Original Article

A low androgen state impairs erectile function by suppressing *EPAC1* in rat penile corpus cavernosum

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Background: Exchange proteins activated by cAMP 1 (*EPAC1*) can promote vasodilatation by regulating endothelial nitric oxide synthase (eNOS) activity through the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway and prevent vascular smooth muscle contraction by restraining the ras homolog gene family, member A/Rho-associated coiled-coil forming protein kinase (RhoA/ROCK) pathway. However, the relationship among *EPAC1*, androgen and erectile function is still unknown. Therefore, we attempted to investigate whether *EPAC1* expresses in penile corpus cavernosum of rats and how *EPAC1* affects erectile function under low androgenic conditions.

Methods: Thirty 8-week-old Sprague-Dawley male rats were randomly divided into six groups (n=5): sham operation (sham), castrated, castrated + testosterone replacement (castrated + T), sham + *EPAC1* over-expression lentivirus (sham + *EPAC1*), castrated + empty lentivirus vector (castrated + empty vector), and castrated + *EPAC1*. Four weeks after the operation, the lentivirus vectors carrying the *EPAC1* gene were injected into the penile corpus cavernosum of the sham + *EPAC1* and castrated + *EPAC1* groups (1×10^8 TU/mL, 20 μL per rat). A week after injection, the ratio of maximum intracavernous pressure to mean arterial pressure (ICPmax/MAP) and the levels of serum testosterone (T), nitric oxide (NO), the active form of RhoA (RhoA-GTP), AKT, phospho-AKT (p-AKT), eNOS, phospho-eNOS (p-eNOS), p-AKT/AKT, p-eNOS/eNOS and *EPAC1* levels were measured.

Results: In comparison to the sham group, ICPmax/MAP and *EPAC1* content in the castrated group were significantly reduced. *EPAC1* is primarily located in the cyto-membrane and cytoplasm of endothelial cells and smooth muscle cells in the rat penile corpus cavernosum. In comparison to the sham group, the T, ICPmax/MAP and NO levels of the castrated group were significantly reduced (P<0.01). Meanwhile, the RhoA-GTP concentration in the castrated + *EPAC1* group was reduced in comparison with the castrated + empty vector group (P<0.01). Compared with the castrated + empty vector group, the p-AKT/AKT, *EPAC1* and p-eNOS/eNOS levels in the castrated + *EPAC1* group were significantly increased (P<0.05).

Conclusions: Androgen deficiency can suppress *EPAC1* expression in the penile corpus cavernosum of rats, while the up-regulation of which can improve the erectile function of castrated rats.

Keywords: Androgen; exchange proteins activated by cAMP 1 (*EPAC1*); erectile dysfunction (ED); penile; RhoA

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Introduction

The International Classification of Diseases, 11th Revision (ICD-11) defines erectile dysfunction (ED) as men are unable or markedly reduced the ability to obtain or maintain a penile erection of sufficient duration or rigidity for sexual activity (1). Among men aged 40 to 80 without comorbidities, the prevalence of ED ranges from 10% to 78% (2). Low androgen status is one of the common etiologies (3).

Androgen is responsible for maintaining the normal function and structure of the penile corpus cavernosum. The low androgen status can reduce the production of nitric oxide (NO) and inhibit erectile function through the protein kinase B/eNOS (AKT/eNOS) pathway (4). Hypertension, diabetes, and low androgen status can significantly increase the bioactivity and expression of the ras homolog gene family, member A/Rho-associated coiled-coil forming protein kinase (RhoA/ROCK) in the penile cavernous tissue of rats (5-8), which enhances the contraction of smooth muscle cells and inhibits the erectile function of rats. The levels of serum T in males peak at the age of thirty and gradually decrease with age. The results confirm a negative correlation between age and serum T. However, to date, the exact mechanism underlying how hypoandrogenism alters ED is still unknown.

As a member of the cyclic-3',5'-adenosine monophosphate (cAMP) effector family, exchange proteins activated by cAMP (*EPACs*) have been proved to affect cell functions dramatically, such as apoptosis, migration and cell proliferation (9-12). *EPACs* also have an essential role in the cardiovascular system (13). *EPACs* participate in the progression of many diseases, such as arteriosclerosis, cardiac hypertrophy, and hypertension (14,15). The *EPACs* consist of two isotypes, exchange proteins activated by cAMP 1 (*EPAC1*) and *EPAC2*. They are expressed in various tissues and regulated by different genes. *EPAC1* is expressed in many organs, especially in the blood vessels, kidneys, and heart and it is regulated by the human *RAPGEF3* gene. *EPAC2* is encoded by the human *RAPGEF4* gene and has tissue-specific expression (16). *EPAC2* is primarily expressed in the central nervous system, adrenal gland, and pancreas, which is considered to be associated with endocrine function (17,18). One study demonstrated that when human airway smooth muscle cells were stimulated with T for 48 hours, the expression of *EPACs* was reduced (19), suggesting a possible relation between *EPACs* and T. *EPAC1* can activate the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, which can promote AKT-dependent eNOS phosphorylation and enhance the activity of eNOS, resulting in the increase of NO and thereby promoting the relaxation of vascular smooth muscle (20,21). RhoA is a kind of guanosine triphosphate (GTP) enzyme that can be converted from an inactive form of RhoA-guanosine diphosphate (GDP) to an active form of RhoA-GTP when activated, and activating downstream signaling effector (22). And *EPAC1* can inhibit RhoA activity and its downstream effector protein ROCK, through ras-related protein 1 (Rap1) signaling to inhibit vasoconstriction (23-25). Many studies have confirmed the expression of *EPAC1* in rat and human arterial endothelium (10,24,25). However, whether *EPAC* is expressed in rat corpus cavernosum and its effect on erectile function under low androgen level has not been reported. Therefore, further research is needed to determine whether *EPAC* is expressed in penile corpus cavernosum of rats and how *EPAC* affects erectile function at low androgen levels.

A protocol was prepared before the study without registration. And we present this article in accordance with the ARRIVE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-314/rc).

Highlight box

Key findings

- Low androgen status can inhibit erectile function by down-regulating the expression of exchange proteins activated by cAMP 1 (*EPAC1*) in penile corpus cavernosum of castrated rats. The up-regulation of the expression of *EPAC1* in penile cavernous tissue can significantly improve erectile function in castrated rats.

What is known and what is new?

- *EPAC1* can promote vasodilatation by regulating endothelial nitric oxide synthase activity through the PI3K/AKT pathway and inhibit vascular smooth muscle contraction by restraining the RhoA/ROCK pathway.
- *EPAC1* is primarily located in the cyto-membrane and cytoplasm of endothelial cells and smooth muscle cells in the rat penile corpus cavernosum. low androgen status can inhibit erectile function by down-regulating the expression of *EPAC1* in penile corpus cavernosum of castrated rats.

What is the implication, and what should change now?

- *EPAC1* may be a potential target for improving erectile function under low androgenic conditions.

Methods

Animals and groups

After the rats were numbered 1–30, 30 eight-week-old

Sprague-Dawley male rats were divided into six groups in a completely random manner (n=5): sham operation (sham), castrated, castrated + testosterone replacement (castrated + T), sham + *EPAC1* over-expression lentivirus (sham + *EPAC1*), castrated + empty lentivirus vector (castrated + empty vector), and castrated + *EPAC1*. After the rats were anesthetized with 1% pentobarbital sodium (30 mg/kg) by intraperitoneal injection, bilateral testes and accessory glands were excised through scrotal incision to establish castrated rat model. T replacement is given subcutaneous injection of T propionate (3 mg/kg) every two days for four weeks. The other groups received equal amounts of vegetable oil (the vehicle for T) in the same way. Four weeks after castration, a rubber band was used to ligate the penile root of the anesthetized rat. The lentivirus vectors carried the *EPAC1* gene (1×10^8 TU/mL, 20 μL per rat, JiKai Gene Company, Shanghai, China) was injected into the middle of the corpus cavernosum of castrated + *EPAC1* and sham + *EPAC1* group rats by a micro syringe. At the same time, the same amount of empty lentivirus was injected into the penis of the castrated + empty vector group rats in the same way, while the rats in the other groups were injected with the same doses of physiological saline. After 3 min of injection, the rubber band tied to the penile root of the rat was removed (3,4). The experimental rats were all raised in the Animal Experiment Center of Southwest Medical University. Room temperature was kept at 22–26 ℃, and the humidity at 40–60%. All rats were free to drink and eat. All experimental procedures were approved by the Animal Experimentation Ethics Committee of the Southwestern Medical University (No. 20211126-001) and followed the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Erectile function assessment the ratio of maximum intracavernous pressure to mean arterial pressure (ICPmax/MAP)

One week after lentivirus transfection, after anesthetizing, the penile corpus cavernosum and the left common carotid artery of animals were punctured with 26 G and 24 G needles infused with heparin, respectively. The two needles and BL420 biofunctional experiment system (Techman Technology Co., Ltd., Chengdu, China) were connected by a pressure transducer to monitor MAP and ICPmax in rats. An incision was made in the abdomen to expose the prostate gland of the rats. After the cavernous nerve was found out, electrical stimulation was performed on it (intensity was 3 V, 5 V, wave amplitude of 5 ms, frequency of 12 Hz, duration of 30 s, interval of 3 min).The ICPmax/MAP was used to erectile function assessment (4).

Detection of serum T and collection of rat samples

After ICPmax/MAP was measured, 3 mL of carotid blood was taken from each rat to detect serum T from 8 a.m. to 10 a.m., and rats were euthanized by injecting an overdosage of anesthetic. The T ELISA Kit (Lengton, Shanghai, China, BPE30610) instructions were used to detect rat serum T by enzyme-linked immunosorbent assay (ELISA). The penis of rats was carefully dissected and only cavernous tissues were retained. The rat penile cavernous tissues were cut into three sections and stored at −80 ℃ until the determination of NO and RhoA-GTP and western blotting. The other two parts of the penis were used for immunohistochemistry (IHC) and immunofluorescence examination (7).

Determination of NO and RhoA-GTP concentrations in rat corpus cavernosum penis

The penile corpus cavernosa of all rats were ground into powder separately, and then the samples were mixed with phosphate buffered saline (PBS; Beyotime, Shanghai, China) uniformly containing phosphatase inhibitors and protease inhibitors in a ratio of 1 to 9. The above operations were performed on ice. Thirty minutes later, the sample was divided into two parts. The two parts were centrifuged separately at 3,100 and 5,000 g at 4 ℃. Ten minutes later, the supernatant of the samples was carefully collected to measure the concentrations of NO and RhoA-GTP. The NO concentration was determined following the NO Colorimetric Assay Kit (Elabscience, Wuhan, China, E-BC-K035-M) instructions. The instructions of the Rat RhoA-GTP ELISA KIT (Ruixinbio, Quanzhou, China, RX300892R) were followed to determine the concentration of RhoA-GTP. The overall protein concentrations were assessed following the Bicinchoninic Acid Assay (BCA) Protein Assay Kit (Beyotime, P0010S) instructions (7).

Fluorescent localization and transfection rate of lentivirus in rat penile cavernosa

Rat penis samples with intact structure were used for frozen sections, and then these frozen sections were stained with diamidino-phenyl-indole (DAPI; Beyotime) and

observed by fluorescence microscopy (Olympus, Tokyo, Japan, BX63). The cells containing green fluorescence protein (GFP) under blue light excitation indicated that the lentiviral vectors carrying the *EPAC1* gene were successfully transfected into these cells. The results were analyzed by Image-Pro Plus 6.0 software (Media Cybernetics Inc., Rockville, MD, USA). The transfection rate was expressed as GFP/DAPI × 100% (3).

IHC of EPAC1 and EPAC2 in rat penile cavernosum

A part of the rat corpus cavernosum tissues were fixed with 4% paraformaldehyde. The sections of tissues embedded with paraffin were used for IHC detection. IHC was performed according as previously described (26,27). The embedded paraffin tissue samples were sectioned, dehydrated, dewaxed, subjected to endogenous peroxidase inhibition, antigen repair and closure. Then the samples were incubated with primary antibody and secondary antibody successively. The primary monoclonal antibodies containing mouse anti-rat *EPAC1* (1:100, Santa Cruz, Dallas, TX, USA, sc-28366) and *EPAC2* (1:100, Santa Cruz, sc-28326). The secondary antibody was goat anti-mouse (1:100, Beyotime, A0216). After 3,3'-diaminobenzidine (DAB; Beyotime) color development, hematoxylin restaining, and dehydration, these samples were fixed with neutral resin. Finally, observing these sections under digital slice scanner (KFBIO, Ningbo, China, KF-PRO-002), the brownish yellow particles indicated the positive expression of proteins. Image-Pro Plus 6.0 software (Media Cybernetics Inc.) was used to count the integrated optical density (IOD) (3,4).

Western blot of EPAC1, EPAC2, eNOS, p-eNOS (Ser1177), AKT, and p-AKT (S473) in rat penile cavernosa

The penis cavernosa were ground into powder and lysed with radio immunoprecipitation assay (RIPA lysis buffer containing phosphatase inhibitors and protease inhibitors (Beyotime) in eppendorf tubes overnight at 4 ℃. The supernatants were transferred to another centrifuge tube after centrifugation. When the total protein concentrations were determined and the loading buffer (Beyotime) was added proportionally, these samples were then heated for 10 minutes at 100 ℃. Then, these denatured proteins were cooled naturally and frozen at −20 ℃ and used for western blot. Samples from different groups were added to corresponding lanes of the SDS electrophoresis gel. The proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Elabscience). Primary antibodies were added and incubated at 4 ℃. The primary antibodies contain mouse anti-rat mouse anti-rat *EPAC1* (1:100, Santa Cruz, sc-28366), EPAC2 (1:100, Santa Cruz, sc-28326) and GAPDH (1:2,000, Beyotime, AF0006) antibodies and rabbit anti-rat phospho-AKT (p-AKT) (1:1,000, CST, Boston, MA, USA, 4060S), AKT (1:1,000, CST, 4685), phospho-eNOS (p-eNOS) (1:1,000, CST, 9570S) and eNOS (1:1,000, CST, 32027S) antibodies. The membranes were incubated with secondary antibody at room temperature for one hour after overnight incubation. The secondary antibodies contain goat anti-rabbit (1:2,000, Beyotime, A0208) and anti-mouse (1:2,000, Beyotime, A0216) antibodies. Finally, the PVDF membranes were placed in a protein imager (Bio-Rad Laboratories Inc., Hercules, CA, USA) after enhanced chemiluminescence (ECL) solution (Beyotime, P0018FS) was uniformly dripped on the surface. Image J (National Institutes of Health, USA) was used to count the ratio of optical density (OD) (4,7).

Statistical analysis

GraphPad Prism 7.0 Software (GraphPad Software, San Diego, CA, USA) to analyze the results. All data were normally distributed (Shapiro-Wilk test) and represented by the mean ± standard deviation. The statistical analysis methods used were one-way ANOVA analysis and Tukey-Kramer test. $P<0.05$ was considered a statistically significant difference.

Results

Body weight, MAP, serum T, ICPmax/MAP in each group

The results of body weight and MAP in each group were not statistically significant. After castration surgery, the serum T level of rats decreased by more than 90% compared to the sham operation group, indicating the successful establishment of a rat castration model. The serum T levels in the castrated (1.22±0.21 nmol/L), castrated + empty vector (1.23±0.16 nmol/L), and castrated + *EPAC1* (1.23±0.18 nmol/L) groups were significantly reduced. In comparison to the sham group (20.76±1.87 nmol/L) castrated + T (22.41±3.71 nmol/L) and sham + *EPAC1* (21.06±2.30 nmol/L) groups (*Table 1*).

The ICPmax/MAP was used as an indicator to evaluate erectile function of rats. In comparison to the sham

Table 1 Body weight, MAP, and serum T in each group

Group (n=5)	Body weight (g)	MAP (mmHg)	T (nmol/L)
Sham	334.70±5.04	107.98±2.53	20.76±1.87
Castrated	332.89±4.66	110.12±2.61	1.22±0.21*
Castrated + T	328.57±5.52	109.47±2.30	22.41±3.71
Sham + *EPAC1*	339.08±3.89	108.54±2.39	21.06±2.30#
Castrated + empty vector	335.23±4.77	109.38±2.44	1.23±0.16*
Castrated + *EPAC1*	335.77±4.22	110.28±2.24	1.23±0.18*

Data were presented as mean ± standard deviation. *, P<0.01 *vs.* the sham group and the castrated + T group. #, P<0.01 *vs.* the castrated group and the castrated + empty vector group. MAP, mean arterial pressure; NO, nitric oxide; T, testosterone.

(0.722±0.019, 3 V; 0.806±0.06, 5 V) group, the ICPmax/MAP in the castrated (0.393±0.003, 3 V; 0.432±0.009, 5 V) group was significantly reduced (P<0.01). In comparison to the castrated + empty vector (0.415±0.032, 3 V; 0.455±0.033, 5 V) group, the ICPmax/MAP of castrated + *EPAC1* (0.653±0.004, 3 V; 0.695±0.123, 5 V) group was significantly increased (P<0.01) (*Figures 1*,*2*).

NO and RhoA-GTP of rats in each group

In comparison to the sham (15.56±1.51 μmol/g) group, the NO in the castrated (4.06±0.59 μmol/g) group was significantly reduced (P<0.01). In comparison to the castrated + empty vector (3.89±0.51 μmol/g) group, the NO of castrated + *EPAC1* (12.40±1.13 μmol/g) group was significantly increased (P<0.01).

The RhoA-GTP content in the castrated (270.87±1.13 μg/g) group was significantly increased compared with the sham (47.29±5.07 μg/g) group (P<0.01). In comparison to the castrated + empty vector (265.87±8.94 μg/g) group, the RhoA-GTP content in the castrated + *EPAC1* group (64.89±5.24 μg/g) was significantly reduced (P<0.01) (*Figure 1*).

Fluorescence distribution and transfection rate of lentivirus in each group

GFP positive indicates that lentivirus were successfully transfected into cells. GFP could be found only in the sham + *EPAC1* group, castrated + *EPAC1* group and castrated + empty vector group. It exists in the cytoplasm of endothelial cells and smooth muscle cells in the rat penile corpus cavernosum. And the transfection rates were as follows: sham + *EPAC1* group, 87.11%±2.19%; castrated + empty vector group, 86.06%±3.44%; and castrated + *EPAC1* group, 86.68%±3.17%. There was no GFP in the remaining groups (*Figure 3*).

The expression and distribution of EPAC1 and EPAC2 in rat penile cavernosa by IHC

EPAC1 is primarily located in the cytomembrane and cytoplasm of endothelial cells and smooth muscle cells in rat penile cavernosa (*Figure 4*). *EPAC1* content in the sham groups was higher than that in the castrated group (P<0.05), and in the castrated + *EPAC1* group was higher than that in the castrated + empty vector group (P<0.05). No positive expression of *EPAC2* was observed.

The expression of EPAC1, EPAC2, eNOS, p-eNOS (Ser1177), AKT, and p-AKT (S473) in rat penile corpus cavernosum by Western blot

In comparison to the sham group, the p-AKT/AKT, *EPAC1* and p-eNOS/eNOS levels in the castrated and the castrated + empty vector groups were significantly reduced (P<0.05). In comparison to the castrated + empty vector group, the p-AKT/AKT, p-eNOS/eNOS and *EPAC1* levels in the castrated + *EPAC1* group were significantly increased (P<0.05) (*Figure 5*). No positive *EPAC2* expression was observed by western blot.

Discussion

In the present study, in comparison to the sham group, the ICPmax/MAP, serum T and NO levels in the castrated group were significantly decreased. At the same time, by supplementing T, the level of those test items in the

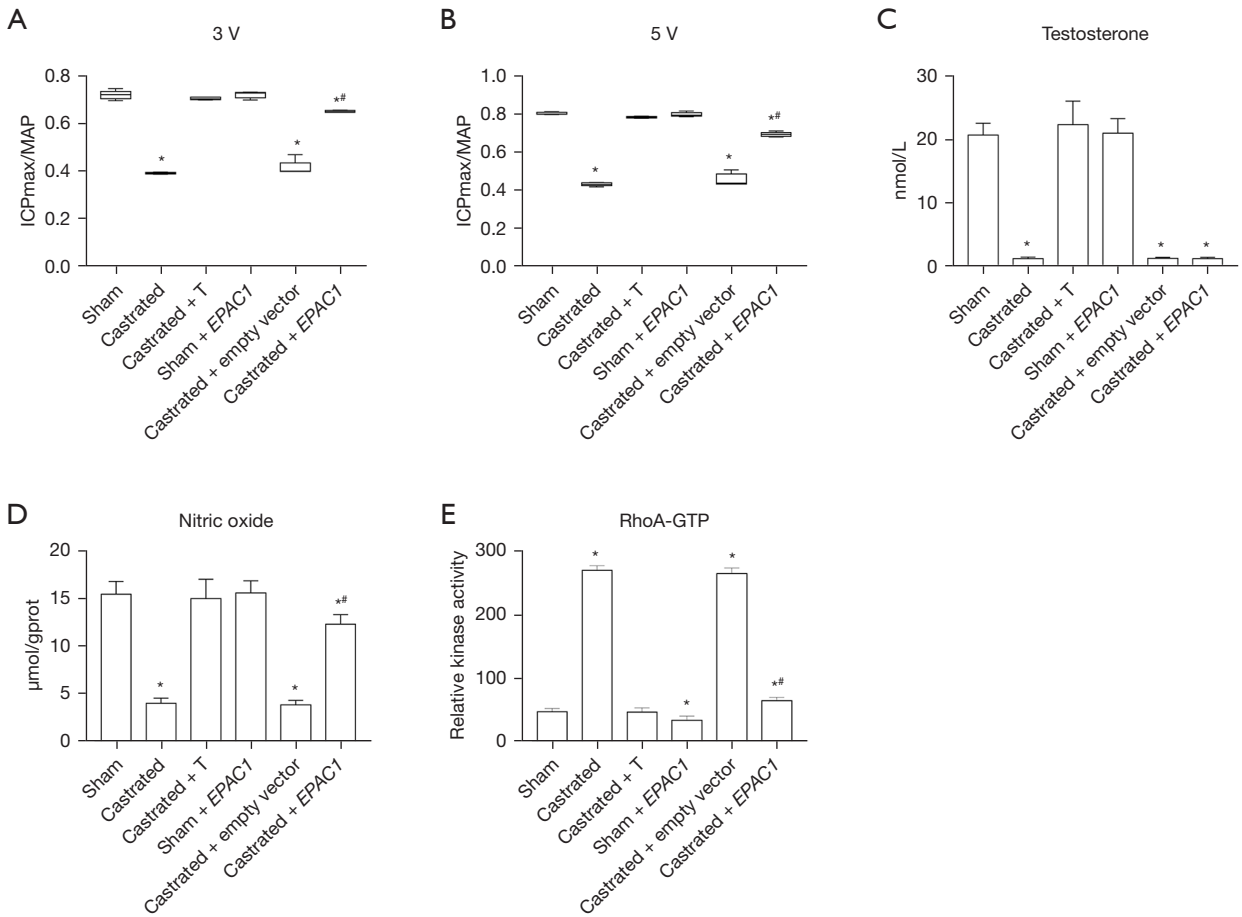

Figure 1 The levels of ICPmax/MAP, serum T, NO and RhoA-GTP. (A) The ratio of ICPmax/MAP at 3 V was impaired in the castrated group. (B) The ratio of ICPmax/MAP at 5 V was impaired in the castrated group. (C) The level of serum T in each group. (D) The level of NO in each group. (E) Activity of the level of RhoA-GTP in each group. *, P<0.01 *vs.* the sham group and the castrated + T group. #, P<0.01 *vs.* the castrated group and the castrated + empty vector group. ICPmax, maximum intracavernous pressure; MAP, mean arterial pressure; RhoA-GTP, the active form of RhoA; NO, nitric oxide; T, testosterone.

castrated + T group was obviously increased in comparison to the castrated group. These results demonstrate that androgen deficiency can impair erectile function in rats by decreasing the level of NO in penile cavernous tissue. These results were in line with previous research (3,4). However, the specific mechanism is unclear.

To our knowledge, this is the first report that *EPAC1* was expressed in the cyto-membrane and the cytoplasm of endothelial cells and smooth muscle cells in penile corpus cavernosum of rats. Studies suggest that *EPAC1* can promote vasodilation by regulating eNOS activity through the PI3K/AKT pathway (20,21). Therefore, *EPAC1* may play a role in the process of penile erection.

In comparison to the sham group and the castrated + T group, the level of ICPmax/MAP, *EPAC1*, p-AKT/AKT, p-eNOS/eNOS and NO in the castrated group was significantly reduced. Therefore, under low androgenic conditions, *EPAC1*/AKT/eNOS/NO signaling pathway in rat penile corpus cavernosum was inhibited, which may be one of the mechanisms that eventually causes ED.

After lentivirus vectors carrying the *EPAC1* gene were transfected into the penile corpus cavernosum, there were no statistically significant differences in the serum T levels between the castrated + *EPAC1* group and the castrated + empty vector group, indicating that the over-expression of *EPAC1* lentivirus has no effect on the serum T level of rats.

On the one hand, in comparison to the castrated + empty vector group, the level of *EPAC1* in penile cavernous

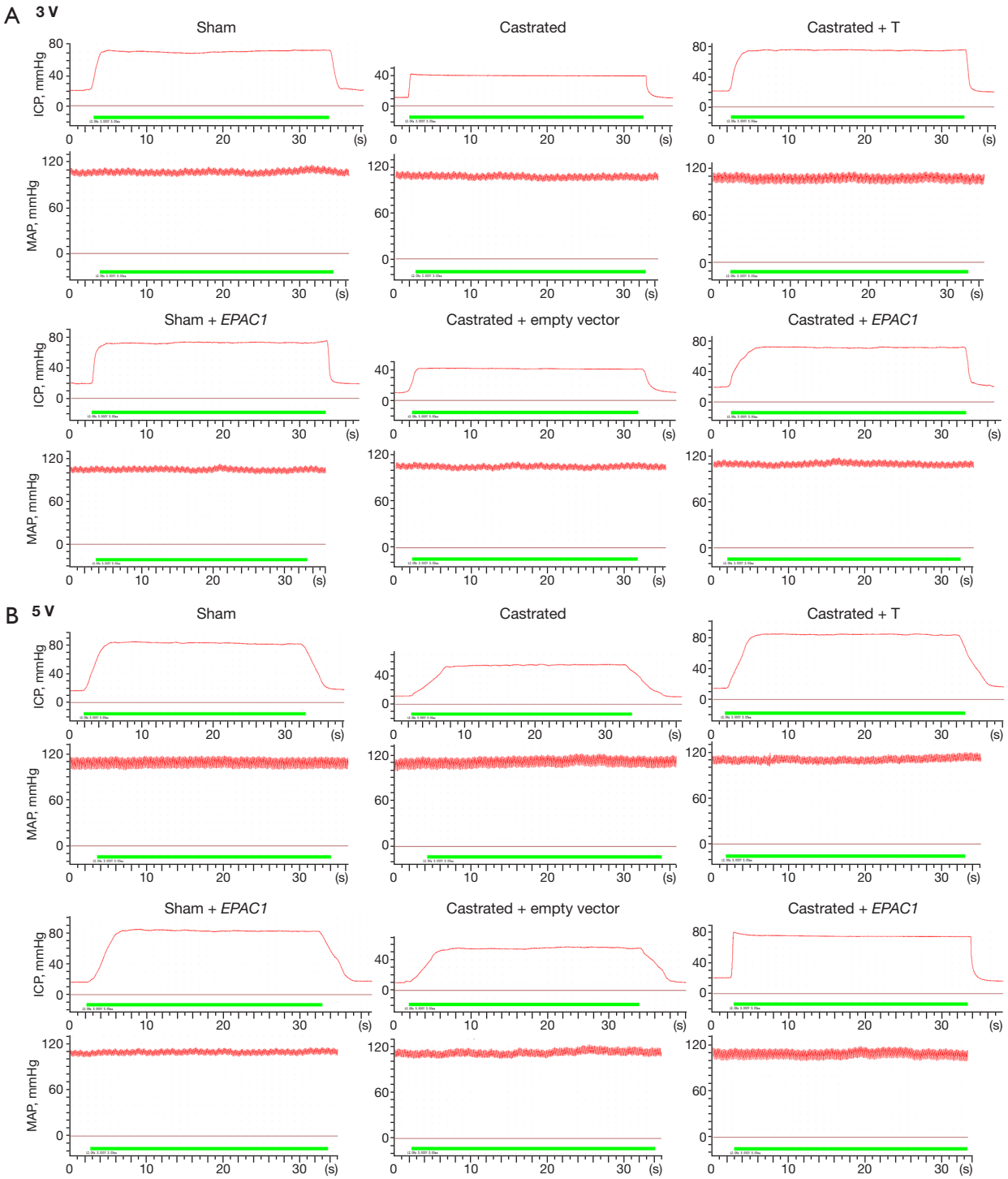

Figure 2 The curves of ICP and MAP in each group. (A) The curves of ICP and MAP during electrical stimulation in 3 V. (B) The curves of ICP and MAP during electrical stimulation in 5 V. The x-axis is the timeline. Red lines: The curves of ICP and MAP during electrical stimulation. Green lines: The duration of electrical stimulation. ICP, intracavernous pressure; MAP, mean arterial pressure; T, testosterone.

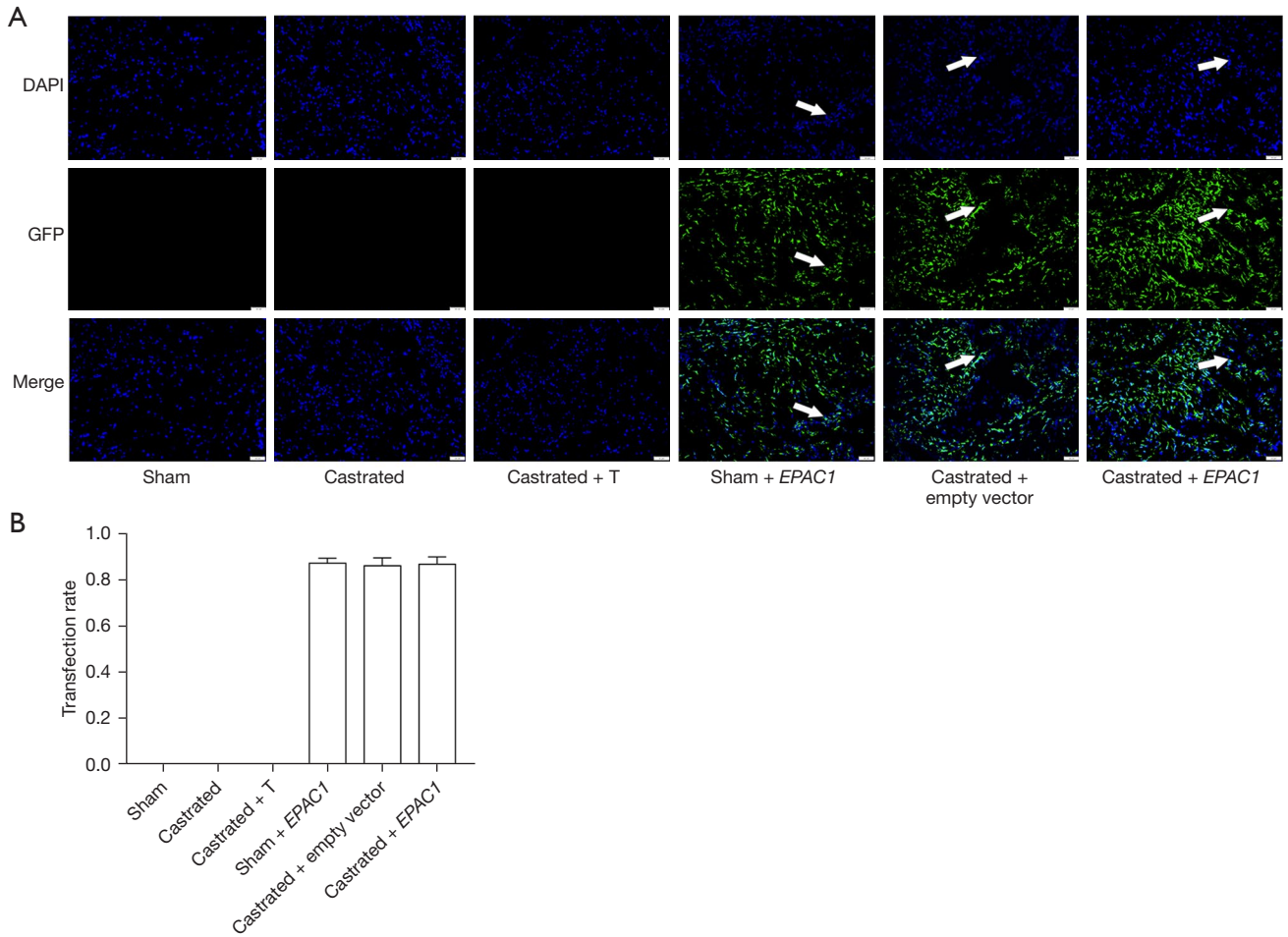

Figure 3 Fluorescence distribution and transfection rate of lentivirus. (A) GFP staining of endothelial cells indicates successful transfection (white arrows). The scale bars is 50 μm. (B) The ratio of transfection in each group. DAPI, diamidino-phenyl-indole; GFP, green fluorescence protein; T, testosterone.

tissue, ICPmax/MAP, p-AKT/AKT, NO, and p-eNOS/eNOS levels in the castrated + *EPAC1* group were also increased significantly. These results suggest that, in the castrated group, the up-regulated content of *EPAC1* in rat penile corpora cavernosum can promote the AKT/eNOS/NO/cyclic guanosine monophosphate (cGMP) signaling pathway to improve NO levels and ICPmax/MAP. As a result, erectile function of castrated rats can be improved by up-regulating *EPAC1* expression.

On the other hand, *EPAC1* inhibits RhoA activity and its downstream effector protein, ROCK, resulting in vasodilation (14-16). Compared to the sham group and the castrated + T group, the RhoA-GTP concentration in penile cavernous tissue of the castrated group was significantly increased. However, in comparison to the castrated + empty vector group, the content of RhoA-GTP in the castrated + *EPAC1* group was dramatically decreased. Therefore, under low androgen conditions, the activity of RhoA-GTP could be inhibited by *EPAC1* over-expression lentiviral vector transfection. This is one of the mechanisms that lead to RhoA/ROCK pathway suppression and erectile function improvement in castrated rats.

This study demonstrates that low androgen can suppress *EPAC1* expression and eNOS activity and activate RhoA-GTP, leading to impaired erectile function. The up-regulation of *EPAC1* expression by transgenic injection in castrated rat penile cavernosa can stimulate the PI3K/AKT pathway, which can increase AKT-dependent eNOS phosphorylation and eNOS activity, leading to an increase in NO production. In addition, it can restrain the RhoA/

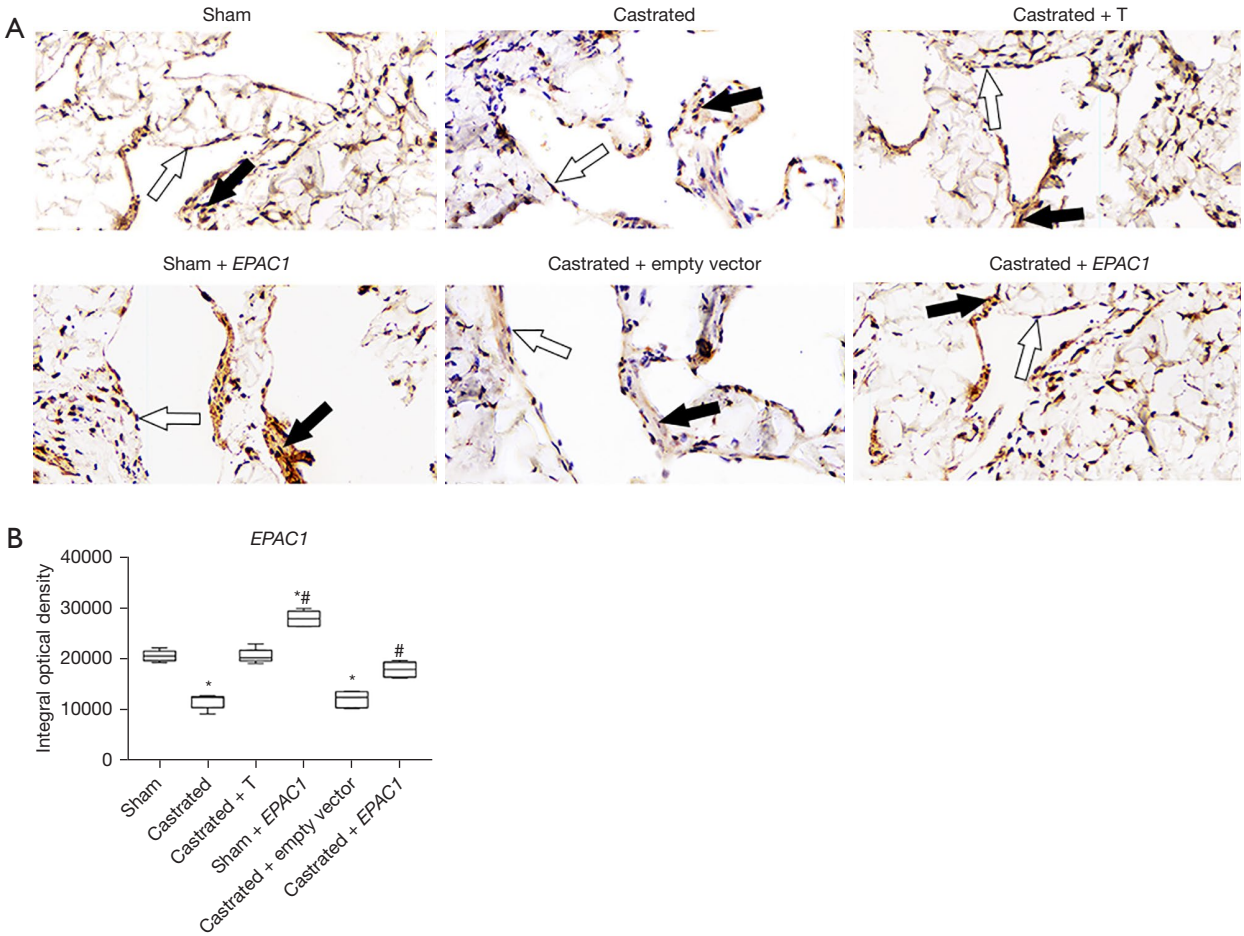

Figure 4 The expression of *EPAC1* in the penile corpus cavernosum of rat examined by immunohistochemistry. (A) *EPAC1* is primarily located in the cytoplasm of endotheliocytes (white arrows) and smooth muscle cells (black arrows) in rat corpus cavernosum penis. The scale bar is 50 μm. (B) The integrated optical density of *EPAC1* in each group. *, P<0.05 *vs.* the sham group and the castrated + T group. #, P<0.05 *vs.* the castrated group and the castrated + empty vector group. T, testosterone.

ROCK pathway by inhibiting RhoA-GTP activity.

This study found that low androgen status can inhibit erectile function by down-regulating the expression of *EPAC1* in penile corpus cavernosum of castrated rats.

The mechanism of down-regulation of *EPAC* in human airway smooth muscle cells stimulated by T is unclear (19). Meanwhile, in our experiment, the expression of *EPAC* was reduced in the penis tissue of castrated rats. This indicates that the relationship between *EPACs* and androgens in different species and tissues is complex and requires further research.

After injection of the lentivirus carrying *EPAC1*, the sham + *EPAC1* group showed a significant increase in *EPAC1* levels compared to the sham group and the castrated + T group (P<0.05), while the activity of RhoA (RhoA-GTP) significantly decreased (P<0.05). However, there were no significant changes in p-AKT/AKT, p-eNOS/eNOS, NO levels, and the erectile function (ICPmax/MAP) of rats did not show any significant changes. It is suggested that the over-expression of *EPAC1* did not significantly affect the erectile function of normal rats.

Conclusions

This study found that low androgen status can inhibit erectile function by down-regulating the expression of *EPAC1* in penile corpus cavernosum of castrated rats. The up-regulation of the expression of *EPAC1* in penile

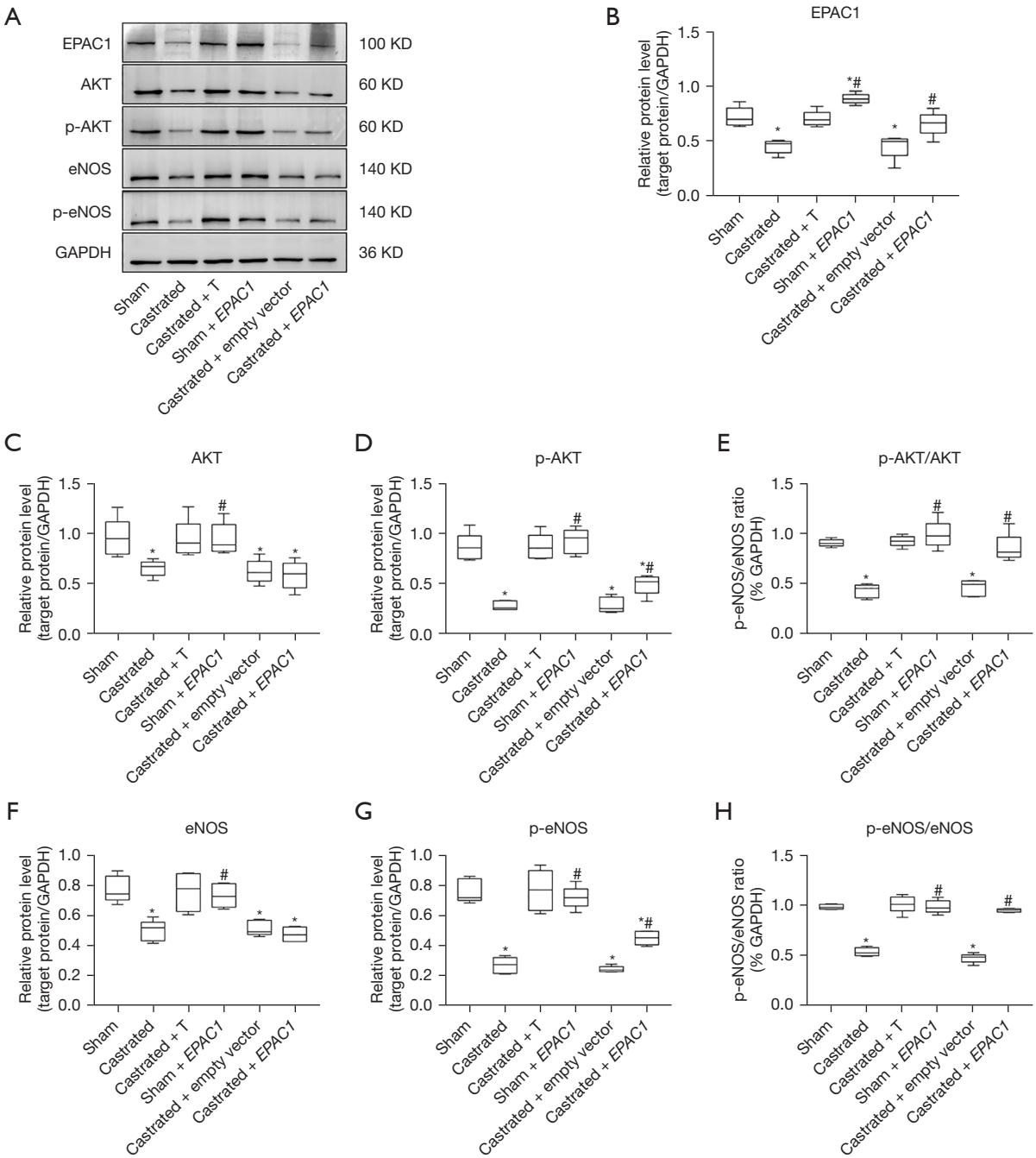

Figure 5 The expression of AKT, p-AKT, eNOS, p-eNOS and *EPAC1* in the rat penile corpus cavernosum by Western blotting. (A) Images of AKT, p-AKT, eNOS, p-eNOS and *EPAC1* by Western blotting. (B-H) The *EPAC1*, AKT, p-AKT, p-AKT/AKT, eNOS, p-eNOS and p-eNOS/eNOS levels in each group. *, P<0.05 *vs.* the sham group and the castrated + T group. #, P<0.05 *vs.* the castrated group and the castrated + empty vector group. AKT, protein kinase B; p-AKT, phospho-AKT; eNOS, endothelial nitric oxide synthase; p-eNOS, phospho-eNOS; T, testosterone.

cavernous tissue can significantly improve erectile function in castrated rats. Therefore, *EPAC1* may be an important target for improving erectile function under low androgenic conditions, which provides an important direction for the clinical treatment of ED requiring maintenance of low androgen status.

This study preliminarily discovered the relation between the expression of *EPAC1* in penile cavernous tissue of rats under low androgen status and erectile function in rats. The exact relation between androgen and *EPAC1* needs to be further validated through experiments on androgen receptor knockout rats. Meanwhile, there is cross-linking between the RhoA/ROCK pathway and the NO/cGMP pathway (25). It is still unclear whether the up-regulation of eNOS in endothelial cells of penile corpus cavernosum after transfection is the direct effect of *EPAC1* or the result of the interaction between *EPAC1* and eNOS after inhibiting RhoA activity. Further study is needed to explore this interaction. The effects of *EPAC1* on ROCK1 and ROCK2 in the RhoA/ROCK pathway and the key downstream molecules (e.g., myosin light chain/phosphorylated myosin light chain) also need further study. This study is applicable to the situation where low androgen levels cause ED while the role of *EPAC1* in penile erection needs further study under other pathological conditions as nerve injury, drugs, psychology, hypertension, diabetes, etc. And whether this study is applicable to humans needs to be further validated.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-23-314/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All experimental procedures were approved by the Animal Experimentation Ethics Committee of the Southwestern Medical University (No. 20211126-001) and followed the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

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