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The myotoxicity of bupivacaine, a ^{31}P n.m.r. investigation

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1 Phosphorus nuclear magnetic resonance (n.m.r.) spectroscopy is a recently introduced method for the non-invasive study of muscle biochemistry.

2 It was shown in the rat that an intramuscular injection of the drug resulted in degeneration that was reflected spectroscopically in a progressive decrease in the intracellular concentrations of phosphocreatine and adenosine triphosphate without a corresponding rise in that of inorganic phosphate. Furthermore, ATP was depleted in the presence of significant levels of phosphocreatine. As regeneration occurred spectra returned to normal and this was complete by day 10.

3 No such spectroscopic effects were demonstrated in man following the use of bupivacaine in an intravenous regional anaesthetic.

4 It is suggested that further studies in man are required before this drug can be administered intramuscularly with confidence.

Introduction

Bupivacaine (Marcain; Duncan, Flockhart and Co. Ltd., London) is an important local anaesthetic that is widely used for epidural anaesthesia, peripheral nerve blocks, intravenous regional anaesthesia and other pain-relieving procedures. It is firmly tissue-bound and this property confers both a long duration of action and low systemic toxicity. However, there is evidence that all local anaesthetics, but especially bupivacaine, is myotoxic – a side effect that has attracted remarkably little attention.

The first demonstration of this myotoxicity was in rats (Sokall, Sonesson & Thesleff, 1968) but since then the process has been confirmed in a variety of animals (Benoit & Belt, 1970; Jirmanová & Thesleff, 1972; Hall-Craggs, 1974; Schultz & Lipton, 1978; Foster & Carlson, 1980). Similar work in man has been impeded by the difficulty of constructing a study that both meets ethical requirements and yet allows the intramuscular injection of local anaesthetic followed by repeated muscle biopsies.

Within the last two years the technique of high resolution phosphorus nuclear magnetic resonance spectroscopy (^{31}P n.m.r.) has been introduced for the non-invasive measurement of the intracellular concentrations of the phosphate-containing metabolites in human muscle. It is based upon the interaction between phosphorus nuclei within tissues when placed in a magnetic field and radio-frequency energy. Signals (resonances) that reflect the intramyocellular levels of phosphocreatine, adenosine triphosphate (ATP) and inorganic phosphate can be recorded painlessly within a few minutes. Furthermore, the intracellular pH can be derived from the spectrum. (For reviews of this technique in biological research see Radda & Seeley, 1979; Gadian & Radda, 1981. For clinical applications see Ross, Radda, Gadian, Rocker, Esiri & Falconer-Smith, 1981; Newman, Bore, Chan, Gadian, Styles, Taylor & Radda, 1982).

This paper describes the use of ^{31}P n.m.r. to investigate non-invasively the myotoxicity of bupivacaine in animals and man.

Methods

Animal studies

Experiments were performed on 20 male Sprague Dawley rats (200–250 g body weight) which were anaesthetized with pentobarbitone sodium B.P. Vet. (Sagatal; May and Baker, Dagenham) 45 mg kg^{-1}, injected intraperitoneally; 3 ml 0.5% plain bupivacaine (pH 5.6) was injected into the calf muscle of one hind limb and an equal volume of normal saline (adjusted to the same pH) was injected into the

contralateral side. The animals were then randomly divided into two groups of 10.

N.m.r. spectra were recorded from both calves of each animal in one group under general anaesthesia in a TMR-32 spectrometer (Oxford Research Systems) on days 1, 2, 3, 5, and 10 following the injection. The instrument incorporated a 1.89 Tesla superconducting magnet with a horizontal bore of 20 cm. During the recording of spectra the rat was secured in the probe in supine position with the hind limb extended and an oval two turn surface coil, 25 × 15 mm diameter and wound from 1 mm diameter insulated copper wire, placed beneath the calf to detect signals.

On each occasion that spectra were recorded, two animals in the second group were killed by cervical dislocation and the calf muscles of both hind limbs quickly excised. Specimens were prepared from the middle third of the muscle belly and fixed in neutral 10% formalin. Following dehydration and embedding in paraffin wax 5 μm-thick transverse and longitudinal sections were cut and stained with haematoxylin and eosin.

Human studies

Following approval by the hospital ethics committee, four freely consenting patients underwent surgery under intravenous regional anaesthesia using 30 ml plain 0.5% bupivacaine. Their clinical details are shown in Table 1. Twenty-four to thirty hours later ^{31}P n.m.r. studies were performed on both upper limbs in the spectrometer previously described. A two turn circular 4 cm diameter surface coil wound from insulated copper wire was placed over the proximal third of the forearm flexor musculature to record signals.

Results

Animal studies

During the first 3 days following the injection of bupivacaine the muscles were pale and oedematous but thereafter their gross appearance was normal. Conversely, the muscles excised from the saline-treated limbs were macroscopically normal throughout the experiment.

Histological examination of the bupivacaine-treated muscle demonstrated a rapid process of degeneration and regeneration. Within 24 h a small cell infiltrate with a polymorphonuclear leukocyte predominance was seen surrounding the shrunken and rounded muscle fibres which were separated by oedema. By day 2, macrophages formed a larger proportion of the inflammatory infiltrate and muscle degeneration was characterized by fragmentation of fibres and loss of clarity of the cross striations. Many fibres displayed central nuclei. Examination the following day revealed the infiltration to be intense and normal architecture was destroyed.

Regeneration was apparent on day 5 when distinct areas of basophilic cytoplasm containing chains of peripheral nuclei were identified. Their diameter had increased (though not to normal size) by day 10 and the small cell infiltration almost resolved.

Muscles excised from the saline-treated limbs displayed no histological changes of degeneration throughout the study.

The n.m.r. spectroscopy performed in parallel with the above histology demonstrated changes in the levels of the intracellular phosphate-containing compounds that were compatible with muscle death followed by regeneration. Furthermore the time course of these changes closely followed that of the histology.

Table 1 Clinical details of patients

Patient	*Age (years)*	*Sex*	*Operation*	*Side*	*Tourniquet time* (min)
1	49	M	Excision dorsal wrist ganglion	Right	25
2	30	F	Excision dorsal wrist ganglion	Left	25
3	41	F	Carpal tunnel decompression	Left	30
4	22	M	Excision dorsal wrist ganglion	Right	35

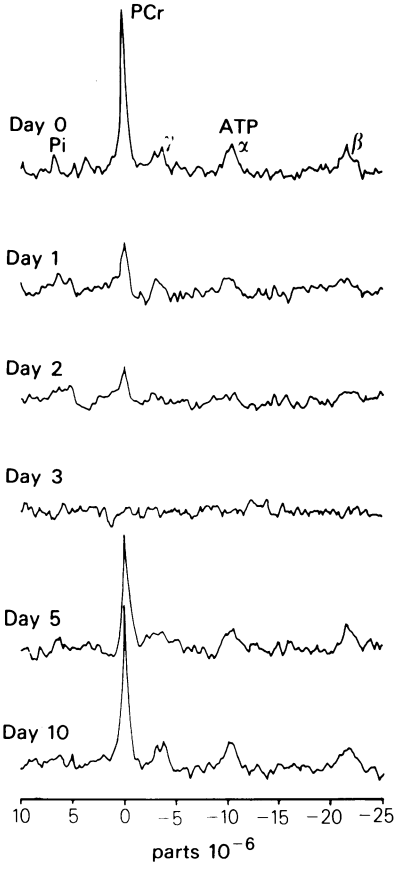

Figure 1 ^{31}P n.m.r. spectra recorded from the calf muscles of a rat before and after the intramuscular injection of bupivacaine. Each spectrum represents the sum of 128 scans recorded using 20 μs radiofrequency pulses of 32.5 MHz at 2 s intervals. Chemical shifts defined as positive in the high-frequency direction with phosphocreatine chosen as the internal chemical shift standard. Broad components of the spectrum eliminated by a line broadening of 12 Hz. The spectra recorded from the control limb remained normal throughout the experiment. PCr = phosphocreatine. Pi = inorganic phosphate α, β and γ refer to the α-, β- and γ-phosphate groups of adenosine triphosphate (ATP).

Following the intramuscular injection of bupivacaine the intensities of the phosphocreatine and ATP signals gradually decreased with no obvious increase in that of inorganic phosphate (Figure 1). ATP was the first metabolite to be completely depleted and this was in the presence of significant levels of phosphocreatine. By 72 h there were no detectable phosphorus-containing compounds in the muscle. Five days after the injection, low amplitude signals were again observed and by day 10 the spectra had returned to normal. In four animals, spectra were also recorded on days 20 and 25 but no further changes were seen.

Spectra recorded from the saline-treated limb were normal and constant throughout the experiment and the ratios of phosphocreatine to ATP and to inorganic phosphate were 2.95 ± 0.35 (s.d.) and 1.50 ± 2.25 respectively. The intracellular pH was 7.06 ± 0.04.

Human studies

Typical spectra recorded from both forearms of a patient are shown in Figure 2. They are normal and no significant difference can be detected. The ratios of phosphocreatine to ATP and to inorganic phosphate in the muscles of the bupivacaine-treated limbs were 3.18 ± 0.3 and 8.75 ± 2.1. In the non-operated limbs these ratios were 3.40 ± 0.4 and 6.50 ± 3.1 respectively. The intracellular pHs were 7.04 ± 0.02

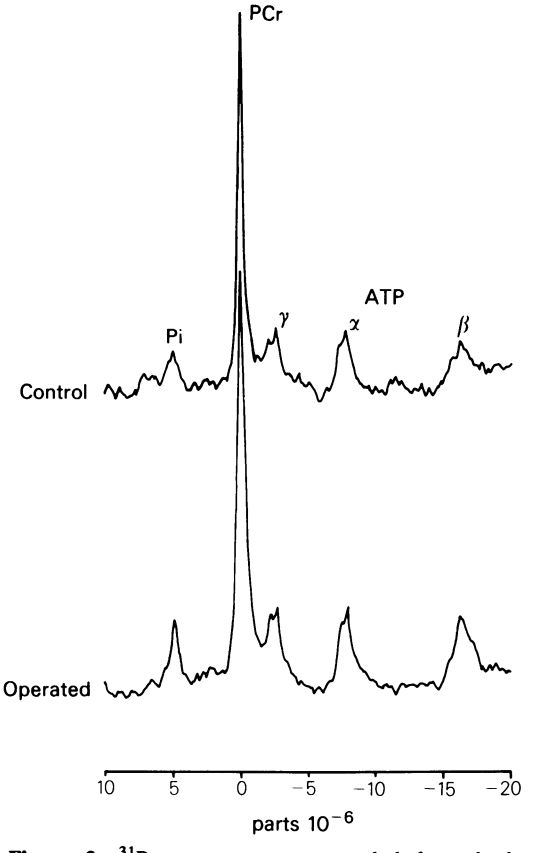

Figure 2 ^{31}P n.m.r. spectra recorded from both forearms of patient 3. Each spectrum represents the sum of 128 scans recorded using 75 μs radiofrequency pulses of 32.5 MHz at 2 s intervals. Internal chemical shift standard, line broadening and peak assignments as in Figure 1.

and 7.06 ± 0.03 respectively. (Normal values 2.9 ± 0.4, 9.3 ± 2.0 and 7.04 ± 0.03, where $n = 20$).

Discussion

The histological studies demonstrated that the intramuscular injection of bupivacaine in rats resulted in marked degeneration followed by rapid regeneration and similar findings have been reported previously (Sokoll *et al.*, 1968; Benoit & Belt, 1970; Hall-Craggs, 1974; Foster & Carlson, 1980). The myotoxicity of other local anaesthetics has also been investigated morphologically including 2% lignocaine, 2% piperocaine, 1% procaine and 0.2–1% tetracaine (Burke, Fedison & Jones 1972; Foster & Carlson 1980). In all cases the pathological process was similar and the amount of degeneration was related to both the concentration of the drug and its relative potency as a local anaesthetic. However, the degree of degeneration was not as profound as that caused by bupivacaine.

The observation of oedema and white cell infiltration soon after the intramuscular injection of the drug gave rise to suggestions that the degeneration was mediated by ischaemia (Luduena, 1969; Hall-Craggs, 1974) but it is more likely that bupivacaine is myotoxic because it interferes with intracellular structures and reactions. Recently it has been shown that amino acid incorporation during protein synthesis in rat muscle was inhibited by the drug (Johnson & Jones, 1978).

This ^{31}P n.m.r. study demonstrated the changes in the levels of the intracellular high energy phosphorus compounds that occurred in the rat following the administration of bupivacaine. These closely followed the time course of the morphological changes and were consistent with myocellular death followed by regeneration. The most important spectroscopic feature was the depletion of ATP in the presence of significant amounts of phosphocreatine. This is because previous n.m.r. studies on both exercising human muscle (Ross *et al.*, 1981) and ischaemic rat muscle (Thulborn, 1981) have shown that ATP levels were maintained more or less constant until all the phosphocreatine was depleted. Only after that point was ATP consumed. This investigation has shown that the myotoxicity of bupivacaine is not simply due to ischaemia but rather that the drug interferes with the production and interconversion of high energy phosphate-containing metabolites.

No changes in the ^{31}P n.m.r. spectra compatible with degeneration were observed in the four patients. Though it is possible that bupivacaine is not myotoxic in man it is far more likely that the lack of effect on phosphate metabolism resulted from dilution of the drug in a large volume of tissue.

In a preliminary study, the mean volume of five adult upper limbs distal to a conventionally placed pneumatic tourniquet was approximately 2000 cm^3 of which 10% was occupied by the skeleton. The dose of 150 mg bupivacaine was therefore distributed in the soft tissues at a concentration of 0.8 mg cm^{-3} which was far lower than the level of 3 mg cm^{-3} in the rat calf muscles (mean volume in 10 animals was 5 cm^3). However, local degeneration has been demonstrated histologically in the soleus muscle following the intramuscular injection of only 2.5 mg bupivacaine (Hall-Craggs & Singh Seyan, 1975) which would have resulted in a muscle concentration of approximately 1 mg cm^{-3}. Other authors (Sokoll *et al.*, 1968; Benoit & Belt, 1970; Libelius, Sonesson, Stamenović & Theslef, 1970) have reported degeneration in the superficial parts of muscles when the same dose was administered into the subcutaneous space. In these cases the intramuscular concentration is not derivable but probably would have been lower than the level of 1 mg cm^{-3} that resulted from direct intramuscular injection (Hall-Craggs & Singh Seyan, 1975).

It can be concluded therefore that provided the maximum permitted dose is not exceeded, bupivacaine probably does not cause muscle degeneration when used as a regional intravenous anaesthetic. However, no comment can be passed on the consequences of its direct intramuscular or deep subcutaneous injection in man.

Though bupivacaine-induced degeneration in animals is followed by rapid regeneration it is not without clinical relevance since the intramuscular administration of the drug is a frequent occurrence. Perhaps the two most common examples of this are dental surgery and the first-aid treatment of muscle strains in athletes. Without more information on the effect of bupivacaine on human muscle it is difficult to assess the importance of these experimental findings and the need for further studies is clear. Nevertheless, these results do suggest that intramuscular injections of bupivacaine, for example in a field block, might be undesirable and may indicate the pathology of persisting muscle pain after such a procedure (Burke *et al.*, 1972).

It is not proposed that bupivacaine be withdrawn from the clinician's armamentarium but rather that thought be given before intramuscular administration and the use of alternative methods and drugs considered. Further efforts should be made to estimate its possible myotoxicity in man.

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