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Pharmacological comparison of anticonvulsant drugs in animal models of persistent pain and anxiety

Gordon Munro*, Helle K. Erichsen, Naheed R. Mirza

NeuroSearch A/S, 93 Pederstrupvej, DK-2750, Ballerup, Denmark

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Abstract

Signs and symptoms of persistent pain are associated with neuronal hyperexcitability within nociceptive pathways. This manifests behaviourally as a decrease in the nociceptive threshold to sensory stimulation, and is closely correlated with altered affective pain processing and increased expression of anxiety-like symptoms. Anticonvulsant drugs can have marked analgesic actions in animals and humans, and some have also been reported to possess anxiolytic-like properties in animals. In the current study, we have compared the antinociceptive actions of diazepam (allosteric $GABA_A$ receptor modulator), gabapentin (binds to $\alpha_2\delta$ Ca^{2+} channel subunit), lamotrigine, riluzole and phenytoin (Na^+ channel blockers), levetiracetam (unknown mechanism), sodium valproate (potentiates GABA-mediated inhibition), ethosuximide (T-type Ca^{2+} channel blocker) and retigabine (K_v7 channel opener) in the rat formalin test, with their anxiolytic actions in the rat conditioned emotional response (CER) model of anxiety. Lamotrigine, gabapentin, riluzole, retigabine and ethosuximide attenuated second phase nociceptive responses in the formalin test. Lamotrigine, gabapentin and riluzole also displayed an anxiolytic-like profile in the CER model. Notably, the minimum doses of these drugs required to attenuate anxiety behaviour were similar to, or considerably lower than those needed to reverse pain-like behaviours. Diazepam was anxiolytic but only attenuated pain-like behaviours at sedative doses. The other drugs tested were inactive in both models. Our data suggests: (i) an antiepileptic mechanism of action per se is not necessarily sufficient for a compound to display antinociceptive and/or anxiolytic actions; and (ii) the combined antinociceptive and anxiolytic-like profiles of lamotrigine, gabapentin and riluzole suggests that these compounds likely modulate both sensory and affective dimensions of pain.

Keywords: Antiepileptic; Conditioned fear; Formalin test; Gabapentin; Lamotrigine; Levetiracetam; Pain affect; Retigabine; Riluzole; Voltage-activated Na^+ channel

1. Introduction

Persistent pain conditions including those induced by peripheral inflammation and nerve injury are characterised by injury-induced neuronal hyperexcitability events within pain transmission pathways. Sensory abnormalities manifesting as allodynia, hyperalgesia and spontaneous pain are routinely observed in human persistent pain conditions as well as in relevant animal models (Dworkin et al., 2003; Blackburn-Munro, 2004). Anticonvulsant drugs typically dampen neuronal hyperexcitability via modulation of ligand-gated (GABA-A and ionotropic glutamate receptors) and voltage-activated (e.g. Na^+ and Ca^{2+}) ion channel function. The latter mechanism of action in particular, is exemplified by the varying success of the newer generation of antiepileptics, lamotrigine and gabapentin, in the clinical treatment of persistent pain symptoms in humans, especially in conditions that are neuropathic in origin (Gilron et al., 2006).

A complicating factor of the chronic unremitting nature of pain, is that it can impact negatively on emotional well-being and future coping strategies in humans, a deleterious

* Corresponding author at: Department of Pharmacology, NeuroSearch A/S, 93 Pederstrupvej, DK-2750 Ballerup, Denmark. Tel.: +45 4460 8333; fax: +45 4460 8080.
E-mail address: gmu@neurosearch.dk (G. Munro).

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consequence of which is a high comorbidity of anxiety and depressive illness (see review by Blackburn-Munro and Blackburn-Munro, 2001). However, classical nociceptive tests performed in animals routinely measure evoked reflex behaviours. These predominantly reflect the sensory-discriminative processing of nociceptive transmission, comprising aspects of stimulus localization, intensity and modality. Measurement of the affective-motivational processing of nociceptive transmission (comprising aspects of emotion which incorporates unpleasantness, fear, distress and arousal) in animals has been less frequently reported, despite a complex interplay existing between these two related dimensions of pain processing (Hunt and Mantyh, 2001). Recently, LaBuda and Fuchs have described a place escape/avoidance paradigm for measuring the affective-motivational component of pain-like behaviour in rats, wherein a conflict between the normal tendency of the animals preference for a dark environment and a potential motivation to avoid noxious hindpaw stimulation occurs (LaBuda and Fuchs, 2000a,b). Subsequently, other groups have shown that anxiety-like behaviours of nerve-injured mice and rats measured in open field and zero maze correlate with hindpaw hypersensitivity in these animals, and might indirectly be interpreted as an index of pain affect (Narita et al., 2006; Hasnie et al., 2007).

The ability to selectively assess drug effects on sensory and affective behaviours in animals might reasonably be expected to translate to improved treatment of pain in humans (see review by Blackburn-Munro, 2004). Thus, we have compared the antinociceptive profiles of various anticonvulsant drugs in a rat model of persistent pain (formalin test), with their anxiolytic profiles in a rat shock-based anxiety model (conditioned emotional response; CER). We chose this model rather than an unconditioned anxiety model such as open field or zero maze for a number of important reasons. Firstly, the pain associated with an unpredictable, preconditioning shock stimulus presented over multiple test sessions engenders anxiety-like behaviours in the test subject. Secondly, unlike other conflict tasks such as the Vogel model, the potential anxiolytic-like activity of a compound can be assessed in the absence of any confounding analgesic effects, since compound testing is conducted under shock-free conditions. Finally, the CER model is sensitive to clinically effective anxiolytics such as benzodiazepines and some antiepileptic drugs (Davis, 1990; Stanhope and Dourish, 1996; Dias et al., 2005; Mirza et al., 2005).

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Harlan Scandinavia, Alleroed, Denmark; total number = 559 rats) were used except for CER experiments. Rats were housed in groups (4–5 per cage according to weight), in a temperature (20 ± 2 °C) and humidity (55 ± 15%) controlled environment with a light-dark cycle of 12:12 h (lights on 06:00 h). Food and water was available ad libitum. The animals were allowed to habituate to the housing facilities for at least one week prior to behavioural testing. For CER experiments, male PVG rats (Harlan, Netherlands; total number = 64 rats) with an initial weight of 200 g were used. Animals were housed and habituated for at least 7 days in Macrolon III cages (20 × 40 × 18 cm, 2 rats/cage) before operant conditioning commenced. Food (Altromin®) was available ad libitum, whereas water was available 15 min every day after CER training sessions (see below) and ad libitum at weekends.

All animal husbandry and experimental procedures were performed according to the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983), in accordance with "Methods and Welfare Considerations in Behavioral Research with Animals" (NIH Publication No. 02-5083, March 2002) and licensed by the Animal Experiments Inspectorate (The Danish Ministry of Justice).

2.2. Formalin test

Assessment of formalin-induced flinching behaviour in normal, uninjured rats (body weight 150–250 g) was made with the use of an Automated Nociception Analyser (University of California, San Diego, CA; Yaksh et al., 2001). Briefly, this involved placing a small C-shaped metal band (10 mm wide × 27 mm long) around the hindpaw of the rat to be tested. Each rat (four rats were included in each testing session) was administered drug or vehicle according to the experimental paradigm being followed, and then placed in a cylindrical acrylic observation chamber (diameter 30.5 cm and height 15 cm). Individual rats were then gently restrained and formalin (5% in saline, 50 µl, s.c.) was injected into the dorsal surface of the hindpaw using a 27G needle. They were then returned to their separate observation chambers, each of which were in turn situated upon an enclosed detection device consisting of two electromagnetic coils designed to produce an electromagnetic field in which movement of the metal band could be detected. The analogue signal was then digitised and a software algorithm applied to enable discrimination of flinching behaviour from other paw movements. A sampling interval of 1 min was used and on the basis of the resulting response patterns three phases of nociceptive behaviour were identified and scored; first phase (0–5 min), interphase (6–15 min) and second phase (16–40 min).

2.3. Rat conditioned emotional response

2.3.1. Apparatus

Eight standard operant chambers (ENV-008, MED Associates, Vermont, USA, 32 × 25 × 25 cm) were used. Each operant chamber was equipped with a 3 watt house light (centre of ceiling) and an operant lever (2 cm above the grid floor) and valve-operated water spout on one wall. A cue light (above the lever and 8 cm from the ceiling) flashed for 0.5 s when a 0.3 ml water reward was delivered. Scrambled footshock (100 ms, 0.4 mA) was delivered by a shocker (42404/5-SS Lafayette Instrument Co, Indiana, USA) connected to the grid floor (E10-10SF, MED Associates, Vermont, USA). The operant chambers were enclosed within sound isolation cubicles (NeuroSearch, Technical Department), and behavioural contingencies, reinforcement scheduling and data collection were controlled by an IBM computer running MED-PC software (v2.06B, MED Associates, Vermont, USA). Rats were trained or tested (as described in detail below and summarized in Table 1) once a day for 4–5 days a week and, 2 h after each session, were allowed 15 min free access to water in their home cages.

2.3.2. Lever press training

Rats were water deprived 24 h prior to the first training session. Initially, one water reward was delivered every 30 s without any requirement for lever pressing. However, during this time, any lever presses that did occur also elicited a reward on an FR1 (fixed ratio) schedule. After 10 min, the free reward ceased and was replaced by incrementing ratio schedules (FR 1, FR 3, FR 5, FR 7 and FR 10) that the animal had to reach in order to attain water reward. The total session time was 6 h, and lever press training was conducted daily until rats completed a minimum of 100 responses during the session. Subsequently, fixed interval (FI) training was initiated. During 30 min sessions, incrementing schedules of FI 1, FI 2, FI 3, FI 5, FI 7, FI 10, FI 20, FI 40 and FI 60 sec were applied, i.e., a reward was only delivered when the lever was pressed after a fixed interval had elapsed. Once rats showed a stable response rate for 3 consecutive sessions, variable interval (VI) training began. Here, rewards were available on average every 60 s (VI 60). The house light remained on throughout all training sessions.