J. Pharm. Pharmacol. 1989, 41: 236–241
Received June 10, 1988

Relaxant Actions of Khellin on Vascular Smooth Muscle

AMALIA UBEDA AND ANGEL VILLAR*

*Departamento de Farmacologia y Farmacotecnica, Unidad Docente de Farmacognosia y Farmacodinamia, Facultad de Farmacia, Avda, Blasco Ibañez 13, 46010 Valencia, Spain, and * Departamento de Farmacologia, Facultad de Farmacia, U.C.M. Madrid, Spain*

Abstract—To clarify the mechanism of the vasodilatory action of khellin on calcium, we have investigated its relaxant action on base line and on K^+ and noradrenaline-induced contractile tensions in rat aorta smooth muscle and on spontaneous contractile activity of rat portal vein. Khellin relaxed both of these preparations with a similar potency, which suggests a non-specific inhibition of calcium flux, without any difference related to the specific calcium channels. We have also studied the capacity of khellin to interfere with the loading and release mechanisms of caffeine and noradrenaline-sensitive calcium stores in Ca-free medium. Khellin's Ca^{2+} loading reduction may be related with its capacity to inhibit calcium influx. Khellin applied during Ca^{2+} release also caused relaxation. We propose that this drug may enhance calcium extrusion or sequestration rather than the calcium release mechanism. These actions on calcium influx and intracellular mobilization can contribute to its vasorelaxant action.

Khellin (5, 8-dimethoxy-2-methylfuranocromone) is an active principle from the plant *Ammi visnaga*, with antispasmodic and coronary vasodilator effects (Anrep et al 1949). Though it has been used in folk medicine since ancient times and experiments have demonstrated its ability to relax smooth muscles in-vivo and in-vitro, its mechanism of action has not been elucidated.

Khellin is known to inhibit contractile responses induced by $BaCl_2$ or calcium ions in the ileum and taenia coli of the guinea-pig, in tracheal chains and in the vas deferens of the rat and rabbit (Simonis et al 1971; Labrid et al 1977; Hemavathi et al 1979).

The final step in the mechanism of vascular relaxation is a decrease in the intracellular concentration of activator free calcium. This increase in the cytoplasmic concentration of the activator ion, which triggers the contractile process in the vascular smooth muscle cells, can be due either to an increased permeability of the cell membrane for extracellular calcium (calcium entry) or to a mobilization of calcium from cellular stores. Vasodilators can act on these processes, thereby causing smooth muscle relaxation. The present study was, therefore, undertaken to provide some information on the effects of khellin on Ca^{2+} movements in smooth muscle. This was studied in rat aortic strips, a tissue in which agonist-induced uptake and release of calcium and the effects of calcium entry blocking compounds have been closely studied (Cauvin et al 1983; Godfraind et al 1986).

Materials and Methods

Animals

Wistar rats (200-300 g) of either sex were stunned and bled. Helically cut strips of approximately 3×30 mm from the descending thoracic aorta (Furchgott & Bhadrakom 1953) and portal veins were mounted in organ baths containing physiological solution. Contractile responses were measured isometrically with a force-displacement transducer (Gould Statham UC2). The strips were maintained under a stable tension of 1g for aorta and 0·5 g for portal vein, during 90-120 min before experiments were started.

Correspondence to: A. Ubeda, Departamento de Farmacología y Farmacotecnica, Facultad de Farmacia, Universidad de Valencia, Avda. Blasco Ibáñez 13, 46010 Valencia, Spain.

Solutions

The physiological solution (PS) was prepared without phosphate ions to minimize Ca^{2+} precipitation during the incubation period (Godfraind 1976). It contained (mM): Na^+ 137, K^+ 5·9, Mg^{2+} 1·25, Ca^{2+} 1·25, Cl^- 132·9, HCO_3^- 15, and glucose 11. The concentration of Ca^{2+} was changed to 0·3 or 7 mM in some experiments. K^+-depolarizing PS (K^+ 80 mM) was obtained by substituting KCl for NaCl on an equimolar basis.

The ionic composition of the Krebs solution in Ca-free medium experiments was as follows (mM): Na^+ 137·4, K^+ 5·9, Mg^{2+} 1·2, Ca^{2+} 2·5, HCO_3^- 15·5, $H_2PO_4^-$ 1·2, Cl^- 134 and glucose 11·5. The Ca-free EDTA (2 mM)— containing solution was prepared by replacing 2·5 mM $CaCl_2$ with equimolar $MgCl_2$ and adding 2 mm $EDTANa_2$ (Itoh et al 1983).

The solutions were aerated with 95% O_2 and 5% CO_2 and the pH adjusted to 7.

Methods

In some experiments khellin and verapamil were added cumulatively to the bath when the contractions evoked by noradrenaline (NA) (10^{-6} M) and K^+-depolarizing PS (80 mM) had reached a steady level. These experiments were performed in the presence of different concentrations of $CaCl_2$. The concentration of antagonist required to inhibit the sustained contraction by 50% (IC50) was calculated from the concentration-inhibition curves.

Ca-free medium contractions procedures are detailed in results.

Drugs

Drugs used were: (-)-noradrenaline-(-)-tartrate (Merck), caffeine (Natra S.A.), verapamil (Knoll) and khellin (Sarsynthèse). Khellin was prepared as stock solution in ethanol. Dilutions were made in distilled water and the final ethanol

concentration in the bath did not significantly affect the results, as we confirmed in parallel experiments.

Statistics

Throughout the paper results are expressed as mean ± s.e.m. Statistical significance was evaluated by Student's *t*-test for paired or unpaired data and differences were considered significant when $P<0{\cdot}05$ (*), $P<0{\cdot}01$ (**), $P<0{\cdot}001$ (***).

Results

Effects on baseline and spontaneous activity

Aortic strips and portal veins were exposed to various concentrations of khellin (2×10^{-5} to $3{\cdot}2\times10^{-4}$ M) to determine whether the drug affected baseline tension and spontaneous mechanical activity. Khellin relaxed the baseline tension (max. 0·035 g, Fig. 1a). Portal veins exhibited spontaneous activity, the amplitude and frequency of which were modified by khellin (Fig. 1b).

Effects on NA-and high K^+-induced contractions

This group of experiments was designed to determine whether khellin could relax already established contractions. Aortic strips were contracted by NA (10^{-6} M) or K^+-depolarizing PS and when the response to either agonist had reached a steady level, antagonists were cumulatively added at 6 min intervals. In these experiments we compared the behaviour of khellin with that of the Ca-entry blocker verapamil together with the influence of various extracellular Ca^{2+} concentrations (the strips were immersed in the high or low Ca^{2+} concentration solution for 30 min before experiments were started).

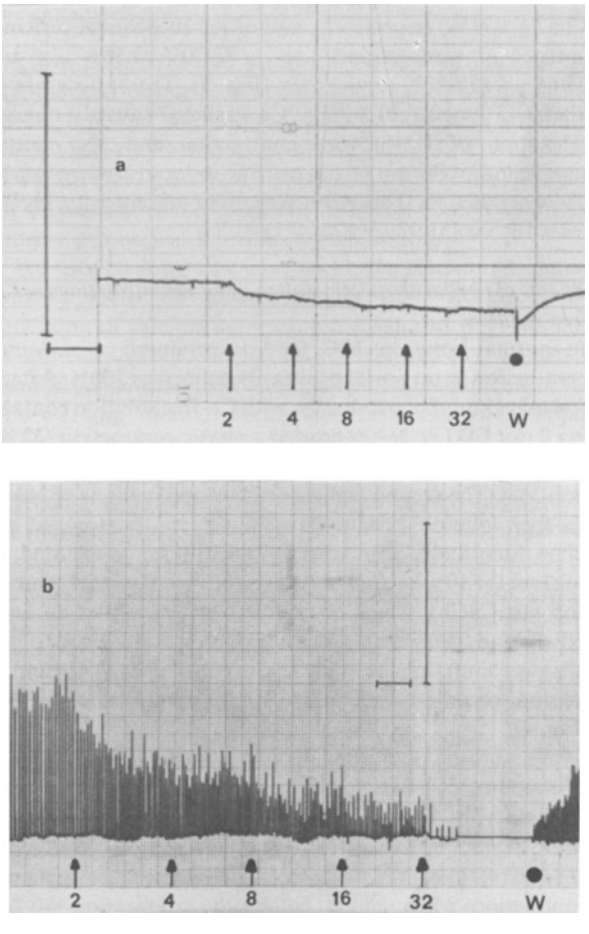

FIG. 1. Effects of khellin ($2\text{–}32\times10^{-5}$ M) on baseline in isolated rat aorta(a). Khellin was cumulatively added at the arrows (W). The vertical and horizontal bars are the contraction amplitude (0·25 g) and the time (10 min) calibration, respectively. Effects of khellin ($2\text{–}32\times10^{-5}$ M) on spontaneous mechanical activity in isolated rat portal vein (b). The vertical and horizontal bars are the contraction amplitude (0·25 g) and the time (3 min) calibration, respectively.

Table 1 shows the IC50 values calculated from cumulative concentration-effect curves for the inhibitory effects of khellin and verapamil. Both drugs produced a dose-dependent relaxation of NA- and K^+-induced contractions but they differed in their potency and behaviour in the presence of different concentrations of Ca^{2+}. Khellin caused total relaxation of both NA- and K^+-contractions and a change in external calcium concentration only slightly modified the IC50 values, (i.e. the concentration-inhibition curves were not significantly shifted).

In contrast, the IC50 of verapamil changed significantly in the presence of different concentrations of Ca^{2+}. A decrease in the concentration of external calcium shifted the curves to the left, whereas increasing it from control levels to 7 mM shifted the curves to the right (IC50 significantly increased). There were also differences between the IC50 of NA– and K^+-induced contractions; verapamil was more effective against the latter and did not cause total relaxation of NA-induced contractions, at the concentrations used ($10^{-8}-3\times10^{-5}$ M).

Effects of khellin on caffeine-induced contractions in Ca–free solution

The amount of Ca^{2+} stored in the muscle cells was estimated from the amplitude of caffeine-induced contractions. First, the strip was treated with Ca-free EDTA-containing solution for 10 min, during which time, caffeine (10 mM) was applied once to deplete the stored Ca^{2+}. Secondly, the tissue was loaded with 2·5 mM Ca^{2+} for 5 min. The amount of Ca^{2+} taken up into the store during this 5 min "Ca loading" period was estimated from the size of the contraction evoked by application of 10 mM caffeine after the tissue had been subsequently washed for 3 or 5 min in Ca-free 2 mM EDTA-containing solution ("Ca washing" period). This experimental protocol (Fig. 2) was used to investigate the effects of khellin on the stored Ca^{2+} (i.e. caffeine-sensitive intracellular Ca^{2+} store) (Endo 1977; Itoh et al 1983).

The amplitude of the caffeine-induced contraction was reproducible in any given preparation with any given procedure. Thus, estimation of the amount of stored Ca^{2+} from the amplitude of caffeine-induced contraction before and after treatment of the tissue with khellin at "Ca loading" and "Ca washing" periods was feasible.

The magnitude of caffeine-induced contractions was decreased by the duration of the "Ca washing" period. However, reduction was slight between 3 and 5 min, which were the times chosen for the experimental procedures.

Khellin, $6{\cdot}4\times10^{-4}$ M, applied during "Ca loading" significantly reduced the amplitude of the caffeine-induced contractions to 61·99 ± 2·14 ($P<0{\cdot}01$). However, when applied during the second procedure (Ca-washing), the amplitude

Table 1. IC50 values calculated from cumulative concentration-inhibition curves on the contractile tension induced by K^+ (80 mM) and noradrenaline (10^{-6} M) in the presence of different extracellular Ca^{2+} concentrations.

Vasodilators		Ca^{2+} concentrations		
		0·3 mM	1·25 mM	7 mM
Khellin ($\times 10^{-4}$M)	K^+	1·44±0·19	1·79±0·21	1·64±0·16
	NA	1·06±0·13	2·28±0·29	1·52±0·27
Verapamil ($\times 10^{-6}$ M)	K^+	0·64±0·08	1·21±0·21	3·73±0·29***
	NA	2·70±0·77	3·16±0·71	8·45±1·32**

Values are mean±S.E. of 6 to 8 experiments.
**$P<0.01$ and ***$P<0.001$; statistically significant difference from the control (1·25 mM Ca^{2+} medium).

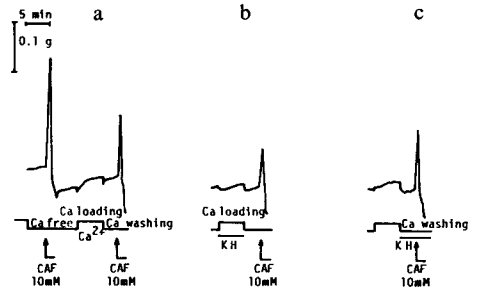

FIG. 2. Effects of khellin (KH) ($6·4 \times 10^{-4}$ M) on the 10 mM caffeine (CAF)-induced contraction, after Ca^{2+} store had been emptied by prior application of caffeine in Ca-free 2 mM EDTA-containing solution. 2·5 mM Ca^{2+} was applied for 5 min (Ca loading). The tissues were rinsed with Ca-free EDTA for 3 or 5 min (Ca washing) and 10 mM caffeine was subsequently applied to test the store size, a) control, b) Khellin during Ca loading, c) Khellin during Ca washing.

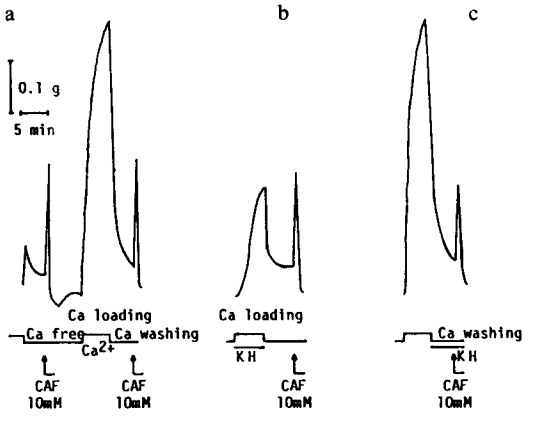

FIG. 3. Effects of khellin (KH) ($3·2 \times 10^{-4}$ M) on the 10 mM caffeine (CAF)-induced contraction. The same experimental procedure described in Fig. 2 was used, but 26 mM K^+ was present instead of 5·9 mM K^+.

was not affected. The addition of khellin $3·2 \times 10^{-4}$ M had no significant effect on either procedure (Fig. 2). This means that khellin affected the replenishment of the stored Ca^{2+} but not the release. We needed a higher concentration of khellin than the IC50 obtained previously to cause relaxation in this experimental protocol (almost threefold).

Using the same experimental procedures we studied the effects of khellin on caffeine-induced contractions in 26 mM K^+-containing solution. This depolarizing medium produced a large contraction during "Ca loading" (Fig. 3) and the amplitude of the caffeine-induced contractions was increased in comparison with the normal medium experiments to 298·74±22·16%. Khellin (3·2 and $6·4 \times 10^{-4}$ M) applied during "Ca loading" showed a significant inhibition of the K^+-induced contraction to 36·71±11·90% and 12·57±1·71%, respectively, and of the subsequent caffeine-induced contraction to 84·70±10·59%* and 54·65±4·15%***, respectively (Fig. 3). The application of khellin ($3·2 \times 10^{-4}$ M) during "Ca washing" caused a quicker relaxation of K^+-induced contraction and also caused significant inhibition of the caffeine-induced contraction to 78·74±7·46%**. This effect was more evident with khellin $6·4 \times 10^{-4}$ M (51·92±9·07%***).

Effects of khellin on noradrenaline-induced contractions in Ca-free solution

In normal solution NA 10^{-6} M produced a sustained contraction in rat aorta, arbitrarily defined as 100%. After a few min (5 min) of incubation with Ca-free solution containing 2 mM EDTA, NA produced a phasic contraction (32%), which declined and reached a steady level (sustained contraction 5%). The second and subsequent applications of NA in Ca-free solution produced sustained contractions of the same amplitude. The muscle strips were re-exposed to calcium for 10 min (Ca loading period) with normal solution (2·5 mM $CaCl_2$), which caused a contraction that declined slowly, and then were washed with Ca-free solution for 5 min (Ca washing); phasic and tonic contractions of similar tension, could be obtained with the re-application of NA (Fig. 4).

The addition of khellin, $3·2 \times 10^{-4}$ M, during the sustained contraction of NA caused complete inhibition, but after washing with Ca-free solution this sustained contraction could be obtained again with the re-application of NA in the absence of khellin. This drug also inhibited the sustained contraction when added before the application of NA (Fig. 4).

To determine in detail the effects of khellin on the NA-releasable Ca^{2+} store, we used an experimental protocol similar to that employed in the caffeine-store experiments. Instead of caffeine we used NA (10^{-6} M) with a Ca loading period of 10 min. Khellin was added during either Ca loading

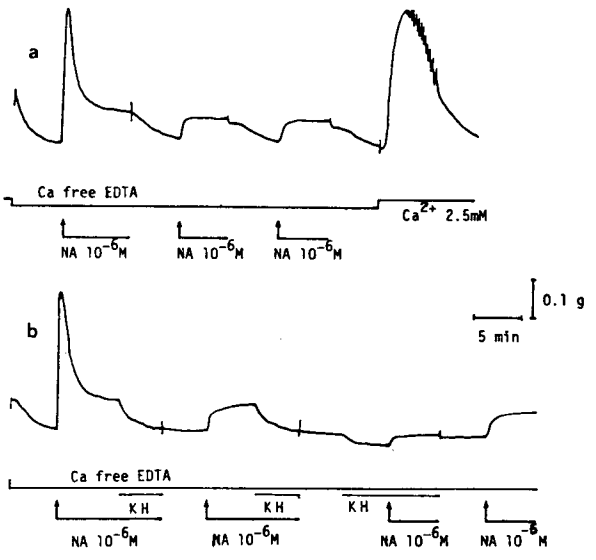

FIG. 4. Effects of khellin (KH) ($3{\cdot}2 \times 10^{-4}$ M) on NA-induced tonic contractions in Ca-free solution (2 mM EDTA). Short-term exposure of the muscle to 2·5 mM Ca^{2+} restored the ability to obtain NA-induced phasic and tonic responses. Panel a) and b) are continuous over time.

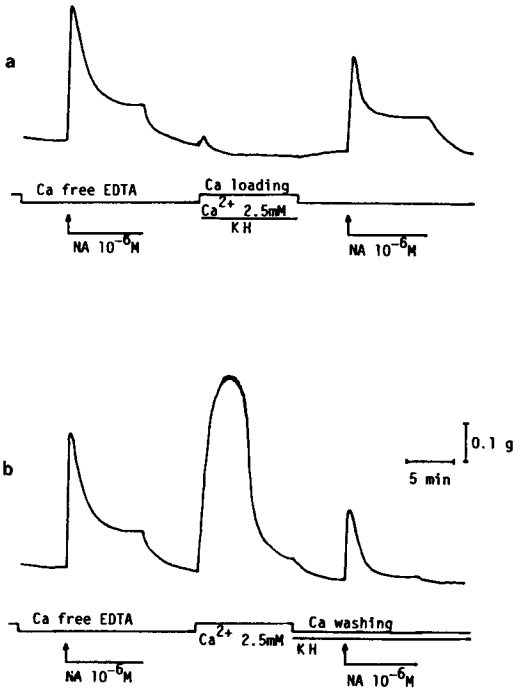

FIG. 5. Effects of khellin (KH) ($3{\cdot}2 \times 10^{-4}$ M) on NA-induced contractions in Ca-free solution (2 mM EDTA). a) khellin applied during Ca loading, b) khellin applied during Ca washing.

or Ca washing (Table 2). Khellin, $3{\cdot}2 \times 10^{-4}$ M, inhibited the NA-induced phasic contraction when added during either period, but the sustained contraction was only inhibited when it was added during Ca washing (Fig. 5). The addition of khellin during Ca washing caused a quicker relaxation of the Ca loading-contraction, similar to that observed with the caffeine protocol in K^+-containing solution.

We tried to determine whether khellin caused this relaxation by inhibition of NA-induced Ca^{2+} release or by activation of calcium decreasing mechanisms (e.g. stimulation of calcium reaccumulation or enhanced calcium extrusion). Khellin was added during different components of the "Ca washing" period: during the first 4 min before application of NA, simultaneously with NA application or during the whole period (Fig. 6).

Khellin addition during the first 4 min caused inhibition of the phasic response to $60{\cdot}53 \pm 7{\cdot}09\%$* but did not affect the sustained component (Fig. 6b). The simultaneous addition of khellin and NA caused little inhibition of the phasic response ($93{\cdot}52 \pm 2{\cdot}15\%$), but abolished the sustained contraction (Fig. 6d). As described previously, the addition of khellin during the whole "Ca washing" period caused marked inhibition of the NA-induced phasic and tonic contractions (Fig. 6c).

The conditioning application of NA (10^{-8} M) during the Ca washing period, caused a significant reduction in both the phasic contraction produced by NA 10^{-6} M to $89{\cdot}36 \pm 7{\cdot}89\%$*** and also in the sustained phase to $79{\cdot}67 \pm 3{\cdot}36\%$** (i.e. the first contraction uses part of the calcium necessary for the phasic and the sustained response) (Fig. 7a). Treatment with khellin ($3{\cdot}2 \times 10^{-4}$ M) during the

Table 2. Effects of khellin on the noradrenaline-induced phasic contraction in Ca-free solution. Rat aorta was incubated with 2·5 mM Ca^{2+} for 10 min (Ca loading), washed with Ca-free solution (2 mM EDTA) for 5 min (Ca washing) and challenged by 10^{-6} M noradrenaline. Khellin ($3{\cdot}2 \times 10^{-4}$ M) was applied during Ca loading or Ca washing.

Applied during:	Ca loading	Ca washing
Control	100	100
Khellin	$73{\cdot}61 \pm 1{\cdot}67$*** (6)	$57{\cdot}29 \pm 2{\cdot}49$**** (7)

Relative contractile tension is shown as mean ± s.e. The number in parentheses indicates the number of experiments.
***$P < 0{\cdot}001$: statistically significant difference from the control.

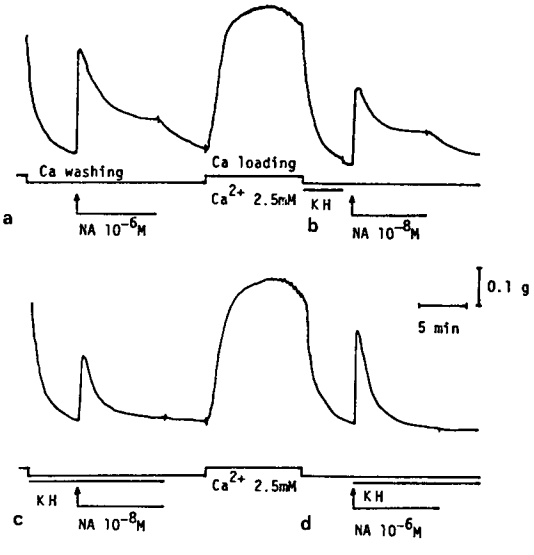

FIG. 6. Effects of khellin (KH) ($3{\cdot}2 \times 10^{-4}$ M) applied during Ca washing. a) Control, b) applied prior addition of NA (first 4 min), c) applied during all Ca washing period, d) applied simultaneously with NA.

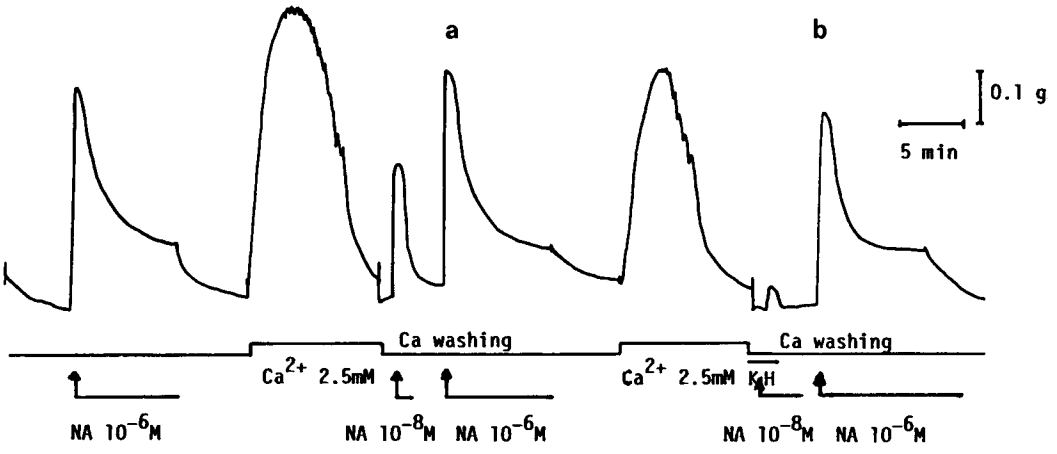

FIG. 7. Effects of conditioning applications of NA (10^{-8} M) on the NA (10^{-6} M)-induced contractions during Ca washing (a). Treatment with khellin (KH) ($3{\cdot}2 \times 10^{-4}$ M) during the first 2 min of Ca washing (b).

first application significantly inhibited to $15{\cdot}55 \pm 4{\cdot}55\%$*** the amplitude of NA (10^{-8} M)-induced contractions and also reduced the NA (10^{-6} M)-induced contraction to $77{\cdot}46 \pm 2{\cdot}57\%$**. However, the sustained contraction was restored (Fig. 7b). These results suggest that the inhibition of 10^{-8} M NA-induced contraction save some calcium that could later be released causing a higher 10^{-6} M NA-induced tonic contraction, i.e. may be a certain recycling between the NA-releasable stores.

Discussion

Khellin was found in this study to be a relaxant of vascular smooth muscle in the concentration range of 10^{-5} to $6{\cdot}4 \times 10^{-4}$ M. Several authors have reported that it antagonizes the contractions produced by $BaCl_2$ and $CaCl_2$ in intestinal and other smooth muscles at similar concentration ranges (Simonis et al 1971; Labrid et al 1977; Hemavathi et al 1979).

In the present study khellin inhibited vascular smooth muscle contractions induced by NA which by binding to specific receptors, causes vascular smooth muscle contraction by increasing intracellular calcium levels. This increase depends on the influx of cations via receptor operated channels (ROC's) and the release of intracellular stored calcium (Heaslip & Rahwan 1982; Godfraind et al 1986). Khellin also caused inhibition of contractions produced by high K^+, which causes depolarization of the cell membrane, allowing Ca^{2+} entry via voltage operated channels (VOC's). Calcium antagonists, like verapamil, are thought to bind directly to these calcium channels and to inhibit Ca^{2+} entry competitively (Cauvin et al 1983).

The actions of khellin suggest that this drug inhibits the pathways of Ca^{2+} influx which are activated by both NA and high K^+, effects which were not modified by changing extracellular Ca^{2+} concentration. In contrast, the inhibitory effects of verapamil were selective for K^+-induced contractions, and were antagonized by raising the external calcium concentration (present study; Gagnon et al 1980; Karaki et al 1984).

Khellin also caused a small relaxation of baseline tension, which could suggest that this drug affects Ca^{2+} leak channels or homeostatic transport systems. Khellin inhibited the spontaneous contractions of rat portal vein, which is consistent with inhibition of Ca^{2+} entry through VOC's, because this spontaneous activity is dependent on extracellular calcium (Sigurdsson et al 1975).

Khellin inhibited the loading of the intracellular stores. This action was more evident on caffeine-sensitive stores in depolarized medium and on NA-sensitive stores, i.e. in conditions of high replenishment. Several authors have reported that in a depolarizing medium there is a greater refilling of the intracellular pools than in a normal medium (Casteels & Droogmans 1981; Van Breeman et al 1986). Khellin only inhibited the NA-induced phasic contraction, which is believed to depend on a rapidly-exchangeable superficial store (Heaslip & Rahwan 1982; Leijten et al 1985). The inhibitory effect caused by khellin may be related to the interference by this drug with the entry of extracellular Ca^{2+}, which is required for the replenishment of the stores (Casteels & Droogmans 1981; Karaki et al 1979).

Both components of NA-induced contractions in Ca-free medium were inhibited by khellin applied during the Ca washing period. Several authors have described these components of the NA-induced contraction in rat and rabbit aorta (Heaslip & Rahwan 1982; Leijten & Van Breemen 1986) and they have been attributed to the existence of two separate, but probably related, stores. The present results suggest that khellin somehow modifies the Ca^{2+} mobilization from these intracellular stores.

Ruckstuhl & Landry (1981) have shown that khellin caused inhibition of cAMP and cGMP phosphodiesterase activities from bovine lung, with higher selectivity for the former. Khellin may relax the NA-induced tonic contraction by an increase in cyclic nucleotide levels, with a mechanism similar to that of glyceryl trinitrate and caffeine. Casteels et al (1981) have shown the relaxant action of these drugs on the tonic component of NA-induced contraction which has been attributed to the acceleration of extrusion of cytoplasmic free calcium through an increase of the cyclic nucleotides (Deth & Lynch 1981; Itoh et al 1983).

At present, khellin's inhibitory actions in Ca-free medium may be related to the following mechanisms: (1) inhibition of the Ca^{2+} release from the intracellular stores; (2) an enhance-

ment of the extrusion of free Ca^{2+} and/or (3) stimulation of sequestration into storage sites. Khellin may act by the above mentioned mechanisms, under the assumption that the Ca^{2+} sensitivity of contractile proteins is not affected by the direct or indirect action (via a second messenger) of khellin.

The present results obtained with repetitive application of NA (10^{-8} and 10^{-6} M) in the presence of khellin indicates that this drug does not inhibit the Ca^{2+} releasing mechanism. Khellin suppressed the first contraction but did not increase the amplitude of the subsequent 10^{-6} M NA-induced contraction. If khellin inhibits the Ca^{2+} release or enhances the sequestration of free Ca^{2+} into the intracellular store, an increase in the amplitude of the second contraction should be observed.

Therefore, the results indicate that khellin may cause an enhancement of the Ca^{2+} extrusion into the extracellular space and/or a sequestration of free cytoplasmic Ca^{2+} into NA-non releaseable Ca^{2+} store, rather than inhibition of Ca^{2+} releasing mechanism induced by caffeine or NA.

We can conclude that khellin seems to have multiple sites of action in addition to the non-specific inhibition of calcium influx. Its action differs from that of calcium antagonists, which cause a specific inhibition of Ca^{2+} entry through VOC's. This drug also shows an intracellular action which may be related to an increase of Ca^{2+} extrusion or sequestration rather than inhibition of Ca^{2+} release. Whether this intracellular action is direct or related to a cyclic nucleotide increase has to be clarified.

Acknowledgements

This work is a part of the doctoral thesis by Amalia Ubeda. This work was supported by a research grant from the Spanish Ministry of Education and Science. A preliminary communication of some of these results was presented at the National Meeting of the Spanish Pharmacological Society.

References

Anrep, G. V., Kenawy, M. R., Barsoum, G. S. (1949) The coronary vasodilator action of khellin. Am. Heart J. 37: 531–542

Casteels, R., Droogmans, G. (1981) Exchange characteristics of the noradrenaline-sensitive calcium store in vascular smooth muscle cells of rabbit ear artery. J. Physiol. 317: 263–279

Casteels, R., Raeymaekers, L., Suzuki, H., Van Eldere, J. (1981) Tension response and ^{45}Ca release in vascular smooth muscle incubated in Ca-free solution. Pflügers Arch. 392: 139–145

Cauvin, C., Loutzenhiser, R., Van Breemen, C. (1983) Mechanisms of calcium antagonist-induced vasodilation. Ann. Rev. Pharmacol. Toxicol. 23: 373–396

Deth, R. C., Lynch, C. J. (1981) Mobilization of a common source of smooth muscle Ca^{2+} by norepinephrine and methylxanthines. Am. J. Physiol. 240: C239–C247

Endo, M. (1977) Calcium release from the sarcoplasmic reticulum. Physiol. Rev. 57: 71–108

Furchgott, R. F., Bhadrakom, S. (1953) Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmacol. Exp. Ther. 108: 124–143

Gagnon, G., Regoli, D., Rioux, F. (1980) Studies on the mechanism of action of various vasodilators. Br. J. Pharmacol. 70: 219–227

Godfraind, T. (1976) Calcium exchange in vascular smooth muscle, action of noradrenaline and lanthanum. J. Physiol. 260: 21–35

Godfraind, T., Miller, R., Wibo, M. (1986) Calcium antagonism and calcium entry blockade. Pharmacol. Rev. 38: 321–416

Heaslip, R. J., Rahwan, R. G. (1982) Evidence for the existence of two distinct pools of intracellular calcium in the rat aorta accessible to mobilization by norepinephrine. J. Pharmacol. Exp. Ther. 221: 7–13

Hemavathi, K. G., Bhatt, J. D., Amin, D. V., Gulati, O. D., Shah, D. S. (1979) Antagonism of calcium-induced contractions by some nonspecific spasmolytics in depolarized smooth muscles. Indian J. Physiol. Pharmacol. 23: 261–268

Itoh, T., Kuriyama, H., Ueno, H. (1983) Mechanism of the nitroglycerine-induced vasodilation in vascular smooth muscle of the rabbit and pig. J. Physiol. 343: 233–252

Karaki, H., Kubota, H., Urakawa, N. (1979) Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. Eur. J. Pharmacol. 56: 237–245

Karaki, H., Nakagawa, H., Urakawa, N. (1984) Comparative effects of verapamil and sodium nitroprusside on contraction and ^{45}Ca uptake in the smooth muscle of rabbit aorta, rat aorta and guinea-pig taenia coli. Br. J. Pharmacol. 81: 393–400

Labrid, C., Dureng, G., Bert, H., Duchene-Marullaz, P. (1977) Antispasmodiques non specifiques et effets contracturants du baryum. J. Pharmacol. 8: 169–184

Leijten, P., Saida, K., Van Breemen, C. (1985) Norepinephrine-induced intracellular Ca^{2+} release from vascular smooth muscle. J. Cardiovasc. Pharmacol. 7 (Suppl 6): S38–S42

Leijten, P., Van Breemen, C. (1986) The relationship between noradrenaline-induced contraction and ^{45}Ca efflux stimulation in rabbit mesenteric artery. Br. J. Pharmacol. 89: 739–747

Ruckstuhl, M., Landry, Y. (1981) Inhibition of lung cyclic AMP- and cyclic GMP-phosphodiesterase by flavonoids and other chromone-like compounds. Biochem. Pharmacol. 30: 697–702

Sigurdsson, S. B., Uvelius, B., Johansson, B. (1975) Relative contribution of superficially bound and extracellular calcium to activation of contraction in isolated rat portal vein. Acta Physiol. Scand. 95: 263–269

Simonis, A. M., Ariëns, E. J., Van Den Broeke, J. J. W. (1971) Non-competitive spasmolytics as antagonists of Ca^{2+} induced smooth muscle contraction. J. Pharm. Pharmacol. 23: 107–110

Van Breemen, C., Lukeman, S., Leijten, P., Yamamoto, H., Loutzenhiser, R. (1986) The role of superficial SR in modulating force development induced by Ca^{2+} entry into arterial smooth muscle. J. Cardiovasc. Pharmacol. 8 (Suppl. 8): S111–S116