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Effects Of 2-Methyl-3-propynylquinazolin-4-(3*H*)-one On Vascular Reactivity In Isolated Porcine Tail Arteries

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Abstract

The vascular effects of 2-methyl-3-propynylquinazolin-4-(3*H*)-one (QUIN) have been studied on isolated porcine tail arteries. QUIN had no effect on resting tension but relaxed, dose-dependently, arteries precontracted with noradrenaline or high-K^+ in the order: NA > high-K^+. Also, QUIN inhibited both intracellular (ICD) and extracellular (ECD) Ca^{2+}-dependent contractions in the order: ICD > ECD. The results suggest that QUIN interferes with vascular Ca^{2+} mobilization.

Key Words: Quinazolinones, Vascular Reactivity, Smooth muscle

Introduction

Quinazolinones are a large group of heterocyclic nitrogen compounds [1, 2] with a wide range of pharmacological activities. Different derivatives have been reported to have cardiotonic, antihypertensive, antiarrhythmic, vasodilator and lipid-lowering properties [3, 4]. In a recent study by Ryu et al [5], a 6-(substituted-phenyl)-amino-5,8-quinazolinedione was reported to have potent inhibitory effect on endothelium-dependent vasorelaxation.

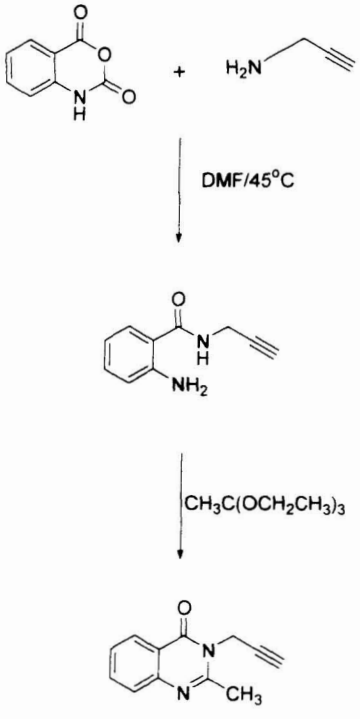
In view of the paucity of information on the cardiovascular actions of quinazolones, the goal of the present study was to investigate some vascular

effects of a quinazolone derivative: 2-methyl-3-propynylquinazolin-4-(3*H*)-one (QUIN) in porcine arterial smooth muscle preparations.

Methods

Synthesis of 2-Methyl-3-propynylquinazolin-4-(3H)-one (QUIN)

The method employed for the synthesis of QUIN is essentially as described previously [1, 2]: briefly, the *o*-amino-*N*-propynylbenzamide was obtained from the ring opening of isatoic anhydride and cyclocondensed in a blanket of nitrogen using triethyl orthoacetate to give the desired compound 2-methyl-3-propynylquinazolin-4-(3H)-one (QUIN) m.p 91-92°C (Lit. [2] 91-93 °C).



Preparation of Arteries:

Segments of pig tails were obtained from a local slaughter house and immediately, placed in physiological salt solution (PSS) of the following composition (mM/l): NaCl, 119.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 24.9; $CaCl_2$, 1.6; $CaNa_2EDTA$, 0.03; Glucose, 11.5. In the laboratory, the tail arteries were carefully dissected out, cleaned of adhering connective tissues and cut into rings (2-3mm). Each arterial ring was suspended between a stainless steel hook and an isometric force transducer (Grass FT.03), in a 20ml organ bath containing PSS. Contractions were displayed on a Grass Model 7 4-channel polygraph.

The PSS was maintained thermostatically at 37°C, pH 7.4 and bubbled continuously with 95% O_2, 5% CO_2 gas mixture, under an initial tension of 1g. The rings were allowed to equilibrate for 1 hour before the start of experiment. Following the equilibration period, the rings were stimulated twice using 80mM K^+ PSS (with rinses and recovery in-between the stimulations). Subsequent contractions during the experiment, were compared with this initial 80mM K^+ contraction. The following experimental protocols were examined: (a) Effect of QUIN on baseline tension (b) Relaxant effect of QUIN on noradrenaline or potassium-induced precontraction (c) Effect of Pharmacologic antagonists on QUIN-induced relaxation and (d) Effect of QUIN on intra- and extra-cellular Ca^{2+}-dependent contractions.

Protocols

Effect of QUIN on baseline tension:

Arterial rings were exposed to cumulative concentrations of QUIN, to examine the possible effect on baseline tension.

Relaxant Effect of QUIN :

Arterial rings were precontracted using $2.3x10^{-5}$M noradrenaline or 40mM K^+ (EC_{70}: concentration producing 70% of maximun contraction). When the contractions were stable, QUIN was added to the bath, cumulatively (a higher

concentration of QUIN was added when the response to the previous concentration was steady).

Effect of QUIN on Intra- and Extracellular Ca^{2+}-dependent Contractions

The procedure employed to assess the effect of QUIN on NA-induced phasic (intracellular) and tonic (extracellular) calcium-dependent contractions is as previously described [6,7,8]: maximum contractions to NA ($1x10^{-4}$M) were obtained in normal PSS and following 30 min exposure to a nominally calcium-free PSS. Following the phasic (intracellular Ca-dependent) contraction, restoration of calcium in the PSS resulted in a tonic (extracellular Ca-dependent) contraction. To assess the influence of QUIN on both components of contraction, varying concentrations of QUIN were applied simultaneously with the change to Ca-free PSS and maintained throughout the duration of both components of contraction.

Effect of drugs on QUIN-induced relaxation:

The influence of various pharmacologic antagonists on QUIN-induced relaxation was examined by estimating the magnitude of the relaxation response induced by $5x10^{-3}$M QUIN first, in the absence and following application of a particular antagonist 20min prior to $2.3x10^{-5}$M NA precontraction (the antagonist remained in the bath for the duration of the response to QUIN).

The following drugs were used: Noradrenaline bitartrate (Levophed, Stirling Drug Inc.); Cimetidine, Indomethacin, Propranolol, Ouabain (Sigma, UK). The drugs were prepared freshly by dissolving in distilled water. Ca-free PSS contained no added $CaCl_2$ with or without 1.0mM EGTA. High-K^+ PSS was prepared by equimolar substitution of NaCl with KCl. All chemicals were of analytical reagent grade.

Statistics

Values are presented as means ± standard error of the mean (S.E.M.) and n represents the number of rats from which tissues were obtained. Comparisons were made where appropriate, by using the Student's t-test ('Microcal Origin' software). A p value less than 0.05 was taken to denote statistical significance in all cases.

Results

Effect of QUIN on baseline tension:

Cumulative addition of QUIN (10^{-7}-10^{-3}M) had no significant effect on baseline tension in all experiments (n=12).

Relaxant Effect of QUIN :

Following pre-contraction induced by $2.3x10^{-5}$M noradrenaline or 40mM K^+, QUIN elicited concentration-dependent relaxation responses. The magnitudes of the contractile responses induced by $2.3x10^{-5}$M noradrenaline or 40mM K^+ were: 865.4±33.5 (n=10) and 882.5±29.4mg (n=10), respectively. The relaxant effect of QUIN was significantly greater in arterial rings pre-contracted with NA (Fig. 1): the IC_{50} values for QUIN-induced relaxation were 5.0 (±0.2)$x10^{-5}$ and 8.0±(0.3)$x10^{-4}$M (respectively) for NA- and K^+-pre-contracted rings.

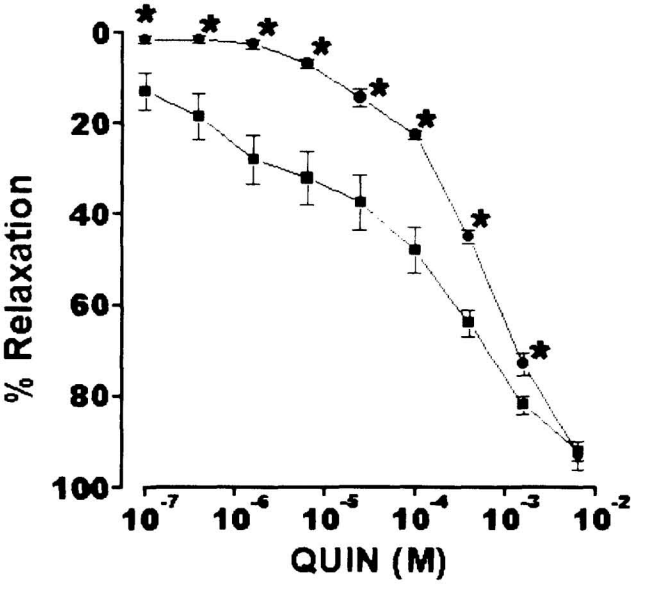


Fig. 1

Relaxation response to QUIN in porcine tail artery rings following precontraction induced by $2.3x10^{-5}$M noradrenaline (■) or 40mM K^+ (●). Means ± SEM; n=10. Asterisks denote statistical significance ($p<0.05$).

Effect of QUIN on Intra- and Extracellular Ca^{2+}-dependent Contractions:

The magnitudes of the phasic and tonic components of $1x10^{-4}$M NA contraction were: 742.5±22.1 and 1625.3±17.9mg, respectively. QUIN (10^{-4}-10^{-3}M) significantly inhibited both components of NA-induced contractions presumed (respectively), to be Intra- and extracellular Ca-dependent. The inhibitory effect of QUIN was significantly greater on Intracellular Ca-dependent contractions (Fig. 2).

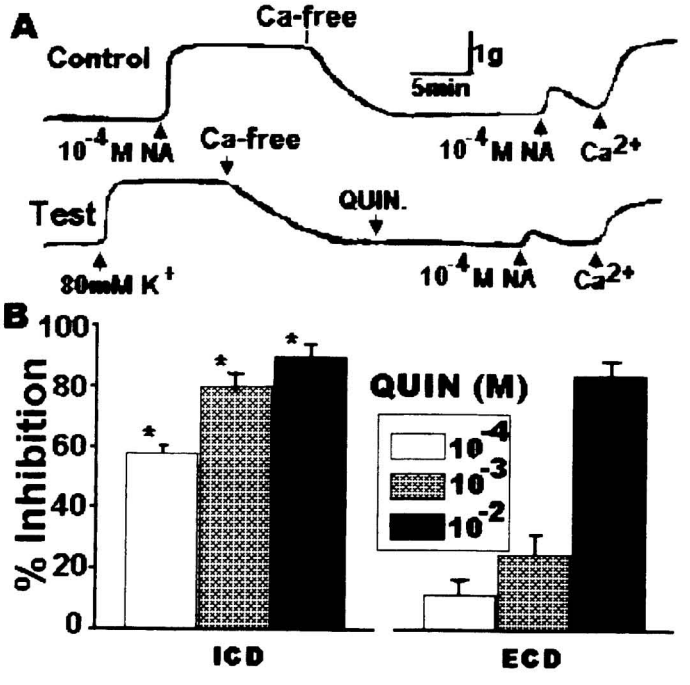


Fig. 2

Representative tracings (A) in a porcine arterial ring preparation, showing the inhibitory effect of QUIN on the phasic (intracellular Ca-dependent) and tonic (extracellular Ca-dependent) noradrenaline-induced contractions. Histogram (B) shows the inhibitory effects of varying concentrations of QUIN on the intracellular (ICD) and extracellular (ECD) Ca-dependent contractions. Means ± SEM; Asterisks denote significant difference ($P<0.05$) from ECD values.

Effect of drugs on QUIN-induced relaxation:

$5x10^{-3}$M QUIN elicited relaxation of $2.3x10^{-5}$M NA-precontracted rings by 87.2±5.4% (n=14). The magnitude of this relaxation response was not significantly altered by exposure of the rings to $1x10^{-4}$M indomethacin (n=6), $1x10^{-3}$M ouabain (n=5), $1x10^{-6}$M propranolol (n=4) and $4x10^{-5}$M cimetidine (n=5).

Discussion

We have observed, in the present study, that 2-methyl-3-propynylquinazolin-4-(3*H*)-one (QUIN) has no contractile effect on resting vascular smooth muscle of porcine tail arteries; however, following precontraction by noradrenaline or high-K^+, a concentration-dependent relaxation response results. The relaxation response induced by QUIN, if present in vivo, may provide a basis for the blood pressure lowering effect of this compound.

The observation that QUIN caused greater relaxation in NA-precontracted rings appears useful in defining its cellular mode of action. NA and high K^+ are two agents commonly employed to study excitation-contraction coupling in vascular smooth muscle [9, 10]: whereas NA contraction involves receptor activation leading to intracellular Ca^{2+} release and influx from the extracellular medium, high K^+ contraction is associated with membrane depolarization and Ca^{2+} influx. The observation that QUIN produced greater inhibition on NA- precontracted rings

suggests a greater effect of the compound on vascular Ca^{2+} metabolism linked to receptor activation.

The experiment represented in fig. 2 shows the influence of QUIN on the intracellular and extracellular Ca^{2+} pools mobilizable by noradrenaline. It is well established that the phasic and tonic contractions induced by NA represent Ca^{2+} mobilization from intracellular and extracellular pools respectively [6, 7, 11]. At all concentrations of QUIN studied, both components of NA contraction were attenuated; however, the inhibitory effect of QUIN on intracellular Ca-dependent contractions was significantly higher than for extracellular Ca-dependent contractions. This suggests a preferential action of QUIN on intracellular Ca^{2+} release mechanisms and may also provide an explanation for the greater inhibitory effect of QUIN in NA-precontracted rings discussed earlier (Fig.1).

The lack of effect of indomethacin, ouabain, propranolol and cimetidine on QUIN-induced relaxation of NA-precontracted arterial rings suggests the non-involvement of cyclooxygenase stimulation, Na^{+}-K^{+} ATPase enzyme activation, β-adrenergic stimulation and histamine H_2-receptor activation as possible mechanisms of QUIN action.

In conclusion, we report that 2-methyl-3-propynylquinazolin-4-(3*H*)-one (QUIN) elicits relaxation of vascular smooth muscle of the porcine tail artery by mechanisms associated with interference with Ca^{2+} supply, particularly, from the intracellular pool.

Acknowledgements

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