Br. J. Pharmac. (1979), **66**, 303–306

THE EFFECT OF POTASSIUM ON FROG STOMACH MUSCLE

F. BAYSAL, S. ÖNDER, M. ÖZGÜL & A. TOYGAR

Department of Pharmacology, Faculty of Medicine (Numune Hospital) Adana, Turkey

1 KCl relaxed strips of frog stomach muscle. Usually, the effect was biphasic, i.e. contraction followed by relaxation at a concentration of 137 mM.

2 The effect was mimicked by K_2SO_4.

3 Ouabain (5 and 10 μg/ml) blocked the relaxations and reversed them to contractions. The tension of strips was not affected.

4 Phenoxybenzamine (1 μg/ml) and procaine (20 μg/ml) inhibited the relaxations, potentiated the contractile component and lowered the tone of the muscle.

5 The relaxation and the tone were inhibited by papaverine (5 μg/ml) but the contractile component was unaffected.

6 It is suggested that potassium-induced relaxations are mediated through the activation of the sodium pump.

Introduction

Potassium chloride induces relaxations in some smooth muscle preparations of warm-blooded animals. Guinea-pig taenia coli that had undergone prolonged cold storage showed hyperpolarization accompanied by relaxation after the addition of potassium (Shibata, Fukuda & Kurahashi, 1973). This inhibitory action of potassium was prevented if the taenia were pretreated with ouabain or incubated in Tyrode solution containing lithium instead of sodium. From these results it was concluded that activation of the electrogenic sodium pump in taenia by potassium elicits these effects. Potassium caused an immediate inhibition of the activity of rabbit myometrium bathed in K^+-free Krebs solution (Johns & Paton, 1974). This effect was abolished by ouabain treatment. The observed inhibition could be produced by the operation of the pump mechanism. Another smooth muscle preparation, the rat anococcygeus muscle treated with acetylcholine, showed dose-dependent relaxations to KCl (Gibson & James, 1977). These authors suggested that this effect may be due to stimulation of inhibitory nerves by potassium ion.

To our knowledge a detailed study of the effects of potassium ion on smooth muscle of cold-blooded animals has not yet been published. Therefore, we have investigated the action of this ion on frog stomach muscle strip pretreated with carbachol.

Methods

Longitudinal muscle strips prepared from the stomach of the pithed frog (*Rana esculenta*) were used in these experiments. The method (Baysal, 1967) was described earlier. Briefly, the stomach was removed and cut open by an incision along the lesser curvature. The mucosa was stripped off. The tissue was then incised two or three times in the direction of the longitudinal muscle. Strips thus obtained (approximately 3 cm long and 0.2 cm wide) were mounted longitudinally in an organ bath filled with a modified Tyrode solution of the following composition (mM): NaCl 135, KCl 2, $CaCl_2$ 3, NaH_2PO_4 0.4, $NaHCO_3$ 12 and dextrose 6. The activity of muscle strips was recorded with an isotonic lever ($\times$ 10 magnification) on a smoked drum. Tension on the strip was 0.5 g. The preparation was allowed to equilibrate for 1 h. The solution was aerated with O_2. Some strips were treated for 10 min with isotonic KCl Tyrode containing different concentrations of KCl. Tissues were then washed with Tyrode solution and allowed to recover for 20 min. In other experiments the tone of the muscle was first raised by placing it in Tyrode solution containing 0.5 μg/ml carbachol for 1 h. Carbachol was tried in the concentration range 0.25 to 1 μg/ml and the most reproducible KCl-induced responses occurred at 0.5 μg/ml. Five strips were used for each concentration. Following carbachol incubation strips were treated with isotonic KCl Tyrode. The concentrations of KCl in different isotonic KCl Tyrode solutions were 13, 27, 54 and 137 mM. In these solutions NaCl was replaced by KCl. Each concentration was tested twice during the control period. Contact time with KCl Tyrode was 5 min. After this, strips were washed with Tyrode solution

0007-1188/79/060303-04 $01.00

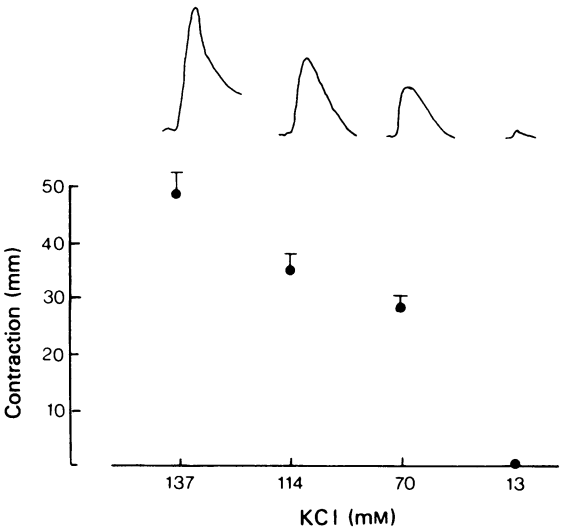

Figure 1 The effect of different KCl Tyrode solutions on frog stomach muscle strips. Points on the graph are mean values (n = 8); Vertical lines show s.e. mean.

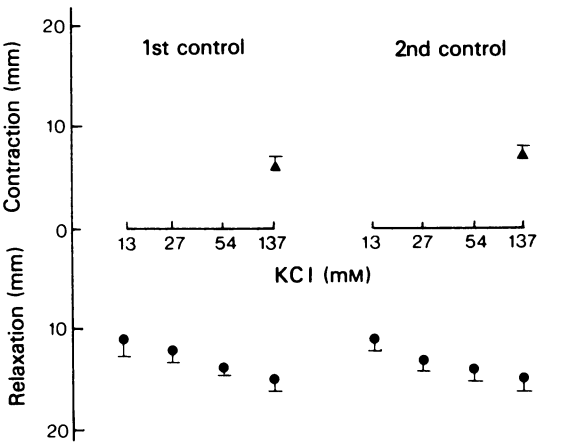

Figure 2 Effects of different isotonic KCl Tyrode solutions on strips pretreated with carbachol. Points are mean values (n = 19); Vertical lines show s.e. mean.

containing carbachol and left for 15 to 20 min. In some experiments we used KCl Tyrode solutions containing 0.5 μg/ml carbachol. No statistically important differences (n = 13) were found between results obtained in KCl Tyrode with carbachol and those of KCl Tyrode without it. The latter solution was considered more suitable for studying the effect of the potassium ion. In a series of experiments the sensitivity of strips to KCl Tyrode was tested again after the first control.

In order to investigate which ion (potassium or chloride) was involved in the action, K_2SO_4 Tyrode was used instead of KCl Tyrode (n = 10) and the effect was compared with that of KCl Tyrode containing 137 mM potassium chloride. K_2SO_4 Tyrode was prepared by complete replacement of NaCl and KCl with K_2SO_4.

Other experiments were carried out in which strips were incubated with ouabain, papaverine, phenoxybenzamine, propranolol or procaine following control responses to KCl Tyrode. The substances were equilibrated for 1 h and kept in the bathing medium throughout the experiment. A separate group of strips was used for each concentration of drugs. In each experimental group, the mean values of KCl-induced effects were calculated and comparisons made by use of Student's t-test. Some results (contraction and relaxation, in mm) were plotted graphically against KCl concentration (mM).

Results

Isotonic KCl Tyrode produced contraction in 8 strips which had not been pretreated with carbachol (Figure 1). The response was dependent on the concentration of KCl.

In the presence of carbachol, a group of strips (n = 19) was treated with isotonic KCl Tyrode solution. KCl relaxed the muscle strips in a dose-dependent manner. However, the same tissue showed a biphasic response, i.e. contraction followed by relaxation at a KCl concentration of 137 mM. After this first control, KCl was tried again on the same strips. No significant differences from the first control were observed (Figure 2).

The responses produced by KCl were mimicked by K_2SO_4 in 10 strips. The same tissues were also treated with 137 mM KCl. The effects of these two potassium salts were not statistically different ($P >$ 0.999).

Ouabain was studied at concentrations of 0.25, 1, 5 and 10 μg/ml. The first two doses failed to elicit any change. However, higher concentrations (5 and 10 μg/ml) inhibited relaxation, increased the contractile component and caused the tissue's responses to resemble those in normal solution (Figure 3). Contractions observed after this treatment were dose-dependent. Spontaneous activity was also inhibited. No important inhibition was observed in muscle tone. The numbers of strips used for each ouabain concentration were respectively 6, 6, 7 and 7.

Some strips were incubated with papaverine (1 and 5 μg/ml). The muscle tension and the responses to KCl were not affected by the smaller dose while 5 μg/ml lowered the tension and depressed relaxations. However, the contractile component showed no significant change with any papaverine concentration. The strip numbers for each concentration were 6 and 11 respectively.

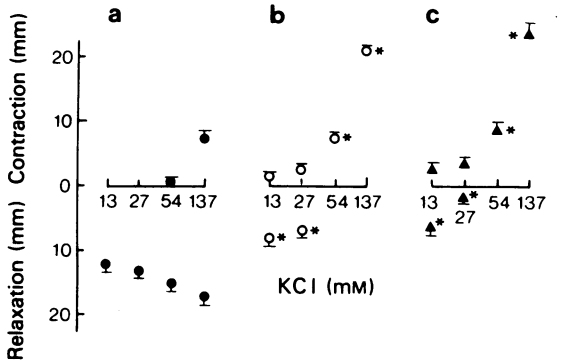

Figure 3 The action of ouabain on KCl-induced effects: (a) control responses (n = 70); (b) in presence of ouabain 5 μg/ml (n = 7); (c) in presence of ouabain 10 μg/ml (n = 7). Vertical lines show s.e. mean. *Indicates significant difference from control values ($P <$ 0.005 and $<$0.001).

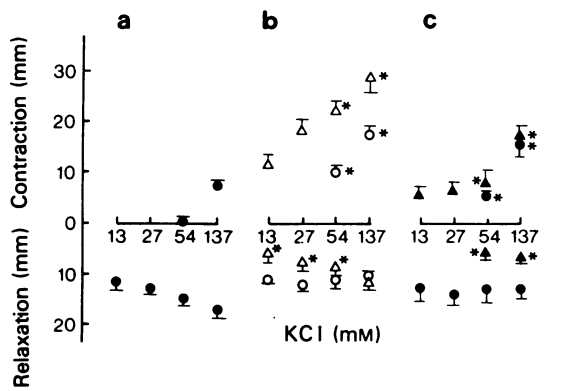

Figure 4 The actions of procaine and phenoxybenzamine on KCl-induced effects in frog stomach strip. (a) Control responses (n = 70); (b) in presence of procaine: (○) 5 μg/ml (n = 5) and (△) 20 μg/ml (n = 7); (c) in presence of phenoxybenzamine: (●) 0.2 μg/ml (n = 5) and (▲) 1.0 μg/ml (n = 8). *Indicates significant difference from control values ($P < 0.02$; $P < 0.005$ and $P < 0.001$).

Procaine was added to the bathing medium at three different concentrations (1, 5 and 20 μg/ml); 7, 5 and 7 strips were used, respectively, for each concentration. All concentrations except 1 μg/ml produced significant effects on KCl-induced responses. The contractile component increased in a significant manner (Figure 4). Relaxation was significantly inhibited only after the highest concentration of procaine (20 μg/ml) which also lowered tone.

No change was observed in the tone of 5 strips treated with 0.2 μg/ml phenoxybenzamine. Relaxations to KCl were not significantly affected despite the increase in the contractile component. A higher concentration of phenoxybenzamine (1 μg/ml, 8 strips) lowered tone, relaxations decreased and the contractile component increased (Figure 4). The responses to adrenaline (2.5 and 5 μg/ml) were significantly blocked only at 1 μg/ml phenoxybenzamine. Concentrations of adrenaline lower than 2.5 μg/ml were ineffective in producing relaxation. In some strips, reproducible responses to adrenaline did not always occur even after these large doses.

Relaxations in response to KCl or adrenaline were unaffected by propranolol treatment (0.2 and 1 μg/ml); 5 and 6 strips were used for each propranolol concentration.

Discussion

The present study indicates that the active ion in KCl is K^+ because similar results are obtained by the treatment of the tissue with another potassium salt, K_2SO_4.

K^+-induced relaxations of frog stomach muscle can be explained by the stimulating action of this ion on the sodium pump, since ouabain inhibited relaxation and reversed it to a contractile response. There is also some evidence that high external potassium can accelerate the activity of this pump (Shibata *et al.*, 1973). A similar hypothesis has been advanced for the effect of potassium on taenia of guinea-pig and rabbit myometrium (Shibata *et al.*, 1973; Johns & Paton, 1974). The concentration of ouabain that we used to obtain a significant action was much higher than the concentration (2 and 10 ng/ml) required to inhibit the sodium pump in rat uterus (Türker, Page & Khairallah, 1967). However, in snail brain 10 μg/ml ouabain was needed to inhibit the pump mechanism and thus reduce the membrane potential (Kerkut, Lambert & Walker, 1973). Similar results also occurred when the potassium concentration was reduced from 4 mM to 0 mM.

The possibility that adrenoceptor activation can explain the relaxation due to potassium was investigated in several ways. Firstly propranolol, a β-adrenoceptor blocking agent, was used. Neither relaxation due to KCl nor to adrenaline was affected by this substance in the concentrations used here. Secondly phenoxybenzamine, an α-adrenoceptor blocking agent, was applied. The high concentration of the latter drug decreased both KCl and adrenaline-induced relaxations. Mean values of the contractile component were significantly increased by low and high doses of this drug. The reductions in KCl as well as in adrenaline-induced relaxations may be due to a nonspecific action, i.e. the inhibition of strip tone, since a similar decrease in KCl-induced relaxation

could be obtained by papaverine treatment. A relaxation could be produced only after large doses of adrenaline. Probably more propranolol is required to block the effect of adrenaline. Thus, it is very unlikely that KCl-induced relaxations are mediated through the activation of adrenoceptors.

Procaine produced results similar to those found with phenoxybenzamine. This supports the idea (Somlyo & Somlyo, 1970) that there is a strong resemblance between the actions of haloalkylamines and local anaesthetics on smooth muscle. Although inhibition of the relaxations produced by both substances can be reasonably explained by their action on tone, the significant potentiation of the contractile component might be due to an effect on membrane calcium fluxes. In other words, the latter action results from a specific effect, not from a decrease in tone, since no significant increase after papaverine treatment in the contractile component was observed despite the inhibition of the muscle tension. In our opinion, the effect of procaine occurred at doses too high for selective action on any neural pathway of the smooth muscle. Nevertheless, this conclusion is not in agreement with the hypothesis advanced by Gibson & James (1977) on potassium-induced relaxations of the rat anococcygeus muscle.

The authors would like to thank Merck, Sharp & Dohme for the generous gift of carbachol. Please send reprint requests to F.B.

References

BAYSAL, F. (1967). Izole kurbağa mide adelesinden hazirlanan bir preparasyonun bazi farmakolojik özellikleri. *A. Ü. Tip Fak. Mec.*, **20**, 533–538.

GIBSON A. & JAMES T.A. (1977). The nature of potassium chloride-induced relaxations of the rat anococcygeus muscle. *Br. J. Pharmac.*, **60**, 141–145.

JOHNS, A. & PATON, D.M. (1974). Inhibitory effect of sodium pumping on spontaneous contractility of rabbit myometrium. *Can. J. Physiol. Pharmac.*, **52**, 786–790.

KERKUT, G.A., LAMBERT, J.D.C. & WALKER, R.J. (1973). The action of acetylcholine and dopamine on a specified snail neurone. In *Drug Receptors*. ed. Rang, H.P., pp. 37–44. London: University Park Press.

SHIBATA, S., FUKUDA, H. & KURAHASHI K. (1973). The inhibitory action of potassium on the mechanical and membranal activities of taenia from guinea-pig caecum. *Life Sci.*, **12**, 425–431.

SOMLYO, A.P. & SOMLYO A.V. (1970). Vascular smooth muscle pharmacology—II. Pharmacology of normal and hypertensive vessels. *Pharmac. Rev.*, **22**, 249–353.

TURKER, R.K., PAGE, I.H. & KHAIRALLAH, P.A. (1967). Angiotensin alteration of sodium fluxes in smooth muscle. *Archs int. Pharmacodyn.*, **165**, 394–404.

(*Received September* 20. 1978.
Revised December 29, 1978.)