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Biphasic inotropic effects of a Ca^{2+} channel activator CGP28392 in rat myocardium: possible relation to intracellular Ca^{2+} release

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1 The inotropic effect of a Ca^{2+}-entry stimulator, CGP28392, (CGP) was compared in rat and frog myocardium in a concentration- and time-dependent manner.

2 Frog preparations exhibited a persistent positive inotropic effect following prolonged treatment with CGP.

3 Compared to amphibian myocardium, rat ventricular muscle exhibited a biphasic time-dependent response to CGP: an initial increase in the twitch tension amplitude of 30% was changed to a reduction of 80% below the control level during prolonged exposure to CGP (stimulation frequency, 0.2 Hz).

4 Following prolonged incubation with CGP, the resting-state contraction was decreased and the negative force-frequency relation was converted into a positive one in rat muscle.

5 Since sarcoplasmic reticulum (SR) is the major source of Ca^{2+} in a rested-state contraction, inhibition by CGP suggests an additional, intracellular action of the Ca^{2+} channel activator on SR-Ca^{2+} release in rat myocardium.

Introduction

Calcium channel agonists belonging to the dihydropyridine family, represent a new type of cardioactive compound (Schramm *et al.*, 1983; Erne *et al.*, 1984; Schramm & Towart, 1985; Reuter *et al.*, 1985). It has been demonstrated in several studies that dihydropyridine Ca^{2+} channel activators (Bay K 8644, CGP28392, the (+)-isomer of 202-791) increase depolarization-induced uptake of $^{45}Ca^{2+}$ and release of [^{3}H]-noradrenaline, increase the $\dot{V}_{max}$ of the slow response action potential and enhance myocardial contractility and calcium current (Schramm *et al.*, 1983; Erne *et al.*, 1984; Thomas *et al.*, 1984; 1985; Renaud *et al.*, 1984; Brown *et al.*, 1984; Hess *et al.*, 1984; Finet & Godfraind, 1985; Kongsamut *et al.*, 1985; Dubé *et al.*, 1985; Schramm & Towart, 1985). The present experiments were undertaken to determine whether stimulation of transmembrane Ca^{2+}-entry by the light-stable activator, CGP 28392 (CGP) (Truog *et al.*, 1984) could affect the anomalous force-frequency relationship in rat myocardium (Langer, 1978), since it is thought that the phenomenon may be causally related to the deficiency of transmembrane Ca^{2+} influx in this species (Nawrath, 1980).

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Methods

Rat myocardium

Wistar rats weighing 250–300 g of either sex were killed by decapitation, their chests opened and hearts rapidly removed. Papillary muscles, 0.2–0.6 mm in diameter and 2–4 mm in length, were isolated and mounted in a perfusion chamber for isometric force measurements. The preparations were superfused at approximately 5 ml min^{-1} in carbogen-saturated Tyrode solution of the following composition (mM): Na^+ 150, K^+ 4.0, Ca^{2+} 2.5, Mg^{2+} 1.0, HCO_3^- 12, $H_2PO_4^-$ 1.8, Cl^- 148.4, and glucose 11; at pH 7.4 and 35 ± 1°C. The tissue was stimulated by bipolar Ag-AgCl electrodes with square wave 3 ms impulses (strength at least twice threshold). Contraction was measured isometrically with a force-displacement transducer attached via a nylon ligature to the tendon of papillary muscle and was monitored by an oscilloscope and recorded on film. After a 2 h equilibration period, tension gradually was applied to the muscle: the final resting tension was 80% of that required to elicit maximum developed tension measured at 0.2 Hz. The membrane potential was recorded by a standard

microelectrode technique (Saxon & Safronova, 1982).

Amphibian myocardium

Frogs (*Rana ridibunda*) weighing 50–100 g of the either sex were killed by destroying the spinal cord. Atrial trabeculae, 0.1–0.15 mm in diameter, were studied by the double sucrose-gap technique described previously (Filippov & Porotikov, 1983). Electrical and mechanical responses were recorded simultaneously from a test 'node' which was 0.2 mm wide. The developed tension was recorded by monitoring the change in intensity of the light passing through the 'node' to a photodiode mounted in the eyepiece of the microscope (Kass, 1980). Stimulation of the preparation and data recording were performed automatically with a computer system SM-3 and CAMAC-modules (Bolon, Poland). Sampling interval was 0.05 ms.

Ringer solution of the following composition (mM) was used: NaCl 110, KCl 2.5, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 2.4, glucose 5.5; isotonic sucrose solution (sucrose dissolved in twice distilled water); pH 7.7 at 18 ± 2°C.

Drugs

The following compounds were used: a Ca^{2+} channel activator CGP28392 (4-[2-(diffluormethoxy)phenyl]-1,4,5,7,tetrahydro-2-methyl-5-oxofuro[3,4-6] pyridine-3 carboxylic acid ethyl ester, (a generous gift of Dr Brunner, CIBA GEIGY, Basel) and nifedipine (Pfeizer). The compounds were dissolved in ethanol. The control muscle studied in parallel received the same concentration of ethanol alone. The drugs were added cumulatively. The results were calculated for each concentration of the drug. Effects are expressed as a percentage of the initial isometric tension. The data are expressed as means ± s.e.mean.

Results

Inotropic effects of CGP28392 (CGP) in rat and frog myocardium.

Concentration-response curve The inotropic effect of CGP in rat papillary muscle was studied in a wide range of concentrations. The effects of CGP (10^{-7}–5×10^{-5} M) on developed tension in muscles stimulated at 0.2 Hz is shown in Figure 1a. A slight increase in the contractility started at 10^{-7} M, but a maximum inotropic effect eliciting an increase in contractility of 30 ± 5% was observed with 5×10^{-6} M CGP ($n = 7$). Increasing the concentration up to 10^{-5} M had no additional effect on the tension amplitude. However, a further increase in the CGP concentration to 5×10^{-5} M resulted in a decline in rat muscle contractility below the control level. This reversal of a positive inotropic action to a negative one is compatible with the dual 'agonistic-antagonistic' nature of the structural analogue of nifedipine and was previously reported for a more effective Ca^{2+} channel activator, Bay K 8644 (Thomas *et al.*, 1984; Freedman & Miller, 1984; Dubé *et al.*, 1985).

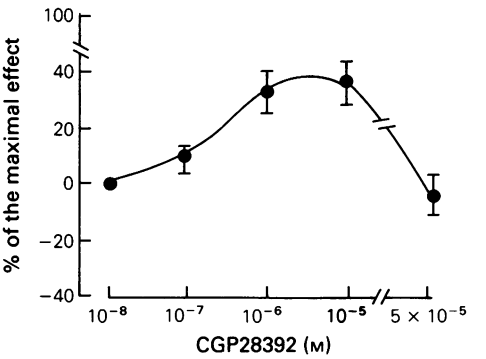

Figure 1 The concentration-response curve for the inotropic effect of CGP 28392 (10^{-8}–5×10^{-5} M) in rat papillary muscles driven at 0.2 Hz. Values represent the mean response of 7 preparations and are given as a percentage of the maximum effect; vertical lines indicate s.e.mean

The peak of the inotropic effects (Figure 1) was reached after 10–15 min and was reversible with washing.

Monophasic time-response curve in frog myocardium Exposure of frog atrial trabeculae to 5×10^{-6}–10^{-5} M CGP ($n = 6$) elicited a sustained increase in the twitch tension amplitude during incubation for 2 h. A typical monophasic positive inotropic response to 10^{-5} M CGP in amphibian myocardium is shown in Figure 2a.

Biphasic time-response curve in rat myocardium In contrast to the frog myocardium, a maximally effective dose of the compound (5×10^{-6} M) produced a biphasic time-dependent inotropic effect in the rat muscle at steady stimulation of 0.2 Hz ($n = 12$). The curve in Figure 2b shows that the initial increase in contractility of 30% was followed by a subsequent decline by more than 80% below the basal level when exposure to CGP at 5×10^{-6} M was prolonged over 2 h. However, an increase in stimulation rate from 0.2 to 2 Hz during this negative inotropic phase was able to restore the depressed contractility to nearly a basal

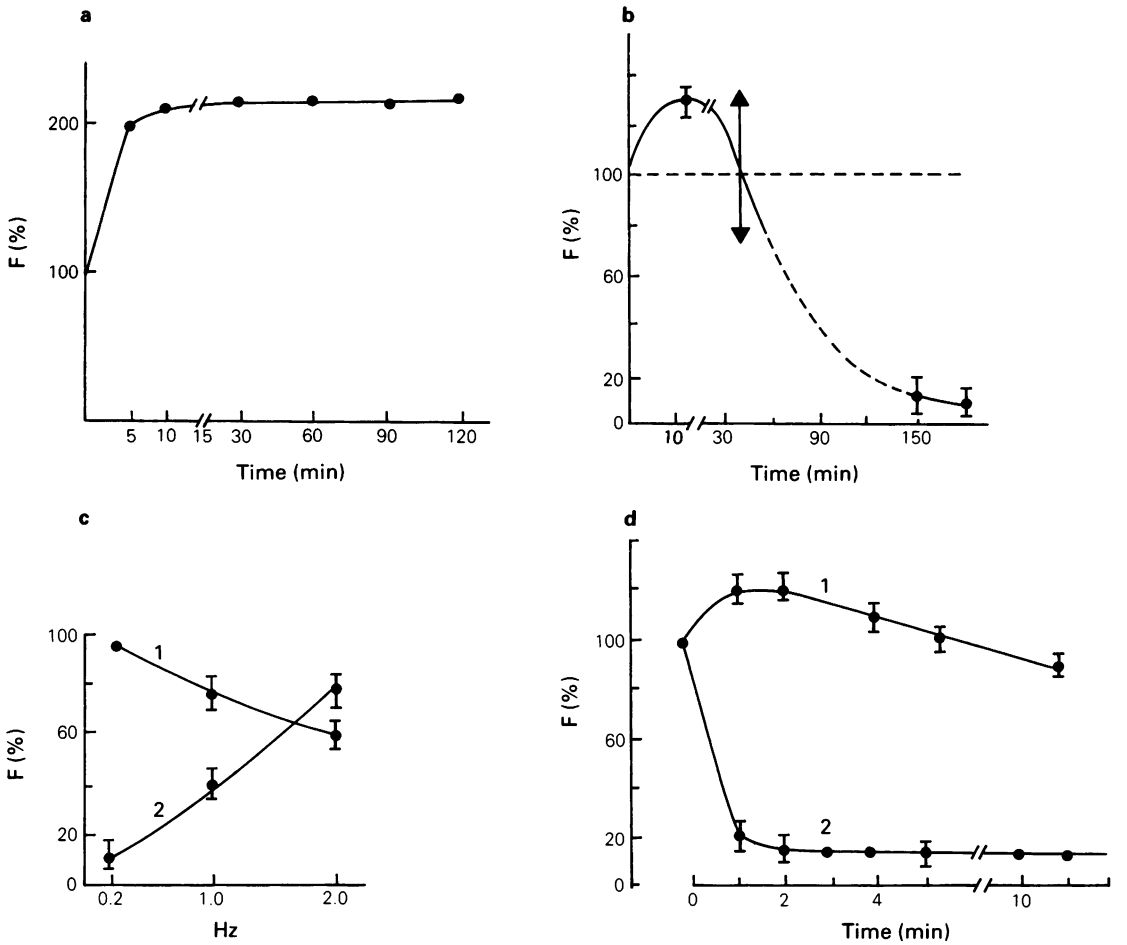

Figure 2 (a) Monophasic mechanical response for 10^{-5} M CGP28392 (2 h treatment) in frog atrial trabeculae. Stimulation frequency was every 20 s. Values are given as percentage of the maximum twitch ($n = 6$). (b) Biphasic mechanical response for 5×10^{-6} M CGP28392 (2.5 h treatment) in rat papillary muscle driven at 0.2 Hz. Values represent the mean response of 12 preparations. (c) Inversion of a negative force staircase to a positive one following long-term exposure (2.5 h) of rat papillary muscle to 5×10^{-6} M CGP28392. Force-frequency relation before (1) and after (2) CGP28392 exposure for 2.5 h. Abscissa scale: stimulation frequency in Hz; ordinate scale: twitch tension amplitude expressed as percentage of the maximal control value at 0.2 Hz. (d) Effect of long-term exposure to CGP28392 (5×10^{-6} M, 2.5 h) on rest-dependent inotropy in rat myocardium. Individual rest intervals were separated by a period of steady stimulation at 1 Hz to reach a stable pre-rest tension. Abscissa scale: duration of rest intervals (min); ordinate scale: amplitude of the post-rest tension expressed as a percentage of the pre-rest steady-state tension amplitude. In all cases vertical lines indicate s.e.mean.

level (Figure 2c, curve 2). Considering the above rate-dependent reversion of twitch amplitude, it should be stressed that the rat ventricular muscle has a negative correlation between developed tension and stimulation frequency (Forester & Mainwood, 1974; Langer, 1978), but prolonged treatment of the rat muscle by CGP reversed the negative force-frequency relation (Figure 2d, curve 1) to a positive one (curve 2).

Depressant effect of CGP28392 on rested-state contraction

In order to study the mechanism of the time-dependent depressant effect of CGP, the change in a rested-

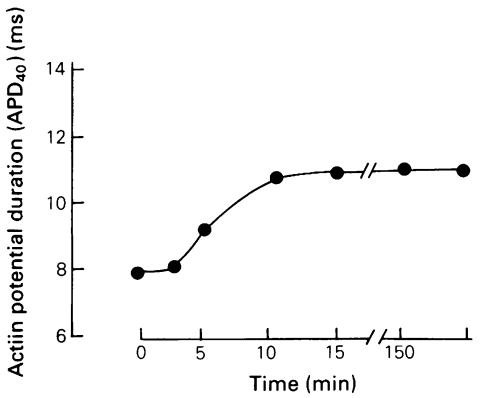

Figure 3 Monophasic time-response curve for action potential (AP) duration in rat papillary muscle measured at 40% repolarization (APD_{40}) following CGP28392 (5.10^{-6} M) incubation for 2.5 h ($n = 4$).

state contraction (the first contraction following the rest period after a steady-state stimulation) was evaluated. As shown by Figure 2d, in rat tissue the first contraction has a large amplitude even after a long (10 min) rest period (curve 1), but its amplitude was dramatically depressed after a short rest period (1 min) following a long-term exposure to CGP (curve 2).

Monophasic effect of CGP28392 on action potential (AP) in rat myocardium

A possible contribution of electrical events to the biphasic inotropic effect of CGP was examined by monitoring AP changes in rat myocardium. Figure 3 illustrates the time course of the effects of CGP (5×10^{-6} M) on action potential duration in rat muscle stimulated at 0.2 Hz ($n = 4$). The prolongation of AP, measured at 40% repolarization, which occurred during the first 10 min of drug exposure did not change during the course of the subsequent incubation for 2 h.

Discussion

This study shows that CGP, a Ca^{2+} channel activator, has a time-dependent biphasic effect in rat ventricular muscle. The first positive inotropic phase presumably relates to the ability of CGP to interact directly with sarcolemmal Ca^{2+} channels and to stimulate Ca^{2+}-entry into myocardial and other cells (Renaud *et al.*, 1984; Erne *et al.*, 1984; Brown *et al.*, 1984; Hess *et al.*, 1984; Thomas *et al.*, 1985). In view of the conventional stimulatory action, the delayed negative inotropy of the Ca^{2+} agonist seems paradoxical. There is no reason to relate the late negative inotropic phase with a dual 'agonistic-antagonistic' nature of this nifedipine derivative since its antagonistic effect occurs at concentrations much higher (5×10^{-5} M) than those required to cause the time-dependent depressant action (5×10^{-6} M). Furthermore, there is no time-dependent change in AP duration, indicating a possible intracellular effect.

The present work also revealed a pronounced inhibition of a rested-state contraction in rat muscle after prolonged treatment with CGP. This type of mechanical activity was shown to be primarily dependent on Ca^{2+} release from sarcoplasmic reticulum (SR) stores. This conclusion is based on the observation of a high sensitivity of rested inotropy to the agent modulating the content of Ca^{2+} in SR. Thus, compounds active at the SR such as caffeine or ryanodine accelerate the decay of rested state inotropy (Bass, 1976; Sutko & Willerson, 1980; Sutko *et al.*, 1986; Bers, 1985), whereas Ca^{2+} overload factors (ouabain, Na^+-free or K^+-free solutions) potentiate a post-rest contraction (Reuter *et al.*, 1984; Sutko *et al.*, 1986). This is in contrast with Ca^{2+} antagonists which are ineffective against rested-state contractions (Lewartowski *et al.*, 1978; McDonald *et al.*, 1981; Reuter *et al.*, 1984; Thomas *et al.*, 1985). Therefore, the inhibition of a rested-state contraction by prolonged treatment with CGP points to an intracellular effect and may relate to a site controlling Ca^{2+} release from internal stores.

The absence of the biphasic time-course of CGP inotropy in amphibian myocardium which has a highly reduced SR system, reinforces the idea of an intracellular action of the Ca^{2+} channel activator in rat papillary muscle.

The changes in rest- and rate-dependent inotropy of rat ventricular muscle produced by long-term incubation with CGP exhibit a close similarity with the action of ryanodine in this and other mammalian species (Sutko & Willerson, 1980; Sutko *et al.*, 1986; Saxon, 1986). According to the latest biochemical data, ryanodine at low concentrations is a highly potent stimulator of Ca^{2+} release from skeletal and cardiac SR (Fleischer *et al.*, 1985; Meissner, 1986) in contrast to the inhibition of Ca^{2+} release previously shown following high concentrations of ryanodine (Sutko & Willerson, 1980). A similar mechanical effect of CGP and ryanodine may be interpreted as a result of an additional intracellular action of the Ca^{2+} channel activator on the SR Ca^{2+} release. The latter effect probably mediated the inversion of the anomalous negative force-frequency relationship of rat papillary muscle, which is characteristic of this species, to a positive one. The biphasic time-dependent inotropy is not a unique finding with CGP. Similar data were obtained previously with Bay K 8644 in perfused rat heart (Armstrong & Ferrandon, 1985). On the basis of

ultrastructural observations it has been suggested that Bay K 8644 is a Ca^{2+}-releasing agent in ventricular myocardium (Howl & Publicover, 1986).

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