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KCl Contractions in the Rat Isolated Seminal Vesicle

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Abstract—Potassium chloride (K^+) produced dose-dependent contractions of the rat isolated seminal vesicle with no sign of tachyphylaxis. The contractile response was biphasic in nature and composed of an early rapid phasic contraction and a slowly developing, but more sustained, tonic response separated by a transient relaxation. Neither cocaine nor depletion of tissue catecholamines by reserpine influenced responses to K^+. Moreover, guanethidine, phentolamine, phenoxybenzamine, atropine and physostigmine all failed to modify responses of the tissue to K^+. Thus, the possibility of K^+ acting via release of endogenous noradrenaline or acetylcholine is excluded. Calcium-free conditions with or without EGTA reduced both the components of K^+-induced contraction. The rate of reduction of both responses was faster in the presence of EGTA with part of the tonic response being resistant even to calcium deprivation in the presence of EGTA. On the other hand, verapamil reduced both responses in a similar manner, whereas nifedipine produced dose-dependent rightward shifts of the concentration-response curves of both the phasic and tonic responses to K^+. However, in the presence of nifedipine, the maximum response of only the phasic contraction was significantly lowered. It is concluded that both phases of KCl contraction in the rat seminal vesicle use extracellular Ca^{2+}, of which some is tightly bound with high affinity, probably to plasma membranes. In addition, two subtypes of voltage-sensitive Ca^{2+} channels may exist, one of which is preferentially sensitive to nifedipine and both are sensitive to verapamil.

Smooth muscles respond to potassium chloride (K^+) with an early rapid phasic contraction followed by a more slowly developing but sustained tonic contraction (Bolton 1979; Högestätt & Anderson 1984). Contractions in response to K^+ are the result of increased membrane permeability to calcium (Ca^{2+}) consequent to membrane depolarization either directly or via the release of endogenous neurotransmitters. It is generally accepted that in smooth muscles, mobilization of Ca^{2+} from extracellular or intracellular sources is necessary to support contraction (Rodger 1985). The contractile responses to K^+ have been extensively investigated in the rat vas deferens (Syson & Huddart 1973; Swamy et al 1976; Triggle et al 1979; Shimodan & Sunano 1981; Hay & Wadsworth 1982). The calcium antagonists verapamil and nifedipine as well as Ca^{2+}-free conditions have been found to inhibit the biphasic response of the vas deferens to K^+, and it was proposed that K^+ uses extracellular Ca^{2+} for contraction. In the present study, the possibility of endogenously released noradrenaline and acetylcholine contributing to this biphasic response is examined in the rat isolated seminal vesicle (Sharif & Gokhale 1986; Sharif et al 1990). The source of activator Ca^{2+} involved in this response is also studied.

Materials and Methods

Preparation of tissues

Seminal vesicles from male Sprague-Dawley rats, 140–160 g, were dissected and prepared as described previously (Sharif & Gokhale 1986). Each was mounted in a 5 mL jacketed organ-bath under a resting tension of 350 mg and bathed in a continuous flow (15 mL min^{-1}) of Krebs solution of the following composition (mM): NaCl 114, KCl 4·7, CaCl 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_2$ 25 and glucose 11·7. The Krebs solution was aerated with 5% CO_2 and maintained at 32°C. Isotonic contractions were recorded via Searle Bioscience T_2 isotonic transducers and displayed on a Searle Bioscience 400 MDR physiograph recorder. The tissues were allowed to equilibrate for at least 60 min before K^+ was added at 20 min intervals and allowed to act for 2 min. Concentration-response curves for K^+ were generated at 60 min intervals by sequential addition of a series of increasing concentrations (0·02–0·16 M). In individual experiments, one horn of a paired preparation was used to study the effects of drugs on responses to K^+ while the other served as a control to test for time-induced changes in sensitivity to K^+ or the effects of vesicles in which drugs were prepared.

Initially control curves for K^+ were obtained in both horns of a paired preparation; later one of them was perfused for 60 min with Krebs solution containing a predetermined concentration of a single drug, in each individual experiment. Responses are expressed as a percentage of the maximum contraction (E_{max}) evaluated by threshold concentration (the concentration necessary to produce the first sign of contraction) and the molar concentration required to produce 50% of the E_{max} (EC50). Estimate of pD_2 (the negative logarithm of the molar concentration producing 50% of the maximum contraction) was determined according to the method of MacKay (1978).

Calcium deprivation and calcium restoration

To examine the effect of Ca^{2+} deprivation and restoration, two successive stable and reproducible contractions to K^+ (100 mM) were first obtained in normal Krebs solution ($[Ca^{2+}]_o = 2·5$ mM) followed by replacement with Ca^{2+}-free Krebs solution either with or without EGTA (0·2 mM) and tissues were allowed to equilibrate for various periods of time before re-exposure to K^+. Tissues were allowed to recover in normal Krebs solution for 20 min between exposures to K^+ in the Ca^{2+}-deprived medium.

Calcium antagonists

After obtaining reproducible contractions to 100 mM K^+, verapamil was applied in increasing concentrations in a cumulative manner (1, 5, 10, 25, 50, 75 and 100 μM). At each concentration, the calcium antagonist was allowed to act for 15 min before the response to K^+ was obtained. Inhibition of contractions by verapamil was used to determine the IC50 (the concentration producing 50% inhibition) and I_{max} (the concentration producing maximum inhibition) values against both the phasic and tonic components of K^+-induced contraction.

With nifedipine, control curves were first determined; tissues were then perfused for 90 min with Krebs solution containing a predetermined concentration of nifedipine (10^{-7}–10^{-5} M).

Reserpine pretreatment

Reserpine (0·5 mg kg^{-1}) was administered intraperitoneally on two consecutive days and animals were killed 24 h after the second injection.

Materials

Acetylcholine chloride (Merck Darmstadt, Germany), atropine sulphate (BDH), cocaine hydrochloride (MacFarlen-Smith Edinburgh, UK), nifedipine (Bayer Leverkusen, Germany), EGTA, (−)-noradrenaline bitartrate, physostigmine salicylate; tyramine hydrochloride and verapamil hydrochloride (Sigma, St Louis, MO), guanethidine sulphate, phentolamine mesylate, reserpine hydrochloride (Ciba-Giegy, Basel, Switzerland) and phenoxybenzamine hydrochloride (SK & F, Welwyn, UK) were used.

Nifedipine was dissolved in water containing ethanol (150 mg mL^{-1}) and polyethylene glycol (150 mg mL^{-1}). The stock solution was stored in the dark to avoid degradation by light.

Statistical analysis

Results are expressed as mean ± s.e.m. In the graphs, standard errors are shown by vertical bars. Student's *t*-test was used for statistical analysis.

Results

Response of rat seminal vesicle to K^+

Potassium chloride (K^+, 2×10^{-2}–$1{\cdot}6\times10^{-1}$ M) produced reproducible concentration-dependent contractions of the rat isolated seminal vesicle with no tachyphylaxis for 4–6 h. The contractile response to K^+ was biphasic with a rapid transient phasic component, followed by a brief fall and then a slowly developing and sustained tonic response. The pD_2 for K^+ was 1·34 ± 0·05 (n = 10).

Effect of adrenergic drugs on K^+-induced contraction

Neither cocaine nor guanethidine influenced the concentration-response curves of either the phasic or tonic components of K^+ contraction.

Seminal vesicles from rats pretreated with reserpine to deplete catecholamines did not respond to a high concentration (0·01 M) of tyramine, an indirectly acting sympathomimetic amine. Reserpine also failed to influence the curves of either components of K^+-induced contraction. In addition, the α-adrenoceptor antagonists phentolamine and phenoxybenzamine (both at a concentration of 10^{-7} M), and the cholinergic drugs atropine (10^{-7} M) and physostigmine (10^{-6} M) failed to modify contractions of the seminal vesicle in response to K^+. At the concentrations used, the α-adrenoceptor antagonists inhibited submaximal responses to noradrenaline, and atropine inhibited while physostigmine potentiated responses to acetylcholine.

Effects of Ca^{2+}-deprivation and restoration on responses to K^+

Exposure of seminal vesicles to Krebs solution without Ca^{2+} added produced significant inhibition of K^+ contraction. Both the phasic and tonic contractions were reduced in a similar manner but with a residual response, in spite of Ca^{2+}-deprivation. However, when EGTA 0·2 mM was added to the Ca^{2+}-free Krebs solution, the phasic response declined rapidly and was eventually blocked (Fig. 1a). The rate of decay of the tonic response developed more slowly with a residual response even in the presence of EGTA (Fig. 1b).

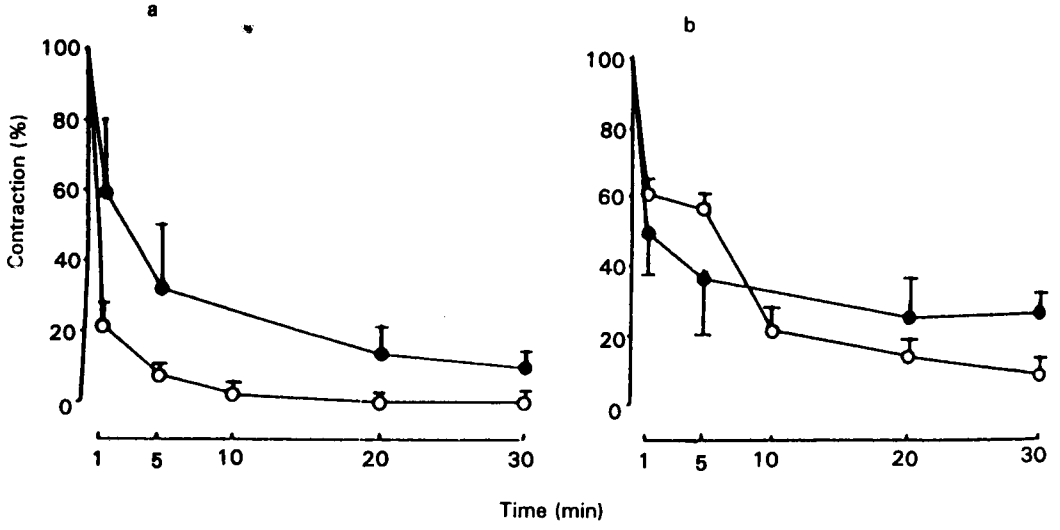

FIG. 1. Reduction of phasic (a) and tonic (b) components of K^+-induced contraction by incubation of tissues in Ca^{2+}-free Krebs (●) and Ca^{2+}-free Krebs containing 0·2 mM EGTA (○) for increasing periods of time. The ordinate shows responses expressed as a percentage of maximum response to K^+ in normal Krebs solution. Each point is the mean of six observations; vertical bars represent ± s.e.m.

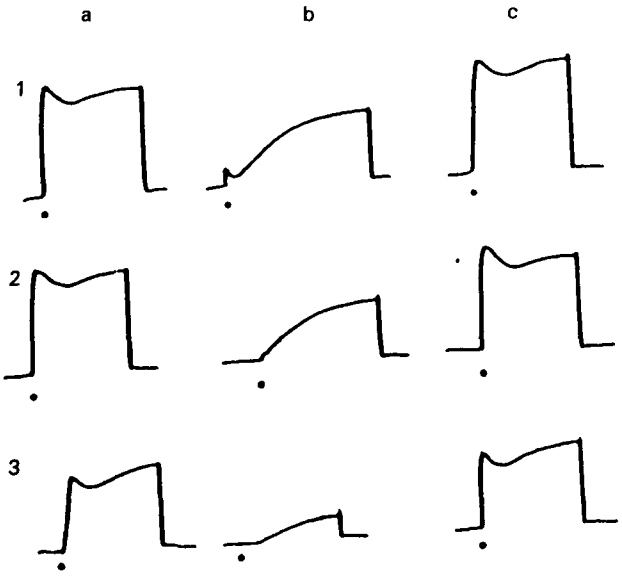

FIG. 2. Sample traces showing responses to K^+ (100 mM) in (a) normal Krebs solution, (b) Ca^{2+}-free Krebs containing 0·2 mM EGTA for 5 min (1), 10 min (2) and 30 min (3). Panel c shows recovery of responses to K^+ (100 mM) obtained after 20 min of re-incubation of tissues in normal Krebs.

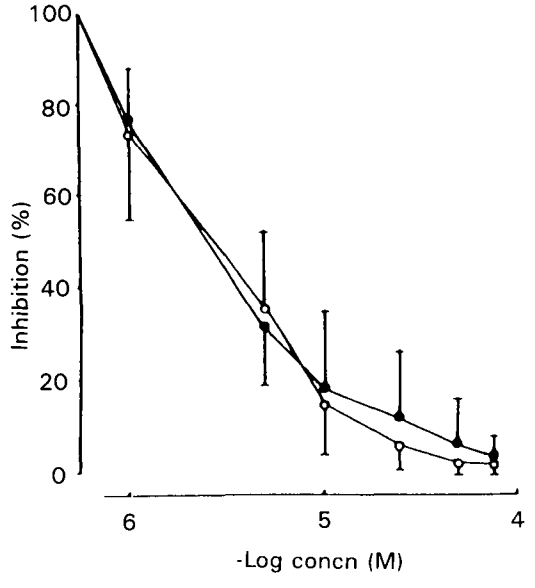

FIG. 3. Inhibition by verapamil of the phasic (●) and tonic (○) responses to K^+ (100 mM). Verapamil was added to the tissue bath in a cumulative manner at 15 min intervals. The ordinate is the response to K^+ in the presence of verapamil expressed as a percentage of the initial maximum response to K^+. Each point is the mean of 6–8 observations. Vertical bars represent ± s.e.m.

Sample traces of responses of the seminal vesicle to K^+ (100 mM) in normal Krebs solution ($[Ca^{2+}]_o = 2{\cdot}5$ mM) and after exposure to Ca^{2+}-free solution in the presence of EGTA (0·2 mM) for 5, 10 and 30 min are shown in Fig. 2. Restoration of full responses to K^+ was always possible and optimal on reintroduction of Ca^{2+}.

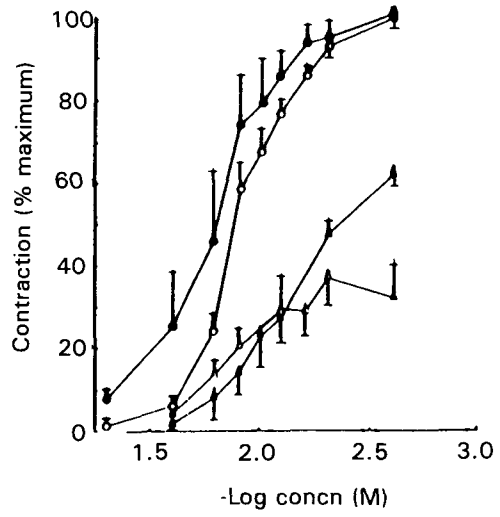

FIG. 4. Mean concentration-response curves of the phasic response to K^+ in the absence of nifedipine (●) and after 90 min incubation with nifedipine (○ 10^{-7}, ▲ 10^{-6} and △ 10^{-5} M). Responses are expressed as a percentage of the maximum response to K^+ in the absence of nifedipine. Vertical bars represent ± s.e.m. (n = 4–6).

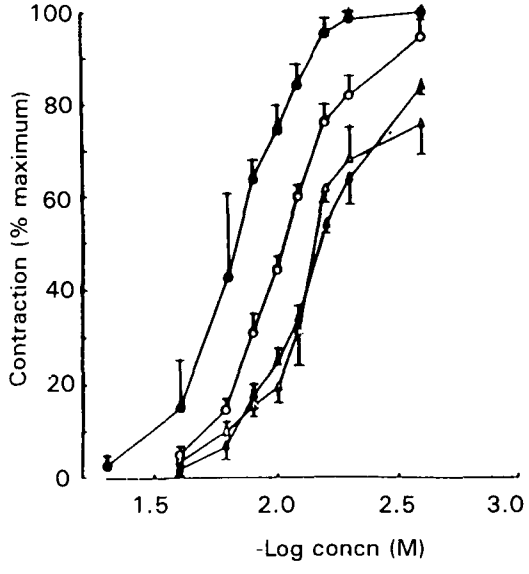

FIG. 5. Mean concentration-response curves of the tonic response to K^+ in the absence of nifedipine (●) and after 90 min incubation with nifedipine (○ 10^{-7}, ▲ 10^{-6} and △ 10^{-5} M). Responses are expressed as a percentage of the maximum response to K^+ in the absence of nifedipine. Vertical bars represent ± s.e.m. (n = 4–6).

Effects of calcium antagonists on responses to K^+

Cumulative addition of increasing concentrations of verapamil (10^{-6}–10^{-4} M) produced progressive inhibition of the contractile effect of K^+ and eventually both the phasic and tonic responses were abolished (Fig. 3). No significant differences were observed between IC50 values or I_{max} values for verapamil against both the responses, indicating no preferential selectivity of verapamil to either components of K^+-induced contraction. On the other hand, a low concentration of nifedipine (10^{-7} M) produced a slight rightward

shift of the concentration-response curve of the phasic response to K^+, but at higher concentrations (10^{-6} and 10^{-5} M) inhibition by nifedipine of the phasic response appeared to be of the non-competitive type with the maximum response to K^+ significantly reduced (Fig. 4). In contrast, displacement of the concentration-response curve for the tonic response by all concentrations of nifedipine was parallel. The lowest concentration of nifedipine (10^{-7} M) produced a rightward shift without significant reduction of the maximum tonic response of K^+, while higher nifedipine concentrations (10^{-6} and 10^{-5} M) produced further rightward displacement of the same magnitude and the maximum phasic contraction was reduced by only about 25% (Fig. 5).

Discussion

In the rat seminal vesicle contractile response to K^+ appears to be mainly due to increased smooth muscle membrane permeability to Ca^{2+} as a result of direct membrane depolarization and not to endogenous neurotransmitter release. This is similar to results in other smooth muscle like vas deferens (Swamy et al 1976; Triggle et al 1979; Shimodan & Sunano 1981; Hay & Wadsworth 1982). Verapamil completely blocked the phasic and tonic responses to K^+, but the two components exhibited differential sensitivity to Ca^{2+}-deprivation in the presence of EGTA, the phasic response attenuating faster than the tonic contraction. Nifedipine produced parallel dose-dependent rightward shifts of concentration-response curves of the tonic contraction but the maximum tonic response to K^+ was only reduced by about 20%. In contrast, rightward displacements of the concentration-response curves for the phasic contraction were non-parallel in the presence of nifedipine which also markedly reduced the maximum response to K^+. This indicates that the phasic component of K^+ contraction is more susceptible to blockade by nifedipine than the tonic component.

Though both the components of K^+-induced contraction use free extracellular Ca^{2+}, the tonic response also relies on Ca^{2+} located extracellularly but tightly bound to cell membranes (Triggle 1985). The latter source of Ca^{2+} seems available to activate contraction when the muscle is depolarized with K^+ (Hay & Wadsworth 1982). This is further substantiated by the observation that nifedipine abolishes the deprivation-resistant tonic component of K^+-induced contraction.

Ca^{2+}-induced Ca^{2+} release should also be considered where a small amount of Ca^{2+} entering the cells triggers the release of stored Ca^{2+} to sustain the excitation-contraction coupling (Saida & Van Breeman 1984) as in skeletal and cardiac muscles (Endo 1977; Fabiato & Fabiato 1975).

In conclusion, the present study shows that both the phasic and tonic responses to K^+ in the rat isolated seminal vesicle use extracellular Ca^+ (free and tightly bound to membranes) and may, partly, rely on mobilization of intracellular Ca^{2+} as a sequence to Ca^{2+}-induced calcium release.

Entry of Ca^{2+} during K^+ contraction occurs through two subtypes of voltage-dependent calcium channels, one of which is preferentially sensitive to nifedipine while both are blocked by verapamil.

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