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EXPERIMENTAL / LABORATORY STUDIES

Reversible Conduction Block in Frog Sciatic Nerve for Three Different Concentrations of Bupivacaine

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Abstract: We examined the effects of various concentrations of the bupivacaine commonly used for spinal anaesthesia on the reversibility of conduction block in isolated frog sciatic nerves measured by the extracellular recording technique. Seventy-two isolated nerves were divided into 3 groups (n = 24), each of which was bathed in a different bupivacaine solution in a range of concentrations (10, 20 or 30 mM for 20 min). In each group, the extracellular action potentials were recorded before exposure to the bupivacaine solution to provide the control data. The extracellular action potentials were recorded after 20 min exposure to the drug by using a BIOPAC MP 100 acquisition system version 3.5.7 (Santa Barbara, USA). The nerves were washed continuously for 3 h with Ringer's solution and action potentials were recorded. The nerves were then soaked overnight at room temperature in Ringer's solution and tested for impulse recovery. The data were analysed with repeated-measures analysis of variance using SPSS 9.05 for Windows. In the presence of 10 mM, 20 mM or 30 mM bupivacaine, the extracellular action potential amplitude decreased by 23.21 ± 12.42%, 28.42 ± 17.51% and 39.45 ± 22.16%, respectively, relative to the control amplitude ($P < 0.05$); it recovered to 89.21 ± 50.00%, 66.43 ± 30.10% and 47.12 ± 37.51% ($P < 0.05$), respectively, after 3-h of wash, and reached 110.31 ± 50.13%, 90.60 ± 43.21% and 130.43 ± 56.32% ($P < 0.05$), respectively, after the overnight soaking process. This study showed that exposing the nerve to high concentrations of bupivacaine causes an reversible impulse blockade and that bupivacaine does not have neurotoxic effects on isolated frog sciatic nerves.

Key Words: Compound action potential, bupivacaine, recovery, cauda equina syndrome

Introduction

Local anaesthetics block the propagation of nerve impulses by binding to receptors on the Na channel and preventing normal functioning (1). This binding appears to involve a single local anaesthetic molecule (2) and to be based on the concentration of local anaesthetic required to bring about 50% inhibition of the Na^+ current.

Bupivacaine hydrochloride (amide local anaesthetic) is the most frequently used local anaesthetic for preoperative and postoperative pain relief in many countries. It is a potent agent capable of producing prolonged anaesthesia. Its long duration of effectiveness plus its tendency to provide more sensory than motor blocking has made it a popular drug for providing prolonged anaesthesia during labour or the postoperative period (3).

Direct exposure of the cauda equina to high concentrations of local anaesthetics during continuous spinal and epidural anaesthesia may have caused the recently reported cases of cauda equina syndrome (4-6).

Since anaesthesiologists cannot predict how cerebrospinal fluid will dilute administered drugs, the appropriate modification to ensure safe practice would be to give concentrations of drugs that in themselves never exceed a safe concentration. It is therefore important to identify a concentration below which the nerve is not irreversibly affected. Thus we sought the effects of various concentrations of the bupivacaine commonly used for spinal anaesthesia on the reversibility of conduction block in isolated frog sciatic nerves.

Materials and Methods

Tissue preparation

Seventy-two *Rana cameroni* frogs weighing 30-40 g were used in the experiments. The sciatic nerves were excised from rapidly decapitated and pithed frogs and maintained in Ringer's solution. This solution was composed of 111.87 mM NaCl, 2.47 mM KCl, 1.08 mM $CaCl_2$ and 2.38 mM $NaHCO_3$. The isolated nerves (n = 72) were randomly divided into 3 groups (n = 24 in each group): group I; 10 mM (0.32% bupivacaine solution), group II; 20 mM (0.65% bupivacaine solution) and group III; 30 mM (0.97% bupivacaine solution). The pH of the Ringer's solution was adjusted to 7.2 and all measurements were recorded with the preparations equilibrated at room temperature (21-23 °C). The bupivacaine hydrochloride used in this experiment was purchased from Sigma Chemical (B-5274). The drug was dissolved in frog Ringer's solution.

Electrophysiological techniques

The experiments were carried out in vitro using extracellular recording techniques (7,8). After 30 min of stabilisation in Ringer's solution, segments of nerve measuring 3-4 cm were placed in a 5 cm x 15 cm Plexiglas nerve chamber containing Ag/AgCl electrodes. The electrodes were 0.5 cm apart. The stimulating voltage was set to produce a maximal compound action potential using single square pulses of supra-maximal strength and 0.5 ms in duration.

Experimental protocols

After the compound nerve action potential (CNAP) had stabilised in Ringer's solution, CNAPs were recorded using a BIOPAC MP100 acquisition system version 3.5.7 (Santa Barbara, USA) from each nerve before exposure to 0.32%, 0.65% or 0.97% bupivacaine solution and these data were treated as the control (experiment I). Each nerve was bathed for 20 min in the bupivacaine solution for each group. After the 20-min drug exposure, CNAPs were recorded (experiment II). Then the nerves were washed (exposed to the drug) continuously with Ringer's solution for 3 h and CNAPs were again recorded (experiment III). The nerves were then removed from the chamber and soaked overnight at room temperature in 100 ml Ringer's solution. The next morning (24-h after the drug exposure), the nerves were replaced in the nerve chamber and were tested for impulse recovery (experiment IV).

Statistical analysis

The same subject groups were observed 4 times using the repeated-measures design. In this design, each subject serves as its own control. After testing normal distribution with the Kolmogorov-Smirnov method, the data were analysed with the repeated-measure analysis of variance by using SPSS 9.05 for Windows. Least significant difference (LSD) was used for post hoc tests. The significance was set at $P < 0.05$.

Results

The amplitude of a given CNAP was defined as the height in millivolts from the peak of positive phase to peak of the negative phase. In the presence of bupivacaine the CNAP amplitude decreased, relative to the control amplitude ($P < 0.05$), but we observed recovery of the CNAP after 3- and 24-h washes in anaesthetic-free Ringer's solution in all groups ($P < 0.05$). In nerves exposed to 10 mM, 20 mM or 30 mM bupivacaine hydrochloride, the extracellular action potential amplitude decreased by 23.21 ± 12.42%, 28.42 ± 17.51% or 39.45 ± 22.16% respectively, relative to the control amplitude ($P < 0.05$). It then recovered to 89.21 ± 50.00%, 66.43 ± 30.10% or 47.12 ± 37.51% ($P < 0.05$), respectively, after 3 h of wash, and reached 110.31 ± 50.13%, 90.60 ± 43.21% or 130.43 ± 56.32% ($P < 0.05$), respectively, after the overnight soak process. (Table). The effects of 10 mM and 30 mM concentrations of bupivacaine applied extracellularly to sciatic nerve action potentials are shown in Figure 1. As seen in this figure, the amplitude of action potential was depressed in these concentrations, but after 3- and 24-h washes the nerves recovered to the control values. Figures 2 and 3 show the mean recovery of the CNAP for different concentrations of bupivacaine after 3- and 24-h washes.

Discussion

This study confirms that the CNAP recorded in the frog sciatic nerve is reversibly eliminated by 20 min exposure to 3 different concentrations of bupivacaine. This reversible inhibition is due to the fact that local anaesthetics block the propagation of nerve impulses by binding to receptors on the sodium channel and preventing normal functioning (1).

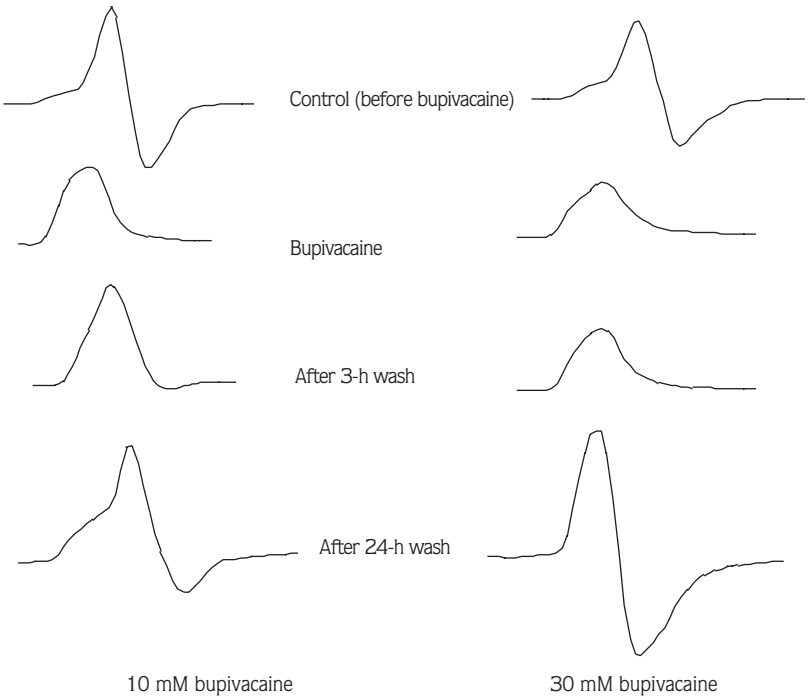

Figure 1. Effects of 10 mM (a) and 30 mM (b) bupivacaine on CNAP and recovery of CNAP after 3-h and 24-h washes

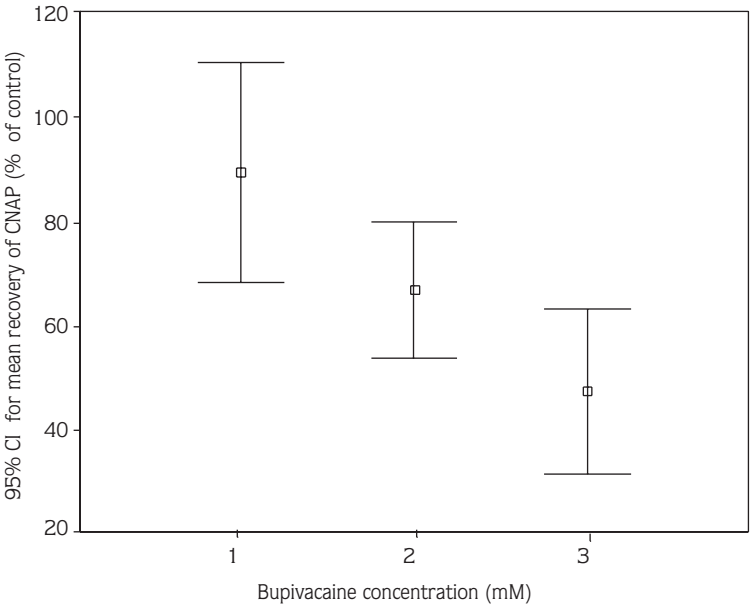

Figure 2. Mean recovery of CNAP after 3-h wash with Ringer's solution for different concentrations of bupivacaine applications. (CI: confidence interval)

Bupivacaine is the most frequently used drug for spinal anaesthesia. In reported cases of cauda equina syndrome after continuous spinal anaesthesia it was suggested that highly concentrated local anaesthetics were responsible (4,5). Studies of spinal canal models show that slow injection through misdirected intrathecal

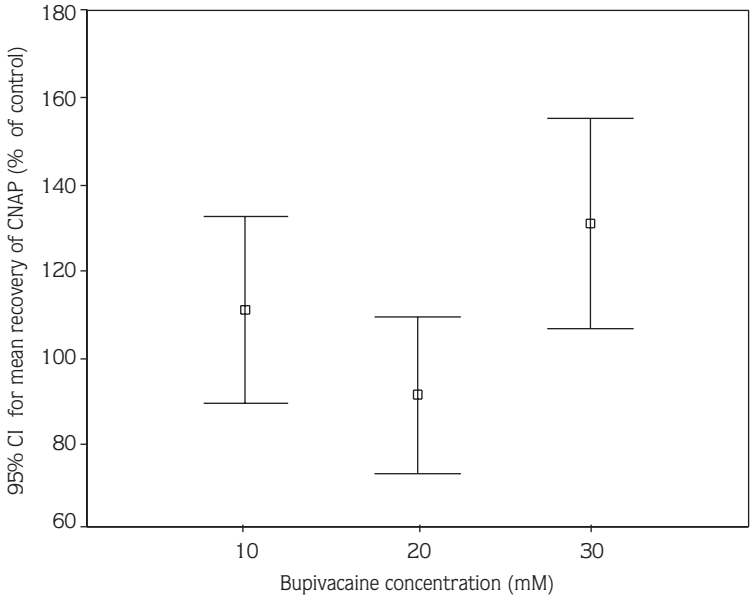

Figure 3. Mean recovery of CNAP after 24-h wash with Ringer's solution for different concentrations of bupivacaine applications. (CI: confidence interval)

catheters may cause nonhomogenous mixing of local anaesthetic with the cerebrospinal fluid (9-11). As a result, the relatively unprotected nerve fibres of the cauda equina may be unintentionally and directly exposed to a high concentration of local anaesthetic.

Bupivacaine is commonly used in 0.5-0.75% concentrations for spinal anaesthesia (12). It has been reported that bupivacaine solution at 0.75% concentration causes partially reversible conduction block (12). Based on this finding, Lambert et al. suggested that bupivacaine could not cause cauda equina syndrome caused by irreversible conduction block.

It is not known whether the effect of bupivacaine on neural substrates at higher concentrations is reversible. To answer this question, we chose a higher dose than that used in clinics as the upper limit in our study.

The present study did not evaluate the potential neurotoxicity of such high concentrations of bupivacaine. Lambert et al. (12) found that nerves exposed to 0.75% bupivacaine for 15 min recovered to nearly 76 ± 3% after 3-h of wash and 44 ± 8% after soaking overnight. In our study, we used a higher bupivacaine dose and a longer exposure than Lambert et al. However, we found that highly concentrated bupivacaine has no toxic effect on frog sciatic nerves, since nerves exposed to 0.97% bupivacaine for 20 min recovered approximately 50% impulse activity during the 3-h wash and nearly completely during the 24-h wash.

In present study, the nerves exposed to 0.32-0.97% bupivacaine showed an apparently greater recovery after the 24-h wash than the control nerves (Table). In addition, Lambert et al. (12) found that nerves exposed

Table. Percentage of CNAP amplitude decrease relative to the control amplitude in the presence of bupivacaine and the recovery of CNAP after 3-h and 24-h washes.

Dose (mM)	Decrease in CNAP amplitude in the presence of bupivacaine	Recovery of CNAP after 3-h wash	Recovery of CNAP after 24-h wash
10	23.21 ± 12.42	89.21 ± 50.00	110.31 ± 50.13
20	28.42 ± 17.51	66.43 ± 30.10	90.60 ± 43.21
30	39.45 ± 22.16	47.12 ± 37.51	130.43 ± 56.32

to 0.06% tetracaine exhibited an apparently greater recovery than the control nerves. This observed greater recovery than in the control nerves remains unexplained.

In conclusion, this study shows that exposing frog myelinated sciatic nerves to high concentrations of bupivacaine causes an reversible impulse blockade and that bupivacaine does not have any neurotoxic effects on isolated frog sciatic nerves.

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