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Additive antinociceptive effects of mixtures of the kappa opioid receptor agonist spiradoline and the cannabinoid receptor agonist CP55940 in rats

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Abstract

Objective—Pain is a significant clinical problem, and there is a need for pharmacotherapies that are more effective with fewer adverse effects. Cannabinoid receptor agonists enhance the antinociceptive effects of mu opioid receptor agonists; it is unclear whether they impact the effects of agonists acting at other opioid receptors. Kappa opioid receptor agonists have antinociceptive effects but their clinical use is precluded by adverse effects; however, their therapeutic potential might be realized if their antinociceptive effects could be selectively enhanced.

Methods—The antinociceptive effects of the cannabinoid receptor agonist CP55940 and the kappa opioid receptor agonist spiradoline, alone and in combination, were studied in rats (n=7) using a warm water tail withdrawal procedure.

Results—Alone CP55940 (0.032–1.0 mg/kg) and spiradoline (1.0–32.0 mg/kg) increased tail withdrawal latency and mixtures of CP55940 and spiradoline (ratios of 1:3, 1:1, and 3:1) produced additive effects.

Conclusions—The antinociceptive effects of a kappa opioid receptor agonist and a cannabinoid receptor agonist were additive. It remains to be determined whether this interaction is selective for antinociception and whether it generalizes to other drugs.

Keywords

antinociception; thermal nociception; cannabinoid receptor agonist; kappa opioid receptor agonist; drug-drug interactions; rats

Introduction

Pain remains a significant clinical challenge (Gaskin and Richard, 2012). Mu opioid receptor agonists are commonly used to treat moderate to severe pain; however, their use is limited by unwanted effects (Benyamin et al., 2008) including overdose and abuse (Atluri et al., 2014). There is a need for safer, more effective pharmacotherapies that have fewer adverse effects than currently available medications.

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Often drugs are used in combination to treat pain, allowing for the possibility that smaller doses can be combined to maintain or improve analgesic effectiveness while reducing or avoiding unwanted effects associated with larger doses of either drug alone (Gilron et al., 2013). Cannabinoid receptor agonists (e.g., delta-9-tetrahydrocannabinol [Δ^9-THC]) have antinociceptive effects (Pertwee, 2001), are used to treat pain (Hosking and Zajicek, 2008; Ware et al., 2010), and enhance the antinociceptive effects of mu opioid receptor agonists (Cichewicz, 2004; Welch, 2009; Li et al., 2008; Maguire et al., 2013; Maguire and France, 2014). It is unclear whether cannabinoid receptor agonists enhance the antinociceptive effects of other drugs, including agonists acting at other opioid receptors.

Kappa opioid receptor agonists such as spiradoline, a potent congener of U50488, have antinociceptive effects (VonVoigtlander and Lewis, 1988) but their use is precluded by adverse effects (Pfeiffer et al., 1986; Walsh et al., 2001). However, their therapeutic potential might be realized if antinociceptive effects could be selectively enhanced, for example, by co-administration with another drug. Kappa receptors appear to play a role in the antinociceptive effects of cannabinoids (Smith et al., 1994; Pugh et al., 1995; Reche et al., 1996; Pugh et al., 1997; Mason et al., 1999), suggesting that mixtures of cannabinoids and kappa opioids might be effective for treating pain. Few studies (e.g., Welch 1993) have investigated interactions between the antinociceptive effects of cannabinoids and kappa opioids and the nature of interactions between these classes of agonists remains unclear.

This study examined the antinociceptive effects of spiradoline and the synthetic cannabinoid receptor agonist CP55940 alone and in combination in rats using a warm water tail withdrawal procedure. If cannabinoid receptor agonists enhance the antinociceptive effects of kappa opioid receptor agonists, mixtures of cannabinoids and kappa opioids might be useful for treating pain.

Methods

Animals

Seven male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), approximately 14 months old were housed under conditions described previously (Maguire et al., 2014). Water was available continuously in the home cage and chow (15–20 g/day; Rat Sterilizable Diet, Harlan Teklad) was provided post-session, maintaining body weight at 420±15 g. Rats had previously received drugs (unpublished data) although not for at least 3 months prior to this study and they had no experience with antinociception procedures. Tests occurred no more than once weekly. Rats were cared for according to local IACUC and National Institutes of Health guidelines.

Apparatus and procedure

Water baths (EW-14576-00, Cole-Parmer, Vernon Hills, IL) maintained water at 40, 50, or 55°C. During tests, approximately 5 cm of the tail was immersed, and the time until the tail was removed was recorded with a stopwatch. A test ended when the tail was completely removed or when the timer reached 20 sec (maximum effect), whichever occurred first. Each temperature was tested once per cycle with approximately 30 sec between tests; the

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order in which temperatures were tested varied across cycles. Rats were weighed, received a vehicle injection, and returned to their home cage; 30 min later tail withdrawal latencies were measured. Immediately after that first test, rats received another injection and were returned to the home cage, followed 30 min later by another test. Sessions lasted until a 20-sec latency was reached with 50°C or for 6 cycles, whichever occurred first.

Dose-effect curves were determined for spiradoline alone then for CP55940 alone. Sessions began with a vehicle test, followed by tests with increasing cumulative doses (1/2-log unit) of spiradoline or CP55940 (Table 1). Dose-effect curves were then determined for mixtures; sessions began with a vehicle test, followed by increasing cumulative doses of spiradoline and CP55940. The ratio (1:1, 3:1, or 1:3) of CP55940 to spiradoline in the mixture remained constant for all cycles of a test session. Doses of mixtures were determined from doses that produced 50% of the maximum possible effect (ED_{50}) for the group mean dose-effect curves for CP55940 and spiradoline (50°C) alone.

Data Analyses

Latencies were expressed as a percentage of maximal possible effect (MPE) as follows: %MPE = [(test latency – control latency)/(20 sec – control latency)] × 100. Control latencies were determined each session for individual subjects during the first test (after vehicle). Because latencies with 40°C were greater than 18 sec (90% MPE) and latencies with 55°C rarely exceeded 7 sec (35% MPE), results with these temperatures were not analyzed. A sigmoidal curve was fitted to the 50°C data for individual rats to determine the slope and ED_{50} of the dose-effect curve, with the minimum and maximum constrained to 0 and 100%, respectively.

The interaction between CP55940 and spiradoline was examined as described by Tallarida (2000; 2006). For each rat, the slope and ED_{50} of dose-effect curves were used to convert doses of CP55940 into spiradoline-equivalent doses. The doses of spiradoline and spiradoline-equivalent doses (CP55490) in each mixture were summed to determine the additive total dose (in spiradoline-equivalent doses) which was used to determine the predicted effect. Predicted effects were averaged across rats and a straight line was fit to the group dose-effect curve using linear regression. Finally, the group dose-effect curve for predicted effects was compared to the group dose-effect curve for observed effects using an extra sum-of-squares F test (Motulsky and Christopoulos 2003). Data for each ratio were analyzed separately. Curve fitting and statistical analyses were conducted using GraphPad Prism version 5.0 (San Diego, CA).

Drugs

CP55940 (2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol, Sigma-Adlrich, St. Louis, MO) was dissolved in a 1:1:18 solution of absolute ethanol, Emulphor-620 (Rhone-Poulenc Inc., Princeton, NJ), and 0.9% saline. When administered alone, spiradoline (U62066; Upjohn, Kalamazoo, MI) was dissolved in 0.9% saline and when administered in a mixture in a 1:1:18 solution containing CP55940. Doses are expressed in terms of the salt; injections (1.0 ml/kg) were administered i.p.

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Results

Mean tail-withdrawal latencies from 50°C water were 6.0 and 4.3 sec following 1:1:18 vehicle and saline injections, respectively. Tail withdrawal latency with 50°C increased with increasing doses of CP55940 or spiradoline (open symbols, left and center panels of Figure 1A), with the group mean (95% CL) ED_{50} of 0.17 (0.08–0.34) mg/kg for CP55940 and 9.56 (3.96–23.07) mg/kg for spiradoline. Mixtures of CP55940 and spiradoline dose-dependently increased tail withdrawal latency (filled symbols, left and center panels of Figure 1A), with larger doses producing at least 75% MPE. Plotted on an isobologram (Figure 1B), results indicate that the ED_{50} for each dose ratio fell near the line of additivity and within the 95% CL. Observed effects for each dose ratio (filled symbols, Figure 1C) were not significantly different from the predicted effects (open symbols; [$F(2,66)=1.03$, $p=.36$ for 1:3; $F(2,52)=0.29$, $p=.75$ for 1:1; and $F(2,52)=1.02$, $p=.37$ for 3:1]).

Discussion

There is a need for pain treatments that are more effective with fewer adverse effects than currently available drugs (e.g., oxycodone). Kappa opioid receptor agonists have antinociceptive effects but their use is precluded by adverse effects (Pfeiffer et al. 1986); the therapeutic potential of kappa receptor agonists might be realized if their antinociceptive effects could be enhanced. The study examined whether a cannabinoid receptor agonist enhances the antinociceptive effects of a kappa opioid receptor agonist.

Consistent with previous studies (Tseng and Craft 2001; Terner et al. 2003), CP55940 and spiradoline dose-dependently increased tail withdrawal latency when administered alone. Moreover, observed effects of mixtures were not different from expected effects based on dose-addition analysis. These data are consistent with a previous report showing that mixtures of selected doses of Δ^9-THC and the kappa opioid receptor agonist U50488 have antinociceptive effects in mice (Welch 1993). To the extent that smaller doses of kappa opioid receptor agonists (i.e., in a mixture) produce fewer and less severe adverse effects, these data suggest that mixtures might be useful for treating pain.

Kappa opioid receptor agonists have aversive effects in nonhuman (Mucha and Herz, 1985) and human (Pfeiffer et al., 1986) subjects which limit their therapeutic use; cannabinoid receptor agonists also have aversive effects (McGregor et al. 1996) and they are abused (Braida et al. 2001). Mixtures of kappa opioid receptor agonists and cannabinoid receptor agonists would not be useful if interactions between the adverse effects of these drugs were additive or greater than additive. However, the therapeutic utility (margin of safety) of kappa receptor agonist might be increased if cannabinoid receptor agonists attenuate the adverse effects of kappa receptor agonists or if the interaction between these classes of drugs, for adverse effects, is less than additive (i.e., sub-additive).

These results indicate that mixtures of the kappa opioid receptor agonist spiradoline and the cannabinoid receptor agonist CP55940 produce additive effects in a warm water tail withdrawal procedure in rats. It remains to be determined whether the nature of the interaction differs with mixtures of other drugs or with effects other than antinociception.

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References

Atluri S, Sudarshan G, Manchikanti L. Assessment of the trends in medical use and misuse of opioid analgesics from 2004 to 2011. Pain physician. 2014; 17:E119–E128. [PubMed: 24658483]

Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, Glaser SE, Vallejo R. Opioid complications and side effects. Pain Physician. 2008; 11:S105–S120. [PubMed: 18443635]

Braida D, Pozzi M, Cavallini R, Sala M. Conditioned place preference induced by the cannabinoid agonist CP 55,940: interaction with the opioid system. Neuroscience. 2001; 104:923–926. [PubMed: 11457579]

Cichewicz DL. Synergistic interactions between cannabinoid and opioid analgesics. Life Sci. 2004; 74:1317–24. [PubMed: 14706563]

Gaskin DJ, Richard P. The economic costs of pain in the United States. J Pain. 2012; 13:715–24. [PubMed: 22607834]

Gilron I, Jensen TS, Dickenson AH. Combination pharmacotherapy for management of chronic pain: from bench to bedside. Lancet Neurol. 2013; 12:1084–95. [PubMed: 24074723]

Hosking RD, Zajicek JP. Therapeutic potential of cannabis in pain medicine. Br J Anaesth. 2008; 101:59–68. [PubMed: 18515270]

Li JX, McMahon LR, Gerak LR, Becker GL, France CP. Interactions between Δ9-tetrahydrocannabinol and μ opioid receptor agonists in rhesus monkeys: discrimination and antinociception. Psychopharmacology (Berl). 2008; 199:199–208. [PubMed: 18470505]

Maguire DR, France CP. Impact of efficacy at the μ-opioid receptor on antinociceptive effects of combinations of μ-opioid receptor agonists and cannabinoid receptor agonists. J Pharmacol Exp Ther. 2014; 351:383–9. [PubMed: 25194020]

Maguire DR, Henson C, France CP. Effects of amphetamine on delay discounting in rats depend upon the manner in which delay is varied. Neuropharm. 2014; 87:173–179.

Maguire DR, Yang W, France CP. Interactions between μ-opioid receptor agonists and cannabinoid receptor agonists in rhesus monkeys: antinociception, drug discrimination, and drug self-administration. J Pharmacol Exp Ther. 2013; 345:354–62. [PubMed: 23536317]

Mason DJ, Lowe J, Welch SP. Cannabinoid modulation of dynorphin A: correlation to cannabinoid-induced antinociception. Eur J Pharmacol. 1999; 378:248–73.

McGregor IS, Issakidis CN, Prior G. Aversive effects of the synthetic cannabinoid CP 55,940 in rats. Pharmacol Biochem Beh. 1996; 53:657–64.

Motulsky, H.; Christopoulos, A. Fitting models to biological data using linear and nonlinear regression: a practical guide to curve fitting. Oxford University Press; 2003.

Mucha RF, Herz A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology (Berl). 1985; 86:274–80. [PubMed: 2994144]

Pertwee RG. Cannabinoid receptors and pain. Prog Neurobiol. 2001; 63:569–611. [PubMed: 11164622]

Pfeiffer A, Brantl V, Herz A, Emrich HM. Psychotomimesis mediated by kappa opiate receptors. Science. 1986; 233:774–6. [PubMed: 3016896]

Pugh G, Abood ME, Welch SP. Antisense oligodeoxynucleotides to the κ-1 receptor block the antinociceptive effects of Δ9-THC in spinal cord. Brain Res. 1995; 689:157–8. [PubMed: 8528701]

Pugh G, Mason DJ, Combs V, Welch SP. Involvement of dynorphin B in the antinociceptive effects of the cannabinoid CP55,940 in the spinal cord. J Pharmacol Exp Ther. 1997; 281:730–7. [PubMed: 9152379]

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Reche I, Fuentes JA, Ruiz-Gayo M. A role for central cannabinoid and opioid systems in peripheral Δ9-tetrahydrocannabinol-induced analgesia in mice. Eur J Pharmacol. 1996; 301:75–81. [PubMed: 8773449]

Smith PB, Welch SP, Martin BR. Interactions between Δ9-tetrahydrocannabinol and k opioids in mice. J Pharmacol Exp Ther. 1994; 268:1381–7. [PubMed: 8138952]

Tallarida, RJ. Drug synergism and dose-effect data analysis. Boca Raton: Chapman Hall/CRC Press; 2000.

Tallarida RJ. An overview of drug combination analysis with isobolograms. J Pharmacol Exp Ther. 2006; 319:1–7. [PubMed: 16670349]

Terner JM, Barrett AC, Cook CD, Picker MJ. Sex differences in (−)-pentazocine antinociception: comparison to morphine and spiradoline in four rat strains using a thermal nociceptive assay. Behav Pharmacol. 2003; 14:77–85. [PubMed: 12576884]

Tseng AH, Craft RM. Sex differences in antinociceptive and motoric effects of cannabinoids. Eur J Pharmacol. 2001; 430:41–7. [PubMed: 11698061]

VonVoigtlander PF, Lewis RA. Analgesic and mechanistic evaluation of spiradoline, a potent kappa opioid. J Pharmacol Exp Ther. 1988; 246:259–62. [PubMed: 2839665]

Walsh SL, Strain EC, Abreu ME, Bigelow GE. Enadoline, a selective kappa opioid agonist: comparison with butorphanol and hydromorphone in humans. Psychopharmacology (Berl). 2001; 157:151–62. [PubMed: 11594439]

Ware MA, Wang T, Shapiro S, Robinson A, Ducruet T, Huynh T, Gamsa A, Bennett GJ, Collet J-P. Smoked cannabis for chronic neuropathic pain: a randomized controlled trial. Can Med Assoc J. 2010; 182:E694–E701. [PubMed: 20805210]

Welch SP. Blockade of cannabinoid-induced antinociception by norbinaltorphimine, but not N, N-diallyl-tyrosine-Aib-phenylalanine-leucine, ICI 174,864 or naloxone in mice. J Pharmacol Exp Ther. 1993; 265:633–40. [PubMed: 8388455]

Welch SP. Interaction of the cannabinoid and opioid systems in the modulation of nociception. Int Rev Psychiatry. 2009; 21:143–51. [PubMed: 19367508]

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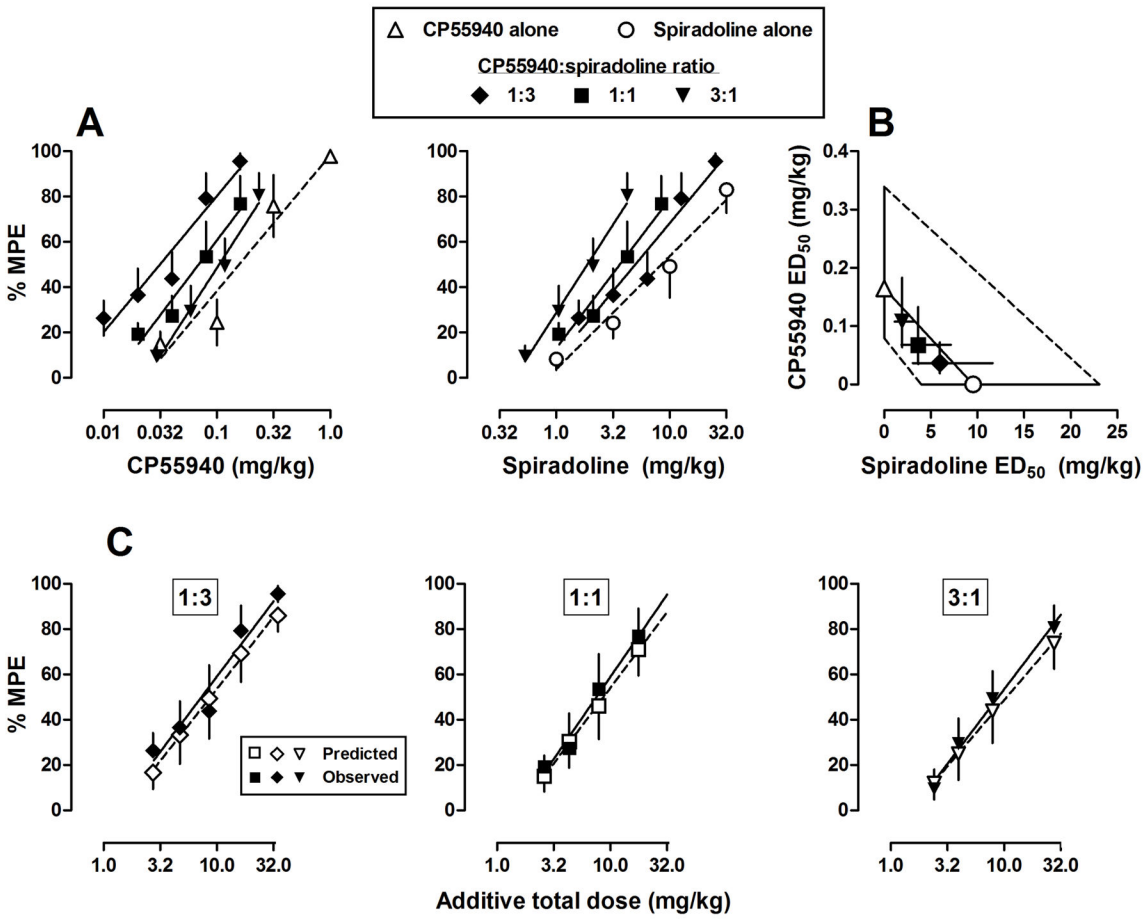

Figure 1.

(A) Antinociceptive effects of cumulative doses of CP55940 and spiradoline alone (open symbols) and in combination (filled symbols) with 50°C water in rats (n = 7) with an inter-injection interval of 30 minutes. The ratio of CP55940 to spiradoline in the mixture, 1:3 (diamonds), 1:1 (squares), and 3:1 (inverted triangles), varied across tests. Abscissae: dose in milligrams per kilogram body weight. Ordinate: % maximum possible effect (MPE) ± 1 SEM (see "Data Analyses" for details). (B) Isobologram for mixtures of CP55940 and spiradoline (same data presented in A). Open symbols indicate the ED_{50} valued for CP55940 alone (triangle) and spiradoline alone (circle); the solid line connecting the open symbols indicates the line of additivity. Filled symbols indicate the ED_{50} values for mixtures of CP55940 and spiradoline (same ratios and symbols presented in A). Abscissae: ED_{50} for spiradoline in milligrams per kilogram body weight (± 95% confidence interval). Ordinate: ED_{50} for CP55940 in milligrams per kilogram body weight (± 95% confidence interval). (C) Comparisons of the predicted effects of the drug mixtures based on the assumption of an additive interaction (open symbols) to the observed effects (filled symbols) for each dose ratio. Abscissae: additive total dose (in spiradoline-equivalent doses) in milligrams per kilogram body weight (see "Data Analyses" for details). Ordinate: % MPE (± 1 SEM).

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Table 1

Doses of CP55940 or spiradoline studied alone and in mixtures.

		Injection	
	Test cycle	**CP55940 (mg/kg)**	**Spiradoline (mg/kg)**
CP99940 alone	1	1:1:18 Vehicle	
	2	0.032	
	3	0.1	
	4	0.32	
	5	1.0	
Spiradoline alone	1		Saline
	2		1.0
	3		3.2
	4		10.0
	5		32.0
1:1[a]	1	1:1:18 Vehicle	
	2	0.01	0.5
	3	0.02	1.1
	4	0.04	2.1
	5	0.08	4.2
	6	0.16	8.4
1:3	1	1:1:18 Vehicle	
	2	0.01	1.6
	3	0.02	3.2
	4	0.04	6.3
	5	0.08	12.7
	6	0.16	25.3
3:1	1	1:1:18 Vehicle	
	2	0.03	0.5
	3	0.06	1.1
	4	0.12	2.1
	5	0.23	4.2

[a] Ratio of CP55940 to spiradoline