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Action of mefloquine on toad isolated rectus abdominis muscle

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Abstract—The effects of mefloquine and quinine on acetylcholine-induced contractures of the toad rectus abdominis muscle have been investigated. Both drugs depressed acetylcholine-induced contractures in a dose-related and non-competitive manner. A partial reversal of the block was observed in the presence of 4-aminopyridine (10, 50 μM) and increased Ca^{2+} content (1·25 ×) of the Ringer solution. In both cases, the mefloquine-induced blockade was more readily reversed than that induced by quinine. Mefloquine and quinine at concentrations greater than 100 and 500 μM, respectively, also elicited a direct contractile response on the muscle. Quantitative differences in their contractile activity have been attributed to the greater lipid solubility and tissue binding affinity of mefloquine.

Mefloquine, a 4-quinolinemethanol derivative structurally related to quinine, is a highly effective antimalarial agent which has been found to be active against chloroquine-resistant strains of *Plasmodium falciparum* (Doberstyn et al 1979). Quinine has been shown to interfere with the contractile activity of skeletal muscle (Grewal & Sharma 1960; Isaacson et al 1970). Whether mefloquine affects muscle in the same way is not known, although it has been noted to cause muscle weakness during antimalarial therapy (Hall et al 1977). We have made a comparative study on the actions of mefloquine and quinine on the toad isolated rectus abdominis muscle. As to be expected from their chemical identity, both drugs affect the muscle in a similar way but quantitative differences are observed.

Methods

The toad (*Bufo melanostictus*) isolated rectus abdominis muscle was mounted in a bath containing Ringer solution of the following composition (mM): NaCl 111, KCl 1·9, $CaCl_2$ 1·4, NaH_2PO_4 0·083, $NaHCO_3$ 2·4, and glucose 0·55. The tissue was aerated with 5% carbon dioxide in oxygen at room temperature (28°C) and equilibrated for 45 min before being challenged with drugs.

The cumulative dose-response curves for acetylcholine added at 90 s intervals were obtained in the absence or presence of mefloquine or quinine added after a 30 min rest. After a further 30 min rest, a final dose-response curve was obtained. No more than three cumulative dose-response curves were obtained with each preparation. Three consecutive control cumulative dose-response curves were also obtained to assess the viability of the tissue. For construction of the cumulative log concentration-response curves, all responses were expressed as a percentage of the maximum height of the control curve.

To assess the effect of increased Ca^{2+}, the Ca^{2+} content of the Ringer solution was increased 1·25× to 1·8 mM and the cumulative curves were obtained as before in the presence of mefloquine (10 μM) or quinine (50 μM). Three consecutive control cumulative dose-response curves were also obtained.

To assess the effect of 4-aminopyridine, the tissue was exposed to the drug (10 or 50 μM) for 10 min after which the cumulative dose-response curve was obtained. After a 30 min rest, 4-aminopyridine and inhibitor were added to the bath and the cumulative dose-response curve repeated. A final curve was obtained 30 min later in the presence of 4-aminopyridine alone. Controls were three successive cumulative dose-response curves obtained in the presence of 4-aminopyridine, with the usual 30 min rest between determinations.

To assess the contractile activity of mefloquine and quinine on the muscle, their cumulative dose response curves were obtained using a 90 s contact time between each addition. In both cases, the maximum response of the muscle could not be recorded because of the limited aqueous solubility of the inhibitors. Thus, the responses were expressed as a percentage of the height attained with the highest concentration of the inhibitor that could be obtained in solution. Two more cumulative dose-responses were repeated subsequently on the same tissue with the usual 30 min rest period to assess muscle responsiveness.

Solutions of erythro-DL-mefloquine hydrochloride (Roche Pharmaceuticals), quinine sulphate BP (Pharmaceutical Department, Republic of Singapore), 4-aminopyridine (98%, Aldrich Chemical Company) and acetylcholine iodide (Sigma Chemical Company) were prepared in distilled water. Appropriate dilutions were made from these solutions. All other chemicals were of analytical grade.

Results

The cumulative log concentration-response curves for acetylcholine in the absence and presence of mefloquine (1, 10, 100, 300 μM) and quinine (10, 50, 100, 1000 μM) showed that both drugs depressed the acetylcholine-induced contractures in a dose related manner and caused the curves to be displaced to the right in a non-parallel manner. That the observed decrease in the maximal response was due to an effect of mefloquine and quinine was validated by a comparison with the three control curves obtained on the same tissue in the absence of inhibitors. These control experiments showed a corresponding decrease of 11·3% (s.e.m. 2·6%) and 14·4% (s.e.m. 2·6%) in the maximal responses of the 2nd and 3rd curves, respectively (n = 6–13 experiments).

An anomaly was noted in the cumulative log concentration-response curves obtained in the presence of mefloquine (100, 300 μM) and quinine (1000 μM) in that the % maximal responses elicited at low acetylcholine concentrations (< 500 μM) were higher than those observed at lower inhibitor concentrations,

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probably as a consequence of the direct contractile effects of mefloquine and quinine at these concentrations.

When the acetylcholine curve in the presence of either 10 μM mefloquine or 50 μM quinine was repeated in Ringer solution of higher (1·25×) Ca^{2+} content, a shift to the left of the control curve (normal Ca^{2+}) with an increase in the maximal response was observed. For mefloquine, the increase in Ca^{2+} content caused the maximal response to rise by 9·1% to 70·3% (s.e.m. 2·8%), for quinine the increase was 7·6% (s.e.m. 4·1%). Control experiments in the Ringer with increased Ca^{2+} showed that the decrease in maximal response (11·3%, s.e.m. 3·66%) was similar to that observed in normal Ringer (n = 6 experiments).

Like increased Ca^{2+}, 4-aminopyridine (10, 50 μM) caused the agonist/antagonist dose-response curve obtained to be displaced to the left of the control (no 4-aminopyridine) with a corresponding increase in the maximal response. For mefloquine, the maximal response in the presence of 10 and 50 μM 4-aminopyridine was 68·1% (s.e.m. 3·4%) and 78% (s.e.m. 3·6%), respectively, corresponding to rises of 6·9% and 16·9% over that of the control. A smaller increase (4·8%, s.e.m. 4·0%) occurred with quinine in the presence of 50 μM 4-aminopyridine (n = 7–16 experiments).

At 10 and 50 μM, 4-aminopyridine did not show any contractile effect on the muscle. Control curves in the presence of 10 μM 4-aminopyridine showed the same loss in maximal response (10·1%, s.e.m. 6·6%) as those obtained in its absence. A greater loss in maximal response (17·2%, s.e.m. 2·1%) was observed with 50 μM 4-aminopyridine. Correction of the results obtained in the presence of quinine or mefloquine for this loss would mean that a greater potentiating effect may be attributed to 50 μM 4-aminopyridine.

Fig. 1 shows the cumulative log concentration-response curves of mefloquine and quinine on the toad rectus abdominis muscle. At 500 μM mefloquine, a contraction height corresponding to 45% of the maximum was observed whereas quinine at the same concentration only caused 7·5% contraction. Thus mefloquine exerts a more potent contractile effect than quinine. Moreover, when the cumulative dose-response curves were repeated a 2nd and 3rd time on the same muscle, the responsiveness of the tissue to mefloquine diminished sharply, with the maximal contraction declining to 3·8% (s.e.m. 2·9%) in the final round. In the case of quinine, a smaller loss in muscle response (56·4%, s.e.m. 10·9%) was observed.

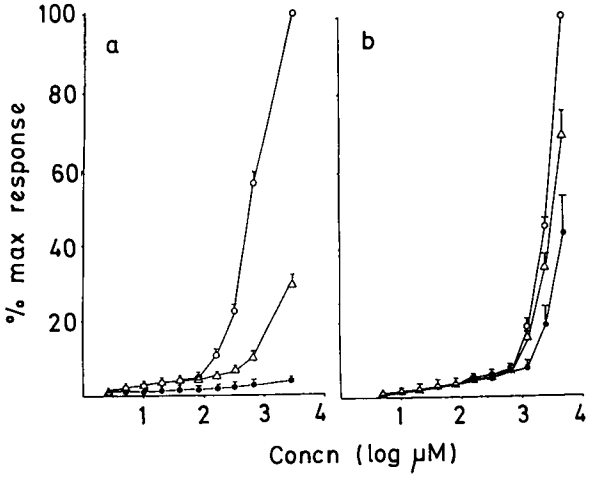

FIG. 1. Cumulative log concentration-response curves for (a) mefloquine-induced and (b) quinine-induced contractures of the toad rectus abdominis muscle. 1st (○), 2nd (△) and 3rd (●) cumulative log concentration-response curves, each done on the same muscle with 30 min interval between each determination are shown (n = 6 experiments).

Discussion

Both mefloquine and quinine depressed the acetylcholine-induced contractures of the toad rectus abdominis, the blockade being characterized by a concentration-dependent and non-parallel shift of the log concentration-response curves to the right of the control, as well as a marked decline in the maximal response. These features of antagonism are those of the non-competitive type and there is a possibility that ion-channel blockade (see Dreyer 1982 for refs) may be involved, bearing in mind that mefloquine, which has been shown to be a stronger anticholinesterase than quinine (Ngiam & Go 1987), was also found by us to be the stronger antagonist.

The blockade imposed by mefloquine and quinine could be partially reversed by increasing the calcium content of the Ringer or by 4-aminopyridine. The reversal effect of calcium has also been reported for chloroquine (Ette et al 1981). It is unlikely that the 4-aminopyridine effect could be due to its action on acetylcholine release at nerve terminals since the muscle was responding to exogenous acetylcholine. A more likely possibility would be its direct enhancement of the excitation-contraction coupling mechanism of the multiply innervated muscle fibres in response to added acetylcholine, not unlike that reported by Khan & Edman (1979) in which 4-aminopyridine potentiated the twitch response of single skeletal muscle fibres from frogs or rats.

At higher concentrations of mefloquine (100 μM) and quinine (500 μM) both drugs elicited a direct contractile response on the muscle. The contractile action of quinine on skeletal muscle is well known and has been attributed to its action on the sarcoplasmic reticulum of causing a release of calcium or inhibition of the calcium transport system, thereby raising the free myoplasmic calcium concentration (Isaacson & Sandow 1967; Gattass & de Meis 1978). Mefloquine may act similarly. The quantitative differences between these two antimalarials could be attributed to the higher lipid solubility of mefloquine (Mu et al 1975) and its marked binding affinity to membrane phospholipids (Chevli & Fitch 1982).

Serum concentrations attained after therapeutic use of mefloquine (Schwartz & Jauch 1982) are comparable with the in-vitro concentrations used in our study. This neuromuscular blocking action could explain the adverse effect of muscle weakness associated with mefloquine and quinine (Martindale 1982).

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