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Ambroxol, a Nav1.8-preferring Na^+ channel blocker, effectively suppresses pain symptoms in animal models of chronic, neuropathic and inflammatory pain

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Abstract

Neuropathic pain affects many patients, and treatment today is far from being perfect. Nav1.8 Na^+ channels, which are expressed by small fibre sensory neurons, are promising targets for novel analgesics. Na^+ channel blockers used today, however, show only limited selectivity for this channel subtype, and can cause dose-limiting side effects. Recently, the secretolytic ambroxol was found to preferentially inhibit Nav1.8 channels. We used this compound as a tool to investigate whether a Nav1.8-preferring blocker can suppress symptoms of chronic, neuropathic and inflammatory pain in animal models. The drug was tested in the formalin paw model, two models of mononeuropathy, and a model of monoarthritis in rats. Ambroxol's effects were compared with those of gabapentin. Ambroxol at a dose of 1 g/kg had to be administered to rats to achieve the plasma levels that are reached in clinical use (for the treatment of infant and acute respiratory distress syndrome). Ambroxol (1 g/kg) was only weakly effective in models for acute pain, but effectively reduced pain symptoms in all other models; in some cases it completely reversed pain behaviour. In most cases the effects were more pronounced than those of gabapentin (at 100 mg/kg). These data show that a Nav1.8-preferring Na^+ channel blocker can effectively suppress pain symptoms in a variety of models for chronic, neuropathic and inflammatory pain at plasma levels, which can be achieved in the clinic.

Keywords: Ambroxol; Na^+ channel; Nav1.8; Neuropathic pain; Inflammatory pain; Chronic pain

1. Introduction

Chronic neuropathic pain can be the consequence of many diseases (like diabetes, herpes zoster infection, cancer, etc.), and despite the fact that major advances have been made for treating this condition, therapy is far from being perfect. Thus, there is still a need for new therapeutic principles. Among the proposed targets for new drugs are Na^+ channels in peripheral sensory neurons, especially Nav1.8 channels (Baker and Wood, 2001). Lai et al. (2002) showed that downregulation of Nav1.8 expression by antisense oligonucleotides suppressed behavioural changes in a rat model of neuropathic pain. Thus, Nav1.8 channel blockers might be promising analgesic agents, and might have a beneficial side effect profile due to sparing other Na^+ channel subtypes. Unfortunately, most of the Na^+ channel blockers available today do not preferentially block Nav1.8 channels, although some show efficacy for treating neuropathic pain (although

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with dose-limiting side effects, e.g. mexiletine; Jarvis and Coukell, 1998).

Ambroxol is a secretolytic compound which was introduced in Germany in 1979 for the treatment of respiratory diseases, and today it is available in more than 50 countries worldwide. In addition to its effects on the respiratory system, recent investigations showed that ambroxol is an effective blocker of neuronal Na^+ channels (Weiser and Wilson, 2002). Interestingly, this drug inhibited tetrodotoxin (TTX)-resistant (Nav1.8) channels more effectively than TTX-sensitive subtypes. We therefore used this compound as a tool to investigate whether a Nav1.8-preferring Na^+ channel blocker might be effective in animal models of chronic, neuropathic and inflammatory pain. Ambroxol's effects were compared with those of gabapentin, a drug widely used for the treatment of neuropathic pain. In addition, pharmacokinetic investigations were performed to ensure that clinically meaningful ambroxol plasma concentrations were achieved.

2. Methods

2.1. Animal care

For the experiments male Wistar rats (tailflick, hotplate, and formalin paw test; weight 200–250 g) or Sprague–Dawley rats were used (all other assays; weight 285–350 g; Harlan Winkelmann, Borchen, Germany). Animals were housed under a 12/12 h light/dark cycle in type IV makrolon cages with softwood granulate bedding. Three animals were housed in a cage. Pelleted food and water were available ad libitum.

Housing, handling and testing of the animals were conducted according to international guidelines, and were approved by the local authorities (''Bezirksregierung Rheinhessen-Pfalz'' and ''Regierungspräsidium Tübingen'')

2.2. Drug administration

In animal experiments ambroxol and gabapentin were administered per os as tylose (50 g/l) suspensions. Ambroxol or vehicle was given at a volume of 4 ml/kg body weight (2 ml/kg for gabapentin). Behavioural testing was performed 60–90 min after ambroxol or the respective vehicle administration. Gabapentin or its vehicle were tested 120 min after administration.

2.3. Models for acute pain

The effects of the compounds on acute analgesia were investigated in the tail flick (D'Amour and Smith, 1941) and the hot plate tests (Woolfe and MacDonald, 1944). To evaluate the tail flick response, animals were placed on the tail flick apparatus (Ugo Basile, Comerio, Italy). An infrared beam was focused on the tail and the latency of the tail flick was assessed. The cut-off time was set to 20 s.

In the hot plate test, rats were placed on a metal plate holding a temperature of 53 °C. The latency of the nocifensive response was measured. The cut-off in these experiments was set at 30 s. Hind paw licking and lifting as well as escape behaviours were regarded as nocifensive responses.

In every experiment two baseline latency responses were determined before drug application. Data are expressed as % maximal possible effect (MPE): MPE = (postdose latency − predose latency)/(cut-off time − predose latency)

2.4. Formalin paw test

Male Wistar rats of 200–250 g body weight were used for the experiments. The formalin test was carried out according to the method described by Carlton and Zhou (1998). Formalin (2% formaldehyde in water, 20 μl) was administered into the plantar region of the right hind-paw. Total numbers of flinches were recorded in 5-min intervals for 60 min. The results are expressed as total number of flinching for the acute (0–10 min) and the late phase (from 15 to 60 min after formalin administration), which is regarded as related to chronic pain.

2.5. Surgery for models of neuropathic pain

Male Sprague–Dawley rats, weighing 185–350 g on the day of surgery, were used for the experiments.

2.6. Partial nerve ligation (PNL)

Partial sciatic nerve injury was applied according to the method by Seltzer et al. (1990). Under anaesthesia (pentobarbital sodium, 60 mg/kg intraperitoneal, i.p.), the rats' left common sciatic nerves were uncovered. About one-third of the nerve diameter was tightly ligated by Vicryl (6/0, Ethicon GmbH, Norderstadt, Germany). Sham operation was applied to the right hindleg. Nineteen days after surgery, when pain-related responses were stable, drug testing was started.

The following schedule was applied for all tests in the models of neuropathic pain: stimuli to test for mechanical allodynia (see below) were applied five times to the contra-, as well as the ipsilateral hindlimb; intervals between test stimuli were approximately 10 min. After establishing baseline (before surgery) and control values (after surgery) doses of test compound and placebo were administered to each rat in a randomized design.