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Inhibitory effects of forskolin and papaverine on nerve conduction partially blocked by tetrodotoxin in the frog sciatic nerve

J.A. Ribeiro & A.M. Sebastião

Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781–Oeiras, Portugal

1 The effects of forskolin, sodium fluoride and papaverine on compound action potentials were investigated in de-sheathed sciatic nerve preparations of the frog.

2 Forskolin decreased in a concentration-dependent manner the amplitude of compound action potentials when nerve conduction was partially blocked by tetrodotoxin (TTX). In the presence of TTX a 50% decrease in the action potential amplitude recorded was obtained with about 2.5 μM forskolin.

3 Sodium fluoride did not modify the amplitude of compound action potentials partially blocked by TTX.

4 Papaverine also decreased the amplitude of compound action potentials partially inhibited by TTX. A 50% decrease in the action potential amplitude recorded in the presence of TTX was obtained with about 10 μM papaverine.

5 The possibility that cyclic AMP modulates axonal excitability by interfering with the entry of sodium through the TTX-sensitive sodium channel is discussed.

Introduction

In a recent paper Ribeiro & Sebastião (1984) produced evidence that R-type adenosine receptors positively coupled to adenylate cyclase are present in peripheral axons and suggested that the activation of these receptors causes a decrease in the entry of sodium during the depolarizing phase of the action potential. This idea is based on the findings that adenosine, adenosine analogues, dibutyryl cyclic AMP and theophylline decrease the amplitude of compound action potentials partially inhibited by tetrodotoxin (TTX) (Ribeiro & Sebastião, 1984), and that adenosine (Roch & Salamin, 1976) and theophylline (Horn & McAffee, 1977) increase cyclic AMP levels in axons. On the other hand, it has been shown that forskolin, a powerful and specific activator of adenylate cyclase (Seamon *et al.*, 1981) by acting directly at the catalytic subunit of the enzyme (see Daly, 1984), increases cyclic AMP levels in frog sciatic nerves (Kilmer & Carlsen, 1984). It therefore seemed of interest to investigate the effect of forskolin on nerve conduction in preparations partially blocked by TTX. The effect of papaverine, a non-xanthine phosphodiesterase inhibitor (Kukovetz & Poch, 1970), on TTX-induced axonal block was also investigated.

Methods

The experiments were carried out at room temperature (22–25°C) on the partially de-sheathed frog sciatic nerve trunk taken from autumn frogs (*Rana ridibunda*). The preparations were mounted in a Perspex chamber in which a Perspex block was fitted with electrodes for stimulating the nerve trunk and for recording the action potentials. The preparations were arranged so that the bathing solution or the solutions containing the drugs could be applied as pulses of 500 μl to the de-sheathed part of the trunk. The tissue as a whole was kept moist because the bottom of the chamber contained the bathing solution and the top was tightly sealed with paraffin wax to prevent evaporation. The technique used for the dissection was that described by the Staff of the Department of Pharmacology of the University of Edinburgh (1968). The nerve was stimulated supramaximally with rectangular pulses of 10 μs duration applied once every 5 s. Throughout the experiments compound action potentials were recorded in the conventional way and photographed.

Solutions and drugs

The bathing solution (pH = 7.0) contained (mM): NaCl 117, KCl 2.5, NaH_2PO_4 1, Na_2HPO_4 1, $MgCl_2$ 1.2 and $CaCl_2$ 1.8. Drugs used were tetrodotoxin (Sankyo), forskolin (Calbiochem), sodium fluoride (BDH) and papaverine (Sigma). Forskolin was made up into a 50 mM stock solution in absolute ethanol, stored in the refrigerator, and dilutions of this stock solution were made on the day of the experiments. The pH of the solutions was readjusted with NaOH where necessary.

Statistics

The significance of the differences between means was calcualted using Student's *t* test. *P* values of 0.05 or less were considered to represent significant differences.

Results

Forskolin

Figure 1 illustrates the effect of forskolin (1–5 μM) on the amplitude and duration of a compound action potential recorded from a frog sciatic nerve. As can be seen, forskolin enhanced in a concentration-dependent manner the inhibitory effect of TTX (80 nM) on action potential amplitude (see also Figure 3). The effect of forskolin could not be attributed to its solvent, ethanol, since the maximum concentration of ethanol (0.02% v/v) present in the forskolin solutions applied to the nerves was inactive on TTX-induced axonal blockade. The full effect of forskolin was usually seen in the first 5–15 min that followed its application to the nerves and was washed out in about 60 min, i.e., following forskolin, TTX decreased the action potential amplitude in a similar way to that observed before using forskolin (Figure 2). This prolonged time for washing out a functional action of forskolin conforms with the results obtained by others (Ram, 1983).

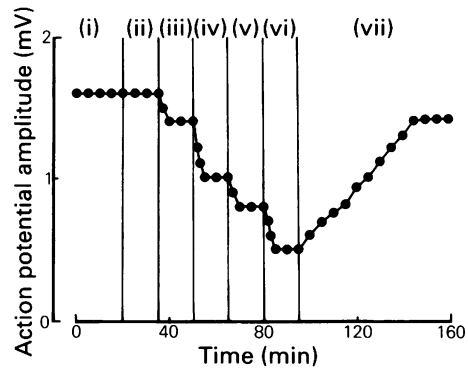

Figure 2 Time course of the effects of different concentrations of forskolin on the amplitude of compound action potentials recorded from a frog sciatic nerve. (i) and (vii) Tetrodotoxin (TTX, (60 nM); (ii) TTX 60 nM) + forskolin (0.5 μM); (iii) TTX (60 nM) + forskolin (1 μM); (iv) TTX (60 nM) + forskolin (2.5 μM); (v) TTX (60 nM) + forskolin (5 μM); (vi) TTX (60 nM) + forskolin (10 μM). Compound action potential amplitude in the bathing solution before applying TTX was 8.8 mV and its value 20 min after (vii) was 8.0 mV.

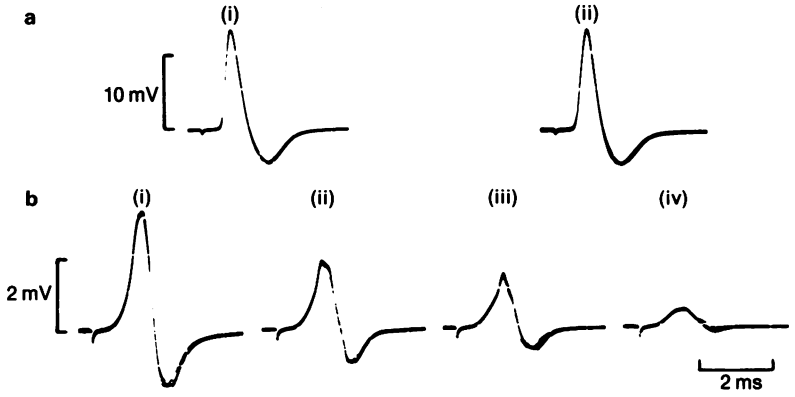

Figure 1 Effect of forskolin on the amplitude and duration of a compound action potential recorded from a frog sciatic nerve: (a) (i) in the bathing solution, before applying tetrodotoxin (TTX) (ii) 30 min after returning to a drug-free bathing solution; (b) (i) effect after 60 min in TTX (80 nM), (ii) effect after 15 min in TTX (80 nM) + fosrskolin (1 μM), (iii) after 15 min in TTX (80 nM) + forskolin (2.5 μM), (iv) after 15 min in TTX (80 nM) + forskolin (5 μM). Each trace consists of six superimposed action potentials. Note the different vertical calibration in (a) and (b). The horizontal calibration refers to both (a) and (b).

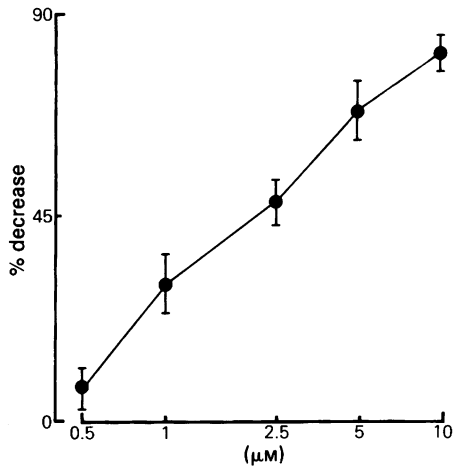

Figure 3 Concentration-response curve for the effect of forskolin on tetrodotoxin (TTX)-induced axonal blockade. The ordinates are percentage decreases in the amplitude of compound action potentials recorded in the presence of TTX 45–80 nM (average action potential amplitude in the presence of TTX was 22 ± 2.4% of the action potential amplitude in the bathing solution). 0% is the action potential amplitude in the presence of TTX, and 100% represents a complete inhibition of action potential amplitude. The vertical bars represent ± s.e.mean. Each point is the average of 5 experiments. The effects are significantly different ($P < 0.05$) from the control (action potential amplitude in TTX) for concentrations of forskolin > 1 μM. Average action potential amplitude in the bathing solution 7.7 ± 1.5 mV.

The concentration-response curve for the inhibitory effect of forskolin (0.5–10 μM) on the amplitude of action potentials partially blocked by TTX (45–80 nM) is shown in Figure 3. The amplitude of the action potentials was inhibited by TTX to 22 ± 2.4% of its initial amplitude; in these conditions, a 50% decrease in the action potential amplitude recorded in the presence of TTX was obtained with about 2.5 μM forskolin (Figure 3).

Forskolin (2.5–25 μM) was without effect on action potential amplitude when applied to axons in the absence of TTX (two experiments).

Sodium fluoride

Fluoride ions activate adenylate cyclase by acting at the regulatory subunit (N-subunit) of the adenylate cyclase (Downs *et al.*, 1980). We decided therefore to test whether sodium fluoride(NaF)affected the axonal blockade induced by TTX. In three experiments in which the amplitude of the action potentials was inhibited by TTX (60–130 nM) to 20 ± 2.8% of their intial value in the bathing solution, NaF (2.5–10 mM) was ineffective on the TTX-induced axonal blockade.

Papaverine

The effect of papaverine (1–25 μM) on the amplitude and duration of a compound action potential recorded from a frog sciatic nerve is illustrated in Figure 4. Papaverine enhanced in a concentration-dependent manner the inhibitory action of TTX on nerve conduction, and the full effect of papaverine was usually seen within 5–15 min after its application to the nerves. The action of papaverine persisted for more than 30 min after returning the preparations to a TTX solution without papaverine; this contrasts with the action of other phosphodiesterase inhibitors (theophylline, caffeine and isobutylmethylxanthine) which can be washed out within that period of time (cf. Ribeiro & sebastião, 1984).

Figure 5 shows the concentration-response curve for the inhibitory action of papaverine (1–25 μM) on the amplitude of compound action potentials partially blocked by TTX (30–80 nM). As can be seen, a 50% decrease in the action potential amplitude recorded in the presence of these concentrations of TTX was obtained with about 10 μM papaverine.

As with forskolin, papaverine (5–25 μM) had no

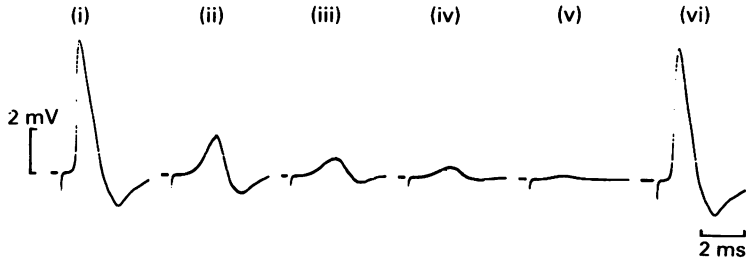

Figure 4 Effect of papaverine on the amplitude and duration of a compound action potential recorded from a frog sciatic nerve: (i) before applying tetrodotoxin (TTX); (ii) effect after 30 min in TTX (80 nM); (iii) effect after 10 min in TTX (80 nM) + papaverine (1 μM); (iv) after 10 min in TTX (80 nM) + papaverine (5 μM); (v) after 10 min in TTX (80 nM) + papaverine (25 μM); (vi) 40 min after returning to a drug-free bathing solution. Each trace consists of six superimposed action potentials.

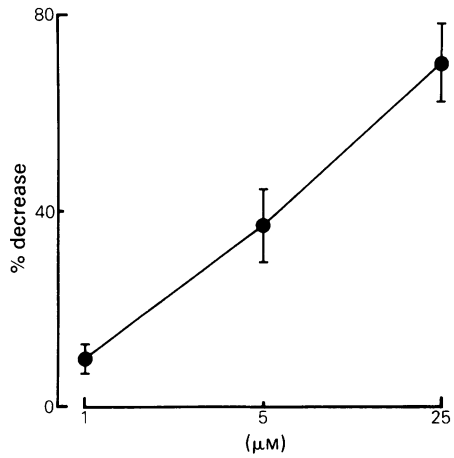

Figure 5 Concentration-response curve for the effect of papaverine on tetrodotoxin (TTX)-induced axonal blockade. The ordinates are percentage decreases in the amplitude of compound action potentials recorded in the presence of TTX 30–80 nM (average action potential amplitude in the presence of TTX was 36 ± 4.3% of the action potential amplitude in the bathing solution). 0% is the action potential amplitude in the presence of TTX and 100% represents a complete inhibition of action potential amplitude. The vertical bars represent ± s.e.mean. Each point is the average of 7 experiments. The effects are significantly different ($P < 0.05$) from the control (action potential amplitude in TTX) for the three concentrations of papaverine. Average action potential amplitude in the bathing solution 4.3 ± 1.3 mV.

effect on action potential amplitude in TTX-free solutions.

Discussion

The present results show that forskolin and papaverine decrease the amplitude of action potentials when nerve conduction is partially inhibited by TTX. The specificity of forskolin as an activator of adenylate cyclase (see Daly, 1984) and the finding that this diterpene, in concentrations similar to those used in the present work, increases cyclic AMP levels in frog sciatic nerves (Kilmer & Carlsen, 1984), strongly suggest that the enhancement of TTX-induced axonal block, caused by forskolin, is mediated by increases in intra-axonal cyclic AMP content. The enhancement of TTX-induced axonal block caused by papaverine may be attributed to its ability to inhibit phosphodiesterases (Kukovetz & Poch, 1970) and thus to increase cyclic AMP levels. However, papaverine is also an inhibitor of the uptake of adenosine (Huang & Daly, 1974), which is released from nerve fibres on electrical stimulation (Maire *et al.*, 1982) and enhances TTX-induced axonal block (Ribeiro & Sebastião, 1984). Thus one cannot preclude the possibility that a papaverine-induced inhibition of adenosine uptake by axonal membranes contributes to the inhibitory action of papaverine on nerve conduction observed in these experiments.

NaF was ineffective on the TTX-induced axonal block. This might indicate that the N-subunit of adenylate cyclase does not contribute to the regulation of cyclic AMP levels in axons. However, it appears that NaF does not activate adenylate cyclase in intact cells (Perkins, 1973) which might explain its absence of effect on the TTX-induced axonal blockade.

The results obtained in the present work, taken together with the findings that the α-subunit of the voltage-sensitive sodium channel can be selectively phosphorylated by a cyclic AMP-dependent protein kinase (Costa *et al.*, 1982) and that the incubation of synaptosomes under phosphorylating conditions causes a decrease in veratridine-stimulated ^{22}Na uptake (Costa & Catterall, 1984), reinforce the idea that increases in cyclic AMP levels in peripheral nerves may modulate axonal excitability by decreasing the entry of sodium through the voltage-sensitive sodium channels (Ribeiro & Sebastião, 1984). This hypothesis is consistent with the theoretical model proposed by Schoffeniels & Dandrifosse (1980) according to which the proteins that control sodium conductance of the axonal membrane can assume two states: the phosphorylated state with low conductance and the dephosphorylated state with high conductance.

In the squid axon the maximum sodium conductance of the membrane ($\bar{g}_{Na}$) was estimated to be about 120 mmho cm^{-2} (see Hodgkin & Huxley, 1952, Table 3), and the sodium conductance (g_{Na}) at the peak of the action potential to be about 30 mmho cm^{-2} (see Hodgkin & Huxley, 1952, Figure 17), which is 25% of the maximum membrane sodium conductance. Assuming that each sodium channel is activated in an all or none fashion, 25% of the maximum sodium conductance at the peak of an action potential implies that only 25% of the total number of the voltage-sensitive sodium channels need to be activated in order to produce a full action potential (see also Schoffeniels & Dandrifosse, 1980). If this applies to the frog axons, the existence of spare voltage-dependent sodium channels in the axonal membranes might explain why it is necessary to decrease the amplitude of the action potentials with TTX, a specific blocker of the voltage-sensitive sodium channels (Narahashi *et al.*, 1964), in order to detect the inhibitory action of dibutyryl cyclic AMP, adenosine, methylxanthines (Ribeiro & Sebastião, 1984), forskolin and papaverine (present work) on nerve conduction.

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