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Pharmacological correlation between the formalin test and the neuropathic pain behavior in different species with chronic constriction injury☆

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Abstract

Research on mechanisms of drug action, and preclinical screening of molecules with a potential activity on neuropathic pain requires extensive animal work. The chronic constriction injury model is one of the best-characterized models of neuropathic pain behavior in rats, but requires extensive time consuming operations and animal handling. The formalin test is easier to perform, and a well validated model. The latter may serve as an effective prescreening test of molecules and may facilitate drug targeting. In the present study the activity of different pharmacological reference compounds was tested in rats and gerbils on the cold plate for animals that had undergone chronic constriction injury and in the second phase of the formalin test. In rats, a comparable outcome in both test conditions was observed for morphine, fentanyl, MK-801 and flunarizine. Clonidine had more activity in the second phase of the formalin test, whereas baclofen, tramadol, amitryptiline, ketamine and topiramate showed more activity in the cold plate. In gerbils, both test conditions yielded comparable results for fentanyl and ketoprofen. Tramadol and CP-96345 tended to have more activity in the second phase of the formalin test, whereas morphine, SR-48968, SR-142801 and R116301 demonstrated more activity in the cold plate test. This study demonstrates a good correlation between the second phase of the formalin test and the cold allodynia in the CCI model for, both for rats and gerbils. Drugs with a proven activity in humans, used as reference compounds, also showed good pharmacological activity in this animal study.

Keywords: Pharmacologic; Rat; Gerbil; Formalin; CCI

1. Introduction

The management of neuropathic pain conditions in humans remains a difficult clinical challenge, because the pathophysiological mechanisms of the induction and the persistence of a neuropathic pain condition are not yet unraveled, and the different pain treatment modalities for neuropathic pain are not always fully efficacious (Finnerup et al., 2005) (Devor and Seltzer, 1999; Bonica and Loeser, 2001). Clinicians nowadays follow treatment algorithms based on the use of analgesics and co-analgesics such as antidepressants, anticonvulsants, local anesthetics and antiarrhythmics (MacPherson, 2000; McCleane, 2004).

Several animal models of neuropathic pain have been developed (Bennett and Xie, 1988; Seltzer et al., 1990; Kim et al., 1995). These models are based on the induction of neuropathic pain behavior after induction of a controlled nerve injury. The different models show various degrees of hypersensitivity to tactile, thermal and chemical stimuli.(Hofmann et al., 2003; De Vry et al., 2004; Decosterd et al., 2004; Dowdall et al., 2005; LaBuda and Little, 2005; Walczak et al., 2006) The chronic constriction injury of the sciatic nerve (Bennett model; CCI model) is one of the best-characterized models of neuropathic pain behavior (Bennett and Xie, 1988). The animals are operated to receive 4 ligatures at the sciatic nerve; they develop an abnormal behavior that is considered to be representative for the neuropathic pain experienced by patients. The preparation of these animals is very time consuming and not all operated animals present the typical abnormal pain behavior in the different test conditions to the same degree, which makes the predictability of the neuropathic pain behavior in these animals difficult (Choi et al., 1994; Desmeules et al., 1995; Kim

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et al., 1997). In contrast to this CCI model, the formalin test is easier to perform and to standardize (Dubuisson and Dennis, 1977; Abbott et al., 1995a). The subplantar injection of formalin results in flinching and licking or biting behavior during an early acute phase, which resembles acute pain, followed by a second delayed phase representative for tonic pain (Dubuisson and Dennis, 1977). This behavior is quite consistent in its presentation. The acute phase is believed to represent peripheral pain pathways, whereas the second phase is indicative of sensitization in central pain conducting pathways comparable to pain pathways activated in the CCI model (Vissers et al., 2004). Since different receptors for neurotransmitters, responsible in the pain transmission in animals, are not necessarily the same as in man, pharmacological evaluation must be performed. (Maggi, 1995). Because not all pathophysiological mechanisms of pain transmission are yet known, the pre-clinical testing in animal models will be important in pharmacological targeting. Therefore, looking to the different activity profiles of different classes of analgesic drugs can instruct us about the underlying pain mechanisms.

In this study, we present the data for several reference drugs tested in rats and gerbils for the second phase of the formalin test, and the cold allodynia in animals following CCI. Furthermore, a pharmacological validation of the formalin test and the CCI model in gerbils was performed. Finally, it was evaluated whether the second phase of the formalin test could be used for pharmacological screening to predict outcome in the cold plate test in CCI animals.

2. Materials and methods

2.1. General conditions

All studies were conducted following the ethical guidelines of the IASP (Zimmermann, 1983) and approved by the Local Animal Care Ethics Committee. Male Sprague Dawley rats (Harlan), weighing 250–280 g, and male adult gerbils (*Meriones unguiculatus*, Crl(MON)BR, Charles River Deutschland, Sulzfeld, Germany) weighing 60–75 g at the start of the experiment, were used. The animals were housed individually in standard rodent cages with sawdust bedding and food and water ad libitum. The housing room was air conditioned with a 12/12 h day/night cycle (lights on 7.00 a.m.). Background noise was produced during the light period by playing a conventional radio station. The same surrounding conditions were used in the laboratory.

2.2. Formalin test in rats

The animals were housed individually in standard plastic observation cages with a wire mesh floor. Rats were habituated in these housings for 30 min. One hour prior to the intraplantar injection of 0.05 ml of 5% formalin in the right hind paw, the animals were injected intraperitoneally (IP) with the test compound and replaced in the individual observation housing. Immediately after the intraplantar injection of formalin, the number of paw flinches and licking or biting were recorded for the first 5 min (early phase) and between 20 to 25 and 40 to 45 min (late phase) following formalin injection. The data of the late phase were summed and defined as formalin second phase. (Vissers et al., 2003, 2004).

2.3. Formalin test in gerbils

The individual test housings were rubber floored and held a back mirror to assist observation. Gerbils were habituated in these housings for 30 min. One hour prior to the intraplantar injection of 0.05 ml of 5% formalin in the right hind paw, the animals were injected IP with the test compound and replaced in the individual observation housing. Evaluation of pain behavior was similar as for the rats.

2.4. Sciatic nerve ligation in rats

The rats were anesthetized by the subcutaneous administration of 1 ml solution containing fentanyl 50 μg and dehydrobenzperidol l5 mg (Thalomonal®) and separately 40 mg/kg IP sodiumpentobarbital. The common sciatic nerve of the left hind paw was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic's trifurcation, 7 mm of nerve was freed and four loose ligatures of 4–0 chromic gut were placed around the sciatic nerve. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted when viewed with a microscope using a 40× magnification. After surgery all animals received 1.25 mg/kg naloxone IP as an antagonist for the Thalamonal® anesthesia to fasten the recovery, prevent further cooling of the animals and to prevent respiratory depression in the absence of further surgical stimulation. After checking for haemostasis, the muscle, the adjacent fascia and the skin were closed with sutures.

2.5. Sciatic nerve ligation in gerbils

The animals were anesthetized with 60 mg/kg IP sodiumpentobarbital. The sciatic nerve was exposed in a similar way as for the rat procedure, and 4 loose chromic catgut ligatures (6/0 Chromic catgut, Ethicon Inc, Somerville, NJ, USA) were placed around the sciatic nerve.(Meert et al., 2003).

2.6. Cold plate test in CCI rats

Cold plate testing was performed in a cage with transparent acrylic walls (height 30 cm) and a metal plate floor of 30 × 30 cm, 7 days after CCI surgery. The surface of the cold plate was cooled by a flow-through cooling apparatus which holds the surface temperature stable on — 0.5±0.5 °C (Julabo F 25®, Juloba Labortechnik, Seelback, Germany). The animal was placed on the cold plate and the duration of lifting of both the left and right hind paw was measured over 5 min. Only rats with a difference in lifting time >25 s between the ligated and non-ligated paw were used for drug testing.

2.7. Cold plate test in CCI gerbils

For testing cold allodynia in gerbils, the animals were placed on a metal plate in a transparent, circular plexiglas cage with

diameter of 190 mm, 7 days after surgery. The temperature (−4 °C) of the cold plate was selected on the basis of a preliminary trial comparing normal animals with CCI ligated animals as described previously (Meert et al., 2003). The number of times that the animal lifted the left or the right hind limb off the platform and the duration of the lifts were separately recorded during a 5 min observation period. Limb movements that were considered a part of the animals' normal movement (walking) were not included in the assessment. Only animals with a lifting time >10 s were used for drug testing.

2.7.1. Administration of the test compounds in CCI operated animals

The CCI operated animals meeting the selection criteria, 7 days after surgery, were injected IP with a test compound and reevaluated 60 min later on the cold plate. The results after treatment were individually compared with those of the selection test. Each animal only received one test compound.

2.8. Drugs tested in the formalin experiments

After habituation (see above), the animals were treated IP with a test compound and 30 min later; the rat or gerbil formalin test was performed. In rats, the tested compounds (see also Table 1) included saline, vehicle solutions, morphine, fentanyl, codeine, tramadol; clonidine; paracetamol; ketamine, MK-801; baclofen; amitryptiline; lamotrigine, carbamazepine, gabapentin and topiramate, suprofen and flunarizine. In gerbils, following compounds were additionally tested: ketoprofen; the tachykinin NK-1 antagonists: R116301, GR-203040; the tachykinin NK-2 antagonist: SR-48968 and the tachykinin NK-3 antagonist: SR-142801. At each dose, the results of 7 animals were collected. The control animals received either an injection with saline or one of the different vehicle solutions used.

2.9. Drugs tested on cold allodynia in CCI animals

After selection, animals with a clear cold allodynia (more than 25 s lifting on the cold plate for rats and 10 s lifting on a cold plate for gerbils) were treated IP with the test compound. One hour later, the cold plate test was repeated. In rats, the tested compounds included saline, vehicle solutions, morphine, fentanyl, tramadol, clonidine, ketamine, MK-801, baclofen, amitryptiline, lamotrigine, carbamazepine, gabapentin and topiramate, suprofen and flunarizine. In gerbils, following compounds were additionally tested: codeine, paracetamol, ketoprofen, the tachykinin NK-1 antagonists: R116301, CP-96345, tachykinin NK-2 antagonist: SR-48968 and tachykinin NK-3 antagonist: SR-142801. At each dose, the results of 5–10 animals were collected. The control animals received either an injection with saline or one of the different vehicle solutions.

2.10. Statistics

For statistical analysis the Fisher exact probability test was used based on the number of active animals tested in the drug treated and control groups (Two tailed; Siegel, 1958). To calculate the correlation between cold allodynia in CCI animals and second phase formalin the Spearman Rank order correlation test was used by Sigmastat version 2.0 (SPSS, Chicago, 1997).

3. Results

3.1. Data from control animals

3.1.1. Formalin testing

Because no differences were observed in control animals between saline and the different vehicles, these data were pooled.

3.1.1.1. Formalin test in rats. For the formalin test in rats, the total time of flinching and licking or biting behavior of the late phase in seconds for the observation periods T 20 to T 25 and T 40 to T 45 min was summed. A total number of 531 control animals were evaluated. These animals reached a mean flinching and licking or biting time of 106.8±1.9 s. Based on an all-or-none criterion, a reduction in pain behavior activity to less than 21 s was chosen for drug activity. This was reached in less than 3% of the controls (See Table 1).

3.1.1.2. Formalin test in gerbils. For the formalin test in gerbils, the total time of flinching and licking or biting of the late phase in seconds for the period T 20 to T 25 and T 40 to T 45 min was taken together. A total number of 548 control animals were evaluated. These animals reached a mean flinching and licking or biting time of 143.7±3.4 s. Activity for drug testing was set here at lower than 45 s; this value was reached by less than 1.5% of the controls. (See Table 1).

3.1.2. Cold plate test

Also, for the cold plate test no differences in control animals between saline and the different vehicle solutions were measured and data were pooled. The total number of tested control animals is described in Table 1.

3.1.2.1. Cold plate test in CCI rats. For the cold plate test in CCI rats, the total time lifting the left hind paw for a period of 5 min was measured before and 1 h after IP drug treatment with the study compounds. A total number of 735 control animals were evaluated. Tested control animals reached a pretreatment mean lifting time of 63.3±1.6 s and a post treatment mean lifting time of 66.9±1.9 s which is 100.4±2.5% of the pretreatment lifting time. The criterion for activity of a test compound was set at a post drug lifting time being <25% of the pretreatment lifting time. Less then 6% controls reached this level. (See Table 1).

3.1.2.2. Cold plate test in CCI gerbils. For the cold plate test in CCI gerbils, the total time lifting the left hind paw for a period of 5 min was taken before and 1 h after IP treatment with the study compound. A total number of 305 vehicle controls were evaluated. Tested control animals reached a pretreatment mean lifting time of 38.2±1.7 s and a post treatment mean lifting time of 31.0±1.9 s which is 90.5±3.5% of the pretreatment time.

Table 1
Overview of all data with different compounds tested: Dose-orderly data are obtained for rats and gerbils for both the second phase of the formalin test in non operated animals and the cold allodynia represented as the lifting time on the cold plate for CCI operated animals

Class	Compound	Dose mg/kg	Rat		Gerbil	
			Bennett Cold plate	Formalin second phase	Bennett Cold plate	Formalin second phase
	Active/tested		44/735	15/531	15/305	7/548
	%		5.9	2.8	5.0	1.2
μ agonists	Morphine	0.01				0
		0.04			20	28*
		0.16		28*	14	43**
		0.63	21	0	57***	71**
		2.5	36	10	74***	57**
		10	73**	80**	100***	71**
		40	100**	100**	100***	100**
	Fentanyl	0.00063			20	
		0.0025			28	
		0.01	14		28	0
		0.04	28	14	28	14
		0.16	43**	57**	100***	71**
		0.63	57**	100**	100***	
	Codeïne	0.16			0	
		2.5		20	14	0
		10		40*	57***	0
		40		60**	100***	0
	Tramadol	0.63		14	14	
		2.5	20	14	0	0
		10	26	43**	43**	0
		40	66**	100**	100***	100**
		2.5	100**	100**		100**
Para-aminophenol derivatives	Paracetamol	0.63			14	
		2.5			0	
		10		0	14	0
		40		14	0	0
		160		100**	71***	14
NMDA antagonists	Ketamine	0.63		0		
		2.5		28*		14
		10	0	0		0
		40	43**	28*		
	MK-801	0.04	16	0		0
		0.16	86**	57**	28	14
		0.63	57**	100**	28	50**
GABA-B agonist	Baclofen	0.63	14	0		
		2.5	28	28*		14
		10	100**	100**		86**
		40	100**	100**		100**
Tricyclic antidepressant	Amytriptiline	0.63		14		
		2.5		0		0
		10	14	43**		14
		40	50**	100**		86**
Anti-epileptics	Lamotrigine	2.5	14	14		0
		10	28	28*		28*
		40	43**	86**		100**
	Carbamazepine	2.5				0
		10	14	0		0
		40	14	43**		57**
	Gabapentin	2.5				0
		10	7	14		43**
		40	28	28*		57**
		160		86**		28*
	Topiramate	0.63		14		0
		2.5		43**		0
		10	0	14		0
		40	66**	0		20*
		80		14		

Table 1 (*continued*)

Class	Compound	Dose mg/kg	Rat		Gerbil	
			Bennett Cold plate	Formalin second phase	Bennett Cold plate	Formalin second phase
NSAID	Suprofen	10	11	0		0
		40	11	0		0
		160		0		0
	Ketoprofen	0.16			20	
		0.63			0	0
		2.5			43***	20*
		10			57***	0
		40			71***	0
Calcium channel blocker	Flunarizine	10	0	0		0
		40	86**	100**		14
Tachykinin NK-1 antagonist	R116301	0.16				
		0.63			0	0
		2.5			10	20*
		10			16	40**
		40			53**	80**
	CP-96345	0.16			0	
		0.63			14	
		2.5			14	20*
		10			57***	0
		40			87***	
	GR-203040	0.63			0	0
		2.5			20	40**
		10			0	20*
		40			0	20*
Tachykinin NK-2 antagonist	SR-48968	2.5			20	0
		10			33	20*
		40			50**	20*
Tachykinin NK-3 antagonist	SR-142801	0.63			11	
		2.5			21	0
		10			33	33*
		40			49**	0

Given for each dose tested are the percentages of animals reaching the activity levels as defined by the all-or-none criterion. Statistical differences were calculated by the Fisher exact probability test for animals tested in a group of 7 animals statistical. Significance is reached at 28% for $p<0.018$ represented as * and at 43% for $p<0.001$ represented as **. For animals tested in a group of 5 animals, statistical significance is reached at 40% for $p<0.005$ represented as * and at 60% for $p<0.0003$ represented as ***. All tested compounds are represented by their generic name and classified in their compound class. No experimental data were obtained for the lines where no data were presented.

The criterion for activity of a test compound was set at a post drug lifting time being <25% of the pretreatment time. Less then 5% controls reached this level. (See Table 1).

3.2. Data from animals in test conditions

Table 1 summarizes all data obtained with the different test compounds from rats and gerbils, both in the cold allodynia testing on the cold plate in CCI animals, and in the second phase of the formalin test. Active doses of each of the experimental compounds are indicated by asterisk in the specific condition. The data obtained, mostly represent dose orderly results, indicating in most of the test compounds that the higher the dose the higher the effect observed.

Statistical analysis: For animals, tested in a group of 7 animals statistical significance is reached at 28% for $p<0.018$ (*) and at 43% for $p<0.001$ (**). For animals tested in a group of 5 animals, statistical significance is reached at 40% for $p<0.005$ (*) and at 60% for $p<0.0003$ (**).

Overall, morphine, fentanyl, tramadol, baclofen and clonidine were active in both tests in rats and in gerbils. Amitryptiline, lamotrigine, carbamazepine and gabapentin were more active in the formalin second phase than on the cold plate test. The reverse was true for codeine, topiramate, ketoprofen, flunarizine, MK-801 and ketamine. Paracetamol was only active in very high dosages. Suprofen was without any effect. Fig. 1 shows the minimal doses of the test compounds providing a statistical significant effect in CCI animals and in the second phase of the formalin test in rats; Spearman Rank order correlation test was obtained between compounds that were active in both test conditions (correlation coefficient $r=0.72$; $p<0.05$). Morphine, fentanyl, MK-801 and flunarizine demonstrated a good correlation between both tests. Clonidine was more active in the formalin second phase, whereas baclofen, tramadol, amitryptiline, ketamine, lamotrigine and topiramate tended to be more active on the cold plate in the CCI operated animals. In rats, codeine and paracetamol were not tested in CCI animals; both compounds were tested in the formalin second phase. Paracetamol was only active in very high doses in the second phase of the formalin test. In CCI rats, carbamazepine and gabapetin showed no activity in the doses tested, whereas, in the formalin second phase, they were active. The NSAID suprofen was not active in the CCI or in the formalin test.

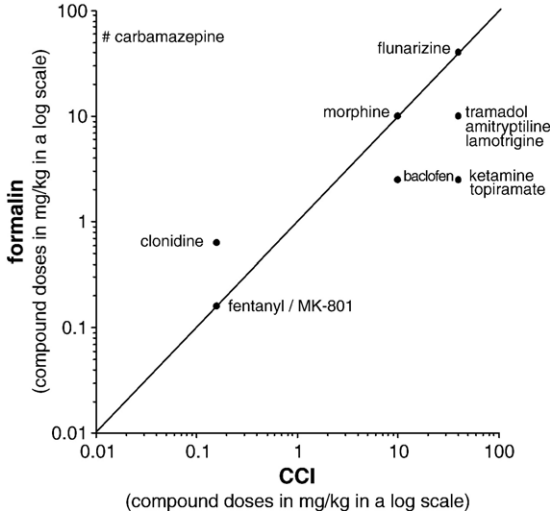

Fig. 1. Shows the correlations of the effect obtained with different experimental compounds for cold allodynia evaluated by the cold plate test in CCI operated rats (*x*-axis) and the second phase of the formalin test in non-operated rats (*y*-axis): the minimal statistical significant active doses for each condition is represented on a dose response logarithmic scale (mg/kg). The Spearman Rank correlation test calculated a correlation coefficient of $r=0.72$ for those tested compounds that were active in both test conditions. (# represent compounds only active in the formalin test, ° represents compounds only active in the CCI test).

Fig. 2 shows the minimal doses of the test compounds providing statistical significant effect in CCI animals and the second phase of the formalin test in gerbils: Spearman Rank order correlation test was obtained between compounds that were active in both test conditions (correlation coefficient $r=0.68$; $p<0.05$). In gerbils, fentanyl and ketoprofen demonstrated a good correlation between both test conditions. CP-96345 and tramadol were more active in the formalin test whereas morphine, R116301, SR-48968 and SR-142801 tended to be more active on the cold plate in CCI operated animals. In gerbils, ketamine, flunarizine and suprofen were not active. Paracetamol was tested in both conditions but only active in the very high dosages. MK-801 and GR-203040 were only active in the formalin test but not in the CCI animals. Codeine demonstrated activity in the CCI model but not in the formalin test. Baclofen, amitryptiline and the antiepileptics showed activity in the formalin second phase. They were not tested in the CCI animals.

4. Discussion

The present study was undertaken to compare the results of reference drugs with a proven efficacy and investigational compounds with a potential efficacy in the treatment of neuropathic pain, on the cold allodynia in CCI operated animals and on the pain behavior in the second phase of the formalin test.

In the acute test conditions, there is a good correlation in pharmacological activity between the second phase of the formalin test and the cold allodynia as evaluated by the total lifting time on the cold plate in CCI rats and gerbils for the reference compounds evaluated except for the NK related agents.

Additionally, this study validates the formalin test in gerbils. It is demonstrated that this test can easily be performed in these animals. The results obtained here are comparable to the results in other rodents (Dubuisson and Dennis, 1977; Abbott et al., 1995b). This validation is important since specific receptors, which are believed to be of importance in induction and maintenance of neuropathic pain states, are species specific. For example, tachykinin NK receptors of rats are different from human tachykinin NK receptors, whereas the tachykinin NK receptor of the gerbil is similar to the human NK receptor (Beresford et al., 1991; Maggi, 1995). Specific clinical relevant drug testing of this receptor must therefore be performed in gerbils and not in rats. Therefore, we additionally evaluated NK receptor antagonists only in gerbils.

In this study a good correlation between both test conditions for morphine, fentanyl, MK-801 and flunarizine was found in rats (see Fig. 1). Clonidine tended to have more activity in the second phase of the formalin test than in the cold plate test in CCI animals. Baclofen, tramadol, amitryptiline, ketamine and topiramate show to be more active in the cold plate test. Carbamazepine, gabapentin, suprofen had no activity at all in the tested doses in the cold plate allodynia in CCI animals.

In gerbils, a good correlation between both test conditions for fentanyl and ketoprofen was observed (see Fig. 2). Tramadol and CP-96345, a selective NK-1 antagonist tended to have more activity in the second phase of the formalin test than in the cold plate test in CCI animals, whereas morphine, the NK-2 antagonist SR-48968, the NK-3 antagonist SR-142801, and the NK-1 antagonist R116301, demonstrated more activity in the cold plate test. Paracetamol was only active in very high doses in the cold plate test.

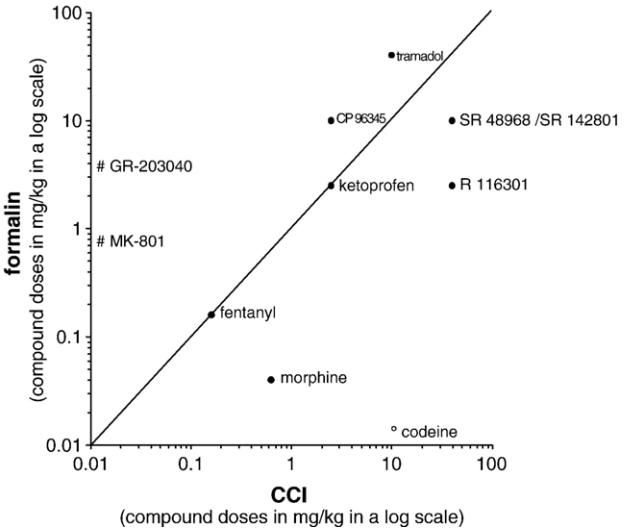

Fig. 2. Shows the correlations of the effect obtained with different experimental compounds for the cold allodynia evaluated by the cold plate test in CCI operated gerbils (*x*-axis) and the second phase of the formalin test in non-operated gerbils (*y*-axis): the minimal statistical significant active doses for each condition is represented on a dose response logarithmic scale. The Spearman Rank correlation test calculated a correlation coefficient of $r=0.68$ for those tested compounds that were active in both test conditions. (# represent compounds only active in the formalin test, ° represents compounds only active in the CCI test).

In the literature, comparable results to these data were found for (1) μ opioid agonists, which show activity in both species and both test conditions (Abbott et al., 1981, 1982; Jazat and Guilbaud, 1991; McLaughlin and Dewey, 1994; McCormack et al., 1998; Tsai et al., 2000; Leung et al., 2001; Oliva et al., 2002), only codeine shows a lower activity in the gerbil because the conversion from codeine to morphine is much slower (Oluyomi et al., 1992); (2) the α2 agonists (Jasmin et al., 1998); (3) the NMDA antagonists (Vaccarino et al., 1993; Lee and Lee, 2001; Berrino et al., 2003; Sawynok and Reid, 2003); (4) baclofen (Shafizadeh et al., 1997; Idanpaan-Heikkila and Guilbaud, 1999; Sabetkasai et al., 1999); (5) the tricyclic antidepressant (Sawynok and Reid, 2001); (6) the antiepileptic/anticovulsant: carbamazepine demonstrates only a partial effect (Idanpaan-Heikkila and Guilbaud, 1999; Blackburn-Munro et al., 2002; Heughan and Sawynok, 2002; Laughlin et al., 2002; Lee et al., 2002; Erichsen et al., 2003) whereas gabapentin is active in the formalin, but less in the CCI model, normally higher doses are needed to see clinical relevant effects (Cesena and Calcutt, 1999); (7) the non-steroidal anti-inflammatory drugs are active in the CCI model (Ossipov et al., 2000); (8) the tachykinin NK antagonists are more active in the CCI model than in the second phase of the formalin test, the NK-1 antagonist demonstrates better effect than NK-3 antagonist (Yamamoto and Yaksh, 1991; Santos and Calixto, 1997; Coudore-Civiale et al., 1998; Henry et al., 1999; Gonzalez et al., 2000).

This is one of the first studies looking for the correlation between the results obtained in different test conditions for standard test compounds of different pharmacological classes. These correlations do not form a substitute for testing the different compounds in individual tests. The correlation between pharmacological activities in different test conditions needs further validation. When interpreting the different test results, the species-specific sensitivity for the individual compounds needs to be taken into consideration. A compound that shows activity in more than one test has a higher chance to be a clinical relevant substance. In this study, all drug testing was done in acute conditions. The evaluation of reference compounds after a more chronic treatment period might be useful to make correlations with human data possible (Kontinen and Meert, 2003). Depending on the evaluated drug classes, a different, well selected, species might be needed to fully elaborate the possible activities of clinical relevant drugs. In order to further evaluate the present results, additional correlations with human clinical results should be obtained.

In conclusion, a correlation between the results obtained with second phase of the formalin test and the cold allodynia in CCI operated animals is found. The results of the tested drugs compared with the published efficacy in human studies, show a correlation between animal and human studies in these specific circumstances (Kontinen and Meert, 2003). Further validation studies are needed to make these correlations clinically applicable.

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