Differential Nerve Blocking Activity of Amino-Ester Local Anaesthetics

J. A. W. Wildsmith, A. J. Gissen, J. Gregus and B. G. Covino

The fibres found in peripheral nerves are classified as types A, B or C according to their sizes and conduction velocities (Ganong, 1983). The most rapidly conducting (A) fibres are the largest in diameter and have thick myelin sheaths, whereas the slowest conducting (C) fibres are the smallest, and are unmyelinated. B fibres are lightly myelinated and intermediate in conduction velocity. Traditionally, it has been believed (de Jong, 1980) that C fibres are more sensitive to the action of local anaesthetics than A fibres, even though the latter are more susceptible to other factors which block reversibly, such as cold and pressure. Clinical observations have shown that, in general, the vegetative modalities of sensation (usually considered to be transmitted by C fibres) are blocked more easily than the more discriminatory modalities or motor function (both transmitted by A fibres). This has supported the older views on axon sensitivity.

Recent laboratory studies, using an in vitro rabbit vagus nerve preparation, found that the sensitivity of these fibre types to blockade by local anaesthetic agents was related directly to their speeds of conduction. A fibres were the most sensitive and C fibres the least (Gissen, Covino and Gregus, 1980; Fink and Cairns, 1983). These studies were concerned primarily with amide type local anaesthetics, whereas most of the early clinical observations on which the traditional views were based were made with the amino-ester agent, procaine (Heinbecker, Bishop and O’Leary, 1934; Sarnoff and Arrowood, 1946). Therefore, the present study was instituted to examine the in vitro differential blocking activity of a series of amino-ester agents.

**MATERIALS AND METHODS**

Albino rabbits of approximately 2 kg weight were sacrificed by air embolism immediately following the administration of sodium thiopental 25 mg i.v. The cervical vagus nerves were removed within 15 min and subsequently kept immersed in HEPES-Liley solution (table I). The nerves were cleaned and desheathed by microdissection before mounting in an airtight chamber (fig. 1). Petroleum

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**SUMMARY**

The in vitro sensitivities to local anaesthetic blockade of A, B and C nerve fibres in rabbit vagus nerves were examined using a series of structurally similar amino-ester agents which varied in lipid solubility and anaesthetic potency. A fibres were found to be the most sensitive and C fibres the least sensitive to conduction blockade with all the agents, provided that equilibrium blockade was allowed to develop. A correlation existed between the intrinsic anaesthetic potency of the various agents and their lipid solubilities. Equipotent concentrations of the drugs blocked C fibres at approximately the same rate, but there were marked variations in the rate at which A fibres were blocked. Amethocaine, an agent of high lipid solubility, blocked A fibres more quickly than C. As lipid solubility decreased through the series studied, so the onset of conduction blockade of A fibres was prolonged. It is suggested that this related to decreasing ability to penetrate the lipid diffusion barriers around A fibres. The traditional view that C fibres were more sensitive to block may have arisen because of confusion between absolute sensitivity and rate of development of conduction blockade.

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jelly seals separated a 1-cm length of the nerve from platinum wire stimulating and recording electrodes. The central section of the chamber (volume 0.25 ml) was perfused with HEPES-Liley solution at 0.5 ml min⁻¹ and the other sections were moistened, but not filled, with the same solution.

**TABLE I. Constituents of the perfusing solutions.** *HEPES = [4-(2 hydroxyethyl)-1-piperazine-ethane sulfonic acid] pK 7.55 at 20°C (7.31 at 37°C)*

<table>
<thead>
<tr>
<th>Constituent</th>
<th>HEPES-Liley</th>
<th>Carbonated-Liley</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (mmol litre⁻¹)</td>
<td>136.8</td>
<td>136.8</td>
</tr>
<tr>
<td>KCl (mmol litre⁻¹)</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>CaCl₂ 2H₂O (mmol litre⁻¹)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>MgCl₂·6H₂O (mmol litre⁻¹)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose (mmol litre⁻¹)</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>NaHCO₃ (mmol litre⁻¹)</td>
<td>—</td>
<td>15.0</td>
</tr>
<tr>
<td>NaH₂PO₄·H₂O (mmol litre⁻¹)</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>HEPES buffer* (mmol litre⁻¹)</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>Aeration</td>
<td>100% oxygen</td>
<td>95% oxygen</td>
</tr>
<tr>
<td>pH (mean ± range)</td>
<td>7.4±0.02</td>
<td>7.4±0.02</td>
</tr>
</tbody>
</table>

**Notes:** pH adjusted by addition of NaOH 0.1 mol litre⁻¹ (approx. 15ml litre⁻¹) to reach stable pH values.

**TABLE II. Chemical formula & indices of liquid solubility.** pKa values from Buchi and Perlia (1971).

<table>
<thead>
<tr>
<th>Drug</th>
<th>pKa</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine</td>
<td>9.02</td>
<td>1.98</td>
<td>0.02</td>
</tr>
<tr>
<td>Chloroprocaine</td>
<td>8.96</td>
<td>—</td>
<td>0.14</td>
</tr>
<tr>
<td>Procainamide</td>
<td>9.26</td>
<td>0.23</td>
<td>—</td>
</tr>
<tr>
<td>Di-MAPA</td>
<td>8.65</td>
<td>0.4</td>
<td>—</td>
</tr>
<tr>
<td>Amethocaine</td>
<td>8.48</td>
<td>4.1</td>
<td></td>
</tr>
</tbody>
</table>

During a control period of 30 min to ensure stability, one stimulator channel was used to stimulate the A fibres at 0.0167 Hz and to trigger the second channel to stimulate C fibres 10 ms later. At various intervals, the second channel was switched to stimulate B fibres specifically. The same stimulus pattern was used during the subsequent application and wash-out of drug solution. Changes caused by the drugs were monitored on the oscilloscope screens but all measurements were made from photographs taken at appropriate intervals, of the traces on the oscilloscope screens.

The effects of various concentrations of the hydrochloride salts of procaine, procainamide (both from ICN Pharmaceuticals, Plainview, NY), chloroprocaine, amethocaine (both from Astra Pharmaceutical Products, Inc., Worcester, MA) and dimethylaminoethylpara-aminobenzoic acid ester (di-MAPA) were studied. Chemical structures and physico-chemical data are shown in table II. The drugs were dissolved in a carbonated-Liley solution (table I) and only one concentration of one drug was applied to individual nerves. Perfusion of
test solution through the chamber at 0.5 ml min⁻¹ continued for 30 min or until changes in all three compound action potentials achieved equilibrium, whichever was the longer. Once stable blockade was achieved, the nerve was washed with HEPES-Liley solution: recovery of the compound action potentials to 90+ % of their control values was required for a valid experiment. All experiments were performed at room temperature.

**FIG. 2.** To illustrate compound action potentials of A, B and C fibres from rabbit vagus nerve (amplification x 500).

Changes caused by drugs were measured in terms of the percent decrease produced in the amplitudes of the three compound action potentials from control values. Absolute potency was assessed by the construction of dose–response curves in which the maximal effect on each fibre type was plotted against the concentration of drug on logarithmic–probit graph paper (Miller and Tainter, 1944). The probit scale allows a sigmoid dose–response relationship to be drawn as a straight line and is illustrated in figure 3, with equivalent percent values. Linear regressions of the log of drug concentration against probit score were derived and solved for the point of 50% depression of the action potential amplitude (ED₅₀). The standard error (SE) of that value was obtained (Miller and Tainter, 1944). Changes of less than 10% or greater than 90% were excluded from the analysis of these results, since we were interested in the central portion of the dose–response relationship.

Linear plots of the changes in compound action potential amplitude with time (rate of blockade) were made also, photographic records being obtained with particular emphasis on the time taken for 20% decreases to occur. That time was plotted against drug concentration, and regression lines derived, to allow comparison of the rates at which A and C fibre blockade developed. Since the compound action potentials of B fibres were not recorded at less than 5-min intervals, this analysis was not performed.

**RESULTS**

Figure 3 illustrates the dose–response relationships (derived by least squares regression analysis of the original data) for each drug on A, B and C fibres drawn on log–probit plot. The slopes of the dose–response relationships were steeper for A and B fibres than for C fibres, but there was little variation, with no trends, in the relative effects of individual drugs on the different fibre types.

The ED₅₀ of each drug for A, B and C fibres calculated from the dose–response relationships is shown in table III, together with its SE and the number of experiments used in the derivation of these data. With each drug studied, the ED₅₀ for A fibres was less than that for B fibres, which was lower than that for C fibres. In every experiment performed the percent decreases in the amplitudes of the compound action potentials were in the order A > B > C, once stable blockade was achieved. There were marked variations in ED₅₀ between drugs, their potency increasing from procainamide, through di-MAPA, procaine and chloroprocaine to amethocaine. This pattern was the same with each fibre type.

Figure 4 shows the percent changes in the compound action potentials for A and C fibres in three individual experiments involving approximately equipotent concentrations of amethocaine, procaine
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FIG. 3. Dose–response relationships for the effect of each drug used on each fibre type. Correlation coefficient for each relationship > 0.87. Desheathed rabbit vagus in vitro; carbonated-Liley solution, 23–26 °C, pH 7.4.

TABLE III. ED₅₀ (mmol litre⁻¹) (and its standard error) for the effect of each drug used on each fibre type.

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Amethocaine</th>
<th>Chloroprocaine</th>
<th>Procaine</th>
<th>Di-MAPA</th>
<th>Procainamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.007 (0.002)</td>
<td>0.17 (0.03)</td>
<td>0.41 (0.07)</td>
<td>1.68 (0.41)</td>
<td>2.90 (1.44)</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>0.008 (0.002)</td>
<td>0.20 (0.04)</td>
<td>0.47 (0.12)</td>
<td>1.83 (0.68)</td>
<td>3.22 (1.56)</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>0.014 (0.007)</td>
<td>0.23 (0.007)</td>
<td>0.71 (0.26)</td>
<td>2.87 (1.60)</td>
<td>5.00 (3.0)</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

and procainamide. The rate of onset of C fibre blockade was of the same order with each drug, but there were obvious differences in the absolute and relative rate of development of A fibre blockade. Amethocaine blocked A fibres faster than C and procaine blocked the two at much the same rate, but with procainamide, A fibre block only started to become apparent after 30 min, and took several hours to stabilize. Figure 5 shows the regression lines of the initial rate of development of A and C fibre blockade (expressed in terms of the time taken for the compound action potentials to decrease by 20%) plotted against concentration for all five drugs. A fibres were blocked faster than C in every experiment with amethocaine, but more slowly than C fibres with di-MAPA and procainamide. There were no consistent differences with either procaine or chloroprocaine.

DISCUSSION

These results confirm earlier work (Gissen, Covino and Gregus, 1980; Fink and Cairns, 1983) that the in vitro sensitivity to conduction blockade by local anaesthetics of the fibres found in rabbit vagus nerve is in the order A > B > C. In this study, sensitivity was examined using a series of agents related to procaine and with molecular structures showing specific variations one from another (table II). These structural differences produced variations in absolute potency that were as expected from standard considerations of the structure–activity relationships of local anaesthetics (Buchi and Perlia, 1971). Potency increases when radicals are added to the aromatic ring structure (procaine to chloroprocaine; di-MAPA to amethocaine) or as the length of the alkyl chains attached to the amine group increases.
Substitution of a nitrogen for an oxygen in the central section of the molecule (procaine to procainamide) reduces potency considerably. These structural changes have possibly their most important physico-chemical effect on lipid solubility which, qualitatively at least, is related to potency. Comparable lipid partition coefficients for all the agents used here are not available, but figure 6 shows data for three of the agents plotted with their ED50 for A and C fibres. An excellent correlation exists between the relative lipid solubility of these agents and their conduction blocking potencies.

Although there were marked differences in overall potency, there were only small differences, with no trends, in the relative effect of each drug on the different fibre types. Major differences were found in the rates at which the fibre types were blocked by equipotent concentrations of each drug. The time scale of C fibre blockade was of the same order with each agent, but development of A fibre blockade varied considerably. Again, this was related to lipid solubility, with the highly soluble agent, amethocaine, blocking A fibres quickly, whereas the low solubility agents procainamide and di-MAPA blocked them very slowly. It was not possible to follow the development of B fibre blockade in detail, but with the agents of low lipid solubility it was obviously intermediate between A and C.
This slow rate of blocking A fibres with some agents was the reason that the drugs used in the study were applied in a carbonated-Liley solution, rather than dissolved in the HEPES-Liley solution in which the nerves were prepared. Carbon dioxide potentiates the action of local anaesthetics by enhancing diffusion and by decreasing intracellular pH, which increases the intracellular concentration of the ionized, active form of the molecule (Catchlove, 1972). Exposure of the preparation used in this study to the same carbonated-Liley solution without drug had no obvious effect on either the amplitude or speed of conduction of the compound action potential of any fibre type, but the absolute potency of the drugs was increased and the rate of blocking was accelerated.

Figure 7 shows C fibre dose–response curves for amethocaine, procaine and procainamide dissolved in HEPES-Liley obtained in some preliminary experiments, compared with those using carbonated-Liley from figure 3. In each case there was some potentiation, with the effect being more marked with the drugs of lower lipid solubility. Figure 8 compares the rate of development of A and C fibre blockade in some individual experiments with equal concentrations of amethocaine, procaine and procainamide in either HEPES-Liley or carbonated-Liley. Again, the potentiation of rate of development was far more marked with the drugs of low lipid solubility. The very slow rate of onset of A fibre blockade in this preparation when the low lipid solubility drugs were dissolved in HEPES-Liley gave rise to doubts as to the viability of the preparation when used over such long periods of time and, therefore, a carbonated solution was used for all drugs, to enable valid comparisons to be made.

A fibres are surrounded by considerably greater barriers to diffusion than are C fibres. The multilayered lipoprotein membranes of the myelin sheath are present along the greater part of their length, but the original view that there are no such coverings at nodes of Ranvier is incorrect. While recent work has confirmed that the myelin sheaths derived from...
adjacent Schwann cells do not overlap, a number of processes from those adjacent Schwann cells were found to interdigitate across the node (Peters and Vaughn, 1970). The lower sensitivity of A fibres to osmotic effects (Dodt, Forke and Zimmerman, 1983) is further evidence of the presence of barriers to diffusion.

A drug of high lipid solubility will pass easily through these lipoprotein diffusion barriers and rapidly block A fibres compared with C fibres. Low lipid solubility drugs will block C fibres without difficulty, since they have relatively few diffusion barriers around them, but will take considerably longer to reach the A fibre axon. Eventually the greater susceptibility of the latter will become apparent. This study has demonstrated this relative effect across a range of drugs with different lipid solubilities, the carbonated solution serving mainly to speed diffusion. During drug washout, the process was reversed. Even with procainamide, the time course of recovery of C fibres was measured in minutes, but for A fibres it took hours.

Another physico-chemical factor besides lipid solubility may be relevant. The pKα of amethocaine is 8.48, that of procainamide 9.26. At physiological pH, approximately 6% of amethocaine will be present as the un-ionized lipid soluble form, whereas for procainamide the figure is 1%. This difference may well contribute to the greater rate of penetration of amethocaine through lipid barriers. The pKα of the other drugs lie between these extremes (table II), although that for di-MAPA is close to that for amethocaine in spite of the fact that it is the second least potent agent in the series. In addition, amethocaine is many times more potent than procainamide than the ratio of their un-ionized concentrations would predict.

The above analysis explains the effects of these agents on this *in vitro* nerve preparation. At first sight, it does not seem to resolve the contradiction between laboratory and clinical work referred to earlier. The earliest laboratory work on differential nerve sensitivity to local anaesthetic was performed in the 1920s (Gasser and Erlanger, 1929) and is usually quoted as having shown that C fibres were more sensitive to cocaine than A fibres. What was actually stated was that “small fibres are blocked before [our italics] larger ones”. Gasser and Erlanger went on to infer that this was the result of nerve susceptibility, but raised the possibility that differences in myelin sheath thickness may have been relevant. Since they were using concentrations of cocaine that completely blocked nerve conduction, they were observing the relative rate of blocking of different fibre types, rather than absolute sensitivity. Similarly, Heinbecker, Bishop and O’Leary (1934) used concentrations of procaine, both *in vitro* and clinically, that produced complete blockade. Clinical situations arise in which evidence of blockade of some functions served by A fibres (light
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Covino and Gregus, 1980) that A fibres are the most marked effect than on C fibres (carrying pain sensations in rabbit vagus nerve and has confirmed the amino-ester local anaesthetics of the nerve fibre (Ford et al., 1983).

sensitive and C fibres the least. The drugs studied A fibres (thought to control voluntary muscle) and can penetrate rapidly the diffusion barriers around A fibres. Application of a high concentration would enable the drug to penetrate these barriers at a faster rate than the circulation removes it, so that complete blockade results. Just to maintain minimal blockade, Sarnoff and Arrowood (1946), the pioneers of differential spinal block, had to use intrathecal infusions of dilute procaine, presumably because of removal of drug by the circulation.

In the extradural space the barriers to drug diffusion are even greater, since the nerve is surrounded by a sheath that is derived from the dura mater. Even a much more lipid soluble drug might penetrate that barrier only at a rate sufficient to block C fibres (Gissen, Covino and Gregus, 1982). Adjustment of dose and frequency of administration of bupivacaine in obstetric extradural practice demonstrates that effect regularly. In clinical practice only the most lipid soluble agents can penetrate nerves fast enough to demonstrate true axon sensitivity.

The most lipid soluble agent to reach clinical use to date is etidocaine, and from an early stage it was noted that: "Motor blockade was strongly intense compared to the marginally effective sensory analgesia" (Bromage, Datta and Dunford, 1974). This fits the theory that a highly lipid soluble agent can penetrate rapidly the diffusion barriers around A fibres (thought to control voluntary muscle) and that their greater sensitivity will result in a more marked effect than on C fibres (carrying pain sensation). Recent in vivo animal work has confirmed that etidocaine can block A fibres faster than C fibres (Ford et al., 1983).

This study has examined the in vitro sensitivity to amino-ester local anaesthetics of the nerve fibre types in rabbit vagus nerve and has confirmed the finding of previous work with amide drugs (Gissen, Covino and Gregus, 1980) that A fibres are the most sensitive and C fibres the least. The drugs studied varied considerably in potency, and in the rate at which A fibre blockade developed. Both effects may be related to lipid solubility—the higher the solubility, the higher the potency and the faster blockade developed.

Relating these results to previous in vivo animal and clinical studies has indicated that the traditional views on the susceptibility of different nerve fibre types may have resulted from confusion of absolute axon sensitivity with rate of penetration of diffusion barriers. It is an interesting speculation worthy of further investigation that, when blockade of only modalities of sensation carried by C fibres is required, a drug of low lipid solubility might have advantages. Procainamide is often thought to have no local anaesthetic activity, and its action has been studied rarely. Newman and Clark (1950) found that it did not produce sciatic nerve blockade (where diffusion barriers are great) in the dog, but that it could last twice as long as procaine when used by infiltration.

Acknowledgements

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References


