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# A further study of the neuromuscular effects of vesamicol (AH5183) and of its enantiomer specificity

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**1** The effects of vesamicol (2-(4-phenylpiperidino) cyclohexanol), an inhibitor of acetylcholine storage, and its two optical isomers have been studied on neuromuscular transmission in rat and frog muscle, and on nerve conduction in frog nerve.

**2** Racemic vesamicol produced a pre-block augmentation of twitch tension that also occurred in directly-stimulated muscle. This effect is thus at least partially due to an increase in muscle contractility.

**3** (−)-Vesamicol was approximately 20 times more potent than (+)-vesamicol in blocking twitches elicited at 1 Hz. This degree of stereoselectivity is similar to that measured for inhibition of acetylcholine uptake by isolated synaptic vesicles. Both enantiomers were equally weak in reducing nerve action potential amplitude in frog nerve.

**4** Further studies with the active isomer, (−)-vesamicol, showed that, like that produced by racemic vesamicol, the neuromuscular block was highly frequency-dependent. The block was not reversed by choline or neostigmine, but was partially reversed by 4- or 3,4-aminopyridine.

**5** Preliminary electrophysiological studies showed that vesamicol reduced miniature endplate potential amplitude in rapidly-stimulated frog nerve-muscle preparations. Addition of lanthanum ions increased the frequency of miniature endplate potentials and led to the appearance of apparently normal-sized potentials amongst those of reduced amplitude.

**6** The results show the close agreement between pharmacological and biochemical observations indicating the suitability of the rat diaphragm as a test model for substances of this nature. The degree of reversibility of the vesamicol-induced neuromuscular block by aminopyridines was unexpected, and it is suggested that in the presence of a drug which greatly increases release, a pool of acetylcholine is capable of being released which is not normally releasable after block of storage by vesamicol. It is also considered possible that the results from the intracellular recording studies may be explained in these terms.

## Introduction

Vesamicol (2-(4-phenylpiperidino) cyclohexanol; AH5183) (Figure 1) is a neuromuscular blocking substance with unusual structure and properties. It is a tertiary amine which was shown to possess skeletal muscle relaxant properties after either oral or parenteral administration (Brittain *et al.*, 1969a, b). A subsequent study on isolated nerve-muscle preparations showed that vesamicol-induced neuromuscular block, like that produced by hemicholinium-3, was highly frequency-dependent. Unlike hemicholinium-3-induced block, block by vesamicol was not reversed by choline (Marshall, 1970). These observations led Marshall (1970) to propose that vesamicol prevented the loading of acetylcholine (ACh) by synaptic vesicles. A biochemi-

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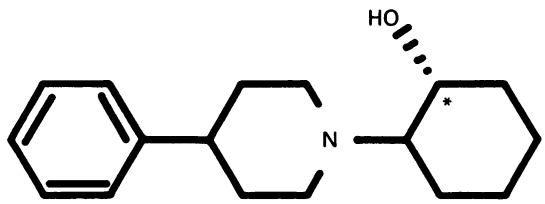


**Figure 1** Structure of vesamicol, showing the hydroxylated asymmetric carbon centre in the cyclohexanol ring of the molecule (starred).

cal study of the effect of vesamicol and other substances on ACh loading by isolated synaptic vesicles confirmed the high potency of vesamicol on this system (Anderson *et al.*, 1983). Thus vesamicol represents a unique class of pharmacological agents with potential usefulness as probes for presynaptic function (see Marshall & Parsons, 1987 for review of recent work.)

In addition to the frequency-dependent block of neuromuscular transmission, vesamicol produces some non-cholinergic effects. These include augmentation of twitch height prior to the neuromuscular block and a weak local anaesthetic action (Marshall, 1970).

The present experiments investigate the actions of vesamicol and its enantiomers on skeletal muscle and somatic nerve preparations in more detail than previously. Studies on isolated vesicles have shown that the (−)-isomer is approximately 25 times more active in blocking ACh transport than the (+)-isomer (Bahr & Parsons, 1986; Bahr & Kaufman, personal communication). This study of the optically resolved forms of vesamicol represents an attempt to correlate biochemical findings with those obtained in an intact functioning system and to obtain information on potential directions for further chemical synthesis aimed at producing more specific compounds acting on the acetylcholine vesicular storage system.

## Methods

### *Skeletal muscle preparations*

*Twitch tension experiments* All twitch tension experiments were performed on the isolated hemidiaphragm preparations of the rat (Bülbring, 1946). In nerve-muscle preparations, the phrenic nerve was stimulated at varying frequencies, with rectangular pulses of 0.2 ms duration and voltage at least twice that required to produce maximal twitches. For direct muscle stimulation, the preparation was first paralysed by the addition of α-bungarotoxin ($2.5\,\mu g\,ml^{-1}$). Direct muscle stimulation was at a rate of 0.1 Hz with rectangular pulses of 5 ms duration and voltage greater than that required to produce maximal twitches.

Preparations were maintained at 32°C in Krebs-Henseleit solution bubbled with 95% $O_2$ and 5% $CO_2$. Tension responses were recorded by strain gauge transducers connected to ink-writing pen recorders.

*Intracellular recording experiments* All experiments were carried out on the isolated sartorius nerve-muscle preparation of the frog (*Rana pipiens*). Intracellular recordings of endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps) were made by use of standard electrophysiological techniques. KCl-filled glass microelectrodes (3 M KCl, 6–10 MΩ resistance) were used to record membrane and transient potentials. Preparations were bathed in a Tris-buffered frog Ringer solution and drugs were applied to the neuromuscular junction area of muscle fibres by local microperfusion (Manthey, 1966; Johnson & Parsons, 1972). In order to record e.p.ps in the absence of muscle contraction, preparations were paralysed by a high magnesium (8 mM), low calcium (0.9 mM) solution, or by addition of tubocurarine ($4 \times 10^{-6}$ M) to the bathing solution.

### *Isolated nerve preparations*

Tests for local anaesthetic activity were carried out on the isolated partially-desheathed sciatic nerve of the frog (*Rana pipiens*). Isolated desheathed nerves were placed in a three-chambered bath with the centre chamber separated from the outer chambers by paraffin wax seals. The nerve was stimulated at one end and the extracellular action potential measured between the two other chambers using a high-gain a.c.-coupled amplifier (Neurolog NL105, Digitimer Ltd.). Drugs were applied by exchanging the fluid in the centre chamber by means of a perfusion pump. Signals were digitized at a rate of 20 kHz by a Data Translation DT 2801A A-D converter and stored for subsequent peak amplitude analysis on an IBM AT microcomputer.

### *Drugs used*

The drugs used were (±)-, (+)- and (−)-vesamicol (their synthesis will be described elsewhere), choline chloride, tubocurarine chloride, neostigmine methylsulphate, 4-aminopyridine (all Sigma), 3,4-diaminopyridine (Koch-Light).

### *Statistics*

Except where stated, results are expressed as mean ± standard error.

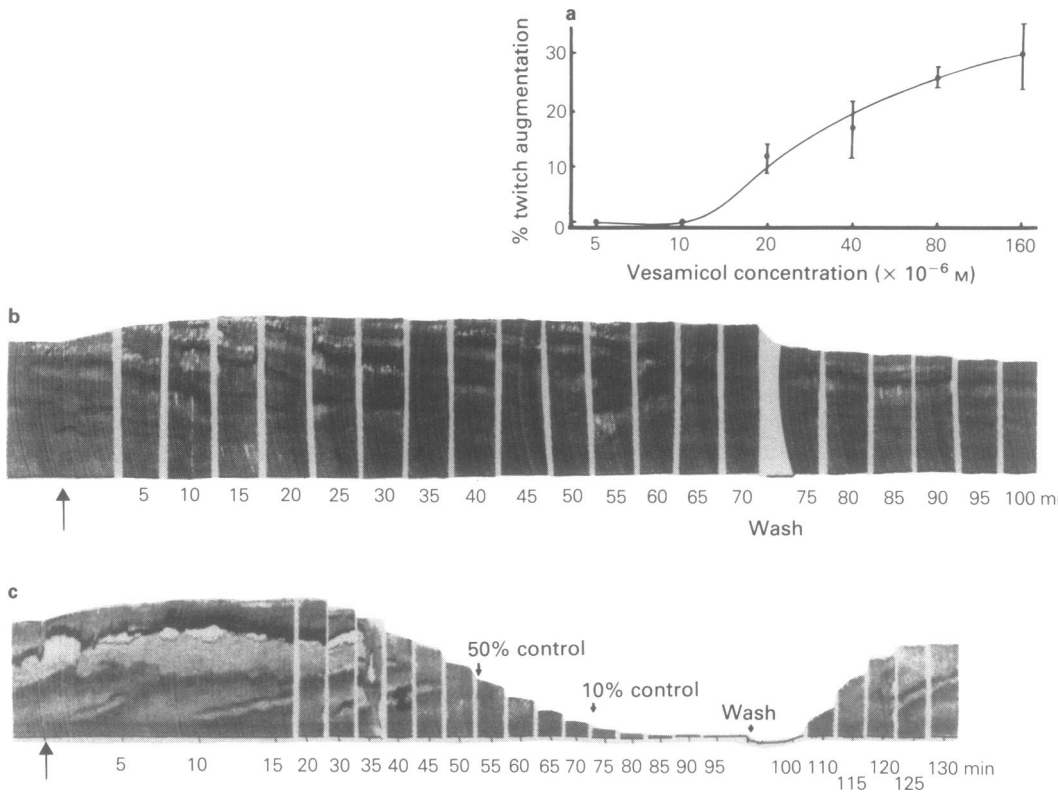


**Figure 2** The augmenting effects of racemic vesamicol on indirectly and directly elicited twitches of the rat hemidiaphragm preparation. In (a) the initial percentage increase in indirectly elicited twitch tension with increasing vesamicol concentration is shown. At each concentration the twitch augmentation was followed by block. In (b) the effects of vesamicol ($3.8 \times 10^{-5}$ M) on the twitch tension of a directly stimulated preparation in which neuromuscular transmission was blocked by α-bungarotoxin is shown. The twitch augmentation was similar to that seen in indirectly stimulated preparations. In (c) the effect of vesamicol ($3.8 \times 10^{-5}$ M) is shown on an indirectly stimulated preparation.

## Results

### *Tension studies with racemic vesamicol on skeletal muscle preparations*

(±)-Vesamicol was tested on directly and indirectly stimulated rat hemidiaphragms to study the mechanism of the twitch augmentation seen with the compound. In preparations stimulated via the phrenic nerve it was observed that the pre-block twitch augmentation was concentration-dependent (Figure 2a). In these experiments the stimulation rate was 0.1 Hz, chosen to keep the frequency-dependent neuromuscular block to a minimum and hence to allow the study of the augmentation of twitches. Thus, at a concentration of $1.5 \times 10^{-4}$ M, vesamicol caused approximately 30% twitch augmentation.

In order to test whether this augmentation was due to an effect on neuromuscular transmission or to an effect on muscle contractility, experiments were carried out in directly stimulated α-bungarotoxin-paralyzed hemidiaphragm preparations. In these experiments vesamicol produced a similar degree of twitch augmentation to that seen in preparations stimulated via the motor nerve (Figure 2).

A second set of experiments with (±)-vesamicol was designed to test whether the neuromuscular block could be reversed by the potassium channel blocking drug 4-aminopyridine, which increases quantal release of transmitter. 4-Aminopyridine ($2 \times 10^{-5}$–$10^{-4}$ M), added during the development of the neuromuscular blocking phase of the action of (±)-vesamicol produced a pronounced although temporary relief of the block. After the augmenting effects of the 4-aminopyridine were over, the block

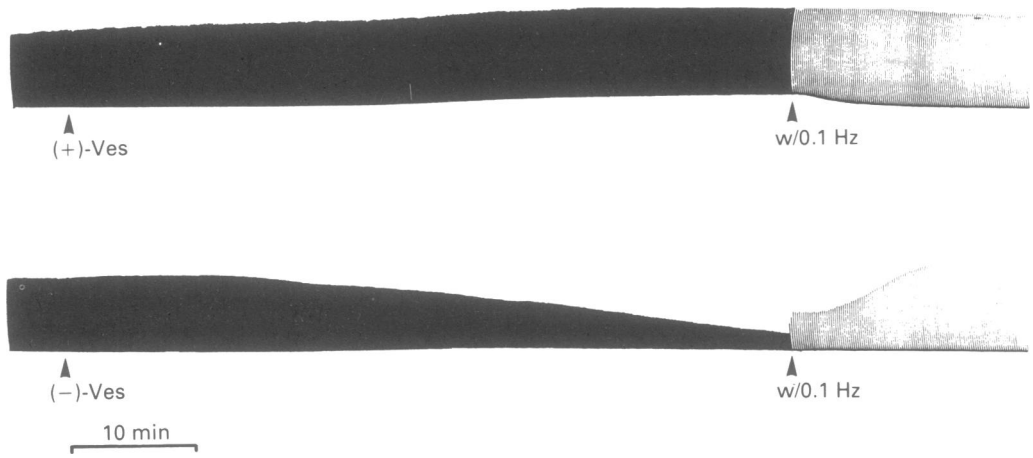


**Figure 3** The effects of (+)- and (−)-vesamicol on indirectly elicited twitches of paired phrenic nerve-hemidiaphragm preparations of the same rat. Both preparations were stimulated at 1 Hz except where indicated by 0.1 Hz. (+)-Vesamicol ((+)-Ves) and (−)-vesamicol ((−)-Ves) were added at the arrows ($5 \times 10^{-6}$ M of both isomers) and allowed to remain in contact with the preparations for 60 min before washout (W) and slowing of the stimulation rate. Note the greater blocking effect of (−)-vesamicol compared to that of (+)-vesamicol. Note also the slight degree of pre-block twitch augmentation and the latent period associated with the action of (−)-vesamicol.

continued to develop at a rate closely similar to that observed before the addition of 4-aminopyridine.

*Tension studies with vesamicol enantiomers on skeletal muscle preparations*

As in the studies carried out on transmitter storage in *Torpedo* isolated synaptic vesicles (Bahr & Parsons, 1986), (−)-vesamicol was more potent than (+)-vesamicol in producing the type of frequency-dependent neuromuscular block that has been ascribed to inhibition of transmitter storage.

Initial experiments were carried out on paired hemidiaphragm preparations from the same rat, set up in separate baths and stimulated via the phrenic nerves at 1 Hz. The same concentration ($5 \times 10^{-6}$ M) of the two isomers was added to each bath and allowed to remain in contact with the tissue for 60 min after which the drugs were washed out and the preparations allowed to recover for at least 60 min. The addition of drugs was then reversed in the two preparations. No significant differences were found ($P > 0.05$) between the effects of first and second additions of the same drug.

In these experiments both (+)- and (−)-vesamicol produced a small degree of twitch augmentation (Figure 3, Table 1) of approximately 10%. In the case of (+)-vesamicol this effect persisted for up to 60 min in some preparations. In other preparations a small degree of twitch block (maximum around 20%) was seen with (+)-vesamicol, after a latent period of about 30 min. In contrast, (−)-vesamicol, after a latent period of about 15 min produced a gradually developing neuromuscular block reaching 75% block after 60 min contact with the tissue (Figure 3, Table 1). Twitch augmentation occurred during the latent period before the onset of the neuromuscular block. The action of each enantiomer was occasionally associated with a small increase in the baseline tension of the preparation.

**Table 1** The effects of (+)- and (−)-vesamicol on indirectly elicited twitches of the rat hemidiaphragm stimulated at 1 Hz

| *Drug* | *Conc*[a] (M) | *% block* (60 min) | *Maximum % augmentation* | *Latent period* (min) | n |
|---|---|---|---|---|---|
| (−)-Vesamicol | $5 \times 10^{-6}$ | 73 ± 7 | 9 ± 3 | 17 ± 4 | 7 |
| (+)-Vesamicol | $5 \times 10^{-6}$ | 5 ± 2 | 8 ± 2 | — | 9 |
| (+)-Vesamicol | $5 \times 10^{-5}$ | 35 ± 8 | 23 ± 1 | 25 ± 4 | 4 |
| (+)-Vesamicol | $10^{-4}$ | 85 ± 6 | 28 ± 3 | 16 ± 4 | 4 |

Experiments were then carried out with (+)-vesamicol in an attempt to find the concentration required to produce an equivalent block to that produced by $5 \times 10^{-6}$ M (−)-vesamicol. Increasing the concentration 10 fold to $5 \times 10^{-5}$ M (+)-vesamicol produced approximately 35% neuromuscular block, after a period of marked twitch augmentation (around 25%) (Table 1). Further increasing the concentration of (+)-vesamicol to $10^{-4}$ M resulted in a block similar to that produced by (−)-vesamicol (about 90% in 60 min, again preceded by twitch augmentation, Figure 4). Experiments with this concentration of (+)-vesamicol were made difficult by the twitch tension occasionally becoming submaximal. This may have been because the high concentration used was approaching that at which local anaesthetic activity is seen (see later).

*Further characterization of the neuromuscular blocking action of (−)-vesamicol*

(−)-Vesamicol, as the biochemically and pharmacologically active isomer of vesamicol, was subjected to further study in order to determine its similarity to the reported actions of racemic vesamicol.

Like racemic vesamicol, (−)-vesamicol produced a neuromuscular block that was markedly frequency-dependent. Thus, at a stimulation frequency of 0.1 Hz, $5 \times 10^{-6}$ M vesamicol, which produced approximately 75% block at a stimulation frequency of 1 Hz, showed no blocking action (Figure 4). It was necessary to increase the concentration 10 fold to $5 \times 10^{-5}$ M in order to see an equivalent block to that seen at the higher frequency (Figure 4). As with (+)-vesamicol, the increase in concentration

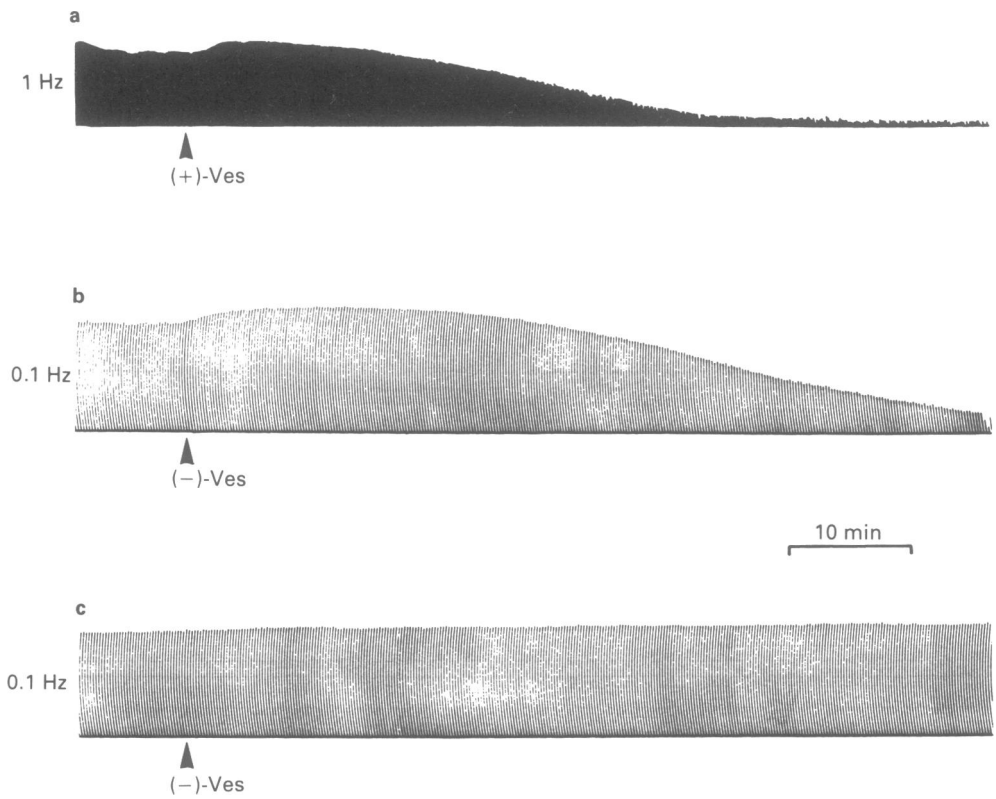


**Figure 4** The effects of concentration and stimulation frequency on the blocking actions of (+)- and (−)-vesamicol on indirectly elicited twitches of the phrenic nerve-hemidiaphragm of the rat. In (a) (+)-vesamicol ((+)-Ves) at a concentration of $10^{-4}$ M produces a similar block to that shown in Figure 3 for (−)-vesamicol at $5 \times 10^{-6}$ M. Note the degree of pre-block twitch augmentation associated with this concentration of the isomer. In (b) and (c) the effects of concentrations of (−)-vesamicol are shown in paired hemidiaphragms from the same rat stimulated at 0.1 Hz. In (c) $5 \times 10^{-6}$ M (−)-vesamicol has no effect on twitches at 0.1 Hz. In (b) $5 \times 10^{-5}$ M (−)-vesamicol produces a similar block at 0.1 Hz to that produced by $5 \times 10^{-6}$ M (−)-vesamicol at 1 Hz (Figure 3). Again note the large degree of twitch augmentation associated with the larger concentration of (−)-vesamicol.

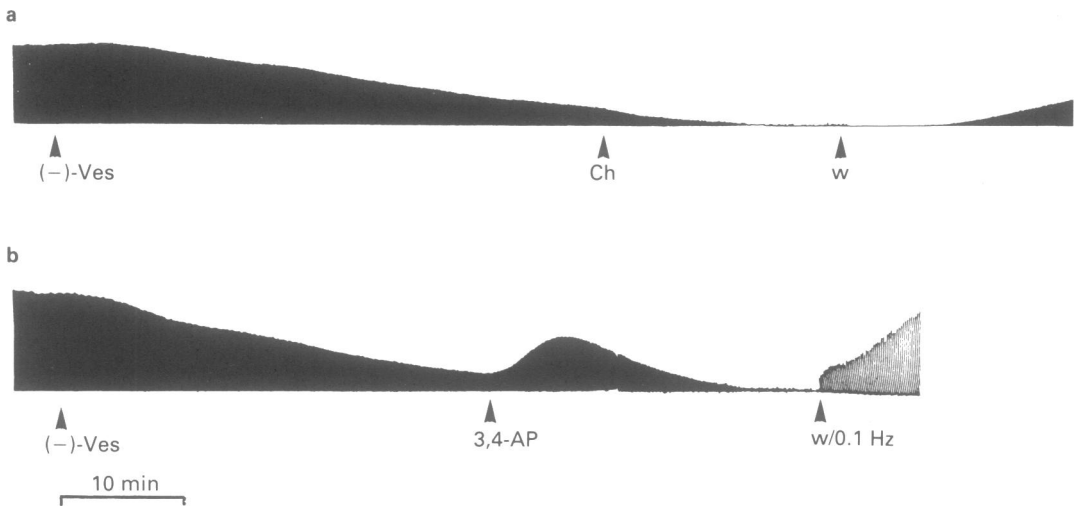


**Figure 5** The effects of choline and 3,4-diaminopyridine on the neuromuscular block produced by (−)-vesamicol. In both (a) and (b) rat phrenic nerve-hemidiaphragm preparations indirectly stimulated at 1 Hz were blocked by $5 \times 10^{-6}$ M (−)-vesamicol ((−)-Ves). In (a) choline (Ch, $3.5 \times 10^{-4}$ M) was ineffective in reversing the effects of (−)-vesamicol. In (b) the block produced by (−)-vesamicol was partially relieved by 3,4-diaminopyridine (3,4-AP, $10^{-4}$ M). Note that after the peak of the reversing effect of 3,4-diaminopyridine, the block due to (−)-vesamicol redevelops, but at a rate similar to that seen before the addition of 3,4-diaminopyridine.

increased the amount of pre-block twitch augmentation (Figure 4).

In a fashion similar to that previously observed with racemic vesamicol, the neuromuscular block produced by (−)-vesamicol ($5 \times 10^{-6}$ M) was not reversed by choline ($3.5 \times 10^{-4}$ M) (Figure 5) and only slightly and transiently reversed by neostigmine ($5 \times 10^{-7}$ M) (not shown). However, 3,4-diaminopyridine ($10^{-4}$ M), produced a marked, albeit transient (5–10 min) reversal of the block (Figure 5). As with racemic vesamicol, the rate of development of the block after the effects of the 3,4-diaminopyridine had diminished was similar to that observed before the addition of the aminopyridine. 3,4-Diaminopyridine ($10^{-4}$ M) also produced a small restoration of tetanic tension in short tetani (50 Hz for 0.2 s) which had faded in tension during the period of stimulation in the presence of vesamicol (not shown). A similar observation of the effect of 3,4-diaminopyridine was made in tetani depressed by hemicholinium-3 ($5 \times 10^{-5}$ M).

*Electrophysiological studies*

Microperfusion of (±)-vesamicol ($10^{-5}$ M) onto end-plate regions of the frog sartorius muscle partially blocked by high magnesium/low calcium solutions or by tubocurarine led to a gradual diminution of e.p.p. amplitude at a stimulation frequency of 1 Hz. The reduction of e.p.p. amplitude was occasionally preceded by an increase in amplitude, leading on one occasion to the firing of a junctional action potential.

In some unparalysed preparations, m.e.p.p. amplitudes were monitored before and after nerve stimulation in the presence of a high concentration of vesamicol ($2 \times 10^{-4}$ M). In these experiments, rapid nerve stimulation (1 Hz for 20 min) in the presence of vesamicol led to a marked reduction in m.e.p.p. amplitude. In one of these experiments, a second microperfusion was started when a large depression of m.e.p.p. amplitude was seen, with a solution containing lanthanum ($10^{-4}$ M). This led to an increase in m.e.p.p. frequency, and to the re-appearance of apparently normal amplitude m.e.p.ps (Figure 6). After this, m.e.p.p. frequency increased so much that it became impossible to measure accurately m.e.p.p. amplitude.

*Local anaesthetic activity*

Extremely large concentrations compared to the neuromuscular blocking concentrations were required for (−)-vesamicol to reduce the amplitude of the gross action potential recorded extracellularly from the partially desheathed frog sciatic nerve preparation. Thus $2 \times 10^{-3}$ M (−)-vesamicol was required to produce an approximately 50%

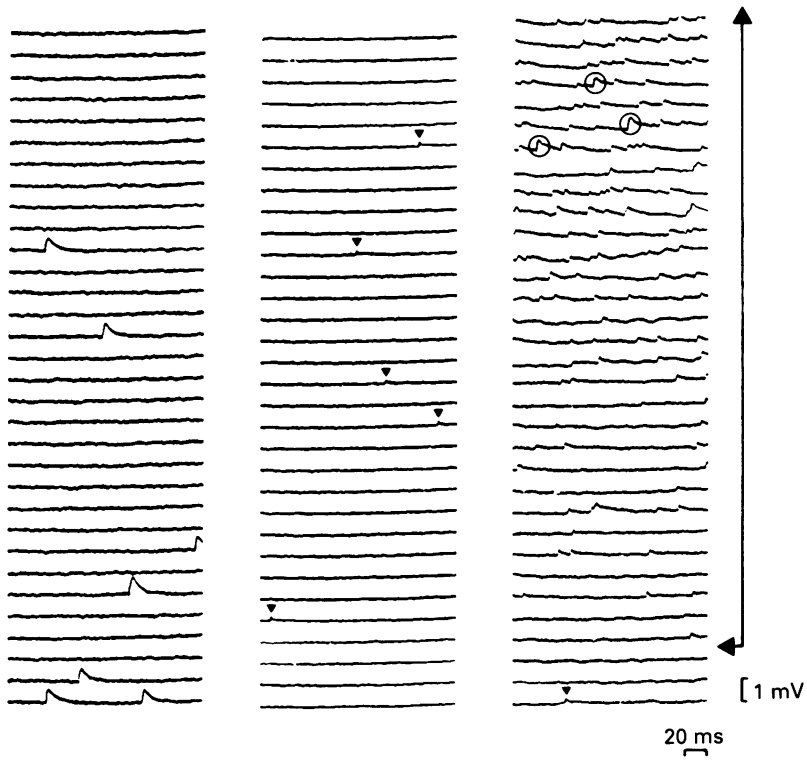


**Figure 6** The effects of (±)-vesamicol on miniature endplate potential (m.e.p.p.) amplitude in the frog sartorius muscle preparation. In the left panel m.e.p.ps are shown before the addition of vesamicol. In the middle panel m.e.p.ps are shown after the addition of vesamicol ($2 \times 10^{-4}$ M) to the preparation and stimulation of the motor nerve at 1 Hz for 20 min. Note the reduction in m.e.p.p. amplitude after stimulation in the presence of vesamicol (examples of low amplitude m.e.p.ps are indicated in this panel by inverted triangles). In the right panel lanthanum ions ($10^{-4}$ M) were microperfused onto the endplate region during the period marked by the arrowed line, resulting in a massive increase in m.e.p.p. frequency. The increase in frequency was associated with the re-appearance of apparently normal-sized m.e.p.ps (circled) along with those reduced in amplitude by the vesamicol.

reduction of amplitude (Figure 7) i.e. 400 times the neuromuscular blocking concentration. (+)-Vesamicol was similar in potency to (−)-vesamicol requiring $1–2 \times 10^{-3}$ M to produce an equivalent effect (Figure 7) i.e. only about 10 times the neuromuscular blocking concentration. For comparison, our previous studies (Dempster, unpublished) showed that lignocaine produces an approximately 50% reduction of nerve action potential amplitude in this preparation at a concentration of $8 \times 10^{-4}$ M.

In contrast to the action of lignocaine, which reaches its maximal effect in about 3 min and is quickly reversed by washing, the effects of both (−)- and (+)-vesamicol were very slow to develop, taking about 15 min to reach full block and being followed by a period of slow and often incomplete recovery after washout (Figure 7).

## Discussion

The results obtained with the vesamicol enantiomers acting on neuromuscular transmission in the intact, functioning hemidiaphragm preparation of the rat correlate well with biochemical data obtained with the isomers acting on the ACh uptake system in isolated synaptic vesicles from *Torpedo californica*. In the isolated vesicle preparation (−)-vesamicol was found to be 22 ± 11 (mean ± s.d., $n = 4$) times more potent than the (+)-isomer (Bahr & Kaufman, personal communication). At the neuromuscular junction the potency ratio between the two isomers was found to be 20. The good agreement between the biochemical and pharmacological data provides strong evidence that the neuromuscular block produced by vesamicol is a manifestation of the inhibi-

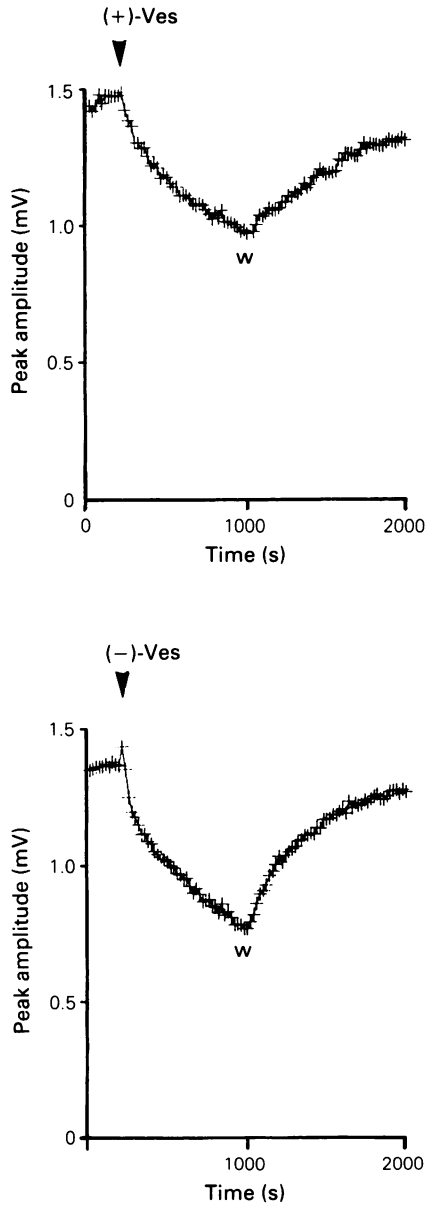


**Figure 7** The effects of (−)- and (+)-vesamicol on extracellularly recorded gross action potential amplitude from the desheathed sciatic nerve of the frog. The nerve was stimulated at 20 s intervals and the peak nerve action potential amplitude recorded and shown as crosses. The vertical scale is calibrated in mV. (−)-Vesamicol ((−)-Ves) and (+)-vesamicol ((+)-Ves) were added at the arrows ($10^{-3}$ M of both isomers at the 10th impulse) and allowed to remain in contact with the nerve for 800 s before washout. Note the similar effects of the two isomers and the slow onset of the local anaesthetic action.

tory action of the compound at the level of the acetylcholine storage system.

The pharmacological results presented here also show that, whilst the primary action of the compound on acetylcholine storage is stereospecific, the other actions of the compound are not. Thus, the twitch augmenting ability and the local anaesthetic activity of the compound were not stereospecific. This may reflect an inherent lack of stereoselectivity of the respective sites of action responsible for these effects. Conversely it may be taken as suggestive evidence that the cyclohexanol ring of the molecule, containing the assymetric centre, is that involved in binding to the synaptic vesicle, whereas the other actions of the compound are a property of the structures of the phenylpiperidino rings. Chemical modification of the compound is being undertaken to address these possibilities.

From our studies we were able to learn more about the non-cholinergic effects of vesamicol on muscle and nerve. Thus, the twitch-augmenting ability of the compound, which is seen prior to the neuromuscular block, is a concentration-dependent phenomenon which is also observed in preparations in which the muscle is stimulated directly after irreversible block of neuromuscular transmission with α-bungarotoxin. It thus appears that the twitch augmenting activity is due, at least in part, to an increase in muscle contractility, although the result does not preclude a concomitant augmentation of neuromuscular transmission. Indeed some evidence was obtained that vesamicol increased e.p.p. amplitude before neuromuscular block. This could have been due to an increase in e.p.p. quantal content. It is possible that such an effect could arise through the compound exerting a weak inhibitory action on potassium channel activity. Alternatively, it could have been due to an anticholinesterase action of vesamicol (Van der Kloot, 1986), although no change in e.p.p. time course was observed.

In terms of the neuromuscular blocking action of both racemic vesamicol and (−)-vesamicol, the ability of 4- and 3,4-aminopyridine to reverse the block temporarily was intriguing. The postulated mechanism of action of vesamicol is that the agent blocks the storage of acetylcholine and that when the preformed stores have been released by nerve stimulation, a reduction of twitch height is observed. This, as we and others have shown previously, is associated with a reduction in the size of the individual quanta released (Van der Kloot, 1986; Whitton *et al.*, 1986). It is assumed that the faster the preformed stores of ACh are released, the faster the block will become apparent. This explains the marked frequency-dependence of the block and the faster onset of block in high calcium-containing solutions than in high magnesium-containing solutions

(Marshall, 1970). The aminopyridines, through their action to block potassium conductance (Pelhate & Pichon, 1974; Kirsch & Narahashi, 1978) greatly increase transmitter release (Molgo *et al.*, 1975; Lundh, 1978; Katz & Miledi, 1979; Molgo *et al.*, 1980; Durant & Marshall, 1980). Thus it would be expected that aminopyridines would, after a transient enhancement of transmission due to increase in quantal content, enhance the rate of the blocking action of vesamicol. In fact, the enhancement was prolonged and the block then redeveloped at the same rate as that observed prior to the addition of the aminopyridines. We postulate that this is related to the observations of Collier *et al.* (1986) at automatic ganglia. They showed that, after vesamicol block, the superior cervical ganglion of the cat was capable of releasing only 15% of the total store of ACh in response to nerve stimulation. This contrasts with the 85% of the store released after block of choline uptake by hemicholinium-3 (Birks & McIntosh, 1964). Collier *et al.* (1986) interpreted their observation in terms of vesamicol blocking transmitter mobilization. We suggest that our results may be explained in similar terms. Thus, if vesamicol not only blocks the storage of ACh by the readily-releasable pool of vesicles that are cycled by nerve stimulation, but also prevents the recruitment of depot store vesicles for release, block will ensue. We further suggest that in the presence of aminopyridines which can increase release many-fold (Katz & Miledi, 1979), the depot store is capable of being released even in the presence of vesamicol. This would explain the apparent 'extra' transmission that is brought into being after the administration of aminopyridines. Whilst our preliminary electrophysiological experiments cannot be regarded as conclusive, we believe that the observation that lanthanum treatment, which greatly increases m.e.p.p. frequency (Blioch *et al.*, 1968; De Bassio *et al.*, 1971; Heuser & Miledi, 1971), led to the immediate appearance of apparently normal-sized m.e.p.ps in a partially vesamicol blocked preparation may lend support to the above idea. It would appear that, despite the rundown of quantal size of m.e.p.ps emanating from the readily-releasable store in the presence of vesamicol, it is possible to induce the release of apparently normal quantal size m.e.p.ps from the depot store. One proviso to this interpretation is that lanthanum alone is capable of inducing 'giant' m.e.p.ps (Heuser & Miledi, 1971), and hence it is possible that the normal-sized m.e.p.ps that we observed after lanthanum treatment were 'giants' reduced in amplitude by vesamicol. However, on the basis of our results, we believe that the vesamicol drug family may represent a potentially useful tool for the study not only of ACh storage phenomena, but also of involvement of different pools of ACh in transmitter release.

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