Effects of ZD6169, a $K_{\text{ATP}}$ Channel Opener, on the Micturition Reflex in the Rat

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ABSTRACT
The effects of ZD6169, a new ATP-sensitive potassium channel opener, on reflex urinary bladder activity were evaluated in urethane-anesthetized female Wistar rats. Continuous transvesical slow infusion cystometrograms (0.04 ml/min) were performed in untreated, capsaicin-pretreated (125 mg/kg s.c., 4 days before experiments) and capsaicin vehicle-pretreated rats. Intravesical infusion of ZD6169 in concentrations of 6, 15, 30, and 300 nM for 2 h at each concentration increased the intercontraction interval and pressure threshold for voiding in a concentration-dependent manner in untreated and vehicle-pretreated rats but not in capsaicin-pretreated animals. The effects appeared within 30 min after administration. ZD6169 did not alter baseline bladder pressure, duration of contractions, or the peak pressure during voiding. Glibenclamide (20 mg/kg i.v.) reversed the effects of ZD6169 (30 nM). During transvesical cystometrograms performed at a fast rate (0.21 ml/min), ZD6169 in concentrations between 6 and 300 nM did not alter the intercontraction interval or pressure threshold for voiding. ZD6169 produced smaller and more variable effects during slow transurethral cystometrograms. Capsaicin, a C-fiber neurotoxin, administered s.c. 4 days before the experiment, produced similar changes and also eliminated the effect of ZD6169. These data suggest that ZD6169 increases the threshold for activation of C-fiber mechanoreceptors in the bladder wall and thereby increases the bladder volume for inducing reflex voiding.

The functions of the lower urinary tract to store and periodically release urine are regulated by neural circuits in the brain and spinal cord (de Groat et al., 1993). In infants these circuits are activated in a reflex manner by mechanoreceptive afferents that respond to bladder distension. In older children and adults, reflex voiding is suppressed and lower urinary tract function is subject to voluntary control mediated by the cerebral cortex (Torrens and Morrison, 1987; de Groat et al., 1993). Disruption of cortical control mechanisms by injury or diseases of the nervous system can lead to the reemergence of involuntary voiding and incontinence in adults (Torrens and Morrison, 1987; Wein, 1992).

Urinary incontinence is treated with various drugs (Wein, 1992; Andersson, 1993), including agents that: 1) directly suppress the bladder smooth muscle; 2) block cholinergic excitatory input to the bladder, e.g., atropine-like drugs; 3) act centrally or peripherally to enhance monoaminergic inhibitory control of the bladder, e.g., imipramine; and (4) act on the afferent limb of the micturition reflex, e.g., capsaicin. Unfortunately, the therapeutic effectiveness of these agents is limited by side effects, by the necessity of using an intravesical route of administration, or because they suppress voluntary as well as involuntary voiding. An ideal drug for the treatment of incontinence would be one that acted selectively on the urinary bladder to promote urine storage, increase bladder capacity, and suppress involuntary voiding without influencing the amplitude of bladder contractions or the efficiency of bladder emptying during voluntary micturition.

Bladder afferent pathways have attracted attention recently as an important target for the pharmacological treatment of bladder hyperreflexia and incontinence (Fowler et al., 1992, 1994; Yoshimura and de Groat, 1997). Experiments in animals as well as clinical studies in patients with neurogenic bladder disorders indicate that certain types of bladder hyperactivity and incontinence are triggered by unmyelinated (C-fiber) bladder afferents (de Groat et al., 1990, 1993; Fowler et al., 1992; Maggi, 1993), whereas normal sensations of bladder filling are carried by Aδ afferent pathways (Torrens and Morrison, 1987; de Groat et al., 1993). Desensitization of C-fiber bladder afferents by intravesical administration of capsaicin, a C-fiber neurotoxin, has been used to treat bladder hyperactivity in patients with multiple sclerosis (Fowler et al., 1992, 1994) and spinal cord injury (Geirsson et al., 1994, 1995; de Ridder et al., 1996; Sedor et al., 1996; Cruz et al., 1997; de Sèze et al., 1998) and also to reduce pain and frequency of voiding in patients with interstitial cystitis (Maggi et al., 1989; Barbanti et al., 1993).

ABBREVIATIONS: ICI, intercontraction interval; CT, contraction time.
Other agents that are potentially useful for the treatment of incontinence are the K<sub>ATP</sub> channel openers, which exert a direct depressant effect on the bladder smooth muscle (Andersson, 1992). Two of these agents, pinacidil and cromakalim, have been shown to suppress the activity of normal and hyperactive bladder smooth muscle following systemic or intravesical administration (Malmgren et al., 1990; Nurse et al., 1991). However, these drugs also produce cardiovascular side effects due to actions on vascular smooth muscle (Donnelly et al., 1990). On the other hand, a new K<sub>ATP</sub> channel opener, ZD6169, administered orally to rats and dogs, selectively increases bladder capacity without altering blood pressure (Howe et al., 1995). ZD6169 in high concentrations directly suppresses bladder smooth muscle in vitro (Li et al., 1995; Chun et al., 1996), However, low doses of ZD6169 administered orally selectively increase bladder capacity in vivo without changing the amplitude of reflex bladder contractions (Howe et al., 1995). This suggests that the drug may alter the threshold for the micturition reflex by increasing the compliance of the bladder or by acting on the nervous system either at the level of the peripheral afferent receptors or in the spinal cord and brain. Capsaicin, the afferent neurotransmitter, elicits a similar increase in bladder capacity without altering the amplitude of bladder contractions (Maggi et al., 1986; Maggi, 1993). An effect of ZD6169 on bladder afferents is also suggested by recent experiments (Pandita et al., 1997) in which orally or intra-arterially administered ZD6169 blocked the bladder hyperactivity induced by intravesical administration of prostaglandin <i>E</i><sub>2</sub>, an agent known to sensitize afferent receptors. ZD6169 also reduced the immediate early gene expression (c-fos) in spinal neurons evoked by intravesical infusion of acetic acid, an irritant substance (Yu et al., 1996; Yu and de Groat, 1998). Capsaicin pretreatment had a similar effect (Birder and de Groat, 1998; Yu and de Groat, 1998).

The present study was undertaken to evaluate the site and mechanism by which ZD6169 alters reflex bladder activity in the urethane-anesthetized rat. The results indicate that ZD6169 administered intravesically in low concentrations increases the intravesical pressure threshold for triggering voiding and lowers the frequency of voiding during constant infusion cystometrograms, but does not alter the amplitude of bladder contractions. Capsaicin administered s.c. 4 days before the experiment produced similar changes and also eliminated the effect of ZD6169. The effects of ZD6169 may be mediated by a depressant action on capsaicin-sensitive mechanoreceptive afferents in the bladder wall. Preliminary reports have appeared in an abstract (Yu and de Groat, 1996).

Materials and Methods

**Animal Preparation.** Experiments were conducted on 68 Wistar adult female rats (250–350 g) anesthetized with urethane (1.2 g/kg s.c.). The trachea was cannulated with a polyethylene tube (PE-205) to facilitate respiration. Body temperature was maintained in the physiological range using a heating lamp.

The bladder was exposed by a midline abdominal incision and intravesical pressure was recorded via a catheter passed either through the urethra (PE-50 tubing) or inserted through a small incision in the fundus of the bladder (PE-50 tubing or a 25-gauge needle). The catheter was connected via a three-way stopcock to a pressure transducer and to a syringe pump used for infusing fluid into the bladder. The recording system was filled with physiological saline solution. After emptying the bladder with gentle manual pressure, a continuous cystometrogram was performed by filling the bladder with a constant infusion of saline. After a 2-h control period, the effects of increasing concentrations of ZD6169 (6, 15, 30, and 300 nM) in saline solution on bladder activity were studied during constant intravesical infusion for 2 h at each concentration under three conditions: 1) slow infusion rate (0.04 ml/min) in untreated animals, 2) fast infusion rate (0.21 ml/min) in untreated animals, and 3) slow infusion rate (0.04 ml/min) in animals pretreated with capsaicin or vehicle 4 days before the experiments. During a micturition reflex the fluid was eliminated through the urethra. The following cystometric parameters were measured: 1) basal pressure (the lowest bladder pressure during filling), 2) pressure threshold (PT, the difference between basal pressure and the pressure immediately before micturition), 3) amplitude of bladder contractions (the difference between basal pressure and peak intravesical pressure during voiding), 4) bladder compliance (volume of infusion fluid during each voiding cycle/pressure threshold minus basal pressure), 5) intercontraction interval (ICI, time between each voiding episode), and 6) contraction time (CT, the duration of each bladder contraction measured at the base of the contraction). Glibenclamide, a K<sub>ATP</sub> channel blocker, was studied in a separate group of rats. In these animals saline was infused intravesically at a slow rate for 2 h, then ZD6169 (30 nM) was infused for another 2 h, after which glibenclamide (20 mg/kg) was administered i.v.

In some rats, capsaicin dissolved in a vehicle containing 10% ethanol, 10% Tween 80, and 80% physiological saline, at a concentration of 20 mg/ml, was administered s.c. in divided doses on 2 consecutive days (total dose 125 mg/kg) starting 6 days before the experiments. This dose of capsaicin can elicit a maximal depletion of Substance P in dorsal root ganglia and markedly reduces Substance P content in the urinary bladder of the rat (Game et al., 1981; Maggi et al., 1987). Control animals received the same volume of vehicle. All injections were conducted under halothane anesthesia. To evaluate the efficiency of capsaicin treatment an eye wipe test was performed in each unanesthetized animal just before the experiment. A drop of 100 μg/ml capsaicin was placed in the eye and the number of defensive forelimb wiping movements was counted (Cheng et al., 1993).

**Statistical Analysis.** All data are expressed as mean ± S.E. Statistical analyses were performed using Student’s <i>t</i> test for paired or unpaired data or ANOVA where applicable.

**Drugs.** The following drugs were obtained from the indicated sources: urethane (Sigma Chemical Co., St. Louis, MO), halothane (Ayerst Labs, Inc., Philadelphia, PA), capsaicin (Sigma), ZD6169 (Zeneca Corporation, Wilmington, DE), and glibenclamide (Research Biochemicals Inc., Natick, MA). ZD6169 was first dissolved in methanol, which was then evaporated under a nitrogen stream. The resulting residue was redissolved in saline.

**Results**

**Effects of ZD6169 on Bladder Activity during Slow Infusion Cystometrograms.** Bladder activity was recorded during slow continuous infusion cystometrograms in 41 female rats with the urethral outlet open to allow voiding (Fig. 1). In 19 animals, saline was infused via a needle inserted into the dome of the bladder (transvesical recording); in 15 animals, infusion was performed via a catheter inserted through the urethra (transurethral recording). Control experiments (<i>n</i> = 5) for transvesical recording were performed by infusing saline into the bladders of untreated rats for 10 h. As shown in Fig. 2, the frequency of bladder contractions remained relatively constant during the experiment. The average frequency measured over the period of 4 to 10 h after the start of the infusion was 11.5 ± 0.7 h, which yields a
calculated voiding volume of 0.21 ml. Assuming a voiding efficiency of 50% in urethane-anesthetized rats (Yoshiyama et al., 1993), this yields a bladder capacity or micturition volume threshold of 0.4 ml, which is similar to the volume threshold reported in other experiments (Yoshiyama et al., 1993). Because the ICI gradually increased in some experiments for several hours after the start of the cystometrograms, a 2-h control period of saline infusion preceded the administration of ZD6169 in all experiments. Bladder activity during the second hour after each concentration of ZD6169 was compared with the activity during the initial 2-h control period in each animal. The ICI and other parameters measured during the 2-h period before drug administration were not significantly different from those observed in the control experiments.

After 2 h of saline infusion, ZD6169 solutions in increasing concentrations (6–300 nM) were infused (2 h at each concentration) to construct a cumulative concentration-response curve. Figure 1 shows the result from one experiment. In this animal, the average peak intravesical pressure during voiding (37.2 cm of H₂O), pressure threshold for evoking micturition (10.9 cm H₂O), ICI (12.2 min), CT (0.5 min), and bladder compliance (0.04 ml/cm of H₂O) during the 2-h control period were relatively consistent. After administration of ZD6169, both the pressure threshold and the ICI were increased in a concentration-dependent manner (range of concentrations, 6–300 nM). On the other hand, bladder CT and peak intravesical pressure did not change. The most prominent effect of ZD6169 was on the ICI which on average (n = 19) increased 32.6 to 89.2% at ZD6169 concentrations between 6 and 300 nM (Fig. 3). Pressure threshold for inducing voiding increased 43.7 ± 6.5, 78.0 ± 12.1, 82.0 ± 10.7, and 82.0 ± 14.6% at ZD6169 concentrations of 6, 15, 30, and 300 nM, respectively. To determine whether the increases in ICI induced by ZD6169 were due to the opening of K_ATP channels, a potent blocker of K_ATP channels, glibenclamide (20 mg/kg), was injected i.v. after infusing ZD6169 (30 nM) intravesically for 2 h (n = 6). ZD6169 increased the ICI by 45.5 ± 18.8% and glibenclamide reversed (P < .05) the effect of ZD6169 (41 ± 5.1% decrease in ICI). Glibenclamide administered to untreated animals (n = 3) did not significantly change the ICI.

In 15 animals, bladder activity was also recorded during continuous transurethral cystometrograms. The ZD6169...
changes in ICI were smaller than in the transvesical infusion experiments and were not concentration-dependent (Fig. 4). Small but significant increases in ICI were noted at concentrations of 30 and 3000 nM but not at 15 and 300 nM (Fig. 4). ZD6169 did not change the peak intravesical pressure or CT.

**Effects of ZD6169 on Bladder Activity during Fast Infusion Cystometrograms.** In six rats, bladder activity was recorded during transvesical infusion at a rate of 0.21 ml/min. Stable ICIs occurred within approximately 30 min at the fast infusion rate in contrast to the slow stabilization (2 h) of bladder activity at slow infusion rates. ZD6169 in a range of concentrations (6–300 nM) did not produce a statistically significant change in ICI (Fig. 5). None of the other cystometrogram parameters were changed by the drug.

**Effects of ZD6169 in Animals Treated with Capsaicin.** To confirm the effectiveness of capsaicin pretreatment, an eye-wipe test (Cheng et al., 1993) was performed in each animal before anesthesia. The number of wiping responses to instillation of one drop of 100 μg/ml capsaicin solution in the eye was 20 ± 4 per 2 min (n = 7) in vehicle-pretreated animals, whereas no wiping responses were detected in capsaicin-pretreated animals (n = 7). In rats pretreated with capsaicin the ICI was considerably longer (20.6 ± 4.8 min) than that observed in vehicle-pretreated animals (9.8 ± 1.8 min) at the same infusion rate (0.04 ml/min; Table 1). The PT in capsaicin-pretreated animals was also higher (12.5 ± 1.3 cm of H2O) than that in vehicle-pretreated animals (7.7 ± 1.6 cm of H2O; Table 1). The peak pressure and CT were not altered by capsaicin pretreatment.

ZD6169 in a range of concentrations (6–300 nM) did not significantly change ICI or PT in capsaicin-pretreated animals, but did alter these parameters in vehicle-pretreated animals (Fig. 6, Table 1). The effects of ZD6169 on ICI and PT were significantly (P < .001) different in capsaicin- and vehicle-pretreated groups (Fig. 6).

**Discussion**

The results of the present study show that transvesical infusion of ZD6169, a new KATP channel opener, can increase bladder capacity and the intravesical pressure threshold for eliciting micturition without changing the amplitude or duration of bladder contractions during voiding. These effects were similar to the responses induced by pretreatment with the C-fiber neurotoxin, capsaicin, and did not occur in capsaicin-pretreated animals. We conclude that ZD6169 acts at some site in the bladder wall to alter the firing of mechano-receptive afferents that trigger reflex voiding.

Previous experiments (Howe et al., 1995) revealed that low oral doses of ZD6169 in the rat and dog increased bladder capacity without changing the amplitude of bladder contractions. The effect of ZD6169 was blocked by glibenclamide, indicating that it was mediated by activation of KATP channels. Although orally administered, ZD6169 could, in theory, change voiding function by acting at various sites including the bladder smooth muscle, as well as in the peripheral and central nervous system. A direct relaxation of the detrusor smooth muscle was suggested (Howe et al., 1995) as a likely mechanism, because high concentrations of the drug can depress the contractions of bladder muscle strips in vitro (Li et al., 1995; Chun et al., 1996) and can increase bladder compliance in the dog in vivo (Howe et al., 1995).

The present experiments provide evidence that ZD6169 acts directly on the bladder in vivo, because the amounts of drug infused into the bladder at the lowest effective concentration (6 nM), even if completely absorbed into the body, would be insufficient to elicit changes in voiding function. For example, it was reported previously (Howe et al., 1995) that the threshold dose of ZD6169 administered orally was 50 μg/kg, whereas in the present experiments the total amount infused intravesically at threshold concentrations was 35 ng/kg, a more than 1000-fold lower amount. This difference is probably even greater because a large percentage of the intravesically administered drug must have been eliminated from the body in the voided fluid. Thus, it seems reasonable to conclude that intravesically administered ZD6169 acts locally in the bladder rather than at some distant site in the peripheral or central nervous system.

It seems unlikely that ZD6169 acts within the bladder on efferent cholinergic pathways or directly on the bladder smooth muscle to change the ICI or bladder capacity, because the drug, even in the highest concentrations, did not reduce the amplitude of the bladder contractions. High concentrations (1 μM) of the drug applied in vitro to bladder muscle strips decrease contraction amplitude (Li et al., 1995); however, these concentrations far exceeded those shown to be effective via intravesical application. Thus, it seems likely
This change, coupled with the increase in PT, indicates (90% after exposure to the maximal concentration of ZD6169. It should increase by 30% when bladder volume increases by 50%. In vehicle-pretreated animals that was significantly different from the V-control (125 mg/kg s.c. 4 days before experiments) or vehicle-pretreated (V-control) but not in capsaicin-pretreated animals. ZD6169 increased PT and ICI in vehicle and untreated animals, but not in capsaicin-pretreated animals (*P < .05, **P < .01). ICI and PT were significantly increased in capsaicin-pretreated animals (V-pretreated) and vehicle-pretreated animals (V-control).

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The similarity in the effects of ZD6169 and capsaicin (Maggi et al., 1986; Maggi, 1993) on voiding function in the rat also supports the view that ZD6169 acts on theafferent limb of the micturition reflex. Capsaicin, a neurotoxin, acts by desensitizing or destroying C-fiber afferents and increases the bladder volume threshold and PT for inducing micturition but does not decrease the amplitude of reflex bladder contractions (Maggi et al., 1986; Maggi, 1993). This suggests that the sensitivity of the mechanoreceptors that initiate micturition was markedly reduced by ZD6169.

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The similarity in the effects of ZD6169 and capsaicin (Maggi et al., 1986; Maggi, 1993) on voiding function in the rat also supports the view that ZD6169 acts on the afferent limb of the micturition reflex. Capsaicin, a neurotoxin, acts by desensitizing or destroying C-fiber afferents and increases the bladder volume threshold and PT for inducing micturition but does not decrease the amplitude of reflex bladder contractions (Maggi et al., 1986; Maggi, 1993). Other similarities between ZD6169 and capsaicin are that both agents have less effect when tested during fast infusion (0.21 ml/min) compared with slow infusion (0.04 ml/min) cystometrograms (Maggi, 1993). They are also less effective when tested using transurethral versus transvesical cystometrograms (Maggi, 1993). This suggests that capsaicin-sensitive C-fiber afferents have a minor role in triggering micturition under abnormal conditions where the bladder is distended rapidly or where a catheter has been inserted through the urethra. Urethral catheters can produce irritative responses (Birder and de Groat, 1992), which may facilitate voiding reflexes and mask the contribution of bladder C-fibers to the initiation of the micturition reflex.

In capsaicin-pretreated animals, ZD6169 did not alter the ICI or PT. This observation provides additional support for the view that ZD6169 acts on capsaicin-sensitive afferents. It seems reasonable to assume that if the contribution of C-fiber afferents to the initiation of micturition is eliminated by capsaicin, then ZD6169 would have no effect. If the drug acted directly on the bladder smooth muscle to decrease contractility and increase compliance then it should still have an effect in capsaicin-treated animals. Because it is likely that Aδ afferents trigger micturition in capsaicin-treated animals (Maggi et al., 1986; Cheng et al., 1993), the failure of ZD6169 to alter voiding in these preparations indicates that ZD6169 does not alter the sensitivity of Aδ afferents and, therefore, must act selectively on a mechano-sensitive (Sengupta and Gebhart, 1994) C-fiber afferent population.

Because glibenclamide blocked the effects of ZD6169 to increase bladder capacity (present study and Howe et al., 1995), it is likely that opening of KATP channels is responsi-
ble for the putative suppression of mechanoreceptor afferent excitability and for the increased threshold for afferent firing. K$_{ATP}$ channels have been identified in neurons (Ashford et al., 1988) and at afferent nerve terminals (Lee et al., 1995), where they function to suppress transmitter release. However, to our knowledge K$_{ATP}$ channels have not been identified in mammalian sensory neurons.

Because K$_{ATP}$ channels are present in bladder smooth muscle, a selective effect of intravesical ZD6169 on afferent receptors without an effect on the smooth muscle is rather unexpected. This suggests either that the channels in the two types of cells have different affinities for ZD6169, the neural channels being much more sensitive, or that the drug has greater access to the afferent nerves. C-fiber afferent terminals are located in the mucosa adjacent to the urothelial surface (Alm et al., 1995) and therefore are likely to be more accessible and subjected to higher concentrations of the drug than the smooth muscle layer, which lies deeper in the bladder wall. A differential sensitivity of the two targets is also possible because in isolated bladder strips, the concentration of ZD6169 required to depress neurally evoked bladder contractions is considerably higher (Li et al., 1995) than the intravesical concentration necessary to change ICI. Therefore, the mechanism in the bladder that controls ICI appears to be considerably more sensitive to ZD6169 than are the mechanisms that mediate neurally evoked contractions of the smooth muscle.

Because ZD6169 evokes changes in reflex bladder activity similar to the delayed depressant effects elicited by intravesical administration of capsaicin, it may provide a new approach for the treatment of neurogenic bladder conditions, such as detrusor hyperreflexia, in multiple sclerosis and spinal cord injury patients (Fowler et al., 1994; Geirsson et al., 1994, 1995; Das et al., 1996; de Ridder et al., 1996; Sedor et al., 1996; Cruz et al., 1997; de Sèze et al., 1998), as well as for the treatment of hypersensitive bladder symptoms in interstitial cystitis (Barbanti et al., 1993; Cruz et al., 1997). In these disorders, intravesical injection of capsaicin has produced beneficial effects. The obvious advantage of ZD6169 over capsaicin is that it can be administered orally rather than intravesically. If ZD6169 affects C-fiber afferent excitability in the bladder, this raises the possibility that other C-fiber afferents involved in various functions throughout the body may be modulated by drugs that activate K$_{ATP}$ channels. Thus, ZD6169 and other K$_{ATP}$ channel openers might be useful in the treatment of conditions in which these afferents are activated to produce pain, inflammation, and reflex responses (e.g., chronic cough; Andersson, 1992).

References


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