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Stereoselective Potencies and Relative Toxicities of γ-Coniceine and *N*-Methylconiine Enantiomers

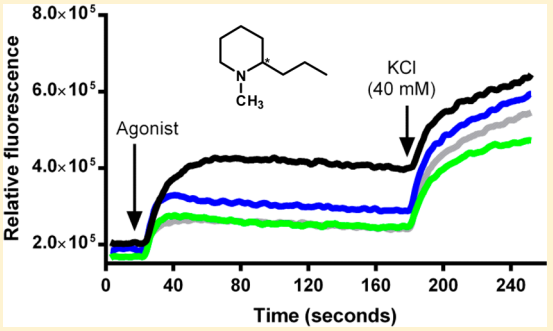
Stephen T. Lee,*[,†] Benedict T. Green,[†] Kevin D. Welch,[†] Glenn T. Jordan,[‡] Qian Zhang,[§] Kip E. Panter,[†] David Hughes,[‡] Cheng-Wei Tom Chang,[§] James A. Pfister,[†] and Dale R. Gardner[†]

[†]Poisonous Plant Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 1150 E. 1400 N., Logan, Utah 84341, United States

[‡]Cincinnati Hills Christian Academy, 11525 Snider Road, Cincinnati, Ohio 45249, United States

[§]Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, Utah 84322-0300, United States

ABSTRACT: γ-Coniceine, coniine, and *N*-methylconiine are toxic alkaloids present in poison hemlock (*Conium maculatum*). We previously reported the comparison of the relative potencies of (+)- and (-)-coniine enantiomers. In this study, we synthesized γ-coniceine and the enantiomers of *N*-methylconiine and determined the biological activity of γ-coniceine and each of the *N*-methylconiine enantiomers *in vitro* and *in vivo*. The relative potencies of these piperidine alkaloids on cells expressing human fetal muscle-type nicotinic acetylcholine receptors had the rank order of γ-coniceine > (−)-*N*-methylconiine > (±)-*N*-methylconiine > (+)-*N*-methylconiine. The relative lethalities of γ-coniceine and (−)-, (±)-, and (+)-*N*-methylconiine *in vivo* using a mouse bioassay were 4.4, 16.1, 17.8, and 19.2 mg/kg, respectively. The results from this study suggest γ-coniceine is a more potent agonist than the enantiomers of *N*-methylconiine and that there is a stereoselective difference in the *in vitro* potencies of the enantiomers of *N*-methylconiine that correlates with the relative toxicities of the enantiomers *in vivo*.



INTRODUCTION

Poison hemlock (*Conium maculatum* L. Umbelliferae) is known worldwide for its toxicity to animals and humans.[1] The principle toxins in *C. maculatum* have been identified as piperidine alkaloids of which γ-coniceine (**1**) and coniine (**2**) are the most prevalent and account for most of the toxicity in the plant.[1,2] However, *N*-methylconiine (**3**), generally considered one of the minor alkaloids in the plant, is a predominant alkaloid in the ripened fruits.[1,3,4] γ-Coniceine (**1**), coniine, (**2**), and *N*-methylconiine (**3**) are also present in other plants, for example, γ-coniceine (**1**) and/or coniine (**2**) have been identified in 12 species in the genus *Aloe*, a genus often promoted for its therapeutic/medicinal properties.[5−8] Interestingly, some of these *Aloe* species have γ-coniceine (**1**) at concentrations higher than those found in poison hemlock.[5]

In adult animals, the three alkaloids have pharmacological properties similar to those of nicotine, although the actions of the hemlock alkaloids are longer lasting.[9,10] γ-Coniceine (**1**), coniine (**2**), and *N*-methylconiine (**3**) (Figure 1) act as agonists at nicotinic acetylcholine receptors (nAChR) with an acute stimulatory phase and then prolonged desensitization of the receptors at higher concentrations.[10] In the body, many of the actions of the three hemlock alkaloids are sensitive to the ganglionic blocking agent hexamethonium, suggesting that the peripheral sites of action of these toxins are autonomic ganglia.[10] One important distinction between the piperidine alkaloids from poison hemlock and the pyridine alkaloid nicotine is the effect of the alkaloids on developing fetuses. Piperidine alkaloids such as coniine cause multiple congenital contracture type defects, while nicotine does not.[11] Recently, we have shown that (−)-coniine (+)-mandelic acid significantly reduced fetal movement for a period of four hours in a goat model, while nicotine was without significant effect on fetal movement.[12] This was attributed to the actions of coniine (**2**) at the fetal muscle-type nAChR in the developing fetus. The potencies and efficacies of γ-coniceine (**1**) and *N*-methylconiine (**3**) at the fetal muscle-type nAChR are not known.

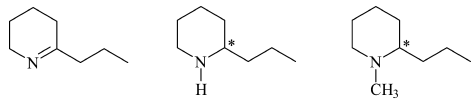


Figure 1. Chemical structures of γ-coniceine (**1**), coniine (**2**), and *N*-methylconiine (**3**). * denotes the asymmetric carbon.

Coniine (**2**) and *N*-methylconiine (**3**), as found in *C. maculatum*, are a mixture of (+)- and (−)-enantiomeric forms.[2] Previously, we reported the separation, isolation, toxicity, and the nAChR agonist potencies of coniine (**2**) enantiomers.[13] In addition, we have also performed similar work with anabasine

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enantiomers from *Nicotiana glauca* and ammodendrine enantiomers from *Lupinus formosus* plants.[14,15] In all cases, we observed differential toxicities between the individual enantiomers.

Our previous results on the toxicities of coniine enantiomers led us to investigate the toxicities of γ-coniceine (**1**) and *N*-methylconiine (**3**) enantiomers. Toxicity information on γ-coniceine (**1**) and *N*-methylconiine (**3**) enantiomers will help to more fully assess the toxicity of poison hemlock and other plants containing these alkaloids (e.g., *Aloe* spp.) as well as comparison of the relative toxicities of these piperidine alkaloids to coniine (**2**). In this study, γ-coniceine (**1**) and the enantiomers of *N*-methylconiine (**3**) were synthesized from readily available commercial reagents. The nAChR agonist potencies of γ-coniceine (**1**) and the enantiomers of *N*-methylconiine (**3**) were assessed using a human tumor cell line (TE-671) expressing fetal muscle-type nAChRs, and the toxicities of the enantiomers were measured in a mouse LD_{50} bioassay.

MATERIALS AND METHODS

Materials. Ammonium hydroxide, *N*,*N*-dimethyl formamide (DMF), and sulfuric acid were obtained from Fisher Scientific (Pittsburgh, PA). 2-Piperidone, *n*-butyryl chloride, and (±)-epibatidine were obtained from Sigma-Aldrich (St Louis, MO). Ammonium acetate was purchased from VWR (Bristol, CT). Fetal bovine serum was obtained from Hyclone, Inc. (Logan, UT). Penicillin/streptomycin was obtained from Invitrogen (Carlsbad, CA). Dulbecco's modified Eagle's medium was from the American Type Culture Collection (ATCC) (Manassas, VA), and the fluorescence dye kits were purchased from Molecular Devices (Sunnyvale, CA). The human rhabdomyosarcoma cell line TE-671 was obtained from ATCC.

γ-Coniceine. γ-Coniceine (**1**) was synthesized as described by Mundy and Larsen.[16] 1-Butanoyl-2-piperidone was prepared by dissolving *n*-butyryl chloride (1.67 g, 15.7 mmol) in 5 mL of dry benzene and then slowly added via syringe to a solution of 2-piperidone (3.10 g, 31.3 mmol) dissolved in 27 mL of dry benzene. The hazy mixture was stirred for several hours and then transferred to a 1-L separatory funnel for workup. The mixture was diluted with 300 mL of CH_2Cl_2, washed (3×) with 10% HCl, and then dried over anhydrous $MgSO_4$. Excess solvent was removed using a rotary evaporator, and the resulting golden colored oil was purified by distillation under reduced pressure to yield 1-butanoyl-2-piperidone (1.19 g, 45%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.87 (t, CH_3), 1.58 (sex, CH_2), 1.75 (m, CH_2−CH_2 of piperidone ring), 2.47 (m, CH_2), 2.79 (t, CH_2), 3.63 ppm (m, CH_2).

γ-Coniceine (**1**) was prepared by slowly adding solid calcium oxide (2.73 g, 48.7 mmol to 1-butanoyl-2-piperidone (2.41 g, 14.3 mmol), in a 50-mL round-bottomed flask assuring thorough mixing. The flask was connected to a distillation column, and the solid mixture was heated under a low flame for 30 min. The snow white powder gradually changed to a light brown/tan color. Heating was gradually increased to distill off the crude product, which was further purified by distillation under reduced pressure to yield γ-coniceine (**1**) (1.04 g, 52%). When freshly distilled, γ-coniceine (**1**) is a faint golden oil that turns red within a few hours at ambient temperature even when protected from exposure to light; therefore, the alkaloid was freshly prepared for toxicity studies. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 0.88 (t, CH_3), 1.51 (m, CH_2−CH_2 of piperidine ring), 1.60 (m, CH_2), 2.06 (m, CH_2 groups attached to ketimine), 3.50 ppm (br t, CH_2 attached to N). ^{13}C NMR ($CDCl_3$, 300 MHz) δ: 13.8, 19.5, 19.7, 21.9, 28.9, 42.9, 48.9, 171.2 ppm.

(±)-, (+)-. and (−)-*N*-Methylconiine. (±)-, (+)-, and (−)-Coniine mandelates were isolated using previously described methods.[13,17] (−)-Coniine (+)-mandelate (814.4 mg, 2.92 mmol) was dissolved in an acetic acid/water/formaldehyde solution (v/v/v 1:1:1, 7 mL) with a catalytic amount of Pd/C and stirred under a hydrogen atmosphere for 48 h. The reaction mixture was then filtered through Celite and the residue washed (methanol, 2 mL). The methanol wash was combined with the filtered acetic acid/water/formaldehyde solution and added to a flask (125 mL, Erlenmeyer) containing Na_2SO_4 (10 g) and $NaHCO_3$ (10 g). Diethyl ether (100 mL) was then added to the acetic acid/water/formaldehyde/Na_2SO_4/$NaHCO_3$ mixture, mixed, allowed to stand for 30 min, and the slurry filtered through glass wool. An additional aliquot of diethyl ether (50 mL) was added to the acetic acid/water/formaldehyde/Na_2SO_4/$NaHCO_3$ mixture and the slurry filtered through the glass wool filter again and added to the previous aliquot of diethyl ether for a total of 150 mL diethyl ether solution. H_2SO_4 (6 M, 0.25 mL, 1.5 mmol) was added to the diethyl ether solution, and then the solution was evaporated to dryness leaving a white solid. Distilled water (20 mL) was added to the white solid and then filtered through a syringe filter resulting in a clear filtrate. The filtrate was added to an ion exchange column (Dowex 1 × 8 (OH^-) 1.5 cm ×5 cm) and eluted with water. *N*-Methylation of coniine (**2**) was confirmed by electrospray ionization-mass spectrometry (ESI-MS) (MH^+ = 142).

The ion exchange eluent was acidified to pH 3 by the dropwise addition of 0.1% formic acid and applied to a 2 g/20 mL Strata X-C SPE cartridge (Phenomenex, Torrance, CA USA) that had been conditioned with MeOH (30 mL) followed by 0.1% formic acid (20 mL). The cartridge was then washed with 0.1% formic acid (30 mL), followed by MeOH (30 mL), and dried under reduced pressure (10 mmHg) for 5 min. The cartridge was eluted twice with NH_3 saturated diethyl ether (30 mL) and the diethyl ether fractions combined. The combined diethyl ether fractions were evaporated under a gentle stream of N_2 at 30 °C to approximately 15 mL and then purged with helium (40 min) to remove NH_3. The diethyl ether solution was further evaporated under a gentle stream of N_2 at 30 °C to approximately 5 mL after which HCl gas was then passed through the diethyl ether solution. As the HCl gas was passed through the diethyl ether solution, a white smoky vapor was emitted. The remaining diethyl ether was evaporated under a gentle stream of N_2 at 30 °C leaving a residue of oily crystals. The residue was crystallized by adding acetone until the residue dissolved. The acetone solution was transferred to a 20 mL screw top vial equipped with a Teflon lined cap (Pierce, Rockford, IL, USA), and an equal volume of hexane was added resulting in a cloudy precipitate. The vial was then capped and heated to approximately 70 °C on a heat block or until all the precipitate dissolved. The heat was then turned off, and the solution was allowed to slowly return to room temperature. As the solution returned to room temperature, fine white needle like crystals formed. The vial was the put into a freezer (−20 °C) for 2 h, removed from the freezer, and the supernatant removed with a disposable pipet. The (−)-*N*-methylconiine-HCl crystals were recrystallized once more with acetone and hexane, washed with hexane, and dried *in vacuo* (87.4 mg, 0.49 mmol, 17% yield): needle-like crystals, mp.155−165 °C; $[\alpha]^{23.5}_D$ = −30.5° (c = 0.56, MeOH). The same procedure was used to obtain (+)-*N*-methlyconiine-HCl from the (+)-coniine (−)-mandelate: needle-like crystals, mp. 159−164 °C; $[\alpha]^{23.5}_D$ = +29.4° (c = 0.60, MeOH). (±)-*N*-Methylconiine-HCl: needle-like crystals, mp. 154−156 °C; $[\alpha]^{23.5}_D$ = +1.74° (c = 0.46, MeOH).

Acute Toxicity Determinations. Known amounts of γ-coniceine (**1**), (±)-*N*-methylconiine-HCl, (+)-*N*-methylconiine-HCl, and (−)-*N*-methylconiine-HCl were dissolved in physiological buffered saline solution at concentrations of 1 mg/mL. The solutions were stored in sterile injection vials for toxicity testing. Seventy-five male Swiss-Webster mice weighing 15 to 20 g (Simonsen Laboratories, Gilroy, CA) were dosed i.v. via the tail vein. Prior to injection, the mice were randomly divided into 3 groups of 25 and maintained under a heat lamp for 15 min to dilate the tail vein. The tail vein was cleaned with 70% ethanol, and i.v. injections were accomplished with a tuberculin syringe equipped with a 1.27-cm-long 27-gauge needle. The volume injected (0.05−0.2 mL) varied depending on the size of the mouse and dose delivered. Time of injection, clinical effects, and time of death were noted. Mice were closely observed for 1 h after the injection. The protocol for animal use in this research was reviewed

and approved by the Institutional Animal Care and Use Committee (IACUC), Utah State University, Logan, Utah.

The LD_{50} for individual alkaloid toxicity was determined by a modified up-and-down method[18] and was calculated using the PROC PROBIT procedures of SAS (SAS Institute Inc., Cary, NC) on a logistic distribution of the survival data. Analysis of variance was used to compare the LD_{50} for the various alkaloids; after a significant F test ($P < 0.05$), the Bonferroni test was used to compare the individual means.

Agonist Actions at Fetal Muscle-Type nAChR. Membrane depolarization responses from the addition of nAChR agonists were measured by changes in fluorescence of a membrane-potential-sensitive dye as previously described.[12,19] Responses were calculated as equal to: $(F_{Max}(\text{compound}) - F_{Basal})/(F_{Max}(\text{calibrant}) - F_{Basal})$, and the depolarizing responses to agonists were normalized to the maximum response generated by (±)-epibatidine, which was considered 100% activation. The normalized data was then curve-fitted with a sigmoidal dose−response equation with variable slope, and the curves of the normalized data were constrained to 0 and 100% of the maximal epibatidine response and graphed with Prism version 6.01 (GraphPad Software, San Diego, CA, USA).[13] The γ-coniceine (**1**) response was decreased (desensitized) at concentrations above 10 μM; therefore, those concentrations of the alkaloid were excluded from the EC_{50} calculation as previously described.[19]

RESULTS AND DISCUSSION

γ-Coniceine (**1**) was prepared using a two step procedure.[16] Acylation of 2-piperidone by reaction with *n*-butyryl chloride to form 1-butanoyl-2-piperidone was followed by *N*-acyllactam rearrangement of 1-butanoyl-2-piperidone in the presence of calcium oxide afforded γ-coniceine (**1**).[20]

(±)-*N*-Methylconiine (**3**), (+)-*N*-methylconiine (**3**), and (−)-*N*-methylconiine (**3**) were prepared by *N*-methyl alkylation at the secondary nitrogen of (±)-, (+)-, and (−)-coniine (**2**), which were previously isolated from (±)-coniine (**2**) using preferential crystallization with (+)- and (−)-mandelic acid.[13] After isolation of the (±)-, (+)-, and (−)-*N*-methylconiine (**3**) from the reaction mixtures, HCl salts of (±)-, (+)-, and (−)-*N*-methylconiine (**3**) were formed by reaction with HCl gas. The measured optical rotations for (+)-*N*-methylconiine (**3**) ($[\alpha]^{23.5}_D = +29.4°$) and (−)-*N*-methylconiine (**3**) ($[\alpha]^{26.1}_D = -30.5°$) were equal and opposite confirming that they were isolated enantiomers, while the measured optical rotation for (±)-*N*-methylconiine (**3**) ($[\alpha]^{23.8}_D = -1.74°$) confirmed it was a near racemic mixture.

The toxicities of γ-coniceine (**1**) and the *N*-methylconiine (**3**) enantiomers were compared using a mouse bioassay. Onset of clinical signs was almost immediate after injection, beginning with piloerection, tail flicking, and rapidly progressing to intention tremors, clonic convulsions, muscular weakness, lateral recumbency, and death. Typically, death occurred within 1−2 min of injection, or recovery was imminent and complete within 1 h. The acute lethality values of the alkaloids, as depicted by their LD_{50} values in mice, were 4.4, 16.1, 17.8, and 19.2 mg/kg for γ-coniceine (**1**), (−)-*N*-methylconiine (**3**), (±)-*N*-methylconiine (**3**), and (+)-*N*-methylconiine (**3**), respectively (Table 1).

The pharmacological potencies of γ-coniceine (**1**) and *N*-methylconiine (**3**) were determined in TE-671 cells. The representative tracings of responses from single wells of each compound from the same experiment are depicted in Figure 2. The TE-671 cells had a stable baseline basal fluorescence during the first 17 s of the experiment. Upon the addition of γ-coniceine (**1**) and *N*-methylconiine (**3**), there were rapid increases in fluorescence associated with changes in membrane potential from the activation of nAChR. After 180 s, a KCl solution was added to the wells to achieve a final concentration of 40 mM, which served as the calibrant for the calculation of the compound responses, as previously described.[15,21]

Table 1. LD_{50} and EC_{50} Values for γ-Coniceine and the Enantiomers of Coniine and *N*-Methylconiine[a]

alkaloid	LD_{50} (SE) (mg/kg)	EC_{50} (95% C.I.) (μM)
γ-coniceine	4.4 a (0.08)	1.3 (0.020−12)
(−)-coniine	7.0 b (0.07)	117 (63.0−197)
(±)-coniine	7.7 b (0.1)	340 (170−683)
(+)-coniine	12.1 c (0.08)	934 (610−1430)
(−)-*N*-Methylconiine	16.1 d (0.3)	105 (49.0−226)
(±)-*N*-methylconiine	17.8 e (0.6)	405 (288−568)
(+)-*N*-methylconiine	19.2 f (0.2)	3000 (1200−7580)

[a]LD_{50} values with different letters indicate significant differences ($P < 0.007$). LD_{50} and EC_{50} data for coniine are from ref 11.

The concentration−effect relationships for γ-coniceine (**1**) and the *N*-methylconiine (**3**) enantiomers on TE-671 cells are displayed in Figure 3, and the EC_{50} values and 95% confidence limits are listed in Table 1. As described above, the responses of the TE-671 cells to the alkaloids were normalized to the maximal epibatidine responses of 1 μM for γ-coniceine (**1**) and 100 μM for *N*-methylconiine (**3**). The relative order of potency for the alkaloids on TE-671 cells was γ-coniceine (**1**) > (−)-*N*-methylconiine (**3**) > (±)-*N*-methylconiine (**3**) > (+)-*N*-methylconiine (**3**). The efficacies of the alkaloids at 1 mM concentration relative to the maximal epibatidine response were 65 ± 32%, 66 ± 6%, 62 ± 6%, and 36 ± 6% for γ-coniceine (**1**) and the (−)-, (±)-, and (+)- enantiomers of *N*-methylconiine (**3**), respectively.

In addition to the data reported in this study regarding the lethality and potency of γ-coniceine (**1**) and *N*-methylconiine (**3**), Table 1 also includes the LD_{50} and EC_{50} values for (±)-coniine (**2**) and (−)- and (+)-coniine (**2**) reported previously.[13] The rank order of lethality (LD_{50}) and potency (EC_{50}) for these alkaloids is γ-coniceine (**1**) > coniine (**2**) > *N*-methylconiine (**3**). This rank order is consistent with the data reported by Bowman and Sanghvi.[10] These data suggest that the presence/absence of the double bond (between the alpha carbon and the nitrogen) in γ-coniceine (**1**)/coniine (**2**) imparts a more critical role in the toxicity of these alkaloids than the absence/presence of the methyl group in coniine (**2**)/*N*-methylconiine (**3**). Additionally, a comparison of the lethality and potency of the enantiomers of coniine (**2**) and *N*-methylconiine (**3**) indicates that the (−)-enantiomers of each alkaloid were more lethal/potent than (+)-enantiomers of their corresponding pair in both cases with the racemic mixtures intermediate in potency.

In addition to acute toxicity, poison hemlock is also reported to cause chronic toxicity and teratogenicity with γ-coniceine (**1**), coniine (**2**), and *N*-methylconiine (**3**) as the principle toxic compounds.[22,23] Chronic toxicity is manifested as birth defects in offspring from pregnant animals exposed to poison hemlock during critical periods of gestation, e.g., days 40−100 in cattle[24,25] and days 30−60 in swine[26,27] and sheep.[28] The proposed mechanism for birth defects due to poison hemlock consumption is reduction in fetal movement due to γ-coniceine (**1**), coniine (**2**), and *N*-methylconiine (**3**) acting as neuromuscular blocking agents.[29] Panter et. al dosed pregnant goats with conium seed on days 30−60 of gestation. They used radio-ultrasound to observe reduction in fetal movement and

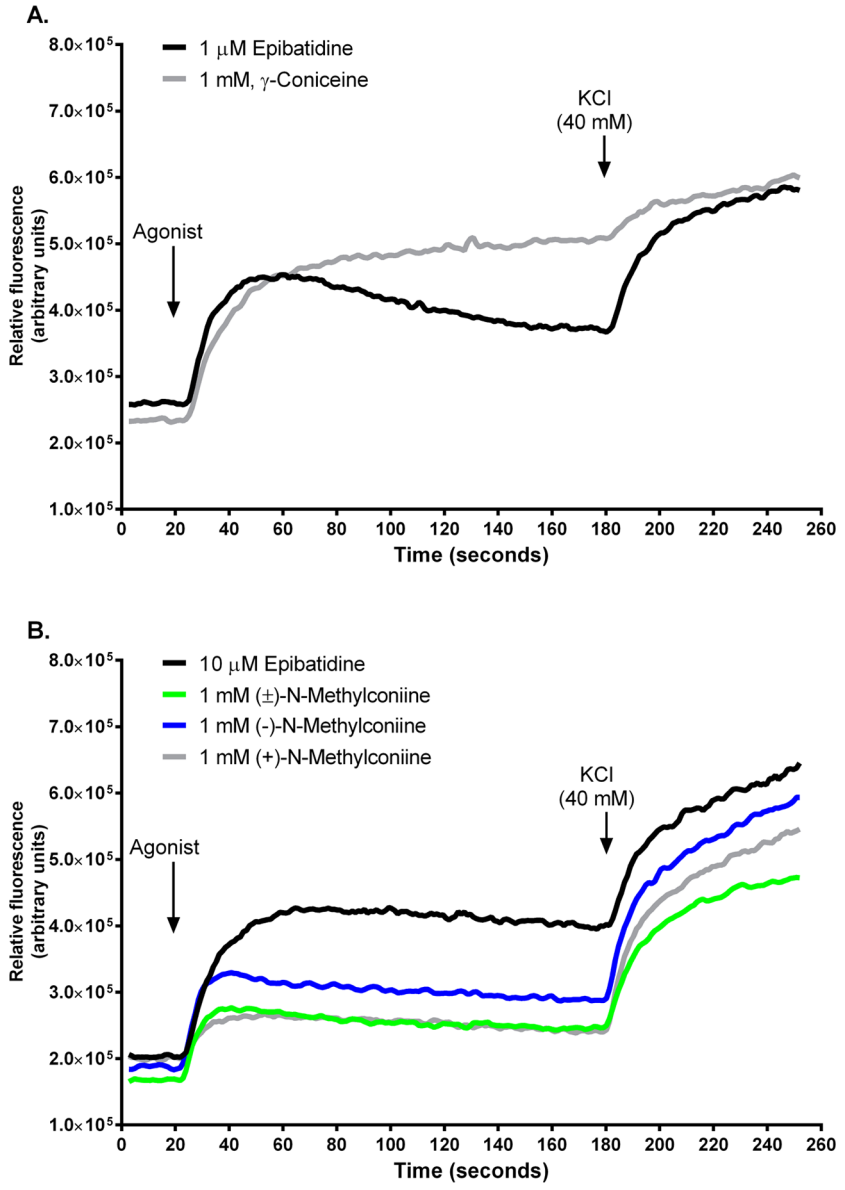

Figure 2. Change in the relative fluorescence of a membrane potential sensing dye in TE-671 cells over 250 s. The traces are representative of a typical epibatidine; γ-coniceine (**1**), panel A, and *N*-methylconiine (**3**), panel B, actions on TE-671 cells. The compounds were added at the times indicated by arrows. Responses represent the concentration of agonist which elicited the maximum membrane depolarization for each compound indicated in the figure legend in its individual 96-well plate experiment.

ultimately observed severe limb, spine, neck defects, and cleft palates in the neonates.[29] Recently, we showed that (−)-coniine was significantly more effective in reducing fetal movement than (+)-coniine over a period of four hours in 40-day pregnant goats.[12] On the basis of previous experiments with coniine (**2**) enantiomers, it is likely that there are also differences in the teratogenic potential of the two *N*-methylconiine (**3**) enantiomers. The relative teratogenic activity of these piperidine alkaloids should be further assessed by measuring the reduction in fetal movement in a pregnant goat model.[29−31]

The results from this study confirm previous structure−activity and receptor-based studies suggesting that the molecular target of coniine is nAChR. Previous work at this laboratory has shown that coniine (**2**) is a nAChR agonist and thus at high concentrations desensitizes the nAChRs to further stimulation, which results in flaccid muscle paralysis in animals.[12] Furthermore, the actions of coniine (**2**) in TE-671 cells can be blocked by the nAChR antagonists α-conotoxin EI and GI providing pharmacological evidence for the receptor-mediated mechanism of action.[12] Other work at this laboratory by Keeler and Balls who screened multiple piperidine alkaloids including γ-coniceine (**1**), coniine (**2**), piperidine, 2-methylpiperidine, 2-ethylpiperidine, 3-methylpiperidine, *N*-methylpiperidine, and 2-piperidine-ethanol has provided structure−activity data.[22] Results from that research suggest that piperidine alkaloids with a carbon side chain of at least three carbons or larger attached to the carbon alpha to the piperidine nitrogen are teratogenic.[22] The presence of a double bond adjacent to the nitrogen such as that found in γ-coniceine (**1**) increases the teratogenic potential of the alkaloids.[22] Results from this work with γ-coniceine (**1**) and *N*-methyl-coniine (**3**) document that they, like coniine, are agonists at fetal muscle-type nAChR and that they have the correct structural features to be teratogens through the inhibition of fetal movement via

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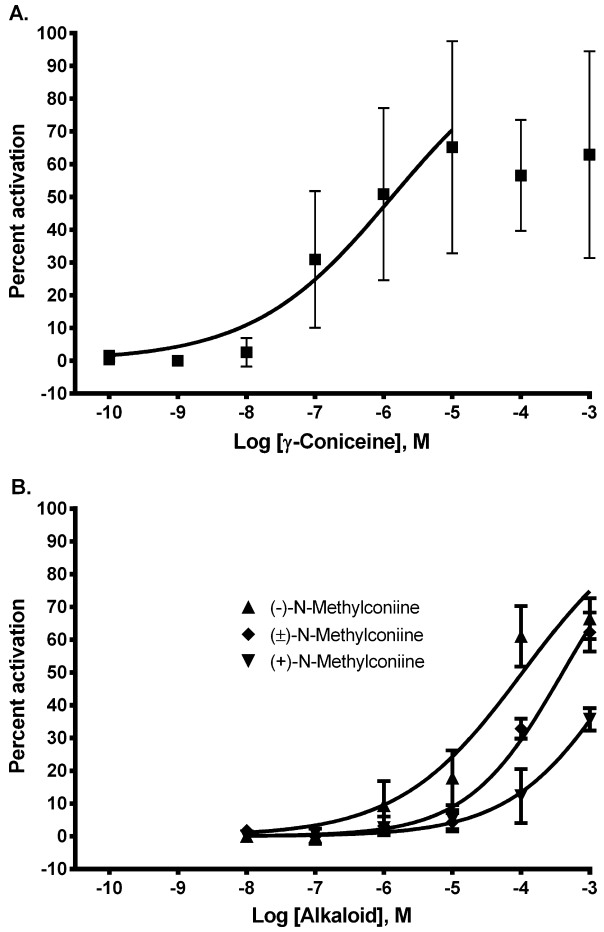

Figure 3. Concentration−effect relationships with best-fit lines for the actions of γ-coniceine (**1**) and *N*-methylconiine (**3**) on membrane potential sensing dye fluorescence in TE-671 cells. Panel A: γ-coniceine (**1**). Panel B: *N*-methylconiine (**3**). In each experiment, the membrane depolarization resulting from the addition of epibatidine (data not shown) and the alkaloids in $\log_{10}$ molar concentrations was measured and displayed as a percentage of the maximal epibatidine response. Each data point represents three experiments of duplicate wells for γ-coniceine (**1**) and six experiments of duplicate wells for *N*-methylconiine (**3**) normalized to the maximum epibatidine response at 1 μM for γ-coniceine (**1**) and 100 μM for *N*-methylconiine (**3**).

the desensitization of the fetal muscle-type nAChR. Moreover, we have recently shown that coniine (**2**) can abolish fetal movement in goats at day 40 of gestation, in an enantiomer-selective manner.[12] These results provide evidence that a receptor-mediated blockade of muscle cell function and inhibition of fetal movement is responsible for multiple congenital type contracture defects in livestock.

In conclusion, the *in vitro* results from this study suggest that γ-coniceine (**1**) is a more potent agonist than *N*-methylconiine (**3**) for nAChR and that (−)-*N*-methylconiine (**3**) is a more potent agonist than (+)-*N*-methylconiine (**3**). This *in vitro* data was corroborated by the *in vivo* acute lethality data, in that γ-coniceine (**1**) was more toxic to mice than *N*-methylconiine (**3**) and that (−)-*N*-methylconiine (**3**) was more toxic to mice than (+)-*N*-methylconiine (**3**). Comparison of previous work with coniine (**2**) suggests that (±)-coniine (**2**) may be a more potent agonist than (±)-*N*-methylconiine (**3**) but that γ-coniceine (**1**) is a much more potent agonist than coniine (**2**) and *N*-methylconiine (**3**). These results indicate that both γ-coniceine (**1**) and *N*-methylconiine (**3**) are agonists at fetal muscle-type nAChR and that binding of *N*-methylconiine (**3**) to the nAChR and toxicity of *N*-methylconiine (**3**) are stereospecific processes.

AUTHOR INFORMATION

Corresponding Author

*Tel: (435) 752-2941. Fax: (435) 753-5681. E-mail: stephen.lee@ars.usda.gov.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

$CDCl_3$, deuterated chloroform; DMF, *N*,*N*-dimethyl formamide; EC_{50}, median effective concentration; ESI-MS, electrospray ionization mass spectroscopy; Hg, mercury; H_2SO_4, sulphuric acid; HCl, hydrochloric acid; IACUC, Institutional Animal Care and Use Committee; i.v., intravenous; KCl, potassium chloride; LD_{50}, median lethal dose; MeOH, methanol; $MgSO_4$, magnesium sulfate; nAChR, nicotinic acetylcholine receptor; NH_3, ammonia; NMR, nuclear magnetic resonance spectroscopy; N_2, nitrogen; Pd/C, palladium on carbon; TE-671, human rhabdomyosarcoma cell line TE-671; $NaHCO_3$, sodium sulfate; Na_2SO_4 (VI), sodium bicarbonate; SPE, solid phase extraction

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