J Anesth (2003) 17:241–245
DOI 10.1007/s00540-003-0184-6

Journal of
Anesthesia

Enhancement of lidocaine-induced epidural anesthesia by deoxyaconitine in the rabbit

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Abstract
Purpose. Aconiti tuber has been used in traditional Oriental medicine to alleviate pain. The antinociceptive property of aconiti tuber is due to the action of its extracted alkaloids such as deoxyaconitine. The purpose of this study was to investigate the effect of epidural deoxyaconitine on epidural lidocaine anesthesia.
Methods. Five adult rabbits were used. Three different combinations of drugs were injected into the epidural space, in the following order: first (combination A), 1.5 ml of 2% lidocaine; second (combination B), 1.5 ml of 2% lidocaine and 150 μg deoxyaconitine; and third (combination C), 3 mg nor-binaltorphimine followed by 1.5 ml of 2% lidocaine and 150 μg deoxyaconitine 30 min later. The latency of onset and the duration of three end-points (sensory loss in the tail, loss of weight-bearing ability, and flaccid paresis of hind limb) were measured.
Results. Onset times for the three end-points were not changed by deoxyaconitine or by nor-binaltorphimine. The duration of sensory loss was 27.0 ± 2.7 min, the duration of loss of weight-bearing ability was 33.0 ± 2.7 min, and the duration of flaccid paresis was 21.0 ± 4.2 min in the combination A group. In the combination B group, deoxyaconitine extended the time of sensory loss by 80%, the time of loss of weight-bearing by 50%, and that of flaccid paresis by 60% compared with the combination A group. In the combination C group, this phenomenon was partially antagonized by pretreatment with nor-binaltorphimine, a κ-opioid antagonist.
Conclusions. Based on our observations, deoxyaconitine enhanced epidural lidocaine anesthesia in the rabbit, and this effect seemed to be partly mediated by κ-opioid receptors.

Key words Aconiti tuber · Epidural anesthesia · Deoxyaconitine · Oriental medicine · Rabbit

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Received: September 4, 2002 / Accepted: June 3, 2003

Introduction

Aconiti tuber (aconite root) has been used in traditional Oriental medicine to alleviate pain [1–3]. Deoxyaconitine, aconitine, mesaconitine, hypaconitine, and similar alkaloidal compounds have been isolated from extracts of aconiti tuber. It is well known that aconiti tuber and its alkaloids have antinociceptive activity on oral administration [4]. Oyama et al. [5] reported that mesaconitine has greater analgesic potency than morphine. Basic investigations have suggested that the antinociceptive action of mesaconitine involves the serotonergic descending inhibitory system [4,6]. Another report suggested that the analgesic effect of processed aconiti tuber was due to the stimulation of a κ-opioid receptor by dynorphine released in the spinal cord [3,7]. Deoxyaconitine has demonstrated antinociceptive action and produces fewer toxic effects, such as arrhythmia and respiratory suppression, compared with the other aconiti tuber alkaloids [8].

Since the discovery of their receptors in the spinal cord, morphine and clonidine have been successfully used epidurally or intrathecally, resulting in good pain relief with reduced dosages and fewer side effects when compared with systemic administration. As the antinociceptive action of aconiti tuber was reported to involve the spinal cord, epidural administration of deoxyaconitine could be effective and beneficial in this regard.

Chen et al. [9] studied the effects and side effects of lappaconitine, one of the alkaloids extracted from aconiti tuber, administered epidurally for postoperative analgesia. They indicated that epidural lappaconitine with bupivacaine was more rapid and more potent than lappaconitine alone in postoperative analgesia. The former produced no side effects and was safer than morphine. In the present study, we investigated the effect of epidural deoxyaconitine on epidural lidocaine anesthesia, and its mechanism of action.

Materials and methods

All experimental procedures were approved and performed in accordance with the guidelines of the Institutional Animal Experimentation Ethics Committee of Shiga University of Medical Science. Five adult, Japanese white rabbits (female, about 4 kg) were obtained and housed individually, with free access to food and water, in standard cages that were maintained with a 12-h light/dark cycle.

Rabbits were anesthetized with a mixture of 3%–5% sevoflurane and oxygen, breathed spontaneously via a mask, and were fixed in a prone position. After removing hair, disinfecting, and administering field block with 1% mepivacaine 0.3 ml, we inserted a lumbar catheter (ϕ, 0.6 × 150 mm) into the epidural space (identified by a loss-of-resistance technique with a 50-mm 19-gauge epidural needle). Sevoflurane anesthesia was maintained throughout this procedure. We assessed all rabbits for paresis after recovery from sevoflurane anesthesia, and rabbits that showed paresis were excluded from the study. To confirm the location of the epidural catheter, 1 ml of 0.5% methylene blue added to 0.5 ml of 2% lidocaine was injected at the end of each experiment. The rabbits were then killed, and the spinal column was dissected and examined.

Deoxyaconitine (Waco Pure Chemical Industries, Osaka, Japan), one of the alkaloids of aconiti tuber, was dissolved in 2% lidocaine to produce a stock solution with a concentration of 1 mg·ml^{-1}. Nor-binaltorphimine (Sigma, St. Louis, MO, USA), a κ-opioid receptor antagonist, was dissolved in saline to produce a stock solution with a concentration of 6 mg·ml^{-1}.

Three hours after the insertion of a lumbar catheter, three different combinations of drugs were injected into the epidural spaces of five rabbits, in the following order: first (combination A), 1.5 ml of 2% lidocaine; second (combination B), 1.5 ml of 2% lidocaine and 150 μg deoxyaconitine; and third (combination C), 3 mg nor-binaltorphimine followed by 1.5 ml of 2% lidocaine and 150 μg deoxyaconitine 30 min later. Each combination was administered more than 2 h after the previous combination: namely, the subsequent combination was started 2 h after the recovery of muscle tone from the previous combination.

To assess the degree of epidural anesthesia, the latency of onset and the duration of three end-points (sensory loss, loss of weight-bearing ability, and flaccid paresis) were measured, as described previously [10]. (i) Nociceptive sensation was assessed using a tail clamp, wherein a rubber-covered surgical clamp was closed over the base of the tail and rotated along its long axis. When any sign of discomfort was noted, the stimulus was terminated. (ii) Loss of weight-bearing ability was noted when the rabbit could no longer spontaneously support its hindquarters, and recovery was readily discernible when the animal was again steady on its hind limbs. (iii) Flaccid paresis was defined as the absence of any discernible tone in either hind limb, and recovery was readily apparent when the animal demonstrated recovery of any muscle tone. Testing of each end-point was assessed at 1-min intervals until onset was identified, and at 5-min intervals thereafter. In every experiment, we monitored blood pressure noninvasively with an automated sphygmomanometer (BP-8800SF; Colin, Hamamatsu, Japan) at 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 min after administration of the drugs.

Statistical analysis

Values for the results were expressed as means ± SD. Statistical analyses were done with repeated measures analysis of variance (ANOVA), followed by the Dunnet test. In all cases, $P < 0.05$ was considered significant. Power analysis performed with an α-error of 5% revealed the β-error to be less than 20%.

Results

The latency of onset was not changed by deoxyaconitine or by nor-binaltorphimine for any of the three end-points. Data are shown in Table 1.

The duration of sensory loss was 27.0 ± 2.7 min in the combination A (control) group ($n = 5$). When 150 μg deoxyaconitine was added (combination B group), the duration was significantly increased, to 49.0 ± 4.2 min. This increase was antagonized, to 33.0 ± 2.7 min, by previous treatment with nor-binaltorphimine (combination C group) (Fig. 1a).

The duration of loss of weight-bearing ability was 33.0 ± 2.7 min in the combination A (control) group ($n = 5$). With deoxyaconitine (combination B group), the duration was significantly increased, to 51.0 ± 2.2 min. This increase was antagonized, to 38.0 ± 2.7 min, by nor-binaltorphimine (combination C group) (Fig. 1b).

The duration of flaccid paresis was 21.0 ± 4.2 min in the combination A (control) group ($n = 5$). With deoxyaconitine (combination B group), the duration was significantly increased, to 35.0 ± 6.1 min. This increase was antagonized, to 24.0 ± 2.2 min, by nor-binaltorphimine (combination C group) (Fig. 1c).

When an animal's leg moved, measurement of blood pressure was difficult. Therefore, the blood pressure was measured only at baseline and while the ancinal was under epidural anesthesia. Blood pressure was decreased by epidural anesthesia in all groups. Systolic blood pressures at 10 min in the combination C group and at 30 min in the combination B group were

Table 1. Onset time after epidural administration of drugs

	Combination A (2% lidocaine only)	Combination B (2% lidocaine with 150 μg deoxyaconitine)	Combination C (nor-binaltorphimine followed by 2% lidocaine with 150 μg deoxyaconitine)
Sensory loss onset (min)	1.0 ± 0.0	1.2 ± 0.4	1.2 ± 0.4
Loss of weight-bearing onset (min)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Flaccid paresis onset (min)	1.6 ± 0.5	1.8 ± 0.4	1.6 ± 0.5

Values are means ± SD ($n = 5$)

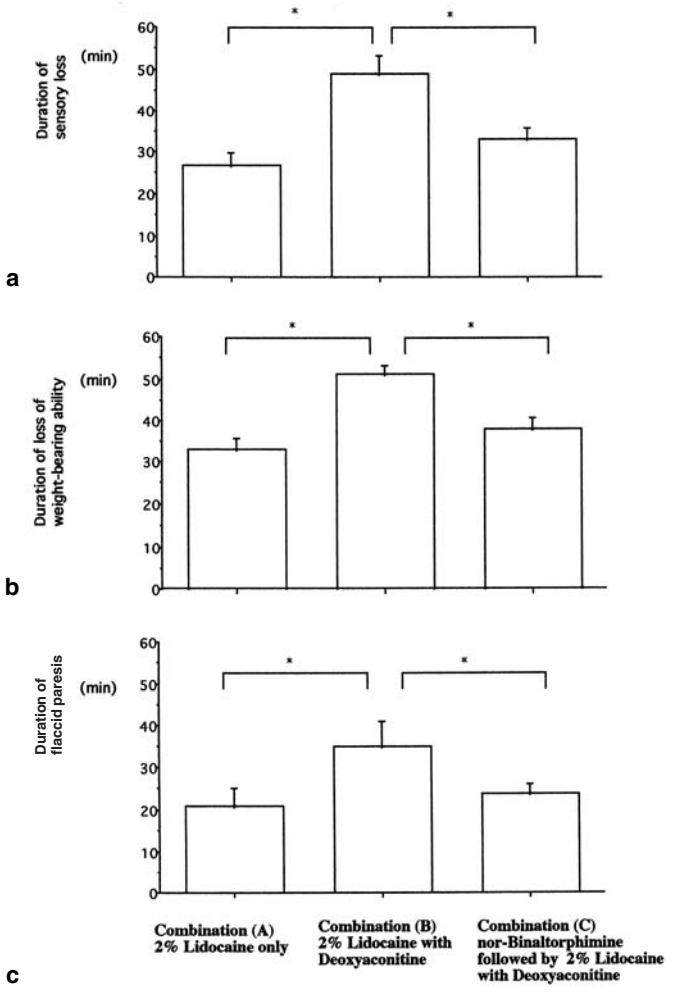

Fig. 1a–c. Duration of sensory loss (**a**), weight-bearing ability (**b**), and flaccid paresis (**c**) after epidural administration of drug. Combination A, 2% lidocaine only; combination B, 2% lidocaine with deoxyaconitine; combination C, nor-binaltorphimine followed by 2% lidocaine with deoxyaconitine. Values are means ± SD ($n = 5$). *Significant differences ($P < 0.05$) between the groups

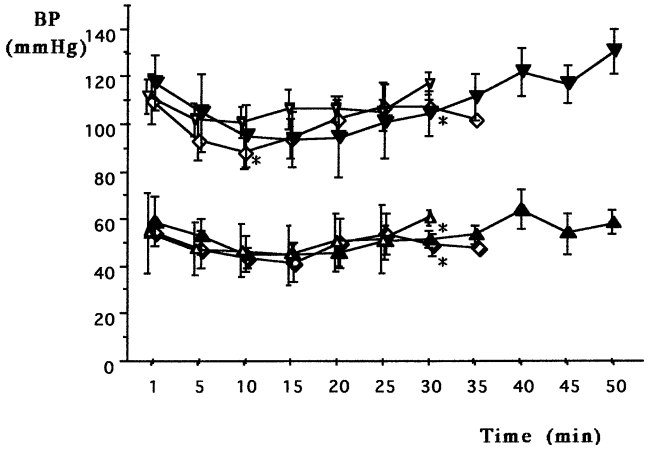

Fig. 2. Time course of blood pressure after epidural administration of drugs. Combination A (*open triangles*); 2% lidocaine only; combination B (*closed triangles*); 2% lidocaine with deoxyaconitine; combination C (*open diamonds*); nor-binaltorphimine followed by 2% lidocaine with deoxyaconitine. Values are means ± SD ($n = 5$). *Significantly different ($P < 0.05$) from combination A

significantly lower than there in the combination A group, and diastolic blood pressure measurements at 30 min in the combination B and combination C groups were also significantly lower than those in the combination A group, but there were no significant differences among the three groups at any other point (Fig. 2).

Discussion

We basically adopted the method of Hughes et al. [10], but in contrast to their single-shot method, we placed a catheter in the epidural space of the rabbit, enabling the drugs to be injected into the same place at the same speed.

We inserted the epidural catheter while the animals were under general anesthesia. The influence of sevoflurane on this experiment was considered to be of little consequence, because recovery from sevoflurane is rapid due to its small blood-gas partition coefficient.

It is reported that oral and intracisternal administration of mesaconitin, another alkaloid extracted from aconiti tuber, induced strong analgesic action in the rat [4]. The analgesic action of intraperitoneal and subcutaneous deoxyaconitine is also known [11]. However,

blood concentrations may exceed toxic levels when these alkaloids are administered in large quantities by an intraperitoneal or subcutaneous route [2,12]. As is the case with morphine, epidural administration of drugs has become popular in clinical practice to achieve satisfactory goals while avoiding systemic side effects. The epidural administration of clonidine, along with its analgesic actions, was reported by Jamali et al. [13] and Eisennach et al. [14]. Enhancement of the analgesic actions of epidurally administered clonidine, when used in combination with local anesthetics, was reported by Mensink et al. [15] and by Carabine et al. [16].

In preliminary experiments, we observed no analgesic action with the epidural administration of 150 μg deoxyaconitine alone. However, with lidocaine 2%, the addition of the same dose of deoxyaconitine extended the time of sensory loss by 80%, the time of loss of weight-bearing by 50%, and that of flaccid paresis by 60% compared with the 2% lidocaine-alone group. This result shows that deoxyaconitine enhanced lidocaine's anesthetic action.

As we did not perform experiments with the repeated administration of lidocaine alone, we can not deny the possibility that repeated lidocaine may have prolonged the duration of the three end-points. But we believe this is not the case, because combination C reversed the duration of the three end-points prolonged by the previously administered combination B. This indicates that the prolongations appeared to be due to deoxyaconitine, and were not due to the cumulative effect of repeated lidocaine.

Blood pressure fell after epidural anesthesia, but there were no differences among the three groups, indicating no effect of blood pressure on the analgesic properties of deoxyaconitine.

The mechanism of the antinociceptive properties of aconiti tuber has not been clarified. Gutser et al. [17] suggested two different mechanisms of antinociceptive action. One is to induce a blockade of neuronal conduction by permanent cell depolarization, the other is to act like local anesthetics.

In our study, deoxyaconitine extended the period of motor paralysis induced by epidural anesthesia with lidocaine. Kimura et al. [1] reported that an alkaloid obtained from aconiti tuber had a neuromuscular blocking action in mice that was not antagonized by neostigmine. This phenomenon may be due to the local anesthetic-like action reported by Gutser et al. [17].

Our results suggest another mode of action of the aconiti tuber alkaloids; that is, via the κ-opioid receptor. In our experiment, the enhancement of epidural anesthesia by deoxyaconitine was partially, but significantly, antagonized by pretreatment with nor-binaltorphimine, a κ-opioid antagonist. This observation is consistent with the findings of a previous study reported by Omiya et al. [3]. They found that the antinociceptive effect of aconiti tuber in mice was antagonized by pretreatment with the κ-opioid antagonist, nor-binaltorphimine, and was abolished by an intrathecal injection of anti-dynorphin antiserum. Their results suggest that the analgesic effect of aconiti tuber was produced via the stimulation of κ-opioid receptors by dynorphin released in the spinal cord.

But, as we know neither the lipid solubility of deoxyaconitine nor its vasoconstricting action, there remains the possibility that the drug might act directly on the brain via elevated blood concentration or that the drug might prolong lidocaine anesthesia by vasoconstriction in the epidural space.

We conclude that deoxyaconitine, an alkaloid obtained from aconiti tuber, enhanced epidural anesthesia with lidocaine in the rabbit, and this effect was probably mediated by κ-opioid receptors.

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