Effects of tacrine, velnacrine (HP029), suronacrine (HP128), and 3,4-diaminopyridine on skeletal neuromuscular transmission *in vitro*

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1 The effects of tacrine (9-amino-1,2,3,4-tetrahydroacridine), velnacrine (HP029, 9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate), suronacrine (HP128, 9-benzylamino-1,2,3,4-tetrahydroacridin-1-ol maleate), and 3,4-diaminopyridine on neuromuscular transmission were compared on isolated nerve-muscle preparations.

2 Tacrine, HP029, and 3,4-diaminopyridine augmented responses of chick biventer cervicis preparations to nerve stimulation, with tacrine and HP029 increasing responses to exogenously applied acetylcholine. HP128 blocked responses to nerve stimulation and to carbachol, but increased responses to acetylcholine.

3 In mouse diaphragm preparations that were partially paralysed by tubocurarine or low calcium solutions, tacrine, HP029, and 3,4-diaminopyridine reversed the twitch block. HP128 deepened the block.

4 In mouse triangularis sterni preparations, tacrine and HP029 prolonged the decay phase of endplate potentials and miniature endplate potentials, but had no effect on quantal content at 36°C; above 10 µM, they reduced endplate potential amplitude. 3,4-Diaminopyridine increased quantal content without affecting the time course of the endplate potentials. HP128 (1–10 µM) had no effect on amplitude or time course of endplate potentials, but reduced their amplitude at higher concentrations.

5 Extracellular recording of nerve terminal currents from triangularis sterni preparations revealed that 3,4-diaminopyridine and HP128 had a selective blocking action on the waveform associated with K⁺ currents, tacrine reduced and prolonged the K⁺-related waveform, and HP029 had nonselective blocking actions only seen at high concentrations.

6 Tacrine and HP029 behave predominantly as anticholinesterase agents, while HP128 has weaker anticholinesterase actions that are masked by cholinceptor blockade. Tacrine and HP128, but not HP029, have some blocking actions on K⁺ currents of mouse motor nerve terminals.

Keywords: Tacrine; aminopyridines; acetylcholine release; inhibition of acetylcholinesterase; potassium currents; nerve action potentials; neuromuscular transmission

Introduction

Since the clinical report of effectiveness of 9-amino-1,2,3,4-tetrahydroacridine (tacrine) in alleviating some of the symptoms of Alzheimer's disease (AD) (Summers *et al.*, 1986), there has been increased interest in the pharmacology of this cholinesterase inhibitor. However, clinical use of tacrine has been associated with liver toxicity (Marx, 1987; Ames *et al.*, 1988), which may limit its clinical usefulness. Therefore, several new analogues, including (±)-9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate (HP029, velnacrine) and (±)-9-(benzylamino)-1,2,3,4-tetrahydroacridin-1-ol-maleate (HP128, suronacrine), have been synthesized (Shutske *et al.*, 1988; 1989). These compounds are structurally similar to tacrine, they inhibit acetylcholinesterase *in vitro* and are active in a model that may be predictive of AD but have less acute toxicity in rats and mice (Shutske *et al.*, 1989). HP029 was well-tolerated in single dose studies with healthy volunteers (Puri *et al.*, 1989).

The ability of tacrine to alleviate some of the symptoms of AD is not simply correlated with its anticholinesterase activity as there are other anticholinesterases, physostigmine for example (Becker & Giacobini, 1988), that are more active as cholinesterase inhibitors but apparently less effective in AD. Therefore, the precise mechanism of action of tacrine in AD is unknown. However, its structural similarity to the diaminopyridines suggests that the clinical efficacy of tacrine could be, in part, due its ability to interfere with the activity of potassium channels (Summers *et al.*, 1986). The ability of tacrine to block potassium channels has been demonstrated in

several studies (e.g. Drukarch *et al.*, 1987; Schauf & Sattin, 1987; Stevens & Cotman, 1987; Rogawski, 1987; Sim & Griffith, 1988; Halliwell & Grove, 1989; Elinder *et al.*, 1989; Harvey & Rowan, 1988; 1990), although it is not known whether the concentrations of tacrine that are effective at blocking potassium channels are clinically relevant.

The purpose of the present study was to investigate the cholinergic actions of the two new tacrine analogues, in comparison to tacrine and 3,4-diaminopyridine (3,4-DAP) at the neuromuscular junction. By using extracellular and intracellular recording techniques, we could estimate the effects of the compounds on nerve terminal action potentials, acetylcholine release, and acetylcholinesterase activity.

Methods

Chick biventer cervicis preparations

Biventer cervicis muscles and associated nerves were removed from 3–14 day old chicks that had been killed by exposure to anaesthetic ether (Ginsborg & Warriner, 1960) and mounted, with a resting tension of approximately 1 g, in 10 ml tissue baths containing a physiological salt solution of the following composition (mM): NaCl 118.4, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25 and glucose 11.1. The solution was maintained at 33°C and pH 7.3, and bubbled with oxygen containing 5% CO₂. Twitches were evoked by stimulating the motor nerve every 10 s with pulses of 0.2 ms duration and a voltage greater than that which produced a maximal twitch. Contractures to exogenously applied acetylcholine (1–3 mM), carbachol (20–40 µM), and KCl (30–50 mM) were obtained

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prior to the addition of the compounds and at the end of the experiment.

Mouse diaphragm nerve-muscle preparations

Twitch tension experiments were performed on the nerve-muscle hemidiaphragm preparations isolated from 15–25 g male mice (Balb C strain) that had been killed by exsanguination after anaesthetizing with ether. The preparations were mounted in 40 ml tissue baths containing the physiological salt solution described above. Experiments were carried out at room temperature and at 36°C. The phrenic nerve was stimulated at 0.1 Hz with rectangular pulses of 0.2 ms duration and voltage greater than that required to produce maximal twitches.

In order to reveal any facilitation of neuromuscular transmission in twitch tension experiments, a 70% reduction in the twitch height was induced by (+)-tubocurarine (1–5 μ M) or by physiological salt solution containing low Ca^{2+} (0.5 mM) and high Mg^{2+} (2.5–5 mM). In these experiments, the concentrations of the compounds tested were increased when the twitch height had stabilized after the previous addition of the compounds.

Mouse triangularis sterni preparations

Experiments were performed on the left triangularis sterni nerve-muscle preparation (McArdle *et al.*, 1981) isolated from 15–25 g male mice (Balb C strain). The complete dissection of the muscle with its three nerves was performed under continuous perfusion with physiological salt solution (aerated with oxygen) of the following composition (mM): NaCl 154, KCl 5, MgCl_2 1.2, CaCl_2 2.5, glucose 11.1 and HEPES 5; the solution was adjusted to pH 7.3 with HCl.

The preparation was pinned thoracic side downwards to the base of a 2–3 ml tissue bath and perfused at a rate of 5–10 ml min⁻¹ with the physiological solution described above to which 15 μ M (+)-tubocurarine (for extracellular recording) or 2–5 mM Mg^{2+} (for intracellular recording) was added to prevent muscle twitching. In all Mg^{2+} experiments, the Ca^{2+} concentration was reduced to 0.5 mM, and a 20 min equilibration period allowed. When Ca^{2+} ions were replaced by Mg^{2+} ions, a solution of the following composition (mM) was used: NaCl 154, KCl 5, MgCl_2 3.7, glucose 11 and HEPES 5 (pH 7.3). Experiments were performed at room temperature (20–26°C), and at 36 $\pm$ 1°C. The intercostal nerves were stimulated via a suction electrode every 2 s with pulses of 50 μ s duration and supramaximal voltage. In experiments where 3,4-DAP and tetraethylammonium (TEA) were used, procaine (10–60 μ M) was used to suppress repetitive activity.

Intracellular recording Neuromuscular junctions were identified by the presence of endplate potentials (e.p.ps) and miniature endplate potentials (m.e.p.ps) with rise times of 1 ms or less. E.p.ps and m.e.p.ps were monitored using conventional intracellular recording techniques. Recordings were made continuously from one endplate area before and throughout application of compounds, each endplate area (and hence each preparation) being exposed once to compounds.

Extracellular recording Presynaptic waveforms were recorded by a glass microelectrode (filled with 2M NaCl, resistance 5–15 M Ω) placed inside the perineural sheath (near endplate areas) of one of the branches of an intercostal nerve (see Mallart, 1985; Penner & Dreyer, 1986; Anderson *et al.*, 1988). The potentials were recorded, amplified and stored as for the intracellular recordings. Usually 20–25 waveforms were recorded at each time period. As the shape of the waveform recorded was very dependent on the electrode position, waveforms were monitored continuously from the same site before and throughout application of drugs. Recording sites were rejected if the signal amplitude fell by more than 10% during the first 20 min prior to the addition of the compounds. With

this protocol, stable recordings could be obtained over a 180 min period in control solutions or in the presence of drugs.

Statistics

Values in the text are mean $\pm$ s.e. of at least four experiments, unless otherwise stated. Differences between means were tested by the Mann-Whitney U-test, $P < 0.05$ and $P < 0.01$ being taken as significant.

Materials

Tacrine was obtained from Aldrich Chemical Co., Gillingham, Dorset. HP029 and HP128 were gifts from Hoechst-Roussel Pharmaceuticals Inc., Somerville, New Jersey, U.S.A. Other drugs were obtained from Sigma Chemical Co., Poole, Dorset.

Results

The effects of tacrine, HP029, HP128, and 3,4-diaminopyridine on chick biventer cervicis preparations

Tacrine, HP029 and 3,4-DAP induced a concentration-dependent increase in the twitch height of indirectly stimulated preparations (Figure 1). The maximal twitch augmentations were 93, 151 and 210% for tacrine (10 μ M), HP029 (30 μ M) and 3,4-DAP (1 mM), respectively. Higher concentrations of tacrine and HP029 caused less augmentation. In contrast, HP128 (1–100 μ M) reduced twitch height in a concentration-dependent way: 10 μ M caused a 40% $\pm$ 5% reduction in twitch height (Figure 2), and 100 μ M abolished the twitch response. HP128 (100 μ M) also induced a large and prolonged contracture of indirectly stimulated preparations.

Tacrine and HP029 (10 μ M) induced a large increase in the response to exogenously applied acetylcholine (1 mM), whereas the responses to carbachol (20–40 μ M) and KCl (30–50 mM) were not significantly different from control (Figure 2a and b). In contrast, 3,4-DAP, at concentrations capable of greatly augmenting indirectly elicited twitches, had no effect on responses to exogenously applied acetylcholine, carbachol or KCl (Figure 2c). HP128 (10 μ M) increased responses to added acetylcholine, but reduced the response to carbachol (Figure 2d). The response to KCl was, however, not significantly different from control (Figure 2d).

Figure 1 Effects of tacrine (●), HP029 (■), and 3,4-diaminopyridine (▲) on the responses of chick biventer cervicis nerve-muscle preparations to indirect muscle stimulation. Compounds were added cumulatively after the twitch height had stabilized in the presence of each concentration. Each point represents the maximum response for that concentration (means, with s.e. shown by vertical bars, $n = 4$).

Figure 2 Effects of tacrine, HP029, HP128, and 3,4-diaminopyridine (3,4-DAP) on the responses of chick biventer cervicis nerve-muscle preparations to indirect muscle stimulation (solid columns), acetylcholine (hatched columns), carbachol (stippled columns), and KCl (open columns). Columns represent the means from 4 preparations; s.e. are shown by the vertical bars. Significantly different from control: * $P < 0.05$; ** $P < 0.01$.

The effects of tacrine, HP029, HP128, and 3,4-diaminopyridine on mouse diaphragm muscle preparations

In order to investigate if the effects of tacrine and analogues were influenced by the amount of acetylcholine released, we carried out experiments where quantal content was high and where quantal content was reduced, using tubocurarine and low Ca^{2+} /high Mg^{2+} , respectively, to prevent muscle twitching. Experiments were also carried out at 36°C and at room temperature to investigate whether the slowing of action potentials at the lower temperature (see Figure 8) could cause any changes in the effects of the compounds.

Figure 3 Reversal of (+)-tubocurarine blockade by tacrine (●), HP029 (■), and 3,4-diaminopyridine (▲) on responses of mouse hemidiaphragm nerve-muscle preparations to indirect muscle stimulation. (a) Experiments carried out at room temperature; (b) experiments carried out at 36°C . Points are means of 4 experiments, and s.e. are indicated by the bars unless smaller than symbols. Preparations were stimulated once every 10 s.

Reversal of tubocurarine Tacrine, HP029 and 3,4-DAP reversed a 70% reduction in twitch height induced by tubocurarine ($1\text{--}5\text{ }\mu\text{M}$) at both room temperature and at 36°C . At room temperature, the threshold concentration for reversal was $0.01\text{ }\mu\text{M}$ for tacrine and HP029, and $0.1\text{ }\mu\text{M}$ for 3,4-DAP. Complete reversal of tubocurarine paralysis was achieved with $0.1\text{ }\mu\text{M}$ of tacrine and about $10\text{ }\mu\text{M}$ of 3,4-DAP (Figure 3a). In the presence of higher concentrations of tacrine ($1\text{--}10\text{ }\mu\text{M}$) and 3,4-DAP ($100\text{ }\mu\text{M}$), augmentation of the maximal twitches could be induced (Figure 3a). The slope of the concentration-effect curve for HP029 was shallower than those for tacrine and 3,4-DAP, and HP029 at $100\text{ }\mu\text{M}$ did not completely reverse the effects of tubocurarine. HP128 ($1\text{--}10\text{ }\mu\text{M}$) failed to reverse the blockade produced by tubocurarine at room temperature and higher concentrations ($30\text{--}100\text{ }\mu\text{M}$) deepened the block.

At 36°C , the threshold concentration was $0.1\text{ }\mu\text{M}$ for tacrine, HP029 and 3,4-DAP (Figure 3b). However, the compounds only partially reversed the effect of tubocurarine, the degree of reversal being less than that observed at room temperature (Figure 3a). HP128 ($1\text{--}10\text{ }\mu\text{M}$) did not reverse the effect of tubocurarine and at $100\text{ }\mu\text{M}$, HP128 deepened the block.

Reversal of low Ca^{2+} /high Mg^{2+} blockade At room temperature, tacrine and HP029 only partially reversed the 70% reduction in twitch height induced by Krebs solution containing low Ca^{2+} (0.5 mM) and high Mg^{2+} ($2.5\text{--}5\text{ mM}$) (Figure 4a). The threshold concentration was around $1\text{ }\mu\text{M}$ for HP029 and $0.1\text{ }\mu\text{M}$ for tacrine, and the maximum recovery was about 60% with both compounds. In contrast, 3,4-DAP ($10\text{ }\mu\text{M}$) produced complete reversal followed by twitch augmentation. At the highest concentration used (1 mM), 3,4-DAP increased twitch height to $215 \pm 5\%$ of control. HP128 ($0.1\text{--}10\text{ }\mu\text{M}$) was unable to reverse the blockade produced by low Ca^{2+} and high Mg^{2+} , and it deepened the block at concentrations above $10\text{ }\mu\text{M}$.

Figure 4 Reversal of low Ca^{2+} and high Mg^{2+} blockade by tacrine (●), HP029 (■), and 3,4-diaminopyridine (▲) on responses of mouse hemidiaphragm nerve-muscle preparations to indirect muscle stimulation. (a) Experiments carried out at room temperature; (b) experiments carried out at 36°C . Points are means of 3–4 experiments, and s.e. are indicated by the bars unless smaller than symbols. Preparations were stimulated once every 10 s.

At 36°C, tacrine and HP029 again only partially reversed the effect of low Ca^{2+} (0.5 mM) and high Mg^{2+} (2.5–5 mM) on indirectly stimulated twitches (Figure 4b). The threshold concentrations for tacrine and 3,4-DAP remained unchanged from those at room temperature, although HP029 was 10 times less active at 36°C (Figure 4). However, the response to 3,4-DAP was quicker in onset but the final twitch height was about half that at room temperature. HP128, once again, was unable to reverse the blockade produced by low Ca^{2+} and high Mg^{2+} and, at concentrations higher than 10 μM , HP128 deepened the blockade.

Intracellular recording

M.e.p.s in control preparations were about 0.3 to 0.6 mV in amplitude and had a time constant of decay of around 0.6–0.8 ms at a resting membrane potential of –60 to –80 mV. Tacrine (1 μM) and HP029 (10 μM) produced 40% and 15% increases in both m.e.p.p. and e.p.p. amplitude, respectively (Figures 5 and 6). However, higher concentrations of tacrine and HP029 reduced the amplitude of m.e.p.s to control levels and of the e.p.s to below control levels. In addition, both tacrine (> 0.1 μM) and HP029 (> 1 μM) increased the decay time of the e.p.s and m.e.p.s in a concentration-dependent manner until time course was 250–300% of control (Figure 5 and 6). Neither tacrine nor HP029 changed quantal content (Figures 5 and 6) or m.e.p.p. frequency, but occasionally, in the presence of higher concentrations of tacrine (10–30 μM), giant m.e.p.s were observed, as previously reported (Thesleff *et al.*, 1990). 3,4-DAP had no effect on m.e.p.p. amplitude or e.p.p. time course (Figure 7). However, 3,4-DAP markedly increased e.p.p. amplitude and quantal content (Figure 7).

HP128 (1–10 μM) did not affect the decay time of e.p.s or m.e.p.s, or the quantal content of e.p.s. However, higher concentrations of HP128 (30–100 μM) greatly reduced the amplitude of e.p.s and m.e.p.s.

Figure 5 (a) Effects of tacrine on m.e.p.p. amplitude (Δ) and on the time constant of decay (○) of m.e.p.s. (b) Effects of tacrine on e.p.p. amplitude (▲), on the time constant decay (●), and on the mean quantal content (■) of e.p.s. Points are means of 3 experiments, and s.e. are indicated by the bars unless smaller than symbols.

Figure 6 (a) Effects of HP029 on m.e.p.p. amplitude (Δ) and on the time constant of decay (○) of m.e.p.s. (b) Effects of HP029 on e.p.p. amplitude (▲), on the time constant decay (●), and on the mean quantal content (■) of e.p.s. Points are means of 3 experiments, and s.e. are indicated by the bars unless smaller than symbols.

Effects of tacrine, HP029, HP128, and 3,4-DAP on perineural waveforms

The perineural waveform is predominantly made up of two negative deflections, usually preceded by a small positivity as the action potential arrives near the nerve endings. The first

Figure 7 (a) Effects of 3,4-diaminopyridine (3,4-DAP) on m.e.p.p. amplitude (Δ) and on the time constant of decay (○) of m.e.p.s. (b) Effects of 3,4-DAP on e.p.p. amplitude (▲), on the time constant decay (●), and on the mean quantal content (■) of e.p.s. Points are means of 3 experiments, and s.e. are indicated by the bars unless smaller than symbols.

Figure 8 Effects of tacrine, HP128, and 3,4-diaminopyridine (3,4-DAP) on perineural waveforms recorded from mouse triangularis sterni preparations. (a) Superimposed recordings from the same site before (Con) and after the addition of 3,4-DAP (the numbers refer to the micromolar concentrations) at 36°C and (b) room temperature. (c) Effects of tacrine on perineural waveforms at 36°C and (d) room temperature. (e) Effects of HP128 on perineural waveforms at 36°C and (f) room temperature. Each record is an average of 20–25 waveforms. The stimulus artefacts have been omitted, for clarity.

negative deflection is associated with the movement of Na^+ ions (inward) at the nodes of Ranvier and heminode, and the second negative deflection is associated with the local circuit current that is generated by the movement of both K^+ (outward) and Ca^{2+} (inward) at the motor nerve terminals (see Brigant & Mallart, 1982; Mallart, 1985; Penner & Dreyer, 1986).

Effects on potassium and calcium currents Tacrine, HP128 and 3,4-DAP at room temperature and 36°C caused a concentration-dependent reduction in the second negative deflection of the perineural waveform (Figures 8 and 9), but did not affect the first negative spike. However, in the presence of higher concentrations of tacrine (300 μM) and HP128 (100 μM), there was a marked reduction on the Na^+ -related component. Additionally, at room temperature, tacrine (100–200 μM) markedly prolonged the duration of the perineural waveform (Figure 8). Similar effects with tacrine and HP128 were also observed in the presence of Ca^{2+} -free salt solution (data not shown). However, 3,4-DAP, even at 100 μM , had no effect on the first negative deflection. HP029 (1–100 μM) did not affect the perineural waveform (Figure 9), and at higher concentrations, it blocked the perineural waveform completely.

Effects on sodium currents In order to investigate further if tacrine and HP128 had any effect on the Na^+ channel activity

Figure 9 Effects of tacrine (●), HP029 (■), HP128 (○), and 3,4-DAP (▲) on the second negative deflection of the perineural waveforms, which corresponds to the nerve terminal K^+ current. (a) Experiments carried out at room temperature; (b) experiments carried out at 36°C. Points are means of 3 experiments, and s.e. are indicated by the bars unless smaller than symbols.

of motor nerves, perineural recordings were carried out in a low Ca^{2+} (0.5 mM) bathing solution and in the presence of maximally effective concentrations of 3,4-DAP (600 μM) and tetraethylammonium (15 mM) to block both voltage-operated and Ca^{2+} -activated K^+ currents in motor nerve terminals. Procaine (50 μM) was used to stop the spontaneous activity produced by the combination of 3,4-DAP and tetraethylammonium; this concentration of procaine had no effect on the first negative deflection. When tacrine (30–200 μM) or HP128 (10–100 μM) was added to the bath solution, the Na^+ spike was blocked in a concentration-dependent way (Figures 10 and 11).

Discussion

The results obtained with tacrine, HP029 and 3,4-DAP on the chick biventer cervicis and the mouse hemidiaphragm preparations show that these compounds can augment neuromuscular transmission. As previously demonstrated biochemically (Shutske *et al.*, 1989), tacrine and analogues have anticholinesterase activity, as they are able to increase the response to exogenously applied acetylcholine on chick biventer cervicis preparations. The anticholinesterase action of tacrine and HP029 explains the increase in m.e.p.p. and e.p.p. amplitude and time to half decay in the triangularis sterni preparation, since similar effects are observed with other anticholinesterase agents. Thus, the anticholinesterase activity of tacrine and HP029 could explain the twitch augmenting action seen in the chick biventer cervicis preparation and the facilitatory activity of these compounds under normal quantal content (reversal of tubocurarine blockade) and under impaired quantal content (reversal of low Ca^{2+} /high Mg^{2+} blockade) in the mouse hemidiaphragm preparation. HP128 did not augment twitch height, which may be a consequence of its anticholinesterase action being weaker than that of

Figure 10 Effect of tacrine on the perineural waveform of a mouse triangularis sterni preparation in the presence of low Ca^{2+} (0.5 mM) bathing solution, 3,4-diaminopyridine (3,4-DAP), tetraethylammonium (TEA), and procaine. (a) Averaged control waveform in the presence of low Ca^{2+} ; (b) after exposure to 3,4-DAP (600 μM), TEA (15 mM) and procaine (50 μM); (c) after tacrine 30 μM ; and (d) after tacrine 200 μM . Each record is an average of 20–25 waveforms. The initial upward deflection is the stimulus artefact.

tacrine or HP029 (Shutske *et al.*, 1989), or HP128 may have additional blocking effects on neuromuscular transmission. HP128 reduced carbachol contractures of the chick biventer cervicis preparation and reduced m.e.p.p. amplitude in the

Figure 11 Effect of HP128 on the perineural waveform of a mouse triangularis sterni preparation in the presence of low Ca^{2+} (0.5 mM) bathing solution, 3,4-diaminopyridine (3,4-DAP), tetraethylammonium (TEA), and procaine. (a) Averaged control waveform in the presence of low Ca^{2+} ; (b) after exposure to 3,4-DAP (600 μM), TEA (15 mM) and procaine (50 μM); (c) after HP128 10 μM ; and (d) after HP128 100 μM . Each record is an average of 20–25 waveforms. The initial upward deflection is the stimulus artefact.

mouse triangularis sterni preparation, indicating a postjunctional blocking activity. 3,4-DAP did not increase the response to exogenously applied acetylcholine on the chick biventer cervicis preparation and did not alter the amplitude and time to half decay of the m.e.p.s on the mouse triangularis sterni preparation, confirming its lack of effect on acetylcholinesterase activity.

However, because of the structural similarity of tacrine and aminopyridines, which are classical K^+ blockers, and the previous demonstration that tacrine can increase quantal content and block K^+ channels in mouse neuromuscular preparations at room temperature (Harvey & Rowan, 1988; 1990), we wanted to investigate if tacrine and analogues were able to block K^+ channels and facilitate acetylcholine release in the triangularis sterni preparation of mouse at a more physiological temperature.

As expected (Harvey & Marshall, 1977a, b; Durant & Marshall, 1980; Molgo *et al.*, 1980; Harvey & Rowan, 1988; 1990), 3,4-DAP markedly increased quantal content, and this increase in acetylcholine release would explain the facilitatory activity and anti-curare actions seen in the twitch experiments. In contrast, tacrine and analogues had no demonstrable presynaptic facilitatory activity at 36°C. Perineural recording showed that 3,4-DAP reduced the second negative deflection, confirming its K^+ blocking activity at motor nerve terminals, as previously reported (Brigant & Mallart, 1982; Harvey & Rowan, 1988; 1990). Tacrine and HP128, but not HP029, were also able to block the K^+ -dependent portion of the nerve terminal waveform. Since this action was also observed in Ca^{2+} -free solution, the effects of tacrine and HP128 on the second negative deflection of the perineural waveform were not via an effect on the Ca^{2+} current. Furthermore, as previous demonstrated (Harvey & Rowan, 1988), tacrine (100–200 μM) caused a marked prolongation of the perineural waveform at room temperature. However, at 36°C, this effect was very small, even at the highest concentrations used. These findings suggest that, at a more physiological temperature, the kinetics of the ion channels and their sensitivity to drugs may be different. This difference might be relevant to the control of transmitter release as tacrine was able to increase transmitter release at room temperature (Harvey & Rowan, 1990) but had no demonstrable facilitatory activity at 36°C.

Additionally, tacrine and HP128 are not very selective for nerve terminal K^+ currents. In the presence of maximally effective concentrations of 3,4-DAP and TEA in a low Ca^{2+} solution, tacrine and HP128 blocked the Na^+ -dependent portion of the nerve terminal waveform. Similar findings with tacrine were seen earlier in unmyelinated invertebrate axons (Schauf & Sattin, 1987; Elinder *et al.*, 1989), although only K^+ channels seem to be affected in another invertebrate (Drukarch *et al.*, 1987). Tacrine also blocks A currents in hippocampal neurones but at high concentrations it blocks the slowly activating K^+ currents and the Na^+ current (Rogawski, 1987). Moreover, most of the effects of tacrine on neuronal K^+ currents require high concentrations of tacrine (30–500 μM), which are much higher than those measured in serum of patients treated with tacrine (Summers *et al.*, 1986).

However, tacrine and analogues may have another action that might be clinically relevant. For example, tacrine, HP029 and HP128 are able to block the neuronal uptake of nor-adrenaline, 5-hydroxytryptamine and dopamine (Drukarch *et al.*, 1987; Shutske *et al.*, 1989). Our results showed that tacrine and analogues have additional blocking effects on neuromuscular transmission, as HP128 reduced carbachol contractures in the chick biventer cervicis preparation, and tacrine, HP029 and HP128, at the highest concentrations used, reduced m.e.p.p. amplitude in the mouse triangularis sterni preparations, indicating a postjunctional blocking activity that could be masking any facilitatory activity of these compounds.

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