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Short communication

Differential cross-tolerance between analgesia produced by α_2-adrenoceptor agonists and receptor subtype selective opioid treatments

Dennis Paul *, Jean G. Tran

Department of Pharmacology, Louisiana State University Medical Center, 1901 Perdido St., New Orleans, LA 70130, USA

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Abstract

Analgesic cross-tolerance between α_2-adrenoceptor and opioid receptor agonists was studied using the mouse tail-flick assay. Mice tolerant to clonidine (0.3 mg/kg s.c.) or xylazine (7 mg/kg s.c.) were cross-tolerant to morphine (5 mg/kg s.c.), nalorphine (70 mg/kg s.c.) and supraspinal [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO; 4 ng i.c.v.), but not *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methanesulfonate (U50,488; 5 mg/kg s.c.), spinal DAMGO (10 ng i.t.), supraspinal [D-Pen^2,D-Pen^5]enkephalin (DPDPE; 9 μg i.c.v.) or spinal DPDPE (700 ng i.t.). In the complimentary studies, mice tolerant to morphine and nalorphine were cross-tolerant to both of the α_2-adrenoceptor agonists, but U50,488 tolerant mice were not. The results suggest differential interactions between α_2-adrenoceptor and μ_1-, μ_2-, δ-, κ_1- and κ_3-opioid analgesic circuitry.

Keywords: DAMGO ([D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin); DPDPE ([D-Pen^2,D-Pen^5]enkephalin); Nalorphine; U50,488; Xylazine; Clonidine

1. Introduction

Administration of α_2-adrenoceptor agonists, either parenterally or intrathecally, produces analgesia (Schmitt et al., 1974; Fielding et al., 1981). This effect is mediated by an action at spinal α_2-adrenoceptors (Schmitt et al., 1974; Spaulding et al., 1979). α_2-Adrenoceptor agonists are commonly used as a veterinary analgesic, and in humans postoperatively and as an adjunct to opiates in chronic cancer pain patients.

Opioids are virtually defined by their analgesic activity. However, the recent identification of multiple opioid receptor subtypes has led to the characterization of neuropharmacologically and neuroanatomically distinct opioid analgesic systems in rodents (Heyman et al., 1988; Millan, 1990; Paul et al., 1989, 1990; Porreca et al., 1987). The μ-opioid selective peptide [D-Ala^2, MePhe^4,Gly(ol)^5]enkephalin (DAMGO) produced analgesia when injected spinally or supraspinally. Analgesia mediated by supraspinal μ_1-opioid receptors and spinal μ_2-opioid receptors were differentiated on the basis of sensitivity of DAMGO analgesia to the μ_1-opioid selective antagonist naloxonazine (Heyman et al., 1988; Paul et al., 1989). Despite the finding that both effects were blocked by β-funaltrexamine, which alkylates both μ_1- and μ_2-opioid receptors, only supraspinal DAMGO analgesia was blocked by naloxonazine (Paul et al., 1989).

Similarly, the δ-opioid receptor selective peptide [D-Pen^2,D-Pen^5]enkephalin (DPDPE) produced analgesia when injected spinally in mice, and at higher doses, supraspinally (Heyman et al., 1988; Paul et al., 1989; Porreca et al., 1987). DPDPE analgesia was sensitive to the δ-opioid receptor antagonists ICI-154,129, ICI-174,864 and naltrindole, but was insensitive to the μ_1-opioid antagonist naloxonazine (Heyman et al., 1988; Paul et al., 1989, 1990; Porreca et al., 1987). Thus, delta receptors mediate analgesia through both spinal and supraspinal mechanisms.

κ-Opioid receptors have been reported to mediate analgesia through spinal and supraspinal sites (Millan,

* Corresponding author. Tel. (504) 568-4740, fax (504) 568-2361.

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1990). Recently, much attention has been given to the characterization of κ-opioid receptor subtypes. The possibility that different κ-opioid subtypes produce analgesia through neuranatomically distinct mechanisms was examined by using spinal and supraspinal injection of the opioid antagonist quadazocine (WIN44,441). This drug was used to challenge analgesia produced by subcutaneous injections of the κ_3-opioid receptor agonists, naloxone benzoylhydrazone and nalorphine, and the κ_1-opioid receptor agonist *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methanesulfonate (U50,488; Paul et al., 1990). U50,488 analgesia was more than 1000-fold more sensitive to i.t. than i.c.v. injections of quadazocine. Conversely, naloxone benzoylhydrazone analgesia was 1000-fold more sensitive to i.c.v. injections of the antagonist. Thus, κ_1-opioid receptor analgesia is mediated spinally whereas κ_3-opioid receptor analgesia has a supraspinal site of action.

Clinically, an important consideration for any analgesic treatment is the development of tolerance with repeated administration. It has been proposed that substitution of one analgesic compound for another may overcome the development of tolerance (e.g. Post et al., 1988). However, dissimilar analgesic agents may be cross-tolerant. Studies that examine cross-tolerance between α_2-adrenoceptor agonists and morphine have produced equivocal results. The α_2-adrenoceptor analgesics, clonidine, guanfacine and xylazine have been demonstrated to be cross-tolerant with morphine (Post et al., 1988; Solomon and Gebhart, 1987; Ossipov et al., 1989). In contrast, others found no cross-tolerance (Spaulding et al., 1979). One potential explanation for this equivocacy is the existence of multiple opioid analgesic systems. It is not known whether analgesia elicited through each of the opioid systems described above is cross-tolerant with α_2 analgesia.

Considering the potential importance of α_2-opioid interactions, the present studies were designed to assess cross-tolerance between the analgesia produced by the α_2-adrenoceptor agonists clonidine and xylazine, and analgesia produced through μ_1-, μ_2-, κ_1-, κ_3-, and δ-opioid receptors.

2. Materials and methods

Male CD-1 mice (25–35 g; Charles River Breeding Laboratories, Wilmington, MA, USA) were maintained on a 12 h light/dark cycle with ad libitum access to Purina Mouse Chow and water. Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections were made under light halothane anesthesia using a Hamilton 10-μl syringe fitted to a 30-gauge needle with PE10 tubing. I.c.v. injections were administered approximately 2 mm caudal and 2 mm lateral to bregma at a depth of 3–4 mm. I.t. injections were by lumbar puncture. Injection volume for i.c.v. and i.t. injections was 1 μl, and for s.c. injections, 1 ml/kg.

Analgesia was determined using the radiant heat tail-flick technique. Baseline latencies (3.5–4.5 s) were determined before experimental treatments as the mean of two trials. To minimize tissue damage, mice were tested at only one post-injection interval on injection days 1, 3 and 5, and a maximal latency of 12 s was used. Post-treatment tail-flick latencies were determined 30 min after s.c. injections and 15 min after i.c.v. or i.t. injections. These post-injection intervals were selected according to our previous work as the time for peak analgesia for the doses of the agonists used in these experiments. Mice that at least doubled their baseline latency were considered to be analgesic. Baseline scores did not change over days.

DAMGO and DPDPE were purchased from Penin-

Table 1
Cross-tolerance among opioids and α_2 agonists

Test drug	Drug on days 1–4					
	Saline	Clonidine	Xylazine	Morphine	U50,488	Nalorphine
Clonidine (0.3 mg/kg)	65	10 [a]		10 [a]	50	10 [a]
Xylazine (7 mg/kg)	60		8 [a]	10 [a]	80	18 [a]
Morphine (5 mg/kg)	64	10 [a]	20 [a]	5 [a]		
U50,488 (5 mg/kg)	58	70	70		5 [a]	
Nalorphine (70 mg/kg)	64	0 [a]	0 [a]			0 [a]
i.c.v. DAMGO (4 ng)	70	20 [a]	11 [a]			
i.t. DAMGO (8 ng)	70	70	70			
i.c.v. DPDPE (9 μg)	67	60	33			
i.t. DPDPE (200 ng)	58	70	70			

Groups of mice ($n \geq 9$) received four daily injections of the drug at the head of each column and on the fifth day were tested with the drug at the beginning of each row. For example, 18% of mice tolerant to nalorphine (last column) were analgesic when tested with xylazine (second row). Data are reported as percent of mice that at least doubled their baseline tail-flick latencies.
[a] $P < 0.05$, Fisher exact test.

sula Laboratories (Belmont, CA, USA), clonidine and nalorphine from Sigma (St. Louis, MO, USA), and xylazine from Research Biochemicals (Natick, MA, USA). Morphine sulphate and U50,488 were obtained from the Research Technologies Branch of NIDA. All drugs were dissolved in saline. DAMGO and DPDPE were administered by both the i.c.v. and i.t. routes, and all other drugs were by the s.c. route. Doses were selected to produce 60–70% analgesia in control mice and were as follows: 7 mg/kg of xylazine; 0.3 mg/kg of clonidine; 5 mg/kg of U50,488; 70 mg/kg of nalorphine; 4 ng i.c.v. DAMGO; 8 ng i.t. DAMGO; 9 μg i.c.v. DPDPE; and 200 ng i.t. DPDPE.

Tolerance to the analgesic effect of clonidine, xylazine, morphine, U50,488 or nalorphine was induced by four daily subcutaneous injections. On the fifth day, mice were injected with the same drug to test for tolerance, or a different drug to test for cross-tolerance. Tolerance development was assessed for each of the subcutaneously administered drugs. Cross-tolerance was assessed for each of the opioid treatments after development of tolerance to one of the α_2-adrenoceptor agonists. Cross-tolerance was also assessed for the two α_2-adrenoceptor agonists after development of tolerance to morphine, U50,488 and nalorphine. Because of deleterious effects of repeated i.c.v. or i.t. injections, DAMGO and DPDPE administered only as test drugs, and two-way cross-tolerance could not be assessed. Planned comparisons were made using the Fisher exact test.

3. Results

Mice tolerant to the α_2-adrenoceptor agonists, clonidine (0.3 mg/kg s.c.) and xylazine (7 mg/kg s.c.), were cross-tolerant to morphine (Table 1). Mice tolerant to morphine were cross-tolerant to clonidine and xylazine. Clonidine and xylazine tolerant animals were also cross-tolerant to μ_1-opioid receptor mediated analgesia (i.c.v. DAMGO, 4 ng) and κ_3-opioid receptor mediated analgesia (s.c. nalorphine, 70 mg/kg). Nalorphine tolerant animals were cross-tolerant to the α_2-adrenoceptor agonists. Animals tolerant to clonidine and xylazine were not cross-tolerant to μ_2- (i.t. DAMGO, 10 mg/kg), κ_1- (s.c. U50,488, 5 mg/kg), supraspinal δ- (i.c.v. DPDPE, 9 μg), or spinal δ-opioid receptor mediated analgesia (i.t. DPDPE, 700 ng).

4. Discussion

The results of these studies demonstrate differential cross-tolerance between the analgesia produced by α_2-adrenoceptor agonists and receptor-selective opioid treatments. The demonstration that clonidine and xylazine analgesia are cross-tolerant with morphine analgesia supports previous work with α_2-adrenoceptor agonists. These findings have been used as evidence for opioid-noradrenergic interactions in the production of analgesia (Post et al., 1988; Solomon and Gebhart, 1987; Spaulding et al., 1979). The finding that the analgesia produced by these two α_2-adrenoceptor agonists are cross-tolerant with nalorphine and i.c.v. DAMGO analgesia implicates an interaction between noradrenergic neurons and κ_3- and μ_1-opioid analgesia. This could be through direct mediation, modulation, or a common final pathway.

Several alternative mechanisms may be responsible for cross-tolerance between opioids and α_2-adrenoceptor agonists. For example, second messenger down-regulation, enzyme induction and associative mechanisms may contribute to development of cross-tolerance between drugs that act at different receptors. The contribution of each of these remains to be investigated.

Considerable evidence implicates α_2-adrenoceptor stimulation by noradrenaline as a mediating event in morphine analgesia (Camarata and Yaksh, 1982; Sawynok, 1989). The α_2-adrenoceptor antagonist yohimbine attenuates morphine analgesia (Ossipov et al., 1989), as does neurotoxic lesions of spinal noradrenergic fibers (Sawynok 1989). Conversely, blockade of noradrenaline reuptake potentiates morphine analgesia (Larsen and Arnt, 1984). Reports of morphine–α_2-adrenoceptor agonist cross-tolerance are consistent with the interpretation that descending noradrenergic fibers are a link in the morphine analgesic circuitry. However, some investigators report symmetric cross-tolerance between morphine and the two α_2-adrenoceptor agonists, whereas others report no cross-tolerance to morphine when rats were made tolerant to α_2-adrenoceptor agonists. Each of these patterns of results has different implications regarding the organization of circuitry responsible for morphine–α_2-adrenoceptor analgesic interactions (Stevens et al., 1988). It is not clear from the present or previous research whether these contradictory results can be explained by differences in species or strain, algesiometric testing procedures, routes of administration, or dosing procedures. However, the present results indicate that cross-tolerance to α_2-adrenoceptor agonists may be more likely in situations where μ_1- and κ_3-opioid receptor analgesic systems are being stimulated.

Conversely, the finding that α_2-adrenoceptor agonists are not cross-tolerant with μ_2-, δ-, or κ_1-opioid receptor mediated analgesia has promising clinical implications. The practice of substituting one analgesic compound for another to overcome the development of tolerance has proved to be of limited value because even dissimilar analgesic agents may be cross-tolerant. However, most previous work has focussed on cross-tolerance between nonselective opioid receptor ago-

nists, such as morphine, and other analgesic compounds. The finding that some of the opioid receptor agonist treatments are not cross-tolerant with α_2-adrenoceptor agonist treatments suggests that if selectivity of the opioid receptor agonist is considered, the problem of cross-tolerance may be obviated.

The delineation of multiple opioid analgesic systems has stimulated considerable research and novel strategies for analgesic treatments. Moreover, the clinical potential of opioid–α_2-adrenoceptor agonist combinations has been proposed for chronic cancer pain patients (Ossipov et al., 1989). The results of the present studies underscore the idea that in order to understand opioid–α_2-adrenoceptor agonist interactions, the issue of multiple opioid analgesic systems must be addressed. For example, if a rotating schedule of opioids and α-adrenoceptor agonists is to be used to retard tolerance development, an opioid receptor agonist that acts at δ-opioid receptors may be more useful than one that stimulates supraspinal μ-opioid receptors.

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