The Journal of Neuroscience, December 1995, 15(12): 8031–8038

Opioid and Adenosine Peripheral Antinociception Are Subject to Tolerance and Withdrawal

K. O. Aley,[3,4] P. G. Green,[3,4] and J. D. Levine[1,2,3,4]

Departments of [1]Anatomy, [2]Medicine, and [3]Oral and Maxillofacial Surgery and [4]Division of Neuroscience, University of California, San Francisco, California 94143

The selective μ-opioid agonist, D-Ala[2],N-Me-Phe[4],Gly[5]-ol-enkephalin (DAMGO), or the selective A_1-adenosine agonist N^6-cyclopentyladenosine (CPA), when coinjected intradermally with prostaglandin E_2 (PGE_2), dose-dependently inhibited PGE_2-induced mechanical hyperalgesia in the rat hindpaw, as determined by the Randall-Selitto paw-withdrawal test. Repeated (hourly × 3) intradermal injections of DAMGO or CPA produced tolerance to the antinociceptive effect of a fourth injection 1 hr later. Furthermore, repeated (hourly × 3) intradermal injections of DAMGO produced cross-tolerance to the antinociceptive effect of CPA, and repeated (hourly × 3) intradermal injection of CPA produced cross-tolerance to the antinociceptive effect of DAMGO. The demonstration of the bidirectional cross-tolerance between the peripheral antinociceptive effects of DAMGO and CPA supports the hypothesis that both these agents produce antinociception by acting on the same cell, presumably the primary afferent nociceptor, and that the development of tolerance involves changes downstream to activation of μ-opioid and A_1-adenosine receptors.

The opioid antagonist naloxone, which had no effect on paw-withdrawal threshold in normal paws, produced withdrawal hyperalgesia in DAMGO-tolerant paws. Furthermore, naloxone elicited a cross-withdrawal hyperalgesia response in CPA-tolerant paws. Similarly, the A_1-adenosine antagonist 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine (PACPX), which had no effect on paw-withdrawal threshold in normal paws, elicited a withdrawal hyperalgesia response in CPA-tolerant paws and cross-withdrawal hyperalgesia response in DAMGO-tolerant paws. These cross-dependence and cross-withdrawal responses suggest that the development of dependence to μ-opioid and A_1-adenosine agonists involves changes in the same second messenger system downstream to both μ-opioid and A_1-adenosine receptor activation.

[Key words: A_1-adenosine, pain, peripheral opioid tolerance, physical dependence, μ-opioid receptor]

The systemic administration of opioids produces potent antinociception in animals, and analgesia in humans, by actions both in the dorsal horn of the spinal cord and at supraspinal sites (Mayer and Price, 1976; Duggan, 1979; Fields and Basbaum, 1978). Clinical and animal studies have shown that opioids also exert antinociceptive effects at peripheral sites in inflamed tissue and inhibit PGE_2-induced hyperalgesia (Ferreira and Nakamura, 1979; Smith et al., 1982; Stein et al., 1988, 1989, 1991; Levine and Taiwo, 1989; Kayser et al., 1990; Parsons and Herz, 1990; Taiwo and Levine, 1990; Wheeler-Aceto and Cowan, 1991; Junien and Wettstein, 1992; Moiniche et al., 1993). There is, at present, a significant interest in the peripheral use of opioids for analgesia, since such use should avoid side-effects (e.g, respiratory depression) that develop to the central action of systemically administered opioids, which often limit their therapeutic use. However, it is not known if peripherally acting opioids would produce tolerance to their analgesic effects which, like for central action, also significantly limits usefulness.

Recent experiments have provided some understanding of mechanisms active in opioid tolerance. Most evidence indicates that tolerance to opioid effects in the CNS is due to a functional uncoupling between the opioid receptor and its second messenger, an inhibitory guanosine triphosphate-binding protein (G-protein), G_i (Collin and Cesselin, 1991), through which opioid agonists act to produce many of their effects (Costa et al., 1988) including antinociception (Levine and Taiwo, 1989). For example, while chronic opioid exposure does not in general alter opioid binding to its receptor, opioid inhibition of adenylyl cyclase activity is decreased after chronic exposure (Polastron et al., 1990). In addition, in other *in vitro* systems chronic administration of morphine results in a loss of the ability of guanine nucleotides to modulate the affinity of the μ-opioid receptor for its ligands, associated with a downregulation of high affinity sites (Puttfarcken et al., 1988; Werling et al., 1989).

A_1-Adenosine agonists [such as N^6-cyclopentyladenosine (CPA)], like μ-opioids, are able to act in the periphery to inhibit prostaglandin E_2 (PGE_2)-induced hyperalgesia (Taiwo and Levine, 1990). A_1-Adenosine receptors are also coupled to an inhibitory G-protein, and A_1-receptor activation also results in inhibition of adenylyl cyclase activity and decreased levels of cAMP (Cooper et al., 1980). Tolerance at the A_1-adenosine receptor occurs following repeated administration of A_1-adenosine agonists (Casati et al., 1994; Tsuchida et al., 1994). However, A_1-adenosine receptor-mediated tolerance has not yet been reported with regard to its antinociceptive effects.

In the present study we determined if tolerance develops with regard to the peripheral antinociception produced by a μ-opioid or an A_1-adenosine agonist. The ability of these agonists to induce cross-tolerance and the ability of opioid and adenosine antagonists to precipitate a withdrawal hyperalgesia was also investigated.

Received June 23, 1995; revised Aug. 9, 1995; accepted Aug. 14, 1995.

We thank Allan Basbaum, Philip Heller, David Reichling, E. Leong Way, and Kimberly Tanner for many helpful discussions during the course of this work. This work was funded by NIH DE08973.

Correspondence should be addressed to Jon D. Levine, M.D., Ph.D., Department of Medicine, Box 0452, University of California, San Francisco, CA 94143.

Table 1. Abbreviations of agents used

Abbreviation	Agent	Action
PGE_2 or E_2	Prostaglandin E_2	Hyperalgesic inflammatory mediator
DAMGO or D	[D-Ala^2,N-Me-Phe^4,gly^5-ol] enkephalin	μ-Opioid receptor agonist
MMI	Morphine methyl iodide	Quaternary salt of morphine
N	Naloxone	Opioid receptor antagonist
CPA	N^6-Cyclopentyl-adenosine	A_1-adenosine receptor agonist
PACPX	1,3-Dipropyl-8-(2-amino-4-chlorphenyl)-xanthine	A_1-adenosine receptor antagonist

Materials and Methods

Animals. Experiments were performed on male Sprague–Dawley rats (250–300 gm; Bantin and Kingman, Fremont, CA). Animals were housed in groups of two or three, under a 12 hr light/12 hr dark cycle. Food and water were available ad libitum. All testing was done between 10:00 and 16:00 hr. Experiments were carried out under approval of the Institutional Animal Care Committee of the University of California, San Francisco.

Behavioral testing. The nociceptive flexion reflex was quantified with a Basile Analgesymeter (Stoelting, Chicago, IL), which applies a linearly increasing mechanical force to the dorsum of the rat's hindpaw. Rats were trained in the test procedure during the week prior to the experiments, a procedure which produces a stable baseline threshold measurement and enhances the ability to detect the action of hyperalgesic agents (Taiwo et al., 1989). On the day of the experiment, baseline threshold was determined. The mean baseline mechanical nociceptive paw-withdrawal threshold in these experiments was 110.4 ± 0.4 gm, n = 340 (mean paw-withdrawal threshold ± SEM). Mechanical threshold was redetermined at different time points (15, 20, and 25 min) after treatments. The mean of these three readings was considered to be the paw-withdrawal threshold following drug administration and this value was used to calculate the percentage change from the baseline threshold.

Drug administration. The drugs used in this study were prostaglandin E_2 (PGE_2, hyperalgesic inflammatory mediator), D-Ala^2,N-Me-Phe^4,Gly^5-ol-enkephalin (DAMGO; μ-opioid receptor agonist), N^6-cyclopentyl-adenosine (CPA, A_1-adenosine receptor agonist), naloxone HCl (opioid receptor antagonist), all from Sigma, St. Louis, MO; 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine (PACPX, A_1-adenosine receptor antagonist) from Research Biochemicals Inc., Natick, MA; and morphine methyliodide (quaternary salt of morphine, which exhibits opioid agonist properties similar to its parent compound, but does not cross the blood brain barrier), a generous gift from Dr. Ivy Carroll, NIDA, Research Triangle Institute. The selection of the drug doses employed in this study was based on dose–response curves determined during this study (DAMGO and CPA) or previously by our laboratory (Levine and Taiwo, 1989; Taiwo and Levine, 1990; Aley et al., 1995). The stock solution of PGE_2 (1 μg/2.5 μl) was prepared in 10% ethanol and further dilutions made in saline. DAMGO, CPA, naloxone, and morphine methyliodide were dissolved in saline. PACPX was dissolved in dimethyl sulfoxide (DMSO, 8 mg/ml) and further dilutions made in saline. Maximum final concentration of ethanol injected was <1%, and the maximum final concentration of DMSO injected was <5%. All drugs were injected intradermally (i.d.) in a volume of 2.5 μl/paw. In addition, morphine methyliodide was also injected subcutaneously. When drug combinations were used, they were administered sequentially as indicated from the same syringe. Antagonists were always injected first. Abbreviations for the drugs administered in this study and their actions, are located in Table 1.

Experimental protocol. Rats used in this study were divided into different groups; details of drug treatments for each group is given in Table 2.

Statistical analysis. Data are presented as mean ± SEM of six or more paws in each of the experimental groups. Statistical significance was determined by analysis of variance (ANOVA) followed by Scheffe's post-hoc test; $p < 0.05$ was considered statistically significant.

Results

Inhibition of PGE_2 hyperalgesia by DAMGO or CPA

Intradermal injection of PGE_2 (100 ng) into the hairy skin of the hindpaw of the rat significantly decreased paw-withdrawal threshold ($p < 0.05$; Fig. 1). Both DAMGO (Fig. 1*A*) and CPA (Fig. 1*B*) dose-dependently inhibited PGE_2-inhibited hyperalgesia (both $p < 0.05$); that is, both produced marked antinociception.

Tolerance to peripheral DAMGO and CPA antinociception

Two or more hourly injections of either DAMGO (1 μg) (Fig. 2*A*) or CPA (1 μg) (Fig. 2*B*) produced a complete tolerance to their antinociceptive effects (*Dx2,D+E2* compared to *E2*, and *CPAx2,CPA+E2* compared to *E2*, both $p > 0.05$). To ensure tolerance for all animals tested, DAMGO or CPA were administered hourly for 3 hr in subsequent experiments. When DAMGO (1 μg) and naloxone (1 μg) were coinjected hourly for 3 hr and at the fourth hour DAMGO plus PGE_2 was injected, DAMGO antinociception was not significantly different from that produced by DAMGO in opioid-naive rats (*(N+D)x3,D+E2* compared to *Dx3,D+E2*, $p > 0.05$; Fig. 2*A*); that is, coinjection of DAMGO with naloxone prevented the development of DAMGO tolerance. Naloxone appears to be acting locally at the site of injection, to inhibit DAMGO antinociception, since DAMGO antinociception is not altered in the paw contralateral to that receiving naloxone (unpublished data). When CPA (1 μg) and PACPX (1 μg) were coinjected hourly for 3 hr and at the fourth hour CPA plus PGE_2 was injected, CPA antinociception was not significantly different from that produced by CPA in CPA-naive rats (*(PACPX+CPA)x3,CPA+E2* compared to *CPAx3,CPA+E2*, $p > 0.05$; Fig. 2*B*); that is, coinjection of PACPX with CPA prevented the development of CPA tolerance.

Cross-tolerance between DAMGO and CPA antinociception

Three hourly injections of DAMGO (1 μg) prior to an injection of CPA plus PGE_2, at the fourth hour, significantly blocked CPA antinociception (*Dx3,CPA+E2* compared to *CPA+E2*, ($p < 0.05$; Fig. 3); that is, cross-tolerance developed between DAMGO and CPA. Similarly, three hourly injections of CPA (1 μg) prior to an injection of DAMGO plus PGE_2 significantly blocked DAMGO antinociception tested at the fourth hour (*CPAx3,D+E2* compared to *D+E2*, $p < 0.05$); that is, cross-tolerance developed between CPA and DAMGO.

Effect of naloxone on DAMGO- and CPA-induced tolerance

The opioid antagonist naloxone (1 μg) blocked DAMGO antinociception (*N+D+E2* compared to *D+E2*, $p < 0.05$; Fig. 4), but not CPA antinociception (*N+CPA+E2* compared to *CPA+E2*, $p > 0.05$; Fig. 4); that is, DAMGO but not CPA is acting through an opioid receptor to induce antinociception. Three hourly injections of DAMGO (1 μg) had no significant effect on basal paw-withdrawal threshold (*Dx3* compared with saline vehicle *Vx3*, $p > 0;05$; Fig. 4). The opioid antagonist naloxone (1 μg) also did not have a significant effect on the

Table 2. Experimental protocols

Group	N	Treatment	Dose(s)
1 a	16	PGE_2	100 ng
b	6	DAMGO + PGE_2	1 ng/100 ng
c	6	DAMGO + PGE_2	10 ng/100 ng
d	6	DAMGO + PGE_2	100 ng/100 ng
e	16	DAMGO + PGE_2	1 μg/100 ng
2 a	6	CPA + PGE_2	1 ng/100 ng
b	6	CPA + PGE_2	10 ng/100 ng
c	6	CPA + PGE_2	100 ng/100 ng
d	12	CPA I PGE_2	1 μg/100 ng
3 a	6	DAMGO × 1, 2nd hr DAMGO + PGE_2	1 μg × 1, 1 μg/100 ng
b	6	DAMGO hourly × 2, 3rd hr DAMGO + PGE_2	1 μg × 2, 1 μg/100 ng
c	6	DAMGO hourly × 3, 4th hr DAMGO + PGE_2	1 μg × 3, 1 μg/100 ng
d	6	DAMGO hourly × 4, 5th hr DAMGO + PGE_2	1 μg × 4, 1 μg/100 ng
4 a	6	CPA × 1, 2nd hr CPA + PGE_2	1 μg × 1, 1 μg/100 ng
b	6	CPA hourly × 2, 3rd hr CPA + PGE_2	1 μg × 2, 1 μg/100 ng
c	6	CPA hourly × 3, 4th hr CPA + PGE_2	1 μg × 3, 1 μg/100 ng
d	6	CPA hourly × 4, 5th hr CPA + PGE_2	1 μg × 4, 1 μg/100 ng
5 a	10	DAMGO hourly × 3, 4th hr CPA + PGE_2	1 μg × 3, 1 μg/100 ng
b	10	CPA hourly × 3, 4th hr DAMGO + PGE_2	1 μg × 3, 1 μg/100 ng
6 a	6	Naloxone + DAMGO + PGE_2 (co-injection)	1 μg/1 μg/100 ng
b	6	Naloxone + CPA + PGE_2 (co-injection)	1 μg/1 μg/100 ng
c	14	DAMGO hourly × 3	1 μg × 3
d	6	Vehicle hourly × 3	2.5 μl × 3
e	6	Vehicle × 3, 4th hr naloxone	2.5 μl × 3, 1 μg
f	6	Naloxone + DAMGO × 3, 4th hr DAMGO + PGE_2	1 μg/1 μg × 3, 1 μg/100 ng
7 a	18	DAMGO hourly × 3, 4th hr naloxone	1 μg × 3, 1 μg
b	6	CPA hourly × 3, 4th hr naloxone	1 μg × 3, 1 μg
c	6	DAMGO hourly × 3, 4th hr DAMGO + naloxone	1 μg × 3, 1 μg/1 μg
d	12	DAMGO hourly × 3, 4th hr CPA + naloxone	1 μg × 3, 1 μg/1 μg
e	6	CPA hourly × 3, 4th hr DAMGO + naloxone	1 μg × 3, 1 μg/1 μg
f	8	CPA hourly × 3, 4th hr CPA + naloxone	1 μg × 3, 1 μg/1 μg
8 a	6	PACPX + CPA + PGE_2 (co-injection)	1 μg/1 μg/100 ng
b	6	PACPX + DAMGO + PGE_2 (co-injection)	1 μg/1 μg/100 ng
c	10	CPA hourly × 3	1 μg × 3
d	6	Vehicle hourly × 3, 4th hr PACPX	2.5 μl × 3, 1 μg
e	6	PACPX + CPA hourly × 3, 4th hr CPA + PGE_2	1 μg/1 μg × 3, 1 μg/100 ng
9 a	8	CPA hourly × 3, PACPX	1 μg × 3, 1 μg
b	8	DAMGO hourly × 3, 4th hr PACPX	1 μg × 3, 1 μg
c	6	CPA hourly × 3, 4th hr CPA + PACPX	1 μg × 3, 1 μg/1 μg
d	6	CPA hourly × 3, 4th hr DMAGO + PACPX	1 μg × 3, 1 μg/1 μg
e	6	DAMGO hourly × 3, 4th hr DAMGO + PACPX	1 μg × 3, 1 μg/1 μg
f	6	DAMGO hourly × 3, 4th hr CPA + PACPX	1 μg × 3, 1 μg/1 μg
10 a	6	Morphine methyl iodide + PGE_2	10 μg/100 ng
b	6	Morphine methyl iodide + PGE_2	100 μg/100 ng
c	6	Morphine methyl iodide (SC) + PGE_2	100 μg(SC)/100 ng

Abbreviations: PGE_2, Prostaglandin E_2 (EP receptor agonist); DAMGO, D-Ala^2,N-Me-Phe^4,gly^5-ol (μ-opioid receptor agonist); CPA, N^6-cyclopentyl adenosine (A_1-adenosine agonist); PACPX, 1,3-dipropyl-8-(2-amino-4-chlorphenyl)-xanthine (A1-adenosine antagonist); SC, subcutaneous (in the neck).

paw-withdrawal threshold of rats treated with three hourly injections of saline vehicle (*Vx3,N* compared with *Vx3*, $p > 0.05$; Fig. 4).

In rats treated with three hourly injections of DAMGO (1 μg), administration of naloxone at the fourth hour produced a significant decrease in paw-withdrawal threshold (i.e., hyperalgesia) (*Dx3,N* compared to *Dx3*, $p < 0.05$; Fig. 5). Similarly, in rats treated with three hourly injections of CPA (1 μg), administration of naloxone at the fourth hour produced a significant decrease in paw-withdrawal threshold (*CPAx3,N* compared with *CPAx3*, $p < 0.05$; Fig. 5). In the DAMGO and CPA treated rats, the paw-withdrawal threshold at the fourth hour, prior to the administration of naloxone, was not significantly different from the basal paw-withdrawal threshold (Figs. 5, 7). Naloxone-induced hyperalgesia in a DAMGO-tolerant paw could be inhibited by coadministration of DAMGO with naloxone at the fourth hour (*Dx3,D+N* compared to *Dx3,N*, $p < 0,05$; Fig. 5). Coadministration of CPA with naloxone did not block naloxone-induced hyperalgesia in DAMGO-tolerant paws (*Dx3,CPA+N* compared with *DAMGOx3,N*, $p > 0.05$; Fig. 5). In CPA-tolerant

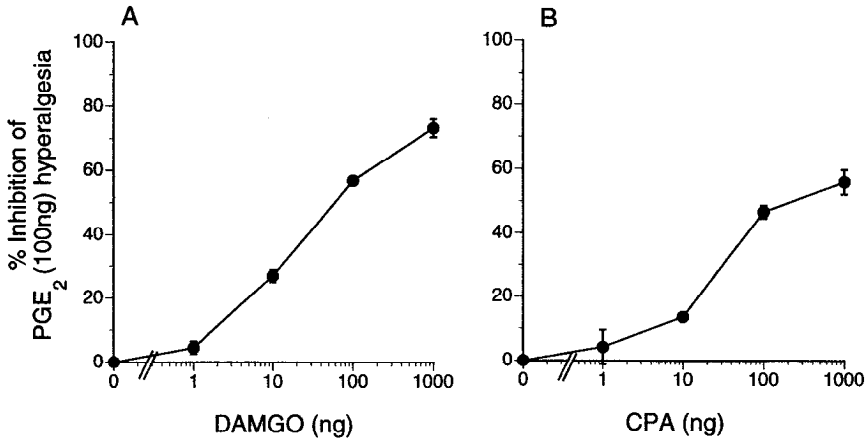

Figure 1. Dose-dependent inhibition of PGE_2-induced hyperalgesia by DAMGO and CPA. Effect of PGE_2 (100 ng, $n = 16$) on paw-withdrawal thresholds and its modification by different doses of: *A*, DAMGO ($n = 6$), or *B*, CPA ($n = 6$). In this and subsequent figures where no error bars seen, they are contained within the symbols.

paws coadministration of CPA with naloxone resulted in cross-withdrawal hyperalgesia (*CPAx3,CPA+N* compared to *CPAx3*, $p < 0.05$), although it was slightly decreased ($p < 0.05$) compared to administration of naloxone alone (*CPAx3,CPA+N* compared with *CPAx3,N*, $p < 0.05$; Fig. 5). However, in CPA-tolerant paws DAMGO did block naloxone-induced withdrawal hyperalgesia (*CPAx3,D+N* compared to *CPAx3,N*, $p < 0.05$; Fig. 5).

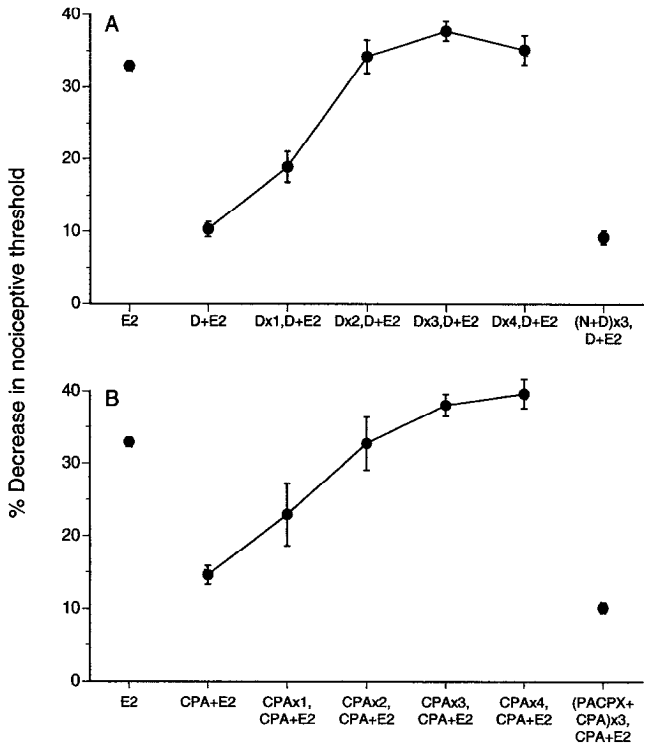

Figure 2. Time course for the development of tolerance to DAMGO- and CPA-induced antinociception and the blockade of the development of tolerance by coadministration of specific receptor antagonists. *A*, Effect of different number of preceding hourly doses of DAMGO (*D*) (1 μg) on DAMGO inhibition of PGE_2 hyperalgesia (*Dx2,D+E2* = DAMGO hourly × 2, then DAMGO plus PGE_2 at the next hour, and similarly for 3 and 4 hr). Naloxone plus DAMGO hourly × 3 and at the fourth hour DAMGO plus PGE_2 (*N+Dx3,D+E2*, $n = 6$). *B*, Effect of different number of preceding hourly doses of CPA (*CPA*) (1 μg) on CPA inhibition of PGE_2 hyperalgesia (*CPAx2,CPA+E2* = CPA hourly × 2, then CPA plus PGE_2 at the next hour, and similarly for 3 and 4 hr). PACPX plus CPA hourly × 3 and at the fourth hour CPA plus PGE_2 (*PACPX+CPAx3,CPA+E2*, $n = 6$).

Effects of PACPX on CPA- and DAMGO-induced tolerance

The A_1-adenosine antagonist PACPX (1 μg) blocked CPA antinociception (*PACPX+CPA+E2* compared to *CPA+E2*, $p < 0.05$; Fig. 6), but not DAMGO antinociception (*PACPX+D+E2* compared to *D+E2*, $p > 0.05$; Fig. 6); that is, CPA but not DAMGO is acting at the A_1-adenosine receptor. Three injections of CPA (1 μg), one every hour, had no significant effect on paw-withdrawal threshold (*CPAx3* compared to *Vx3*, $p > 0;05$; Fig. 6). PACPX (1 μg) also did not have a significant effect on the paw-withdrawal threshold of rats treated with three hourly injections of saline vehicle (*Vx3,PACPX* compared to *Vx3*, $p > 0.05$; Fig. 6).

In rats treated with three hourly injections of CPA (1 μg), administration of PACPX at the fourth hour produced a significant decrease in paw-withdrawal threshold (i.e., hyperalgesia) (*CPAx3,PACPX* compared to *CPAx3*, $p < 0.05$, Figs. 7). Similarly, after three hourly injections of DAMGO (1 μg), administration of PACPX at the fourth hour produced a significant decrease in paw-withdrawal threshold (i.e., hyperalgesia) (*Dx3,PACPX*, compared to *Dx3*, $p < 0.05$; Fig. 7). The paw-withdrawal threshold in the CPA- and DAMGO-treated rats, at

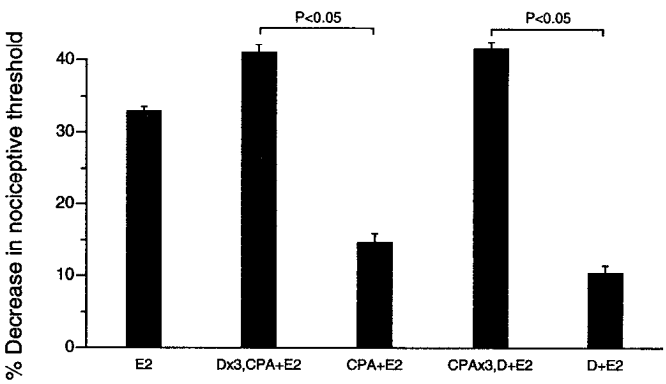

Figure 3. Bidirectional cross-tolerance develops between DAMGO- and CPA-induced antinociception. Effect of PGE_2 (*E2*, $n = 16$); DAMGO hourly × 3 and at the fourth hour CPA plus PGE_2 (*Dx3,CPA+E2*, $n = 10$) compared to DAMGO and PGE_2 (*D+E2*, $n = 16$); and CPA hourly × 3 and at the fourth hour DAMGO plus PGE_2 (*CPAx3,D+E2*, $n = 10$) compared to CPA plus PGE_2 (*CPA+E2*, $n = 10$) on mechanical paw-withdrawal threshold in the rat.

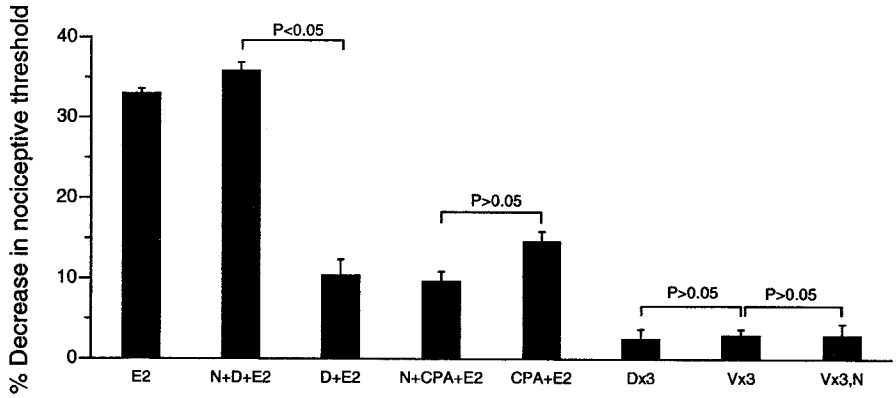

Figure 4. Naloxone blocks DAMGO- but not CPA-induced antinociception. Effect of PGE_2 (*E2*, $n = 16$), DAMGO and PGE_2 (*D+E2*, $n = 16$), naloxone, DAMGO and PGE_2 (*N+D+E2*, $n = 6$), CPA and PGE_2 (*CPA+E2*, $n = 12$), naloxone, CPA and PGE_2 (*N+CPA+E2*, $n = 6$), DAMGO hourly × 3 (*Dx3*, $n = 14$), Saline (2.5 μl/paw) hourly × 3 (*Sx3*, $n = 6$), saline (2.5 μl/paw) hourly × 3 and at the fourth hour naloxone (*Vx3,N*, $n = 6$).

the fourth hour prior to the administration of PACPX, was not statistically significant from the basal paw-withdrawal threshold (Figs. 5, 7). PACPX-induced withdrawal hyperalgesia could be inhibited by coadministration of CPA with PACPX at the fourth hour (*CPAx3,CPA+PACPX* compared to *Dx3,PACPX*, $p < 0.05$; Fig. 7). However, coadministration of DAMGO with PACPX did not block PACPX-induced withdrawal hyperalgesia in CPA-tolerant paws (*CPAx3,D+PACPX* compared to *CPAx3,PACPX*, $p > 0.05$). In DAMGO-tolerant paws coadministration of DAMGO with PACPX did not block PACPX-induced withdrawal hyperalgesia (*Dx3,D+PACPX*, compared to *Dx3*, $p < 0.05$; Fig. 7). However, in DAMGO-tolerant paws CPA did block PACPX-induced withdrawal hyperalgesia (*Dx3,CPA+PACPX* compared to *Dx3,PACPX*, $p < 0.05$; Fig. 7).

Effect of morphine methyliodide on PGE_2-hyperalgesia

To confirm that the antinociceptive effect of intradermally administered opioid was due to its peripheral action, the effect of a quaternary salt of morphine on PGE_2-induced hyperalgesia was evaluated. Morphine methyliodide (MMI) (10 and 100 μg) was antinociceptive (*E2* compared with *MMI(10μg)+E2* and *E2* compared to *MMI(100μg)+E2*, both $p < 0.05$, Fig. 8). However, subcutaneous (in the neck) injection of morphine methyliodide failed to inhibit PGE_2-induced hyperalgesia (100 μg, *MMI(100μg,SC)+E2* compared to *E2*) ($p > 0.05$).

Discussion

In this study, we found that tolerance develops to the peripheral antinociceptive effects of DAMGO and CPA after repeated administration of these agents in the hindpaw of the rat. Since the development of tolerance is blocked by coadministration of naloxone (but not PACPX) with DAMGO or of PACPX with CPA (but not DAMGO), we suggest that DAMGO is acting at an opioid receptor (presumably μ) and CPA is acting at an A_1-adenosine receptor to produce tolerance. In a previous study, repeated subcutaneous (i.e., systemic) administration of morphine failed to produce tolerance to the antinociceptive activity of intraplantar (i.e., local peripheral) morphine administration (Ferreira et al., 1984). The lack of observed tolerance to peripheral morphine in that study may be due to the difference in the route of drug administration used to induce tolerance compared to our study.

Following injection into the hindpaw, DAMGO and CPA probably exert their antinociceptive effects locally, since the doses administered are too low to have an effect in the CNS (Taiwo and Levine, 1990). This hypothesis is supported by the observation that the quaternary compound morphine methyliodide, which does not as readily enter the CNS, produced antinociception following intradermal administration into the hindpaw, but not when the same dose was administered systemically (subcutaneously at a distant site).

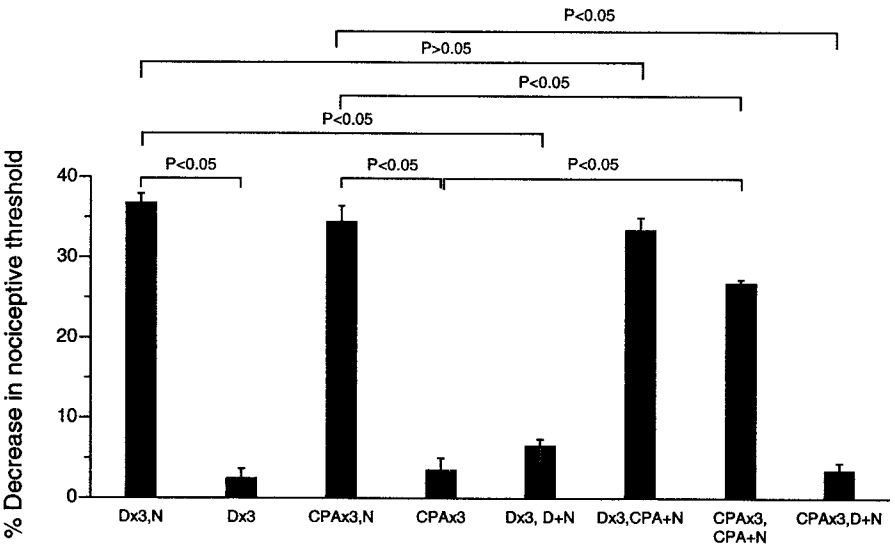

Figure 5. Naloxone induces withdrawal hyperalgesia in DAMGO- and CPA-tolerant paws. Effect of DAMGO hourly × 3 and at the fourth hour naloxone (*Dx3,N*, $n = 18$), CPA hourly × 3 and at the fourth hour naloxone (*CPAx3,N*, $n = 6$), DAMGO hourly × 3 and at the fourth hour DAMGO plus naloxone (*Dx3,D+N*, $n = 6$), DAMGO hourly × 3 and at the fourth hour CPA plus naloxone (*Dx3,CPA+N*, $n = 12$), CPA hourly × 3 and at the fourth hour DAMGO plus naloxone (*CPAx3,D+N*, $n = 6$), and CPA hourly × 3 and at the fourth hour CPA plus naloxone (*CPAx3,CPA+N*, $n = 8$) on mechanical paw-withdrawal threshold in the rat.

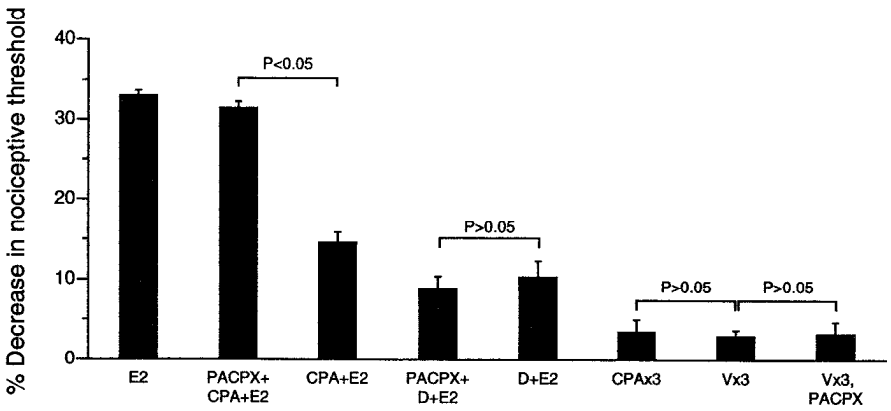

Figure 6. PACPX blocks CPA- but not DAMGO-induced antinociception. Effect of PGE_2 (*E2*, $n = 16$), CPA and PGE_2 (*CPA+E2*, $n = 12$), PACPX, CPA and PGE_2 (*PACPX+CPA+E2*, $n = 6$), DAMGO and PGE_2 (*D+E2*, $n = 16$), PACPX, DAMGO and PGE_2 (*PACPX+D+E2*, $n = 6$), CPA hourly $\times$ 3 (*CPAx3*, $n = 10$), saline (2.5 μl) hourly $\times$ 3 (*Sx3*, $n = 6$), saline (2.5 μl/paw) hourly $\times$ 3 and at the fourth hour PACPX (*Vx3,PACPX*, $n = 6$).

Our data also indicate that there is cross-tolerance between DAMGO and CPA since in the DAMGO-tolerant paw CPA was not antinociceptive, and in the CPA-tolerant paw DAMGO was not antinociceptive. The bidirectional cross-tolerance between DAMGO and CPA suggests that opioid and A_1-adenosine receptors on the same cell, possibly the primary afferent nociceptor, and are coupled to a common second messenger system that participates in the development of tolerance. There is now considerable evidence that cAMP mediates the hyperalgesic effects of PGE_2 (Ferreira and Nakamura, 1979; Taiwo and Levine, 1991; Hingtgen et al., 1995) as well as a sensitization of primary afferent neurons (Pitchford and Levine, 1991; Vasko et al., 1994; Wang et al., 1995). For example, PGE_2 raises immunoreactive cAMP levels in sensory neuron cultures, which results in an enhanced release of primary afferent neurotransmitter peptides (evoked by bradykinin or capsaicin) (Hingtgen et al., 1995). Increased cAMP levels will also produce an increase in the phosphorylation of intracellular proteins, including ion channels, thereby increasing neuronal excitability. In fact, several studies have demonstrated that both cAMP and prostaglandins enhance sensory neuron excitation produced by bradykinin or capsaicin (Martin et al., 1987; Dray et al., 1992; Ouseph et al., 1995). Therefore, it is of note that it has been shown that both opioids and A_1-adenosine antinociceptive agents act on receptors coupled to G-proteins (Ferreira, 1981; Taiwo and Levine, 1991; Ingram and Williams, 1994), which *decrease* cAMP levels (VanCaulker et al., 1979; Hamprecht and VanCaulker, 1985). These results suggest that primary afferent induced hyperalgesia and antinociception involve modulation of a common second messenger pathway (Hingtgen et al., 1995).

An alternative explanation for the observed cross-tolerance is that repeated DAMGO administration could release endogenous adenosine (to act at and tolerize A_1-receptors). This alternative explanation is unlikely for two reasons. First, while endogenous opioids can be released in peripheral tissues, this only occurs in the setting of inflammation (Stein et al., 1990), and opioids, such as DAMGO, are anti-inflammatory in peripheral tissues. Second, since PACPX did not affect DAMGO antinociception (suggesting endogenous adenosine is not involved) and naloxone did not affect CPA antinociception (suggesting that endogenous opioids are not involved), it is unlikely that cross-tolerance between DAMGO and CPA is due to release of endogenous ligands.

Tolerance to the effects of opioids is commonly associated with the development of dependence on the opioid, most often evident by the presence of an abstinence-induced or an antagonist-precipitated withdrawal (Way, 1993). For example, after development of tolerance to the antinociceptive effect of morphine, hyperalgesia might result if the opioid is discontinued or if naloxone were administered. We found that both in DAMGO- and CPA-tolerant paws, a withdrawal response is induced by the selective opioid and A_1-adenosine antagonists respectively, and also that a cross-withdrawal response is observed (Figs. 5, 7). Antagonist-induced withdrawal is thought to result from displacement of the agonist from its receptor binding sites. However, the observation of cross-withdrawal suggests that dependence in this model is not maintained solely by receptor occu-

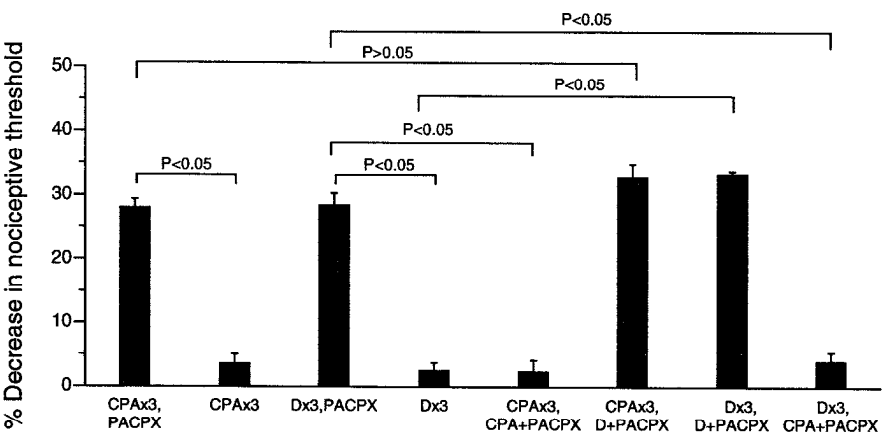

Figure 7. PACPX induces withdrawal hyperalgesia in CPA- and DAMGO-tolerant paws. Effect of CPA hourly $\times$ 3 and at the fourth hour PACPX (*CPAx3,PACPX*, $n = 8$), DAMGO hourly $\times$ 3 and at the fourth hour PACPX (*Dx3,PACPX*, $n = 8$), CPA hourly $\times$ 3 and at the fourth hour CPA plus PACPX (*CPAx3,CPA+PACPX*, $n = 6$), CPA hourly $\times$ 3 and at the fourth hour DAMGO plus PACPX (*CPAx3,D+PACPX*, $n = 6$), DAMGO hourly $\times$ 3 and at the fourth hour DAMGO plus PACPX (*Dx3,D+PACPX*, $n = 6$), DAMGO hourly $\times$ 3 and at the fourth hour CPA plus PACPX (*Dx3,CPA+PACPX*, $n = 6$) on mechanical paw-withdrawal threshold in the rat.

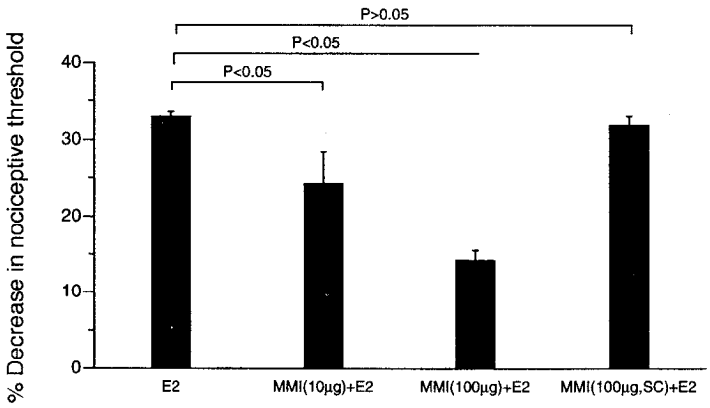

Figure 8. Quaternary morphine inhibits PGE_2-induced hyperalgesia. Effect of PGE_2 (*E2*, $n = 16$), morphine methyliodide (10 μg) plus PGE_2 (*MMI(10μg)+E2*, $n = 6$), morphine methyliodide (100 μg) plus PGE_2 (*MMI(100μg)+E2*, $n = 6$), and morphine methyliodide 100 μg s.c. plus PGE_2 (*MMI(100μg,SC)+E2*, $n = 6$) on mechanical paw-withdrawal threshold in the rat.

pancy. A recent model of opioid dependence suggested by Wang and colleagues (1994) is of interest in this regard. These workers hypothesize that μ-opioid agonists produce dependence by inducing a spontaneously active phosphorylated state of the μ-opioid receptor at which naloxone can act as an inverse agonist. Thus, cross-tolerance may be a result of phosphorylation of both the A_1-adenosine as well as the μ-opioid receptor following chronic exposure to either CPA or DAMGO.

DAMGO failed to block PACPX-induced withdrawal hyperalgesia in DAMGO-tolerant paws (Fig. 7). Similarly, CPA administered with naloxone in CPA-tolerant paws resulted in a marked naloxone-induced cross-withdrawal (Fig. 5); however, there was a small, but statistically significant, reduction compared to naloxone alone. This small difference may be due to an incomplete tolerance for CPA agonism produced following three hourly injections.

In conclusion, we report that both μ-opioid and A_1-adenosine agonists produce tolerance to their peripheral antinociceptive effects on repeated administration. Furthermore, we suggest that both μ-opioid and A_1-adenosine receptors share a common second messenger pathway in the induction of tolerance since there is cross-tolerance between agonists acting at these two receptors. Finally the observation of naloxone- and PACPX-induced withdrawal in both opioid- and CPA-tolerant paws, supports the hypothesis of the development of a cross-dependence as well as a cross-tolerance for the peripheral antinociceptive effects of μ-opioid and A_1-adenosine agonists. The failure of the opioid agonist to block the withdrawal effect of the A_1-adenosine antagonist and the failure of the adenosine agonist to block the withdrawal effect of opioid antagonist, irrespective of the agonist (μ or A_1) used to induce tolerance, suggests that it is not necessary for an agonist to occupy a receptor for an antagonist to be able to precipitate withdrawal and thus that there must be changes in the action of the antagonist at that receptor (e.g., phosphorylation of the receptor so that the antagonist can now act as an inverse agonist; Wang et al., 1994). This suggests that the changes associated with dependence may involve cellular mechanisms distinct from those underlying tolerance. Further studies are in progress to investigate the specific second messenger pathways in the primary afferent underlying these phenomena.

References

Aley KO, Khasar SG, Levine JD (1995) Multiple second messenger systems act sequentially to mediate rolipram-induced prolongation of prostaglandin E_2-induced mechanical hyperalgesia in the rat. Neuroscience 64:769–776.

Casati C, Monopoli A, Dionisotti S, Zocchi C, Bonizzoni E, Ongini E (1994) Repeated administration of selective adenosine A_1 and A_2 receptor agonist in the spontaneously hypertensive rat: tolerance develops to A_1-mediated hemodynamic effects. J Pharmacol Exp Ther 268:1506–1511.

Collin E, Cesselin F (1991) Neurobiological mechanisms of opioid tolerance and dependence. Clin Neuropharmacol 14:465–488.

Cooper DMF, Londos C, Rodbell R (1980) Adenosine receptor mediated inhibition of rat cerebral cortical adenylate cyclase by a GTP-dependent process. Mol Pharmacol 18:598–601.

Costa T, Klinz FJ, Vachon L, Herz A (1988) Opioid receptors are coupled tightly to G proteins but loosely to adenylate cyclase in NG108-15 cell membranes. Mol Pharmacol 34:744–754.

Cox BM, Ginsburg M, Osman OH (1968) Acute tolerance to narcotic analgesic drugs in rats. Br J Pharmacol 33:245–256.

Dray A, Patel IA, Perkins MN, Rueff A (1992) Bradykinin-induced activation of nociceptors: receptor and mechanistic studies on the neonatal rat spinal cord-tail preparation *in vitro*. Br J Pharmacol 107: 1129–1134.

Duggan AW (1979) Morphine and enkephalins at the spinal cord. Adv Pain Res Ther 3:449–458.

Ebersolt C, Premont J, Prochiantz A, Perez M, Bockaert J (1983) Inhibition of brain adenylate adenylate cyclase by A_1 adenosine receptors: pharmacological characteristics and locations. Brain Res 267: 123–129.

Ferreira SH (1981) Local control of inflammatory pain. Agents Actions 11:636–638.

Ferreira SH, Nakamura M (1979) Prostaglandin hyperalgesia, a cAMP/Ca^{2+}-dependent process. Prostaglandins 18:179–190.

Ferreira SH, Lorenzetti BB, Rae GA (1984) Is methylnalorphinium the prototype of an ideal peripheral analgesic? Eur J Pharmacol 99:23–29.

Fields HL, Basbaum AI (1978) Brainstem control of spinal pain-transmission neurons. Annu Rev Physiol 4:451–462.

Hamprecht B, VanCaulker P (1985) Nomenclature of adenosine receptors. Trends Pharmacol Sci 6:151–154.

Harada H, Ueda H, katada T, Ui M, Satoh M (1990) Phosphorylated μ-opioid receptor purified from rat brain lacks functional coupling with G_i, a GTP-binding protein in reconstituted lipid vesicles. Neurosci Lett 113:47–49.

Hingtgen C, Waite K, Vasko M (1995) Prostaglandins facilitate peptide release from rat sensory neurons by activating the adenosine 3′,5′-cyclic monophosphate transduction cascade. J Neuroscience 15: 5411–5419.

Ingram SL, Williams JT (1994) Opioid inhibition of Ih via adenylyl cyclase. Neuron 13:179–186.

Junien JL, Wettstein JG (1992) Role of opioids in peripheral analgesia. Life Sci 51:2009–2018.

Kayser V, Gobeaux D, Lombard MC, Guilbaud G, Besson JM (1990) Potent and long lasting antinociceptive effects after injection of low doses of a mu-opioid receptor agonist, fentanyl, into the brachial plexus sheath of the rat. Pain 42:215–225.

Law PY, Wu J, Koehler E, Loh HH (1985) Demonstration and characterization of opiate inhibition of the striatal adenylate cyclase activity. J Neurochem 36:1834–1846.

Law PY, Hom DS, Loh HH (1991) Effect of chronic D-Ala2. D-Leu5-enkephalin or pertussis toxin treatment on the high-affinity state of delta-opioid receptor in neuroblastoma × glioma NG108-15 hybrid cells. J Pharmacol Exp Ther 256:710–716.

Levine JD, Taiwo YO (1989) Involvement of the mu-opiate receptor in peripheral analgesia. Neuroscience 32:571–575.

Mankman MH, Dvorkin B, Crain SM (1988) Modulation of adenylate cyclase activity of mouse spinal cord-ganglion explants by opioids, serotonin and pertussis toxin. Brain Res 445:303–313.

Martin HA, Basbaum AI, Kwiat GC, Goetzl EJ, Levine JD (1987) Leukotriene and prostaglandin sensitization of cutaneous high-threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. Neuroscience 22:651–659.

Mayer DJ, Price DD (1976) Central nervous system mechanisms of analgesia. Pain 2:379–404.

Moiniche S, Dahl JB, Kehlet H (1993) Peripheral antinociceptive effects of morphine after burn injury. Acta Anaesthesiol Scand 37:710–712.

Ouseph AK, Khasar SG, Levine JD (1995) Multiple second messenger systems act sequentially to mediate rolipram-induced prolongation of prostaglandin E2-induced mechanical hyperalgesia in rat. Neuroscience 64:769–776.

Parsons CG, Herz A (1990) Peripheral opioid receptors mediating antinociception in inflammation. Evidence for activation by enkephalin-like opioid peptides after cold water swim stress. J Pharmacol Exp Ther 255:795–802.

Pitchford S, Levine JD (1991) Prostaglandins sensitize nociceptors in cell culture. Neurosci Lett 132:105–108.

Polastron J, Boyer MJ, Quertermont Y, Thouvenot JP, Meunier JC, Jauzac P (1990) Mu-opioid receptors and not kappa-opioid receptors are coupled to the adenylate cyclase in the cerebellum. J Neurochem 54:562–570.

Puttfarcken PS, Werling LL, Cox BM (1988) Effects of chronic morphine exposure on opioid inhibition of adenylyl cyclase in 7315c cell membranes: a useful model for the study of tolerance at mu opioid receptors. Mol Pharmacol 33:520–527.

Sharma SK, Nirenberg M, Klee WA (1975) Morphine receptors as regulators of adenylate cyclase activity. Proc Natl Acad Sci USA 72: 590–594.

Smith TW, Buchan P, Parsons DN, Wilkinson S (1982) Peripheral antinociceptive effects of N-methyl morphine. Life Sci 31:1205–1208.

Stein C (1991) Peripheral analgesic actions of opioids. J Pain Symptom Management 6:119–124.

Stein C, Millan MJ, Yassouridis A, Herz A (1988) Antinociceptive effects of mu- and kappa-agonists in inflammation are enhanced by a peripheral opioid receptor-specific mechanism. Eur J Pharmacol 155:255–264.

Stein C, Millan MJ, Shippenberg TS, Peter K, Herz A (1989) Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J Pharmacol Exp Ther 248:1269–1275.

Stein C, Comisel K, Haimerl E, Yassouridis A, Lehrberger K, Herz A, Peter K (1991) Analgesic effect of intraarticular morphine after arthroscopic knee surgery. N Engl J Med 325:1123–1126.

Taiwo YO, Levine JD (1990) Direct cutaneous hyperalgesia induced by adenosine. Neuroscience 38:757–762.

Taiwo YO, Levine JD (1991) Further confirmation of the role of adenyl cyclase and of cAMP-dependent protein kinase in primary afferent hyperalgesia. Neuroscience 44:131–135.

Taiwo YO, Coderre TJ, Levine JD (1989) The contribution of training to sensitivity in the nociceptive paw-withdrawal test. Brain Res 487: 148–151.

Tsuchida A, Thompson R, Olsson RA, Downey JM (1994) The anti-infarct effect of an adenosine A1-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischaemic preconditioning in rabbit heart. J Mol Cell Cardiol 26:303–311.

VanCaulker P, Muller M, Hamprecht B (1979) Adenosine regulates via two different types of receptors the accumulation of cAMP in cultured brain cells. J Neurochem 33:999–1005.

Vasko MR, Campbell WB, Waite KJ (1994) Prostaglandin E2 enhances bradykinin-stimulated release of neuropeptides from rat sensory neurons in culture. J Neurosci 14:7–4997.

Wang Z, Bilsky EJ, Porreca F, Sadee W (1994) Constitutive μ-opioid receptor activation as a regulatory mechanism, underlying narcotic tolerance and dependence. Life Sci 54:339–350.

Wang J-F, Khasar SG, Ahlgren SC, Levine JD (1995) Sensitization of C-fibers by prostaglandin E_2 in the rat is inhibited by guanosine 5′-O-(2-thiodiphosphate), 2′ · 5′-dideoxyadenosine and WIPTIDE. Neuroscience, in press.

Way EL (1993) Opioid tolerance and physical dependence and their relationship. Handb Exp Pharmacol 104:573–596.

Werling LL, McMahon PN, Cox BM (1989) Selective changes in mu-opioid receptor properties induced by chronic morphine exposure. Proc Natl Acad Sci USA 86:6393–6397.

Wheeler-Aceto H, Cowan A (1991) Involvement of a peripheral component in the antinociceptive effects of opioids in a model of tonic pain. Nida Res Monogr 105:399–400.