European Journal of Pharmacology 673 (2011) 20–24

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and Analgesia

Spinal anesthesia with diphenhydramine and pheniramine in rats☆

Ching-Hsia Hung [a], Chin-Chen Chu [b], Yu-Chung Chen [c], Yu-Wen Chen [b,d,*], Zong-Ying Li [d], Jhi-Joung Wang [b]

[a] Institute & Department of Physical Therapy, National Cheng Kung University, Tainan, Taiwan
[b] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[c] Division of Physical Therapy, Department of Physical Medicine and Rehabilitation, Cheng Hsin General Hospital, Taipei, Taiwan
[d] Department of Physical Therapy, College of Health Care, China Medical University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 30 August 2011
Received in revised form 30 September 2011
Accepted 11 October 2011
Available online 25 October 2011

Keywords:
Diphenhydramine
Pheniramine
Lidocaine
Spinal anesthesia

ABSTRACT

The aim of this study was to evaluate the local anesthetic effects of pheniramine and diphenhydramine, two histamine H_1 receptor antagonists, on spinal anesthesia and their comparison with lidocaine, a commonly used local anesthetic. After rats were injected intrathecally with diphenhydramine and pheniramine, the dose–response curves were obtained. The potency and duration of diphenhydramine and pheniramine on spinal anesthesia were compared with lidocaine. We showed that diphenhydramine and pheniramine produced dose-dependent spinal blockades in motor function, proprioception, and nociception. On a 50% effective dose (ED_{50}) basis, the rank of potency of drugs was diphenhydramine = pheniramine > lidocaine ($p<0.05$ for the differences). In equianesthetic doses (ED_{25}, ED_{50}, and ED_{75}), the block duration caused by diphenhydramine was longer than that caused by pheniramine or lidocaine ($p<0.01$ for the differences). Diphenhydramine, but not pheniramine or lidocaine, elicited longer duration of sensory block than that of motor block at the same dose of 1.75 μmol. These preclinical data reported that diphenhydramine with a more sensory-selective action over motor blockade demonstrated more potent and longer-lasting spinal blockades, compared with pheniramine or lidocaine.

1. Introduction

Diphenhydramine and pheniramine, two histamine H_1 receptor antagonists, are widely used antihistaminics (Estelle and Simons, 1999; Pullman et al., 1975; Sharma and Hamelin, 2003) and have antipruritic effects (Pavlidakey et al., 2009). Diphenhydramine is a first generation antihistamine mainly used to treat allergies and may act as an antiemetic, sedative and hypnotic (Pavlidakey et al., 2009; Shepherd, 2011). Diphenhydramine also has the local anesthetic properties (Steffen et al., 1956), and has been used successfully as a local cutaneous anesthetic when allergies to other local anesthetic agents exist (Pollack and Swindle, 1989). From that time onwards, there is a growing body of evidence that diphenhydramine had topical ocular and dermal local anesthetic properties (Green et al., 1994; Pavlidakey et al., 2009; Suffridge et al., 2009).

Diphenhydramine has been shown to have the characteristic of the blockade of Na^+ currents (Kim et al., 2000; Kuo et al., 2000), which is one of the major mechanisms of local anesthesia, produces spinal anesthesia, peripheral nerve block, and infiltrative cutaneous analgesia (McLure and Rubin, 2005). However, to the best of our knowledge, no study of diphenhydramine or pheniramine on spinal anesthesia has been reported to date. Spinal anesthesia is a relatively simple technique, which brings competent surgical conditions by the injection of a small amount of local anesthetic with easy landmarks, giving a wide popularity to this practice (Vandermeersch et al., 1991). The aim of this study was to evaluate the spinal anesthesia following intrathecal injections of diphenhydramine and pheniramine by testing motor function, proprioception, and nociception on rats. Lidocaine, a known local anesthetic, was used as control.

2. Materials and methods

2.1. Animals

The experiment was approved by the Institutional Animal Care and Use Committee of China Medical University, Taiwan on 11 January 2010 and conformed to the recommendations and policies of the International Association for the Study of Pain (IASP). Two hundred and sixteen male Sprague–Dawley rats weighting 300–350 g were obtained from the National Laboratory Animal Centre in Taiwan, and then housed in a climate controlled room maintained at 21 °C with approximately 50% relative humidity. Lighting was on a 12-h light/dark cycle (light on at 6:00 AM), with food and water available *ad libitum* up to time of the experiment.

☆ Conflicts of interest: There is no conflict of interests for all authors.

* Corresponding author at: Department of Physical Therapy, China Medical University, No.91 Hsueh-Shih Road, Taichung 40402, Taiwan. Tel.: +886 4 22053366x7327; fax: +886 4 22065051.
E-mail address: cywhwok@mail.cmu.edu.tw (Y.-W. Chen).

0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.10.019

2.2. Drugs

Diphenhydramine HCl, pheniramine maleate, and lidocaine HCl monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs in stock were freshly prepared in 5% dextrose as solution before intrathecal injections. After injections, the low pH of these plain solutions (range, 6.0–6.5) is likely to be buffered quickly by the cerebral spinal fluid (pH 7.4).

2.3. Experimental protocols

Three specific experiments were carried out. In experiment 1, the effects of diphenhydramine (0.30, 0.60, 0.90, 1.50, 1.75 μmol), pheniramine (0.40, 0.75, 0.90, 1.50, 1.90 μmol), lidocaine (0.50, 0.75, 1.00, 1.50, 2.50 μmol), and vehicle (5% dextrose) on spinal block were evaluated (n = 8 rats for each dose of each drug). In experiment 2, the spinal block effect of diphenhydramine or pheniramine was compared with that of lidocaine at the same dose of 1.75 μmol (n = 8 rats for each dose of each drug). In experiment 3, on an equipotent basis (ED_{25}, ED_{50} and ED_{75}), the duration of lidocaine on spinal anesthesia was compared with that of diphenhydramine or pheniramine (n = 8 rats for each dose of each drug).

2.4. Spinal anesthesia by intrathecal injections of drugs

All animals were injected intrathecally one time in this study. Lumbar puncture was done on conscious rats. Before the intrathecal injections, local anesthesia was given. Following an optimal flexion of the rat lumbar spine under prone position, each 50-μl of 0.5% lidocaine was injected into the right- and left-side of paraspinal space (0.5 cm in depth) which was 0.5 cm away from the mid-point of the longitudinal line of L4–5 intervertebral space (Chen et al., 2010b; Leung et al., 2010). Two minutes later, a 27-gauge needle attached to a 50-μl syringe (Hamilton, Reno, Nevada) was inserted into the mid-line of the L4–5 intervertebral space until a tail-flick indicated entrance into the intrathecal space. The intrathecal injection volume used in the present study (50 μl) is larger than is commonly used but not exceptionally large, since intrathecal injection volumes as high as 100 μl have been used in the rat to show a long-lasting spinal anesthesia effect by amitriptyline and diphenidol (Leung et al., 2010; Sudoh et al., 2003). Fifty microliters of drug was injected and the rat was observed for the development of spinal blockade, indicated by paralysis of both hind limbs. Rats, which demonstrated unilateral blockade, were excluded from the study and sacrificed by using an over dose of isoflurane.

2.5. Neurobehavioral evaluation

After intrathecal injection of drug, three neurobehavioral evaluations, which consisted of evaluations of motor, proprioception, and nociception, were conducted (Chen et al., 2007; Chen et al., 2010a). For consistency, an experienced investigator (Dr. Hung), who was blinded to the identity of the injected drugs, was responsible for handling of all rats and behavioral evaluations. Rats were evaluated before medication and at 1, 5, and 10 min afterwards, then again at 10-min interval until 1 h and at 15 min interval until 2 h. The magnitude of spinal blockade in motor function, proprioception, and nociception was described as the percentage of possible effect (% PE). The maximum blockade in a time course of spinal anesthesia of drugs was described as the percent of maximal possible effect (% MPE).

In brief, nociception was evaluated by the withdrawal reflex or vocalization elicited by pinching a skin fold over each rat's back at 1 cm from the proximal part of the tail, the lateral metatarsus of bilateral hind limbs, and the dorsal part of the mid-tail. At each testing time, only one pinch was given to each of the four testing sites, and the time interval between stimulations at different sites was around 2 s. The nociceptive blockade was graded as 4 (normal or 0% MPE), 3 (25% MPE), 2 (50% MPE), 1 (75% MPE), and 0 (absent or 100% MPE) (Chen et al., 2004; Hung et al., 2009).

Proprioception evaluation was based on the resting posture and postural reactions ('tactile placing' and 'hopping'). Hopping response was performed by lifting the front half of the animal off the ground and lifting one hind limb at a time off the ground so that the animal was standing on just one limb. Then, the animal was moved laterally, which normally evoked a prompt hopping response with the weight-bearing limb in the direction of movement to prevent the animal from falling. A predominantly motor impairment caused a prompt but weaker than normal response. Conversely, with a predominantly proprioceptive blockade, delayed hopping was followed by greater lateral hops to prevent falling over or, in this case of complete block, no hopping at all. The functional deficit was graded as 3 (normal or 0% MPE), 2 (slightly impaired), 1 (severely impaired), and 0 (completely impaired or 100% MPE) (Chen et al., 2004; Hung et al., 2009).

Motor function was evaluated by measuring 'the extensor postural thrust' of the right hind limb of each rat. The extensor thrust was measured as the gram force, which resisted contacting the platform by the rat heel applied to a digital platform balance (Mettler Toledo, PB 1502-S, Switzerland). The reduction in this force, representing reduced extensor muscle tone, was considered as a deficit of motor function and expressed as a percentage of the control force. The pre-injection control value was considered as 0% motor block or 0% maximal possible effect (% MPE). A force less than 20 g (also referred to as the weight of the 'flaccid limb') was interpreted as the absence of extensor postural thrust or a 100% motor block or 100% MPE (Leung et al., 2010; Thalhammer et al., 1995).

2.6. Effective doses (EDs)

After intrathecally injecting the rats with four different doses of each drug (n = 8 for each dose of each drug), the dose–response curve was constructed. The curve was then fitted using a SAS Nonlinear (NLIN) Procedures (SAS Institute Inc., Carey, NC), and the value of 50% effective dose (ED_{50}), defined as the dose that caused 50% spinal anesthesia, were obtained (Chen et al., 2011a; Minkin and Kundhal, 1999). The ED_{25} or ED_{75} of drug was obtained by the same curve-fitting (SAS NLIN Procedures) which was used to derive the ED_{50} (Chen et al., 2011c; Minkin and Kundhal, 1999). The full recovery time, defined as the interval from injection to full recovery, caused by each drug (n = 8 rats for each dose of each drug) was evaluated on an equipotent basis (ED_{25}, ED_{50} and ED_{75}). In this study, we also evaluated the % MPE, complete blockade time, time to full recovery, area under curves (AUCs) of motor, proprioception and nociception for diphenhydramine, pheniramine, and lidocaine at the same dose of 1.75 μmol. The AUC of spinal blockade of drug was obtained by using Kinetica v 2.0.1 (MicroPharm International, USA) (Chen et al., 2011b).

2.7. Statistical analysis

Data are presented as means ± S.E.M. or ED_{50} values with 95% confidence interval (95% CI). Values were evaluated by either 1-way (experiments 1 and 2) or 2-way (experiment 3) analysis of variance (ANOVA) followed by pairwise Tukey's honest significance difference (HSD) test. A statistical software, SPSS for Windows (version 17.0, SPSS, Inc, Chicago, IL, USA), was used, and a P value less than 0.05 was considered statistically significant.

3. Results

3.1. The spinal blockade of diphenhydramine and pheniramine

Diphenhydramine and pheniramine, as well as lidocaine, displayed dose-dependent effects on spinal anesthesia in rats (Fig. 1). Intrathecal injection of 5% dextrose (vehicle) produced no spinal anesthetic effects