IN VIVO DETERMINATION OF CONCENTRATION-EFFECT CURVES OF LOCAL ANAESTHETICS IN MAN

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SUMMARY

In clinical use, the concentrations of local anaesthetics at the site of action are unknown. With the method described here, concentrations of local anaesthetics can be predetermined and kept constant at the site of action. In six volunteers, a blister was raised on the ventral surface of the forearm. After removal of the epithelium, the blister base was rinsed continuously with carbogenated Tyrode's solution with and without increasing concentrations of bupivacaine (Carbostesin) for 15 min each. The effects of bupivacaine were determined by changes in the perception (tactile sensation) of drops falling on the blister base from increasing heights. The minimal height at which the drops were just perceived characterized the threshold of perception. With increasing bupivacaine concentrations, threshold increased until the drops were no longer perceived, at a median concentration of 2.48 mmol litre⁻¹ (range 1.24-3.10 mmol litre⁻¹). After the blister base was rinsed with Tyrode's solution, threshold of perception reached baseline values, which was in accordance with an intact blister base. (Br. J. Anaesth. 1993; 70: 552-555)

KEY WORDS
Anaesthetics, local. Pharmacodynamics: threshold concentration.

The concentrations of local anaesthetics at the site of action are unknown in clinical use [1]. Thus concentration-effect curves have been derived exclusively in animals by dissecting and bathing isolated nerves [2]. The present experiments aimed to develop a simple method in man by which concentrations of local anaesthetics could be predetermined and kept constant at the site of action. The method described here fulfilled these criteria and allowed us to test the sensitivity of neural structures to local anaesthetics in man.

SUBJECTS AND METHODS

After approval by the local Ethics Committee and informed consent had been obtained, we studied six healthy male volunteers (aged 26-30 yr) in the early afternoon. Room temperature was kept constant at 20-22 °C.

A blister (0.5 cm diameter) was raised on the ventral surface of the forearm using an appropriate suction device and water at 38-40 °C [3]. After the blister base was uncovered under sterile conditions, it was sealed in an open chamber (fig. 1) and rinsed continuously with carbogenated Tyrode's solution (flow 1.5 ml min⁻¹) with and without increasing concentrations of bupivacaine (Carbostesin). The solutions used were warmed to keep the blister base temperature within ±2 °C of the surrounding skin temperature as measured by infra-red telethermometry. The pH of the solutions was 7.3-7.5. The effects of bupivacaine were determined by means of changes in the perception of drops of equal size falling on the blister base from increasing heights. The minimal height at which the drops were just perceived characterized the perception threshold and was quantified by a millimetre scale connected with a support. The perception threshold was evaluated by the method of limits [4, 5]—that is, as the mean intensity of the last stimulus less than and the first greater than threshold.

After the blister base had been rinsed continuously with Tyrode's solution for 15 min, the perception threshold was evaluated in each volunteer (baseline). For this purpose, drops of the same composition were released on the blister base from increasing heights. The volunteers were asked to report if the drops touching the blister base were perceived or not. In the same way, the effect of each drug concentration was determined.

Fig. 1. Schematic diagram of the blister base surrounded by an open chamber. By appropriate arrangement of the inflow and outflow cannula, the blister base was covered by a liquid phase of about 1 mm thickness. h = Height.
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Fig. 2. A typical experimental programme. Bupivacaine concentrations were increased by 0.62 mmol and the corresponding perception thresholds (release height) evaluated (upper graph). The local anaesthetic effect of each concentration was expressed by the percent changes of perception threshold, the baseline threshold (Tyrode's solution; 0.6 cm) being set arbitrarily at 0, the threshold at maximal blocking effect (4.5 cm) at 100. Thus the blocking effects are expressed in percent of the maximal effect (relative blocking effect) (lower graph).

Starting with the smallest bupivacaine concentration (0.62 mmol litre\(^{-1}\)), the concentrations were increased stepwise. Each concentration was maintained until perception threshold repeatedly remained constant, thus demonstrating an equilibrium between the concentration at the site of action with that of the bathing solution. As the local anaesthetic effect reached a plateau within 2 min after administration, periods of 15 min for each concentration seemed to be adequate. The smallest bupivacaine concentration above which threshold remained constant was defined as the minimal blocking concentration. This definition relies on the premise that drops released from greater heights would be perceived via mechanoreceptors outside the blister base. To compare the perception threshold at the end of the experiment with the baseline value, the blister base was rinsed with Tyrode's solution again.

In one volunteer a wedge-shaped piece of the blister base and the surrounding skin was excised. It was examined to see if the epithelium was disconnected from the underlying tissues. The material was fixed by immersion in 1.25% glutaraldehyde and 1% paraformaldehyde in phosphate buffer 0.1 mol litre\(^{-1}\) at pH 7.4. Dehydration was by passage through the ascending acetone series, and the block was embedded in Spurr's medium. Semithin sections were stained with toluidine-blue.

Data analysis

Each threshold was determined four times and mean values calculated. The local anaesthetic effect was expressed by relating the percent changes in perception threshold against drug concentrations, the baseline threshold (Tyrode's solution) being arbitrarily set at 0, the threshold at maximal blocking effect at 100.

RESULTS

The results of a typical experiment are illustrated in figure 2. While the blister base was rinsed with Tyrode's solution, drops released from a height above 0.6 cm were just perceived, so that the perception threshold was 0.6 cm (upper graph). Note that the perception threshold increased as the bupivacaine concentrations increased; that is, to evoke the same minimal perception the release height had to be increased. As the perception threshold (4.5 cm) did not change with large concentrations (15.18 mmol litre\(^{-1}\)), the smallest concentration with the same threshold was defined as minimal blocking concentration (2.48 mmol litre\(^{-1}\)). At the end of the experiment, the blister base was rinsed with Tyrode's solution again, the threshold of perception being identical with baseline (0.6 cm). By relating blocking concentration of bupivacaine (X-axis) against drug effect expressed as percent of maximal

Fig. 3. Concentration-effect curves of bupivacaine derived from the blister base in man at pH 7.4 and normal skin temperature (right). The blocking concentrations for various degrees of blocking effect (75%, 50%, 25%) were interpolated, as in the experiments on the isolated aortic nerve of cats (pH 7.4, 38 °C [2]) depicted on the left for comparison. X-axis: blocking concentration (logarithmic scale); Y-axis: relative blocking effect (100 = threshold at maximal blocking effect). The concentration-effect curves are similar, but the blocking concentrations in the blister base are 45-fold greater than in the isolated nerve preparation.
Absence of morphological damage and identical heat trauma should be avoided, which could sensitize exposure time was 15 min to ensure drug equivalence was reached within 2 (block)-3 (recovery) min, for each drug concentration, a concentration equilibrium was established within 2 min. After the blister base was rinsed with Tyrode’s solution, in each volunteer at the end of the experiments, perception threshold reached baseline values within 3 min. In five volunteers, the wound healed by primary intention and in one it healed by secondary intention with scar formation.

Histological examination of one blister periphery revealed that the cover of the blister consisted of epithelial layer exclusively, while the blister base was intact dermis.

**DISCUSSION**

The validity of our method rests on the assumption that the bupivacaine concentration in the bathing solution equals that at its site of action. Three observations support this assumption: (1) The diffusion distance between blister base and site of action was very small, as evident from anatomical and pharmacological findings. In the cantharides blister [6], the epithelium as a diffusion barrier was removed completely by uncovering the blister base. Thus mechanoreceptors sensing tactile stimuli, located at the tip of intermediate epithelial ridges and in the papillary ridges of the dermis [7,8], are located within 100 μm from the blister base [7]. Also, the rapid establishment of plateau effects within 2–3 min (block and recovery from block, respectively) suggests diffusion distances less than 200 μm [1,2]. That the blocking effect of bupivacaine was abolished within 3 min after the blister base was rinsed with Tyrode’s solution is in accordance with earlier findings in isolated nerves [2]. The absence of an epithelial barrier and perineural binding structures may explain this rapid recovery. (2) Short diffusion distances guarantee concentration equilibrium only when more drug molecules are transported to the site of action than diffuse away. Even with maximal blood flow (8000 ml min⁻¹; [9]) in human skin (surface area 1.7 m²), blood flow under the blister base (0.2 cm²) is thought to be 0.1 ml min⁻¹, only a fraction of the 1.5 ml min⁻¹ used in our experiments. Consequently, blister base and underlying tissues are provided with bupivacaine in abundance. (3) Although the maximal bupivacaine effect was reached within 2 (block)-3 (recovery) min, exposure time was 15 min to ensure drug equilibrium.

During generation of the blister, care was taken to keep the water temperature at 38–40 °C, as painful heat trauma should be avoided, which could sensitize and thus decrease the threshold of perception [10]. Absence of morphological damage and identical perception thresholds with Tyrode’s solution before and after the blistering procedure suggest integrity of the blister base. In an effort to rank the results of the blister base method, we have compared our data with those determined in a “classical” manner—that is, in animals, by dissecting and bathing isolated nerves. As the information sensed by mechanoreceptors travels along A-fibres (conduction velocity 40–80 m s⁻¹ [8]), blocking experiments in the aortic nerve of cats (A-fibres, mainly A-δ-fibres) were viewed for comparison. In these experiments [2], concentration-effect curves and minimal blocking concentrations (see left part of figure 3) were measured by bathing the nerve in increasing concentrations of bupivacaine (pH 7.4, 38 °C) and simultaneous recording of nerve activity. Surprisingly, the curves are similar in shape, and slope. Obviously, the same alterations in concentration induce the same alterations in effect characterizing similar drug-“receptor” interactions of the two preparations [11]. Also, interindividual variability does not differ, although one volunteer seemed to be conspicuously sensitive.

In contrast, however, the blocking concentrations derived from the present experiments were 45-fold greater than the minimal blocking concentrations in the aortic nerve of cats (fig. 3). Also, in experiments performed in isolated perfused vein segments of humans, the blocking concentrations were greater than in the isolated perfused nerve of the cat, by a factor of 30 [12]. There are two possible explanations for this difference:

(1) Axons are blocked, as probably occurs clinically. In this case, great blocking concentrations may be necessary because of short exposure lengths (less than three nodes of Ranvier). This assumption is based on recent experiments in isolated frog sciatic nerves [13], in which an inverse relation between blocking length and local anaesthetic concentration was found; that is, the smaller the exposure length, the greater the blocking concentration. As the nerves supplying the mechanoreceptors in the skin run perpendicularly up to the epithelium [7] and these receptors are located close to the blister base; the depth of local anaesthetic penetration into the dermis equals the exposure length of axons.

(2) Mechanoreceptors located close to the blister base and thus easily accessible for bupivacaine are blocked. As receptors are said to be less sensitive to local anaesthetics than nerve fibres (see figure 5 in [14]), they are blocked only with large concentrations. Receptor block does not seem to be involved in local anaesthesia under clinical circumstances.

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**REFERENCES**