QUINACRINE IS A PROSTAGLANDIN ANTAGONIST

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Summary: Quinacrine, an anti-malarial with local anaesthetic properties, because of its fluorescence characteristics and its ability to combine with chromosomes and biological membranes has been widely used as a "probe". The sites with which it combines in Torpedo marmorata electric organs have many of the characteristics of specific receptors. Using rat vascular and gastric smooth muscle we have shown that quinacrine can competitively antagonise the actions of prostaglandin E2. We suggest that the biological sites to which quinacrine binds can normally be occupied by prostaglandins.

Introduction

Quinacrine is an anti-malarial drug with local anaesthetic properties (1). Its fluorescence characteristics, its ability to bind to chromosomes (2.3) and to other membranes (4,5) and its inhibition of oxidative phosphorylation and mitochondrial ATPase activity (6,7) have led to its extensive use as a chromosome and membrane "probe". Using the electric organs of Torpedo marmorata, Grunhagen & Changeux (8,9) have shown that quinacrine binds to a specific allosteric receptor which can modulate the behaviour of the cholinergic receptor site. Surprisingly little interest has been shown in the question as to what natural compounds might be capable of combining with the sites to which quinacrine becomes attached. We have shown that a group of anti-malarial and/or local anaesthetic substances (including chloroquine, quinine and procaine) behave as competitive prostaglandin antagonists (10,11,12). Quinacrine is related to chloroquine and quinine both in structure and properties. It too is a prostaglandin antagonist. We propose that the membrane sites, and possibly the nucleic acid sites with which quinacrine interacts are normally sites to which prostaglandins can bind.

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Materials and Methods

Most of the experiments were carried out on the perfused superior mesenteric artery of the male rat as previously described in detail (13,14). Under ether anaesthesia the artery was cannulated and its vascular bed dissected out and mounted in an organ bath. Using a peristaltic pump the preparation was perfused with Krebs-bicarbonate buffer at a flow rate of 3 ml/minute. Perfusion pressure was recorded via a side arm off the arterial cannula. Test injections into another side arm of pressor agents dissolved in 0.1 ml buffer caused brief vasoconstriction and hence elevation of pressure. We have previously shown that both the baseline pressure and the responses to a fixed dose of pressor agent remain stable for many hours. Actual doses of pressor agents used, each sufficient to give a 40-60% maximal pressor response were 10 ng noradrenaline as the bitartrate, 7 micromol of potassium chloride, 1 ng of angiotensin II and 25 ng of synthetic arginine vasopressin. The pressor effect of potassium injections is not dependent on release of noradrenaline from nerve endings since it persists even after complete abolition of adrenergic effects by phenoxybenzamine. Responses to potassium and vasopressin are rapidly abolished by the use of a calcium-free buffer and presumably depend on stimulation of calcium entry from outside. Responses to noradrenaline and angiotensin remain at 60-80% of their original level on removal of extracellular calcium and presumably largely depend on calcium release from intracellular stores. In two experiments the effects of quinacrine on contractile responses of rat stomach strips were investigated.

We tested the actions of quinacrine on pressor responses to noradrenaline, and potassium ions (6 experiments each) and to vasopressin and angiotensin (2 experiments each). Once the preparation had stabilized, three test injections of the pressor agent to be used were given: the mean amplitude of these responses was taken as 100% and subsequent results were expressed as percentages of this. Progressively increasing concentrations of quinacrine were then added to the buffer each being present for 10 minutes: at the end of that time the response to the same fixed dose of the pressor agent being used was tested. Preliminary experiments had shown that changes in response to a given concentration of quinacrine reached a plateau within 5 minutes and thereafter no changes occurred in spite of maintenance of the same concentration of quinacrine for prolonged periods.

Results

Within the 10^{-6}M range quinacrine caused a weak but consistent increase in the amplitude of pressor responses but in the 10^{-5}M range consistent inhibition was obtained (fig. 1). Quinacrine was rather more effective against noradrenaline than against potassium. In two experiments each we showed that the inhibition of angiotensin responses was similar to that of noradrenaline ones while that of vasopressin responses was similar to that of potassium ones.

We have previously shown that a characteristic of the inhibitory actions of prostaglandin antagonists on vasoconstriction is that the adding of

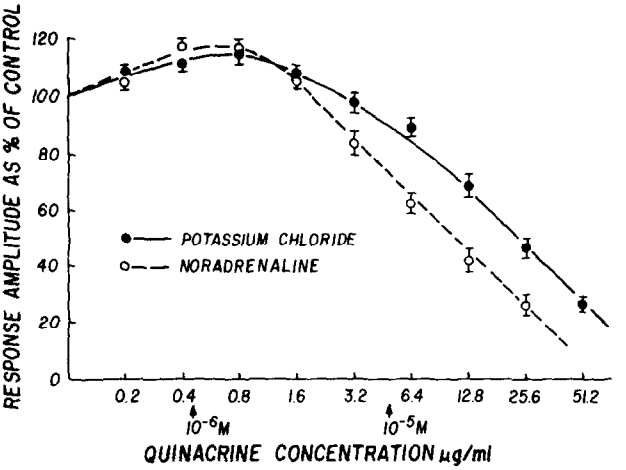

Fig. 1: The effects of increasing concentrations of quinacrine in the perfusion fluid on the amplitude of the pressor responses to fixed doses of noradrenaline (10 ng) and potassium ions (7 µmol) injected in 0.1 ml buffer. Results are expressed as percentages of the responses to three pressor injections prior to addition of quinacrine to the buffer. Each pressor agent was tested on 6 preparations and the results show the mean ±SEM at each point.

indomethacin (5 µg/ml) to the buffer partially to inhibit endogenous prostaglandin synthesis moves the inhibitory line to the left while the addition of exogenous prostaglandin E2 (5 ng/ml) pushes it to the right (10,15). In three experiments each (results not shown) using noradrenaline as the pressor agent we demonstrated that the inhibitory action of quinacrine is influenced in a similar way by indomethacin and prostaglandin E2. Finally we completely blocked both endogenous prostaglandin synthesis and vascular responsiveness by adding a high concentration of indomethacin (64 µg/ml) to the buffer. Responses to noradrenaline were then partially restored by adding either 1 or 5 ng/ml prostaglandin E2 to the buffer. At this stage three test injections of noradrenaline were given and quinacrine added to the buffer in increasing concentrations as before. Comparing the linear sections of the graph (fig. 2) it can be seen that the inhibitory line with 5 ng/ml prostaglandin E2 is parallel to and to the right of that with 1 ng/ml.

Isolated strips of rat stomach were mounted in an organ bath and

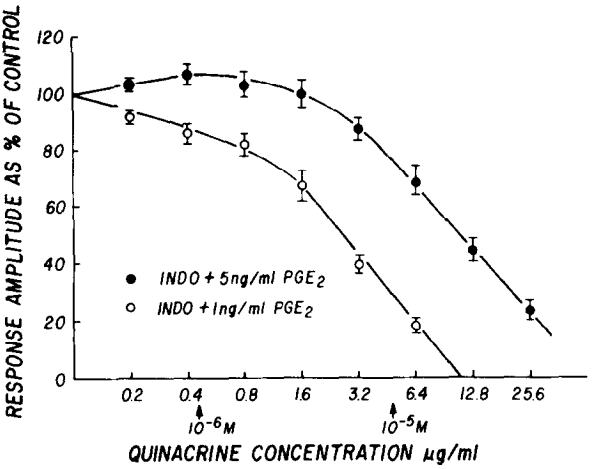

Fig. 2: The effects of increasing concentrations of quinacrine in the perfusion fluid on the amplitude of responses to 10 ng noradrenaline when the perfusing buffer contained 64 µg/ml indomethacin and either 1 or 5 ng/ml prostaglandin E2. The indomethacin abolished endogenous prostaglandin synthesis and also vascular responsiveness. Responsiveness was partially restored by the exogenous prostaglandin, three control injections of noradrenalir were given, and then quinacrine was added to the buffer. 6 experiments were done with each prostaglandin concentration and the results are shown as mean ±SEM.

responses measured using an isometric recording system. Dose/response curves using increasing concentrations of prostaglandin E2 were obtained in control buffer and in buffer containing 5 or 10 µg/ml quinacrine. In two experiments quinacrine moved the dose/response curve progressively to the right but did not change the amplitude of the maximal response to the prostaglandin.

Discussion

A peculiarity of the action of drugs which inhibit prostaglandin synthesis on the rat mesenteric preparation is that responses to all vasoconstrictors appear to be blocked. The drug concentration required to produce a 50% inhibition of pressor response is approximately the same irrespective of the pressor agent used (14). Some antagonists of prostaglandin action such as local anaesthetics and methyl xanthines behave in a

similar way (10,15). A distinction between prostaglandin antagonists and inhibitors of synthesis can be made by using sufficient indomethacin in the perfusing buffer to block both endogenous prostaglandin synthesis and vascular reactivity. Normal vascular reactivity can then be restored by adding exogenous prostaglandin E2 to the buffer even with the indomethacin still present (14). In this situation inhibitors of prostaglandin synthesis have no effect because that synthesis is already blocked but prostaglandin antagonists still inhibit responses to pressor agents.

Our results strongly suggest that quinacrine, like other local anaesthetics and anti-malarials, is a prostaglandin antagonist, probably of a competitive type. The evidence is as follows: 1. It is able to inhibit responses to noradrenaline, angiotensin, potassium ions and vasopressin. 2. Partial inhibition of prostaglandin synthesis by indomethacin moves the dose/response curve to the left while addition of extra prostaglandin E2 moves it to the right. 3. It antagonises pressor responses to noradrenaline in preparations treated with a high dose of indomethacin to abolish endogenous prostaglandin synthesis and vascular reactivity whose responsiveness was restored by exogenous prostaglandin E2. 4. In the indomethacin-E2 treated preparations the dose/response curve with 5 ng/ml E2 was parallel and to the right of that with 1 ng/ml E2. 5. Quinacrine in a concentration of either 5 or 10 μg/ml moves the dose/response curve relating the effect of prostaglandin E2 on rat stomach strip contractions progressively to the right.

In concentrations in the 10^{-6}M range quinacrine caused a slight but consistent enhancement of the pressor responses. At the moment we have no explanation for this.

Since quinacrine is a prostaglandin antagonist, the binding sites on chromosomes and membranes with which quinacrine interacts may also be occupied by prostaglandins. Prostaglandins may play an important part in regulating membrane and nucleic acid function.

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