Specific Antagonism by Glibenclamide of Negative Inotropic Effects of Potassium Channel Openers in Canine Atrial Muscle

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Abstract—The mode of antagonism by glibenclamide, a potassium channel blocker, of the negative inotropic effects of potassium channel openers, cromakalim, pinacidil and nicorandil, was investigated in canine atrial muscle. Glibenclamide shifted the concentration-negative inotropic effect curves for cromakalim, pinacidil and nicorandil to the right without affecting the basal force of contraction. Schild analysis yielded uniform pA2 values of 6.06–6.35 for glibenclamide against the three potassium channel openers. The force of contraction of atrial muscles previously reduced by cromakalim was also antagonized by increasing concentrations of glibenclamide. Glibenclamide affected neither the concentration-negative inotropic effect curves for carbachol, an opener of the muscarinic receptor-coupled potassium channel, nor those for nifedipine, a calcium channel blocker. From these results, it became evident that glibenclamide behaved as a pharmacological antagonist of cromakalim, pinacidil and nicorandil in cardiac inotropy. The antagonism seems to involve competition of glibenclamide and these potassium channel openers, presumably at the ATP-sensitive channel in canine right atrial muscles.

Potassium channel openers such as nicorandil, pinacidil and cromakalim (1) all produce a negative inotropic effect in high doses or concentrations (2–7 for nicorandil; 5, 6, 8 for pinacidil; 5, 6, 9 for cromakalim). This effect is thought to entirely result from the limited calcium influx due to the abbreviation of cardiac action potentials that results from the increased potassium conductance produced by these drugs but not due to calcium channel blockade (5, 6). Indeed with high concentrations of nicorandil (4), pinacidil (10) or cromakalim (11), the duration of cardiac action potentials is abbreviated to such an extent that they resemble neural action potentials. However, these drugs do not directly block the calcium current in cardiac muscle (12). Recent data (11,13–17) have indicated that the target in cardiac muscle for potassium channel openers is the ATP-sensitive (or -regulated) potassium channel and their action is blocked by glibenclamide, an oral hypoglycemic drug whose mechanism of action in releasing insulin is thought to be blocking of the ATP-sensitive potassium channel in pancreatic B-cells (18, 19). From these data, it is evident that potassium channel openers and glibenclamide interfere with each other at the ATP-sensitive potassium channel in cardiac muscle. However, it has not been fully elucidated whether this interaction occurs in such a way that glibenclamide and potassium channel openers compete for the same binding sites or receptors at the ATP-sensitive potassium channel or in more complex manners. Recent studies (20–22) have indicated that it is likely that glibenclamide and potassium channel openers compete for the same receptors at the presumed ATP-sensitive potassium channel in vascular smooth muscle. We designed the present experiments to further address this issue in canine atrial muscle. As our previous studies (5, 6) have demonstrated that the negative inotropic effect of three potassium channel openers faithfully reflect their opening action on the
presumed ATP-sensitive channel in atrial muscle, we investigated how their negative inotropic effect would be modified by glibenclamide in the present study.

**Materials and Methods**

**Preparation and experimental procedures:** Hearts were excised from mongrel dogs of either sex, weighing 5 to 13 kg, anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Trabecular muscles of the right atrial wall were isolated from the heart in oxygenated cold (ca. 7°C) Krebs-Henseleit solution and mounted in 20-ml organ baths. The composition of the solution was as follows: 118 mM NaCl, 24.9 mM NaHCO3, 4.7 mM KCl, 1.2 mM KH2PO4, 2.5 mM CaCl2, 1.2 mM MgSO4, 11.1 mM glucose, 0.057 mM ascorbic acid, 0.027 mM Na2EDTA. The solution was equilibrated with 95% O2 and 5% CO2 at a temperature of 37°C (pH 7.4). Muscles were stretched to a resting tension of about 5 mN and stimulated by square pulses of twice the threshold voltage and 5-msec duration at a frequency of 0.5 Hz. During an equilibration period of about 1 hr, the length of the muscle was adjusted to give the maximum contractile force. The force of isometric contraction was recorded on a thermal pen-writing oscillograph (NEC San-ei, Recti-Horiz-8K) by means of strain-gauge transducers (Shikoh, UL-10230). The concentration of pinacidil, nicorandil and carbachol were increased at 5 min intervals and those of cromakalim and nifedipine at 10 and 30-min intervals, respectively. Usually 4-8 muscles were isolated from each heart and run in parallel, one of them being used as the control. Glibenclamide was administered 20 min before dosing any one of the potassium channel openers.

**Drugs and chemicals:** Drugs and chemicals used were as follows: cromakalim (Beecham Research Laboratories), pinacidil (Shionogi), nicorandil (Chugai), nifedipine (in ampoules at a concentration of 100 μg/ml or 289 nM, Bayer), carbachol chloride (Sigma), glibenclamide (Hoechst), atropine sulphate (Wako). Glibenclamide was dissolved in dimethylsulfoxide to give a concentration of 10 mM. Cromakalim was dissolved in 70% ethanol to give a concentration of 10 mM. Pinacidil was dissolved in 0.1 N HCl to give a concentration of 200 mM. Other drugs were dissolved in distilled water in the desired concentrations. These stock solutions were diluted to the desired concentrations with distilled water.

**Analytical procedures:** The concentration-negative inotropic effect curves for potassium channel openers, carbachol or nifedipine were expressed as the % decrease in the basal force and computer-fitted to a logistic equation:

\[ E = M \times A^p / (A^p + K^p) \]

where E is the normalized effect, M is the maximum effect of each drug, A is the drug concentration, K is the EC50 value of each drug and p is the slope parameter (23). EC50 values were presented as pD2 (pD2=−log EC50).

The antagonism by glibenclamide of the negative inotropic effects of the three potassium channel openers was analyzed in the following way: Schild analysis (24) was performed in the concentration range of glibenclamide that produced parallel rightward shifts of the concentration-negative inotropic effect curves of cromakalim, pinacidil or nicorandil.

**Statistical analysis:** Experimental values are given as the mean or the mean±S.E.M. Statistical significance of differences between mean values was estimated by Student's t-test. A t-test for the paired comparison was used when it was applicable. A P value smaller than 0.05 was considered to be significant.

**Results**

Antagonism by glibenclamide of the negative inotropic effects of potassium channel openers: As in the previous studies (5, 6), cromakalim (10−7 to 10−4 M), pinacidil (10−7 to 3×10−4 M) and nicorandil (10−6 to 3×10−3 M) caused a concentration-dependent decrease in the force of contraction (up to 90%) in canine right atrial muscles. Figures 1a, 2a and 3a show the modification by glibenclamide of the concentration-negative inotropic effect curves for cromakalim, pinacidil and nicorandil, respectively. Glibenclamide (3×10−7 to 3×10−5 M) per se did not change the basal force of contraction. These concentrations of glibenclamide antagonized the negative inotropic effects of the three potas-
Fig. 1. The antagonism by glibenclamide of the negative inotropic effect of cromakalim in canine atrial muscles. (a) (○) Control, (□) $3 \times 10^{-7}$ M, (■) $10^{-6}$ M, (△) $10^{-5}$ M, (▲) $3 \times 10^{-5}$ M glibenclamide. (b) Schild plot of the antagonism of the negative inotropic effects of cromakalim by glibenclamide. $pA_2$ value, 6.08; the slope of regression line, -0.85; the correlation coefficient, -0.81 (n=26).

Fig. 2. The antagonism by glibenclamide of the negative inotropic effect of pinacidil in canine atrial muscles. (a) (○) Control, (□) $3 \times 10^{-7}$ M, (■) $10^{-6}$ M, (△) $10^{-5}$ M, (▲) $3 \times 10^{-5}$ M glibenclamide. (b) Schild plot of the antagonism of the negative inotropic effects of pinacidil by glibenclamide. $pA_2$ value, 6.06; the slope of regression line, -1.24; the correlation coefficient, -0.90 (n=31).

Glibenclamide at $3 \times 10^{-7}$ to $3 \times 10^{-5}$ M shifted the concentration-negative inotropic effect curves for cromakalim and pinacidil in a parallel manner to the right; the maxima and slope parameters remained nearly unchanged (Figs. 1 and 2, Table 1). In the presence of $3 \times 10^{-6}$ to $3 \times 10^{-5}$ M glibenclamide, however, the curves for nicorandil were shifted to the right and downward (Fig. 3, Table 1). In the presence of $3 \times 10^{-5}$ M glibenclamide, nicorandil ($10^{-3}$ to $10^{-2}$ M) produced a small but distinct positive inotropic effect. Since the concentration-negative inotropic effect curves for cromakalim and pinacidil were shifted to the right in a parallel manner by $3 \times 10^{-7}$ to $3 \times 10^{-5}$ M glibenclamide, the data obtained with these concentrations of glibenclamide were subjected to Schild analysis. Each Schild regression was linear and had a slope of unity (Figs. 1b and 2b). This analysis yielded $pA_2$ values of 6.08 and 6.06 for glibenclamide against cromakalim and pinacidil, respectively. The data points constituting the curves for nicorandil which were shifted almost in a parallel manner by glibenclamide ($3 \times 10^{-7}$ to $3 \times 10^{-6}$ M) were also subjected to Schild analysis. The $pA_2$ value for glibenclamide against nicorandil thus obtained was 6.35 (Fig. 3b).
No modification by glibenclamide of the negative inotropic effects of carbachol and nifedipine: Nifedipine (10^-8 to 3 × 10^-6 M) produced a negative inotropic effect leading to complete abolition of contractions. Carbachol also caused a concentration-dependent decrease in the force of contraction (up to 90%), which was completely blocked by atropine (Fig. 4). The pD2 values of nifedipine and carbachol in producing a negative inotropic effect in the absence and presence of 10^-6 and 10^-5 M glibenclamide are summarized in Table 1. In contrast to the three potassium channel openers, the concentration-negative inotropic effect curves for nifedipine or carbachol were not affected by glibenclamide at all.

Effect of glibenclamide in the presence of negative inotropism produced by cromakalim: In the presence of cromakalim (10^-6 and 10^-5 M), the force of contraction of atrial muscles remained reduced. Under these conditions, the negative inotropic effect of cromakalim was reduced by increasing concentrations of glibenclamide. The negative inotropic effect of 10^-6 M cromakalim was abolished by 3 × 10^-5 M and 10^-4 M glibenclamide, but that of 10^-5 M cromakalim was only partly reversed by those concentrations of glibenclamide (Fig. 5).
Table 1. Influences of glibenclamide on the negative inotropic effects of potassium channel openers, nifedipine and carbachol

|                         | Control | 3×10⁻² | 10⁻⁶ | 3×10⁻⁶ | 10⁻⁵ | 3×10⁻⁵ |
|-------------------------|---------|--------|------|--------|------|--------|
| Cromakalim              |         |        |      |        |      |        |
| Max                     | (5)     | (5)    | (6)  | (5)    | (5)  | (6)    |
| Max                     | 91.3±1.7| 91.2±1.7| 82.9±3.2* | 89.4±2.3 | 84.5±5.6 | 88.9±3.2 |
| pD₂                     | 5.95±0.08| 5.77±0.18| 5.59±0.16** | 5.21±0.2** | 4.79±0.11** | 4.49±0.17** |
| Pinacidil               | (7)     | (7)    | (7)  | (5)    | (8)  | (6)    |
| Max                     | 88.7±2.4| 82.5±4.5| 90.5±2.2 | 86.6±3.4 | 79.0±4.3 | 76.0±4.2* |
| pD₂                     | 5.25±0.10| 5.21±0.07| 5.02±0.11* | 4.68±0.16** | 3.89±0.16** | 3.37±0.15** |
| Nicorandil              | (6)     | (5)    | (6)  | (7)    | (3)  |        |
| Max                     | 87.4±1.8| 79.4±4.2| 73.3±8.0 | 61.4±6.0** | 27.9±12.3* | no analysis |
| pD₂                     | 3.92±0.08| 3.78±0.07| 3.46±0.18* | 3.00±0.13** | 2.34±0.51* | no analysis |
| Nifedipine              | (6)     | (7)    | (5)  |        |      |        |
| Max                     | 96.1±1.4|          | 94.6±2.8 |          | 92.7±4.9 |          |
| pD₂                     | 7.46±0.08|          | 7.42±0.13 |          | 7.24±0.10 |          |
| Carbachol               | (6)     | (9)    | (9)  |        |      |        |
| Max                     | 87.8±0.1|          | 90.8±1.0 |          | 89.1±1.2 |          |
| pD₂                     | 6.97±0.08|          | 6.77±0.12 |          | 6.79±0.15 |          |

Maximum effects (Max) and pD₂ values were obtained by computer fitting the concentration-effect curves to the logistic equation. *P<0.05, **P<0.01, compared with control values. Numbers of the muscles subject to the statistical analysis are given in parentheses.
Discussion

As in the previous experiments (5, 6), the three potassium channel openers, i.e., nicorandil, pinacidil and cromakalim, all produced a negative inotropic effect whose maxima remained about 90% as against 100% with the calcium channel blocker nifedipine in canine atrial muscle. The negative inotropic effects of the three potassium channel openers were all “specifically” antagonized by glibenclamide; the negative inotropic effects of nifedipine and carbachol were not affected at all by glibenclamide. The negative inotropic effect of carbachol has been understood to result from the abbreviation of atrial action potentials due to opening of the potassium channel coupled to muscarinic receptors (25, 26). Indeed, as was the case with the three potassium channel openers, the maximum negative inotropic effect of carbachol remained about 90%. Glibenclamide per se did not affect the basal force of contraction. Thus, in the present experiments, glibenclamide behaved as a pharmacological antagonist as has been shown in its antagonism of the vascular relaxant effect of potassium channel openers (20, 21). Schind analysis of the glibenclamide antagonism of the negative inotropic effects of the three potassium channel openers yielded uniform pA2 values of 6.06–6.35 for glibenclamide against the three potassium channel openers. Thus, it is highly likely that glibenclamide and the three potassium channel openers compete for the same receptors at the presumed ATP-sensitive channel in canine atrial muscle.

The pA2 values for glibenclamide determined in canine atrial muscle were smaller by about one log unit than its pA2 values (7.17–7.22) determined in guinea pig pulmonary arteries against the potassium channel openers, cromakalim, pinacidil and RP 49356 (21). The dissociation constants of glibenclamide estimated from its pA2 values determined in the present study, i.e., 6.06–6.35,
are larger by two orders of magnitude than its binding constants (K_d) obtained with [3H] glibenclamide and its putative receptor solubilized from brain membrane (27). The dissociation constants of glibenclamide estimated in the present study are also larger by two orders of magnitude than its IC50 values in blocking the ATP-sensitive potassium channel in pancreatic B-cells (19, 28). Thus, there would be several subtypes of receptors for glibenclamide or potassium channel openers. The dissociation constants of glibenclamide estimated in the present study are at variance with its K_d values determined with guinea pig and chicken microsomes (29) or its concentrations in antagonizing the effect of ATP depletion in guinea pig cardiac cells (29); the values were in the nanomolar range. However, 100 or 200 nM glibenclamide was needed to antagonize the effect of pinacidil in opening the ATP-sensitive potassium channel in guinea pig ventricular cells (15, 16). At present, the reason for the discrepancy in results on cardiac tissue remains to be elucidated.

In our previous study (6), the negative inotropic effects of the three potassium channel openers were all antagonized by tetraethylammonium (TEA) in a competitive manner in its low concentrations and in a non-competitive manner in its highest concentration. Schild analysis of the competitive antagonism yielded pA_2 values of 3.47–3.66, and the analysis of the non-competitive antagonism gave pK_a values of 3.47–3.89 for TEA against the three potassium channel openers. The negative inotropic effect of pinacidil was also antagonized by tetrabutylammonium (TBA), and Schild analysis yielded a pA_2 value of 4.70 for TBA against pinacidil. Based on these data, we have speculated that quaternary ammonium compounds prevent potassium channel openers from binding to the same sites or closely related sites at the presumed ATP-sensitive potassium channel in canine atrial muscle (6). However, unlike the case with glibenclamide, quaternary ammonium compounds produced a positive inotropic effect while antagonizing the three potassium channel openers. Thus, as has already been shown, quaternary ammonium compounds close the I_K and I_K1 potassium channels and thereby increase the duration of cardiac action potentials leading to positive inotropy in addition to antagonizing the action of potassium channel openers. In contrast, the presumed ATP-sensitive channel susceptible to the opening action of potassium channel openers would remain closed under physiological conditions since intracellular concentrations of ATP are high enough.

In the presence of 3 x 10^{-5} M glibenclamide, nicorandil (10^{-3} to 10^{-2} M) produced a small but distinct positive inotropic effect. In our previous study (30) nicorandil at these high concentrations inhibited the cyclic AMP phosphodiesterase activity in canine cardiac muscle. Thus, the small positive inotropic effect of nicorandil seen in the presence of glibenclamide may have been due to the accumulation of cyclic AMP via inhibition of cyclic AMP phosphodiesterase by nicorandil (7).

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