Dihydropyridine Ca^{2+} agonists and channel blockers interact in the opposite manner with photogenerated unpaired electrons

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Interaction of Ca^{2+}-channel antagonists (felodipine, ryocidil, verapamil, diltiazem) and agonists (dihydropyridine derivatives Bay K 8644 and CGP 28392) was studied by the methods of absorption spectroscopy Ca^{2+}-channel antagonists were found to act as electron donors, the agonists being electron acceptors in the interaction with dye free radicals in solution Redox transitions in channel-forming protein were proposed as a possible mechanism of the modulation of channel activity by the compounds tested

Ca^{2+} agonist *Ca^{2+} antagonist* *Photoreduction* *Electron donor* *Electron acceptor*

1. INTRODUCTION

Ca-channel blockers are widely used in the treatment of cardiovascular disorders and as effective tools in studying functional organization of Ca^{2+} channels. Ca^{2+} antagonists comprise a chemically heterogeneous group of compounds including the most effective dihydropyridine derivatives (nitrendipine, felodipine, ryocidil) and less potent agents (verapamil and diltiazem) [1]. Different receptor sites have been revealed for these diverse chemical compounds [2].

The discovery of 'Ca agonists' such as Bay K 8644 and CGP 28392 [3,4] which increase divalent cation influx provides new possibilities for channel investigation. These dihydropyridines replace Ca-channel blockers in a competing manner and introduce some difficulties into the interpretation of blocker action as a mechanical plug [5] This urges us to search for another mechanism of action.

Studying the differences in the physicochemical properties of the antagonists and agonists may also provide a key to their action and channel operation. We investigated redox properties of Ca-channel modulators in their reactions with excited dyes in solution. Opposite redox characteristics of channel modulators were found: the antagonists behave as electron donors whereas the agonists act as electron acceptors in the reactions with dye free radicals. This leads to the suggestion that the mechanism underlying the regulation of Ca-channel activity may involve a modulator interaction with some redox states of the channel-forming protein.

2. MATERIALS AND METHODS

The compounds that donate electrons to the dye cation radicals accelerate the rate of myoglobin or hemin photoreduction sensitized by eosin [6]. The sample contained 1×10^{-5} M whale myoglobin [7], 1×10^{-5} M eosin (Chemapol) and the tested Ca-channel modulator dissolved in 2 ml bidistilled water, pH 7.5. After thorough deaeration in a sealed quartz cuvette the sample was exposed to light from a tungsten lamp and the absorption spectra were registered as a function of illumination time.

The hydrophobic substances were dissolved in a

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dimethyl sulfoxide (DMSO)/water mixture (4:1) where hemin served as an electron acceptor and erythrosin sensitized its photoreduction.

The electron acceptors accelerated the disappearance of dye anion radicals. This reaction was followed by flash photolysis To enhance anion-radical production 1×10^{-4} M NADH (Reanal) was added to the sample as a primary electron donor.

The drugs tested were: felodipine (Hassle, Molndal, Sweden), kindly provided by Dr Bostrom; Bay K 8644 (Bayer AG, FRG), a generous gift from Dr M. Schramm, CGP 28392 (CIBA-Geigy, Basel), kindly supplied by Professor H. Brunner and Dr M. Meier; diltiazem (Tanabe Pharmaceutical), kindly presented by Dr Hiroshi Ono; verapamil (Serva), ryocidil (Riga, USSR).

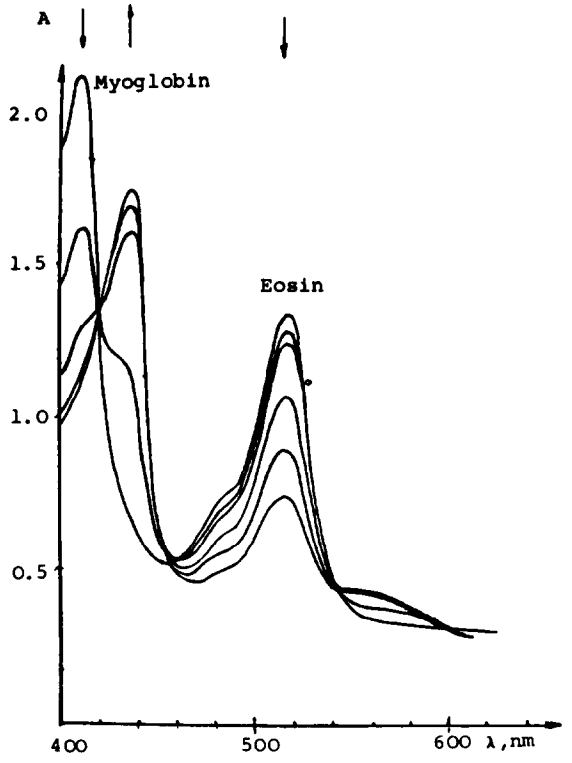

Fig 1 Myoglobin reduction by ryocidil photosensitized by eosin Mb, $1\,3 \times 10^{-5}$ M; eosin, $1\,3 \times 10^{-5}$ M, ryocidil, 1×10^{-4} M in 2 ml bidistilled water, pH 7 5 The GS-18 filter (USSR) transmits light at wavelengths longer than 500 nm Each trace was made after 1 min illumination Deaerated sample

3. RESULTS

Fig.1 shows the changes in the absorption spectra of the sample containing myoglobin, eosin and ryocidil as a result of its illumination with continuous light. The spectra were registered at 1 min illumination intervals. The Soret band of oxidized Mb^{3+} at λ_m = 409 nm decreased and appeared at λ_m = 434 nm that is indicative of anaerobic myoglobin reduction. The process was practically complete after 5 min illumination. The eosin absorption band at 517 nm diminished under the light.

Both complete myoglobin reduction and eosin bleaching were commonly observed when the sample contained an efficient electron donor. In the control only 20% of the myoglobin content was photoreduced by eosin which remained practically unchanged

Fig.2 shows the time course of myoglobin reduction in the presence of widely used Ca^{2+}-channel antagonists Diltiazem and verapamil reduced Mb^{3+} 3-times slower than the dihydropyridine ryocidil. Felodipine, one of the most efficient Ca^{2+} antagonists [8], was poorly soluble in water and was therefore tested in a DMSO system with erythrosin as sensitizer and hemin serving as electron acceptor To compare felodipine with previously studied blockers, one of them, ryocidil,

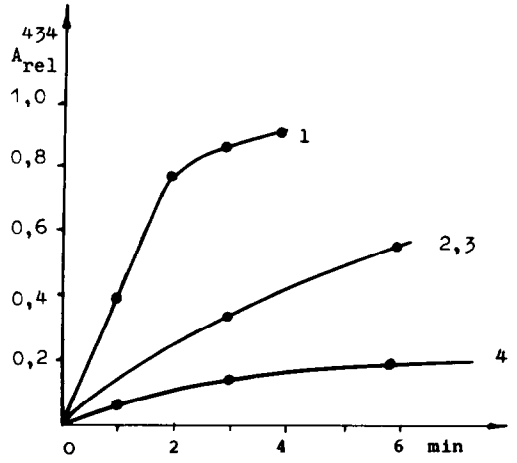

Fig.2 Time course of myoglobin photoreduction by Ca^{2+}-channel blockers: (1) ryocidil (1×10^{-4} M), (2) diltiazem (1×10^{-4} M), (3) verapamil (1×10^{-4} M), (4) control Other conditions as in fig 1

was also tested in the same medium and showed 1.5-times slower hemin photoreduction

All the Ca^{2+} antagonists studied revealed electron donor properties and could be arranged in the order: felodipine > ryocidil > diltiazem = verapamil, according to their ability to promote myoglobin photoreduction.

As shown recently, chemical modification of the dihydropyridine Ca^{2+} antagonist nifedipine resulted in compounds Bay K 8644 and CGP 28392 possessing agonist properties [3,4]. Despite the similarity of their chemical structure with channel blockers these new compounds did not promote myoglobin or hemin photoreduction. This suggests that the agonists do not behave as irreversible electron donors in the steady light experiments. Therefore, their influence on transient dye radical kinetics was studied by flash photolysis.

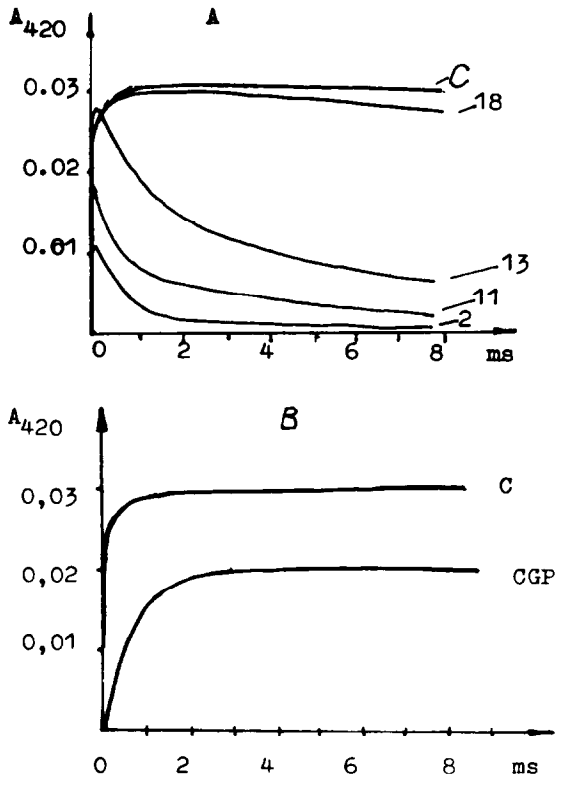

Fig 3 Eosin anion-radical kinetics under the influence of Ca^{2+} agonists (A) Bay K 8644 (1×10^{-5} M) in eosin (1×10^{-5} M) and NADH (1×10^{-4} M) water solution The figures indicate the number of flashes (B) CGP 28392 (2×10^{-4} M) (C) Control.

NADH added to deaerated eosin solution highly enhanced anion-radical production upon a light flash. After a fast rise, the anion-radical concentration was seen to remain stable on a 10 ms scale (fig.3). In the presence of Bay K 8644 in the sample the first light flashes revealed a low yield of anion radicals and their fast decay (fig.3A). Consecutive flashes resulted in progressive enhancement of radical concentration and prolongation of radical lifetime so that after 20 flashes the anion-radical kinetics approached those of the control.

This is a common way of action of many effective electron acceptors: they primarily oxidize the triplet state and thus diminish the anion-radical yield Along with the triplet oxidation, anion-radical oxidation takes place resulting in their fast decay Further illumination of the sample leads to gradual reduction of the electron acceptors, their concentration decreasing and anion-radical kinetics approaching those of the control.

Another Ca^{2+} agonist, CGP 28392, slowed down the anion-radical rise time and diminished their yield without affecting anion-radical disappearance (fig.3B), which suggests that CGP 28392 oxidizing activity decreased under the flash.

4 DISCUSSION

The main result from this work is the observation of opposite redox activity of Ca^{2+}-channel modulators. It was found that all the Ca^{2+} antagonists tested exhibited electron donor properties in myoglobin reduction photosensitized by eosin. Their ability to accelerate the reaction decreased in the following order: felodipine > ryocidil > diltiazem = verapamil These data are in agreement with physiological findings that the low concentration of felodipine (3×10^{-8} M) and a higher one of diltiazem and verapamil (10^{-6} M) act in producing maximum inhibition of smooth muscle contraction [8,9]

On the other hand, Ca^{2+}-channel agonists did not change myoglobin photoreduction compared to the control indicating that the compounds were not electron donors in the test system used. Moreover, Bay K 8644 behaved as a strong electron acceptor in the reaction with dye anion radicals The agonist diminished the quantum yield and lifetime of anion radicals and both gradually

returned to the control value during the course of Bay K 8644 reduction.

To our knowledge this is the first observation of different reactions of Ca-channel modulators with unpaired electrons in which Ca^{2+}-channel antagonists behave as electron donors while agonists act as electron acceptors. This finding raises the question as to whether some free-radical states are involved in the channel function. The possible formation of unpaired electrons may be contributed by S-S/-SH transitions in the channel-forming protein thus indicating the locus of the channel-modulators' action. This is in line with the recent observation that SH reagents modify the affinity of some dihydropyridines to Ca^{2+}-channel receptors in T-tubule membranes of skeletal muscle [10]. We believe that the interaction with dye radicals may provide a useful method for screening the potent channel modulators and may offer a clue for evaluating the mechanism of Ca^{2+}-channel functioning.

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