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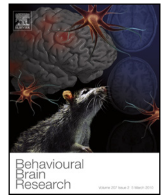

Research report

Pharmacological characterization of intraplantar Complete Freund's Adjuvant-induced burrowing deficits

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HIGHLIGHTS

- Burrowing impairments after intraplantar CFA injection unmask spontaneous pain.
- Known analgesics (NSAIDs and an anti-NGF antibody) reinstate burrowing performance.
- Opioid efficacy is masked by sedative motor impairing side effects.
- Burrowing is not driven by an instinct to find shelter or biased by trait anxiety.
- Burrowing is an objective read-out for pain in the rat intraplantar CFA pain model.

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ABSTRACT

Background: It has recently been suggested that non-reflex behavioral readouts, such as burrowing, may be used to evaluate the efficacy of analgesics in rodent models of pain.
Objective: To confirm whether intraplantar Complete Freund's Adjuvant (CFA)-induced pain reliably results in burrowing deficits which can be ameliorated by clinically efficacious analgesics as previously suggested.
Methods: Uni- or bilateral intraplantar CFA injections were performed in male Wistar Han rats. The time- and concentration-response of burrowing deficits and the ability of various analgesics to reinstate burrowing performance were studied. An anxiolytic was also tested to evaluate the motivational cue that drives this behavior.
Results: Burrowing deficits were dependent on the concentration of CFA injected, most pronounced 24 h after CFA injections and even more pronounced after bilateral compared with unilateral injections. Celecoxib and ibuprofen reversed CFA-induced burrowing deficits whereas indomethacin failed to significantly reinstate burrowing performance. Morphine and tramadol failed to reinstate burrowing performance, but sedation was observed in control rats at doses thought to be efficacious. An antibody directed against the nerve growth factor significantly improved CFA-induced burrowing deficits. Neither gabapentin nor the anxiolytic diazepam reinstated burrowing performance and the opportunity to find shelter did not modify burrowing performance.
Conclusion: Burrowing is an innate behavior reliably exhibited by rats. It is suppressed in a model of inflammatory pain and differently reinstated by clinically efficacious analgesics that lack motor impairing side effects, but not an anxiolytic, suggesting that this assay is suitable for the assessment of analgesic efficacy of novel drugs.

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1. Introduction

Preclinical behavioral readouts for the assessment of pain usually depend on reflex paw withdrawal measures in response to mechanical or thermal stimuli. The predictive validity of such assays to determine analgesic effects of drugs has been critically evaluated and the extrapolation of such animal data to human pain states controversially discussed [1,2]. A major criticism is that reflex withdrawal-based paradigms do not measure the global impact of pain and furthermore do not address ethological validity in rodent

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International; ANOVA, analysis of variance; CFA, Complete Freund's adjuvant; CHO DG44, Chinese hamster ovary- DG44 cells; COX, cyclooxygenase; IASP, International Association for the Study of Pain; IgG, immunoglobulin G; IgG1, immunoglobulin G 1; i.p., intraperitoneal; i.v., intravenous; mAB, monoclonal antibody; NGF, nerve growth factor; NGFB, nerve growth factor beta; p.o., per os; p75NTR, p75 neurotrophin receptor; s.c., subcutaneous; TRKA, tyrosine kinase receptor tropomyosin-related kinase A.

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measures of pain [3]. Instead, they measure an elicited response to an external stimulus, which may have little relationship to spontaneous ongoing pain [4], an important symptom in the majority of patients with chronic pain [5]. Consequently, alternative non-reflex behavioral readouts have recently been developed and proposed to be implemented for the evaluation of analgesic efficacy in preclinical pain models. In such behavioral assays, analgesic drugs reinstate innate behaviors which are suppressed by pain. However, the development, refinement and validation of behavioral assays based on spontaneous rodent behavior, as well as their reproducibility across labs is essential and these factors need to be addressed in future studies. The latter is especially important before considering replacing reflex withdrawal-based paradigms. Additionally, the predictive validity of such assays needs to be carefully assessed and previous studies have begun to compare innate behaviors such as burrowing with more classical withdrawal measures [9,14], as well as other non-evoked measures including weight bearing and exploratory behavior [16,24].

It has been suggested that studying improvement of innate behaviors suppressed by pain bears the advantage that compounds which impair motor function should not appear as false positives [3]. Additionally, it has been claimed that unlike withdrawal-based assays the global impact of pain and ethological validity are addressed [3]. In this regard, pain reduced innate behaviors such as exploratory and locomotor activity are of interest since it has been demonstrated that these behaviors can be reinstated by clinically efficacious analgesics [6–8]. Burrowing has been described as an ancient and ethologically relevant behavior [9,10] in which a rodent moves a substrate out of a container via coordinated hind- and forelimb movements [11]. This behavior is altered by various pain states and can be reinstated by clinically efficacious analgesics [3,9,12–16]. It has been argued that the pain detected in burrowing assays is spontaneous, rather than evoked pain, as previous groups showed no correlation between the amount of substrate burrowed and an evoked paw withdrawal measure [9,14]. Burrowing is extremely easy to measure objectively in laboratory rodents by weighing the amount of substrate, such as sand, left in the burrow at the end of the test period [9,10,15,16]. It is consistent and highly reproducible within the same animal [9]. Demonstration of this behavior is indicative of the global ”wellbeing” of a rodent [3,11] since it is also affected by a range of brain lesions and disorders, including prion disease [11], as well as post-laparotomy pain [12], peripheral inflammatory and neuropathic pain [3,14,17], intestinal inflammation [13] and after immune system activation [18,19], as well as mice fed under high fat dietary conditions [20]. Measuring the effect of persistent pain on the wellbeing of rodents may thus offer an effective way to assess the global effect of pain and analgesics in the preclinical setting [3].

The aim of the present study was to confirm and expand on previous studies which suggest that burrowing is impaired by inflammatory pain states and is sensitive to drugs with proven clinical analgesic efficacy [3,9,15,16]. We demonstrate that inflammatory pain induced by intraplantar CFA injection impairs burrowing performance and that burrowing performance can be reinstated by analgesics with well described clinical efficacy, similar to a previous report that used intra-articular CFA injections to induce sub-acute inflammatory knee pain [15,16]. This supports previous studies indicating that burrowing provides an effective way to assess the global effect of pain and analgesic efficacy in rodent models [3,9]. In order to study whether improvement of the CFA-induced burrowing impairment is mediated by attenuation of the inflammatory pain state and not just an epiphenomenon of another symptom domain such as anxiety, which often accompanies chronic pain in patients [5] we also evaluated the effect of diazepam, which lacks analgesic properties.

2. Methods

2.1. Chemicals and drugs

Unless otherwise stated, all chemicals and drugs were purchased from Sigma–Aldrich (Taufkirchen, Germany). Celecoxib (LKT Laboratories, St. Paul, MN, USA) and indomethacin (both 3, 10 and 30 mg/kg) were suspended in a solution of 0.5% Natrosol and 0.1% tween-80 (9:1) and administered per os (p.o.) two hours before burrowing performance was assessed. Ibuprofen (30 mg/kg), morphine hydrochloride (0.3, 1, 3 mg/kg; Caesar & Loretz, Hilden, Germany), tramadol (10, 30, 100 mg/kg) and gabapentin (10, 30, 100 mg/kg; Tokyo Chemical Industry, Tokyo, Japan) were solved in saline and respectively administered subcutaneously (s.c.) in the interscapular area 90 min, s.c. one hour, p.o. one hour or intraperitoneally (i.p.) immediately before burrowing performance was measured. Diazepam (Ratiopharm, Ulm, Germany) was a ready to use solution further diluted with saline (to 0.3, 1 and 3 mg/kg doses) and administered i.p. 30 min before burrowing performance was assessed. Doses and routes of administration were determined based on pilot data and published burrowing studies [9,24].

2.2. Antibody generation

To generate the antibody directed against the nerve growth factor (NGF), the anti-NGF variable domains were extracted from the patent application WO 2004/058184 A2 (applicant Rinat Neuroscience Corp.). The variable domain of the heavy chain was fused to a human IgG1 backbone with the double mutant L234A, L235A [21] by cloning it into a pOptiVec (Invitrogen) which encodes the CH1, CH2 and CH3 domain. The kappa light chain of the antibody was generated by cloning the variable domain into a pcDNA3 vector (Invitrogen) which encodes the kappa constant chain. CHO DG44 cells were stably transfected with both vectors and the antibody was purified from the cell culture media with the affinity medium MabSelect (GE Healthcare Europe, Freiburg, Germany). The dissociation constant of the purified antibody towards mouse NGF (R&D Sytems, Wiesbaden, Germany) is 37 pM and was determined by multi-cycle kinetic analysis (data not shown). The measurement was performed in the Biacore T200 with the BIAevaluation software and the human IgG capture kit (GE Healthcare Europe, Freiburg, Germany). The yield antibody was solved in saline and administered intravenous (i.v.) and burrowing performance assessed twice, 6 h and 24 h after administration, corresponding to 24 h and 48 h after intraplantar sham or CFA injections.

2.3. Animals

All animal protocols were approved by the Local Animal Care and Use Committee and were in accordance with local guides for animal use and AAALAC regulations as well as the IASP guiding principles for pain research in animals. Our report is also in line with the ARRIVE guidelines for reporting in vivo experiments. Adult male Wistar Han rats (200–220 g) were purchased from Charles River Laboratories (Sulzfeld, Germany) and group housed (4 per cage) in open top cages under controlled environmental conditions (22–24 °C, 40% relative humidity, 12 h light/dark cycle) with woodchip bedding and a red shelter; tap water and standard rodent chow were available ad libitum. After arrival all animals were allowed to adapt to the new environment for at least one week and were allowed to habituate to the experimental room for at least one hour before burrowing performance was assessed (between 8 am and 1 pm).

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