Journal of Korean Medical Science
Vol. 8, No. 1, 53-59 February 1993

Actions of Potassium Channel Openers in Rat Detrusor Urinae

Jeoung Hee Ha*, M.D., Kwang Youn Lee, M.D., and Won Joon Kim, M.D.

Department of Pharmacology, Yeungnam university, College of Medicine, Taegu, 705-035, Korea

This study was performed to investigate the action of potassium channel openers on the mechanical activity of detrusor muscle isolated from rats. Detrusor muscle strips, 15 mm in length, were myographied isometrically in an isolated organ bath.

P 1060, RP 49356 and BRL 38277, potassium channel activators, reduced the basal tone and diminished the phasic activity of detrusor concentration-dependently. P 1060, RP 49356 and BRL 38227 suppressed the maximal responses to bethanechol and shifted the concentration-response curves of bethanechol-induced contraction to the right. RP 49356 and BRL 38227 reduced the contraction by low (20 mM) concentration of potassium. P 1060, however, diminished the high (80 mM) and low (20 mM) concentration of potassium-induced contraction. Glibenclamide, an inhibitor of ATP-dependent potassium channel, antagonized the suppressive action of P 1060, RP 49356 and BRL 38227 on the basal tone. Apamin or procaine did not antagonize it significantly. Based on these results, it is suggested that the relaxation of detrusor muscle strip caused by P 1060, RP 49356 and BRL 38227 may predominantly involve opening of the same potassium channel, i.e., the ATP-dependent potassium channel.

Key Words: *P 1060, RP 49356, BRL 38227, glibenclamide, detrusor muscle, urinary bladder*

INTRODUCTION

Many drugs have been used in an attempt to treat patients with hyperactive detrusor. Various anticholinergic agents, the most widely employed drugs, have limited efficacy and/or systemic anticholinergic side effects. Agents with a direct relaxant effect on the smooth muscle have also been explored, among which the calcium channel blockers have a reasonably well defined mechanism of action.

P 1060, RP 49356 and BRL 38227 are potassium channel openers of cyanoguanidine, tetrahydrothiopyrane and benzopy ran derivatives, respectively, with potent vasodilator properties (Edwards et al., 1990). This has been ascribed to opening of the potassium channel in the cell membrane, efflux of potassium, membrane hyperpolarization and subsequent vascular smooth muscle relaxation (Hamillton et al., 1986; Weston et al., 1988; Cook 1988; Edwards et al., 1990; Longmore et al., 1990). Recently, several investigations have revealed the ability of potassium channel openers to relax the smooth muscles of trachea (Allen et al., 1986), uterus (Hollingsworth et al., 1987; Piper et al., 1989) and vas deferens (Soares-da-Silva et al., 1990). Moreover, this new therapeutic principle was suggested to be beneficial in the treatment of detrusor hyperactivity (Malmgren et al., 1989; Malmgren et al., 1990; Fujii et al., 1990; Grant et al., 1991).

The aim of the present study was to observe the effects of potassium channel openers and their interaction with potassium channel blockers on the mechanical activity of rat detrusor urinae using isolated strips.

Address for correspondence: *Jeoung Hee Ha, Department of Pharmacology, Yeungnam University, College of Medicine, Taegu, 705-035, Korea.* *This paper was partially supported by the non-directed fund, Korea Research Foundation, 1991*

MATERIALS AND METHODS

Sprague-Dawley rats weighing 200-250 g were sacrificed by decapitation. The urinary bladder was

isolated, and the surrounding adipose tissue and the mucosa were cleared. The detrusor muscle was dissected into horizontal strips, approximately 2 mm wide and 10mm long. Both ends were tied with silk ligatures, and each preparation was attached to a holder and mounted in an isolated muscle bath. Each bath contained 1 ml of physiologic salt solution (PSS) bubbled with 95% O_2 and 5% CO_2 mixture resulting in a pH of 7.4 at 37°C. The PSS used in this experiment had the following composition (mM): NaCl 136.9, KCl 2.68, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $MgCl_2$ 1.05, $CaCl_2$ 1.84, Glucose 5.5.

One end of the muscle strip was connected to a force displacement transducer (FT-03, Grass). Isometric tension was recorded on a polygraph (79E, Grass). Under an initial tension of 3 g, preparations were perfused with PSS for 60min. and then equilibrated for 60-90 min. in the muscle bath.

Stock solutions of BRL 38227 (Merck, donated), P 1060 (Leo, donated), RP 49356 (Rhone Poulenc, donated), and glibenclamide (Sigma) 10^{-3}M in absolute ethanol were stored in -20°C, and then diluted with distilled water for use. Apamin (Sigma), bethanechol (Eisai, donated from Hwan-In pharmaceuticals), ethylenediamine tetraacetic acid (EDTA) disodium salt dihydrate (Fluka AG), potassium chloride (Shinyo) and procaine hydrochloride (Kukjeon) were dissolved into distilled water.

The significance of the difference between two means was assessed by Student's t-test. A difference between means was assumed to be significant when $p < 0.05$. Some data were fitted to Michaelis-Menten equation by Multifit® (Macintosh version 2.01, Day Computing) to calculate EC_{50} and Emax.

RESULTS

P 1060, RP 49356 and BRL 38227 reduced the basal tone and diminished the spontaneous phasic contraction of detrusor muscle strips isolated from rat urinary bladder in a concentration dependent manner (Fig 1,2). EC_{50} (nM) of RP 49356, 170±51 was significantly higher than those of P 1060 and BRL 38227, 20±4 and 40±13 ($p < 0.05$), respectively. Emax (%) of P 1060, RP 49356 and BRL 38227 were 77±2.7, 78±3.7, and 79±3.7, respectively (Table 1).

P 1060, RP 49356 and BRL 38227 suppressed the bethanechol induced contraction and shifted the concentration-response curves to the right (Fig 3,4). EC_{50} (mM) of bethanechol in the presence of 10^{-6}M P 1060 and BRL 38227, 5.8±0.90 and 7.5±0.75 were significantly higher than that of the control, 1.3±0.12

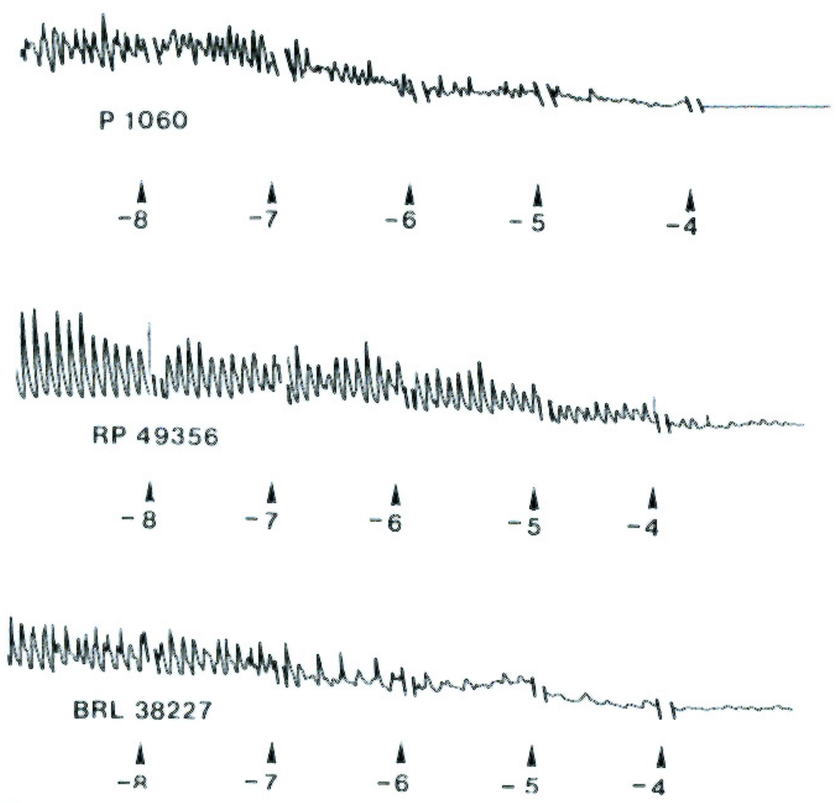

Fig. 1. Original recordings of cumulative concentration response of P 1060, RP 49356 and BRL 38227 in rat detrusor urinae. -8, -7, -6, -5 and -4 mean the concentrations of drug 10^{-8}M, 10^{-7}M, 10^{-6}M, 10^{-5}M and 10^{-4}M, respectively.

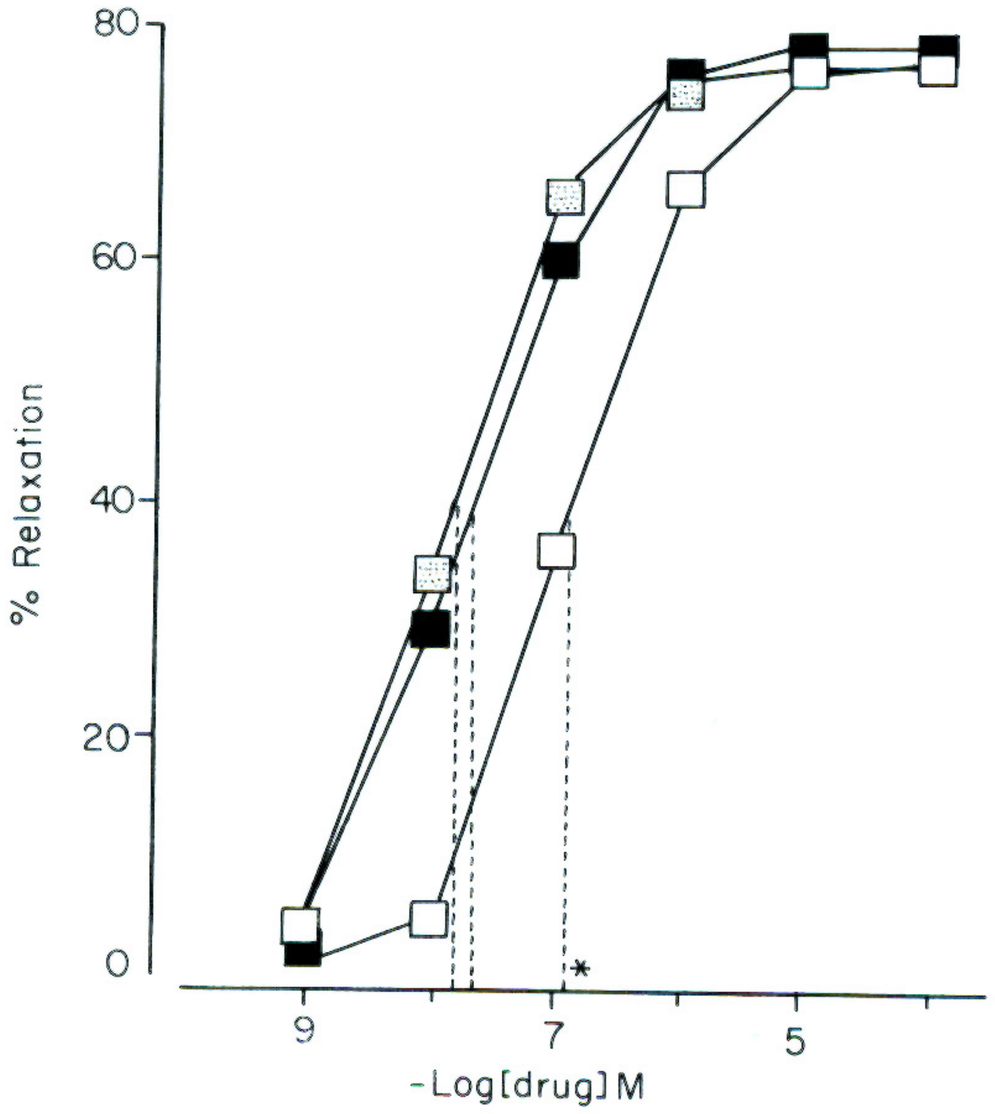

Fig. 2. Concentration-response curves of P 1060 (▨), RP 49356 (□) and BRL 38227 (■) on isolated rat detrusor urinae. Data were fitted to the Michaelis-Menten equation to calculate EC_{50} and Emax. Intersections of the dotted lines with curves and abscissa indicate the 1/2Emax and EC_{50}, respectively. Each point represents a mean of seven experiments, expressed as percent relaxation which means the proportional reduction of basal tone from normal equilibrated state to the level of passive tension obtained by the addition of EDTA 20 mM.
*$p < 0.05$: Significantly different from counterpart.

Table 1. EC_{50} and Emax of P 1060, RP 49356 and BRL 38227 on the basal tone of the isolated detrusor muscle strips of rat

Drug	EC_{50} (nM)	Emax (%)
P 1060	20±4	77±2.7
RP 49356	170±51*	78±3.8
BRL 38227	40±13	79±3.7

Values are expressed as mean±SE of EC_{50} and Emax calculated by the curve-fitting (to Michaelis-Menten equation) of the concentration-response curves of P 1060, RP 49356 and BRL 38227.
*$p<0.05$; significantly different from counterpart ($n=7$ for each group)

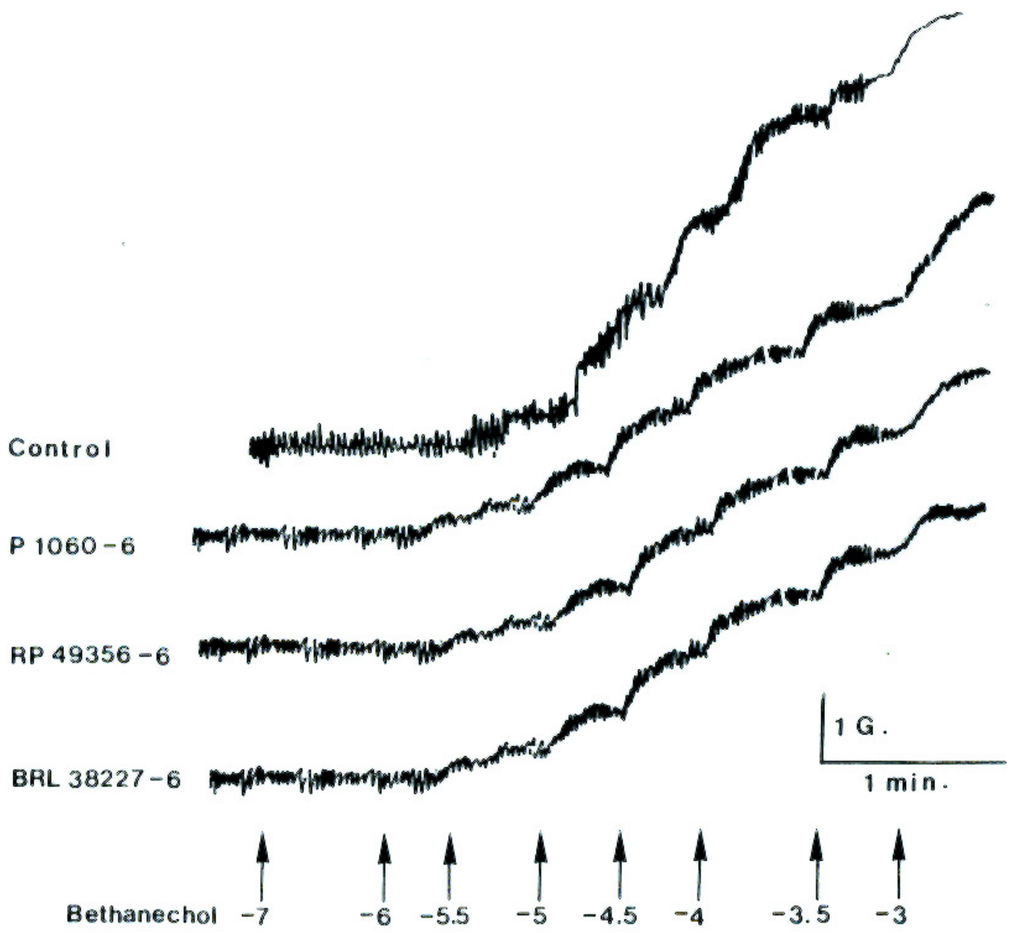

Fig. 3. Original recordings of cumulative concentration responses of bethanechol in the absence and presence of P 1060, RP 49356 and BRL 38227 10^{-6}M in rat detrusor urinae. P 1060-6, RP 49356-6 and BRL 38227-6 mean the concentrations of P 1060 10^{-6}M, RP 49356 10^{-6}M and BRL 38227 10^{-6}M, respectively. Bethanechol-7, -6, -5.5, -5, -4.5, -4, -3.5 and -3 mean the concentrations of bethanechol 10^{-7}M, 10^{-6}M, $10^{-5.5}$M, 10^{-5}M, $10^{-4.5}$M, 10^{-4}M, $10^{-3.5}$M and 10^{-3}M, respectively.

($p<0.05$). EC_{50} (mM) of bethanechol in the group of 10^{-6}M RP 49356, 2.8±0.60, was significantly higher than that of the control, 1.3±0.12 ($p<0.05$). Emax (%) of bethanechol in the presence of 10^{-6}M P 1060, RP 49356 and BRL 38227, 79±2.3, 75±2.8 and 71±2.1 were significantly lower than that of the control, 98±1.5 ($p<0.05$, Table 2).

The contractile response induced by 20 mM potassium chloride was reduced in the presence of 10^{-6}M BRL 38227 and 10^{-6}M RP 49356 significantly ($p<0.05$), but contracture by 80 mM potassium was not affected. However, 10_6M P 1060 diminished the

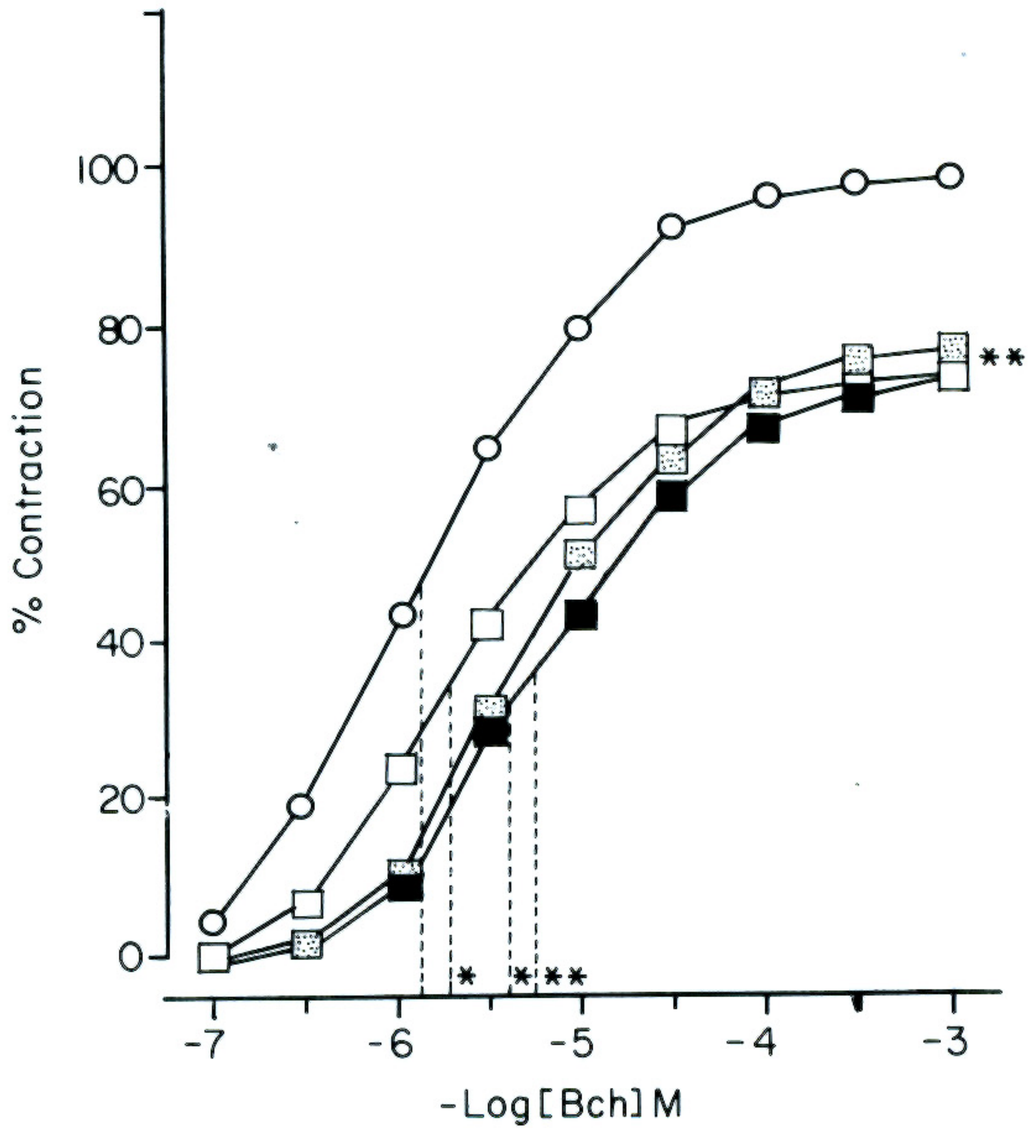

Fig. 4. Cumulative concentration-responses of bethanechol on the contractility of detrusor muscle strip in the absence (○) and presence of 10^{-6}M P 1060 (▨), RP 49356 (□) and BRL 38227 (■). Data were fitted to the Michaelis-Menten equation to calculate EC_{50} and Emax. Intersections of the dotted lines with curves and abscissa indicate the 1/2Emax and EC_{50}, respectively. Each point represents a mean of seven experiments, expressed as percent of maximal response of control.
*$p<0.05$, **$p<0.01$: Significantly different from control.

Table 2. EC_{50} and Emax of bethanechol in the presence of P 1060, RP 49356 and BRL 38227 on the basal tone of the isolated detrusor muscle strips of rat

Drug	EC_{50} (μM)	Emax (%)
Control	1.3±0.12	98±1.5
P 1060-6	5.8±0.90*	79±2.3*
RP 49356-6	2.8±0.60*	75±2.8*
BRL 38227-6	7.5±0.75*	71±2.1*

Values are expressed as mean±SE of EC_{50} and Emax calculated by the curve-fitting (to Michaelis-Menten equation) of the concentration-response curves of bethanechol.
P 1060 10^{-6}M, RP 49356 10^{-6}M and BRL 38227 10^{-6}M, respectively.
*$p<0.05$, significantly different from control ($n=7$ for each group)

20 and 80 mM potassium-induced response significantly ($p<0.05$, Fig 5,6).

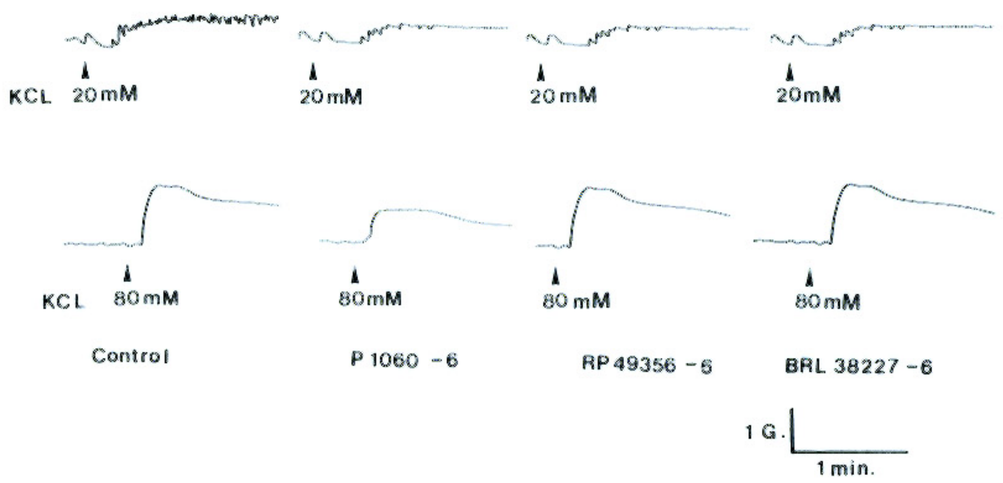

Fig. 5. Original recordings of contractions elicited by potassium chloride (20 and 80mM) in the absence and presence of P 1060, RP 49356 and BRL 38227 10^{-6}M in rat detrusor urinae. P 1060-6, RP 49356-6 and BRL 38227-6 mean the concentrations of P 1060 10^{-6}M, RP 49356 10^{-6}M and BRL 38227 10^{-6}M, respectively.

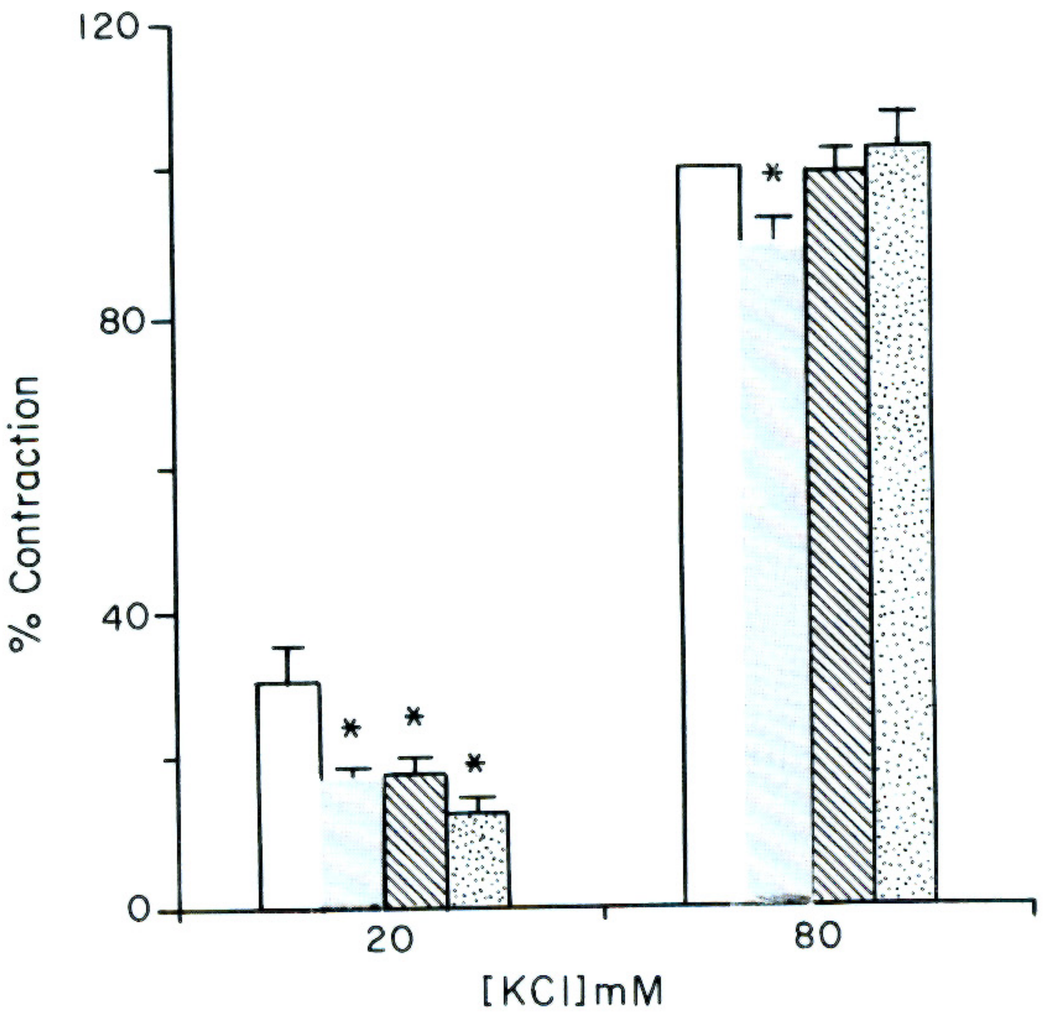

Fig. 6. Effect of 10^{-5}M P 1060 (■), RP 49356 (▨) and BRL 38227 (▩) on the potassium chloride-induced detrusor contraction. Each column represents a mean of seven experiments, expressed as % of maximal response of control. Vertical bar represents SE.
*$p < 0.05$: Significantly different from control (□).

10^{-6}M and 10^{-5}M glibenclamide antagonized the suppressive action of P 1060, RP 49356 and BRL 38227 on the basal tone (Fig 7). EC_{50} (μM) of 1060, RP 49356 and BRL 38227 in the presence of 10 M glibenclamide, 0.70 ± 0.065, 3.67 ± 0.854 and 0.85 ± 0.097 ($p < 0.05$), respectively, were significantly higher than those of the control, 0.02 ± 0.005, 0.18 ± 0.055 and 0.05 ± 0.016, respectively. Emax (%) of P 1060 in the group of 10^{-5}M and 10 M glibenclamide, 60.72 ± 7.2 ($p < 0.05$) and 43 ± 3.8 ($p < 0.05$) were significantly lower than those of the control, 77 ± 2.8. Emax (%) of RP 49356 and BRL 38227 in the presence of 10 M glibenclamide, 54 ± 2.7 and 61 ± 1.3 are significantly lower than those of the control, 73 ± 3.6 and 78 ± 3.8 ($p < 0.05$, Table 3). 10^{-5}M procaine did not inhibit but showed a tendency of enhancing the relaxant effects of P 1060, RP 49356 and BRL 38227 (Fig 8, Table 3). 10^{-5}M and 10^{-5}M apamin showed a tendency of inhibition on the relaxant action of P 1060, RP 49356 and BRL 38227 (Fig 9, Table 3).

DISCUSSION

At the present study, we used BRL 38227, a new benzopyran derivative, P 1060, a cyanoguanidine derviative and RP 49356, a tetrahydrothiopyrane derivative. The results in this paper have shown that the potassium channel openers which have different chemical structures produced a similar inhibition on the bethanechol-induced contraction in rat detrusor urinae.

When potassium was the spasmogen, there was an evidence of selective inhibition of the response to a lower concentration of potassium (20 mM), whereas the response to the nigher concentration (80 mM) was

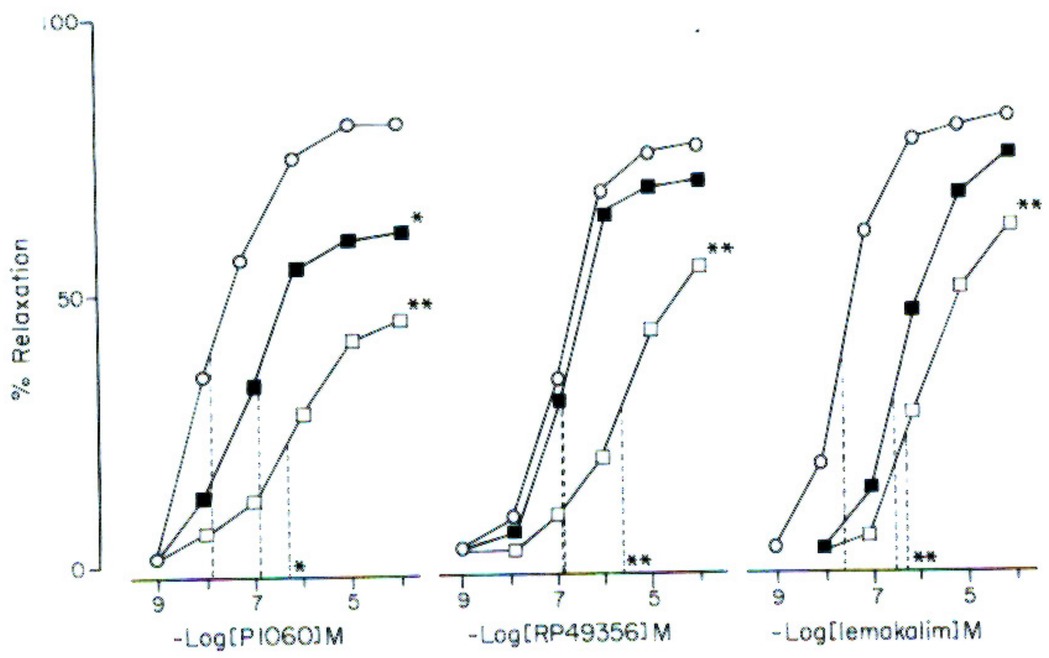

Fig. 8. Concentration-response curves of P 1060, RP 49356 and BRL 38227 in the absence (○) and presence of 10 M procaine (●) on isolated rat detrusor urinae. Data were fitted to the Michaelis-Menten equation to calculate EC_{50} and Emax. Intersections of the dotted lines with curves and abscissa indicate the 1/2Emax and EC_{50}, respectively. Each point represents mean of seven experiments, expressed as % relaxation which means the proportional reduction of basal tone from normal equilibrated state to the level of passive tension obtained by the addition of EDTA 20 mM.

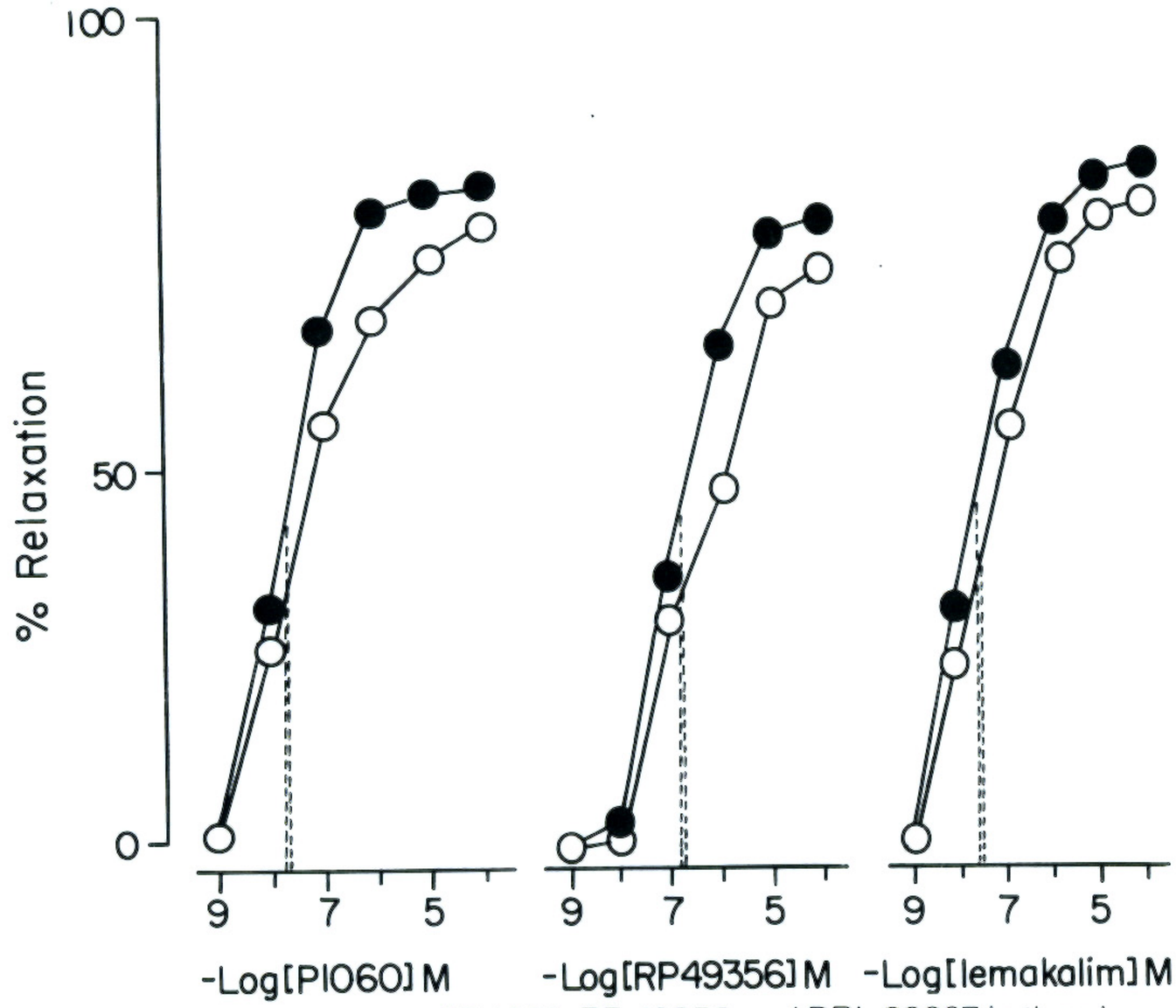

Fig. 7. Concentration-response curves of P 1060, RP 49356 and BRL 38227 in the absence (○) and presence of 10^{-6}M (□) and 10^{-5}M (■) glibenclamide on isolated rat detrusor urinae. Data were fitted to the Michaelis-Menten equation to calculate EC_{50} and Emax. Intersections of the dotted lines with curves and abscissa indicate the 1/2Emax and EC_{50}, respectively. Each point represents a mean of seven experiments, expressed as percentage relaxation which means the proportional reduction of basal tone from normal equilibrated state to the level of passive tensions obtained by the addition of EDTA 20mM.
*$p < 0.05$, **$p < 0.01$: Significantly different from control.

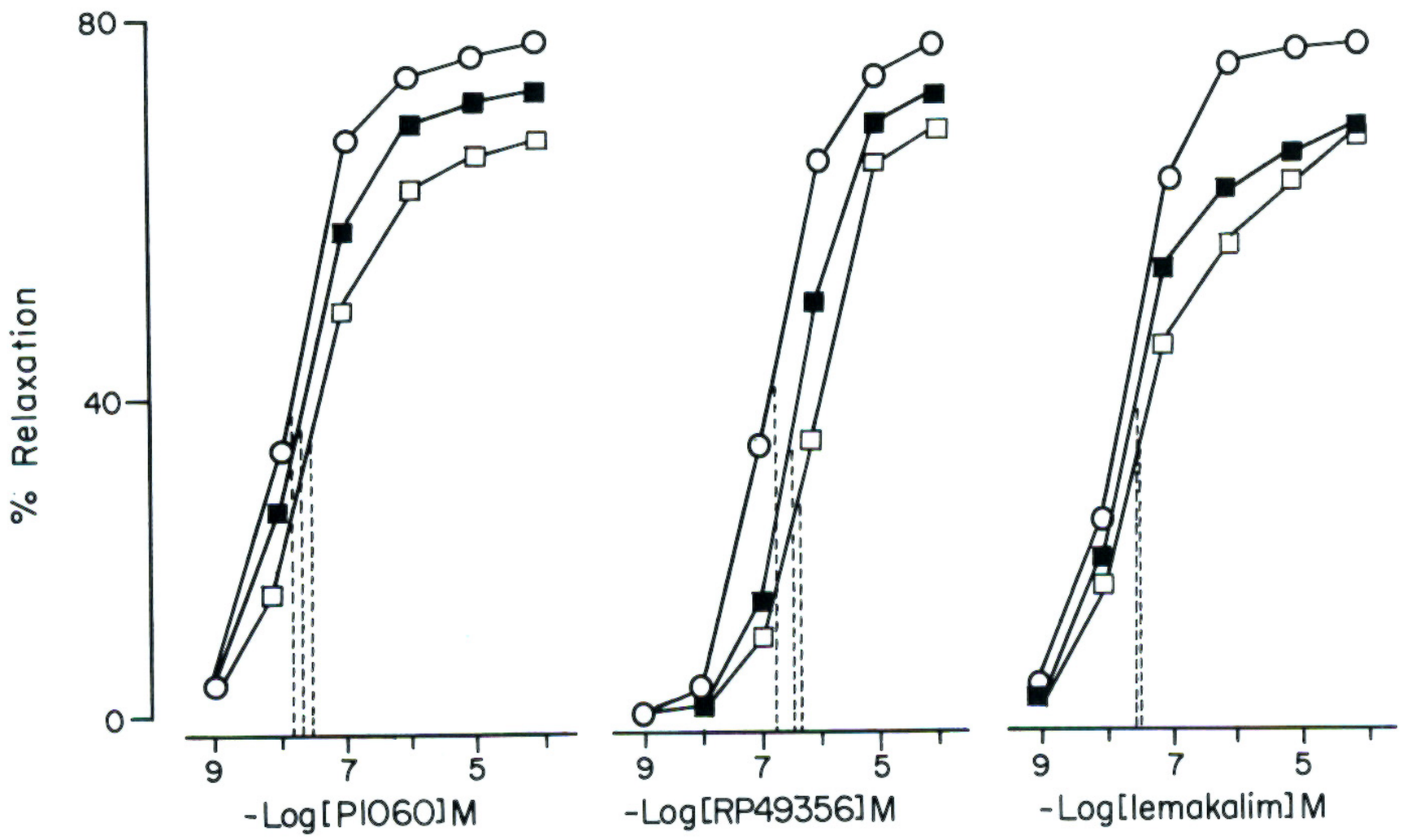

Fig. 9. Concentration-response curves of P 1060, RP 49356 and BRL 38227 in the absence (○) and presence of 10^{-5}M (□) and 10^{-6}M apamin (■) on isolated rat detrusor urinae. Data were fitted to the Michaelis-Menten equation to calculate EC_{50} and Emax. Intersections of the dotted lines with curevs and abscissa indicate the 1/2Emax and EC_{50}, respectively. Each point represents a mean of seven experiments, expressed as % relaxation which means the proportional reduction of basal tone from normal equilibrated state to the level of passive tension obtained by the addition of EDTA 20 mM.

Table 3. EC_{50} and Emax of P 1060, RP 49356 and BRL 38227 in the presence of glibenclamide, procaine and apamin of the isolated detrusor muscle strips of rats

	EC_{50} (μM)			Emax (%)		
Group	P 1060	RP 49356	BRL 38227	P 1060	RP 49356	BRL 38227
Control	0.02±0.005	0.18±0.055	0.05±0.016	77±2.8	73±3.6	78±3.8
Gly-6	0.17±0.128	0.19±0.068	0.60±0.306	60±7.2*	68±3.8	74±6.8
Gly-5	0.70±0.065*	3.67±0.854*	0.85±0.097*	43±3.8*	54±2.7*	61±1.3*
Pro-3	0.03±0.010	0.25±0.124	0.04±0.004	79±4.1	77±6.2	84±1.0
Apa-6	0.03±0.009	0.48±0.211	0.05±0.033	75±3.5	71±5.4	66±5.5
Apa-5	0.05±0.020	0.70±0.286	0.06±0.038	70±8.0	68±5.1	67±4.6

Values are expressed as mean±SE of EC_{50} and Emax calculated by the curve-fitting (to Michaelis-Menten equation) of the concentration-response curves of P 1060, RP 49356 and BRL 38227. Gly-6, Gly-5, Pro-3, Apa-6 and Apa-5 mean the concentrations of glibenclamide 10^{-6}M, glibenclmide 10^{-5}M, procaine 10^{-3}M, apamine 10^{-6}M and apamin 10^{-5}M, respectively.
*$p<0.05$; significantly different ($n=7$ for each group)

relatively unaffected. Such an inhibitory effect was more clearly seen when the spasmolytic effect of RP 49356 or BRL 38227 against potassium induced contraction was studied.

The explanation that RP 49356 and BRL 38227 are potassium channel opening drugs is consistent with their ability to inhibit the contractions produced by low concentrations of potassium selectively but not the responses to higher concentrations.

In the presence of RP 49356 and BRL 38227, the membrane potential of the smooth muscle cells will move toward equilibrium potential (EK). Adding 20 mM potassium, EK decreased but is still more negative than the potential at which the voltage operated Ca^{2+} channel opens and the tissue will relax (if already exposed to 20 mM potassium) or fail to contract (if exposed to potassium channel opening drug prior to exposure to potassium) (hamilton et al., 1986). Adding 80 mM potassium, the membrance potential will remain at a level of depolarization which ensures that the voltage operated Ca^{2+} channel remains open. Thus no relaxation or failure to contract will be observed. However, P 1060 inhibited spasms with both concentrations of potassium (20 and 80 mM), suggesting it to have an additional mechanism of action in high concentration (Piper et al., 1989; Anabuki et al., 1990).

A striking feature of the inhibitory action of the potassium channel openers, BRL 38227, RP 49356 and P 1060 was its ability to inhibit the spontaneous mechanical activity and the basal tone. EC_{50} of RP 49356 was smaller than that of P 1060 and BRL 38227 and Emax of the three drugs were similar. These results suggested RP 49356 had a weaker potency than BRL 38227 and P 1060.

To investigate the types of potassium channel present in smooth muscle, potassium channel inhibitors have been used as tools although there is considerable overlap in the channels each inhibitor can block, and there are few, if any, compounds which selectively block a single class of potassium channel (Cook 1988). There is, at present, little detailed information about the potassium channels present in detrusor muscle. The best studied bladder is that of guinea pigs (Fujii et al., 1990; Grant et al., 1991). Detrusor muscle of guinea pig was relaxed by cromakalim (BRL 34915); this effect was antagonized by glibenclamide, blockade of the ATP-sensitive potassium channel, and procaine, blockade of the delayed rectifier potassium channel. These reports suggested that the channel opened by cromakalim in guinea pigs may be similar to the ATP-sensitive potassium channels that have been demonstrated in vascular smooth muscle. They also suggest that the channels are similar to the delayed rectifier potassium channels that have been demonstrated in many smooth muscles. In guinea pigs, the inhibitory action of cromakalim on detrusor urinae was reported to be poorly affected by apamin, blockade of the low conductance Ca^{2+}-activated potassium channel. An apamin-sensitive type was found in guinea-pig taenia caeci (Weir et al., 1986).

When the tissues were treated with glibenclamide, in this study, antagonism was observed against the relaxant responses to P 1060, RP 49356 and BRL 38227. The relaxation caused by these drugs was comparatively less affected by apamin. These results are in agreement with the studies using guinea pigs (Fujii et al., 1990; Grant et al., 1991). Inconsistent with the guinea pig data (Fujii et al., 1990), however, the relaxation by potassium channel openers was hardly

affected by procaine, blockade of the delayed rectifier potassium channel. Observing these results, we guessed that species-difference might exist in the potassium channel of detrusor urinae.

In summary, P 1060, RP 49356 and BRL 38227 reduced the basal tone and phasic activity and inhibited the agonist- and potassium-induced contraction of detrusor urinae. Glibenclamide is a common antagonist of the muscular relaxant action of P 1060, RP 49356 and BRL 38227.

From these results, it is suggested that P 1060, RP 49356 and BRL 38227 are good relaxants of the hyperactive detrusor muscle; such relaxant action of the three drugs may predominanlty involve the opening of the same potassium channel, the ATP-sensitive potassium channel.

Further investigations for the clinical application of the potassium channel openers will be valuable for an establishment of a therapeutic remedy for the hyperactive detrusor.

REFERENCES

Allen SL, Boyle JP, Cortijo J, Foster RW, Morgan GP and Small RC: *Electrical and mechanical effects of BRL 34915 in guinea pig isolated trachealis. Br J Pharmacol 89:395-405, 1986.*

Anabuki J, Hori M, Ozaki H, Kato I and Karaki H: *Mechanisms of pinacidil induced vasodilation. Eur J Pharmacol 190:373-379, 1990.*

Cook NS: *The pharmacology of potassium channels and their therapeutic potential. TIPS 9:21-28, 1988.*

Edwards G and Weston AH: *Structure-activity relationships of potassium channel openers. TIPS 11:417-423, 1990.*

Fujii K, Foster CD, Brading AF and Parekh AB: *Potassium channel blockers and the effects of cromakalim on the smooth muscle of the guinea pig bladder. Br J Pharmacol 99:779-785, 1990.*

Grant TL and Zuzack JS: *Effects of potassium channel blockers and cromakalim (BRL 34915) on the mechanical activity of guinea pig detrusor smooth muscle. J Pharma Exp Ther 259:1158-1164, 1991.*

Hamilton TC, Weir, SW and Weston, AH: *Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity In rat portal vein. Br J Pharmac 88:103-111, 1986.*

Hollingsworth M, Amedee T, Edwards D, Mironneau J, Savineau JP, Small RC and Weston AH: *The relaxant action of BRL 34915 in rat uterus. Br J Pharmacol 91:803-813, 1987.*

Longmore J, Newgreen DT and Weston AH: *Effects of cromakalim, RP 49356, diazoxide, glibenclamide and galanin in rat portal vein. Eur J Pharmacol 190:75-84, 1990.*

Malmgren Andersson KE, Sjogren C and Andersson PO: *Effects of pinacidil and cromakalim (BRL 34915) on bladder function in rats with detrusor instability. J Urol 142:1134-1138, 1989.*

Malmgren A, Andersson KE, Andersson PO, Fovaeus M and Sjogren C: *Effects of cromakalim (BRL 34915) and pinacidil on normal and hypertrophied rat detrusor in vitro. J Urol 143:828-834, 1990.*

Piper I and Hollingsworth M: *Cromakalim, RP 49356, Pinacidil and Minoxidil sulfate in the rat uterus and their antagonism by glibenclamide. Br J Pharmacol 98 (suppl):807, 1989.*

Soares-da-Silva P and Fernandes MH: *Inhibition by the putative potassium channel opener pinacidil of the electrically-evoked release of endogenous dopamine and noradrenaline in the rat vas deferens. Naunyn-Schmiedebergs Arch Pharmacol 342:415-421, 1990.*

Weir SW and Weston AH: *Effect of apamin on responses to BRL 34915, nicorandil and other relaxants in the guinea-pig taenia caeci. Br J Pharmac 88:113-120, 1986.*

Weston AH, Southern JS, Bray KM, Newgreen DT and Taylor SG: *The mode of action of pinacidil and its analogs P 1060 and P 1368: results of studies in rat blood vessels. J Cardiovascular Pharmacol 12 (suppl.2):S10-S16, 1988.*