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EFFECTS OF METHYLECGONIDINE ON ACETYLCHOLINE-INDUCED BRONCHOCONSTRICTION AND INDICATORS OF LUNG INJURY IN GUINEA PIGS[1]

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Abstract. The fumarate salt of methylecgonidine (MEG; anhydroecgonine methylester), a pyrolysis product of cocaine, has previously been shown to antagonize contractions of guinea pig isolated trachea induced by acetylcholine (ACh) and other spasmogenics. We determined the effects of MEG fumarate on ACh-induced bronchoconstriction *in vivo*. Specific airway conductance (SGaw) was measured in guinea pigs receiving 30 - 300 mg/kg s.c. MEG fumarate and exposed one hour later to nebulized ACh (0.2 - 3.2%; by inhalation). MEG fumarate did not induce any changes in SGaw; neither did it antagonize dose-dependent decreases in SGaw induced by ACh. However, tremors, salivation, startle and increased numbers of fecal boli were observed after MEG administration. Thus, unlike antagonism of ACh-induced contractions of guinea pig isolated trachea observed *in vitro*, MEG fumarate does not antagonize ACh-induced bronchoconstriction *in vivo*, even at doses which induced changes in grossly-observable behavior. Inhalation of a condensation aerosol of MEG base induced lung damage as evidenced by the presence of blood and higher levels of protein and lactate dehydrogenase in the lung lavage fluid of MEG-treated animals than of control animals. Aerosols of MEG fumarate, on the other hand, did not induce lung damage when inhaled. These results extend previous observations that MEG base may contribute to detrimental pulmonary effects of crack smoking.

Key Words: acetylcholine, cocaine, methylecgonidine, bronchoconstriction, lung injury, guinea pig

Introduction

Methylecgonidine (MEG) is produced when cocaine base ("crack") is heated under conditions similar to those found in crack pipes (1,2,3). The amount of MEG inhaled during crack smoking is likely to depend on several factors, including the efficiency of inhalation and conditions under which the crack is heated; amounts ranging from trace to 5% have been observed (1,3). Nevertheless, MEG has been detected in significant amounts in the

[1]All animals used in this study were maintained in accordance with the guidelines of the Animal Care and Use Committee, NYU and of the "Guide for Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication No. (NIH)85-23, revised 1985.

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urine of crack smokers suggesting that it is inhaled and absorbed (2); only negligible amounts are detected in the urine of subjects given cocaine by other routes (2). Condensation aerosols of MEG base induce bronchoconstriction in guinea pigs (4) and squirrel monkeys (5). The mechanism of this bronchoconstriction is unclear, but may involve a reflexive event induced by the alkaline challenge posed by the base on pulmonary tissue. Unlike MEG base, only relatively high, acidic concentrations of nebulized fumarate salt of MEG induces bronchoconstriction (4); such acidity is also likely to provoke irritation and bronchoconstriction by reflex mechanisms. Intravenous administration of MEG fumarate also does not induce bronchoconstriction in squirrel monkeys, though it is not without effects on cardiovascular parameters (5 and Wood *et al.* unpublished observations). Interestingly, MEG fumarate relaxes guinea pig isolated tracheal rings that have been precontracted with ACh and also noncompetitively antagonizes contractions induced by ACh, carbachol, histamine and potassium chloride, but not barium chloride in this tissue (6,7). This finding led to the suggestion that MEG fumarate, or its derivatives, may have utility in the management of asthma (7). The mechanism by which MEG induces its effects *in vitro* or *in vivo* is currently unknown; indeed, receptor binding studies (National Institute of Mental Health/Novascreen®) revealed that MEG has negligible or only low affinity for muscarinic (M_1, M_2, M_3) histaminic (H_1, H_2), serotonergic ($5HT_1$, $5HT_{1\alpha}$, $5HT_2$, $5HT_3$), α- or β-adrenergic receptors and other neurotransmitter or peptidergic sites.

To further examine whether MEG fumarate may be of interest from the point of view of development as an antiasthmatic agent, we examined whether MEG fumarate antagonizes ACh-induced bronchoconstriction in guinea pigs *in vivo*, circumventing any local airway irritant effect of the compound by administering it systemically. Additionally, to extend our previous findings that MEG base induces bronchoconstriction (4) and to determine whether it may contribute to the detrimental pulmonary effects of crack smoking (8,9), we examined whether lung damage is induced in guinea pigs breathing atmospheres containing condensation aerosols of MEG base or nebulized MEG fumarate.

Methods

Thirty-four adult male guinea pigs (Hartley; Crl:(HA)BR; Charles River, Kingston, NY) weighing 250-350 g were housed, except during experimental sessions, singly in a temperature- and humidity-controlled vivarium (23 °C; 48% humidity) with a 12/12 hr light/dark cycle and allowed food (Purina Guinea Pig Chow) and water *ad libitum*. Twenty-four animals were drug-naive at the start of experiments; these animals were used to determine effects of MEG on indicators of lung injury. For studies of MEG on ACh-induced bronchoconstriction, 10 guinea pigs were used that had previously been exposed to increasing concentrations of nebulized ACh (described below) then administered saline or MEG fumarate (10 mg/kg, s.c.) and reexposed 1 hour later to ACh. At least 7 days elapsed before their use in this experiment.

Bronchoconstriction: During experimental sessions, which were conducted no more than once per week and lasted no more than 2 hours, 10 animals were placed singly in transfer cages containing a layer of bedding, in which they were temporarily housed prior to being placed in a whole-body plethysmograph as described by Agrawal (10). Animals were injected s.c. with saline (1.0 or 3.0 ml/kg) or MEG fumarate (30 and 100 mg/kg in a volume of 1.0 ml/kg; 300 mg/kg was administered in a volume of 3.0 ml/kg because of the solubility limit of MEG fumarate). Three animals did not receive any dose of MEG, but acted as

concurrent saline-treated controls; 3 received saline and MEG fumarate; 4 animals did not receive saline, but received at least 2 doses of MEG fumarate which was synthesized as previously described (4). After injection, animals were returned to their transfer cage and 60-min later placed in the plethysmograph for measurement of SGaw. Any signs observed during the 60-min after injection were noted. Only 1 dose of MEG or saline was given per week and animals were returned to their home cages after experimental sessions.

After measuring baseline SGaw as previously described (4), guinea pigs were exposed to aerosols of phosphate buffered saline (PBS; Dulbecco's, Life Technologies Inc., Grand Island NY) or ACh (0.2-3.2%). Aerosols were produced by nebulization with a DeVilbiss 646 nebulizer (DeVilbiss Healthcare, Somerset PA) operated at 10 psi. This yielded particles with mass median aerodynamic diameters (MMAD) of 3.25 μm, geometric standard deviation (GSD) 2.02. Animals were exposed for 30 sec to PBS and SGaw was measured at the end of exposure; 3.5 min later, animals were exposed in the same manner to doubling concentrations of ACh chloride (i.e., 0.2%, 0.4%, 0.8%, etc.; Sigma, St. Louis, MO; dissolved in PBS). SGaw was measured following each 30-sec exposure; once a 50% or greater decrease in SGaw was observed, guinea pigs were removed from the plethysmograph and returned to their home cage. For each guinea pig, SGaw measured after exposure to PBS or ACh was expressed as a percent of the baseline SGaw.

Lung injury: Six drug-naive guinea pigs were exposed to 1% MEG fumarate nebulized with two 3-jet Collison nebulizers (BGI, Waltham MA) operated at 30 psi, yielding a MMAD of 0.57 μm with a GSD of 1.83. This concentration of MEG fumarate was the highest that could be attained and still produce a particle size of approximately 1.0 μm, comparable to that of the condensation aerosols of MEG base (see below). The concentration of MEG fumarate in air was approximately 0.03 mg/l, much lower than that which could be achieved with MEG base (see below). Thus, MEG fumarate was administered for 6 hours to achieve a delivered dose which could approach that produced by inhalation of MEG base. Animals were exposed in an acrylic chamber measuring 58.4x30.5x30.5 cm with 8 compartments that were separated by wire mesh. Each animal was placed into 1 compartment and could move freely within the confines of the space. The chamber humidity was 40-60%; the aerosol was equilibrated in a 0.82 liter cylinder (10.2 cm ID) before introduction to the exposure chamber. Humidified air was delivered under the same conditions to 6 control animals. Condensation aerosols of MEG base were generated and characterized as previously described (3,4). MEG base was delivered at concentrations of 0 (air), 4.32 ± 0.085 (MMAD 1.50 μm; GSD 2.18), 7.30 ± 0.121 (MMAD 1.25 μm; GSD 2.23) and 16.12 ± 0.433 mg/l (MMAD 1.61 μm; GSD 2.51). Three animals were each exposed for 1-min at each concentration in the plethysmograph and then returned to their home cages.

Approximately 24 hours after exposure to air or aerosols of MEG fumarate or MEG base, guinea pigs were anesthetized by i.m. injection of a solution containing ketamine hydrochloride (100 mg/kg; Bristol Lab., Syracuse, NY) and xylazine (15 mg/kg; Haver Lockhart, Shawnee, KS). After animals were anesthetized (determined by absence of blink and pedal reflexes) they were exsanguinated, their lungs lavaged and lavage fluid assayed as previously described (11). Briefly, 4 aliquots of 7.0 ml of PBS (37 °C) were introduced via a tracheal cannula. The first 2 aliquots were combined and an aliquot removed for total (manual counting on a hemocytometer) and differential (made on Giesma-stained cells adhered to glass slides by centrifugation) cell counts; the remainder was centrifuged (10 min at 400*g*). The supernatant was removed and assayed for total protein and lactate dehydrogenase (LDH) using commercially available kits (BioRAD, Richmond, CA; Sigma).

Results

No differences in baseline SGaw were observed between animals receiving subcutaneous administration of saline or of 30-300 mg/kg MEG fumarate (Table I). The effects of nebulized PBS and ACh (0.2-3.2%) in guinea pigs receiving saline or MEG fumarate (30-300 mg/kg, s.c.) on SGaw are also presented in Table I. The mean SGaw following PBS administration was ≥92% of baseline levels regardless of treatment conditions. Decreases in SGaw were observed in all animals following exposure to ACh, although animals differed in their sensitivity to ACh. For example, following saline administration, 3 animals received 1.6% ACh before exhibiting a >50% decrease in SGaw, 1 animal received 0.8% ACh, 1 animal 0.4% ACh and in the other animal a >50% decrease in SGaw was observed after exposure to 0.2% ACh. Similar differences in sensitivity to ACh were observed in animals treated with 30-300 mg/kg MEG fumarate. Analysis of covariance confirmed a significant interaction between dose of ACh and decrease in SGaw ($F=10.63$; $p=0.002$; d.f.=1). However, as suggested by the data presented in Table I, there was no significant effect of MEG fumarate at any dose on ACh-induced decreases in SGaw ($F=0.595$; $p=0.62$; d.f.=3).

TABLE I

SGaw in Guinea Pigs Breathing Air or Atmospheres Containing Nebulized PBS or ACh Following Subcutaneous Administration of Saline or MEG Fumarate

	MEG fumarate dose (mg/kg s.c.)			
	0	30	100	300
	Mean ± SEM (n) SGaw [$sec^{-1}(cmH_2O)^{-1}$]			
Air	0.29 ± 0.009 (6)	0.29 ± 0.017 (5)	0.28 ± 0.010 (5)	0.29 ± 0.007 (5)
	Mean ± SEM (n) SGaw [percent baseline]			
PBS	97.0 ± 5.95 (6)	94.5 ± 1.29 (5)	93.9 ± 5.67 (5)	92.6 ± 8.00 (5)
ACh 0.2%	84.9 ± 13.04 (6)	75.4 ± 5.26 (5)	64.5 ± 17.58 (5)	72.7 ± 16.42 (5)
ACh 0.4%	80.8 ± 16.22 (5)	60.2 ± 14.68 (5)	71.9 ± 27.03 (3)	67.0 ± 21.36 (3)
ACh 0.8%	65.3 ± 18.90 (4)	39.0 ± 20.56 (3)	23.5 ± 6.76 (2)	68.9 (1)
ACh 1.6%	17.2 ± 3.36 (3)	80.1 (1)	-	14.3 (1)
ACh 3.2%	-	49.4 (1)	-	-

While subcutaneously administered MEG fumarate did not antagonize the effects of ACh, guinea pigs receiving MEG fumarate by this route displayed signs which included: a large number of fecal boli in the transfer cage (20-40 at 300 mg/kg s.c. MEG fumarate, compared to 0-3 for saline-treated animals), salivation, tremors and startle responses when the cage was opened. These signs appeared within 30 min of injection and were most apparent in animals receiving 300 mg/kg MEG fumarate s.c. Animals exposed to aerosols of MEG fumarate also defecated at a rate higher than that of control animals; however, its incidence could not be quantified because of coprophagia. No other signs were observed in these

animals. Animals exposed to aerosols of MEG base did not defecate, but were flaccid and exhibited signs of labored breathing after exposure to the highest concentration.

The effects of aerosols of MEG fumarate and MEG base on LDH and other indicators of lung injury are shown in Table II. MEG base induced significant (oneway ANOVA) changes in protein ($F_{[3,8]}$=13.58; p=0.0017) and LDH ($F_{[3,8]}$=21.97; p=0.0003) levels within the bronchoalveolar lavage fluid. This was attributable solely to the highest concentration of MEG base (simultaneous comparisons, p<0.01). Blood was present in the lavage fluid from 1 animal exposed to 4.32 ± 0.085 mg/l MEG base and 3 animals exposed to 16.12 ± 0.433 mg/l. Inhalation of MEG fumarate did not induce changes indicative of lung damage.

TABLE II

Bronchoalveolar Lavage Fluid Parameters from Guinea Pigs Breathing Air or Aerosols of MEG Base or MEG Fumarate

	n	Viability (%)	LDH (unit/ml)	Protein (µg/ml)	Neutrophils (%)
MEG base (mg/l)					
0 (Air)	3	91.3 ± 1.20	28.1 ± 3.33	133.0 ± 27.42	1.45 ± 0.78
4.32 ± 0.048	3	92.7 ± 0.67	37.7 ± 5.67	190.7 ± 54.87	2.33 ± 1.26
7.30 ± 0.121	3	91.7 ± 2.91	48.9 ± 12.27	197.0 ± 67.09	0.67 ± 0.00
16.12 ± 0.433	3	92.3 ± 0.33	134.2 ± 25.89[a]	1248.3 ± 277.58[a]	5.22 ± 1.42
MEG fumarate (mg/l)					
0 (Air)	6	89.3 ± 3.05	45.2 ± 3.17	141.5 ± 8.27	3.72 ± 1.78
0.03	6	91.2 ± 1.89	47.4 ± 8.89	276.0 ± 123.89	1.56 ± 0.39

[a]Significantly different than air control (p<0.01)

Discussion

The study of the effects of MEG fumarate on ACh-induced bronchoconstriction in guinea pigs was prompted by the finding that MEG fumarate antagonizes contractions of isolated tracheal rings induced by ACh and other spasmogenics *in vitro* (6,7). This finding led to the suggestion that MEG fumarate, or derivatives, may be developed as an antiasthmatic agent (7). In the present study, however, s.c. administration of MEG fumarate neither induced an increase in airway conductance nor did it antagonize the bronchoconstrictor effects of ACh. Thus, we fail to find support for use of MEG fumarate as an antiasthmatic agent when given systemically even at doses that in guinea pigs produce grossly observable signs that the compound was affecting function. Interestingly, these signs, including salivation, tremors and defecation, more closely resemble those expected of a cholinomimetic agent than one exhibiting anticholinergic effects, as MEG fumarate displays *in vitro*; how MEG fumarate induces these effects *in vivo* or *in vitro* are unknown.

Inhalation of aerosols of MEG base by guinea pigs induces bronchoconstriction (4). The present study extends this finding and indicates that inhalation of MEG base also induces lung injury as evidenced by blood, increased LDH (a general marker of cell injury; 11) and protein (a measure of the transudation of serum protein; 11) in the bronchoalveolar lavage fluid. The actual dose of MEG base that was received by guinea pigs is unknown and is dependent upon many factors, not least of which is the pattern of deposition of the inhaled particles; different species exposed to the same particle atmosphere may not show comparable deposition patterns (13). Guinea pigs are nasal obligate breathers and the pattern of deposition in these subjects will be different than that of an orally breathing human where there is likely greater penetration into the lung (13). Thus, an equivalent dose of MEG base may be achieved in humans breathing a lower concentration. Persistent changes in pulmonary function, possibly resulting from damage to the pulmonary epithelia or pulmonary-capillary membrane, have been implicated by studies of humans with self-reported histories of crack abuse (8,9,12). MEG, produced during the pyrolysis of crack cocaine, may contribute to such changes.

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