2. Materials and Methods

2.1. Animals

2.1.1. Raising and Care

All experiments were carried out in male Wistar rats bred at Freie Universität Berlin, Berlin, Germany weighting 150-180 g. Animals were housed in groups of three in cages lined with ground corn cob bedding. Laboratory rodent chow (ssniff, rat and mouse, Spezialdiäten GmbH, Soest, Germany) and tap water were available *ad libitum.* Room temperature was maintained at 22 ± 0.5 °C with a relative humidity between 60 and 65%. All experiments were performed in the light phase of a 12 h/12 h (8 a.m./8 p.m.) light-dark cycle. The guidelines on ethical standards of the International Association for the Study of Pain (1985) and of the Animal Care Committee of the State of Berlin were followed.

2.1.2. Preparation for experiment

Five days before experiments rats were removed from a general animal room to the experimental room where they were kept individually in cages. Animals were handled once a day for 4 days always following the same schedule before any testing was performed to avoid stress and anxiety. Separate groups of rats (n = 6-13 per group) were used for each treatment (a dose of a drug) including a control group. All experiments were performed by the experimenter blinded to the treatments using coded syringes or bottles containing the compounds.

2.2. Drugs and immunoreagents

2.2.1. Opioid receptor antagonists

Naloxone hydrochloride (NLX), a nonselective opioid receptor antagonist, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH$_2$ (CTOP), a selective μ-opioid receptor antagonist, Naltrindole hydrochloride (NTI), a selective δ-opioid receptor antagonist, and Nor-Binaltorphimine dihydrochloride (nor-BNI), a selective κ-opioid receptor antagonist were used. Antagonists were obtained from Sigma (Taufkirchen,

Germany). NLX, NTI and nor-BNI were dissolved in physiological saline (0.9 % NaCl) whereas CTOP was dissolved in sterile water.

2.2.2. Monoclonal antibodies against opioid peptides

Rabbit anti-rat-β-Endorphin IgG (anti-β-END), rabbit anti-rat-methionine-Enkephalin IgG (anti-Met-ENK), and rabbit anti-porcine cross reacting with rat Dynorphin-A (1-17) IgG (anti-DYN) were used. Antibodies were obtained from Peninsula Laboratories (San Carlos, CA, USA) and were dissolved in sterile water.

2.2.3. Monoclonal antibodies against adhesion molecules

Mouse anti-rat-α4 (CD49d) $IgG_{1\kappa}$, Clone Number: TA-2 (anti-α4), mouse anti-rat-β2 chain (CD18) $IgG_{1\kappa}$, Clone Number: WT.3 (anti-β2), mouse anti-rat-intercellular adhesion molecule-1 (CD54) $IgG_{1\kappa}$, Clone Number: 1A29 (anti-ICAM-1), and mouse anti-rat-platelet endothelial cell adhesion molecule-1 (CD31) $IgG_{1\kappa}$, Clone Number: TLD-3A12 (anti-PECAM-1) were used. The TA-2 mAb is directed against the α4 subunit and therefore recognizes two integrins sharing this subunit α4/β1 (VLA-4) and α4/β7. Because α4/β7 primarily mediates lymphocyte migration to gut and associated lymphoid tissues [74] the TA-2 mAb most probably targets α4/β1 (VLA-4) in our modell, as documented by others [49, 51]. Anti-α4 was obtained from Endogen (Woburn, MA, USA). Anti-β2, anti-ICAM-1 and anti-PECAM-1 were obtained from PharMingen (Europe). Antibodies were dissolved in 0.9 % NaCl.

2.2.4. Other compounds

Corticotropin releasing factor (Human, Rat) (CRF), CRF receptor antagonist [9-41] (α-helical CRF), fucoidin (a L- and P-selectin blocker), anti-rabbit IgG (as control for anti-β-END, anti-Met-ENK, anti-DYN), anti-mouse IgG (as control for anti-α4, anti-β2, anti- ICAM-1, anti-PECAM-1), halothane, and modified Freund's complete adjuvant (FCA) containing 0,1% heat killed and dried *Mycobacterium butyricum* in 85% Marcol 52 and 15% Aracel A mannide monooleate emulsifier were used. Compounds were obtained from Sigma (Taufkirchen, Germany) (CRF, α-helical CRF, fucoidin, rabbit IgG and mouse IgG), Willi Rüsch Hospital Vertriebs Gmbh (Böblingen, Germany) (halothane), and Calbiochem (La Jolla, CA, USA) (FCA). CRF, α-helical CRF and

rabbit IgG were dissolved in sterile water while fucoidin and mouse IgG were dissolved in 0.9 % NaCl.

2.3. Anesthesia

2.3.1. Brief anesthesia

For brief anesthesia rats were taken out of their cages and placed in a glass chamber that contained a piece of paper soaked with halothane. Above the halothane soaked paper a perforated ceramic plate through which the halothane could evaporate and on which the rat could sit was placed. A glass lid covered the chamber to keep rat and halothane inside. After the rat fell asleep it was removed from the chamber and intraplantar (i.pl.) injections were performed.

2.3.2. Deep anesthesia

For deep anesthesia rats were taken out of their cages and underwent brief anesthesia (see 2.3.1.). Then their mouth and nose were covered by a mouth piece attached to a tube that was connected to an anesthesia machine („Tiberius", Drägerwerk, Lübeck, Germany) which provided a mixture of halothane (1.0-3.0 l/min) and oxygen (0.8-1.0 l/min). When the rat was asleep intraveneous (i.v.) injections were performed.

2.4. Injections

2.4.1. Intraplantar

I.pl. injections into the rat's hindpaw either unilateral into the inflamed paw or bilateral into both paws using a 1 ml syringe attached to a 26 G needle (Omnifix-F, B. Braun, Melsungen AG, Melsungen, Germany) were made under brief anesthesia. Injections were made in volumes of 0.1 ml for single and 0.15-0.2 ml for combined injections.

2.4.2. Subcutaneous

S.c. injections were made without anesthesia. Rats were gently held under soft paper wadding and injections were made into a skinfold in the neck in a volume of 0.1 ml

(single injections) or in 0.2 ml (combined injections) using a 1 ml syringe attached to a 26 G needle (see 2.4.1.).

2.4.3. Intraveneous

Intraveneous (i.v.) injections into a tail vein in a volume of 0.6 ml using a 1 ml syringe attached to a 26 G needle (see 2.4.1.) were performed under deep anesthesia (see 2.3.2). Before injections the rat's tail was held in a small glass container filled with warm water (38 °C - 45 °C) so the veins could be seen easier.

2.5. Inflammation

2.5.1. Induction of inflammation

The inflammatory agent used was FCA. Under brief anesthesia rats received an i.pl. injection of 0.15 ml FCA into the right hindpaw. In this model rats develop a local inflammation which remains confined to the inoculated paw up to 6 days [75]. The advantage of this model is that the contralateral noninjected paw serves as a control which substantially decreases the number of animals used. All experiments were performed at 6 h or 4 days after inoculation with FCA.

2.5.2. Evaluation of inflammation

To evaluate the degree of inflammation two parameters were employed: swelling (increased paw volume; PV) and hyperthermia (elevated paw surface temperature; PT). In both cases the noninflamed paw served as a control based on former studies which have shown no signs of inflammation in noninjected paws of unilaterally FCA treated rats as compared with rats not treated with FCA [64, 70].

2.5.2.1. Paw volume

The PV was measured with a plethysmometer (Ugo Basile, Comerio, Italy). Rats were taken out of their cages and held under soft paper wadding. Each hindpaw was submerged to the tibiotarsal joint into a saline-filled Perspex cell of the plethysmometer. The volume of displacement, which is equal to the PV, was

indicated on a digital display. For each paw measurements were done twice and the average calculated.

2.5.2.2. Paw temperature

The PT was determined using a contact thermometer (Cooper Instrument Corporation, Middlefield, CT). Rats were removed from their cages and held under soft paper. The thermoprobe was placed on the dorsal surface of the paw and the temperature read from a digital display. For each paw measurements were made twice and the average calculated.

2.6. Algesiometry

To determine nociceptive thresholds and to evaluate antinociception we used the paw pressure algesiometer (modified Randall – Selitto test [76], Ugo Basile, Comerio, Italy). Animals were taken out of their cages and held under soft paper wadding. Their hindpaw was placed on a teflon block and a wedge-shaped, blunt piston was placed on the dorsal surface of the paw (on an area of 1.75 mm^2) exerting an incremental pressure by means of an automated gauge. The pressure to elicit paw withdrawal, the paw pressure threshold (PPT), was determined. A cutoff of 250 g was employed to avoid tissue damage. Three consecutive trials, separated by intervals of 10 s were conducted and the average determined. The same procedure was performed on the contralateral paw. To preclude „order" effects the paws were tested alternately between subjects.

2.7. Cold water swim stress test

Intrinsic opioid antinociceptive mechanisms can be activated by certain stressful environmental stimuli [65]. One of these stimuli is CWS. For CWS stress rats were taken from their cages and placed into a metal container containing water at 2-4°C to swim for 1 min. A CWS duration of 1 min was chosen based on former experiments which have shown that 1 min of swimming produces an opioid specific elevation of PPT in inflamed paws and does not significantly change PPT in noninflamed paws [70,] [72]. Afterwards, rats were removed from the water and dried with a soft paper towel. PPT measurements were performed repeatedly thereafter. Noninflamed paws served

as controls based on former studies which have shown no CWS-induced antinociception in noninflamed paws of unilaterally FCA treated rats as compared with rats not treated with FCA [70].

2.8. Experimental protocols

2.8.1. Evaluation of inflammation at 6 h and 4 days

In this experiment the inflammatory parameters swelling, hyperthermia and nociceptive thresholds were examined at 6 h and 4 days after FCA. Rats (n = 7 per time point) were removed from their cages and PPT (see 2.6.), PV (see 2.5.2.1.) and PT (see 2.5.2.2.) were measured. Noninflamed paws served as control (see 2.5.2., 2.7.).

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

Effects of CWS on PPT of inflamed and noninflamed hindpaws were examined at 6 h or at 4 days after FCA. Baseline PPT were taken and rats were returned to their home cages. After completing all baseline measurements 4 groups of rats (n = 7 per group) were exposed to CWS. The PPT were reevaluated at 1, 5 and 10 min after CWS.

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

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

First, to examine whether CWS-induced antinociception at 6 h after FCA was brought about by a local opioid-receptor specific mechanism we used the nonselective opioid receptor antagonist (-)-naloxone (NLX). Following baseline PPT recordings 7 groups of rats (n = 5-13 per group) received i.pl. either 0.9 % NaCl (control) or different doses of NLX (0.14, 0.28, 0.56, 1.125, 1.4, 2.25 µg) into inflamed paws to asses its dose-response relationships. NLX or saline were injected 5 min before CWS and

PPT were reevaluated 1 min after CWS (at the time of maximum PPT elevation). Only unilateral injections were performed because CWS induces antinociception only in inflamed paws similar to present and previous studies [9].

Then we sought to clarify which types of opioid receptors are involved in CWS-induced antinociception at 6 h after FCA using receptor selective antagonists. After baseline PPT recordings μ- (CTOP), δ- (NTI) and κ- (nor-BNI) selective opioid receptor antagonists were injected i.pl into inflamed paws. Rats were given the following doses: Five groups (n = 6-7 per group) received 0.5, 1, 2, 3, or 4 μg of CTOP, another 5 groups (n = 6-9 per group) were injected with 25, 37.5, 50, 75, or 100 μg of NTI and 4 groups (n = 6-7 per group) were given 12.5, 25, 37.5, or 50 μg of nor-BNI. Control groups (n = 6-9 per group) received sterile water (CTOP experiment) or 0.9 % NaCl (NTI and nor-BNI experiments). Injections were performed 5 min prior to CWS and PPT were reevaluated at 1 min after CWS. Dosages, time of administration and PPT measurements were based on former and present preliminary experiments.

A separate group of animals received a combined injection of the three opioid receptor antagonists CTOP, NTI and nor-BNI at 6 h after FCA and the effect of this treatment on CWS-induced antinociception was examined. Following baseline PPT recordings rats (n = 6-7 per group) were injected concomitantly with the most effective doses of CTOP (2 μg), NTI (50 μg) and nor-BNI (37.5 μg) i.pl. into inflamed paws. Combined injections were performed using a 1 ml syringe filled with 50 μl of each antagonist so a total volume of 150 μl was administered. A control group received equivalent amounts of 0.9 % NaCl. Injections were made 5 min before CWS and 1 min after CWS PPT were reevaluated.

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

In this experiment we examined the contribution of endogeneous opioid peptides β-END, Met-ENK, and DYN to CWS-induced antinociception at 6 h after FCA. Rats were removed from their cages and baseline PPT were taken. Five min before CWS anti-β-END, anti-Met-ENK or anti-DYN were injected i.pl. into inflamed paws. To establish dose-response relationships 6 groups (n = 5-9 per group) received different

doses of anti-β-END (0.25, 0.5, 1, 2, 3, 4 μg), 4 groups (n = 7-8 per group) were injected with various doses of anti-Met-ENK (0.06, 0.25, 1, 4 μg) and 5 groups (n = 6-8 per group) were given different doses of anti-DYN (1, 4, 6, 8, 16 μg). Control groups (n = 8-10 per group) received rabbit IgG in the dose equivalent to the highest dose of anti-opioid peptide antibodies. One minute after CWS PPT were reevaluated. All doses and times of administration were based on former [70] and present preliminary experiments.

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

To evaluate the endogeneous agent triggering opioid peptide release and consequently intrinsic opioid antinociception the effect of the CRF receptor antagonist α-helical CRF on CWS-induced antinociception at 6 h after FCA was examined. Following baseline PPT recordings 6 groups of rats (n = 5-8 per group) received either sterile water (control) or different doses of α-helical CRF (2, 8, 16, 32, 64 ng) i.pl. into inflamed paw. Five min later the rats were exposed to CWS and 1 min after CWS PPT were reevaluated. Dosages and times of administraton were based on former [72] and present preliminary experiments [77].

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

This experiment was performed to examine whether central opioid receptors contribute to CWS-induced antinociception at 6 h after FCA. For this purpose NLX was administered s.c. in doses that activate both peripheral and central opioid receptors [77]. After establishing baseline PPT rats (n = 6-7 per goup) were injected with either different doses of NLX (0.06, 0.25, 1, 2 mg/kg) or 0.9 % NaCl (control) s.c. 5 min prior to CWS. PPT were reevaluated at 1 min after CWS. Dosages and times of administration were based on previous and present preliminary experiments.

2.8.5. Peripheral opioid antinociception at 4 days after induction of inflammation

In this experiment we tested the effects of NLX, nor-BNI and anti-Met-ENK on CWS-induced antinociception at 4 days after FCA. This was to confirm that differences in

the involvement of opioid receptors and opioid peptides in CWS-induced antinociception between 6 h (present study) and 4 days [9, 70] after FCA were not due to different experimental conditions (e.g. different dosages). The contribution of β-END and of μ- and δ-receptors to CWS-induced antinociception at 4 days was shown previously [70]. Following baseline PPT recordings compounds were administered i.pl. into inflamed paws. Rats were injected with 18 μg of NLX (n = 9), 37.5 μg of nor-BNI (n = 7) or 0.25 μg of anti-Met-ENK (n = 7) 5 min prior to CWS. Control groups (n = 7-8 per group) received 0.9 % NaCl (NLX and nor-BNI experiments) or rabbit IgG (anti-Met-ENK experiment). One minute after CWS PPT were reevaluated. The dose of NLX was based on former experiments [71]. Nor-BNI and anti-Met-ENK were injected in doses found to be most effective at 6 h after FCA (present study).

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

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

The time-course and dose-dependency of CRF-induced antinociception in inflamed and noninflamed paw at 6 h after induction of inflammation were examined in this experiment. Rats were removed from their cages and baseline PPT were recorded. Then 5 groups received different doses of CRF (0.5, 1.5, 3, 4, 6 ng) i.pl. into inflamed and noninflamed paws. Controls (n = 13) were injected with sterile water. PPT were reevaluated at 5 and 10 min after injection of CRF. Dose-response relationships of i.pl. CRF were examined at the time of the peak effect i.e. at 5 min after CRF. The time-course of testing was based on the former studies [57, 64].

2.8.6.2. Effects of local injections of the opioid receptor antagonist naloxone on corticotropin releasing factor-induced antinociception

This experiment was performed to examine whether CRF-induced antinociception in inflamed paws is mediated by opioid receptors using the nonselective opioid receptor antagonist NLX. Following baseline recordings 3 groups of rats (n = 6-7 per group) received different doses of NLX (9, 35, 140 ng) concomitantly with CRF (6 ng). This dose of CRF was the most effective in producing antinociception found in experiment 2.8.6.1.. Injections were made i.pl. into inflamed paws in a volume of 0.2 ml.

Unilateral injections were performed because CRF induces antinociception only in inflamed paws according to present (see section 2.8.6.1) and previous studies [64]. Control groups received 0.9 % NaCl concomitantly with CRF in an equivalent volume. PPT were reevaluated at 5 min after injections.

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

To confirm a peripheral site of action the most effective doses of opioid receptor antagonists, anti-opioid peptides, α-helical CRF and CRF (found in experiments 2.8.3.1., 2.8.3.2., 2.8.3.3., 2.8.6.) were injected s.c. (into an animal's back) 6 h after FCA. Rats were taken from their cages, baseline PPT were measured and the following compounds were administered: 1.125 µg of NLX (n = 8), 2 µg of CTOP (n = 6), 50 µg of NTI (n = 9), 37.5 µg of nor-BNI (n = 6), 2 µg of anti-β-END (n = 6), 0.25 µg of anti-Met-ENK (n = 10), 8 µg of anti-DYN (n = 6), 8 ng of α-helical CRF (n = 6) or 6 ng of CRF (n = 6). CTOP, NTI and nor-BNI were administered as single injections or concomitantly (see 2.8.3.1.). Control groups (n = 6-8 per group) received saline (NLX, NTI, nor-BNI experiments), sterile water (CTOP, α-helical CRF, CRF experiments) or rabbit IgG (anti-β-END, anti-Met-ENK, anti-DYN experiments). Animals treated with opioid receptor antagonists, anti-opioid peptides and α-helical CRF were subjected to CWS 5 min after injections. PPT were reevaluated at 1 min after CWS or 5 min after administration of CRF.

2.8.8. Contribution of adhesion molecules to swim stress induced-antinociception at 6 h after induction of infammation

The effect of adhesion molecule blockade on CWS was evaluated. In addition, the effect of such treatments on macroscopic inflammation was assessed. Immediately before induction of inflammation rats (n = 5-8 per group) received the following substances i.v.: fucoidin (10 mg/kg), a polysacchride that blocks L- and P-selectin, or monoclonal antibodies against the integrins α4 (anti-α4; 4, 8 mg/kg) and β2 (anti-β2; 2, 4, 8 mg/kg), the Ig superfamily members ICAM-1 (anti-ICAM-1; 2, 4, 8 mg/kg) and PECAM-1 (anti-PECAM-1; 1, 2.5, 5, 10 mg/kg). Control groups (n = 5-8 per group) received mouse IgG in equivalent dosages and volumes. Six hours later baseline

PPT were taken and PT was measured. Rats were then subjected to CWS, and 1 min later PPT were reevaluated. PV was measured thereafter. Doses and times of administration were based on pilot experiments and on a previous study [57].

2.8.9. Contribution of intercellular adhesion molcule-1 to corticotropin releasing factor-induced antinociception at 6 h after induction of inflammation

The effects of ICAM-1 blockade on CRF-induced antinociception at 6 h after FCA were assessed. Rats (n = 6-8 per group) were injected i.v. with either 4 mg/kg of anti-ICAM-1 or mouse IgG immediately before induction of inflammation. Six hours later baseline PPT were evaluated and rats received CRF (4 ng) i.pl. into both hindpaws. PPT were reevaluated 5 min after CRF.

2.9. Statistical analysis

In all experiments values were normally distributed with equal variances, therefore parametric tests were used. Differences were considered significant if $p < 0.05$. The power of the performed tests was α with 0.800.

A) For independent data, to compare effects between groups of different individuals, the following tests were used:

i) The unpaired t-test (t-test) was employed to examine differences between two groups at one time point in the mean values. Groups of rats treated with drugs or immunoreagents were compared with control groups with the null hypothesis that there are no differences in PPT, PV and PT between the two groups.

ii) One way analysis of variance (ANOVA) was performed to examine differences between the means of more than two experimental groups. In our experiments PPT of several groups which received different doses of a drug were compared with each other with the null hypothesis that there are no differences among the groups.

B) For dependent data to test changes in the same individual before and after one or more treatments or changes in condition the following tests were used:

i) The paired t-test was performed to test the effect of a treatment at one or between two time points. In one set of experiments inflamed and noninflamed paws of the same individuals were compared with the null hypothesis that there are no differences in PPT, PV and PT. In another set of experiments changes before and after treatment (e.g. before and after CWS or CRF injection) were examined with the null hypothesis that there are no changes in PTT.

ii) One way repeated measures ANOVA was performed to examine the differences at more than two time points of the same individuals. In our experiments the same group of rats was examined at up to 4 time points (i.e. before and 3 time points after either CWS or injection of CFR) with the null hypothesis that there are no changes in PPT over time.

C) To determine exactly the differences between particular groups and the level of significance as a multiple comparison procedure (post-hoc-test) the Dunnett's test was used. This test employs multiple comparisons vs. a control group, and was used following one way ANOVA or one way repeated measures ANOVA. In one set of experiments rats which had received different doses of a drug were compared to their controls. In another set of experiments the changes in one group of rats over time were compared to baseline PPT (i.e. the PPT taken before CWS or CRF).

D) To examine a dose dependency of various treatments a linear regression was performed to test the zero slope hypothesis. The test followed one way ANOVA if this detected a significant effect.

For all statistical evaluations the computer program *SigmaStat version 2.0* was used. Calculations were performed with the computer program *excel version 7.0*.

3.0. Descriptive statistics

All data are expressed as means ± SEM.. PPT are given as raw values or, in case of adhesion molecule experiments, as percentages of controls (%control) according to the following formula: $(PPT_{drug\ treated\ group} \times 100)$ / mean $PPT_{control}$. PV and PT values are also given as percentages of controls (%control) according to the following formulas: $(PV\ or\ PT_{drug\ treated\ group} \times 100)$ / mean PV or $PT_{control}$. Statistical examination in all experiments was performed using raw data.

Inflammatory parameters (hyperalgesia, edema, hyperthermia) are presented as bar chart graphs. The effects of CWS on PPT at 6 h and 4 days after induction of inflammation are shown in a table. The results from i.pl. CRF examining changes over time are shown as line and scatter plots where PPT elevation is plotted against the different time points after treatment where the time point "0" represents baseline values. All dose-response relationships are presented as line and scatter plots. To construct the dose-response curve, PPT elevation is plotted against the different doses, a dose of "0" representing the control group. In dose-response experiments the most effective dose of each opioid receptor antagonist, anti-opioid peptide and α-

helical CRF was tested against its own baseline PPT. For clarity of graphs baseline PPT of a representative group is presented as a dashed line. This is justified because there were no significant differences in baseline PPT between groups. The combined injection of the most effective doses of opioid receptor antagonists is presented as a bar chart graph where baseline of a representative group was chosen for simplicity. Again, this was justified because there were no sgnificant differences in baseline PPT between groups. Only inflamed paws are shown. To summarize all results from opioid receptor antagonists, anti-opioid peptides and α-helical CRF experiments results of inflamed paws are finally presented together as two bar chart graphs including only one representative control group and baseline values of a representative group. The effects of s.c. injections at 6 h and of i.pl. injections at 4 days after FCA are put in tables. Representative control groups were chosen for simplicity. Adhesion molecule data are shown as bar chart graphs. Data are expressed as a % of control where control group represents 100%. Each group treated with fucoidin or anti-adhesion molecules was compared with its individual control group. Graphs show only results obtained in inflamed paws, each group of rats is represented by one bar. Control groups are presented as dashed lines. Data from noninflamed paw are shown in a table where as control data of one representative control group is presented. The effects of fucoidin and anti-adhesion molecules on baseline PPT, PV and PT are shown in a table. Baseline PPT are given in raw values. Representative control groups were chosen for simplicity. PV and PT measurements are expressed as a % control that represents 100%. Each group treated with fucoidin or anti-adhesion molecules was compared with its respective control. The results of the effects of anti-ICAM-1 on CRF-induced antinociception are shown in bar chart graphs where, for clarity of graphs, baseline of a representative group is presented. This is justified because there were no significant differences in baseline PPT between groups.

All graphs were created using the computer program *SigmaPlot versions 4.01* and *8.0.*