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Epinephrine as adjuvant for propranolol produces a marked peripheral action in intensifying and prolonging analgesia in response to local dorsal cutaneous noxious pinprick in rats

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ABSTRACT

The aim of this study was to evaluate the effect of epinephrine as additive for propranolol as an infiltrative anesthetic. Using a rat model of cutaneous trunci muscle reflex (CTMR), we tested the effect of co-administration of epinephrine with propranolol on infiltrative cutaneous analgesia. Bupivacaine, a long-lasting local anesthetic, was used as control. Subcutaneous propranolol and bupivacaine elicited a dose-dependent local anesthetic effect on infiltrative cutaneous analgesia. On the 50% effective dose (ED_{50}) basis, the relative potency was bupivacaine [2.05 (1.95–2.21) μmol/kg] > propranolol [9.21 (9.08–9.42) μmol/kg] ($P < 0.01$ for each comparison). Subcutaneous epinephrine (0.012 μmol/kg) did not produce cutaneous analgesia. Mixtures of epinephrine (0.012 μmol/kg) with drugs (propranolol or bupivacaine) at ED_{50} or ED_{95}, respectively, intensified and prolonged drug action on infiltrative cutaneous analgesia. Intraperitoneal injection of combined drugs (propranolol or bupivacaine) at ED_{95} with epinephrine (0.012 μmol/kg) exhibited no cutaneous analgesia. We concluded that propranolol was less potent but produced a similar duration of action when compared to bupivacaine on infiltrative cutaneous analgesia. Epinephrine as adjuvant for propranolol or bupivacaine enhanced the potency and extended the duration of action on infiltrative cutaneous analgesia.

1. Introduction

Propranolol as the first clinically useful β-adrenergic receptor antagonist was discovered in 1964 and introduced to the clinical practice in the treatment of cardiovascular diseases (Frullani et al., 1970; Matthews and Baker, 1982). Until now, numerous indications for the use of propranolol included the therapy of essential tremor (Alkondon et al., 1986; Winkler and Young, 1974), cardiac arrhythmias (Matthews and Baker, 1982), hyperthrophic obstructive cardiomyopathy (Hess et al., 1983), angina pectoris (Frishman et al., 1989; Zimmermann et al., 2010), hypertension (Frishman et al., 1989), migraine (Linde and Rossnagel, 2004), infantile haemangiomas (Leaute-Labreze et al., 2008; Siegfried et al., 2008), many neuropsychiatric disorders (Tchivileva et al., 2010), dental anxiety (Heaton et al., 2010) and pulmonary hemangioma (Emiralioglu et al., 2014). Because propranolol can block neuronal voltage-gated sodium channels (Fabritz et al., 2014; Wang et al., 2010), it therefore has been shown to have a local (termed topical) anesthetic effect (Frullani et al., 1970; Leszczynska and Kau, 1992; Zimmermann et al., 2010). For instance, propranolol blocked the sciatic nerve in mice (Leszczynska and Kau, 1992). Our previous studies demonstrated that propranolol was similar to lidocaine at producing spinal anesthesia and elicited the longer action of spinal blockade than lidocaine in rats (Chen et al., 2011b, 2011c).

Surgery and postoperative pain control are frequently performed through injection of the long-acting local anesthetic (Job et al., 1979; Khan et al., 2002). However; the technique is limited due to the short duration of analgesia or anesthesia (Cameron and Cross, 1985). For this reason, bupivacaine is chosen for infiltration

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because of its longer duration of effective analgesia (Hannibal et al., 1996). Additionally, epinephrine is often added to the local anesthetics to prolong the duration of central and peripheral neuraxial blockades (Scott et al., 1972), but it is important to limit the dosage of epinephrine seriously to prevent side effects. The optimal concentrations of epinephrine may vary based on the anesthetic agent and its injection site (Gessler et al., 2001; Liu et al., 1995; Scott et al., 1972). We presume that the addition of epinephrine may enhance and prolong propranolol cutaneous analgesia. The purpose of this study was to evaluate the local anesthetic potency and duration of propranolol following the addition of epinephrine. Bupivacaine, a long-acting local anesthetic, was used as a control.

2. Materials and methods

2.1. Animals

The experimental protocol was approved *via* the Institutional Animal Care and Use Committee of China Medical University (Taichung, Taiwan) and conducted according to IASP ethical guidelines (Zimmermann, 1983). Male Sprague-Dawley rats, each weighing 205 − 255 g, were obtained from the National Laboratory Animal Centre (Taipei, Taiwan) and kept in the animal housing facilities at China Medical University, with controlled humidity (approximately 50% relative humidity), room temperature (22 °C), and a 12-hour (6:00 a.m. to 6:00 p.m.) light/dark cycle.

2.2. Drugs

Bupivacaine HCl and (±)-Propranolol HCl, and (±)-epinephrine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in saline (0.9% NaCl) before injection.

2.3. Groups and design

Four investigations were carried out. In investigation 1, cutaneous analgesia of propranolol (40.0, 26.7, 6.7, 3.3 μmol/kg) and bupivacaine (9.0, 4.0, 1.3, 0.8 μmol/kg) in a dose-related fashion was constructed to obtain the 50% effective dose (ED_{50}) ($n=8$ for each group). In investigation 2, the effects of propranolol at 40 μmol/kg, bupivacaine at 9 μmol/kg, epinephrine at 0.012 μmol/kg and saline (vehicle) were performed on infiltrative cutaneous analgesia ($n=8$ for each group). Epinephrine (0.012 μmol/kg) and saline elicited no cutaneous analgesia. This dose of epinephrine was chosen according to our previous study (Chen et al., 2008). In investigation 3, the %MPE (percent of maximal possible effect), duration, and area under the curves (AUCs) of drug (ED_{50} or ED_{95}) alone or co-administration of drug (ED_{50} or ED_{95}) with epinephrine (0.012 μmol/kg) were evaluated on infiltrative cutaneous analgesia ($n=8$ for each group). In investigation 4, two control groups were further tested to rule out the possibility of systemic effect of drugs on infiltrative cutaneous analgesia. One group ($n=8$ for each group) received intraperitoneal injection of testing drug (bupivacaine or propranolol) at a dose of $2 \times ED_{95}$; another group ($n=8$ for each group) received intraperitoneal injection of co-administration of epinephrine (0.012 μmol/kg) with drug (bupivacaine or propranolol) at ED_{95}.

2.4. Subcutaneous injection

Before the experiments, rats were handled daily up to 7 days to minimize the stress on the animals during the study and generally improve their experimental performance. On the day before subcutaneous injection, the hair on the rats' dorsal surface of the thoracolumbar region (10×6 cm^2) was mechanically shaved. Subcutaneous injection was carried out as reported previously (Chen et al., 2011a, 2011d). In brief, drugs dissolved in saline were injected subcutaneously using a 30-gauge needle in unanesthetized rats at the dorsal surface of the thoracolumbar region. The total volume of injection was 0.6 mL. In order to reduce the numbers of experimental animals used, the back of rat was further divided into left and right parts, either of which, after a washout period of 1 week, received one drug injection. After subcutaneous injection, a wheal, a circular elevation of the skin, approximately 2 cm in diameter occurred. The wheal was marked with ink within 30 s after injection.

2.5. Neurobehavioral evaluation

For consistency, an experienced investigator who was blinded to the drug injected was responsible for neurobehavioral assessment. Cutaneous analgesia was evaluated according to the cutaneous trunci muscle reflex (CTMR), characterized by the reflex movement of the skin over the back elicited through twitches of the lateral thoracispinal muscle in response to local dorsal cutaneous stimulation (Bulbring and Wajda, 1945; Khodorova and Strichartz, 2000). A Von Frey filament (No.15; Somedic Sales AB, Stockholm, Sweden), to which the cut end of an 18-gauge needle was affixed, was applied to elicit the standardized nociceptive stimulation (19 ± 1 g) without producing skin damage (Chen et al., 2012a, 2012b). After observing a CTMR response to the pinprick applied outside the wheal and on the contralateral side, we applied six pinpricks with a frequency of 1 Hz inside the wheal and scored the number to which the rat failed to react. Each drug's cutaneous analgesia was examined quantitatively as the number of times the pinprick failed to elicit a response, with, for instance, the complete absence of six responses was defined as complete nociceptive/sensory block (100% of possible effect; 100% PE). During the test, the maximum blockade in a time course of cutaneous analgesia of the drug was described as the %MPE (maximal possible effect). The test of six pinpricks was applied at 0, 2 and 5 min after injection, every 5 min after injection for the first 30 min afterwards, then again every 10 min after injection for 30–60 min, and every 15–60 min thereafter until the CTMR completely recovered from the block. Each drug's duration of action was defined as the time from drug injection (i.e., time = 0) to full recovery of CTMR (no analgesic effect or 0% MPE) (Khan et al., 2002; Khodorova and Strichartz, 2000).

2.6. The ED_{50} and AUCs

The dose–response curves were constructed after subcutaneously injecting the rats with different doses of each drug. These curves were then fitted by using the computer-derived SAS NLIN Procedures (SAS Institute Inc., Carey, NC), and the values of ED_{50} and ED_{95}, defined as the dose that produced 50% and 95% cutaneous analgesia, respectively, was obtained (Leung et al., 2013a, 2013b). Furthermore, we also assessed the %MPE, complete blockade time, time to full recovery, AUCs of drug (ED_{50} or ED_{95}) alone or co-administration of drug (ED_{50} or ED_{95}) and epinephrine (0.012 μmol/kg). The AUCs were obtained by using *Kinetica version 2.0.1* (InnaPhase Corporation, Philadelphia, PA).

2.7. Statistical analysis

Values are presented as mean ± S.E.M. or ED_{50} and ED_{95} values with 95% confidence interval (95% CI). Data were analyzed by Student's *t*-test or 1-way analysis of variance (ANOVA) followed by pairwise Tukey's honest significance difference (HSD) test. A statistical