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A selective inhibitor of cAMP-specific phosphodiesterase, Ro 20-1724, has no effect on the quantal release of acetylcholine from the mouse phrenic nerve

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Abstract—The indirect twitch response of the mouse isolated phrenic nerve-diaphragm preparation, partially paralysed by tubocurarine, was restored only by about 10% by Ro 20-1724 at 2 to 280 μM. The solvent vehicle, dimethylsulphoxide, also showed the same effect to a similar extent. Intracellular recordings with glass microelectrodes revealed that Ro 20-1724 (40 μM) affected neither the resting membrane potential, the amplitude and frequency of miniature endplate potentials nor the amplitude of nerve-impulse evoked endplate potentials recorded in curarized preparations. The result indicates that Ro 20-1724 at a concentration four times the IC50 of phosphodiesterase inhibition has no effect on the quantal release of acetylcholine from a mammalian motor nerve and suggests that cAMP has no modulatory effect on the transmitter release.

It has been proposed that adenosine 3′,5′-cyclic monophosphate (cAMP) mediates or modulates the neuronal activity and release of neurotransmitter in sympathetic ganglia, cerebellum and probably also in other neural systems (see Briggs & McAfee 1982). In the vertebrate skeletal muscle neuromuscular junction, an enhancement of the frequency of miniature endplate potentials (m.e.p.p's) or the quantal content of evoked end-plate potentials (e.p.p's) and an induction of antidromic repetitive nerve activity by lipid soluble analogues of cAMP, adenylate cyclase activators and by phosphodiesterase (PDE) inhibitors have been taken as evidence to support the physiological role of cAMP (Goldberg & Singer 1969; Jacobs & Shinnick 1973; Jacobs & McNiece 1977; Singer & Goldberg 1970; Standaert et al 1976; Standaert & Dretchen 1979, 1981; Wilson 1974). However, this view is not generally accepted (Ginsborg & Hirst 1972; Häggblad & Heilbron 1983; Quastel & Hackett 1971). On the other hand, Silinsky & Vogel (1986) found that an adenylated cyclase inhibitor (MDL 12,330A; *N*-(*cis*-2-phenylcyclopentyl)azacyclo-tridecan-2-imine HCl) increased both m.e.p.p. frequency and evoked quantal release of acetylcholine and arrived at the opposite conclusion that an increase in cAMP at a strategic cellular locus decreases ACh release (Silinsky 1984). Since most of the pharmacological agents used are either methylxanthines or aminopyridine derivatives, which have multiple pharmacological actions, the pharmacological effects observed are not necessarily the result of the intervention of cAMP.

In this experiment, we studied the effect of Ro 20-1724 ((±)-4-(3-butoxy-4-methoxybenzyl)-2-imiadazolidinone), a selective inhibitor of the cAMP-specific low K_m (high affinity) PDE but with chemical structure different from others so far studied (Bergstrand et al 1977; Prasad et al 1975) on the quantal release of ACh in the mouse isolated phrenic nerve-diaphragm preparations.

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Materials and methods

The phrenic nerve-diaphragm preparation, isolated from 20–25 g ICR mice of either sex, was incubated in 20 mL Tyrode solution (mM: NaCl 137, KCl 2·8, $CaCl_2$ 1·8, $MgCl_2$ 1·2, $NaHCO_3$ 11·9, NaH_2PO_4 0·33, glucose 11·2) at 37 ± 0·5 °C and oxygenated with 95% O_2 and 5% CO_2. Indirect twitch responses were evoked by stimulation of the nerve with supramaximal single pulses (0·05 ms) at 0·1 Hz and recorded isometrically on a Gould recorder (2200s) via a force-displacement transducer (BG25).

Intracellular recordings were performed with 5 to 10 MΩ glass microelectrodes filled with 3 M KCl. Resting membrane potential, e.p.p's evoked at 1 Hz in tubocurarine (2·5 μM)-immobilized preparations and m.e.p.p.'s in normal Tyrode solution were recorded and analysed by a computer program. Ro 20-1724 was kindly supplied by Drs H. Gutman and P. Wever, Hofmann-La Roche & Co., Basel, and dissolved in dimethylsulphoxide at 80 mM, as a stock solution.

Data are shown as mean ± s.e. and were analysed by Student's *t*-test.

Results

Effects on the twitch response. Ro 20-1724 had no effect on the twitch response to indirect stimulation (0·1 Hz) of the normal mouse phrenic nerve-diaphragm preparation. When the preparation was partially blocked with tubocurarine to sensitize the neuromuscular transmission to the effect of pharmacological agents, Ro 20-1724 added at 40 μM to the organ bath slightly restored the twitch response followed by a depression in 1 to 2 min and then regained the facilitation by about 12% in 30 min (Fig. 1). When only dimethylsulphoxide was added to the organ bath at final concentration of 0·05% v/v, equivalent to that used for solution of Ro 20-1724, the same facilitatory

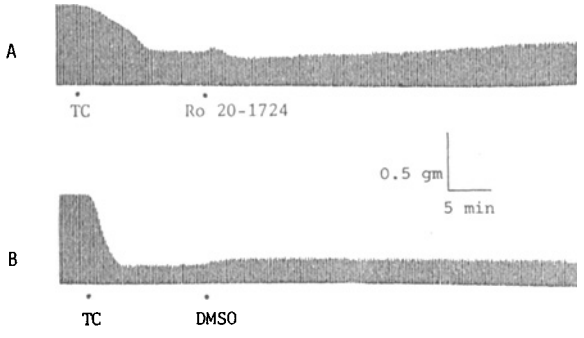

FIG. 1. Effects of Ro 20-1724 and vehicle on the indirect twitch response of curarized mouse phrenic nerve-diaphragms. The indirect twitch response was evoked by stimulation of the nerve at 0·1 Hz and first partially paralysed by (+)-tubocurarine (Tc; 0·86 μM), Ro 20-1724 (40 μM) restored the response to the same extent as dimethylsulphoxide (DMSO) (0·05%) at steady state.

effect, except for the temporary depression, was observed. No appreciable effect due to Ro 20-1724 was discernible at the range of concentrations tested, 2 to 280 μM.

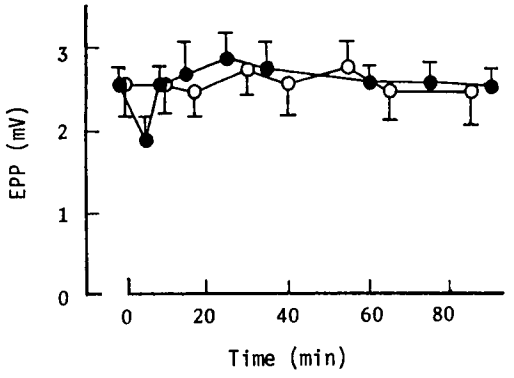

FIG. 2. The time course of the effects of Ro 20-1724 and vehicle on the amplitude of e.p.p.'s. The e.p.p.'s were evoked at 1 Hz in (+)-tubocurarine (2·5 μM) immobilized muscles. The concentrations used were 40 μM for Ro 20-1724 (●) and 0·05% for dimethylsulphoxide (○).

Effect on the quantal release. Neither the resting membrane potential nor the amplitude or frequency of m.e.p.p's was affected by Ro 20-1724 or dimethylsulphoxide, indicating that postsynaptic site is not functionally affected. The amplitude of e.p.p.'s evoked at 1 Hz in the presence of tubocurarine to immobilize the muscle was also unaffected by dimethylsulphoxide (0·05% v/v). Ro 20-1724 (40 μM) caused a slight transient depression of e.p.p. amplitude which regained the original value thereafter for more than 60 min (Fig. 2). Evidently, Ro 20-1724 did not have the capacity to increase the release of transmitter from the motor nerve terminal.

Discussion

cAMP-specific PDE has been demonstrated in the axon of mammalian sciatic nerve and is transported to the nerve terminal by rapid axonal transport (Bray et al 1971; Curley et al 1984). Ro 20-1724 inhibits this enzyme selectively with an IC50 of 9 μM (Bergstrand et al 1977). One may therefore anticipate a reasonable inhibition of the cAMP-specific PDE in motor nerve terminals in the present experiment at 40 to 280 μM, 4 to 30 times higher than the IC50 to result in an accumulation of cAMP. In contrast to most other pharmacological agents used for elucidating the role of cAMP in the motor nerve terminal, Ro 20-1724 is devoid of effect except for the transient depression in the present experiment after the correction of the effect due to dimethylsulphoxide. An increase of quantal release, however, was reported by Dryden et al (1984) in the same muscle preparation. Another batch of Ro 20-1724 was kindly provided by Hoffman-La Roche & Co and also was proved ineffective.

Ro 20-1724 and most other agents used in previous experiments are practically water insoluble and have to be dissolved first in organic solvents. Some organic solvents such as ethanol and dimethylsulphoxide, however, have been known to facilitate acetylcholine release at the neuromuscular junction (Gage 1965; Gandiha & Marshall 1972; Geron & Meiri 1985). Such an effect with the vehicle might be unrecognized.

Methylxanthine derivatives are the most widely used inhibitors of PDE. It is known, however, that methylxanthines act as adenosine receptor antagonists at a concentration much lower than that as PDE inhibitors. The adenosine receptor antagonist has been shown to increase acetylcholine release by antagonizing the effect of endogenous adenosine (Chiou et al 1987). In addition, methylxanthines also have significant effect on intracellular Ca^{2+} metabolism (Tsien 1977). Etazolate (SQ 20009), a non-xanthine PDE inhibitor containing an aminopyridine moiety, was shown to enhance markedly the transmitter release (Jacob & McNiece 1977). The mechanism underlying this effect, however, could be attributed to an aminopyridine like effect rather than to an inhibition of PDE (Chang et al unpublished).

In conclusion, the ineffectiveness of the selective inhibitor of high affinity, cAMP-specific PDE, Ro 20-1724, which has a chemical structure different from other PDE inhibitor, suggests that cAMP is not involved in the regulation of ACh release from the vertebrate neuromuscular system.

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