3. Results

3.1. Evaluation of inflammation at 6 h and 4 days

Both at 6 h and 4 days after i.pl. FCA inflammation was confined to the inoculated paws and characterized by hyperalgesia (decreased PPT), swelling (increased paw volume), hyperthermia (elevated paw temperature) ($p < 0.001$, paired t-test; Fig. 4). PPT and PT of inflamed paws were not significantly different between 6 h and 4 days ($p > 0.05$, t-test), whereas PV of inflamed paws at 4 days was significantly higher than at 6 h ($p < 0.001$, t-test).

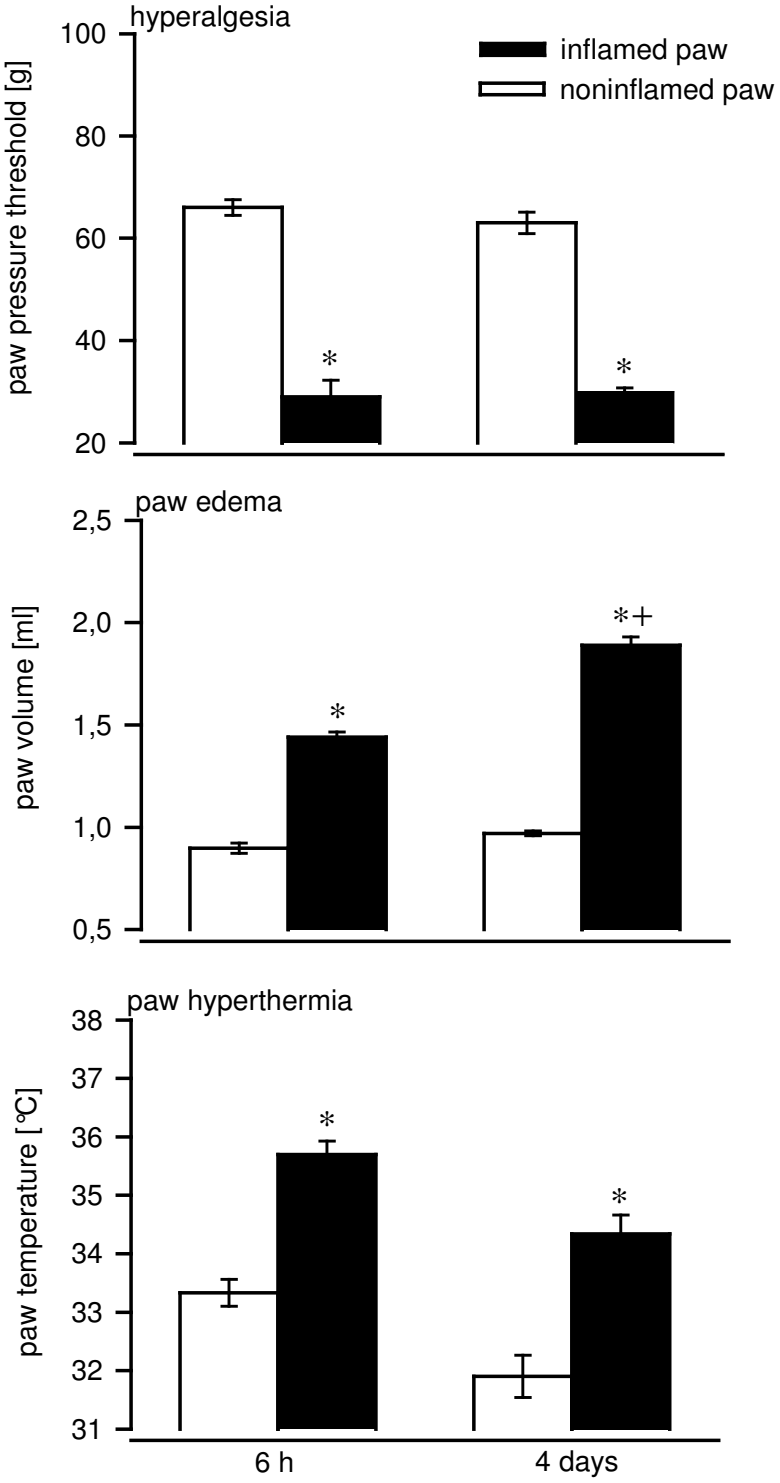

Figure 4. Assessment of inflammation at 6 h and 4 days after injection of FCA into one hindpaw. * indicates a statistically significant difference compared to respective noninflamed paw ($p < 0.001$, paired t-test). + indicates a statistically significant difference compared to inflamed paw at 6 h ($p < 0.001$, t-test). Data are expressed as means ± SEM.

3.2. Effects of the duration of inflammation on swim stress-induced antinociception

Both at 6 h and 4 days exposure of rats to CWS produced antinociception in inflamed but not in noninflamed paws ($p < 0.001$ and $p > 0.05$, paired t-test, respectively; Table 1). At both time points after FCA maximum antinociception was measured at 1 min ($p < 0.05$, Dunnett's test; Table 1) and returned to baseline levels at 5-10 min after CWS ($p > 0.05$, Dunnett's test; not shown). Therefore, further experiments were performed at 1 min after CWS. In one of four experiments CWS-induced antinociception was significantly higher at 4 days compared with 6 h after FCA ($p < 0.05$, t-test; Table 1).

Table 1. Effects of CWS on PPT at 6 h and 4 days after induction of inflammation

Paw pressure threshold [g]				
	6 h		4 days	
	inflamed	noninflamed	inflamed	noninflamed
BL	25 ± 2.2	60 ± 1.1	25 ± 1.5	62 ± 1.7
CWS	103 ± 5,1* +	60 ± 1,3	126 ± 9,4*	64 ± 1,3

PPT were measured before (baseline; BL) and at 1 min after CWS. * indicates a statistically significant difference compared with respective baseline ($p < 0.001$, paired t-test). + indicates a statistically significant difference compared with inflamed paw at 4 days ($p < 0.001$, t-test). Data are expressed as means ± SEM.

3.3. Peripheral intrinsic opioid antinociception at 6 h after induction of inflammation

3.3.1. Effects of local administration of opioid receptor antagonists on swim stress-induced antinociception

Intraplantar injection of NLX (0.14 - 1.125 μg) and antagonists selective for μ- (CTOP, 0.5 - 2 μg), δ- (NTI, 25 - 50 μg) and κ- (norBNI, 12.5 - 37.5 μg) opioid receptors dose-dependently decreased CWS-induced antinociception ($p < 0.001$, ANOVA, linear regression; Figs. 5 – 8). The most effective doses of each antagonist

did not completely abolish this antinociception, i.e. the remaining PPT were significantly higher than baseline PPT ($p < 0.001$, paired t-test, Figs. 5 - 8, Fig. 10). Higher doses of NLX (1.4 - 2.25 μg), CTOP (3 - 4 μg), NTI (75 - 100 μg) and norBNI (50 μg) produced less inhibition of stress-induced antinociception ($p > 0.05$, Dunnett's test; Figs. 5 - 8). No significant changes were observed in noninflamed paws ($p > 0.05$, ANOVA; Figs. 5 - 8).

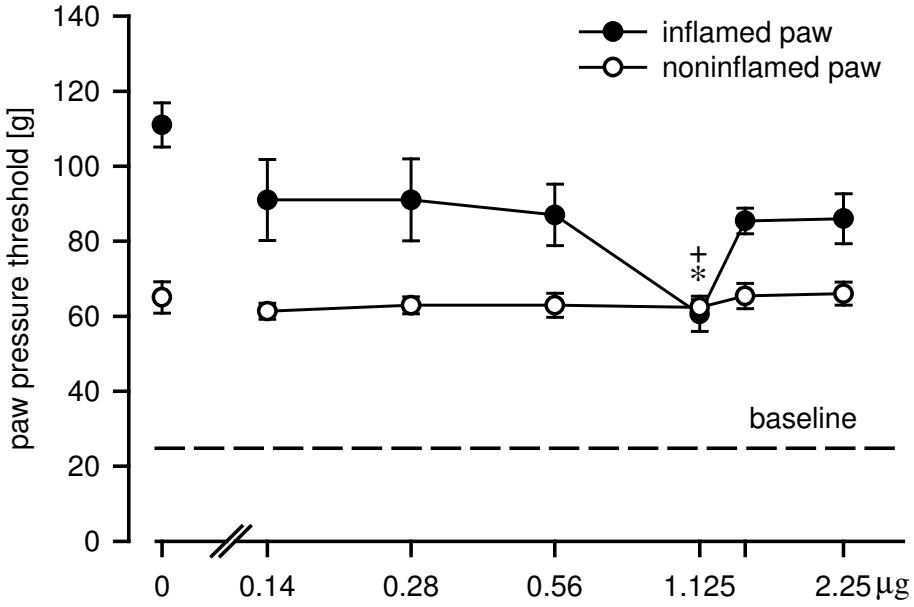

Figure 5. Dose-response effects of intraplantar nonselective opioid receptor antagonist NLX on CWS-induced antinociception at 6 h after induction of inflammation. NLX (0.14 – 2.25 μg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (25 ± 1.6 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

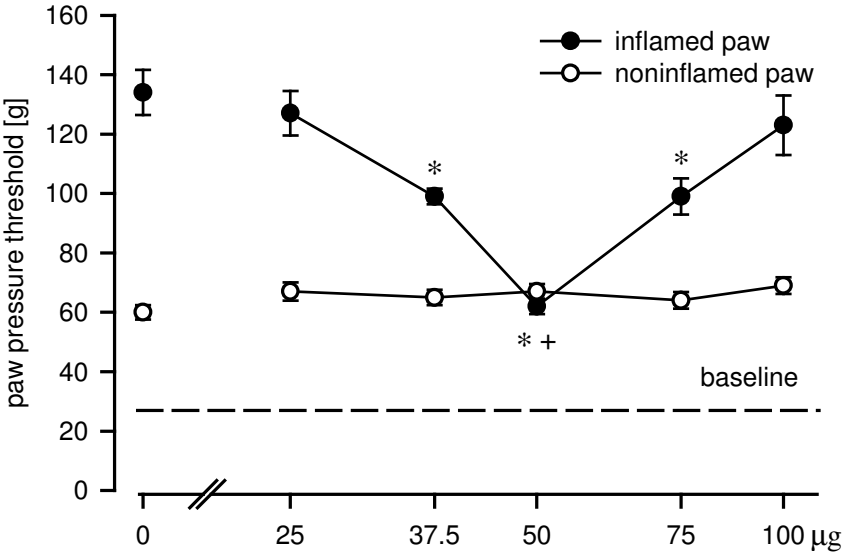

Figure 6. Dose-response effects of intraplantar selective opioid receptor antagonist CTOP on CWS-induced antinociception at 6 h after induction of inflammation. CTOP (0.5 – 4 µg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (24 ± 1.8 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

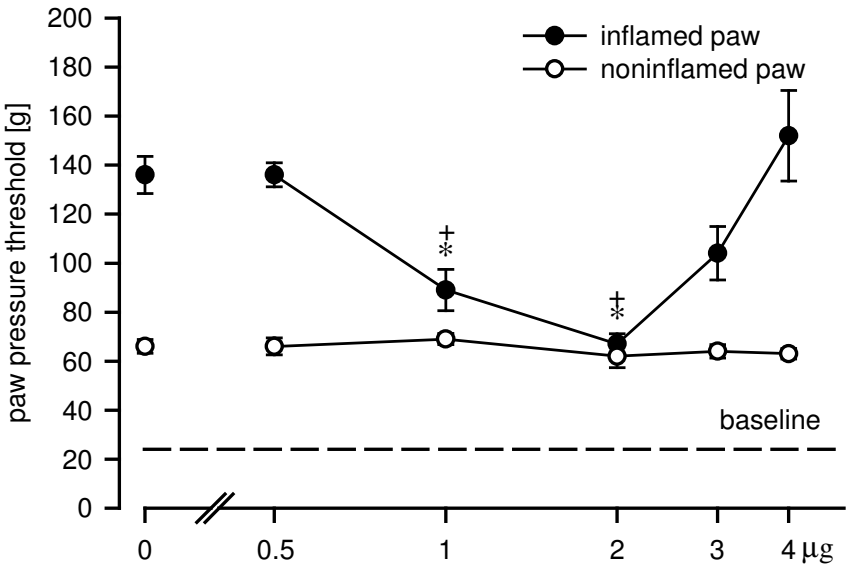

Figure 7. Dose-response effects of intraplantar selective opioid receptor antagonist NTI on CWS-induced antinociception at 6 h after induction of inflammation. NTI (25 – 100 µg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (27 ± 1.65 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

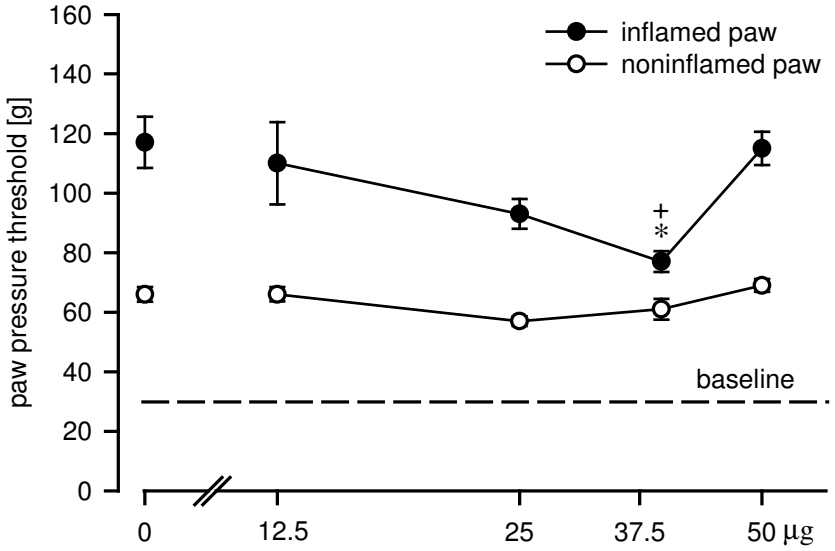

Figure 8. Dose-response effects of intraplantar selective opioid receptor antagonist nor-BNI on CWS-induced antinociception at 6 h after induction of inflammation. Nor-BNI (12 – 50 µg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (30 ± 1.99 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

Concomitant i.pl. injection of CTOP (2 µg), NTI (50 µg) and nor-BNI (37.5 µg) in the doses that were the most effective when antagonists were injected separately significantly decreased CWS-induced antinociception ($p < 0.001$, t-test; Fig. 9). This treatment did not completely abolish this antinociception i.e. the remaining PPT were significantly higher than baseline PPT ($p < 0.001$, paired t-test; Fig. 9). The effect of combined injection of antagonists was not significantly different compared with the effect of each antagonist given alone ($p > 0.05$, ANOVA; compare Fig. 9 with Fig. 10).

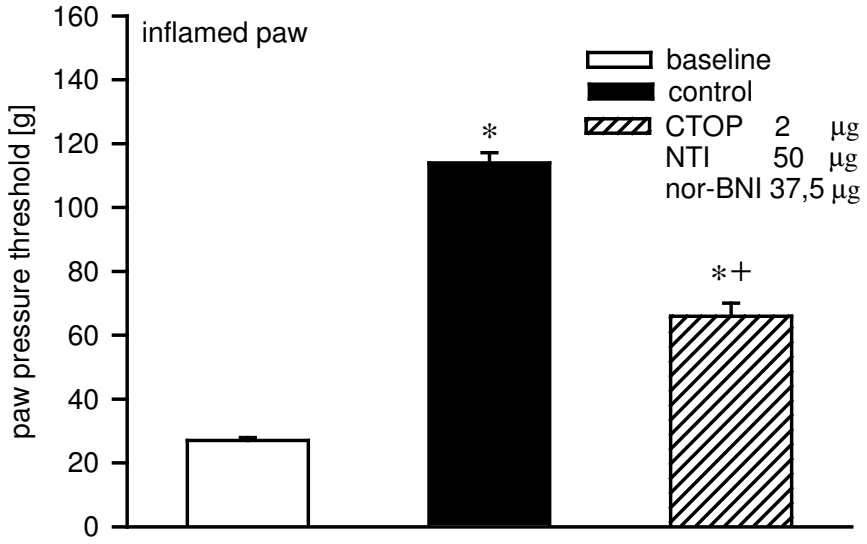

Figure 9. The effect of the concomitant intraplantar injection of CTOP, NTI and nor-BNI on CWS-induced antinociception at 6 h after induction of inflammation. * indicates a statistically significant difference compared with respective baseline ($p < 0.001$, paired t-test). + indicates a statistically significant difference compared with respective control ($p < 0.001$, t-test). Data are expressed as means ± SEM.

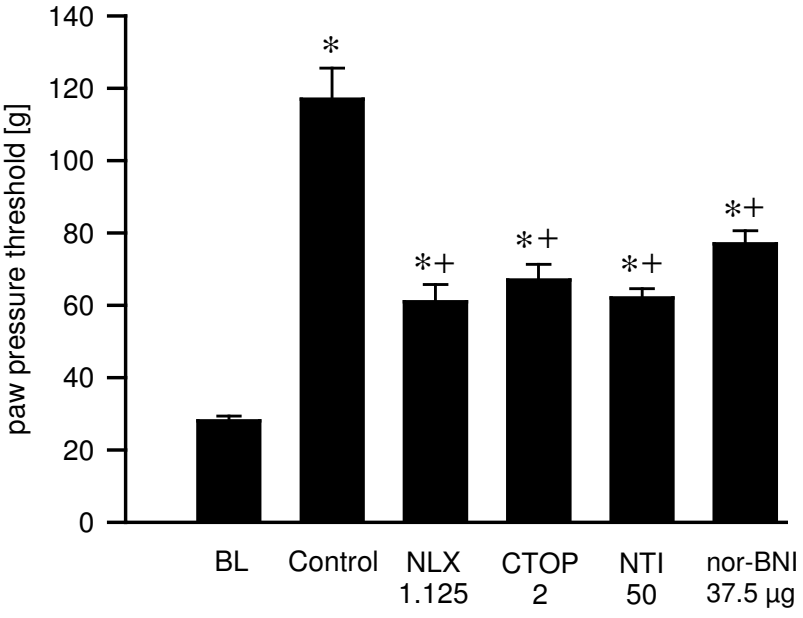

Figure 10. Summary of the effects of the most effective intraplantar doses of opioid receptor antagonists on CWS-induced antinociception at 6 h after induction of inflammation. Representative baseline values (BL) and one representative control group were chosen for simplicity. * indicates a statistically significant difference compared with respective baseline ($p < 0.001$, paired t-test). + indicates a statistically significant difference compared with respective control group ($p < 0.001$, t-test). Data are expressed as means ± SEM.

3.3.2. Effects of local administration of antibodies against opioid peptides on swim stress-induced antinociception

Intraplantar injection of anti-β-END (0.25 - 2 μg), anti-Met-ENK (0.06 - 1 μg) or anti-DYN (1 - 8 μg) dose-dependently decreased CWS-induced antinociception (anti- β-END, anti-Met-ENK, $p < 0.001$; anti-DYN, $p < 0.01$; ANOVA, linear regression) (Figs. 11 – 13). The most effective doses of each antibody did not completely abolish this antinociception, i.e. the remaining PPT were significantly higher than baseline PPT ($p < 0.001$, paired t-test) (Figs. 11 - 13, Fig. 15). Higher doses of anti-β-END (3 μg), anti-Met-ENK (4 μg) and anti-DYN (16 μg) produced less inhibition of CWS-induced antinociception ($p > 0.05$, Dunnett's test; Figs. 11 - 13). No significant changes were observed in noninflamed paws ($p > 0,05$, ANOVA; Figs. 11 - 13).

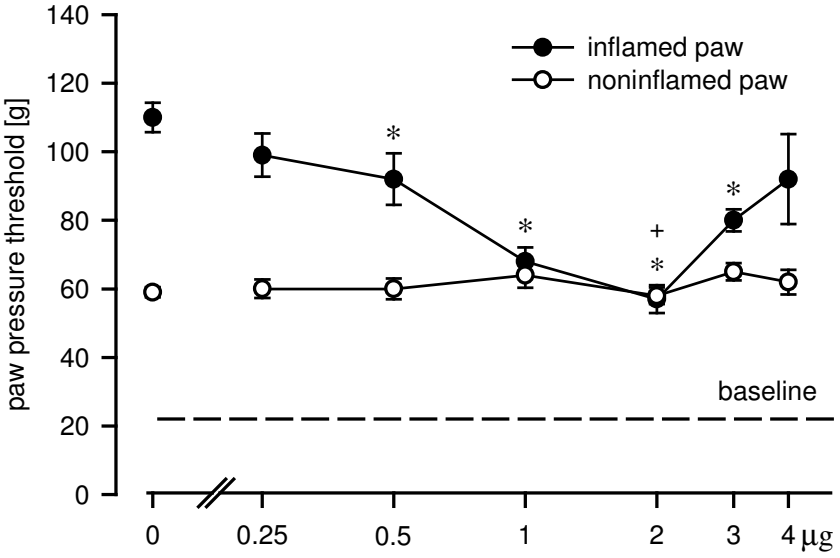

Figure 11. Dose-response effects of intraplantar antibody against β-END on CWS-induced antinociception at 6 h after induction of inflammation. Anti-β-END (0.2 – 4 μg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (22 ± 1.0 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

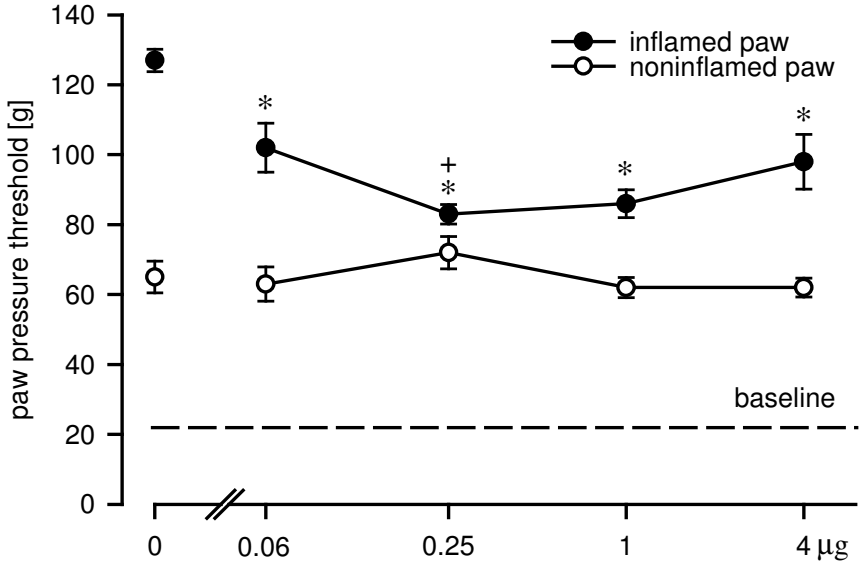

Figure 12. Dose-response effects of intraplantar antibody against Met-ENK on CWS-induced antinociception at 6 h after induction of inflammation. Anti-Met-ENK (0.06 – 4 µg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (23 ± 1.3 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

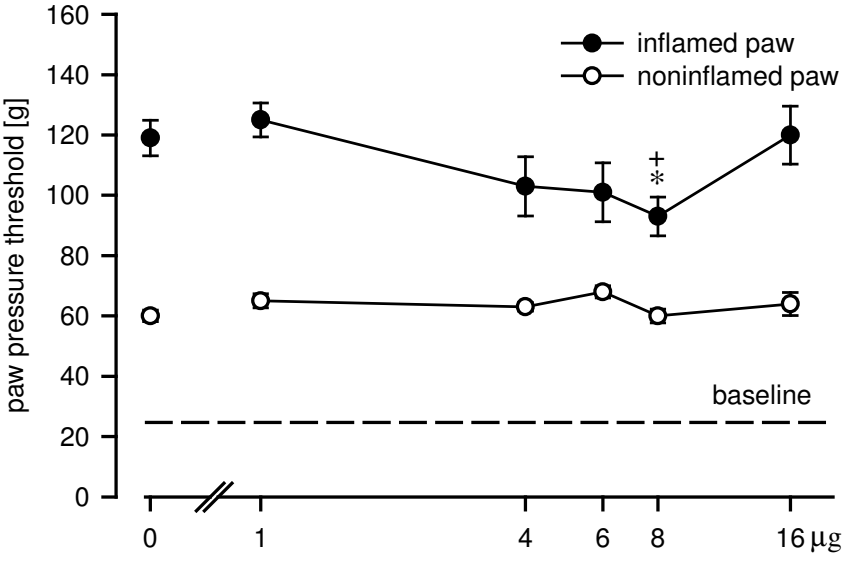

Figure 13. Dose-response effects of intraplantar antibody against DYN on CWS-induced antinociception at 6 h after induction of inflammation. Anti-DYN (1 – 16 µg) ($p < 0.01$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (25 ± 1.1 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

3.3.3. Effects of local administration of corticotropin releasing factor receptor antagonist on swim stress-induced antinociception

Intraplantar injection of the CRF receptor antagonist α-helical CRF (3 - 16 ng) dose-dependently decreased CWS-induced antinociception ($p < 0.01$, ANOVA, linear regression; Fig. 14). Its most effective dose did not completely abolish this antinociception, i.e. the remaining PPT were significantly higher than baseline PPT ($p < 0.001$, paired t-test) (Fig. 14, 15). Higher doses of α-helical CRF (32 - 64 ng) produced less inhibition of CWS-induced antinociception (Fig. 14). No significant changes were observed in noninflamed paws ($p > 0.05$, ANOVA; Fig. 14).

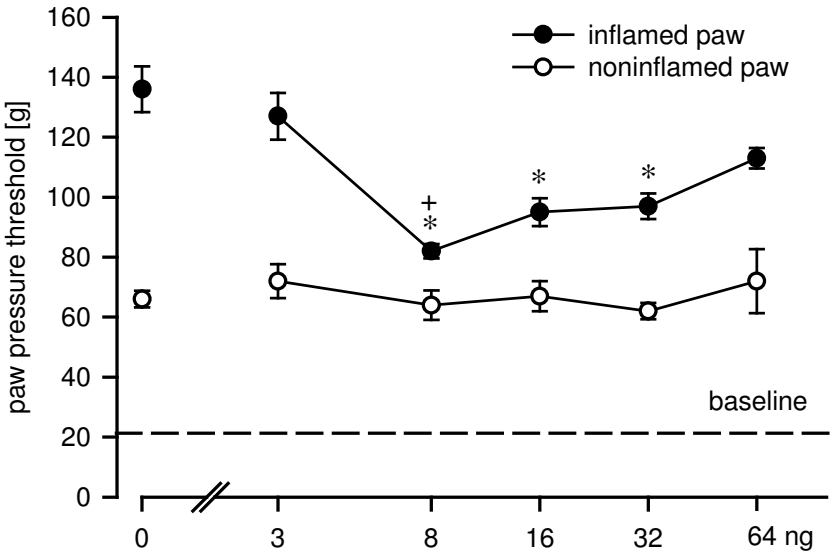

Figure 14. Dose-response effects of intraplantar CRF receptor antagonist α-helical CRF on CWS-induced antinociception at 6 h after induction of inflammation. α-helical CRF (3 - 64 ng) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). + indicates a statistically significant difference compared with baseline (21.5 ± 1.8 g; dashed line) ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

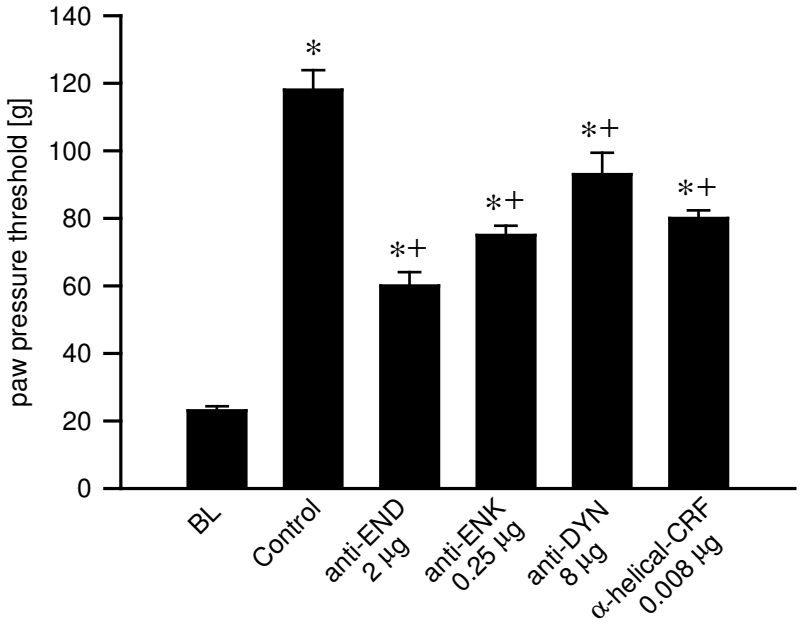

Figure 15. Summary of the effects of the most effective intraplantar doses of antibodies against opioid peptides and α-helical CRF on CWS-induced antinociception at 6 h after induction of inflammation. Representative baseline values (BL) and one representative control group were chosen for simplicity. * indicates a statistically significant difference compared with respective baseline ($p < 0.001$, paired t-test). + indicates a statistically significant difference compared with respective control group ($p < 0.001$, t-test). Data are expressed as means ± SEM.

3.4. Central intrinsic opioid antinociception at 6 h after induction of inflammation

Subcutaneous injection of peripherally and centrally acting doses of NLX (0.06 - 2 mg/kg) dose-dependently blocked CWS-induced antinociception completely ($p < 0.001$, ANOVA, linear regression; Fig. 16). The effect of NLX (2 mg/kg s.c.) was not significantly different from baseline PPT ($p > 0.05$, paired t-test; Fig. 16). No significant changes were observed in noninflamed paws ($p > 0.05$, ANOVA; Fig. 16).

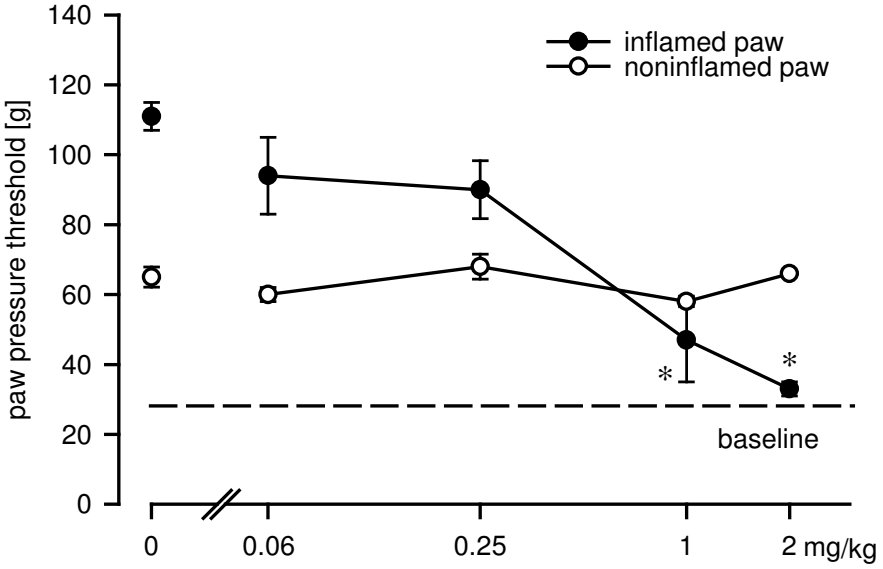

Figure 16. Dose-response effects of subcutaneous nonselective opioid receptor antagonist NLX on CWS-induced antinociception at 6 h after induction of inflammation. NLX (0.06 – 2 mg) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). Data are expressed as means ± SEM.

3.5. Peripheral intrinsic opioid antinociception at 4 days after induction of inflammation

Intraplantar injection of NLX (18 μg) in a peripherally selective dose [70] completely blocked CWS-induced antinociception, i.e. its effect was not significantly different from baseline PPT ($p > 0.05$, paired t-test; Table 2). In contrast, i.pl. nor-BNI (37.5 μg) and anti-Met-ENK (0.25 μg) did not significantly change CWS-induced antinociception ($p > 0.05$, t-test; Table 2). In a previous study we found that higher i.pl. doses of nor-BNI (50 - 400 μg) and anti-Met-ENK (1 - 8 μg) did not produce significant effects either while i.pl. anti-β-END (0.25 - 1 μg) and selective μ- and δ-receptor antagonists completely inhibited CWS-induced antinociception [70]. No changes were observed in noninflamed paws ($P > 0.05$, paired t-test; Table 2).

Table 2. Effects of intraplantar NLX, nor-BNI, and anti-Met-ENK on CWS-induced antinociception at 4 days after induction of inflammation

Treatment (dose)		Paw pressure threshold [g]	
		inflamed paw	noninflamed paw
Control	BL	23 ± 1.6	60 ± 1.7
	CWS	119 ± 5.4 *	65 ± 2.6
NLX (18 µg)	BL	32 ± 1.7	65 ± 3.0
	CWS	38 ± 3.2	64 ± 3.8
Nor-BNI (37.5 µg)	BL	33 ± 1.1	65 ± 1.5
	CWS	133 ± 6.6 *	70 ± 3.8
Anti-Met-ENK (0.25 µg)	BL	22 ± 2.5	61 ± 2.0
	CWS	172 ± 14.4 *	69 ± 5.4

PPT were measured before (baseline; BL) and at 1 min after CWS. * indicates a statistically significant difference compared with respective baseline ($p < 0.001$, paired t-test). Data are expressed as means ± SEM.

3.6. Peripheral corticotropin releasing factor-induced antinociception at 6 h after induction of inflammation

3.6.1. Effects of local injection of corticotropin releasing factor on nociceptive thresholds

Intraplantar CRF (0.5 - 6 ng) produced dose-dependent antinociception in inflamed but not in noninflamed paws ($p < 0.001$ and $p > 0.05$, respectively; ANOVA, linear regression; Fig. 17 B). Maximum antinociceptive effects were observed at 5 min and returned to baseline levels at 10 min after injection ($P < 0.05$ and $P > 0.05$, respectively, Dunnett's test; Fig. 17 A).

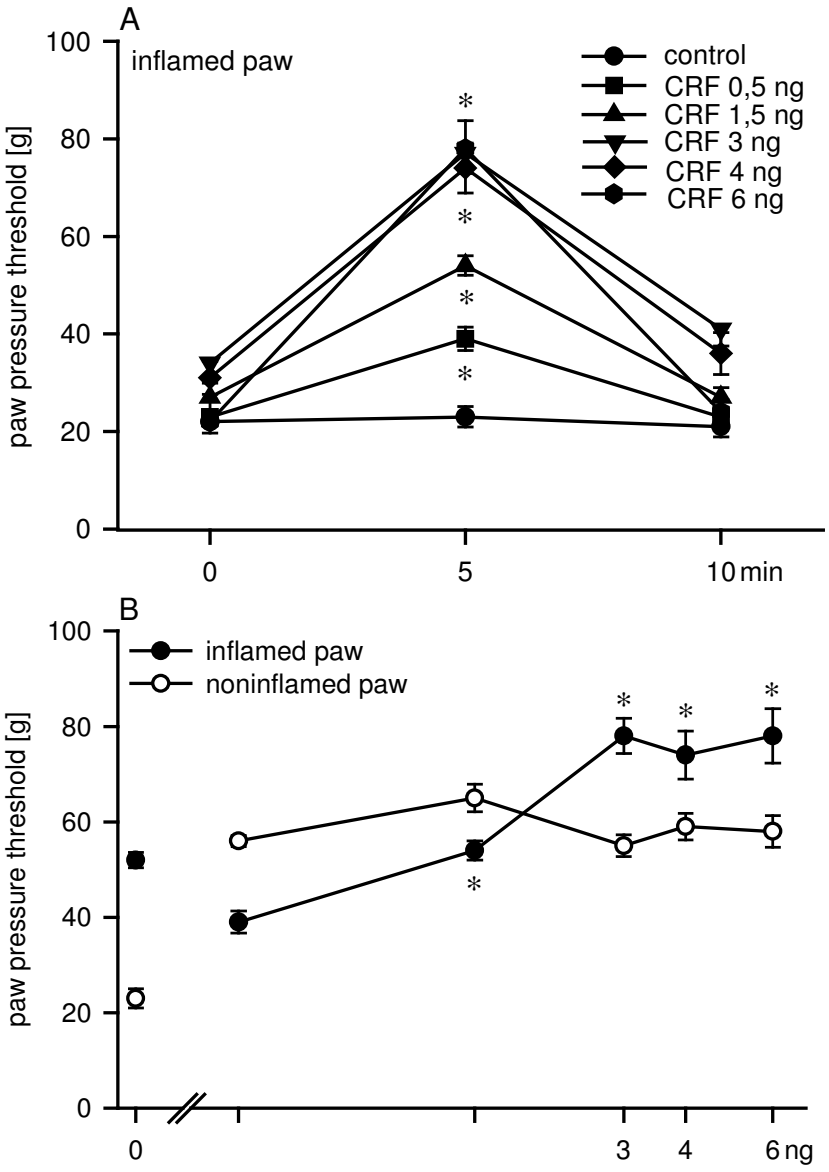

Figure 17. Effects of intraplantar CRF on PPT at 6 h after induction of inflammation. **A.** Dose-response effects at 5 min after CRF. ∗ indicates a statistically significant difference compared with respective control group ("0" dose) ($p < 0.05$ Dunnett's test). Inflamed paw ($p < 0.001$, ANOVA, linear regression). Noninflamed paw ($p > 0.05$, ANOVA). **B.** Time-course in inflamed paws. ∗ indicates a statistically significant difference compared with respective baseline ("0" time point) and with control group (at 5 min) ($p < 0.05$ Dunnett's test).Data are expressed as means ± SEM.

3.6.2. Effects of local injection of opioid receptor antagonist naloxone on corticotropin releasing factor-induced antinociception

NLX (9 - 140 ng) injected i.pl. concomitantly with CRF (6 ng) dose-dependently blocked CRF-induced antinociception in inflamed paws ($p < 0.001$, ANOVA, linear regression; Fig. 18). The effect of the most effective dose of NLX (140 ng) was not significantly different from baseline PPT ($p > 0.05$, paired t-test; Fig 18). No significant changes were observed in noninflamed paws ($p > 0.05$, ANOVA; Fig. 18). CRF-induced antinociception at 4 days after FCA was characterized earlier [64].

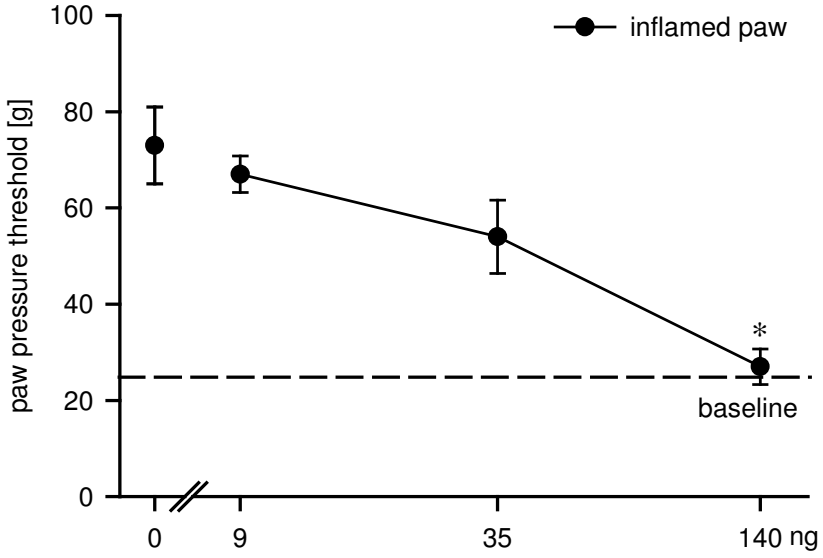

Figure 18. Dose-response effects of intraplantar nonselective opioid receptor antagonist NLX on intraplantar CRF (6 ng)-induced antinociception at 6 h after induction of inflammation. NLX (9 – 140 ng) ($p < 0.001$, ANOVA, linear regression). * indicates a statistically significant difference compared with the respective control group ("0" dose) ($p < 0.05$, Dunnett's test). Dashed line represents baseline paw pressure threshold of representative group and is 25 ± 1.4 g. Data are expressed as means ± SEM.

3.7. Confirmation of a peripheral site of action in intrinsic opioid antinociception at 6 h

Subcutaneous injections of the most effective i.pl. doses of NLX, CTOP, NTI and norBNI injected separately, CTOP, NTI and norBNI injected concomitantly, antibodies against each opioid peptide, and α-helical CRF had no significant effects on CWS-induced antinociception ($p > 0.05$, t-test; Table 3). Also, s.c. administration of the most effective i.pl. dose of CRF did not significantly change PPT in inflamed paws ($p > 0.05$, t-test; Table 3). No significant changes were observed after any of these treatments in noninflamed paws ($p > 0.05$, paired t-test; Table 3).

Table 3. The effect of subcutaeous injections of opioid receptor antagonists and antibodies against opioid peptides on CWS-induced analgesia and of CRF on PPT at 6 h after induction inflammation

Treatment (dose)	Paw pressure threshold [g]	
	inflamed paw	noninflamed paw
Control	99 ± 2.5	59 ± 0.9
NLX (1.125 μg)	92 ± 9.5	60 ± 1.7
CTOP (2 μg)	107 ± 4.1	61 ± 1.9
NTI (50 μg)	103 ± 7.0	61 ± 2.5
Nor-BNI (37.5 μg)	106 ± 6.9	57 ± 2.6
Control	115 ± 1.5	63 ± 2.5
Anti-β-END (2 μg)	112 ± 3.2	62 ± 3.5
Anti-Met-ENK (0.25 μg)	122 ± 9.9	63 ± 2.7
Anti-Dyn (8 μg)	115 ± 3.2	64 ± 2.8
Control	23 ± 1.7	61 ± 2.5
CRF (6 ng)	26 ± 2.3	57 ± 2.1

Data are expressed as means ± SEM.

3.8. Contribution of adhesion molecules to intrinsic opioid antinociception at 6 h after induction of inflammation

3.8.1. Effects of blockade of selectins, α4 and β2 integrins, intercellular adhesion molecle-1 and platelet endothelial cell adhesion molecule-1 on inflammation

Fucoidin, anti-α4 (4 – 8 mg/kg), anti-β2 (2 – 8 mg/kg), anti-ICAM-1 (2 – 8 mg/kg) and anti-PECAM-1 (1 – 10 mg/kg) had no significant influence on hyperalgesia , i.e. baseline PPT at 6 h after injection with FCA and anti-adhesion molecules but before CWS ($p > 0.05$, t-test; Table 4). Paw volume was significantly decreased by fucoidin (2.0 ± 0.05 ml vs. 1.7 ± 0.08 ml, control vs. fucoidin; $p < 0.01$, t-test; Table 4), and anti-ICAM-1 (1.6 ± 0.05 ml vs. 1.3 ± 0.03 ml, control vs. anti-ICAM-1, 4 mg/kg; $p < 0.001$, t-test; Table 4). Paw temperature was slightly decreased by anti-ICAM-1 (35.1 ± 0.14 °C vs. 34.2 ± 0.02 °C, control vs. anti-ICAM-1 8mg/kg, $p < 0.001$; Table 4). Other treatments with anti-α4, anti-β2, anti-ICAM-1 (2 mg/kg), and anti-PECAM-1 did not significantly change these parameters of inflammation ($p > 0.05$, t-test; Table 4). None of anti-adhesion molecule treatments caused significant changes in noninflamed paws ($p > 0.05$, t-test; Table 4).

3.8.2. Effects of blockade of selectins, α4 and β2 integrins, intercellular adhesion molecle-1 and platelet endothelial cell adhesion molecule-1 on swim stress-induced antinociception

The concomitant blockade of L- and P-selectins by fucoidin (10 mg/kg) completely abolished CWS-induced antinociception, because the effects of fucoidin were not significantly different from those of 1.125 µg i.pl. NLX (61 ± 4.7 g vs. 63 ± 4.5 g, NLX vs. fucoidin; $p < 0.05$, t-test; compare Fig. 5 with Fig. 19). Also, single blockade of IgSF member ICAM-1 by anti-ICAM-1 in a dose of 4 mg/kg markedly decreased CWS-induced antinociception (117 ± 6.1 g vs. 82 ± 4.3 g, control vs. anti-ICAM-1; $p < 0.001$, t-test; Fig. 19). The effect was slightly but significantly less compared with that of 1.125 µg i.pl. NLX (61 ± 4.7 g vs. 82 ± 4.8 g, NLX vs. anti-ICAM-1; $p < 0.05$, t-test; compare Fig. 5 with Fig. 19). A slight but significant decrease of CWS-induced antinociception was also observed after single blockade of integrin β2 by 4 mg/kg of

anti-β2 (116 ± 6.1 g vs. 101 ± 3.2 g, control vs. anti-β2; $p < 0.05$, t-test; Fig 19). This effect was significantly different from that of i.pl. naloxone in a dose of 1.125 µg (61 ± 4.7 g vs. 101 ± 3.2 g, NLX vs. anti-β2; $p < 0.05$, t-test; compare Fig. 5 with Fig. 19). The single blockade of integrins by other doses of anti-α4 (4 – 8 mg), anti-β2 (2 and 8 mg/kg), or IgSF members by other doses of anti-ICAM-1 (2 and 8 mg/kg), or anti-PECAM-1 (1 – 10 mg/kg), respectively, did not significantly change CWS-induced antinociception ($p > 0.05$, t-test, Fig. 19). No significant changes were observed in noninflamed paws after any treatment ($p > 0.05$, paired t-test; compare Table 4 with Table 5).

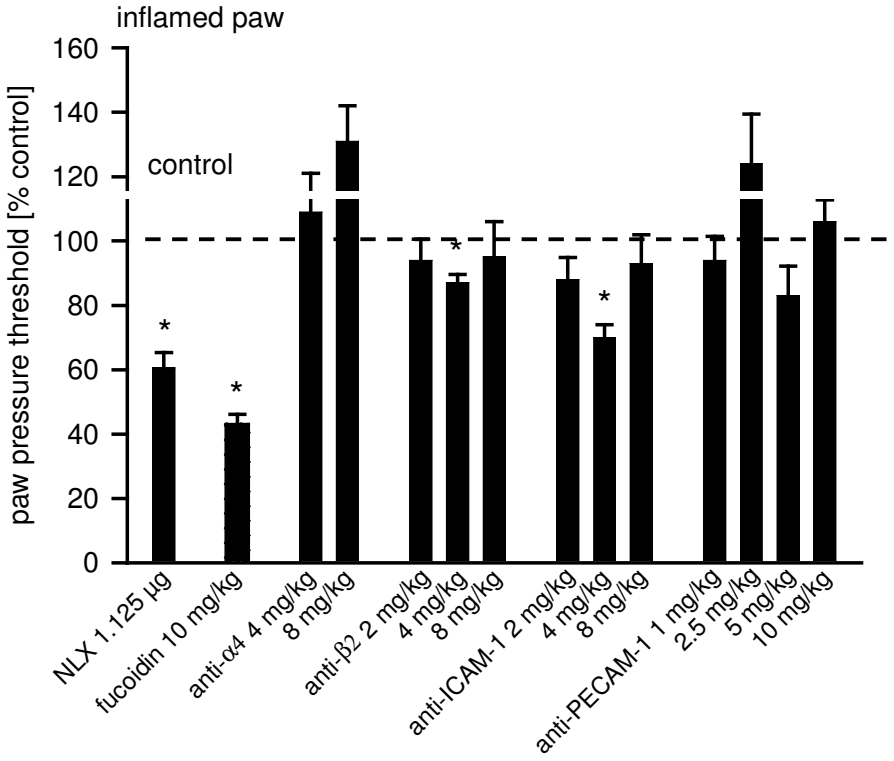

Figure 19. Effects of blockade of selectins (by fucoidin; 10 mg/kg, i.v.), integrins α4 (by anti-α4; 4 - 8 mg/kg, i.v), β2 (by anti-β2; 2 - 8 mg/kg, i.v.), and ICAM-1 (anti-ICAM-1; 2 -8 mg/kg, i.v) and PECAM-1 (anti-PECAM-1; 2 - 10 mg/kg, i.v) on CWS-induced antinociception at 6 h after induction of inflammation. * indicates a statistically significant difference compared with respective control group ($p < 0{,}001$, t-test). Dashed line represents representative control group (100 %). Data are expressed as a percentage of control and are means ± SEM.

Treatment/dose	Baseline PPT [g]		Paw volume [% control]		Paw temperature [% control]	
	Inflamed paw	Noninflamed paw	Inflamed paw	Noninflamed paw	Inflamed paw	Noninflamed paw
Control	40 ± 5.2 *	72 ± 2.6				
Fucoidin						
10 mg	45 ± 4.4 *	69 ± 6.6	84 ± 3.8 * +	95 ± 3.7	98 ± 0.5 *	100 ± 1.1
Anti-VLA-4						
4mg	31 ± 7.7 *	76 ± 2.6	97 ± 2.5 *	100 ± 0.5	100 ± 1.1 *	98 ± 0.7
8mg	27 ± 5.5 *	70 ± 2.0	106 ± 3.3 *	108 ± 3.3	ND	ND
Anti-CD18						
2 mg	26 ± 3.7 *	61 ± 1.8	101 ± 1.5 *	104 ± 3.0	ND	ND
4 mg	25 ± 2.0 *	60 ± 1.4	98 ± 3.7 *	102 ± 4.1	98 ± 0.7 *	97 ± 1.0
8 mg	26 ± 1.3 *	52 ± 2.4	105 ± 5.3 *	93 ± 3.8	ND	ND
Anti-ICAM-1						
2 mg	33 ± 5.2 *	64 ± 3.2	87 ± 4.9 *	107 ± 4.6	ND	ND
4 mg	25 ± 0.9 *	56 ± 1.3	79 ± 1.9 * +	100 ± 1.4	98 ± 1.1 *	98 ± 1.0
8 mg	38 ± 4.4 *	62 ± 5.7	95 ± 3.2 *	101 ± 2.4	97 ± 0.2 * +	100 ± 0.8
Anti-PECAM-1						
1 mg	32 ± 2.2 *	68 ± 3.4	95 ± 2.8 *	95 ± 2.3	100 ± 0.7 *	100 ± 0.4
2,5 mg	22 ± 1.3 *	56 ± 0.9	105 ± 2.4 *	108 ± 2.4	102 ± 0.4 *	102 ± 0.6
5 mg	33 ± 2.4 *	58 ± 1.0	101 ± 1.4 *	106 ± 1.9	101 ± 0.5 *	102 ± 0.8
10 mg	24 ± 2.8 *	62 ± 2.6	108 ± 2.7 *	104 ± 2.5	101 ± 1.1 *	100 ± 0.8

* indicates a statistically significant difference compared with noninflamed paw ($p < 0,05$, paired t-test); + indicates statistically significant difference compared with respective control ($p < 0,05$, t-test). A representative control goup was chosen for simplicity. PV and PT values are expressed as a %control, control goup presenting 100%.Values are expressed as means ± SEM. ND, not determined.

Table 5. Effects of adhesion molecule blockade on PPT in noninflamed paws after CWS at 6 h after induction of inflammation

Treatment/dose	Paw pressure threshold [g]
	noninflamed paw
Control	65 ± 2.7
Fucoidin	
10 mg	67 ± 3.0
Anti-α4	
4 mg	71 ± 4.3
8 mg	70 ± 6.8
Anti-β2	
2 mg	62 ± 3.1
4 mg	61 ± 1.7
8 mg	53 ± 3.7
Anti-ICAM-1	
2 mg	62 ± 2.9
4 mg	56 ± 2.7
8 mg	73 ± 11.0
Anti-PECAM-1	
1 mg	70 ± 3.8
2.5 mg	60 ± 4.9
5 mg	63 ± 1.7
10 mg	62 ± 2.6

Data are expressed as means ± SEM.

3.8.3. Effects of blockade of intercellular adhesion molecule-1 on corticotropin releasing factor-induced antinociception

Anti-ICAM-1 treatment (4 mg/kg) did not significantly change hyperalgesia (i.e. baseline PPT at 6 h after FCA and anti-ICAM-1 but before CRF) ($p < 0.05$, t-test; Fig. 20). Anti-ICAM-1 (4 mg/kg) substantially decreased CRF-induced antinociception ($p < 0.01$, t-test; Fig. 20). The effect was slightly but significantly less compared to the effect of 140 ng i.pl. NLX ($p < 0.001$, t-test; 27 ± 3.7 g vs. 53 ± 2.6 g, , NLX vs. anti-

ICAM-1; compare Fig. 17, B with Fig. 20). No significant changes were observed in noninflamed paws ($p < 0.05$, t-test; Fig. 20).

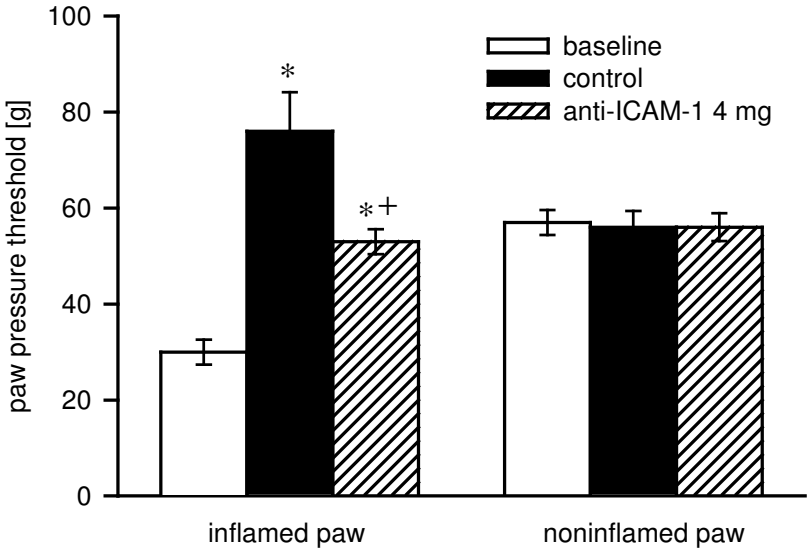

Figure 20. Effects of ICAM-1 blockade (by anti-ICAM-1; 4 mg/kg, i.v.) on CRF (4 ng, i.pl.)-induced antinociception at 6 h after induction of inflammation. * indicates a statistically significant difference compared to respective control ($p < 0{,}001$, t-test). + indicates a statistically significant difference compared to respective baseline ($p < 0{,}001$, paired t-test). Baseline of a representative group is shown. Data are expressed as means ± SEM.