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Short- and long-term effects of silodosin, a selective α_{1A}-adrenoceptor antagonist, on ejaculatory function in rats

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OBJECTIVE

To investigate the short- and long-term effects of silodosin, a selective α_{1A}-adrenoceptor antagonist, on spontaneous seminal emission by isolated rats and on the properties of α_1-adrenoceptor subtypes in the rat seminal vesicle, as silodosin produces a relatively high incidence rate of abnormal ejaculation and chronic administration of receptor antagonists causes an up-regulation in the targeted receptor.

MATERIALS AND METHODS

Rats were treated with two doses (0.1 and 3 mg/kg/day) of silodosin orally for 3 or 30 days. Spontaneous seminal emission was studied during the 3-day observation period before completing treatment. The expression levels of α_{1A}, α_{1B} and α_{1D}-adrenoceptor mRNAs in the rat seminal vesicle and prostate were quantified by real-time reverse transcription-polymerase chain reaction using SYBR Green I.

RESULTS

The administration of two doses of silodosin for 3 or 30 days caused a significant dose-dependent reduction in the number of ejaculatory plugs and in their dry weight. However, in rats receiving the low dose of silodosin the inhibitory effect of the drug on spontaneous seminal emission diminished significantly with chronic usage over time. Although short-term administration of silodosin did not affect expression levels of any α_1-adrenoceptor subtype mRNAs in the rat seminal vesicle and prostate, long-term administration of silodosin caused a significant up-regulation in the mRNA expression of α_{1A}-adrenoceptor in a tissue-dependent manner.

CONCLUSION

Silodosin-induced up-regulation of α_{1A}-adrenoceptor mRNA in the rat seminal vesicle might indicate potential differences in the inhibitory effect of this drug on ejaculatory function with chronic usage over time.

KEYWORDS

silodosin, seminal emission, α_1-adrenoceptor, rat

INTRODUCTION

It is well accepted that prostatic smooth muscle contraction is mediated by the α_{1A}-adrenoceptor subtype [1], and thus several selective α_{1A}-adrenoceptor antagonists that do not affect vascular smooth muscle have been developed. Silodosin, a novel selective α_{1A}-adrenoceptor antagonist, has been shown to have beneficial effects on the symptoms associated with BPH, but minimal effects on blood pressure [2].

However, α_{1A}-adrenoceptors are widely distributed in all the organs participating in the emission phase of ejaculation, e.g. the seminal vesicles and vas deferens [3,4], suggesting that α_{1A}-adrenoceptors might be important in the emission phase. Silodosin produces a relatively high incidence of abnormal ejaculation [2], which might be related to the high α_{1A}-adrenoceptor selectivity of this drug. A recent study showed that ejaculatory dysfunction caused by α_1-adrenoceptor antagonists is not retrograde ejaculation but failure of emission [4].

We previously reported that the long-term administration of doxazosin, a subtype-nonselective α_1-adrenoceptor antagonist, causes a significant up-regulation in the expression levels of α_1-adrenoceptor subtype mRNAs in the rat genitourinary tract [5,6], and suggested that this change in the receptor might be one mechanism to explain the reduction in the effectiveness of α_1-adrenoceptor antagonists with chronic usage over time, as reported by several investigators [7,8]. Given the extensive use of subtype-selective α_{1A}-adrenoceptor antagonists, further studies are needed to determine whether long-term administration of these drugs causes similar changes in the organs participating in ejaculation. Therefore, in the present study we examined the short- and long-term effects of silodosin on spontaneous seminal emission, which occurs daily in rats while isolated from females, and on the expression levels of α_1-adrenoceptor subtype mRNAs in the rat seminal vesicle.

MATERIALS AND METHODS

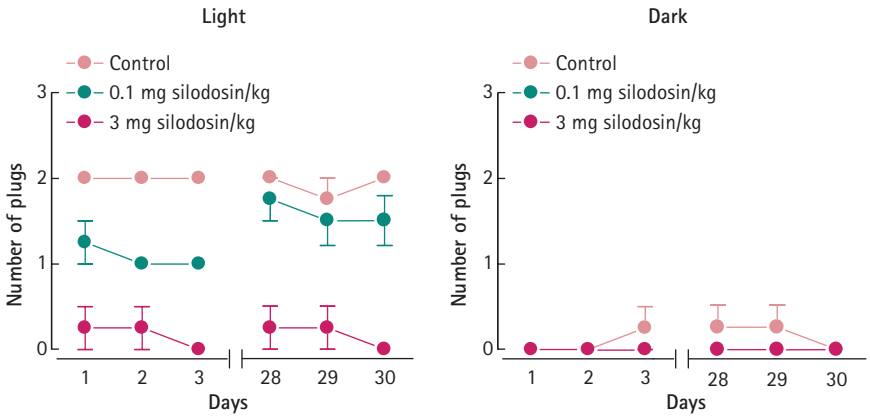
Male Sprague-Dawley rats (10 weeks old) were housed four per cage, except during tests for spontaneous seminal emission, when they were placed individually in wire-

TABLE 1 Sequences of oligonucleotides used as primers

mRNA	Accession no.*	Sequence (5′→3′)	Length†
α_{1A}-Adrenoceptor	NM_017191		
Sense		CGAGTCTACGTAGTAGCC	203
Antisense		GTCTTGGCAGCTTTCTTC	
α_{1B}-Adrenoceptor	NM_016991		
Sense		ATCGTGGCCAAGAGGACC	201
Antisense		TTTGGCTGCTTTCTTTTC	
α_{1D}-Adrenoceptor	NM_024483		
Sense		CGCGTGTACGTGGTCGCAC	219
Antisense		CTTGGCAGCCTTTTTC	
β-Actin	NM_031144		
Sense		AGATGACCCAGATCATGTTTGAGA	86
Antisense		ACCAGAGGCATACAGGGACAA	
GAPDH	NM_017008		
Sense		GCCAGCCTCGTCTCATAGACA	75
Antisense		TGGTAACCAGGCGTCCGATA	

**GenBank accession number of cDNA and corresponding gene, available at http://www.ncbi.nlm.nih.gov/.*
†Amplicon length in base pairs.

FIG. 1. Spontaneous seminal emission during the light and dark phases of the 3-day observation period before completing treatment. Each point is the mean (SEM) for 4 rats.



bottomed cages. Lighting was controlled on a 12-h light/dark cycle. All the animal studies were approved by Institutional Animal Care and Use Committee, Kumamoto University. Silodosin was kindly donated by Kissei Pharmaceutical Company (Matsumoto, Japan). Rats were administered orally with two doses (0.1 and 3 mg/kg/day) of silodosin suspended in a 0.5% methylcellulose aqueous solution for 3 or 30 days. The oral doses of silodosin were chosen at 0.1 and 3 mg/kg/day because they produced dose-dependent inhibition of the phenylephrine-induced increase in intraurethral pressure in rats [9], and no severe effects on the central nervous, cardiovascular, respiratory, or reproductive systems in rats [10]. The single daily dosage schedule was also based on a previous study showing that α_1-adrenoceptor occupancy by silodosin in the rat prostate was maintained at relatively high levels until 24 h later, despite a short elimination half-life of 1.3 h [11]. Control rats were given the vehicle alone.

After completing the treatment, rats were killed by decapitation, and the seminal vesicles and prostates were harvested. The prostates were separated into ventral and dorsolateral portions. The dissected tissues were frozen in liquid nitrogen, and stored at −80 °C until assayed.

To prevent genital autogrooming and consumption of seminal material, plastic corsets covered with cotton gauze were fitted around the thorax and upper abdomen of the rat [12,13]. Coagulated seminal plugs, which dropped through the wire bottoms of the cage onto collecting trays or were found adhering to the penis, were collected during the light and dark phases of the 3-day observation period before completing the treatment. The seminal plugs were counted, dried at room temperature for 7 days and weighed.

Using cDNA prepared by reverse transcription (RT) of RNA extracted from frozen tissues, expression levels of α_{1A}, α_{1B} and α_{1D}-adrenoceptor mRNAs were quantified by real-time RT-PCR using SYBR Green I, as previously described [14]. Sequences of oligonucleotides used as primers for all three α_1-adrenoceptor subtypes, β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are summarized in Table 1. Immediately after the amplification, melt-curve protocols were used to ensure that primer-dimers and other nonspecific products had been minimized or eliminated. RT-PCR data were analysed with the iCycler iQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). In brief, an initial copy number of the target gene was calculated from its standard curve and normalized against an initial copy number of the housekeeping gene, which also was calculated from its standard curve.

One-way ANOVA was used to statistically analyse differences among the groups, and, where differences were found, Scheffé's *F*-test was used to analyse significance, with $P < 0.05$ considered to indicate statistical significance.

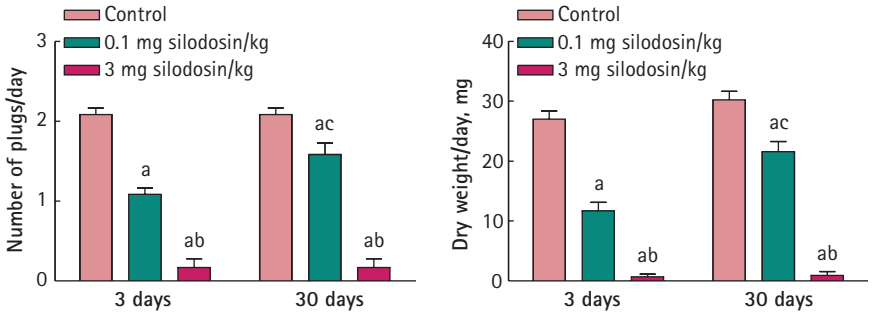
RESULTS

During tests for spontaneous seminal emission, coagulated seminal plugs were collected twice each day, i.e. immediately before the lights were turned on or off. The number of ejaculatory plugs emitted was constant during the 3-day observation period in each group (Fig. 1); most of the seminal plugs were emitted during the light phase (Fig. 1).

The administration of two doses (0.1 and 3 mg/kg/day) of silodosin for 3 or 30 days caused a significant dose-dependent

reduction in the daily mean number of ejaculatory plugs emitted and in their dry weight (Fig. 2). The results of spontaneous seminal emission test in rats that received the low dose of silodosin showed that the number of plugs and their dry weight in 30-day treated rats were significantly higher than those in 3-day treated rats (Fig. 2). However, there was no significant duration-related difference in the number of plugs and their dry weight in rats that received the high dose of silodosin between the 3- and 30-day studies (Fig. 2).

FIG. 2. Daily mean number of ejaculatory plugs emitted and their dry weight during the 3-day observation period before completing treatment. Each bar is the mean (SEM) for 4 rats. [a]P < 0.05 vs control rats within the same treatment duration group; [b]P < 0.05 vs treated rats that received 0.1 mg silodosin/kg within the same treatment duration group; [c]P < 0.05 vs treated rats that received silodosin for 3 days within the same dose group.



The expression levels of β-actin and GAPDH mRNAs had similar distribution profiles in the rat seminal vesicle and prostate. The relative expression of GAPDH mRNA levels normalized against β-actin showed a low variability between the tissues studied (data not shown), suggesting that they are suitable housekeeping genes for normalization.

The relative expression levels of α_{1A}, α_{1B} and α_{1D}-adrenoceptor mRNAs normalized against β-actin in rat tissues are shown in Fig. 3. RT-PCR data indicated that all three α_1-adrenoceptor subtypes were expressed in all tissues studied, and that α_{1A}-adrenoceptor mRNA was the significantly predominant gene transcript in all tissues studied. Although administration of low or high doses of silodosin for 3 days did not affect expression levels of any α_1-adrenoceptor subtype mRNAs in the rat seminal vesicle and prostate, administration of two doses of silodosin for 30 days caused a significant dose-dependent up-regulation in the mRNA expression of α_{1A}-adrenoceptor in the rat seminal vesicle. The results of PCR studies in the rat prostate are different from those in the rat seminal vesicle. In the ventral and dorsolateral portions of the rat prostate administration of the low dose of silodosin for 30 days did not affect expression levels of any α_1-adrenoceptor subtype mRNAs, whereas administration of the high dose of silodosin for 30 days caused an up-regulation in the mRNA expression of α_{1A}-adrenoceptor. Similar expression profiles of α_{1A}, α_{1B} and α_{1D}-adrenoceptor mRNAs in the rat seminal vesicle and prostate were obtained with data normalized against GAPDH (data not shown).

DISCUSSION

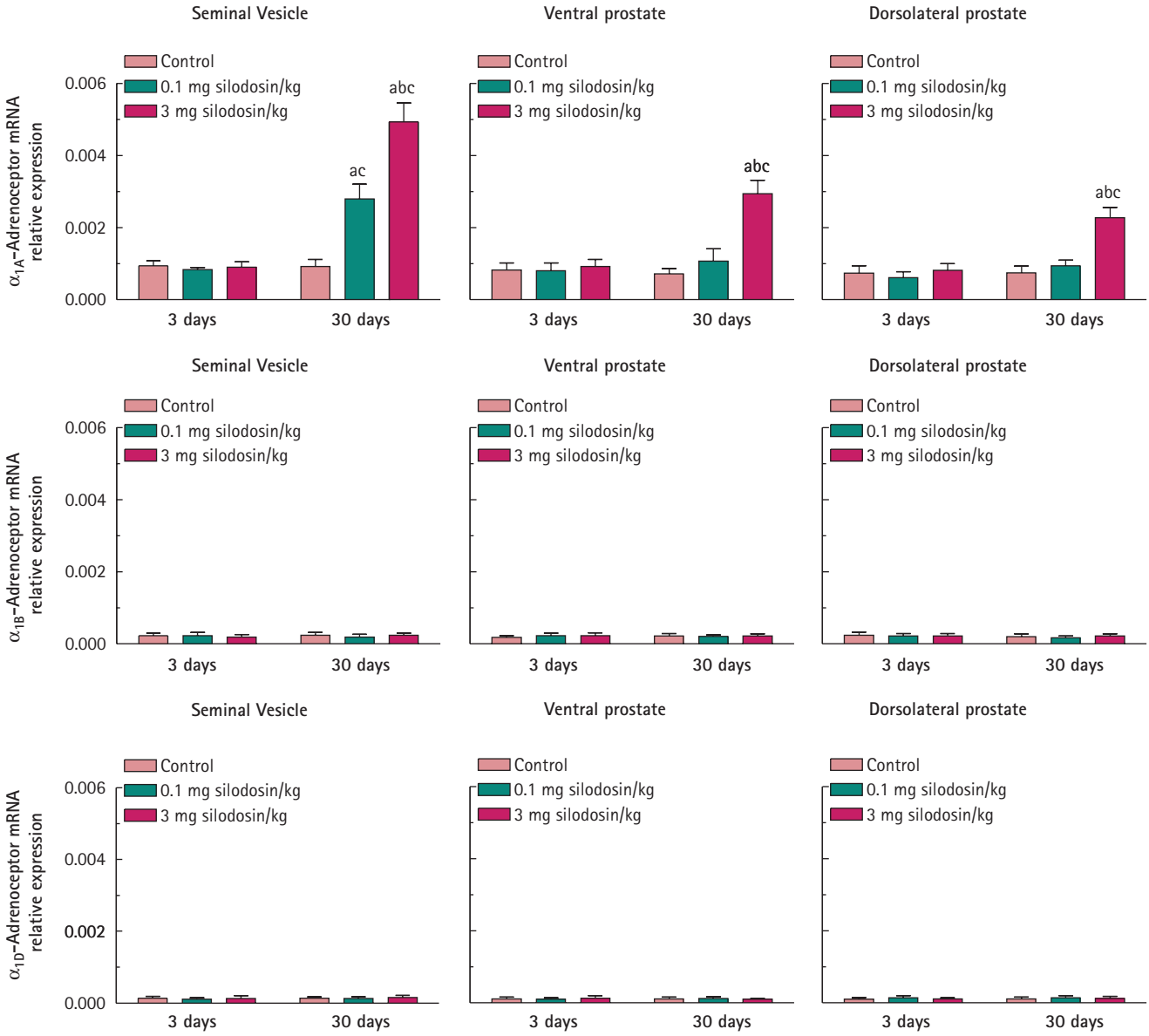
To our knowledge the current study is the first to show that the long-term administration of silodosin causes a significant up-regulation in the mRNA expression of α_{1A}-adrenoceptor in the rat seminal vesicle and prostate in a tissue-dependent manner. These changes in the rat seminal vesicle might account for the reduction in the inhibitory effect of this drug on spontaneous seminal emission with chronic usage over time. If similar receptor changes occur in the human seminal vesicle, the prevalence and incidence of clinically observed abnormal ejaculation with selective α_{1A}-adrenoceptor antagonists might decline over time. This might be one mechanism to explain a low discontinuation rate for abnormal ejaculation with silodosin reported by several investigators [2].

In the human seminal vesicle, the α_{1A}-adrenoceptor is the predominant subtype expressed [4], suggesting that α_{1A}-adrenoceptor-mediated contractions of the seminal vesicle might be important in the emission phase of ejaculation. Thus, antagonists with selectivity for α_{1A}-adrenoceptors such as silodosin and tamsulosin might affect this first phase of ejaculation. Additional effects of these drugs on the serotonergic and dopaminergic brain receptors, both of which are involved in the central control of ejaculation, have been proposed [15,16]. However, it is unlikely that the abnormal ejaculation associated with silodosin and tamsulosin is due to CNS effects, because these drugs have a low potential to cross the blood–brain barrier (BBB) [11,17]. On the other hand, ageing of the cerebral microcirculation results in significant alteration in the BBB [18]. Thus, in elderly patients silodosin and tamsulosin might produce an occupancy of not only α_{1A}-adrenoceptors but also these nonadrenergic brain receptors. At present it is not known whether changes in the BBB contribute to the differences in the response to α_1-adrenoceptor antagonists with ageing.

A recent study [19] showed that α_{1A}-adrenoceptor 'knockout' mice have a somewhat, and $\alpha_{1A/B/D}$-adrenoceptor triple 'knockout' mice a markedly, reduced fertility rate with no abnormality in sperm function. This suggests that the α_{1B} and α_{1D} subtype, as well as α_{1A} subtype, might contribute to ejaculation in mice. However, in humans the pharmacological blockade of all three α_1-adrenoceptor subtypes via oral antagonists such as doxazosin or terazosin fails to cause abnormal ejaculation [20], which could be due to their low occupancy of α_{1A}-adrenoceptors in the seminal vesicle. Silodosin, which produces fairly selective and sustained occupancy of α_{1A}-adrenoceptors [11], might inhibit the α_{1A}-adrenoceptor-mediated contractions of the seminal vesicle more potently than the α_{1A}- and α_{1D}-adrenoceptor-mediated prostate and urethral contractions.

The oral doses of silodosin produced dose-dependent inhibition of the phenylephrine-induced increase in intraurethral pressure in rats, and the doses to produce 50% inhibition of this response were 0.011 and 0.057 mg/kg at 4 and 12 h after its oral administration, respectively [9,21]. Thus, our data obtained with rats that received the low dose (0.1 mg/kg/day) of silodosin might have clinical significance. In the present study, at the low dose of silodosin, the inhibitory effect of this drug on spontaneous seminal emission diminished significantly with chronic usage

FIG. 3. Relative expression of α_{1A}, α_{1B} and α_{1D}-adrenoceptor mRNA in the rat seminal vesicle and prostate, normalized against β-actin. Each bar is the mean (SEM) for 4 rats. [a]P < 0.05 vs control rats within the same treatment duration group; [b]P < 0.05 vs treated rats that received 0.1 mg silodosin/kg within the same treatment duration group; [c]P < 0.05 vs treated rats that received silodosin for 3 days within the same dose group.



over time. This contention is further supported by a 3-year clinical trial of tamsulosin, a selective $\alpha_{1A/D}$-adrenoceptor antagonist, in which abnormal ejaculation occurred during the beginning of treatment with hardly any new cases thereafter, and its prevalence appeared to decline over time [22].

The present RT-PCR data showed that long-term administration of the low dose of silodosin caused a significant up-regulation in the mRNA expression of α_{1A}-adrenoceptor in the rat seminal vesicle, but not in the prostate. These findings indicate that α_{1A}-adrenoceptor mRNA in the rat seminal vesicle might be more susceptible to chronic silodosin treatment than those in the rat prostate. Because long-term administration of receptor antagonists causes an up-regulation in the targeted receptor [5,6], it is conceivable that chronic treatment with α_1-adrenoceptor antagonists might lead to a reduced therapeutic response to these drugs over time in patients with BPH. However, in the rat prostate the high dose of silodosin was required to induce up-regulation of α_{1A}-adrenoceptor mRNA. Thus, it is suggested that these effects of silodosin on prostatic α_1-adrenoceptors at the molecular level are of no clinical relevance. This corresponds with clinical trial data showing that the efficacy of silodosin for treating LUTS associated with BPH was sustained for 1 year [23].

There are some reports that evaluated the long-term effectiveness of α_1-adrenoceptor

antagonists. In a 3-year clinical trial of tamsulosin, only 9% of patients discontinued due to insufficient therapeutic response in the first year, whereas the discontinuation rate for this reason was 27% after 3 years and gradually increased with time [22]. In another study of the long-term effects of three α_1-adrenoceptor antagonists, the proportion of patients in whom α_1-adrenoceptor antagonist treatment ultimately failed and who required re-treatment was 38% after 3 and 54% after 5 years of follow-up [24]. However, the clinical efficacy of silodosin for the first year is not disputed [23], whereas little is known about the efficacy in the longer term (>3 years). Thus, further investigations are needed to conclusively evaluate the mechanism of action and ability to maintain long-term effectiveness.

In conclusion, our data showed that long-term administration of the low dose of silodosin caused a significant up-regulation of α_{1A}-adrenoceptor mRNA in the rat seminal vesicle, but not in the prostate, which might account for the reduction in the inhibitory effect of this drug on ejaculatory function with chronic usage over time, and the ability to maintain long-term effectiveness. These findings might provide a new insight into the efficacy and adverse effects of long-term administration of selective α_{1A}-adrenoceptor antagonists when they are used to treat LUTS secondary to BPH.

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CONFLICT OF INTEREST

None declared.

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Abbreviations: **RT**, reverse transcription; **GAPDH**, glyceraldehyde-3-phosphate dehydrogenase; **BBB**, blood–brain barrier.