DIFFERENTIAL SENSITIVITY OF A AND C NERVE FIBRES TO LONG-ACTING AMIDE LOCAL ANAESTHETICS

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SUMMARY

The differential sensitivities of A, A δ and C fibres of rat vagus nerve to bupivacaine, etidocaine and AL-381 were studied in vitro. In A fibres, at 35–37 °C, 50 μmole litre⁻¹ of the drugs had similar effects on the action potential amplitude, while at greater concentrations (100 and 200 μmole litre⁻¹) the greatest mean depression of amplitude was seen with etidocaine (n.s.). AL-381 had the most marked effect on the A δ potentials, and it appeared that it was about twice as potent as the others in blocking these fibres. Etidocaine 100 μmole litre⁻¹ was more depressant than the same dose of bupivacaine. The C fibres were blocked most rapidly by AL-381, while etidocaine had the least effect.

The sensitivity of different nerve fibres to blockade by locally acting anaesthetics may depend on a number of factors related to the fibres themselves, the drugs or the surrounding tissue. The concept that the small myelinated fibres are more susceptible than the larger fibres to blockade by local anaesthetics (Gasser and Erlanger, 1929) has been questioned, and recently a reverse fibre sensitivity was found in rabbit sciatic and vagus nerves, at 22 °C (Gissen, Covino and Gregus, 1980). Small myelinated A δ fibres (1–4 μm) are blocked by smaller concentrations of local anaesthetics (procaine) than even the smaller unmyelinated C fibres (Nathan and Sears, 1961). Myelinated B fibres, on the other hand, are more sensitive than C fibres to lignocaine (Heavner and de Jong, 1974), 2-chloroprocaine and bupivacaine (Rosenberg et al., 1980).

Bupivacaine is about three times as potent on B and C fibres as the poorly lipid-soluble 2-chloroprocaine in vitro (Rosenberg et al., 1980). At 22 °C bupivacaine and the even more lipid-soluble etidocaine seem to be equipotent in rabbit A fibres (Gissen, Covino and Gregus, 1980). However, clinically they affect motor nerve fibres differently; etidocaine produces a more profound motor block (Bridenbaugh et al., 1973). To find out whether motor and pain fibres would exhibit differential sensitivity to different local anaesthetics under controlled experimental conditions, the effects of bupivacaine, etidocaine and AL-381 (new experimental amide local anaesthetic) on action potentials in A and C fibres were investigated.

MATERIALS AND METHODS

Nerves and electrical stimulation

Young Sprague–Dawley rats were anaesthetized with diethyl ether and killed by cervical dislocation. The vagus nerve was rapidly removed from the thoracic and cervical region and immersed in oxygenated (95% oxygen, 5% carbon dioxide) Ringer’s solution. The nerve sheath was left intact and the trunk was carefully fixed on a paraffin plate in a thermoregulated nerve chamber, filled with buffer. The composition of the Ringer’s solution was (mmole litre⁻¹): sodium chloride 136, potassium chloride 5.6, calcium chloride 2.2, magnesium chloride 1.3, sodium bicarbonate 16.2, sodium dihydrogen phosphate 1.3, D-glucose 11.0 (pH 7.4 unit, 37 °C).

From another series of rats, phrenic nerves were excised and handled similarly. The phrenic nerve contains predominantly A motor fibres. The nerve chamber, electrical stimulation and technique of recording have been described in detail previously (Rosenberg et al., 1980).

The action potentials were elicited by supramaximal stimulation of 0.15 ms duration (A fibres 1–4 V, C fibres 10–20 V). The vagus nerve is a mixed nerve (Paintal, 1953) and the individual action potentials of A, A δ and C fibres were identified according to the speed of conduction, as measured on the oscilloscope (Coppin and Jack, 1972). In some instances both A and C action potentials were visible simultaneously, but for optimal supramaximal (two times the voltage that caused a maximal amplitude) stimu-
lation, the action potentials of A and C fibres were studied separately in the same nerve trunk. Each nerve was used on two occasions only; the second occasion following recovery and sufficient time for adaptation.

Drugs and test procedure

The local anaesthetics were bupivacaine hydrochloride, etidocaine hydrochloride (Astra Pharm. Comp., Södertälje, Sweden) and AL-381 (Apothekernes Laboratorium, Oslo, Norway). AL-381 is a new amide local anaesthetic, which has been tested only experimentally, but seems to possess characteristics similar to those of bupivacaine (table I).

Solutions of 50, 100 or 200 μmole litre⁻¹ were prepared immediately before each experiment in fresh oxygenated Ringer’s solution. The experimental local anaesthetic AL-381 was studied at a concentration of 25 μmole litre⁻¹ also. Before starting the experiments and the recording of baseline action potentials the nerves were allowed a period of adaptation (about 30 min at 35–37°C). During the recording the stimulation frequency was 1 Hz; between recordings and during washing it was 0.1 Hz. The nerves were exposed to the drug solution for 20 min by perfusing (5 ml min⁻¹) the chamber continuously. After the exposure period the solution was changed to a drug-free solution, and the recovery of the action potential was recorded at regular intervals for up to 45 min. The experiment was considered acceptable when the amplitude of the action potential returned to more than 85% of its baseline value.

Action potential amplitude (peak to peak) and latency (duration from stimulation artefact to the beginning of the action potential) were measured from an ink-jet recording, which was produced subsequently at a speed 100 times slower than the original magnetic recording.

| Table I. Molecular weight (MW), pKₐ, relative lipid solubility and toxicity of the local anaesthetics (modified from Covino and Vassallo, 1976) |
|-----------------|-----------------|-----------------|-----------------|
|                 | MW  | pKₐ  | Relative lipid solubility | Toxicty in mice (LD₅₀) (mg kg⁻¹) |
|                 |     |      |                             | i.v. | s.c.          |
| Bupivacaine     | 325 | 8.1  | 28                          | 6.4 | 45.0          |
| Etiocaine       | 313 | 7.7  | 141                         | 6.7 | 99.0          |
| AL-381          | 280 | 8.0  | ≈28                         | 13.5| 145.0         |

RESULTS

To allow valid comparisons the effects on the action potential amplitude are reported at 4 and 8 min of drug exposure. Although the total exposure (perfusion) time was 20 min, action potentials, although clearly depressed, were only observed for the whole period in association with etidocaine 50 μmole litre⁻¹ in C fibres, and AL-381 25 or 50 μmole litre⁻¹ in A β fibres.

Effect on A fibres

A δ. AL-381 50 μmole litre⁻¹ abolished the action potential in 4 min. Etiocaine depressed the action potential more than bupivacaine, this difference being clearly evident with 100 μmole litre⁻¹. Etiocaine blocked these fibres within 4 min, while with bupivacaine 57% and 13% of the amplitude height remained at 4 min and 8 min respectively (fig. 1). AL-381 25 μmole litre⁻¹ decreased the action potential amplitude to a mean value of 26% at 4 min and 19% at 8 min.

The latency (duration from stimulation artefact to the beginning of the action potential) was a little longer with bupivacaine 50 nmole litre⁻¹ (mean 133%) compared with etidocaine (124%) at 4 min. In the first minutes, when AL-381 had not yet abolished the amplitude, the effect on latency was of the same order as that of the other drugs.

Effect on C fibres

The C fibre action potential was most readily blocked by AL-381. With 50 μmole litre⁻¹ depression at 4 min was similar with the three drugs, but by 8 min AL-381 had abolished the action potential. The effect of AL-381 25 μmole litre⁻¹ was similar to that of etidocaine 50 μmole litre⁻¹: at 4 min the amplitude was 62%, and at 8 min it was 47% of the control amplitude. At a concentration of 100 μmole litre⁻¹ there were no differences between the effects
DIFFERENTIAL BLOCK BY AMIDE LOCAL ANAESTHETICS

FIG. 1. Effects of 50, 100 and 200 μmole litre⁻¹ of bupivacaine, etidocaine and AL-381 on the action potential amplitude of Aβ, Aδ and C fibres of rat vagus nerve, and the compound action potential of rat phrenic nerve. The mean effects at 4 and 8 min are shown (± SEM). Three to five experiments were undertaken at each concentration. With each concentration there was total depression of some of the potentials: these are marked on the x-axis with short vertical lines.

Differential block by amide local anaesthetics

of AL-381 and bupivacaine. Both abolished the action potential in 8 min. However, with etidocaine the amplitude had been decreased to only 30% of control at that time. When the concentration was increased to 200 μmole litre⁻¹ AL-381 caused total blockade of C fibres within 4 min, while with bupivacaine and etidocaine 22% and 38% respectively of the action potential amplitudes were unblocked.

No action potentials were observed at 8 min with any of the drugs at 200 μmole litre⁻¹. The effect of the drugs on the latency could be compared only at the smallest concentration. At 4 min the increase in latency was approximately equal; bupivacaine 112%, etidocaine 110% and AL-381 113%.

DISCUSSION

Previously, it was demonstrated that B fibres (myelinated preganglionic) of rabbit sympathetic trunk were more susceptible than thinner C fibres (unmyelinated) to bupivacaine and 2-chloroprocaine, at 35.7–37.2°C (Rosenberg et al., 1980). In the present study with rat nerves, the fibre sensitivity varied with the drug studied and also, to some extent, with the concentration studied. There were some clear differences in the effect on the amplitude of the action potential of the three local anaesthetics. AL-381 was the most potent blocker of the Aδ fibres, as 25 μmole litre⁻¹ depressed these potentials to a mean value of less than half that resulting from 50 μmole litre⁻¹ of bupivacaine or etidocaine by 4 min, and to the same mean value after 8 min exposure.

Another difference was seen in the effect on the amplitude of the C fibre action potentials. AL-381 had the most marked effects, at 4 and 8 min,
25 μmole litre⁻¹ being comparable to 50 μmole litre⁻¹ of the other drugs. At greater concentrations the effect of AL-381 on C fibres was similar to that of bupivacaine, which at 100 μmole litre⁻¹ was about twice as effective as etidocaine. Surprisingly, etidocaine 50 μmole litre⁻¹ (4 min) caused a more marked decrease in the amplitudes of the C fibres than 100 μmole litre⁻¹. This was possibly a result of a limited diffusion of the drug to randomly distributed unmyelinated axons of the isolated vagus nerve. At 8 min depression was clearly concentration dependent.

The effect of the local anaesthetics on the large A fibres also varied with the concentrations used. At the lowest concentrations all had similar action, while at 100 and 200 μmole litre⁻¹ there was a tendency to a greater mean decrease in amplitude with etidocaine and bupivacaine than with AL-381. The compound action potential of the phrenic nerve probably comprises principally the fastest A fibre potentials. It was interesting to note that, while AL-381 and etidocaine affected Aβ fibres and the compound action potential of the phrenic nerve equally, bupivacaine had a slower effect on the latter potential. This difference between etidocaine and bupivacaine may be a result of differences in lipid solubility and pKₐ (table I). However, as the lipid solubility and pKₐ of AL-381 and bupivacaine are similar, the reason for the differences shown between these drugs must lie in their ability to penetrate through the various barriers protecting the nerve cell. From a theoretical point of view, it may be concluded that the new amide local anaesthetic AL-381, having physical characteristics similar to those of bupivacaine, would be a good agent with which to block pain impulses. However, as shown in these experiments, myelinated fibres known to transmit part of the pressure impulse (Aβ) were not blocked as effectively as by bupivacaine. It is possible that, as with bupivacaine, adequate motor blockade requires more concentrated solutions of the drug (Bromage, 1978).

The results do not agree with those of Gissen, Covino and Gregus (1980), who did not find any differential effects on sensory and motor fibres between bupivacaine and etidocaine. Neither do we agree with their conclusion that the largest A fibres would be blocked at the smallest concentrations of bupivacaine, followed by B fibres, and that C fibres would be the most resistant to conduction block. The main reason for the disparity with the present results may be found in the experimental temperature, which differed by about 15°C. Hypothermia potentiates the effect of local anaesthetics on nerve conduction (Rosenberg and Heavner, 1980). For instance, lignocaine 100 μmole litre⁻¹ at 37°C depressed the compound action potential amplitude (rat sciatic nerve) to about 78%, while at 17°C the same concentration totally abolished the action potential.

The results, particularly with etidocaine and AL-381, agree with those of Franz and Perry (1974), who showed that small myelinated fibres (Aδ) are blocked more quickly than large myelinated fibres. Also, the onset of the block in unmyelinated fibres (C) was shown to be slower or equal to that of small myelinated fibres, depending on the concentration of the local anaesthetic. This last concept was evident in our experiments with etidocaine when the effects of 50 and 100 μmole litre⁻¹ were compared. At 50 μmole litre⁻¹ Aδ and C fibres were blocked about equally, but 100 μmole litre⁻¹ had a more marked effect on the C fibre potential.

The results with bupivacaine and etidocaine also agree with the clinical characteristics of these agents. Bupivacaine causes a better sensory block (analgesia) and etidocaine causes more profound motor blockade (Bridenbaugh et al., 1973).

It appears that AL-381 may be a more effective blocker of pain fibres than bupivacaine and it may be a useful drug for regional analgesia.

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**SENSIBILITE DIFFERENTIELLES DES FIBRES A ET C AUX ANESTHESIQUES LOCAUX A FONCTION AMIDE D'ACTION LONGUE**

**SENSIBILIDAD DIFERENCIAL DE LAS FIBRAS NERVIOSAS A Y C A LOS ANESTÉTICOS LOCALES DE AMIDAS DE LARGA DURACIÓN**

**RESUME**

Les sensitivities individuelles des fibres A beta, A delta et C d'une nerf vague de rat à la bupivacaine, à l'étidocaine et à l'AL-381 ont été étudiées in vitro. Dans les fibres A beta, à 35–37°C, 50 μmol/l des agents avaient des effets semblables sur l'amplitude du potentiel d'action, tandis qu'à des concentrations plus importantes (100 et 200 μmol/l) la dépression d'amplitude moyenne la plus importante survenait avec l'étidocaine (n.s.). L'AL 381 avait l'effet le plus marqué sur les potentiels A delta et il apparaissait environ deux fois aussi puissant que les autres pour bloquer ces fibres. A 100 μmol/l l'étidocaine était plus dépressive que la bupivacaine. Les fibres C étaient bloquées plus rapidement par l'AL 381, alors que l'étidocaine avait l'effet le moins important.

**SUMARIO**

Se llevaron a cabo estudios in vitro de las sensibilidades diferenciales de las fibras Aβ, Aδ y C del nervio vago del ratón a la bupivacaina, a la etidocaina y al AL-381. En las fibras Aβ, a los 35–37°C, 50 μmol/l de las substancias tenían efectos semejantes en la amplitud potencial de acción, mientras que en concentraciones mayores (100 y 200 μmol/l), la depresión promedio de amplitud más importante se registró con la etidocaina (n.s.). El AL-381 tuvo el efecto más marcado en los potenciales Aδ, y pareció tener un efecto dos veces mayor que el de los demás en lo que se refiere al bloqueo de esas fibras. Una dosis de 100 μmol/l de etidocaina era más sedativa que la misma dosis de bupivacaina. El AL-381 produjo un bloqueo de las fibras C más rápido mientras que la etidocaina resultaba menos efectiva.

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**ZUSAMMENFASSUNG**

In vitro wurde die unterschiedliche Empfindlichkeit von Aß-, Aδ- und C-Fasern des Vagusnervs der Ratte auf Bupivacain, Etidocain und AL-381 untersucht. Bei Aß-Fasern hatten bei 35–37°C 50 μmol/l der Medikamente ähnliche Effekte auf die Aktionspotential-Amplitude, während bei höheren Konzentrationen (100 und 200 μmol/l) die größte mittlere Dämpfung der Amplitude bei Etidocain beobachtet wurde (n.s.). AL-381 hatte den deutlichsten Effekt auf Aß-Potentiale; bei der Blockierung dieser Fasern schien es etwa zweimal so wirksam wie die anderen Medikamente. Bei 100 μmol/l wirkte Etidocain stärker blockierend als Bupivacain. Die C-Fasern wurden am schnellsten durch AL-381 blockiert, während Etidocain den geringsten Effekt hatte.

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**RESUMEN**

Se llevaron a cabo estudios in vitro de las sensibilidades diferenciales de las fibras Aβ, Aδ y C del nervio vago del ratón a la bupivacaina, a la etidocaina y al AL-381. En las fibras Aβ, a los 35–37°C, 50 μmol/l de las substancias tenían efectos semejantes en la amplitud potencial de acción, mientras que en concentraciones mayores (100 y 200 μmol/l), la depresión promedio de amplitud más importante se registró con la etidocaina (n.s.). El AL-381 tuvo el efecto más marcado en los potenciales Aδ, y pareció tener un efecto dos veces mayor que el de los demás en lo que se refiere al bloqueo de esas fibras. Una dosis de 100 μmol/l de etidocaina era más sedativa que la misma dosis de bupivacaina. El AL-381 produjo un bloqueo de las fibras C más rápido mientras que la etidocaina resultaba menos efectiva.