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

Pascual Medina, Gloria Segarra, María Dolores Mauricio, José M. Vila, Pascual Chuan, and Salvador Lluch

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 dihydroxyridine-sensitive Ca²⁺ 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 nonspecific inhibitor of large- and intermediate-conductance Ca²⁺-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 Ca²⁺-activated K⁺ channels, apamin (10⁻⁶ M), a blocker of small-conductance Ca²⁺-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 Ca²⁺ 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 Ca²⁺ entry through L-type voltage-dependent Ca²⁺ channels.

© 2010 Elsevier Inc. 0090-4295/10/$36.00 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\(_2\)PO\(_4\), 1.2; MgCl\(_2\)·6H\(_2\)O, 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 elicited contractile responses that were abolished by tetrodotoxin (10\(^{-6}\) M) or prazosin (10\(^{-6}\) M).

When electrically induced phasic contractions were stable (after 10-20 minutes) tetraethylammonium (TEA, 10\(^{-3}\) M), glibenclamide (10\(^{-5}\) M), charybdotoxin (10\(^{-7}\) M), apamin (10\(^{-6}\) M), and glibenclamide (10\(^{-5}\) 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, charybdotoxin, tetraethylammonium bromide (TEA), glibenclamide, 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 \(\mu\)L. Stock solutions of the drugs were freshly prepared every day and kept on ice throughout the experiment.

**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), charybdotoxin (10\(^{-7}\) M), or apamin (10\(^{-6}\) M) had no effect on vas deferens rings under resting conditions.
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 \( \alpha_1 \) 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 \times 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^{-4} 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>
<thead>
<tr>
<th>Table 1.</th>
<th>pD(_2) values and maximal contractions elicited by norepinephrine alone (control) and in the presence of glibenclamide, TEA, iberiotoxin, charybdotoxin, or apamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>pD(_2) ± SEM</td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>5.03 ± 0.09</td>
</tr>
<tr>
<td>Glibenclamide (10^{-5} M) (n = 6)</td>
<td>4.97 ± 0.11</td>
</tr>
<tr>
<td>TEA (10^{-3} M) (n = 6)</td>
<td>5.38 ± 0.08*</td>
</tr>
<tr>
<td>Iberiotoxin (10^{-7} M) (n = 7)</td>
<td>4.95 ± 0.09</td>
</tr>
<tr>
<td>Charybdotoxin (10^{-7} M) (n = 7)</td>
<td>5.35 ± 0.08*</td>
</tr>
<tr>
<td>Apamin (10^{-6} M) (n = 7)</td>
<td>4.89 ± 0.12</td>
</tr>
</tbody>
</table>

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

COMMENTS

The present study demonstrates that neurally released and exogenously applied norepinephrine activates charybdotoxin-sensitive, but iberiotoxin-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 \( \alpha_1 \)-adrenoceptors involved in the adrenergic activation of longitudinal or circular muscle of the human vas deferens, with predominance of \( \alpha_{1L} \)-subtype in longitudinal muscle and \( \alpha_{1A} \)-subtype in circular muscle.\(^{17} \)
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. The present experiments indicate that the ATP-sensitive and Ca²⁺-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 Ca²⁺-activated K⁺ channels are involved in maintenance of the resting membrane potential in smooth muscle or nerve endings of the vas deferens.

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. This action may be caused by prolongation of the action potential duration by block of K⁺ channels, delaying repolarization and increasing the availability of Ca²⁺. The fact that TEA, a nonspecific K⁺ channel blocker, and charybdotoxin, an inhibitor of both large and intermediate conductance Ca²⁺-activated K⁺ channels, increased contractions due to both electrical field stimulation and norepinephrine suggests that Ca²⁺-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 Ca²⁺-activated K⁺ channels. After Ca²⁺-activated K⁺ channel blockade, hyperpolarization is prevented, intracellular Ca²⁺ is increased, and norepinephrine-induced contractions are enhanced. This most likely occurs because of an increase in intracellular Ca²⁺ and membrane depolarization. In the present experiments the addition of nifedipine to the vas deferens segments significantly decreased the potentiation induced by Ca²⁺-activated K⁺ channel inhibitors. Under these conditions, both TEA and charybdotoxin were practically without effect on neurogenic- and norepinephrine-induced contractions. This indicates that Ca²⁺ influx through dihydropyridine-sensitive Ca²⁺ channels was increased by blockade of TEA- and charybdotoxin-sensitive K⁺ channels. Thus, although not active under resting conditions, Ca²⁺-activated K⁺ channels may participate in the regulation of vas deferens contractions during adrenergic stimulation.

Glibenclamide, an inhibitor of ATP-sensitive K⁺ channels, apamin, a specific blocker of small conductance Ca²⁺-activated K⁺ channels, or iberiotoxin, a specific blocker of large conductance Ca²⁺-activated K⁺ channels, had no effects on adrenergic-mediated contractions of the human vas deferens. Thus, large and small conductance Ca²⁺-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 Ca²⁺-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 Ca2+ influx through dihydropyridine-sensitive Ca2+ 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 Ca2+-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 α1A-adrenoceptors are required for normal contraction of the vas deferens and consequent sperm ejaculation. Therefore, the contractile dysfuncition of the vas deferens induced by the loss of functioning α1A-adrenoceptors can explain the side effects observed in patients being treated with an α1A-adrenoceptor blocker by benign prostatic hyperplasia as well as prostatitis. Possible manipulation of Ca2+-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).

References


