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Research paper

Cutaneous synergistic analgesia of bupivacaine in combination with dopamine in rats

Jann-Inn Tzeng[a], Jieh-Neng Wang[b], Jhi-Joung Wang[c], Yu-Wen Chen[c,d,1], Ching-Hsia Hung[e,f,*,1]

[a] Department of Anesthesiology, Chi-Mei Medical Center, Yong Kang, Tainan, Taiwan
[b] Department of Pediatrics, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[c] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[d] Department of Physical Therapy, College of Health Care, China Medical University, Taichung, Taiwan
[e] Department of Physical Therapy, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[f] Institute of Allied Health Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan

HIGHLIGHTS

- Dopamine produced dose-dependent cutaneous analgesia.
- Dopamine was less potent than bupivacaine at producing cutaneous analgesia.
- Dopamine elicited a similar duration of cutaneous analgesia compared with bupivacaine.
- Dopamine combined with bupivacaine produced a synergistic analgesic effect.

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ABSTRACT

The main goal of the study was to investigate the interaction between bupivacaine and dopamine on local analgesia. After the blockade of the cutaneous trunci muscle reflex (CTMR) responses, which occurred following the drugs were subcutaneously injected in rats, the cutaneous analgesic effect of dopamine in a dosage-dependent fashion was compared to that of bupivacaine. Drug-drug interactions were evaluated by isobolographic methods. We showed the dose-dependent effects of dopamine on infiltrative cutaneous analgesia. On the 50% effective dose (ED_{50}) basis, the rank of drug potency was bupivacaine (1.99 [1.92–2.09] μmol/kg) greater than dopamine (190 [181–203] μmol/kg) ($P < 0.01$). At the equianalgesic doses (ED_{25}, ED_{50}, and ED_{75}), dopamine elicited a similar duration of cutaneous analgesia compared with bupivacaine. The addition of dopamine to the bupivacaine solution exhibited a synergistic effect. Our pre-clinical data showed that dopamine produced a dose-dependent effect in producing cutaneous analgesia. When compared with bupivacaine, dopamine produced a lesser potency with a similar duration of cutaneous analgesia. Dopamine added to the bupivacaine preparation resulted in a synergistic analgesic effect.

Infiltrative administration of local anesthetic agents is commonly performed for clinical procedures of laparoscopic surgery [8] and for treating postoperative pain following inguinal hernia repair [32] due to limited side effects [19]. However, the technique is restrained by the short duration of analgesia or anesthesia in clinical applications [6]. In order to satisfy the procedures in long-haul surgeries, the addition of epinephrine to the local anesthetic preparations [30] or the long-acting local anesthetic bupivacaine [15] was employed. Conversely, it is important to restrict the epinephrine concentrations severely to prevent adverse effects.

Essentially, dopamine had a vasoconstrictive effect in the human skin [25]. Catecholamines (i.e., dopamine, epinephrine, and norepinephrine) were synthesized while tyrosine was transported into the nerve terminals [28]. It has been known that the subcutaneous injection of epinephrine caused a transient blockade of sensory/nociceptive function in rats [11,12,20,36]. Additionally, epinephrine was used as a vasoconstrictor to restrict the systemic

* Corresponding author at: Department of Physical Therapy, College of Medicine, National Cheng Kung University, No.1 Ta-Hsueh Road, Tainan, Taiwan.
E-mail address: chhung@mail.ncku.edu.tw (C.-H. Hung).
[1] These two authors contributed equally to this work.

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Table 1
The 50% effective dose (ED_{50}), ED_{25}, and ED_{75} of bupivacaine and dopamine on infiltrative cutaneous analgesia in rats.

Drug	ED_{25} (95% CI)	ED_{50} (95% CI)	ED_{75} (95% CI)
Bupivacaine	1.31 (1.24–1.40)	1.99 (1.92–2.09)	3.75 (3.65–3.90)
Dopamine	130 (122–142)	190 (181–203)	240 (225–261)

The EDs of drugs (μmol/kg) were constructed from Fig. 1A. CI = confidence interval. The potency of drug (ED_{50}) was bupivacaine > dopamine ($P < 0.01$, for each comparison) via using one-way analysis of variance and pairwise Tukey's honest significant difference test for paired comparisons.

absorption [24,30], which therefore enhanced the action of local anesthetic agents during medical, dental, or surgical procedures [2,5]. By using an isobologram analysis, the co-administration of epinephrine with bupivacaine exhibited a synergistic effect on infiltrative cutaneous analgesia in rats [11].

Moreover, the isobolographic analysis provided a basis for examining if the biological responses elicited through the mixture of the two drugs provoke antagonistic, synergistic, or additive reactions [33]. Because dopamine is a vasoconstrictor [25], it may potentiate the local anesthetic action (i.e., infiltrative cutaneous analgesia). Based on the reports, we aimed to (1) investigate the analgesic potency of local anesthetic bupivacaine versus dopamine and (2) examine the cutaneous analgesic interaction between bupivacaine and dopamine by isobologram in rats. Bupivacaine, a long-lasting local anesthetic, was used as a control.

The experimental protocols were approved through the Standing Committee on Animals of National Cheng Kung University (Tainan, Taiwan). Animals were treated in accordance with the recommendations and policies for experimental animal use of the International Association for the Study of Pain (IASP). Male Sprague-Dawley rats (205–255 g at delivery) were obtained from National Cheng Kung University and kept in plastic cages with soft bedding with access to food and water ad libitum, maintained on a 12-h light/12-h dark cycle, and controlled humidity (approximately 50% relative humidity) with room temperature (22 °C).

Dopamine HCl and bupivacaine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and were dissolved in normal saline (0.9% NaCl) as solution freshly before injection.

Five studies were designed (n = 8 in each group). In Study 1, cutaneous analgesia following subcutaneous dopamine (82, 164, 246, and 355 μmol/kg) and bupivacaine (1.02, 1.36, 2.73, and 9.82 μmol/kg) in a dose-dependent manner was tested. In Study 2, the duration, %MPE (percentage of maximum possible effect), and area under the curves (AUCs) of dopamine (355 μmol/kg), bupivacaine (9.82 μmol/kg) and saline (vehicle) on infiltrative cutaneous analgesia were estimated. Saline (vehicle) group elicited no cutaneous analgesia. In Study 3, at the equipotent doses [50% effective dose (ED_{50}), ED_{25} and ED_{75}], the duration of cutaneous analgesia of dopamine was compared to that of bupivacaine. In Study 4, isobolographic analyses were used to analyze the mixtures of dopamine and bupivacaine in producing cutaneous analgesia. In Study 5, two control groups were performed to exclude the possible systemic effect of drugs from local/cutaneous analgesia. One group received intraperitoneal injection of drugs (dopamine or bupivacaine) at a higher dose of $2 \times ED_{75}$; another group underwent intraperitoneal injection of the co-administration of dopamine (ED_{50}) with bupivacaine (ED_{50}).

The hairs on the dorsal surface of the rats' thoracolumbar area (6 cm × 10 cm) were mechanically shaved on the day before injection. The protocol of the subcutaneous injection was performed as described previously [12,16]. In brief, drugs dissolved in saline (0.6 mL) were subcutaneously injected using a 30-gauge needle at the dorsal sites of the thoracolumbar areas of the un-anesthetized rats. After the rats were injected, a circular elevation of the skin, also known as a wheal, approximately 2 cm in diameter appeared and was marked with ink within one minute. Cutaneous analgesia was defined as the blockade of the cutaneous trunci muscle reflex (CTMR), which is characterized by the reflex movement of the skin over the back evoked by twitches of the underlying lateral thoracospinal muscles in response to the local dorsal skin stimulus [34].

The CTMR block was evaluated by a pinprick reaction using a von Frey filament (No.15; Somedic Sales AB, Stockholm, Sweden) with an 18-ga needle [36,37]. After seeing a CTMR response to the pinprick applied on the contralateral side and outside the wheal, we applied six pinpricks with a frequency of 2 Hz inside the wheal. The cutaneous analgesia of each drug was examined quantitatively and scored the number to which the rat failed to react. Each drug's cutaneous analgesia was quantitatively recorded as the number of times the stimulus failed to induce a CTMR response and was described as the percentage of possible effect (%PE). For example, the absence of six responses after six stimuli was defined as complete nociceptive/sensory block (100% PE), which was calculated as follows:

$$\%PE = ((\text{number of stimuli that elicited no response})/6) \times 100\%.$$

During the period of the drug action, the maximum value of %PE was presented as the percentage of maximal possible effect (%MPE). For experimental consistency, a trained experimenter who was blinded to the drug treatments, was responsible for handling all the animals and behavioral assessments. The pinprick tests were performed 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. The duration of action was defined as from the injection time (i.e., time = 0) to the complete recovery of CTMR (no analgesia or 0% PE).

The dose-response curves were obtained from the %MPE of each dose of drugs (bupivacaine or dopamine) after subcutaneously injecting the rats with four doses of drugs. The value of the ED_{50}, which was defined as a dose that elicited 50% sensory/nociceptive blockade, was constructed by using the SAS NLIN Procedures (SAS Institute Inc., Carey, NC) [17,35]. The values of AUCs of nociceptive/sensory blockades were constructed by using Kinetica version 2.0.1 (InnaPhase Corporation, Philadelphia, PA).

Isobolographic methods (version 1.27, Pharm Tools Pro, McCary Group, Wynnewood, PA) were used to investigate the drug–drug interactions [9,33]. In regards to construct the experimental value of ED_{50}, the dose–response curve of the combined drugs at five equipotent doses (e.g., bupivacaine combined with dopamine at a ratio of ED_{50} vs. ED_{50}) was performed and calculated by using SAS NLIN Procedures (SAS Institute Inc., Carey, NC). Then, the ED_{50} of combined drugs was evaluated by using the isobologram methods described by Tallarida [33]. The difference between the experimental ED_{50} value (calculated from the dose–response curve of combined drugs) and the theoretical ED_{50} value (obtained from the theoretical additive line by computer simulation) was examined [10,22].

Data are expressed as mean ± S.E.M. or ED_{50} values with 95% confidence interval (95% CI). The values were analyzed by using the Student's *t*-test and one-way or two-way analysis of variance (ANOVA) followed by pairwise Tukey's honest significance difference (HSD) test. The statistical software, SPSS for Windows (version 17.0, SPSS, Inc., Chicago, IL, USA), was used. The differences between groups were set to be significant at a value of $P < 0.05$.

Dopamine and bupivacaine both exhibited dose-dependent cutaneous analgesia in rats (Fig. 1A). The values of ED_{25}s, ED_{50}s, and ED_{75}s of dopamine and bupivacaine are shown in Table 1. On an equipotent (ED_{50}) basis, the rank of drug potency was bupivacaine greater than dopamine ($P < 0.01$; Table 1). In Fig. 1B, dopamine and bupivacaine produced a complete sensory/nociceptive block-