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W. TUGANOWSKI

# EFFECTS OF CALMIDAZOLIUM, CARBACHOL AND DERIVATIVES OF CYCLIC GMP ON THE LONGITUDINAL INTERNAL RESISTIVITY IN RABBIT ATRIAL TRABECULAE

Department of Physiology, Silesian School of Medicine, Katowice, Poland

The effects of calmidazolium, carbachol and membrane permeable derivatives of cGMP (dipalmitoyl cGMP and 8-Bromo cGMP) on the longitudinal internal resistivity ($R_i$) were studied in the rabbit atrial trabeculae by means of electrophysiological recording techniques and histological planimetry. Calmidazolium as well as carbachol decreased $R_i$ whereas cGMP-derivatives enhanced this resistivity. The effect of calmidazolium suggested that calmodulin reduced the cell coupling under control conditions. Carbachol decreased the Ca-inward current, and probably it prevented the calmodulin activation. The action of the nucleotides showed that cGMP did not mediate the cholinergic effect on the cell coupling. The possible interaction between calmodulin and cGMP was discussed.

Key words: *Atrial myocardium — Cell coupling — Calmodulin — Acetylcholine — cyclic GMP*

## INTRODUCTION

Intercellular coupling in the heart muscle is done by gap-junctional channels, called connexons, which link directly the cytoplasm of the neighbouring cells (1). The connexons are sensitive to some chemical signals such as cyclic AMP, intracellular $Ca^{2+}$ — concentration ($Ca_i$) and calmodulin (CaM).

Cyclic AMP decreases intercellular or juntional resistance, and it enhances diffusional permeability of the connexons (2—4) probably due to a direct phosphorylation of the channel protein (5, 6).

The elevation of $Ca_i$ results in an increase in the junctional resistance leading to the partial or total decoupling (7). In various tissues this effect is probably mediated by CaM or CaM-like proteins (8). CaM may be also involved in the modulation of the cardiac cell coupling. This view is supportcd by the following facts: i) calmidazolium (CDZ), a potent inhibitor of CaM

(9), counteracts the Ca-induced uncoupling (10); ii) CDZ reduces the longitudinal internal resistance ($r_i$) in the sinus node strips (11); iii) trifluoperazine, another inhibitor of CaM prevents the increase in the junctional resistance by Ca-loading solutions (10).

The effects of acetylcholine and cGMP on the cardiac cell coupling are still controversial. One paper reports that carbachol (CCh), a stable analogue of acetylcholine, increases the junctional resistance between cardiac cells (12). Other authors have not found any effect of the cholinergic stimulation on the cardiac cell coupling (3, 4, 13).

The effects of membrane permeable derivatives of cGMP are also equivocal. Cyclic GMP decreases the cell coupling in cardiac cell pairs (12) and in sinus node (14) but this nucleotide does not evoke any effect in other cardiac specimens (3, 4, 13).

In the present study the effects od CDZ, CCh and two permeable derivatives od cGMP — dipalmitoyl cGMP (dp cGMP) and 8-Bromo cGMP (8-Br cGMP) — on the cell coupling expressed as a longitudinal internal specific resistance, or resistivity, ($R_i$) were examined in atrial trabeculae. These experiments were undertaken in order to answer the following questions: i) whether or not CaM influenced the cell coupling; ii) whether or not cGMP mediated the cholinergic stimulation; and iii) whether or not the effects of cGMP and CaM were interrelated.

## MATERIAL AND METHODS

Muscular trabeculae (6—8 mm long, 0.5 mm in diameter) were isolated from the right auricles and mounted in a Perspex chamber comprising two compartments connected by a channel (2 mm long, 0.5 mm in diameter) (Fig. 1). The trabeculae were placed in the chamber such that their free-running portions passed through the channel. The preparations were superfused with warmed (303°K) Tyrode solution (NaCl 137, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 11, glucose 5.5 mmol/l) saturated by 95% $O_2$+5% $CO_2$ gas mixture. The solution was pumped into the closed right-hand compartment, passed through the channel and outflowed from the opened left-hand compartment at a rate of 2.5 ml/min.

The trabeculae were stimulated with square-wave pulses (0.5 Hz, 1—2 ms duration, double threshold voltage) delivered from Ag/AgCl electrodes placed in the left-hand compartment. Transmembrane potentials were recorded in the proximity of the channel left outlet by means of floating microelectrodes. Extracellular (bipolar) action potentials were recorded with a pair of Ag/AgCl electrodes placed on the ends of the channel. These electrodes could be shunted with a resistor $r_s$ (*Fig. 1*).

After the experiment, the portion of the specimen that passed through the channel was fixed in Bouin's fluid, embedded in paraffin, sliced transversely and stained by Azan method. In histological sections the ratio of the muscular tissue area ($A_m$) to the total stained area was determined planimetrically.

The slices sampled from the ends and from the center of the same specimen showed that the proportion of the $A_m$ to non-muscular tissue area ($A_n$) was constant in all preparations examined.

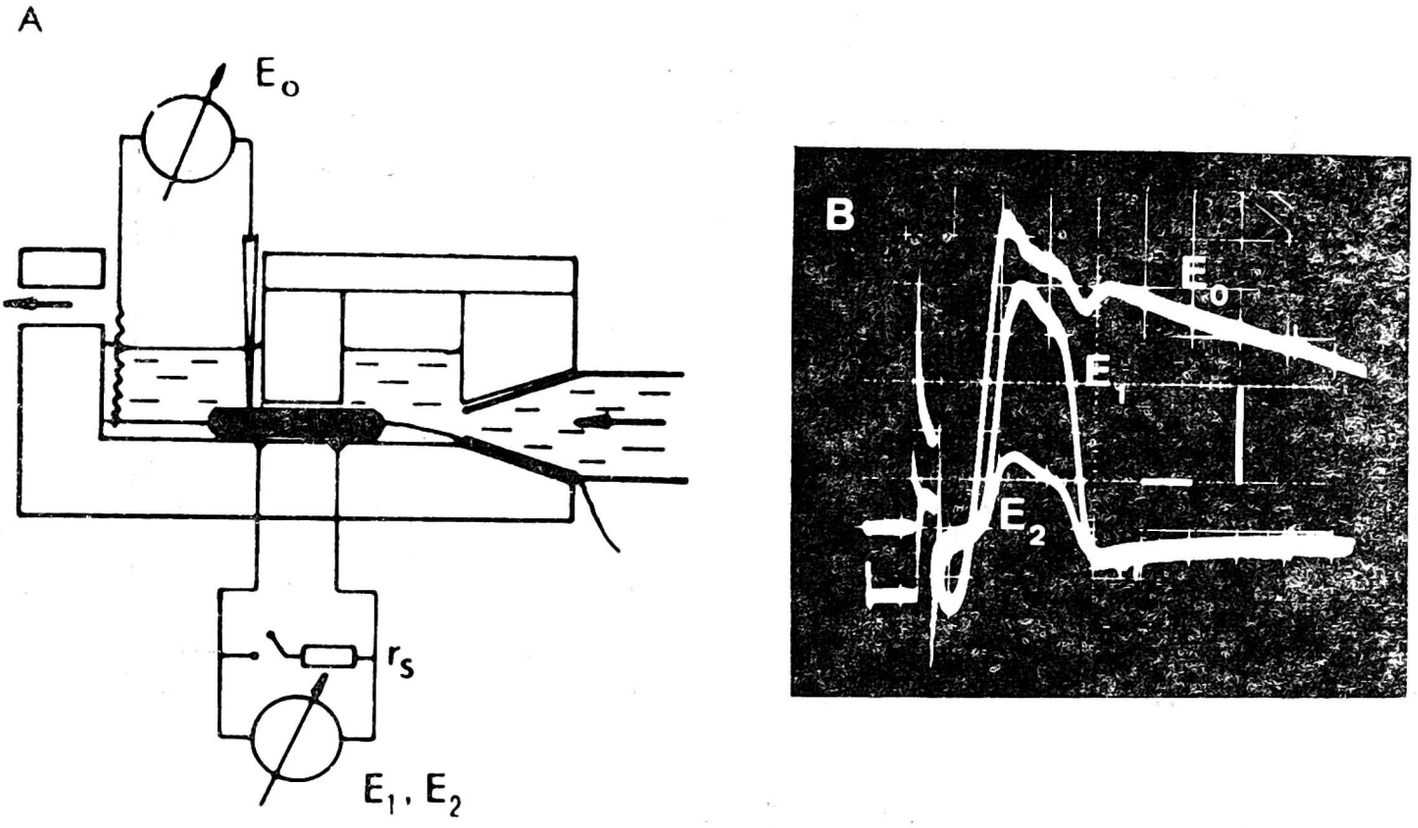


*Fig. 1.* A — experimental set-up, B — electrical activity of the atrial trabecula. All symbols are explained in the text. Vertical bar: 25 mV for $E_0$, 10 mV for $E_1$ and $E_2$; horizontal bar: 2 ms.

(n = 30). Thus, the channel cross-sectional area (A) could be considered as a sum of $A_m$, $A_n$ and $A_e$ (extracellular fluid area). Hence, $A_m + A_n = A - A_e$ or $A_m = f(A - A_e)$, where f is the fraction of $A_m$ in the total stained area.

$R_i$ was calculated from $r_i$, $r_e$ (longitudinal external resistance) and $A_e$ assuming that the resistivity of the extracellular fluid ($R_e$) was 59Ω cm, i. e., the resistivity of Tyrode solution measured in a conductivity cell at 303°K. The $r_i$ and $r_e$ were determined by a method described elsewhere (11, 15). Briefly, the transmembrane action potentials ($E_0$) and bipolar action potentials before ($E_1$) and after ($E_2$) shunting with $r_s$ were recorded (Fig. 1). The longitudinal resistances (in ohms per unit length) were calculated from the formulas:

$$r_i = r_s \times E_0 \left(\frac{1}{E_2} - \frac{1}{E_1}\right) \text{ and } r_e = r_s \times \frac{E_0(E_1 - E_2)}{E_2(E_0 - E_1)};$$ Ae was calculated from the Ohm's

$$\text{law: } A_e = \frac{R_e}{r_e}, \text{ thus } R_i = r_i \times A_m = r_i \times f(A - A_e).$$

Although the microelectrode impalements were performed in the close vicinity of the left-hand extracellular electrode, the bipolar electrograms were sometimes delayed (0.1—0.3 ms) in respect to $E_0$ (*Fig. 1*). This delay, however, did not influence the measurements of $r_i$ and $r_e$ because of the homogeneity of the trabecular muscle ($E_0$ recorded from various sites of the specimen had in practice the same value).

The biphasic course of $E_1$ enabled the measurement of the conduction velocity (Θ). This value equalled the channel length divided by the conduction time (a delay between the onset of the rapid upstroke and the end of the rapid downstroke).

The following drugs dissolved in Tyrode solution were used: CCh, CDZ, atropine sulfate, 8-Br cGMP-Na (Sigma, St. Louis, MO, USA) and dp cGMP-Na (Calbiochem, San Diego, CA, USA). CDZ and dp cGMP were dissolved initially in dimethylsulfoxide (DMSO; Sigma, St. Louis,

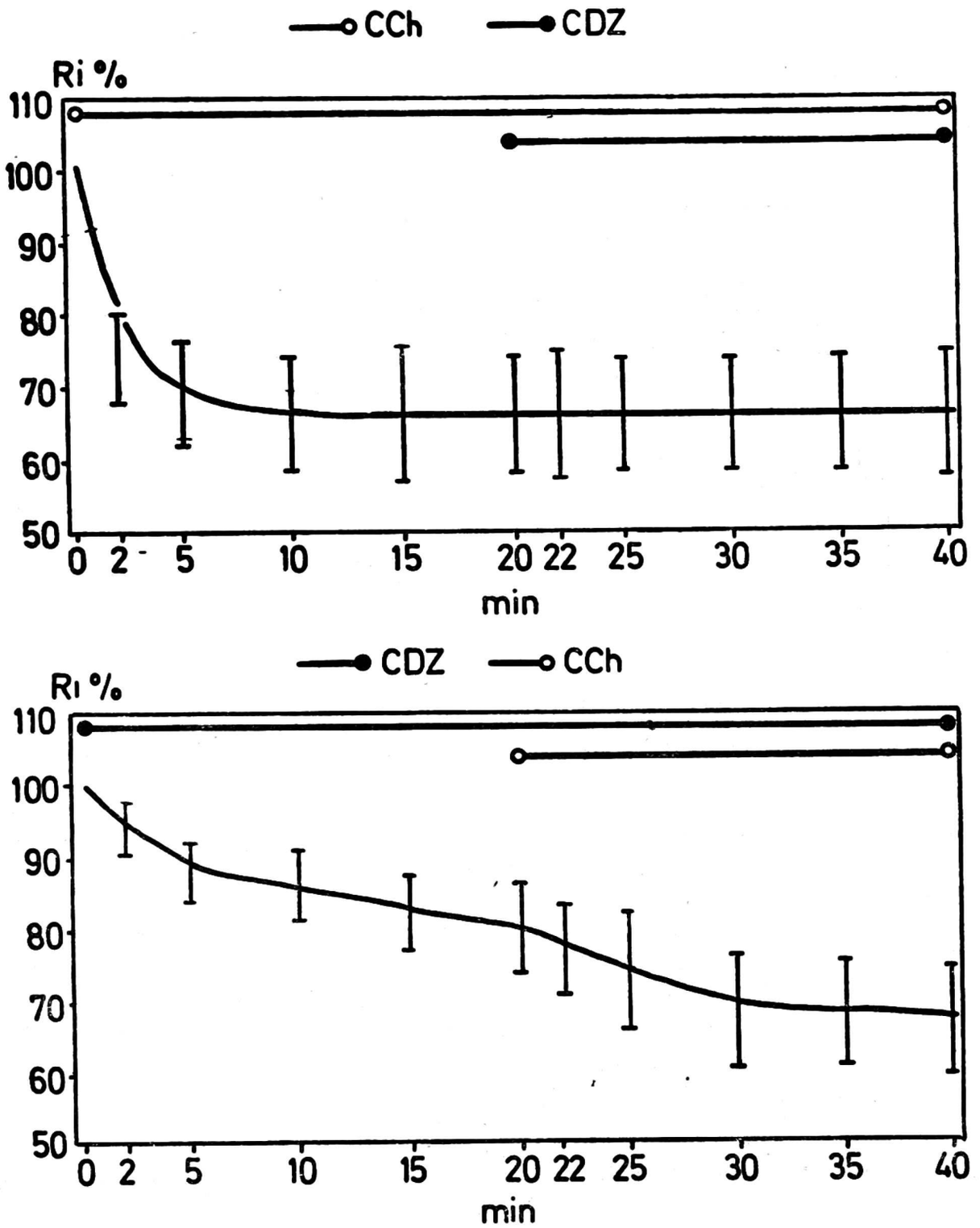


*Fig. 2.* Effects of CCh (1 μmol/l) and CDZ (1 μmol/l) on $R_i$ in the atrial trabeculae. the data are expressed as percents of the respective pretreatment control. The points and bars are the mean of differences ±S. D. of 10 experiments. Each value is significant ($p < 0.001$) vs. the control. The mean ±S. D. control values of $R_i$ are 208±25 Ω cm (upper diagram) and 204±31 Ω cm (lower diagram).

MO, USA). The final concentration of DMSO in Tyrode fluid did not exceed 0.1 vol%. DMSO at this concentration did not influence $R_i$ (5 experiments).

In the control experiments the specimens were superfused with Tyrode solution for 2 h. In other experimental groups the trabeculae were superfused initially with Tyrode solution for 20 min, and subsequently with drug-containing solutions.

The paired t-test was used for comparison of the $R_i$ values before and after the addition of the drugs.

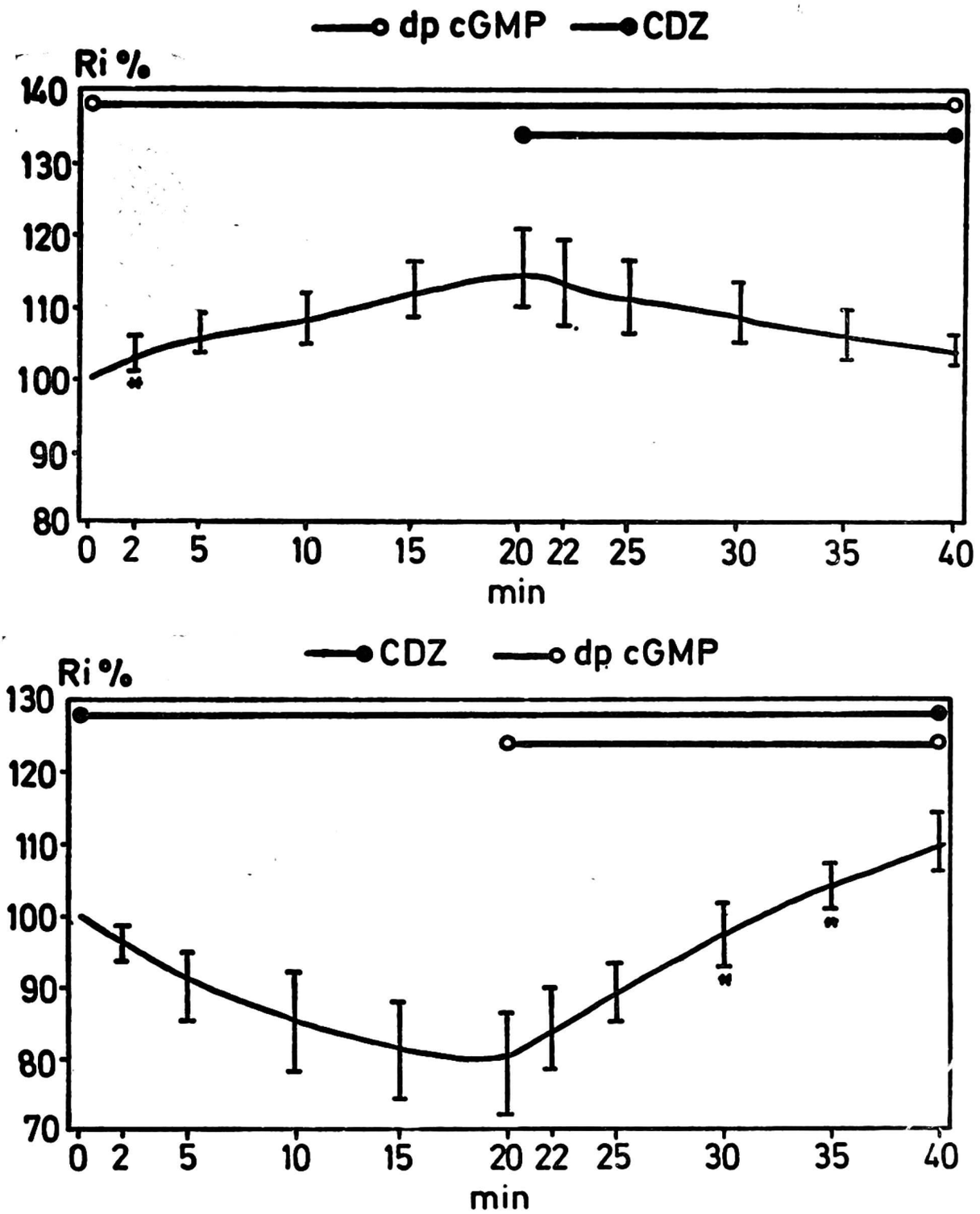


*Fig. 3.* Effects of dp cGMP (10 μmol/l) and CDZ (1 μmol/l) on $R_i$ in the atrial trabeculae. The data are expressed as percents of the respective pretreatment control. The points and bars are the mean of differences ±S. D. of 10 experiments. Asterisk denotes $p < 0.01$; unmarked values are highly significant ($p < 0.001$) vs. the control. The mean ±S. D. control values of $R_i$ are 214±24 Ωcm (upper diagram) and 207±24 Ωcm (lower diagram).

## RESULTS

The results of the control experiments are shown in *Table 1*. Under control conditions the value of $R_i$ was stable for 2 h of the experiment. After thit period, $R_i$ was 1.2±6.5% (mean ± S. D.; n = 30; p = 0.2) higher than as

the beginning of the experiment. It should be also noted that the present value of $R_i$ was in agreement with the results obtained in arterially perfused rabbit papillary muscles (16, 17).

The value of $R_i$ was dependent on driving rate. In group of 10 experiments the mean ±S. D. control value of $R_i$ was 212±28 Ω cm at 0.5 Hz. An increase in the stimulation frequency to 2.5 Hz enhanced $R_i$ by 14±6.2% (mean ± S. D.; $p < 0.001$) within 10—15 min.

*Table 1.* Planimetric and electrical parameters measured in rabbit atrial trabeculae. Each value is the mean (upper row) ±S. D. (lower row) of 30 experiments.

| | $A_e^*$ mm² | $E_0$ mV | $E_l$ mV | $r_i$ kΩ/cm | $r_e$ kΩ/cm | $R_i$ Ωcm | Θ cm/s |
|---|---|---|---|---|---|---|---|
| 0.82 | 0.072 | 101 | 29 | 207 | 81 | 209 | 40 |
| 0.04 | 0.009 | 4.3 | 6.5 | 26 | 11 | 30 | 3.8 |

* when A = 0.196 mm²; f — fraction of the muscular tissue area in total stained area; $A_e$ — extracellular fluid area; $E_0$ — transmembrane action potential amplitude; $E_l$ — extracellular action potential amplitude before shunting; $r_i$ — longitudinal internal resistance; $r_e$ — longitudinal external resistance; $R_i$ — longitudinal internal resistivity; Θ — conduction velocity.

*Table 2.* Effects of calmidazolium (CDZ) and carbachol (C Ch) on the longitudinal internal resistivity ($R_i$) in atrial trabeculae. Each value in upper rows is the mean ±S. D. of $R_i$ (in Ωcm); each value in lower rows is the mean of differences ±S. D. expressed as percent of the respective control.

| | | | μmol/l | | | | |
|---|---|---|---|---|---|---|---|
| drug | n | control | 0.1 | 1.0 | 2.5 | 10 | 100 |
| CDZ | 5 | 221±25 | 206±26<br>−7± 3 | 175±21<br>−21± 6 | 177±28<br>−20± 7 | | |
| CCh | 5 | 213±28 | 179±17<br>−16± 6 | 140±17<br>−34±10 | | 125±9<br>−41±6 | 121±8<br>−43±4 |

The effects of increasing concentrations of CDZ and CCh on $R_i$ are shown in *Table 2*. The exposure to each concentration of either drug was 30 min.

In 10 experiments CCh (1 μmol/l) and atropine (1 μmol/l) were used in order to identify the type of cholinergic receptor. The control value of $R_i$ was 211±26 Ω cm (mean ± S. D.). Superfusion with CCh reduced $R_i$ by 32±7% ($p < 0.001$) within 5 min. Subsequent addition of atropine to the CCh-containing Tyrode abolished this effect. Then, atropine increased $R_i$ to 14±1.8% ($p < 0.001$) above control within the next 5 min.

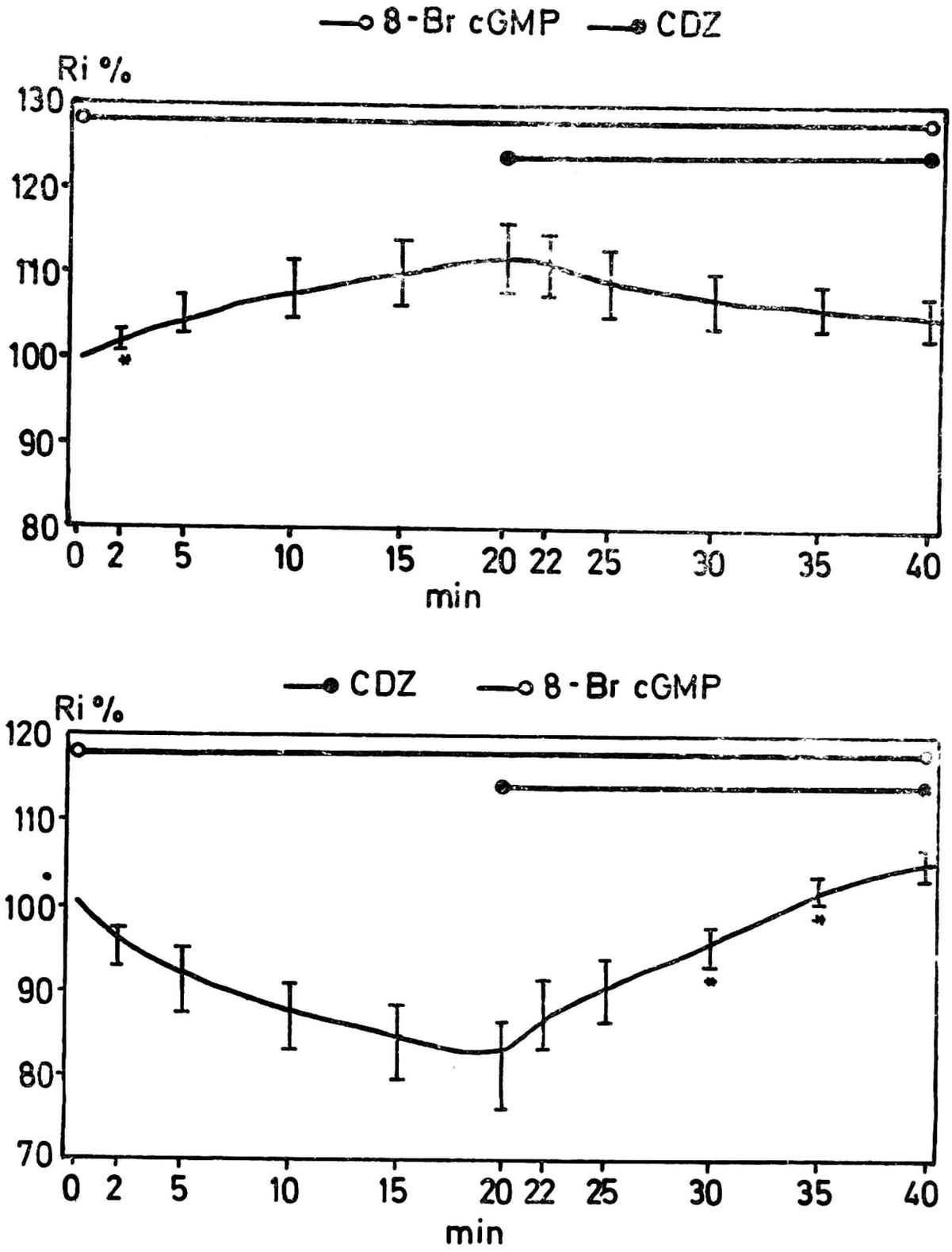


*Fig. 4*. Effects of 8-Br cGMP (10 μmol/l) and CDZ (1 μmol/l) on $R_i$ in the atrial trabeculae. The data are expressed as percents of the respective pretreatment control. The points and bars are the mean of differences ±S. D. of 10 experiments. Asterisk denotes $p < 0.01$; unmarked values are highly significant ($p < 0.001$) vs. the control. The mean ±S. D. control values are 218±30 Ωcm (upper diagram) and 220±33 Ωcm (lower diagram).

In remaining experimental groups the interaction between CCh and CDZ, dp cGMP and CDZ and 8-Br cGMP and CDZ was examined. The results of these experiments are shown in Figs. 2—4.

## DISCUSSION

As results from Table 2 and Figs. 2—4 CDZ reduces $R_i$ in the atrial trabeculae. This finding suggests that the intercellular coupling in atrial muscle may be depressed by CaM under control conditions, i. e., in stimulated specimens superfused with normal Tyrode solution. A similar effect was also showed in spontaneously active sinus node strips (11). This basal depression of cell-to-cell coupling is probably dependent on $Ca_i$. One may assume that under control conditions the Ca-inward current is sufficient to activate CaM, and thereby to produce a partial decoupling in atrial cells. The increase in $R_i$ at higher stimulation rate may be also evoked by an elevated Ca-influx.

The effect of CCh on cell-to-cell coupling in auricle may be also the Ca--dependent phenomenon. CCh reduced $R_i$ acting via muscarinic receptors. Activation of these receptors diminishes the Ca-current (18, 19). In that way, CCh may prevent the activation of CaM, and thereby it may increase the cell coupling in the auricle. However, CCh enhanced the peak effect of CDZ on $R_i$. Thus, one may assume that some pool of $Ca_i$ acts on the cell coupling in a CaM-independent manner.

Two used derivatives of cGMP significantly enhance $R_i$. These findings show that cGMP cannot mediate the effect of acetylcholine on the cell coupling in the atrial trabeculae. On the other hand, however, the effect of cGMP mimicks the depressive action of CaM on the cell coupling. Thus, the question arises whether or not their effects are interrelated. There are a few possibilities of such relation — cGMP and CaM may act in series, in parallel or in combined manner.

It seems that CaM cannot mediate the effect of cGMP since CDZ does not prevent the increase in $R_i$ after the addition of cGMP-derivatives to the CDZ-containing Tyrode. On the other hand, cGMP cannot mediate the effect of CaM because the addition of CDZ to the cGMP-containing Tyrode decreases $R_i$. Thus, one may suppose that these mediators do not act in series or in combined mode.

CaM and cGMP may compete for the same site responsible for the cell decoupling. Under such conditions CDZ should increase the number of the sites available to cGMP, and thereby it should increase the effect of this nucleotide on $R_i$. However, CDZ added to the cGMP-containing Tyrode reduces the value of $R_i$.

In the additive parallel or independent action, the effect of CaM should augment the effect of cGMP or vice versa. On the other hand, the blockade of one factor should reduce the total effect. These requirements are fulfilled by the following facts: i) the derivatives of cGMP enhance the basal effect of CaM on $R_i$, ii) CDZ added to the cGMP-containing Tyrode reduces $R_i$,

iii) the absolute increase in $R_i$ (basal depression + effect of cGMP) is greater than the effect of the nucleotide added to the CDZ-containing Tyrode.

To sum up, the present results suggest that CaM and cGMP may act on the atrial cell coupling in the additive parallel manner. One may assume that CaM-activated and cGMP-activated phosphodiestereases are responsible for this action (20, 21). Phosphodiesterases lower the cAMP content in the atrial cell, and thereby they may decrease the intercellular coupling.

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Author's address: Witold Tuganowski, Department of Physiology, Silesian School of Medicine 40-762 Katowice ul. Medyków 18, Poland