Modulation of Adrenergic Responses of Human Vas Deferens by K^+ Channel Inhibitors

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OBJECTIVES The present study was designed to evaluate the role of K^+ channels in the adrenergic responses of human vas deferens as well as the intervention of dihydropyridine-sensitive Ca^{2+} channels on modulation of adrenergic responses by K^+ channel inhibitors.

METHODS Ring segments of the epididymal part of the vas deferens were taken from 32 elective vasectomies and mounted in organ baths for isometric recording of tension. We then studied the effects of K^+ channel blockers on neurogenic and norepinephrine-induced contractile responses.

RESULTS Addition of tetraethylammonium (TEA, 10^{-3} M), a nonspecific K^+ channel blocker, or charybdotoxin (10^{-7} M), a nonselective inhibitor of large- and intermediate-conductance Ca^{2+}-activated K^+ channel, increased the contractile responses to norepinephrine and electrical field stimulation-induced contractions ($P < .01$), whereas iberiotoxin (10^{-7} M), a selective blocker of large-conductance Ca^{2+}-activated K^+ channels, apamin (10^{-6} M), a blocker of small-conductance Ca^{2+}-activated K^+ channels, or glibenclamide (10^{-5} M), an inhibitor of ATP-sensitive K^+ channels, were without effect. TEA- and charybdotoxin-induced potentiation of contractions elicited by electrical field stimulation and norepinephrine was blocked by L-type Ca^{2+} channel blocker nifedipine (10^{-6} M).

CONCLUSIONS The results suggest that charybdotoxin-sensitive, but iberiotoxin-insensitive, K^+ channels are activated by stimulation with norepinephrine and electrical field stimulation to counteract the adrenergic-induced contractions of human vas deferens. Thus, inhibition of these channels increases significantly the contraction, an effect that appears to be mediated by an increase in Ca^{2+} entry through L-type voltage-dependent Ca^{2+} channels. UROLOGY 76: 1518.e7–1518.e12, 2010. © 2010 Elsevier Inc.

There are several types of K^+ channels in visceral smooth muscle cells that play an important role in the modulation of excitability and in action potential generation in these cells. Activation of K^+ channels of smooth muscle cells causes membrane hyperpolarization, closure of voltage-dependent Ca^{2+} channels and relaxation.[1] Conversely, blockade of K^+ channels causes membrane potential depolarization, opening of voltage-dependent Ca^{2+} channels, and contraction.[1,2]

Many electrophysiological and functional studies have been performed to identify and characterize K^+ channels in vas deferens from different animal species.[3-7] The motor innervation of the human vas deferens is mainly noradrenergic,[8,9] and the contraction is mediated by neurally released norepinephrine and stimulation of postjunctional α_1 adrenoceptors.[10] In contrast, the contraction of vas deferens from rat, rabbit, and guinea pig in response to sympathetic nerve stimulation has been shown to be biphasic, ATP inducing the initial fast phase and norepinephrine the later slower phase.[11,12] These differences between human and other species demonstrate the need to study the human vas deferens function whenever possible.

In human vas deferens, large conductance Ca^{2+}-activated K^+ channels have been reported in both smooth muscle[13] and epithelial cells.[14] Furthermore, it has been previously demonstrated that sildenafil, a phosphodiesterase 5 inhibitor, has inhibitory effects on smooth muscle of human vas deferens by activation of presynaptic large-conductance Ca^{2+}-activated K^+ channels.[15] Thus, a link between adrenergic contraction of vas deferens and modulation of K^+ channels is becoming apparent.

The present study was designed to examine the contribution of K^+ channels on neurogenic- and norepinephrine-induced contractions in the epididymal portion of the human vas deferens. To examine the possibility that K^+ channel blockers may facilitate Ca^{2+} entry

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Submitted: February 1, 2010, accepted: July 17, 2010

doi:10.1016/j.urology.2010.07.475

through dihydropyridine Ca^{2+} channels, experiments were performed in the presence of the Ca^{2+} channel blocker nifedipine.

MATERIAL AND METHODS

Segments (15-20 mm in length) of the epididymal part of the vas deferens were taken from 32 men (aged 30-44 years) who were sterilized by elective vasectomy. The study was approved by the Ethics Committee of our institution and informed consent was obtained from each subject before the study. The specimens were placed in chilled isotonic NaCl, and were divided into ring preparations 3-4 mm in length.

Ring preparations were suspended between 2 L-shaped stainless steel pins. One pin was fixed while the other was connected to a strain gauge (Grass FT03; Astro-Med, West Warwick, RI). Changes in isometric force were recorded by use of Chart v3.4/s software and a MacLab/8e data acquisition system (ADInstruments). Each preparation was set up in a 4-mL bath containing modified Krebs–Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6; KH_2PO_4 1.2; $MgCl_2{\cdot}6H_2O$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1; and disodium EDTA, 0.01. The solution was equilibrated with 95% O_2 and 5% CO_2. The preparations were allowed to equilibrate for 1 hour and during this time tension was adjusted to a tension of 19.6 mN.

Electrical field stimulation was provided by a Grass S88 stimulator (Astro-Med) via 2 platinum electrodes positioned 1 cm apart on each side and parallel to the axis of the ring. Single square wave pulses (20 Hz, 0.25-ms pulse duration, at a supramaximal voltage of 20 V/cm) were used. The train duration was 5 seconds and the stimulation interval 180 seconds. The stimulation parameters used elicit contractile responses that were abolished by tetrodotoxin (10^{-6} M) or prazosin (10^{-6} M).[16]

When electrically induced phasic contractions were stable (after 10-20 minutes) tetraethylammonium (TEA, 10^{-3} M), glibenclamide (10^{-5} M), charybdotoxin (10^{-7} M), iberiotoxin (10^{-7} M), or apamin (10^{-6} M) were added to the preparations and the effects of electrical field stimulation were recorded.

Concentration–response curves to norepinephrine were determined in a cumulative manner, and control (in the absence of K^+ channel blocker) and experimental (in the presence of K^+ channel blocker) results were obtained from separate preparations.

In another group of experiments, the preparations were preincubated with the dihydropyridine Ca^{2+} channel blocker nifedipine (10^{-6} M) for 20 minutes before the addition of the K^+ channel blocker and response to electrical field stimulation and concentration–response curves to norepinephrine were determined under control and experimental conditions.

Drugs

The following drugs were used: norepinephrine hydrochloride, prazosin hydrochloride, tetrodotoxin, tetraethylammonium bromide (TEA), charybdotoxin, iberiotoxin, apamin, glibenclamide and nifedipine (Sigma, Chemical Co., St. Louis, MO). All drugs were dissolved in Krebs' solution, except for norepinephrine, which was prepared in Krebs' solution with ascorbic acid (10^{-6} M), nifedipine, which was dissolved initially in ethanol, and glibenclamide which was prepared in dimethyl sulphoxide and further diluted in Krebs' solution to the appropriate final concentration. Drugs were added to the organ bath in volumes of less than 70 μL. Stock solutions of the drugs were freshly prepared every day and kept on ice throughout the experiment.

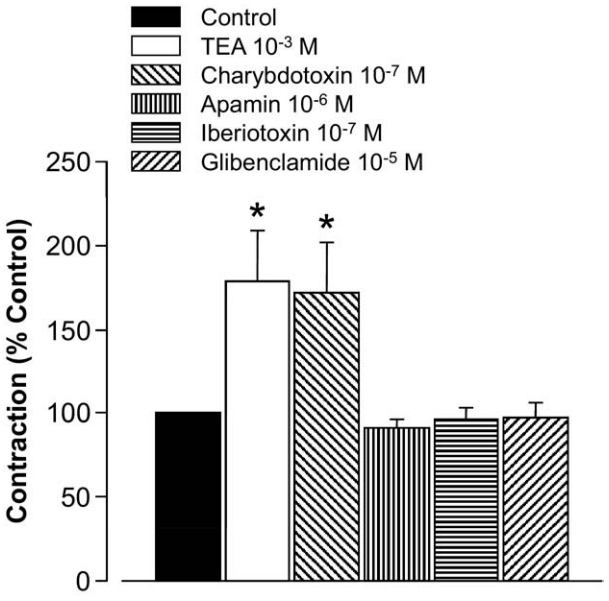

Figure 1. Contractile effects of electrical field stimulation (20 V/cm, 0.25 ms pulse duration, 20 Hz, train duration 5 seconds, stimulation interval 180 seconds) on human vas deferens in the absence (control, n = 10) and in the presence of TEA (10^{-3} M, n = 7), charybdotoxin (10^{-7} M, n = 7), apamin (10^{-6} M, n = 7), iberiotoxin (10^{-7} M, n = 7), and glibenclamide (10^{-5} M, n = 7). Values are means ± SEM. $*P < .01$.

Data Analysis

The contractile responses were measured as absolute values (mN) or as percentages of control responses of the maximum of the phasic contractions. All values are expressed as means ± standard error of the mean (SEM). pD_2 values (negative logarithm of the molar concentration at which half-maximum contraction occurs) were determined from individual concentration–response curves by nonlinear regression analysis. The number of rings taken from each subject varied from 4 to 7. The responses obtained in each subject were averaged to yield a single value. Therefore, all n values are presented as the number of subjects. For statistical comparison, one-way analysis of variance (ANOVA), and then Bonferroni's test was performed. Statistical significance was accepted at $P < .05$.

RESULTS

Segments of the epididymal portion of the vas deferens equilibrated for 60 minutes at a basal tension of (19.6 mN) did not generate any contractile activity during the period of equilibration. Application of TEA (10^{-3} M), glibenclamide (10^{-5} M), iberiotoxin (10^{-7} M), charybdotoxin (10^{-7} M), or apamin (10^{-6} M) had no effect on vas deferens rings under resting conditions.

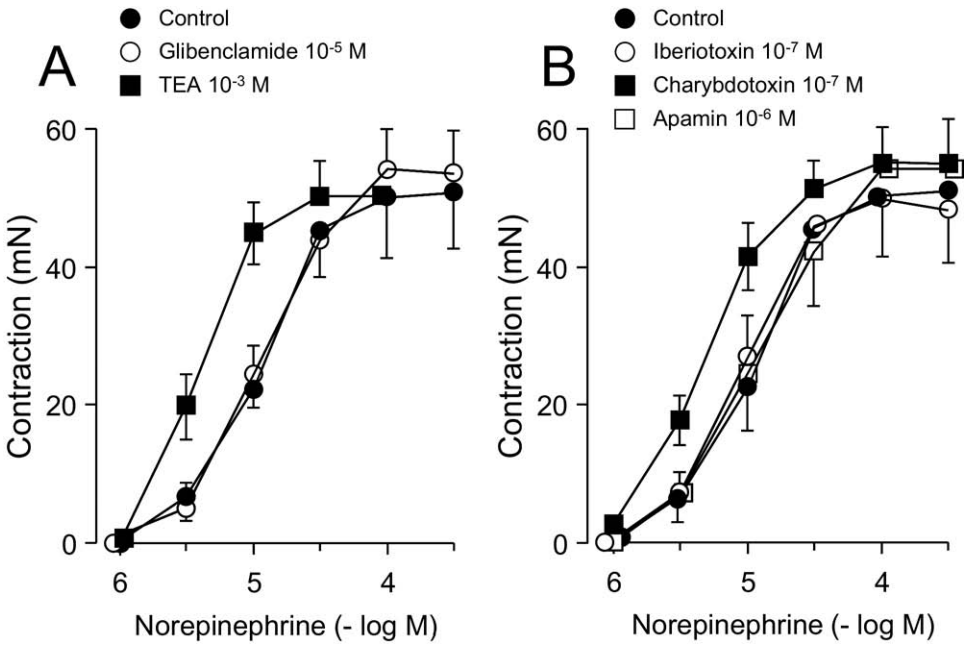

Figure 2. (**A**) Concentration–response curves to norepinephrine in the absence (control, n = 8) and in the presence of glibenclamide (10^{-5} M, n = 6) or TEA (10^{-3} M, n = 6). (**B**) Concentration–response curves to norepinephrine in the absence and in the presence of iberiotoxin (10^{-7} M, n = 7), charybdotoxin (10^{-7} M, n = 7), or apamin (10^{-7} M, n = 7). Values are shown as the means ± SEM.

Electrical stimulation induced increases in tension (45.1 ± 3.9 mN) in all the experiments which were abolished by tetrodotoxin (10^{-6} M) and prazosin (10^{-6} M), thus indicating that the effect was due to the release of norepinephrine from adrenergic nerves acting on α_1 adrenoceptors (results not shown). The presence of TEA (10^{-3} M) or charybdotoxin (10^{-7} M) significantly augmented electrical field stimulation induced contractions, 79% and 71%, respectively (Figure 1). Apamin (10^{-6} M), iberiotoxin (10^{-7} M), or glibenclamide (10^{-5} M) did not modify ($P > .05$) the contractile responses to electrical field stimulation (Figure 1).

Cumulative addition of norepinephrine (10^{-6}-3 × 10^{-4} M) induced repetitive phasic, concentration-dependent contractions with a pD_2 value of 5.03 ± 0.09. Pretreatment with either glibenclamide (10^{-5} M), iberiotoxin (10^{-7} M), or apamin (10^{-6} M) did not affect the contractions induced by norepinephrine (Figure 2). We observed that both TEA (10^{-3} M) and charybdotoxin (10^{-7} M) significantly potentiated the contractile effects of norepinephrine (Figure 2); the pD_2 values for norepinephrine increased but maximal responses remained unchanged (Table 1).

The dihydropyridine Ca^{2+} channel blocker nifedipine (10^{-6} M) caused a 38% reduction in the contractions to electrical field stimulation and a 40% reduction in the maximal response to norepinephrine and decreased the pD_2 value from 5.03 ± 0.09 to 4.49 ± 0.08 ($P < .05$) (Figure 3). In the presence of nifedipine (10^{-6} M), the TEA- and charybdotoxin-induced potentiation of contractions to electrical stimulation and norepinephrine was abolished (Figure 3).

Table 1. pD_2 values and maximal contractions elicited by norepinephrine alone (control) and in the presence of glibenclamide, TEA, iberiotoxin, charybdotoxin, or apamin

Norepinephrine	pD_2 ± SEM	Maximal responses (mN ± SEM)
Control (n = 8)	5.03 ± 0.09	49.7 ± 7.8
Glibenclamide (10^{-5} M) (n = 6)	4.97 ± 0.11	52.4 ± 6.1
TEA (10^{-3} M) (n = 6)	5.38 ± 0.08*	49.3 ± 5.0
Iberiotoxin (10^{-7} M) (n = 7)	4.95 ± 0.09	49.1 ± 5.6
Charybdotoxin (10^{-7} M) (n = 7)	5.35 ± 0.08*	50.7 ± 6.8
Apamin (10^{-6} M) (n = 7)	4.89 ± 0.12	48.0 ± 6.4

Values are mean ± SEM. *n*, number of subjects.
* $P < .05$ *versus* control rings.

COMMENT

The present study demonstrates that neurally released and exogenously applied norepinephrine activates charybdotoxin-sensitive, but iberotoxin-insensitive, K^+ channels in the epididymal portion of human vas deferens. This effect may limit smooth muscle contraction in response to adrenergic stimulation. It is important to note that our observations are for circular muscle. There is evidence suggesting differences in the pharmacologic characteristics of the α_1-adrenoceptors involved in the adrenergic activation of longitudinal or circular muscle of the human vas deferens, with predominance of α_{1L}-subype in longitudinal muscle and α_{1A}-subtype in circular muscle.[17]

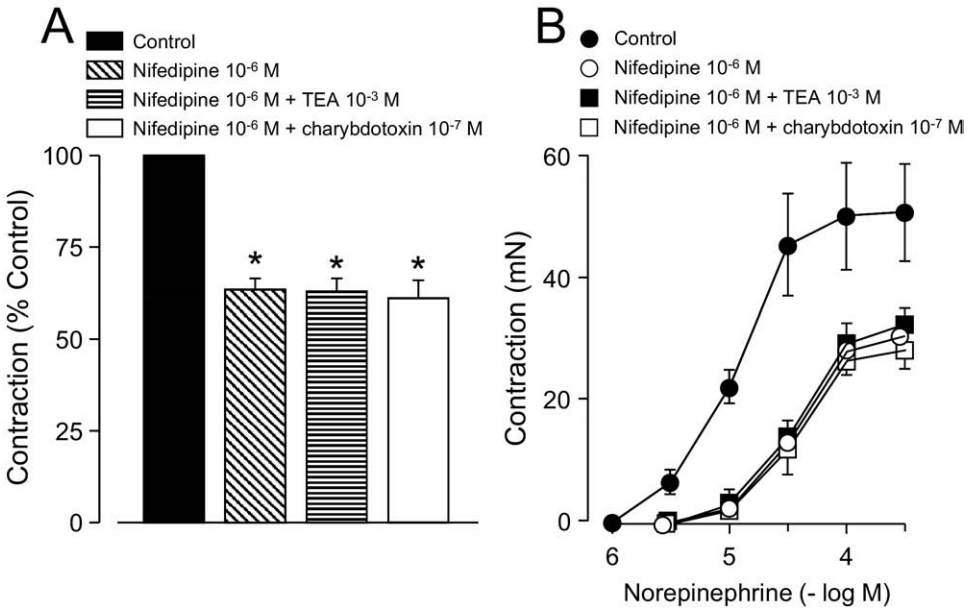

Figure 3. (**A**) Bar graph of contractile responses to electrical field stimulation in the absence (control) and in the presence of nifedipine (10^{-6} M; n = 8), and nifedipine together with TEA (10^{-3} M; n = 6) or charybdotoxin (10^{-7} M; n = 6). (**B**) Concentration–response curves to norepinephrine in the absence (control; n = 8) and the presence of nifedipine (10^{-6} M; n = 6) and in the presence of nifedipine together with TEA (10^{-3} M; n = 6), and charybdotoxin (10^{-7} M; n = 6). *$P < .05$ *versus* control.

K^+ channels are present in virtually all mammalian cell types and some of them are believed to play an important role in controlling the resting membrane potential.[18] The present experiments indicate that the ATP-sensitive and Ca^{2+}-activated K^+ channel blockers used had no effect on human vas deferens under resting conditions. These results confirm previous studies in rat vas deferens indicating that neither ATP-sensitive nor Ca^{2+}-activated K^+ channels are involved in maintenance of the resting membrane potential in smooth muscle or nerve endings of the vas deferens.[19]

In the present study, TEA and charybdotoxin significantly increased the nerve stimulation- and norepinephrine-evoked contraction. It is well known that K^+ channel blockers, such as TEA and charybdotoxin potentiate the stimulation-evoked release of norepinephrine.[20,21] This action may be caused by prolongation of the action potential duration by block of K^+ channels, delaying repolarization and increasing the availability of Ca^{2+}. The fact that TEA, a nonspecific K^+ channel blocker,[22] and charybdotoxin, an inhibitor of both large and intermediate conductance Ca^{2+}-activated K^+ channels,[23] increased contractions due to both electrical field stimulation and norepinephrine suggests that Ca^{2+}-activated K^+ channels modulate the action of the adrenergic neurotransmitter on smooth muscle rather than the release of neurotransmitter.

The norepinephrine-induced contraction would be expected to evoke the opening of Ca^{2+}-activated K^+ channels.[1] After Ca^{2+}-activated K^+ channel blockade, hyperpolarization is prevented, intracellular Ca^{2+} is increased, and norepinephrine-induced contractions are enhanced. This most likely occurs because of an increase in intracellular Ca^{2+} and membrane depolarization.[1,24] In the present experiments the addition of nifedipine to the vas deferens segments significantly decreased the potentiation induced by Ca^{2+}-activated K^+ channel inhibitors. Under these conditions, both TEA and charybdotoxin were practically without effect on neurogenic- and norepinephrine-induced contractions. This indicates that Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels was increased by blockade of TEA- and charybdotoxin-sensitive K^+ channels. Thus, although not active under resting conditions, Ca^{2+}-activated K^+ channels may participate in the regulation of vas deferens contractions during adrenergic stimulation.

Glibenclamide, an inhibitor of ATP-sensitive K^+ channels,[25] apamin, a specific blocker of small conductance Ca^{2+}-activated K^+ channels[26] or iberiotoxin, a specific blocker of large conductance Ca^{2+}-activated K^+ channels[27] had no effects on adrenergic-mediated contractions of the human vas deferens. Thus, large and small conductance Ca^{2+}-activated K^+ channels and ATP-sensitive K^+ channels do not appear to be activated in association with adrenergic neurotransmitter in human vas deferens. The adrenergic response of the human vas deferens is potentiated by charybdotoxin, but not by iberiotoxin or apamin, a characteristic feature of intermediate Ca^{2+}-activated K^+ channels.

It is noteworthy that regional variation in adrenergic responses of the vas deferens was examined in various experimental animals, and norepinephrine was reported to be more responsible for contraction in the epididymal

portion than in the prostatic portion of the vas deferens.[28] The samples used in the present study were removed from proximal portions of vas deferens within 3 cm of the ductus epididymis in healthy fertile men undergoing vasectomy. Because a regional variation in vas deferens responses, the effects of K^+ channel blockers on adrenergic contractions demonstrated in our study only represent one portion of the vas deferens and may not necessarily reflect total vas deferens reactivity to adrenergic stimulation.

CONCLUSIONS

The findings of the present study support the view that charybdotoxin-sensitive, iberiotoxin-insensitive, K^+ channels modulate the adrenergic contractile responses in human vas deferens via Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels. An important function of the vas deferens is the transport of spermatozoa from the epididymis for inclusion in the semen. Although the present studies provide evidence of a link between Ca^{2+}-activated K^+ channels and adrenergic contraction of human vas deferens, future studies are necessary to elucidate the potential therapeutic modification of vas deferens motility, via pharmacologic intervention of these channels. Increasing adrenergic motor function of the vas deferens has been demonstrated to be successful on the treatment of idiopathic oligospermia induced by aperistaltic vas deferens.[29] Sambe et al.,[30] have shown that α_{1A}-adrenoceptors are required for normal contraction of the vas deferens and consequent sperm ejaculation. Therefore, the contractile dysfunction of the vas deferens induced by the loss of functioning α_{1A}-adrenoceptors can explain the side effects observed in patients being treated with an α_{1A}-adrenoceptor blocker by benign prostatic hyperplasia as well as prostatitis. Possible manipulation of Ca^{2+}-activated K^+ channels sensitive to charybdotoxin may enable clinical investigators to increase or improve functional adrenergic contractions of the vas deferens and increase sperm transport.

Acknowledgments. This work was supported by Ministerio de Ciencia e Innovación and Conselleria de Sanidad (BFU 2009-12909), Generalitat Valenciana (AP-043/09, AP-074/10).

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