, effect of phenobarbital, rats □ Binding, serum protein—warfarin and dicumarol, effect of phenobarbital, rats □ Warfarin—serum protein binding, effect of phenobarbital, rats □ Dicumarol—serum protein binding, effect of phenobarbital, rats □ Anticoagulants—warfarin and dicumarol, serum protein binding, effect of phenobarbital, rats □ Enzyme inducers—phenobarbital, effect on serum protein binding of warfarin and dicumarol, rats

The total clearance of warfarin by the body is directly proportional to the free fraction of this drug in serum of rats (1, 2) and humans (3). There are pronounced interindividual differences in free fraction values of warfarin (4) and corresponding differences in the total clearance of this drug by the body (2, 3). Ongoing studies in rats[1] indicate that this is true also for the other major coumarin anticoagulant, dicumarol. There is no relationship between the free fraction values of either warfarin (2) or dicumarol (data in this report) and the concentration of albumin in serum.

Recent studies showed that endogenous inhibitors in uremic as well as in normal human serum cause a decrease in the protein binding of warfarin and other drugs (5). Therefore, interindividual differences in the serum protein binding of warfarin and dicumarol may be caused by corresponding differences in the serum concentration of such inhibitors. Treatment with an enzyme inducer such as phenobarbital may change the serum protein binding of warfarin, dicumarol, and other drugs by changing the concentration of endogenous inhibitors in serum. This situation could result from a change in the formation or elimination kinetics of the inhibitors due to increased activity of certain enzyme systems. Accordingly, a study was initiated to determine the effect of treatment with phenobarbital on the protein binding of warfarin and dicumarol in the serum of rats.

EXPERIMENTAL

Forty-eight adult male Sprague–Dawley rats, ~350 g, were used for an initial screening study. About 3 ml of blood was obtained from the tail artery of each animal, and serum was separated. The free fraction of racemic warfarin in these serum samples was determined by equilibrium dialysis as described previously (2). Based on the results of this screening study, 20 rats were selected and classified into 10 pairs with very small intrapair and large interpair differences in the warfarin free fraction value.

Four days later, one member of each pair received phenobarbital, 75 mg/kg/day ip, for 4 days while the other member received injections of normal saline solution. One day later, the animals were sacrificed by removing all blood from the aorta under ether anesthesia. The liver was also removed, compressed slightly between paper tissue to remove remaining blood, and weighed. Serum was separated and used to determine the free fraction values for warfarin (2) and dicumarol (6) by equilibrium dialysis. The concentration of total protein in serum was determined by the method of Gornall *et al.* (7) with crystalline rat albumin as the standard, and the fraction of albumin was determined by electrophoresis using a commercial[2] serum protein electrophoresis system.

[1] To be published.

[2] Gelman Instrument Co., Ann Arbor, Mich.