Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 512 (2005) 61–66

www.elsevier.com/locate/ejphar

Effects of solifenacin succinate (YM905) on detrusor overactivity in conscious cerebral infarcted rats

Masanori Suzuki*, Akiyoshi Ohtake, Taiji Yoshino, Hironori Yuyama, Aska Hayashi, Masashi Ukai, Hiroko Okutsu, Yukiko Noguchi, Shuichi Sato, Masao Sasamata

Applied Pharmacology Research, Pharmacology Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

Received 4 November 2004; received in revised form 11 February 2005; accepted 18 February 2005
Available online 17 March 2005

Abstract

Solifenacin succinate [YM905, (+)-(1*S*,3′*R*)-quinuclidin-3′-yl 1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate monosuccinate] is a novel muscarinic receptor antagonist. We examined the effects of solifenacin and two other muscarinic receptor antagonists, tolterodine and propiverine, on detrusor overactivity in cerebral infarcted rats. Evaluation was done under conscious conditions using cystometry 1 day after middle cerebral artery occlusion. The cerebral infarcted rats showed decreases in bladder capacity and voided volume and an increase in residual volume, but no change in micturition pressure. Solifenacin increased bladder capacity and voided volume at doses of 0.03 mg/kg i.v. or more. Tolterodine increased bladder capacity and voided volume at 0.03 and 0.1 mg/kg i.v., while propiverine increased bladder capacity and voided volume at 1 mg/kg i.v. and at 0.3 and 1 mg/kg i.v., respectively. In contrast, none of the three drugs affected residual volume or micturition pressure. These results suggest that solifenacin may improve detrusor overactivity without causing urinary retention and may be a promising drug in the treatment of patients with overactive bladder syndrome.

Keywords: Solifenacin; Muscarinic receptor antagonist; Detrusor overactivity; Cerebral infarction

1. Introduction

The apparently contradictory functions of the bladder, broadly divided into the storage and excretion of urine, are under both sympathetic and parasympathetic nervous system control (Andersson, 1993). The best known of the neurotransmitters involved in micturition is acetylcholine, which is released from parasympathetic nerve endings. Five muscarinic receptor subtypes (M_1–M_5) have been identified by both molecular biological and pharmacological investigations (Caulfield and Birdsall, 1998), of which the muscarinic M_2 and M_3 receptor subtypes are well known to be located postsynaptically on detrusor smooth muscle (Wang et al., 1995; Eglen et al., 1994, 1996; Ehlert et al., 1997). Muscarinic M_3 receptors are a minority population in this tissue, but have been shown to play a predominant role in mediating detrusor smooth muscle contraction (Eglen et al., 1994, 1996; Ehlert et al., 1997). The treatment of patients with overactive bladder syndrome symptoms such as urgency, frequency and incontinence therefore generally involves the use of antimuscarinic drugs, primarily oxybutynin (Madersbacher et al., 1999), tolterodine (Hegde et al., 2004) and propiverine (Madersbacher et al., 1999).

Solifenacin succinate [YM905, (+)-(1*S*,3′*R*)-quinuclidin-3′-yl 1-phenyl-1,2,3,4-tetrahydroisoquinoline-2-carboxylate monosuccinate] is a newly synthesized muscarinic receptor antagonist with affinity for the muscarinic M_3 receptor. Solifenacin shows inhibitory effects on muscarinic M_3 receptor-mediated intracellular Ca^{2+} mobilization in bladder smooth muscle cells isolated from guinea pigs (Ikeda et al., 2002), rats (Ohtake et al., 2004) and cynomolgus monkeys (Kobayashi et al., 2004), and on contraction in rat bladder strips (Ikeda et al., 2002). Further, in vivo studies indicate that solifenacin inhibits carbachol-induced intravesical

* Corresponding author. Tel.: +81 298 63 6637; fax: +81 298 54 1616.
E-mail address: suzuki_m@yamanouchi.co.jp (M. Suzuki).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.02.023

pressure elevation with greater selectivity for urinary bladder over salivary gland in rats (Ikeda et al., 2002; Ohtake et al., 2004). This compound is therefore expected to serve as a therapeutic drug in the treatment of overactive bladder syndrome.

Urinary frequency and incontinence are often observed in patients with cerebral infarction (Tsuchida et al., 1983; Griffiths et al., 1994; Khan et al., 1990). Neuronal damage in the forebrain can induce detrusor overactivity, which is probably attributable to impairment of the inhibitory neuronal system toward the pontine micturition center in the brainstem. From a rat model of detrusor overactivity induced by middle cerebral artery occlusion (Ishiura, 1996), this overactivity has been shown to involve glutamate, dopamine (Yokoyama et al., 1999, 2002) and γ-aminobutyric acid (GABA) receptors (Kanie et al., 2000) in the central nervous system. It has also been demonstrated that antimuscarinic drugs ameliorate this detrusor overactivity (Ishiura, 1996; Nakada et al., 2000). In the present study, we evaluated the effects of a new muscarinic receptor antagonist, solifenacin, on detrusor overactivity in the cerebral infarcted rat model.

2. Materials and methods

2.1. Middle cerebral artery occlusion

Male Sprague–Dawley rats (270–320 g; Charles River, Kanagawa, Japan) were housed in cages with free access to food and water until use. After anesthesia with pentobarbital sodium (50 mg/kg i.p.), the bladder was exposed via a midline incision in the abdomen and a polyethylene catheter (PE-50, Becton Dickinson and Co., Sparks, MD, USA) with a collar for cystometry was inserted through its superior aspect and fixed in place. In the same session, a polyethylene catheter (PE-50) for drug administration was inserted into the common jugular vein, which was exposed via a midline incision in the neck. Two or three days later, cerebral ischemia was induced according to the intraluminal suture occlusion method described by Longa et al. (1989). Briefly, the rat was anesthetized with 0.5–1% halothane (Fluothane®, Takeda Pharmaceuticals, Osaka, Japan) in a gas mixture (70% N_2O+30% O_2) and a midline incision was made in the neck. The external and common carotid arteries were ligated, and the occipital and pterygopalatine arteries were cauterized with a bipolar coagulator (T-45, Keisei Medical, Tokyo, Japan). A 3–0 nylon monofilament suture (Nicho Kogyo, Tokyo, Japan) with its tip rounded by heating near a flame was introduced through the external carotid artery into the internal carotid artery and advanced approximately 17 mm intracranially from the common carotid artery bifurcation to block the origin of the middle cerebral artery. When the intraluminal suture occlusion procedure was finished, anesthesia was discontinued and the animal was allowed to waken. Sham-operated rats underwent midline incision in the neck without the intraluminal suture occlusion procedure. The animals were then kept individually, with free access to food but no access to water until cystometric examination.

2.2. Neurological deficit

One day after middle cerebral artery occlusion, neurological deficits were measured according to a modification of the method of Garcia et al. (1995), and the presence of deficits was used to indirectly determine whether middle cerebral artery occlusion had induced cerebral infarction. Minimum score under this method is 4, representing severe deficit, while the maximum score is 18 for normal animals. The study protocol required the exclusion of animals showing no or only a mild neurological deficits (score: 14–18), but no animals were in fact excluded for this reason.

2.3. Cystometry

After the measurement of neurological deficits, cystometry was performed as previously described (Kaidoh et al., 2002). Briefly, conscious rats showing a moderate to severe neurological deficit (score: 4–13) were placed in a restraining cage (Ballman Cage, KN-326 Type 3, Natsume, Tokyo, Japan). The bladder catheter was connected to a pump (STC525, Terumo, Tokyo, Japan) for the continuous infusion of saline and to a pressure transducer (TP-400, Nihon Kohden, Tokyo, Japan) for the measurement of intravesical pressure. Intravesical pressure was recorded by infusing saline into the bladder at rates of 3–7 ml/h (infarcted rats) or 6–9 ml/h (sham-operated rats), and the volume of urine voided from the urethral meatus was measured to determine the voided volume. To facilitate drug evaluation, only those animals showing urinary frequency (bladder capacity <1 ml) were eligible for study. The bladder was emptied by drainage of urine through the catheter and then continuously re-infused with saline. After stable voiding cycles were established, each rat received a single intravenous administration of test drug at a volume of 1 ml/kg. The cystometric parameters measured were the voided volume (volume of urine voided) and micturition pressure (maximum bladder pressure during voiding). Bladder capacity and residual volume were calculated according to the following equations: bladder capacity= residual volume at previous void+volume of saline infused up to the time of voiding and residual volume=bladder capacity−voided volume. To examine the effects of drugs, the mean cystometric values of the voiding cycles closest and second closest to a point 30 min after drug administration were analyzed.

2.4. Drugs

Solifenacin succinate, tolterodine tartrate and propiverine hydrochloride were prepared at Yamanouchi Pharmaceutical

Table 1
Cystometric parameters in sham-operated and cerebral infarcted rats

Group	Number	Bladder capacity (ml)	Voided volume (ml)	Residual volume (ml)	Micturition pressure (mm Hg)
Sham	8	1.32±0.08	1.20±0.09	0.12±0.02	26.0±1.5
Cerebral infarcted	8	0.83±0.03[a]	0.54±0.03[a]	0.29±0.04[a]	23.6±1.0

Saline (vehicle) was intravenously given at a volume of 1 ml/kg 1 day after cerebral artery occlusion or sham operation. Each value represents the mean±S.E.M.

[a] $P<0.01$: significant difference to the sham-operated group (Student's *t*-test).

(Tokyo, Japan). All drugs were dissolved in saline and serially diluted to desired concentrations.

2.5. Statistics

Cystometric values were expressed as the mean±S.E.M. of 8 animals and neurological deficit scores as the median of 8 animals. Statistical differences between the sham-operated and cerebral infarcted groups were analyzed using Student's *t*-test and statistical differences in the effect of drugs from the cerebral infarct control group were analyzed using Dunnett's multiple comparison test. Statistical analysis of neurological deficit scores was done using Steel's test. Differences with a $P<0.05$ were considered statistically significant. All data analyses were performed using the SAS statistical software (SAS Institute, Cary, NC, USA).

3. Results

Cystometric parameters in the cerebral infarcted and sham-operated rats are shown in Table 1. The cerebral infarcted rats showed a significant decrease in bladder capacity and voided volume (0.83±0.03 ml and 0.54±0.03 ml, respectively) compared to the sham-operated rats (1.32±0.08 ml and 1.20±0.09 ml, respectively). On the other hand, the cerebral infarcted rats showed a significant increase in residual volume (0.29±0.04 ml) compared with the sham-operated rats (0.12±0.02 ml). There was no significant change in micturition pressure between the sham-operated (26.0±1.5 mm Hg) and cerebral infarcted groups (23.6±1.0 mm Hg). The cerebral infarcted rats exhibited deficits in terms of forelimb flexion and circling.

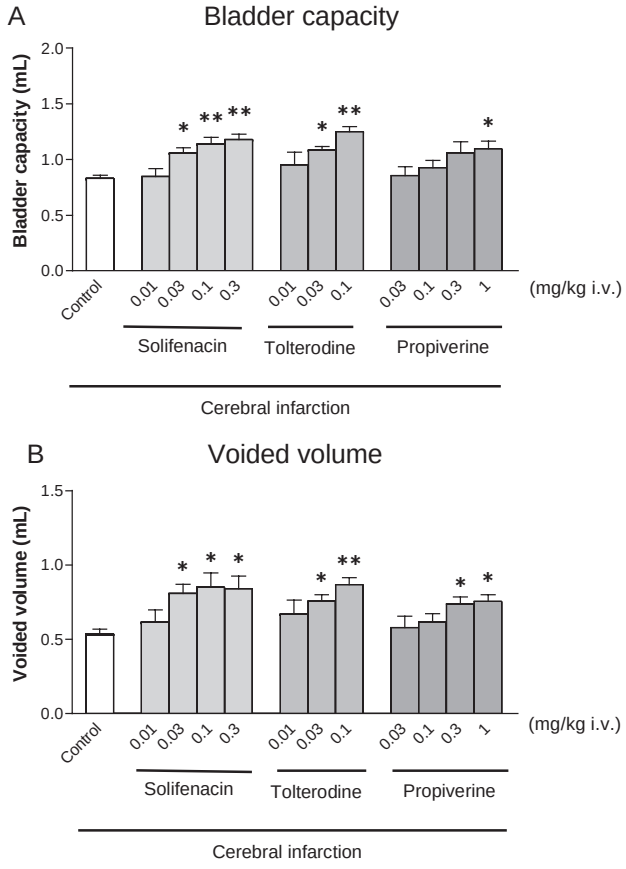

Fig. 2. Effects of solifenacin, tolterodine and propiverine on bladder capacity and voided volume in cerebral infarcted rats. The test drugs were intravenously administered 1 day after cerebral artery occlusion. Each column represents the mean±S.E.M. of 8 rats. *$P<0.05$, **$P<0.01$: significant difference to the control group (Dunnett's multiple comparison test).

Pre-values for bladder capacity, voided volume, residual volume and micturition pressure in the cerebral infarcted rats were 0.81±0.01 ml, 0.58±0.02 ml, 0.23±0.01 ml and 24.3±0.6 mm Hg (n=96), respectively. Pre-value for the neurological deficit score in the cerebral infarcted rats was 11.0 (median, n=96).

The effects of solifenacin, tolterodine and propiverine on bladder capacity and voided volume in the cerebral infarcted rats are shown in Figs. 1 and 2, and on residual volume and micturition pressure in Table 2. Solifenacin (0.01−0.3 mg/kg i.v.) dose-dependently increased bladder capacity and voided volume at doses of 0.03 mg/kg i.v. or more, but did not affect residual volume or micturition

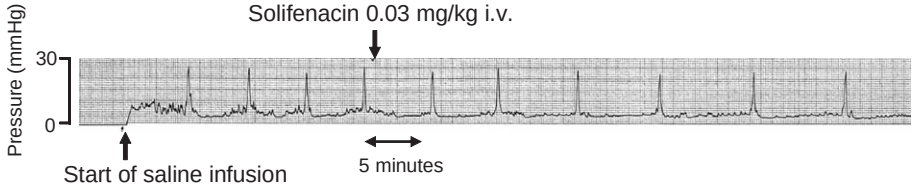

Fig. 1. Representative cystometry in a cerebral infarcted rat. Solifenacin (0.03 mg/kg i.v.) prolonged micturition intervals.

Table 2
Effects of solifenacin, tolterodine and propiverine on residual volume and micturition pressure in cerebral infarcted rats

Drug	Dose (mg/kg i.v.)	Number	Residual volume (ml)	Micturition pressure (mm Hg)
Control	Saline	8	0.29±0.04	23.6±1.0
Solifenacin	0.01	8	0.22±0.03	24.9±1.0
	0.03	8	0.25±0.04	24.3±1.5
	0.1	8	0.28±0.05	22.0±3.0
	0.3	8	0.33±0.07	22.8±2.1
Tolterodine	0.01	8	0.28±0.07	23.2±1.5
	0.03	8	0.33±0.04	20.7±2.4
	0.1	8	0.38±0.04	21.3±1.3
Propiverine	0.03	8	0.28±0.04	22.7±1.7
	0.1	8	0.31±0.04	25.6±2.4
	0.3	8	0.32±0.07	23.2±2.8
	1	8	0.34±0.04	24.7±2.3

The test drugs were intravenously administered 1 day after cerebral artery occlusion. Each value represents the mean±S.E.M. No significant difference was observed compared with control group (Dunnett's multiple comparison test).

pressure at any dose tested. Tolterodine (0.01−0.1 mg/kg i.v) also dose-dependently increased bladder capacity and voided volume at 0.03 and 0.1 mg/kg i.v., but did not affect residual volume or micturition pressure at any dose tested. Further, propiverine (0.03−1 mg/kg i.v.) tended to increase bladder capacity at 0.3 mg/kg i.v. (p=0.09) and significantly increased it at 1 mg/kg i.v., and dose-dependently increased voided volume at 0.3 and 1 mg/kg i.v., but did not affect residual volume or micturition pressure at any dose tested.

4. Discussion

In the present study, we examined the effects of the novel muscarinic receptor antagonist solifenacin on detrusor overactivity using cystometry in a rat model of cerebral infarction induced by middle cerebral artery occlusion. The results suggested that solifenacin improves detrusor overactivity without causing urinary retention and that it may be useful in the treatment of overactive bladder syndrome.

With regard to the model used, Ishiura (1996) have demonstrated that oxybutynin, a muscarinic receptor antagonist, increased bladder capacity in cerebral infarcted rats but not in normal rats. The reason for the failure of antimuscarinic drugs to increase bladder capacity in normal rats is unclear. One possibility is that the bladder is sufficiently relaxing during filling in normal conscious conditions such that antimuscarinic drugs fail to induce further relaxation. In fact, not only antimuscarinic drugs but also calcium antagonists (Nakamura et al., 1999) and β-adrenoceptor agonists (Kaidoh et al., 2002) show amelioratory effects on bladder function in cerebral infarcted but not normal rats. Rat models of cerebral infarction are thus considered to be useful for evaluating new drugs.

As in previous investigations (Yokoyama et al., 1999, 2002; Kaidoh et al., 2002; Pehrson et al., 2003), the cerebral infarcted rats had a smaller bladder capacity and a smaller voided volume than the sham-operated rats. In the present study, animals showing no or a mild neurological deficit or having a bladder capacity of ≥1 ml were excluded, meaning that only animals suffering from detrusor overactivity accompanying cerebral infarction were eligible.

Given that cerebral infarcted rats may serve as a model for human disease, drug-induced reversal of small bladder capacity may be of clinical interest. Our results showed that solifenacin dose-dependently increased bladder capacity in line with other antimuscarinic drugs, namely tolterodine and propiverine, both of which are widely used for the treatment of overactive bladder (Hegde et al., 2004; Madersbacher et al., 1999). Solifenacin significantly increased bladder capacity by 37% at a dose of 0.1 mg/kg i.v., an amount similar to that by tolterodine at 0.03 mg/kg i.v. (31%). Propiverine increased it by 33% at a dose of 1 mg/kg i.v. These results suggest solifenacin could exhibit improving effect on detrusor overactivity at lower doses than propiverine. Further, these are considered to reflect our previous findings that solifenacin at 0.1 mg/kg i.v. and tolterodine at 0.03 mg/kg i.v. inhibited carbachol-induced intravesical pressure elevation to a similar degree (about 45% inhibition) in rats (Ohtake et al., 2004). On the other hand, the most common adverse effect of antimuscrinic drugs in clinical is dry mouth (xerostomia) due to blockade of muscarinic M_3 receptors in salivary gland. Solifenacin's amount of salivary inhibition at 0.1 mg/kg i.v. (8%) was less than that of tolterodine at 0.03 mg/kg i.v. (35%) in rats suggesting that solifenacin may provide symptomatic relief in the treatment of overactive bladder with less dry mouth than tolterodine.

Although it is well known that muscarinic M_2 and M_3 receptor subtypes are located on bladder smooth muscle, muscarinic M_3 receptors have been proven to play a predominant role in mediating bladder smooth muscle (Wang et al., 1995; Eglen et al., 1994, 1996; Ehlert et al., 1997). Ohtake et al. (2004) previously demonstrated that solifenacin inhibited intravesical pressure in rats, indicating its muscarinic M_3 receptor antagonistic activity, and at effective doses (0.03 mg/kg i.v. or more) that are consistent with those in the present cerebral infarcted rats (0.03 mg/kg i.v. or more). The effects of solifenacin on bladder function in the present study are therefore likely to derive from its muscarinic M_3 receptor antagonistic activity.

In the present study, cerebral infarction induced no change in micturition pressure. This is in agreement with a previous finding (Nakamura et al., 1999) that micturition pressure was unchanged 1 day after middle cerebral artery occlusion in rats. Compounds possessing muscarinic receptor antagonistic activity are known to suppress the parasympathetic bladder contraction response, and thereby to not only increase bladder capacity but also decrease micturition pressure (Sjögren, 1976). However, we observed

that solifenacin, tolterodine and propiverine in this model showed no effects on micturition pressure at doses at which they showed an amelioratory effect on bladder capacity. Because they also did not increase residual volume at the same doses, their amelioratory effects on bladder capacity are considered to be due to increased voiding volume. The results suggest that the amelioratory effects of solifenacin on detrusor overactivity operate over a wide dose range without causing urinary retention.

The site of action of solfenicin is also of interest. In this model, micturition function involves not only the cholinergic system (Nakada et al., 2000) but also the glutamatergic, dopaminergic (Yokoyama et al., 1999, 2002) and GABAergic systems (Kanie et al., 2000) in the central nervous system. Binding assay showed that solifenacin has low affinity for $GABA_A$ receptors and dopamine receptors at the high concentration of 10^{-5} M (data not shown). pK_i values of the affinity of solifenacin for the recombinant human muscarinic M_1, M_2 and M_3 receptor subtypes are 7.6, 6.9 and 8.0, respectively (Ikeda et al., 2002), and hence while its selectivity for the M_3 over the M_2 subtype is apparent, that over the M_1 subtype is only marginal. Although muscarinic M_1 receptors play a role in the forebrain inhibitory mechanisms involved in the micturition reflex (Yokoyama et al., 2001), the functional roles of muscarinic M_3 receptors, if any, in those areas of the central nervous system which are important for voiding remain to our knowledge unclear. Further investigations are needed to determine whether muscarinic M_3 receptor antagonists in the central nervous system are involved in bladder function. On the other hand, in vitro study showed that solifenacin inhibited contraction in urinary bladder strips, suggesting that it has direct effects on muscarinic receptors of bladder smooth muscle, at least in part (Ikeda et al., 2002). The finding that solifenacin was scarcely detected in the brain of mice and rats (data not shown) is obtained and is therefore probably not permeable through the blood–brain barrier further suggests that its effects on bladder function are more likely to occur peripherally.

In conclusion, solifenacin ameliorated the detrusor overactivity induced by cerebral infarction without causing urinary retention in a rat model. These findings suggest that solifenacin may be a promising drug in patients with overactive bladder syndrome including urgency frequency and incontinence.

References

Andersson, K.E., 1993. Pharmacology of lower urinary tract smooth muscles and penile erectile tissues. Pharmacol. Rev. 45, 253–308.

Caulfield, M.P., Birdsall, N.J., 1998. International Union of Pharmacology: XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50, 279–290.

Eglen, R.M., Reddy, H., Watson, N., John-Challiss, R.A., 1994. Muscarinic acetylcholine receptor subtypes in smooth muscle. Trends Pharmacol. Sci. 15, 114–119.

Eglen, R.M., Hegde, S.S., Watson, N., 1996. Muscarinic receptor subtypes and smooth muscle function. Pharmacol. Rev. 48, 531–565.

Ehlert, F.J., Ostrom, R.S., Sawyer, G.W., 1997. Subtypes of the muscarinic receptor in smooth muscle. Life Sci. 61, 1729–1740.

Garcia, J.H., Wagner, S., Liu, K.F., Hu, X.J., 1995. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 26, 627–634.

Griffiths, D.J., McCracken, P.N., Harrison, G.M., Gormley, E.A., Moore, K., Hooper, R., McEwan, A.J., Triscott, J., 1994. Cerebral aetiology of urinary urge incontinence in elderly people. Age Ageing 23, 246–250.

Hegde, S.S., Mammen, M., Jasper, J.R., 2004. Antimuscarinics for the treatment of overactive bladder: current options and emerging therapies. Curr. Opin. Investig. Drugs 5, 40–44.

Ikeda, K., Kobayashi, S., Suzuki, M., Miyata, K., Takeuchi, M., Yamada, T., Honda, K., 2002. M_3 receptor antagonism by the novel antimuscarinic agent solifenacin in the urinary bladder and salivary gland. Naunyn-Schmiedeberg's Arch. Pharmacol. 366, 97–103.

Ishiura, Y., 1996. Experimental study of voiding dysfunction induced by cerebral infarction in rats. Jpn. J. Urol. 87, 1221–1230.

Kaidoh, K., Igawa, Y., Takeda, H., Yamazaki, Y., Akahane, S., Miyata, H., Ajisawa, Y., Nishizawa, O., Andersson, K.E., 2002. Effects of selective β2 and β3-adrenoceptor agonists on detrusor hyperreflexia in conscious cerebral infarcted rats. J. Urol. 168, 1247–1252.

Kanie, S., Yokoyama, O., Komatsu, K., Kodama, K., Yotsuyanagi, S., Niikura, S., Nagasaka, Y., Miyamoto, K.I., Namiki, M., 2000. GABAergic contribution to rat bladder hyperactivity after middle cerebral artery occlusion. Am. J. Physiol. 279, R1230–R1238.

Khan, Z., Starer, P., Yang, W.C., Bhola, A., 1990. Analysis of voiding disorders in patients with cerebrovascular accidents. Urology 35, 265–270.

Kobayashi, S., Ikeda, K., Miyata, K., 2004. Comparison of in vitro selectivity profiles of solifenacin succinate (YM905) and current antimuscarinic drugs in bladder and salivary glands: a Ca^{2+} mobilization study in monkey cells. Life Sci. 74, 843–853.

Longa, E.Z., Weinstein, P.R., Carlson, S., Cummins, R., 1989. Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20, 84–91.

Madersbacher, H., Halaska, M., Voigt, R., Alloussi, S., Hofner, K., 1999. A placebo-controlled, multicentre study comparing the tolerability and efficacy of propiverine and oxybutynin in patients with urgency and urge incontinence. BJU Int. 84, 646–651.

Nakada, Y., Yokoyama, O., Komatsu, K., Kodama, K., Yotsuyanagi, S., Niikura, S., Nagasaka, Y., Namiki, M., 2000. Effects of aniracetam on bladder overactivity in rats with cerebral infarction. J. Pharmacol. Exp. Ther. 293, 921–928.

Nakamura, Y., Yokoyama, O., Komatsu, K., Mita, E., Namiki, M., Kontani, H., 1999. Effects of nifedipine on bladder overactivity in rats with cerebral infarction. J. Urol. 162, 1502–1507.

Ohtake, A., Ukai, M., Hatanaka, T., Kobayashi, S., Ikeda, K., Sato, S., Miyata, K., Sasamata, M., 2004. In vitro and in vivo tissue selectivity profile of solifenacin succinate (YM905) for urinary bladder over salivary gland in rats. Eur. J. Pharmacol. 492, 243–250.

Pehrson, R., Stenman, E., Andersson, K.E., 2003. Effects of tramadol on rat detrusor overactivity induced by experimental cerebral infarction. Eur. Urol. 44, 495–499.

Sjögren, C., 1976. The effects of some anticholinergic compounds on the infused urinary bladder of anaesthetized rabbits. Acta Pharm. Toxicol. 39, 167–176.

Tsuchida, S., Noto, H., Yamaguchi, O., Itoh, M., 1983. Urodynamic studies on hemiplegic patients after cerebrovascular accident. Urology 21, 315–318.

Wang, P., Luthin, G.R., Ruggieri, M.R., 1995. Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. J. Pharmacol. Exp. Ther. 273, 959–966.

Yokoyama, O., Yoshiyama, M., Namiki, M., de Groat, W.C., 1999. Glutamatergic and dopaminergic contributions to rat bladder

hyperactivity after cerebral artery occlusion. Am. J. Physiol. 276, R935–R942.

Yokoyama, O., Ootsuka, N., Komatsu, K., Kodama, K., Yotsuyanagi, S., Niikura, S., Nagasaka, Y., Nakada, Y., Kanie, S., Namiki, M., 2001. Forebrain muscarinic control of micturition reflex in rats. Neuropharmacology 41, 629–638.

Yokoyama, O., Yoshiyama, M., Namiki, M., de Groat, W.C., 2002. Changes in dopaminergic and glutamatergic excitatory mechanisms of micturition reflex after middle cerebral artery occlusion in conscious rats. Exp. Neurol. 173, 129–135.