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Contraction Induced Either by Iso-osmolar or Hyper-osmolar Potassium-rich Solutions Influences Relaxant Responses to Pinacidil and Verapamil in Rat Isolated Aorta

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Abstract—Relaxant responses of pinacidil and verapamil were studied in rat isolated aorta contracted by either iso-osmolar or hyper-osmolar potassium-rich solutions. Relaxant response profiles of pinacidil and verapamil in rat isolated aorta contracted by 124 mM K^+ hyper-osmolar Krebs solutions showed marked reductions in inhibiting E_{max} values and substantial increases in corresponding IC50 values in comparison with results obtained at iso-osmolar conditions. Changes in the slopes of the fitted log concentration–relaxation curves were also observed, whereas pinacidil relaxation curves obtained after initial contraction induced by 30 mM K^+ Krebs solutions were only slightly influenced by osmolarity, a definite decrease in E_{max} occurred for verapamil at hyper-osmolar conditions. Initial tension development was much slower and maximal tension lower when induced by 124 mM K^+ in hyper-osmolar compared with iso-osmolar Krebs solutions. After incubation in Ca^{2+}-deprived EGTA-containing Krebs solutions the maximal tension produced by 124 mM K^+ iso-osmolar Krebs solution in rat aorta was nearly 95% reduced, whereas it was only reduced by 50% at hyper-osmolar conditions. Hyper-osmolarity as established by direct addition of potassium chloride to Krebs solutions in order to induce contraction in vascular smooth muscle could influence the in-vitro action profile, potency and intrinsic activity of the two vascular relaxant drugs.

Potassium-rich Krebs solutions are widely used to contract smooth muscle cells in the assessment of the in-vitro effect of vasodilator drugs, such as calcium-channel blockers. Depolarization and thus contraction of the smooth muscle cells by high K^+-Krebs solutions have also been used in the assessment of the new hyperpolarizing potassium-channel openers such as cromakalim and pinacidil (Bray et al 1987; Meisheri et al 1990). Increase of the K^+-concentration in Krebs bathing solutions is generally done by either adding KCl to the solution, and thus making it hyper-osmolar (Ishida et al 1980; Bray et al 1987) or by replacing an equal molar amount of NaCl by KCl in order to keep the solution iso-osmolar (Massingham 1973; Karaki et al 1988).

The effect of both diltiazem on rat aorta smooth muscle and pinacidil on guinea-pig airway smooth muscle has previously been reported to be influenced by the osmolarity of the K^+ solution used for contracting the preparations (Magliola & Jones 1987; Nielsen-Kudsk et al 1990).

Since experiments with new putative vasodilating drugs are widely carried out on the rat isolated aorta contracted by high K^+ solution, we found it is of interest to study the combined influence of osmolarity and K^+ concentration in the Krebs solution on the relaxant responses of a potassium-channel opener, pinacidil, and a calcium-channel blocking agent, verapamil, on the isolated smooth muscle cells of rat aorta.

Materials and Methods

Rat isolated aorta-preparations

Wistar rats of either sex, 300–400 g, were killed by a blow to the neck. The thoracic aorta was isolated and cut into tubular segments (length 3 mm). These were transferred to temperature-regulated (37·0°C) 5 mL organ baths containing Krebs solution (composition in mM: NaCl 118·0, KCl 4·6, $CaCl_2$ 2·5, $MgSO_4$ 1·15, $NaHCO_3$ 24·9, KH_2PO_4 1·15, glucose 5·5, pH = 7·4) aerated with 95% O_2–5% CO_2. Each preparation was mounted in a precision myograph (Nielsen-Kudsk et al 1986) and suspended under a passive tension of 3·0 g. Before the experiments, the preparations were allowed to equilibrate for 1 h during frequent exchange of the bathing Krebs solution.

Experiments

The relaxant activity of pinacidil and verapamil was assessed from concentration–effect curves, obtained with the preparations precontracted by either 30 mM K^+-iso- or hyper-osmolar Krebs solutions or by 124 mM K^+-iso- or hyper-osmolar Krebs solutions. In the iso-osmolar K^+-solutions an equal amount of NaCl was replaced by KCl. The final KCl concentration includes the K^+ already present in the solution. In the hyper-osmolar K^+ solution an extra small volume of KCl (4060 mM) was added to the normal Krebs solution.

Pinacidil and verapamil were added cumulatively to the organ baths when stable contractions to the K^+ solutions had developed. The total extra volume added to the organ bath in this manner was 200 μL. The tension was allowed to stabilize before the concentration of pinacidil or verapamil was increased. The tension produced by 124 mM K^+ iso- or hyper-osmolar solution was compared before and after 1-h incubation in a Ca^{2+}-deprived EGTA (1 mM)-containing solution, which was exchanged every 15 min. After the

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Table 1. Contraction tension and the related times for induction as elicited by 124 mM K^+ iso-osmolar, 124 mM K^+ hyper-osmolar, 30 mM K^+ iso-osmolar or 30 mM K^+ hyper-osmolar solutions in rat isolated aorta (n = 5–8).

Solution	Tension level (g ± s.e.)	Time (min ± s.e.)
124 Iso-osmolar	1·8 ± 0·2	17·2 ± 1·1
124 Hyper-osmolar	1·3 ± 0·1*	31·8 ± 1·9*
30 Iso-osmolar	1·0 ± 0·1*	12·9 ± 0·6#
30 Hyper-osmolar	0·9 ± 0·1*#	12·6 ± 1·1#

*$P < 0·05$ compared with 124 mM K^+ iso-osmolar, #$P < 0·05$ compared with 124 mM K^+ hyper-osmolar.

secondary tension had developed the effects of papaverine (10^{-4} M) were measured after another 20 min.

Data analysis

Mean tension values ± s.e. were calculated for each of the four K^+ solutions and in the Ca^{2+}-deprived EGTA-containing solution experiment. Relaxant mean values ± s.e. were calculated for each concentration of pinacidil and verapamil. Continuous sigmoid log concentration–effect curves and the corresponding IC50, E_{max} and Hill exponent (S) parameters were obtained from the mean data by nonlinear iterative regression analysis. Differences between the calculated parameters were evaluated statistically by Student's *t*-test for paired or unpaired data. A *P* value of 0·05 was considered as the limit of statistical significance.

Drugs

Pinacidil (Leo Pharmaceutical Products, Copenhagen, Denmark) was dissolved in 0·1 M HCl and further diluted with 0·9% NaCl (saline). Verapamil (GEA, Copenhagen, Denmark) was dissolved in saline. Papaverine (Sigma Chemical Co., St Louis, MO, USA) was dissolved in ethanol, and EGTA (Sigma Chemical Co., St Louis, MO, USA) was dissolved in the Krebs solutions.

Results

The maximal and stable tension levels produced by the different high-K^+ Krebs solutions and the time needed to reach these levels are given in Table 1. In the experiment using Ca^{2+}-deprived EGTA-containing solutions, the tension developed by the 124 mM K^+ iso-osmolar solution was reduced by 93·6 ± 1·7% (n = 5) when rechallenged with 124 mM K^+ iso-osmolar solution, while the tension developed when rechallenged with 124 mM K^+ hyper-osmolar solution was only reduced by 45·2 ± 10·0% (n = 5), which was significantly less. The persisting tension of the hyper-osmolar solution, was not influenced by the addition of papaverine (10^{-4} M) to the bathing solution.

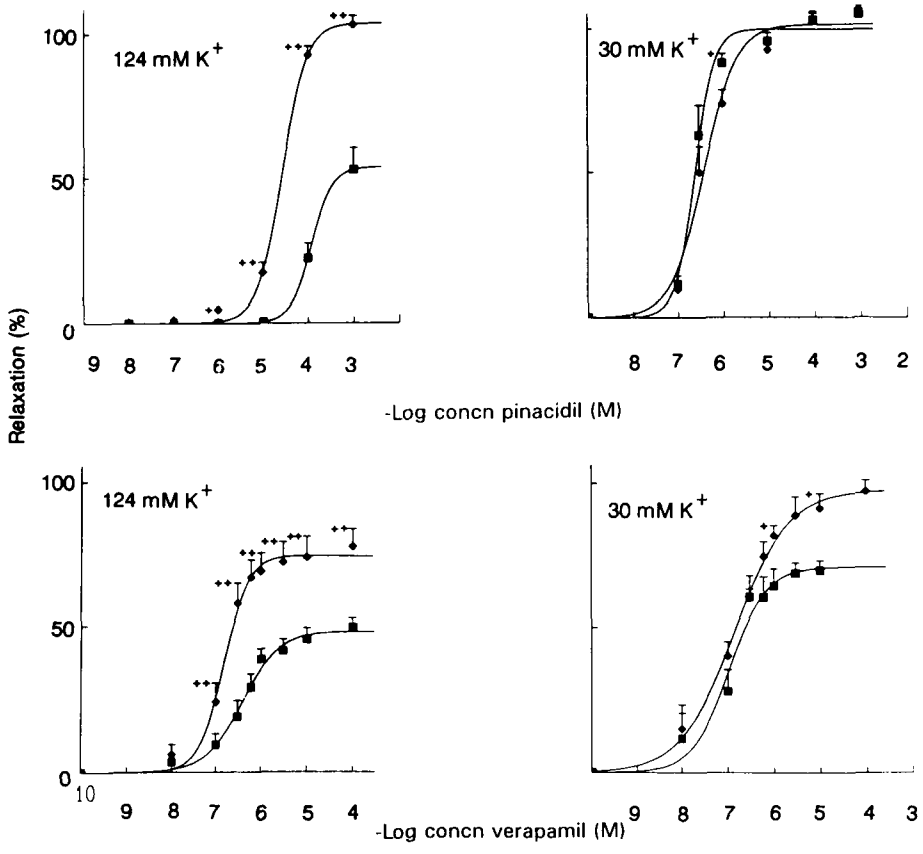

FIG. 1. Log concentration–relaxation plots and fitted curves for pinacidil and verapamil effects on rat isolated aorta in-vitro contracted by 30 or 124 mM K^+ in either iso- (◆) or hyper-osmolar (■) Krebs solutions. The curves were obtained by iterative, nonlinear regression analysis of the experimental data using a hyberbolic Hill equation as the model function. Vertical bars represent s.e. The derived dynamic parameters are stated in Table 1 (n = 5–7). †$P < 0·05$, ††$P < 0·01$.

Table 2. Inhibiting E_{max} values, IC50 values and Hill coefficient ($\pm$s.e., n = 5–7) for the relaxing effects of pinacidil and verapamil on rat aorta ring preparations contracted by 124 or 30 mM potassium in either iso-osmolar or hyper-osmolar K^+-enriched Krebs solutions. The results were derived by iterative, nonlinear regression analysis of the experimental data plotted in Fig. 1.

	124 mM K^+-enriched Krebs		30 mM K^+-enriched Krebs	
	Iso-osmolar	Hyper-osmolar	Iso-osmolar	Hyper-osmolar
Pinacidil				
E_{max} (%)	103·7±2·6	53·9±0·01	101·3±4·2	99·6±2·9
IC50 (μM)	26·9±2·9	120±2·8	0·389±0·07	0·25±0·03
Hill coefficient	1·60±0·16	1·88±0·03	1·19±0·27	1·99±0·39
Verapamil				
E_{max} (%)	75·5±1·7	49·0±1·7	97·5±2·1	70·9±4·3
IC50 (μM)	0·16±0·01	0·41±0·05	0·12±0·02	0·11±0·003
Hill coefficient	1·57±0·20	1·06±0·14	0·74±0·06	1·09±0·29

The concentration–effect curves of the relaxant effects of pinacidil and verapamil are shown in Fig. 1; IC50, E_{max} and the slope of the curves (S) are stated in Table 2. The IC50 values for pinacidil were increased when 124 mM K^+ Krebs solutions were used instead of 30 mM K^+ Krebs solutions and the E_{max} values of pinacidil were significantly reduced when rat aorta was contracted by 124 mM K^+ hyper-osmolar solutions instead of 124 mM K^+ iso-osmolar solutions, but pinacidil was able to relax rat aorta completely, except when contracted by 124 mM K^+ hyper-osmolar solution, E_{max} being about 50%. The time needed for a stable tension level before adding the next pinacidil concentration was 10 min, regardless of which solution was used as contracting medium.

The potency and intrinsic activity of verapamil was dependent on the osmolarity of the solution used to contract the isolated aorta. When 124 mM K^+ were used, E_{max} was reduced from 75·5±1·7% (iso-osmolar) to 49·0±1·7% (hyper-osmolar) and the IC50 displayed a 2·6-fold shift to the right when hyper-osmolar solutions were used. Unlike pinacidil, verapamil was less efficient when rat aorta was contracted by 30 mM K^+ hyper-osmolar solutions instead of 30 mM K^+ iso-osmolar solutions. The time needed for a stable tension level before adding the next verapamil concentration was 30 min, regardless of which solution was used as the contraction medium.

Discussion

The present study shows that not only the tension level and time to attain this level, produced by high K^+-rich solutions, is influenced by the osmolarity and the concentration of K^+ in these solutions, but also the relaxant effects of pinacidil and verapamil on rat isolated aorta are influenced.

The tension levels produced by 124 mM K^+ iso-osmolar solution, were significantly larger and developed significantly more rapidly than those produced by 124 mM K^+ hyper-osmolar solution. This indicates an additional mode of action of the 124 mM K^+ hyper-osmolar solution. In agreement with this, previous reports have shown that hyper-osmolar solutions with or without high K^+ concentrations can induce a contraction of rat aorta smooth muscle (Kent et al 1983; Magliola & Jones 1987). The mechanism behind this has, however, not been clarified. Kent et al (1983) suggested that contractile response to hyper-osmolar sucrose solutions in rat aorta might be due to alteration in the intracellular calcium and similar conclusions were made when rat aorta was contracted by 100 mM K^+ hyper-osmolar solution (Magliola & Jones 1987). Other authors have provided evidence that hyper-osmolar sucrose solutions produce cell shrinkage, due to dehydration, which might contribute to the contraction of smooth muscle cell preparations as seen in in-vitro experiments (Kirkpatrick et al 1980). To elucidate the importance of extracellular Ca^{2+} on the action of 124 mM K^+-iso- or hyper-osmolar Krebs solutions, experiments with Ca^{2+}-deprived EGTA-containing Krebs solutions were performed. The result could be interpreted as if only about 50% of the tension produced by 124 mM K^+ hyper-osmolar solution, was influenced by influx of extracellular Ca^{2+} since the tension level was reduced by approximately 50% after incubation in a Ca^{2+}-deprived EGTA-containing medium for 1 h, whereas the tension produced by 124 mM K^+ iso-osmolar solutions was reduced by 93·6% in agreement with previous reports (Winquist et al 1984; Rico et al 1990). Furthermore, in agreement with this, verapamil, a calcium-influx blocking drug, was only able to reduce tension produced by 124 mM K^+ hyper-osmolar solutions by approximately 50% (E_{max} in Table 1). Addition of papaverine (10^{-4} M) did not reduce the tension further after 20 min. The Na^+ concentration in the 124 mM K^+ iso-osmolar solution is very low, but the influence of this low Na^+ content on membrane potential is only about 1·5% (calculated by the Goldmann constant field equation). This could influence the results, but only to a limited extent. When 30 mM K^+ solutions were used, the tension and the time to attain this was similar, indicating that only when very high K^+ concentrations are used is the composition of the potassium-rich solution of importance.

The relaxant effects of pinacidil and verapamil in the present study, were influenced by osmolarity and the concentration of K^+ in the solutions. In agreement with the present results which showed a very reduced potency of pinacidil under 124 mM K^+ conditions, Mikkelsen & Pedersen (1982) showed that pinacidil was by far the less potent drug in inhibiting 127 mM K^+ iso-osmolar-induced rat aorta con-

tractions compared with the calcium antagonist nifedipine, although they were able to relax rat aorta completely with pinacidil 10^{-3} M, as in the present study. Thus pinacidil is able to relax rat isolated aorta contracted by 124 mM K^+ iso-osmolar solutions completely, and must therefore have an additional mode of action besides opening of potassium channels. Others have found, in contrast to the present and previous results (Nielsen-Kudsk et al 1990), that pinacidil was unable to relax rat aorta contracted by 80 mM K^+ hyper-osmolar solutions (Bray et al 1987).

The E_{max} values for verapamil were decreased under hyper-osmolar conditions and complete relaxation was only observed when 30 mM K^+ iso-osmolar solution was used. The IC50 values for verapamil were enhanced when 124 mM K^+ hyper-osmolar solution was used, while the IC50 values for verapamil alone was slightly different when the other solutions were used. In agreement with this, Koch et al (1988) found that verapamil (10^{-5} M) only reduced the tension level in rat aorta contracted by 80 mM K^+ iso-osmolar solutions by approximately 80%. Others found an IC50 value of approximately 0·3 μM for verapamil when rat aorta was contracted by 45 mM K^+ iso-osmolar solutions (Massingham 1973), while Ishida et al (1980) found an IC50 value of approximately 0·1 μM for verapamil when rat aorta was contracted with 40 mM K^+ hyper-osmolar solutions. Thus it seems that the E_{max} value of verapamil is influenced by the osmolarity and concentration of K^+ in the Krebs solutions.

In conclusion, the present study has shown that the tension and the time to attain this tension produced by high K^+ solutions in rat aorta smooth muscle preparations is influenced by the osmolarity and the concentration of K^+ in the solution. Furthermore, the relaxant responses to pinacidil and verapamil is influenced by the osmolarity and the concentration of K^+ in the solution inducing rat aorta contraction.

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