Potentiation of local lignocaine-induced sensory block by calcium channel blockers in rats

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Summary

We have studied the effects of three different types of calcium channel blockers (verapamil, diltiazem, and nicardipine) on local lignocaine sensory block. The standardized tail flick test was used to measure the duration and degree of lignocaine-induced conduction block in rats. After obtaining baseline tail flick latencies (mean 3.2 s), two 100-μl doses of 0.3 % lignocaine alone, a combination of verapamil 25, 100 or 200 μg, diltiazem 25, 100 or 200 μg, or nicardipine 0.5, 1.0 or 2.0 μg, and a large dose of calcium channel blockers (verapamil 200 μg, diltiazem 200 μg or nicardipine 2.0 μg) were injected on opposite sites of the tail base and the tail flick test was performed every 5 min for 45 min. A large dose of the calcium channel blockers showed no prolongation of tail flick latencies. Administration of 0.3 % lignocaine alone produced a significant increase in tail flick thresholds and the peak effect of the percentage maximum possible effect (% MPE) was demonstrated at 5 min after drug injection (mean % MPE 28.8 %; \( P < 0.01 \) vs baseline). Co-administration of 0.3 % lignocaine and three doses of verapamil produced significant increases in area under the curve (AUC) in a dose-dependent fashion. Mean AUC values for 0.3 % lignocaine alone and a combination of verapamil 25, 100 or 200 μg were 217.5, 502.5, 529.1 and 1600.3, respectively. Almost similar patterns of augmentation in AUC values were demonstrated after addition of different doses of diltiazem or nicardipine to 0.3 % lignocaine. We conclude that the use of mixtures of local anaesthetic and calcium channel blocker potentiated lignocaine sensory block at the level of the peripheral nerves. (Br. J. Anaesth. 1996; 77: 243–247).

Key words

Anaesthetics local, lignocaine. Calcium channel block, verapamil. Calcium channel block, diltiazem. Calcium channel block, nicardipine. Rat.

The primary action of local anaesthetics reversibly blocks conduction of nerve impulses by preventing increases in the permeability of the nerve membrane to sodium ions. Calcium movement is essential for normal sensory processing and plays a role in axonal conduction and synaptic transmission. Recently it has been reported that local anaesthetics depress influx of calcium ions at concentrations required to block nerve conduction [1, 2].

Calcium channel blockers block sodium in addition to calcium channels [3] and have local anaesthetic actions [4, 5]. Interaction between calcium channel blockers and local anaesthetic agents is now well documented [6, 7]. We have also reported that intrathecally administered verapamil potentiated the depth and duration of spinal anaesthesia with lignocaine [8]. Therefore, we hypothesized that co-administration of calcium channel blocking agents would increase the analgesic effects of local anaesthetics.

In this study we examined if calcium channel blockers could potentiate and prolong sensory block with lignocaine at peripheral nerves in rats.

Materials and methods

The study was approved by our Animal Care and Use Committee. Male Sprague–Dawley rats, weighing 250–300 g, were used in the experiments. Animals were housed in a temperature-controlled (21 ± 1 °C) room with a 21-h light-dark cycle (lights on 07:00 to 19:00) and given free access to food and water. To reduce the effects of handling on nociceptive responses, all animals were handled and trained in the test situation for at least 3 days before testing. A maximum of three experiments was conducted on each rat with a minimum of 3 days between studies.

nociceptive test

To assess the thermal nociceptive threshold, the standardized tail flick test was performed, using a restrain box, by monitoring latency to withdraw from a heat source (a 50-W projection lamp bulb) focused on a distal segment of the tail (KN-205E, Natsume, Japan). A single control switch simultaneously activated the light and a timer. The time interval between switching on the light to flick of the tail was recorded as tail flick latency. The location on the tail stimulated was varied systematically so that...
the same portion of the tail was not exposed repeatedly to the light source. A 10-s cut-off time was used in the tail flick tests to minimize tissue damage. Mean baseline tail flick latency was 3.2 (2.8–3.9) s in the experiment.

DRUGS AND INJECTION

The drugs used were lignocaine hydrochloride (Fujisawa, Osaka, Japan), verapamil hydrochloride (MW 491.07; Sigma, St Louis, MO, USA), diltiazem hydrochloride (MW 450.99; Tanabe, Osaka, Japan) and nicardipine hydrochloride (MW 515.99; Yamanouchi, Tokyo, Japan). Drugs were freshly dissolved in sterile physiological saline in concentrations that allowed local injection at the root of the tail in 100-μl volumes. Animals were placed in individual plastic cylinders with an opening to allow the tail to protrude. After determining baseline tail flick latencies, 0.3 % lignocaine alone, or combined with verapamil 25, 100 or 200 μg, diltiazem 25, 100 or 200 μg, or nicardipine 0.5, 1.0 or 2.0 μg and verapamil 200 μg, diltiazem 200 μg or nicardipine 2.0 μg alone were injected bilaterally at the base of the tail in 200-μl volumes, in a randomized manner. Using the modified methods of Grant and colleagues [9], two 100-μl of volumes of each drugs were injected directly into the base of the animal’s tail on opposite sides of the midline using a 30-gauge needle attached to a 100-μl syringe (Hamilton, Reno, NV, USA). Accurate bilateral injections were noted to result in slight circumferential swelling of the tail root, as described in an original report. Tail flick latencies were evaluated 5, 10, 15, 20, 30 and 45 min after administration of the drug at the root of the tail.

The pH values of the solutions of 0.3 % lignocaine, calcium channel blockers and mixtures of 0.3 % lignocaine and calcium channel blocker in physiological saline were analysed by an ABL3 (Radiometer, Copenhagen, Denmark). The pH of a 100-μl solution of verapamil, 200 μg, diltiazem 200 μg, nicardipine 2.0 μg, 0.3 % lignocaine-200-μg verapamil, 0.3 % lignocaine-200 μg diltiazem, and 0.3 % lignocaine-2.0 μg nicardipine were 5.81, 5.37, 5.28, 6.25, 6.01 and 5.59, respectively.

CARDIOVASCULAR VARIABLES AND TAIL TEMPERATURE

To examine cardiovascular variables and temperature in the tail, arterial pressure, heart rate and skin temperature at the middle of the tail were measured before and after administration of 0.3 % lignocaine–200 μg verapamil in the four animals in which an arterial catheter had been inserted via the internal carotid artery.

STATISTICAL ANALYSIS

The response for the tail flick test was calculated as percentage maximum possible effects (% MPE): % MPE = (post-drug latency – baseline latency)/(cut-off time – baseline latency) × 100 %. The area under the curve (AUC) of % MPE vs time was calculated using the trapezoidal rule in order to express the overall magnitude and duration of effect for the tail flick test. Differences in % MPE and AUC between groups, and within-group differences between baseline values and those after administration of the drugs, were tested using Friedman’s analysis of variance (ANOVA) by ranks and the Mann-Whitney U test. P < 0.05 was considered statistically significant. All data are expressed as mean (SEM).

Results

Animals were able to move their tail at random throughout the experiments. Lignocaine 0.3 % alone, administered at the base of the tail, produced
Calcium channel blockers and local lignocaine sensory block

Significant prolongation of tail flick latency. The peak effect was observed 5 min after drug administration. A large dose of calcium channel blocker (verapamil 200 μg, diltiazem 200 μg or nicardipine 2.0 μg) alone did not produce prolongation of tail flick latency compared with each pre-drug value.

In contrast, co-administration of lignocaine and the three different doses of verapamil produced a dose-dependent increase in tail flick latencies and the duration of sensory block increased with increasing concentration of verapamil compared with lignocaine alone (fig. 1).

A similar pattern of augmentation in % MPE and AUC after addition of different doses of diltiazem or nicardipine to 0.3 % lignocaine resulted in an increase in % MPE and AUC, which were approximately dose-dependent increases in the tail flick test (figs. 2–4).

There were no significant changes in mean arterial pressure, heart rate or tail temperature after local injection of the drugs at the base of the tail (data not shown).

Discussion

We have demonstrated that local sensory block of lignocaine was potentiated and prolonged by concomitantly administered L-type calcium channel blockers (which alone exert no influence on local sensory block) in a dose- and time-related manner.

Local anaesthetics such as lignocaine, procaine, amylcaine or cinchocaine have been shown to inhibit calcium uptake and the calcium ion-activated ATPase activity of sarcoplasmic reticulum vesicles [10–12]. This ability of local anaesthetic agents to inhibit calcium efflux from the sarcoplasmic reticulum was explained by incorporation of these amphophilic drugs into the lipid layer [13, 14], interaction with calcium ion binding sites on membrane phospholipids, or direct interaction with a calcium channel [15, 16].

Calcium channel blockers block sodium channels in addition to calcium channels in a dose-dependent manner and have local anaesthetic actions. Kraynack, Lawson and Gintautas demonstrated that a blocking action of the fast sodium channel by verapamil was almost equal to that of procaine, although the mode of pharmacological action of these drugs was different. It has also been demonstrated that simultaneous administration of an amide local anaesthetic agent and the calcium channel blocker, verapamil, has more marked cardiovascular depressant effect than local anaesthetic alone [6, 7]. In this study, a large dose of calcium channel blocker alone, administered into the base of the tail, produced no significant antinociceptive effects. It is therefore

![Figure 3](image1.png)

Figure 3  Changes in percentage maximum possible effect (% MPE) after injection of 0.3 % lignocaine (∆, n = 8), nicardipine 2.0 μg (∆, n = 5) and a mixture of 0.3 % lignocaine and nicardipine 2.0 μg (●, n = 7), 1.0 μg (●, n = 5) or 0.5 μg (▲, n = 5). Data are mean, SEM. *P < 0.05, **P < 0.01 compared with control (C) (pre-injection) value.

![Figure 4](image2.png)

Figure 4  Area under the curve (AUC) of % MPE x time (0–45 min) after injection of 0.3 % lignocaine alone, and combined with verapamil 25, 100 or 200 μg, diltiazem 25, 100 or 200 μg or nicardipine 0.5, 1.0 or 2.0 μg. Data are mean, SEM. *P < 0.05, **P < 0.01 compared with 0.3 % lignocaine alone.
unlikely that the local anaesthetic activity of calcium channel blocking agents accounts for the increase in tail flick latency observed in the study, although little is known of the specific mechanism of augmentation of lignocaine sensory block by calcium channel blockers.

Several investigators have used *in vivo* animal models to evaluate local anaesthetic-induced conduction block. However, some of these studies have assessed sensory loss using non-quantitative methods. Analgesia tests in the rat using thermal stimulation such as tail flick or hot plate have been used for many years. These tests are useful for evaluation of systemic or neuroaxial analgesics but have limited use for local anaesthetics because they cannot clearly differentiate between motor and sensory block. A recent report has indicated that local anaesthetic injection directly into the base of the tail is a simple and reliable *in vivo* laboratory method using the standard tail flick test for comparing sensory block produced by different local anaesthetics [9]. These injections produce no motor block as tail motion is controlled by ligaments originating in muscles proximal to the level of the injection sites. Thus, the model enabled us to assess independently local sensory block produced by the injected drugs. In our study, all animals were able to move their tail at random after injection of the drugs.

It has been known widely that systemic administration of calcium channel blocker causes vasodilatation and cardiac depression, which result in hypotension and bradycardia [17]. No significant circulatory changes were observed in that study. Another possibility that could explain the observed effects would be a pharmacokinetic interaction between the drug solutions, such as changes in the pH of the tissue of the injection sites. Concomitant administration of lignocaine and calcium channel blockers might produce pharmacodynamic changes in tail temperature or regional blood flow. It is also not enough to explain the fact that local lignocaine sensory block was potentiated by co-administration of large dose of calcium channel blockers alone did not produce a significant change in tail flick latency, as described above.

In this study we used three different calcium channel blockers with different structures: d-phenylalkylamine (verapamil), benzoiazepine (diltiazem) and dihydropyridine (nicardipine). ID50 values for maximum amplitude of the calcium ion in tail flick latency observed in the study, although little is known of the specific mechanism of augmentation of lignocaine sensory block by calcium channel blockers. In conclusion, we have demonstrated that co-administration of a calcium channel blocker potentiated the degree and duration of local lignocaine sensory block. Most local anaesthetic drugs can be used for peripheral nerve block and the choice of drug is determined primarily by the required duration of anaesthesia. The duration of sensory analgesia is prolonged significantly when vasoconstrictors, usually adrenaline, are frequently added to local anaesthetic solutions. However, addition of a vasoconstrictor may cause cardiovascular disturbances, including cardiac arrhythmia or hypertension. The use of mixtures of local anaesthetic and calcium channel blocker may offer clinical advantages, with peripheral nerve block for chronic pain management. However, more study is required before co-administration of lignocaine and calcium channel blockers can be considered for clinical use.

References
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