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Effects of quazodine (MJ 1988) on contractions of the isolated hemidiaphragm of the rat and the biventer cervicis muscle of the chick

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Summary

1. The effects of quazodine and theophylline have been studied on the rat hemidiaphragm and chick biventer cervicis preparations *in vitro*.

2. Theophylline and quazodine enhanced maximal twitches and contractural responses to acetylcholine and carbachol.

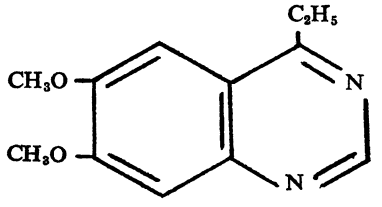
3. These actions on contractility were exerted directly upon the muscle fibres and were dependent upon the concentration of calcium ions in the bathing solution.

4. In addition, quazodine enhanced the neuromuscular blocking activity of tubocurarine, probably by a prejunctional action.

5. Theophylline potentiated acetylcholine to a greater extent than it potentiated carbachol thus suggesting that theophylline possesses anticholinesterase activity.

Introduction

Quazodine (6,7-dimethoxy-4-ethylquinazoline ; MJ 1988) possesses cardiac stimulant, bronchodilator and vasodilator activity and induces relaxation in a number of isolated smooth muscle preparations (Lish, Cox, Dungan & Robbins, 1964 ; Carr, Cooper, Daggett, Lish, Nugent & Powers, 1967 ; Aviado, Folle & Pisanty, 1967 ; Parratt & Winslow, 1971a, 1971b, 1972). Studies in broken cell preparations have shown that the compound inhibits the phosphodiesterase responsible for destroying cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Amer & Browder, 1971).



QUAZODINE

The reported actions of quazodine resemble those of theophylline, quazodine being the more potent compound both in whole tissue and as a phosphodiesterase inhibitor *in vitro*. The methylxanthines produce pronounced effects on skeletal muscle (for reviews see Sandow, 1964, 1965) and it was therefore of interest to study the effects of quazodine on this tissue. This paper describes its effects on isolated preparations of the rat hemidiaphragm and chick biventer cervicis muscle.

Methods

Rat-diaphragm

Phrenic nerve-hemidiaphragm preparations from male rats weighing 200 to 400 g were set up according to the method of Bülbring (1946) in 50 ml organ baths containing Krebs solution at 32° C and continually gassed with 95% oxygen and 5% carbon dioxide. The composition of the Krebs solution was as follows (in g/litre): NaCl, 6·9; KCl, 0·34; $NaHCO_3$, 2·1; $MgCl_2$, 0·11; NaH_2PO_4, 0·15; $CaCl_2$, 0·6; glucose, 1·0.

Preparations were indirectly stimulated via the phrenic nerve, with rectangular pulses of 100 μs duration and twice the amplitude necessary to produce a maximal twitch. Direct stimulation with pulses of 1 ms duration was applied between one platinum wire, to which the diaphragm was secured, and another situated in the bathing solution. In experiments using direct stimulation, tubocurarine, in a concentration sufficient to block neuromuscular transmission completely (3 μg/ml, 4×10^{-6}M), was added to the reservoir of Krebs solution. A resting tension of 2–3 g was applied to the tissue and isometric contractions were recorded with a strain gauge (Grass FT03) coupled to a pen recorder (Grass Polygraph) or to a dual beam cathode ray oscilloscope (Nihon Kohden VC 7A). In some experiments qualitative comparisons of the rates of development of tension (dT/dt) of the contractions were obtained with a Grass Polygraph Differentiator. Accurate measurements of dT/dt could not be obtained because the frequency response of the recording system as a whole (about 80 Hz) was too low. In other experiments gross muscle action potentials from indirectly stimulated preparations were recorded between two platinum pins inserted in the muscle and displayed upon one beam of the oscilloscope; the other beam was used to display twitch tension. In experiments in which latency between stimulation and contraction was recorded, the oscilloscope was adjusted to a fast time base; one beam was used to display stimulus artifact and the other to display tension.

Modification of the bathing medium

In some directly stimulated preparations the normal Krebs solution surrounding the diaphragm was changed to a solution containing double the normal calcium concentration, or to a solution in which the calcium chloride had been omitted. The tissue was then allowed to equilibrate for periods of 10 to 45 min, i.e., until a new steady level of twitch tension was reached.

Chick biventer cervicis

Chicks, aged 1–15 days, were killed by ether inhalation. The biventer cervicis muscle preparation was set up according to the method of Ginsborg & Warriner (1960) in a 50 ml organ bath containing Krebs solution at 35° C and continuously gassed with 95% oxygen and 5% carbon dioxide. A tension of 0·5 to 1·5 g was applied to the tissue. Indirect stimulation was effected by means of electrodes which encircled the muscle tendon, with rectangular pulses of 200 μs duration and twice the amplitude required to produce a maximal twitch. Direct stimulation was applied in the presence of (+)-tubocurarine (10 μg/ml, $1{\cdot}45\times10^{-5}$M) with the same electrodes positioned close to the muscle belly. Pulses of 1 ms duration were used at a strength sufficient to elicit contractions equal in amplitude to

indirectly elicited maximal twitches. Isometric contractions were recorded with a strain gauge (Grass FT03) and either a Grass or Devices 2 channel pen recorder. The second channel was used to record the differential of the tension with respect to time (dT/dt).

The drugs used were quazodine (Mead Johnson & Co.), theophylline hydrate, acetylcholine chloride and carbamyl choline chloride (B.D.H.), and (+)-tubocurarine chloride (Burroughs Wellcome). All drugs were dissolved in 0·9% w/v NaCl solution (saline) and the concentrations refer to the cations or the bases.

Results

Rat hemidiaphragm preparation

In eighteen directly stimulated preparations, quazodine produced a reversible increase in the tension of maximal twitches evoked at a frequency of 0·1 Hz. The smallest effective concentration was 20 μg/ml (9×10^{-5}M) and the effect was dose-dependent within the range 20–800 μg/ml (9×10^{-5}–$3{\cdot}6\times10^{-3}$M), with concentrations of 800 μg/ml producing an increase of 81–110%. The increase in twitch tension reached its peak from 15 to 20 min after the addition of the drug and was sustained for the duration of drug contact. When twitch tension was displayed on one, and stimulus artifact on the other beam of the oscilloscope it was seen that quazodine did not change the latency between stimulus artifact and onset of tension development. The increase in twitch tension was associated with a small increase

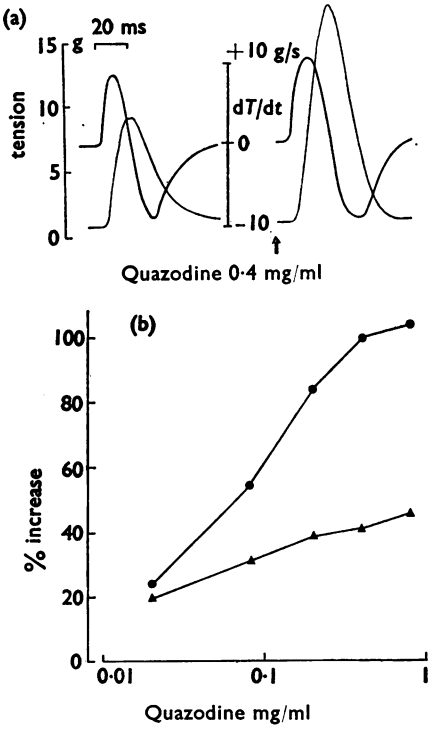


FIG. 1. Effect of quazodine on indirectly elicited twitches of rat-hemidiaphragm preparation. Frequency of stimulation was 0·1 Hz. (a) Oscilloscope records of tension and rate of tension development (dT/dt) before and 15 min after quazodine (0·4 mg/ml, $1{\cdot}8\times10^{-3}$M). (b) Percentage increases in tension (●), and in time to peak tension (▲).

in the maximum rate of rise of tension ($+dT/dt$), an increase in the time to peak tension (up to 50%) and an increase in twitch duration, measured from onset of twitch to half relaxation (up to 30%). Figure 1 shows oscilloscope records of the changes produced by quazodine and graphs of the effects of quazodine on peak tension development and on time to peak tension. In fifteen preparations stimulated indirectly at a frequency of 0·1 Hz the effect of quazodine upon twitch tension did not differ from the effect in preparations stimulated directly.

In five preparations the frequency of direct stimulation of the preparations was increased from 0·1 to 1 Hz. The augmentation of twitch amplitude induced by quazodine (100 μg/ml) was followed by a secondary period of depression during which a slowly developing contracture occurred. Recovery from both the contracture and the twitch depression occurred after washout and was more rapid if the stimulation frequency was lowered to 0·1 Hz.

The effects of quazodine and theophylline upon twitch tension were compared in five preparations stimulated directly at 0·1 Hz. Log dose-response lines to quazodine (50–400 μg/ml, $2{\cdot}2\times10^{-4}$–$1{\cdot}8\times10^{-3}$M) and theophylline (100–600 μg/ml, $5{\cdot}5\times10^{-4}$–$3{\cdot}3\times10^{-3}$M) were parallel, with quazodine $3{\cdot}05\pm0{\cdot}6$ times as potent on a weight basis and $3{\cdot}8\pm0{\cdot}7$ times as potent on a molar basis as theophylline.

Effect of calcium

The effect of Ca^{++} upon the response to quazodine was examined in rat hemidiaphragm preparations stimulated directly at a frequency of 0·1 Hz. In each experiment the effect of quazodine (200 μg/ml, 9×10^{-4}M) was first examined in standard Krebs and then, after a period of equilibration, in modified Krebs solution. When the concentration of calcium was doubled the tension-enhancing activity of quazodine was increased by 50–300% in five experiments. On the other hand complete lack of calcium caused a 30–70% reduction in four experiments and a complete lack of response to quazodine in one other experiment.

Chick biventer cervicis preparation

In fifteen preparations stimulated indirectly at 0·1 Hz, quazodine (50–300 μg/ml, $2{\cdot}2\times10^{-4}$–$1{\cdot}3\times10^{-3}$M) increased twitch tension by up to 113%. This response to quazodine began within 30 s of its addition to the organ bath, reached a peak within 15 to 20 min and then, following washout, returned to control levels within 5 to 10 minutes. Concentrations of quazodine above 100 μg/ml produced contracture of the preparations which reached a peak within 4 min of addition and which was sustained until quazodine was washed from the bath. In other indirectly stimulated preparations, theophylline (0·1–1·0 mg/ml, $5{\cdot}5\times10^{-4}$–$5{\cdot}5\times10^{-3}$M) exerted effects similar to those of quazodine, the highest dose increasing twitch tension by up to 135%. However, in only one preparation did theophylline produce a contractural response. In six experiments dose-response relationships for quazodine and theophylline were compared. Log dose-response lines were parallel, with quazodine $1{\cdot}42\pm0{\cdot}14$ times as potent on a weight basis and $1{\cdot}77\pm0{\cdot}17$ times as potent on a molar basis as theophylline.

The increases in twitch tension in response to quazodine or theophylline in the chick biventer cervicis preparation were associated with larger increases in maximum rate of rise of tension ($+dT/dt$) than those occurring with the rat diaphragm. The possibility that facilitation of neuromuscular transmission contributed to this

effect on tension development was examined by comparing the effect of theophylline in directly and indirectly stimulated biventer cervicis preparations. Theophylline (400 μg/ml) caused an increase of $44 \pm 4\%$ in twitch tension in eight indirectly stimulated preparations and an increase of $48 \pm 7\%$ in five directly stimulated preparations. There is no significant difference between these means.

Quazodine and theophylline, at concentrations effective in enhancing twitch tension, increased the contractile response to acetylcholine (10–20 μg/ml, $6{\cdot}5 \times 10^{-5}$–$1{\cdot}3 \times 10^{-4}$M) or to carbachol (2–4 μg/ml, $1{\cdot}3 \times 10^{-5}$–$2{\cdot}7 \times 10^{-5}$M). Figure 2 summarizes the effects of different concentrations of quazodine on responses of the biventer to electrical stimulation, to acetylcholine or to carbachol, and shows that

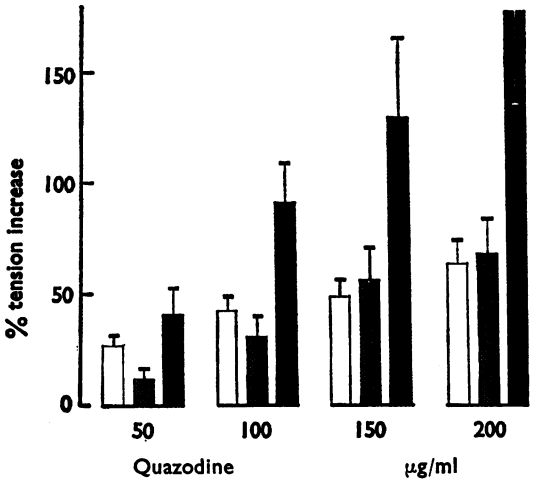


FIG. 2. Chick biventer cervicis muscle. Per cent increases induced by quazodine (50–200 μg/ml, $2{\cdot}3 \times 10^{-4}$–9×10^{-4}M) on the tension of maximal twitches elicited indirectly at 0·1 Hz (open columns) and contractures in response to acetylcholine (10 μg/ml, $6{\cdot}5 \times 10^{-5}$M; shaded columns) and carbachol (4 μg/ml, $2{\cdot}7 \times 10^{-5}$M; closed columns). Each column represents the mean ± S.E. of nine results.

progressively higher doses of quazodine enhanced responses to carbachol to a greater extent than responses to acetylcholine. This apparently greater potentiation of carbachol may be explained by the observation that in the chick biventer cervicis preparation the dose-response curve for carbachol is steeper than that for acetylcholine (Marshall, 1971). In fact when full dose-response lines were plotted from the results of seven further experiments, quazodine (200 μg/ml) caused parallel shifts of the log dose-response lines for acetylcholine and for carbachol corresponding to potentiation by $1{\cdot}34 \pm 0{\cdot}12$ and $1{\cdot}41 \pm 0{\cdot}08$ fold respectively, and these factors are not significantly different. In a further six experiments theophylline at a concentration of 400 μg/ml (equieffective with 200 μg/ml of quazodine in enhancing twitch tension) was significantly more effective in potentiating acetylcholine than in potentiating carbachol (factors of $3{\cdot}34 \pm 0{\cdot}10$ and $1{\cdot}35 \pm 0{\cdot}10$ respectively; $P < 0{\cdot}001$). Effects of quazodine and theophylline on the dose-response relationships of acetylcholine and carbachol are shown in Figure 3.

Partially curarized preparations

In five indirectly stimulated rat hemidiaphragm and in seven chick biventer cervicis preparations, quazodine (100–200 μg/ml) augmented the partial block

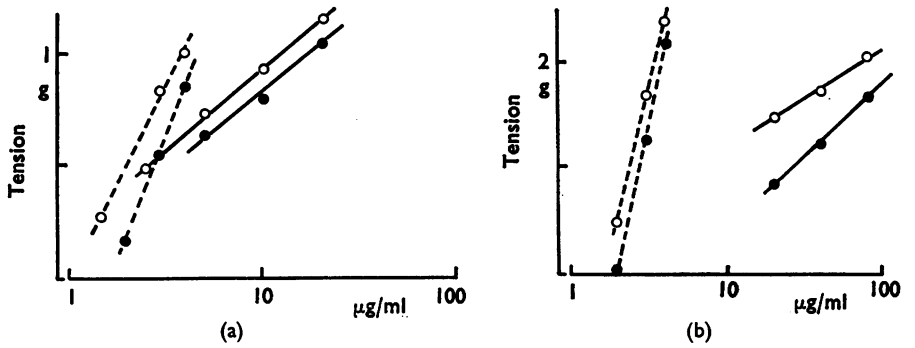


FIG. 3. Chick biventer cervicis muscle. Log dose-response lines from two experiments for contractural responses to carbachol (interrupted lines) and acetylcholine (continuous lines) in the absence (○) and presence (●) of quazodine (100 µg/ml, $4{\cdot}5\times10^{-4}$M; left diagram) and theophylline (200 µg/ml, $1{\cdot}1\times10^{-3}$M; right diagram).

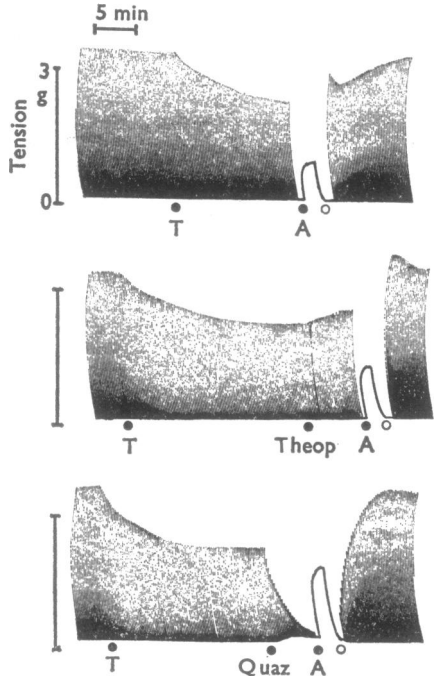


FIG. 4. Chick biventer cervicis muscle. Effects of theophylline and quazodine on twitches elicited indirectly at 0·1 Hz and on acetylcholine induced contractures in partially curarized preparations. Tubocurarine (1 µg/ml, $1{\cdot}4\times10^{-6}$M) was injected at T. Stimulation was temporarily stopped and at A, acetylcholine (40 µg/ml, $2{\cdot}6\times10^{-4}$M) was injected and at ○ the bath was washed. Uppermost trace, control. Middle trace, effects of theophylline (Theop; 1 mg/ml, $5{\cdot}5\times10^{-3}$M). Lowest tract, effects of quazodine (Quaz; 1 mg/ml, $4{\cdot}4\times10^{-3}$M). Theophylline enhanced the partially blocked twitches and the acetylcholine contracture. Quazodine enhanced the acetylcholine contracture but markedly increased the depth of tubocurarine block. Control records identical with that of the uppermost trace were recorded after theophylline and quazodine were washed out, but have been omitted from the figure.

induced by previous addition of tubocurarine (0·5–2·0 µg/ml). In three of these hemidiaphragm preparations the effect of quazodine (100–400 µg/ml) on the gross muscle action potential was examined. Quazodine reduced the amplitude of the

action potentials by 12 to 59%. Despite the deepening by quazodine of the tubocurarine block in the chick muscle, the response to acetylcholine (10–20 μg/ml) was simultaneously increased. In four of the same partially curarized preparations theophylline enhanced twitch tension and augmented acetylcholine-induced contractures. Figure 4 contrasts these effects of quazodine and theophylline.

Discussion

Quazodine, like theophylline, enhanced both directly and indirectly elicited twitches of the rat and chicken muscles. Since there was little or no difference between the magnitude of the effects whether the stimulation was direct or indirect, it can be concluded that the enhancement of contractility with both drugs was due to direct actions on the muscle fibres and was independent of any action on neuromuscular transmission.

In the rat diaphragm the augmented twitch produced by either drug was associated with an increase in the time to reach peak tension, although there was no detectable change in the time of onset of tension development; the maximum rate of rise of tension ($+dT/dt$) was slightly increased. In the chick muscle, similar effects were produced except that the maximum rate of rise of tension associated with the augmented twitches appeared to be more pronounced than that in the rat muscle. These changes in the time-course of the twitches suggest that an increased rate of activation of the contractile elements and a prolongation of the active state contribute to the enhancement of twitch tension.

The effect of quazodine on contractility, like that of methylxanthines (Bianchi, 1961; Sandow, 1965), probably involves mobilization of calcium ions so that more are made available to the contractile elements, because the effects on twitch tension were enhanced by calcium excess and inhibited by calcium lack. Twitch enhancement may result from prolongation of the muscle action potential, with a resultant increase in calcium release during the spike, or depression of the mechanical threshold for activation of the contractile elements. Caffeine exerts both actions (Sandow, 1965, 1970; Heistracher & Hunt, 1969) and quazodine and theophylline may do likewise. The compounds may also act by modifying calcium uptake and binding activities of the sarcoplasmic reticulum. Increased uptake of calcium during relaxation may result in more calcium being released by the action potential and thus twitch enhancement. In this respect the ability of quazodine and theophylline to inhibit cyclic nucleotide phosphodiesterase in cardiac broken cell preparations, thereby causing accumulation of cyclic AMP (Sutherland, Robison & Butcher, 1968; Amer & Browder, 1971) could be important, since there is evidence, albeit conflicting, that cyclic AMP facilitates calcium uptake into myocardial sarcoplasmic reticulum (Shinebourne & White, 1970; Sulakhe & Dhalla, 1970).

Conversely quazodine and theophylline may inhibit the calcium uptake process and storage capacities of the sarcoplasmic reticulum in skeletal muscle. Such effects, which are exerted by caffeine (Weber & Herz, 1968; Fuchs, 1969; Thorpe & Seeman, 1971) would easily explain the shared ability of caffeine, theophylline and quazodine to enhance twitches (by prolonging active state) and in higher doses to induce contractures (by release of calcium into the myoplasm). Presumably such effects on the sarcoplasmic reticulum do not involve phosphodiesterase inhibition since, under conditions where caffeine reduces sarcoplasmic reticulum-calcium affinity, cyclic AMP is ineffective (Weber, 1968).

According to Breckenridge, Burn & Matchinsky (1967), Goldberg & Singer (1969) and Ginsborg & Hirst (1972), theophylline exerts a pre-junctional facilitatory action on neuromuscular transmission. However, in the present experiments the increase produced by theophylline in the tension of the twitches of partially curarized muscles was no greater than that attributable to its direct action on the muscle fibres, and no evidence was therefore obtained in support of a facilitatory action on transmission. Despite this, in the chick muscle theophylline potentiated acetylcholine to a greater extent than it potentiated carbachol. The increase in potency of carbachol is explicable in terms of theophylline's ability to enhance muscle contractility. The extra increase in potency of acetylcholine suggests a weak anticholinesterase action. However, if acetylcholinesterase were inhibited, an anticurare effect would be expected and this was not observed. It therefore seems likely that theophylline exerts a weak inhibitory action against the butyrylcholinesterase of chick muscle, and that this accounts for its ability to potentiate acetylcholine to a greater extent than carbachol.

In contrast to theophylline, quazodine augmented the neuromuscular block produced by tubocurarine. During the increased depth of block, responses to acetylcholine or carbachol in the chick muscle were nevertheless increased in size, as in the non-curarized muscle. This result indicates that quazodine impairs neuromuscular transmission by a prejunctional action, probably by depressing acetylcholine output from the nerve endings. It is likely that this depressant action on transmission is also exerted in non-curarized muscle, but presumably it is too weak to depress transmitter output below the level necessary to evoke the critical degree of endplate potential that triggers contraction, so that only the post-junctional enhancement of contractility is evident. However, when the safety margin in transmission is lost as a result of partial curarization, the preparation is very sensitive to agents that influence the transmission process, and under these circumstances the inhibitory action of quazodine on transmission becomes evident. No evidence is available to account for the prejunctional inhibitory action of quazodine on neuromuscular transmission.

Possible clinical uses have been proposed for the cardiovascular actions of quazodine (Aviado, 1965; Parratt & Winslow, 1972). The concentrations of quazodine necessary to affect skeletal muscle are high and blood concentrations approaching them are unlikely to be produced by the doses necessary to affect the heart. However, caution may be advisable should neuromuscular blocking drugs have to be administered to patients being treated with quazodine.

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