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Effects of the facilitatory compounds catechol, guanidine, noradrenaline and phencyclidine on presynaptic currents of mouse motor nerve terminals

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Summary. Catechol, guanidine, noradrenaline, and phencyclidine can increase acetylcholine release at neuromuscular junctions. To determine if they act by affecting nerve terminal action potentials, the electrical activity of the terminal regions of motor nerves was recorded with an extracellular electrode inserted in the perineural sheaths of nerves in the mouse triangularis sterni preparation. Catechol (from 10 μM) and guanidine (from 1 mM) produced a selective reduction in the component of the perineural waveform associated with voltage-dependent K^+ currents, without significant effects on Na^+, Ca^{2+}, or Ca^{2+}-activated K^+ currents. A selective block of K^+ channels in nerve terminals would cause a prolonged depolarization and hence a large influx of Ca^{2+} to trigger acetylcholine release; this could explain the facilitatory effects of guanidine and catechol. Noradrenaline produced a slight increase in the amplitude of the perineural waveform. This is consistent with hyperpolarization of the resting membrane potential of the nerve, which could lead to facilitation of acetylcholine release. Phencyclidine blocked Na^+- and K^+-related portions of the signal.

Key words: Neuromuscular junction – Acetylcholine release – Motor nerve terminals – Potassium channels – Presynaptic currents

Introduction

Augmentation of neuromuscular transmission is usually revealed by the reversal of the twitch blockade induced by a drug like tubocurarine. The best known mechanism for producing augmentation is inhibition of acetylcholinesterase, but there are also a number of compounds that act prejunctionally at the neuromuscular junction to facilitate the evoked release of acetylcholine. Such compounds include aminopyridines, tetraethylammonium (TEA), guanidine, catechol, and noradrenaline (for review see Bowman 1980). Of these, the aminopyridines and TEA are thought to act by blocking K^+ channels: depolarization of the nerve terminal is thus prolonged, allowing a greater amount of Ca^{2+} to enter and release more acetylcholine per nerve impulse. The mechanisms of action of guanidine, catechol, and noradrenaline at the neuromuscular junction are not known.

Although most presynaptic terminals are too small for conventional electrophysiological techniques to be used, the electrical activity of motor nerve terminals can be recorded with an extracellular electrode. The extracellular waveform has components that have been ascribed to Na^+, K^+, Ca^{2+}, and Ca^{2+}-activated K^+ currents (Brigant and Mallart 1982; Mallart 1985a, b). Use of this technique has confirmed that aminopyridines and TEA do block K^+ currents in motor nerve terminals, and also revealed that TEA is relatively selective for the Ca^{2+}-activated K^+ current (Brigant and Mallart 1982; Mallart 1985a, b). We have used the extracellular recording technique to investigate whether the facilitatory effects of catechol (Otsuka and Nonomura 1963; Gallagher and Blaber 1973), guanidine (Otsuka and Endo 1960; Kamenskaya et al. 1975; Teräväinen and Larsen 1975), noradrenaline (Jenkinson et al. 1968; Kuba 1970), and phencyclidine (Albuquerque et al. 1981) are related to changes in the electrical properties of motor nerves.

Methods

Mouse triangularis sterni preparation. Experiments were performed on the left triangularis sterni nerve-muscle preparation (McArdle et al. 1981) isolated from 17–22 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 containing 5% carbon dioxide) of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; glucose, 11.1; and $NaHCO_3$, 25, to buffer at pH 7.3.

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 with the physiological solution described above, to which d-tubocurarine (14–28 μM) was added to abolish postsynaptic activity. Experiments were performed at 18–22°C. The intercostal nerves were stimulated via a suction electrode every 2 s with pulses of 50–100 μs duration and supramaximal voltage.

Presynaptic waveforms were recorded by a glass microelectrode (filled with 2 M NaCl, resistance 5–15 MΩ) placed inside the perineural sheath (near endplate areas) of one of the branches of an intercostal nerve (see Mallart 1985a). The potential difference between the silver/silver chloride reference electrode in the bath and the recording electrode was measured by a high impedance unity gain

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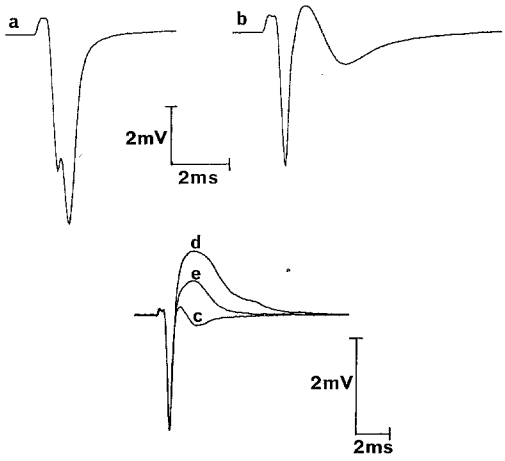

Fig. 1. Effects of 3,4-diaminopyridine and TEA on averaged perineural waveforms. *a:* Control; *b:* same site after 15 min application of 2 mM 3,4-diaminopyridine. *c–e:* Perineural waveforms recorded from a different preparation in the presence of 200 μM 3,4-diaminopyridine (**c**), and a combination of 200 μM 3,4-diaminopyridine with 1 mM TEA (**d**). 200 μM Cd^{2+} reduced the large positive deflection produced by TEA and 3,4-diaminopyridine (**e**)

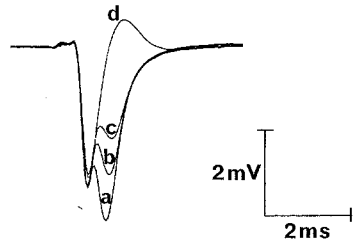

Fig. 2. Effects of catechol on averaged perineural waveforms. *a:* Control; *b:* 5 min after 50 μM catechol; *c:* 10 min after 100 μM catechol; *d:* 5 min after 500 μM catechol

electrometer (W-P Instruments, model M-701), displayed on a dual beam storage oscilloscope and simultaneously stored on FM tape (Racal 4DS). Usually 20–25 waveforms were recorded at each time period, and the average waveform was computed. 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; in control experiments, waveforms were found to vary by less than 10% in a 90 min recording period. With the exception of phencyclidine, all drugs were added to the reservoir containing the solution for perfusion. In some experiments, phencyclidine was added to 20 ml of physiological solution which was recycled with aeration throughout the experiment; recycling of solution in control experiments did not significantly alter the configuration of the waveform. Unless stated otherwise, three preparations were used for each experimental condition, and values quoted are means ± SEM.

Materials. Catechol was obtained from Aldrich Chemical Co., Gillingham, Dorset, UK; guanidine, noradrenaline (hydrochloride and bitartrate), TEA (chloride) and 3,4-diaminopyridine from Sigma Chemical Co., Poole, Dorset, UK; and phencyclidine was a gift from Professor D. Lodge, London. Noradrenaline and catechol were dissolved in acid saline immediately before each experiment.

Results

As previously described by Mallart (1985a) and Penner and Dreyer (1986), typical waveforms recorded from the perineural sheath have a small positive (upward) component followed by a large biphasic negative (downward) component (Fig. 1a). The first negative component is associated with nodal inward Na^+ current, which produces an outward current at the nerve endings. The second negative component can be abolished by TEA and 3,4-diaminopyridine (Fig. 1b), and is associated with an outward K^+ current at the nerve terminals. In the presence of 3,4-diaminopyridine, there is a small, delayed negative deflection (Fig. 1c), which can be enhanced by raising the Ca^{2+} concentration and which is abolished by TEA (Fig. 1d). This component corresponds to a Ca^{2+}-activated K^+ current (Mallart 1985b). In the presence of TEA and 3,4-diaminopyridine, prolonged positive waveforms that are sensitive to Cd^{2+} and some other calcium channel blockers are produced (Fig. 1d, e).

Catechol

Catechol caused progressive, concentration-dependent changes in perineural waveforms. At 10–50 μM, catechol induced a selective fall in the amplitude of the second negative waveform, i.e. the one associated with K^+ currents at the nerve terminals (Fig. 2a, b). The effect was obvious within 3–5 min of adding the catechol and it equilibrated in about 10 min. With 10 μM catechol, the second negative component was reduced to 62 ± 3% of control. An increase in catechol concentration to 100 μM brought about a further reduction in this component of the waveform (Fig. 2c). When the concentration was increased to 0.5–1 mM, the second negative waveform was abolished and a small positive waveform took its place (Fig. 2d). This positive waveform was stable for at least 20–30 min, and could be reduced by the addition of 0.2 mM $CdCl_2$. The effects of catechol were readily reversed by washing.

The effects of catechol are similar to those of 3,4-diaminopyridine. Although this implies that catechol blocks K^+ channels, it should be remembered that the interpretation of extracellularly recorded waveforms can be ambiguous because the signal recorded is the sum of inward and outward currents in the vicinity of the electrode. Hence, the decrease seen in the presence of catechol could be due to either a block of outward K^+ currents or an increase of the inward Ca^{2+} current. Further experiments were performed to distinguish between these possibilities.

Waveforms recorded from a preparation equilibrated in the absence of Ca^{2+} (no added $CaCl_2$ plus 1 mM EGTA) often have a second negative component that is smaller than normal. This may result from the loss of the contribution of a Ca^{2+}-activated K^+ current. However, in the absence of Ca^{2+}, catechol (400 μM) still abolished the second negative component without changing the amplitude of the first negative component (Fig. 3).

When experiments were performed with solutions containing 6 mM $CaCl_2$ to enhance the contribution of the Ca^{2+}-activated K^+ current, catechol (1 mM) blocked the second negative component and induced a delayed negative

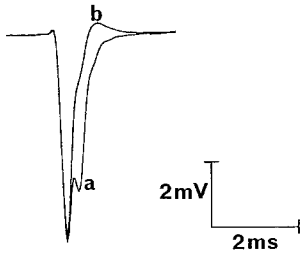

Fig. 3. Effects of catechol on averaged perineural waveforms in the absence of Ca^{2+}. *a:* Control waveforms recorded after 60 min equilibration in physiological solution containing 1 mM EGTA and no added Ca^{2+}; *b:* same site 10 min after 400 μM catechol

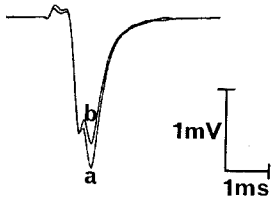

Fig. 4. Averaged perineural waveforms recorded before (*a*), and at the same site 10 min after 1 mM guanidine (*b*)

component, which was abolished either by changing the perfusing solution to one containing 2.5 mM $MgCl_2$ but no Ca^{2+}, or by the addition of 1 mM TEA. However, on preparations in which 3,4-diaminopyridine (400 μM) was used to block voltage-dependent K^+ channels and reveal the delayed negative component, the addition of catechol (0.1 – 0.5 mM) did not alter the waveform.

As described earlier, prolonged positive waveforms that are sensitive to Cd^{2+} are produced in the presence of TEA and 3,4-diaminopyridine. Catechol (1 mM) did not increase such waveforms.

Guanidine

At 0.1 mM, guanidine had little effect on perineural waveforms, but at 1 mM and higher it reduced the second negative component (Fig. 4). The decrease developed over 10 – 20 min and was not reversed by washout of guanidine for 30 min. In some experiments, there was a fall in the first negative component but this was always much less than that of the second component. The average changes produced by 1 mM guanidine were 20 ± 11% and 39 ± 11% decreases in the first and second components, respectively. The second negative component was greatly reduced, but not abolished, by 5 mM guanidine. Subsequent addition of 0.2 mM 3,4-diaminopyridine abolished the remainder of this component and revealed a positive component that was enhanced by addition of 1 mM TEA.

Guanidine (1 mM) also produced a selective reduction in the second negative component in the absence of Ca^{2+}. Thus, 1 mM guanidine reduced the second negative component by 30 – 35%, with no change in the first negative component. When tested in the presence of 0.4 mM 3,4-diaminopyridine, guanidine (1 – 10 mM) had little effect on the amplitude of the positive or negative components, but it increased the duration of the delayed negative component by 10 – 15%. Guanidine (1 – 5 mM) did not increase the

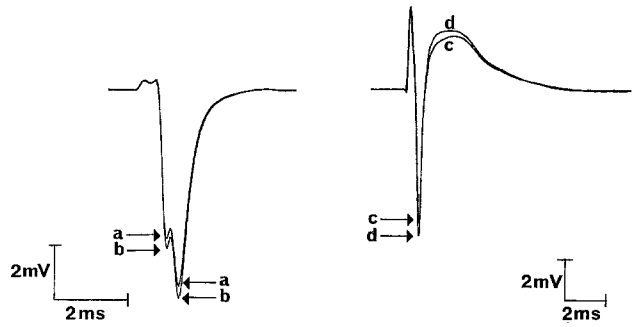

Fig. 5. Effect of noradrenaline on averaged perineural waveforms. *a:* Control; *b:* same site 2 min after 10 μM noradrenaline. *c:* Control waveforms recorded from a different preparation in the presence of 1 mM TEA and 200 μM 3,4-diaminopyridine; *d:* same site (as c) 10 min after 100 μM noradrenaline

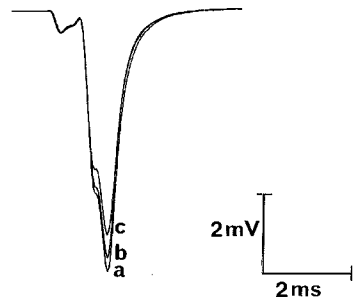

Fig. 6. Effect of phencyclidine on averaged perineural waveforms. *a:* Control; *b:* 15 min after 0.1 μM phencyclidine; *c:* 15 min after 10 μM phencyclidine

prolonged positive waveforms recorded in TEA plus 3,4-diaminopyridine.

Noradrenaline

10 μM Noradrenaline produced a small and short-lived increase (lasting about 10 min) in the amplitude of both negative components of the perineural waveform (Fig. 5a, b). The increase in the first negative spike averaged 6 ± 2%. Addition of 100 μM noradrenaline increased the amplitude of the waveform further, by 6 ± 3%, and the waveform remained increased for at least 20 min. No selective reduction in the amplitude of the second negative component was seen in the presence of noradrenaline (10 – 110 μM).

Similar results were obtained when the effects of noradrenaline were assessed in the presence of 0.2 mM 3,4-diaminopyridine and 1 mM TEA. 10 μM Noradrenaline caused a small increase (averaging 5%) in the initial negative component that was accompanied by an increase in the amplitude of the prolonged positive component; 110 μM noradrenaline produced a 9% increase in the negative component and a 15% increase in the delayed positive component (Fig. 5c, d).

Phencyclidine

At concentrations of 0.1 μM and above, phencyclidine slowly reduced the amplitudes of both negative components of the perineural waveform. Larger decreases occurred in the presence of 1, 10 and 100 μM phencyclidine (Fig. 6). The amplitude of the signal recovered after washout of the phencyclidine.

Discussion

Catechol (Otsuka and Nonomura 1963; Gallagher and Blaber 1973), guanidine (Otsuka and Endo 1960), noradrenaline (Jenkinson et al. 1968; Kuba 1970) and phencyclidine (Albuquerque et al. 1981) are known to be able to increase the amount of acetylcholine released in response to single nerve stimulation (i.e. the quantal content) in a variety of neuromuscular preparations. These effects are presumably a consequence of an increased influx of Ca^{2+} into nerve terminals during an action potential, but the underlying mechanisms are unclear. The main possibilities are a direct action on Ca^{2+} channels, an increase in the duration of action potentials following block of K^+ channels, and an overall increase in the amplitude of the nerve terminal action potential. By using the extracellular recording technique with mammalian motor nerves (Brigant and Mallart 1982; Mallart 1985a), these possibilities can be distinguished.

The compounds generally produced different changes to nerve terminal waveforms; hence, they will be discussed separately. Catechol and guanidine both caused a reduction in the second negative phase of the extracellularly recorded waveform. As the waveform recorded by an extracellular electrode is the algebraic sum of inward and outward currents in the vicinity of the electrode, this effect could be due to a decrease in the outward K^+ current or to an increase in the inward Ca^{2+} current at the nerve endings. A direct enhancement of the inward Ca^{2+} current by catechol or guanidine is unlikely because they still produced the same effect in the absence of external Ca^{2+}, and because neither compound affected the prolonged Ca^{2+} current seen after abolition of K^+ conductances by TEA and 3,4-diaminopyridine. Thus, the probable mechanism of action for catechol and guanidine is block of K^+ channels. As the compounds did not reduce the delayed negative waveform associated with Ca^{2+}-activated K^+ channels, they must be blocking voltage-dependent channels. This is consistent with the lack of effect of the compounds when the voltage-dependent K^+ channels have been blocked by prior addition of 3,4-diaminopyridine.

Catechol has been shown to block selectively a fast voltage-dependent K^+ current in cell bodies of neurones isolated from dorsal root ganglia of bull-frogs (Ito and Maeno 1986). As a consequence of this effect, the duration of action potentials increases in the presence of catechol. A similar effect at nerve terminals would allow the voltage-dependent Ca^{2+} channels to open for longer and hence, for more transmitter to be released in response to each action potential. However, catechol is unlikely to be acting selectively on a specific subpopulation of K^+ channels at the neuromuscular junction because it completely removes the component of perineural waveforms associated with voltage-dependent K^+ currents, and because it does not induce repetitive firing as shown by a blocker of transient K^+ channels (Anderson and Harvey 1988).

Guanidine was about 100 times less potent than catechol and its effects were poorly reversed by washing; this is in agreement with previous studies on acetylcholine release (Otsuka and Nonomura 1963; Kamenskaya et al. 1975). Although guanidine has been shown several times to increase the evoked release of acetylcholine at neuromuscular junctions while having little or no effect on spontaneous release (e.g. Otsuka and Endo 1960; Kamenskaya et al. 1975; Teräväinen and Larsen 1975), a number of mechanisms have been suggested. These include a lengthening of the duration of presynaptic action potentials (Kamenskaya et al. 1975), an effect on presynaptic binding sites for divalent cations (Teräväinen and Larsen 1975; Matthews and Wickelgren 1977), and an intracellular action to increase the availability of acetylcholine (Farley et al. 1979). Guanidine can broaden action potentials in mouse skeletal muscle fibres (Kamenskaya et al. 1975) and in giant axons of the lamprey (Matthews and Wickelgren 1977). The results from the present experiments are consistent with guanidine blocking K^+ currents in motor nerve terminals, an action that would lead to prolongation of the depolarization induced by action potentials. There was no evidence for actions directly on Ca^{2+} currents, which is consistent with the finding that guanidine has little or no effect on the amount of acetylcholine released by K^+-induced depolarization (Kamenskaya et al. 1975; Matthews and Wickelgren 1977).

Phencyclidine has been reported to increase quantal content in a frog neuromuscular preparation, and also to block some K^+ channels (Albuquerque et al. 1981; Tourneur et al. 1982; Blaustein and Ickowicz 1983; Bartschat and Blaustein 1986; Aguayo and Albuquerque 1987). Hence, it was of interest to determine if it had any effect on nerve terminal action potentials. It is not easy to study the prejunctional effects of phencyclidine with conventional electrophysiological approaches because its ability to block receptor ion channels obscures its action on acetylcholine release. However, phencyclidine reduced both negative components of the perineural waveform, indicating that it could affect Na^+ channels in addition to any action on K^+ channels. As any change in the operation of the neuronal Na^+ channels will alter the depolarization of the nerve terminals and hence the opening of the K^+ and Ca^{2+} channels, phencyclidine cannot be used as a tool to explore the possible subtypes of K^+ channels in mammalian motor nerve terminals. Previously, phencyclidine has been shown to block Na^+ channels as well as K^+ channels in neuroblastoma cells (Tourneur et al. 1982), in cardiac ventricular cells (Hadley and Hume 1986), and in spinal neurones in culture (Aguayo and Albuquerque 1987).

While the facilitatory effects of catechol and guanidine can be ascribed to block of K^+ conductances, the facilitatory actions of noradrenaline are harder to elucidate. Noradrenaline acts prejunctionally to increase the quantal content of e.p.p.s and frequency of m.e.p.p.s in frog and rat muscle preparations (Jenkinson et al. 1968; Kuba 1970). These effects are mediated by α-adrenoceptors because they are blocked by α- but not β-adrenoceptor antagonists. This is in contrast to the presynaptic facilitatory effects of catecholamines in sympathetic ganglia, where β-adrenoceptors are involved (Kuba and Kumamoto 1986).

Results from perineural recordings indicate that noradrenaline does not have a selective effect on Na^+ or K^+ currents. Focal extracellular recordings from neuromuscular junctions in the rat diaphragm preparation also showed that noradrenaline had little effect on the nerve terminal waveforms (Kuba 1970). Although the extracellular recording technique cannot be used to measure steady changes in resting membrane potential, the increased amplitude of all components of the perineural waveform found in the present experiments could be a result of hyperpolarization of the motor nerve ending. Such hyperpolarization would increase

quantal content (Hubbard and Willis 1962). Noradrenaline can cause hyperpolarization of frog motor neurones (Wohlberg et al. 1986). As has been pointed out by others, however, hyperpolarization would not be expected to be associated with an increase in m.e.p.p. frequency (Jenkinson et al. 1968; Kuba 1970). Perhaps this dilemma could be resolved if noradrenaline produced two separate effects: one on evoked transmitter release mediated by hyperpolarization, and another on spontaneous transmitter release, presumably via an increase in the free concentration of Ca^{2+} within the nerve terminal. The mechanisms underlying these effects remain unknown. As they are not mediated by β-adrenoceptors, they presumably do not involve increases in cyclic AMP; it is tempting to speculate that they may involve an increase in phosphatidyl inositol metabolism, as shown for α-adrenoceptors at other sites.

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