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Effects of Novel Potassium Channel Opener KR-30450 and its Metabolite KR-30818 on the Smooth Muscle of the Guinea Pig

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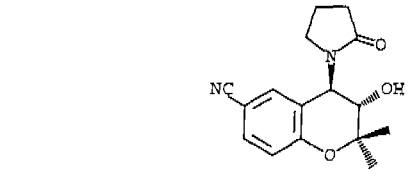
Abstract – The effect of potassium channel openers, KR-30450, KR-30818 and lemakalim have been compared against several spasmogens in guinea pig bronchi. In guinea pig bronchi, KR-30450 had a greater relaxant effect than lemakalim and KR-30818 against tone induced by histamine 10^{-5}M (EC_{50}, μM: KR-30450, 0.108±0.007; KR-30818, 0.403±0.023; lemakalim, 0.968±0.036) and prostaglandin $F_{2\alpha}$ 3×10^{-6} M (EC_{50}, μM: KR-30450, 0.018±0.001; KR-30818, 0.028±0.003; lemakalim, 0.138±0.019). Relaxant effect of KR-30450 and KR-30818 were significantly reduced by 20 min pretreatment of tissues with 10^{-6} M glibenclamide, a selective blocker of ATP-sensitive potassium channel. Against acetylcholine-induced tone in guinea pig bronchi, however, these compounds had little effect. In summary, KR-30450 and KR-30818 showed greater relaxant effect than lemakalim in guinea pig bronchi (KR-30450>KR-30818>lemakalim). These relaxant actions are suggested to be mediated at least in part by a mechanism which involves the opening of ATP-sensitive potassium channel.

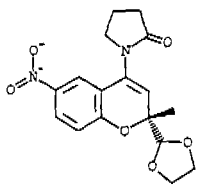
Keywords □ KR-30450, KR-30818, lemakalim, guinea pig, bronchi, potassium channel

ATP sensitive potassium channels (K_{ATP}) represent a class of channels which are gated in part by ATP, with normal intracellular ATP levels being associated with a low open probability (Noma, 1983; Cook and Hales, 1984). K_{ATP} exists in numerous tissues, including smooth muscle, cardiac myocyte, skeletal muscle, pancreas and brain (Spruce *et al.*, 1985; Treherne and Ashford, 1991). The physiological function of K_{ATP} in various tissues is being actively studied and this work has been greatly aided by the development of selective channel blockers and openers of this channel (Atwal, 1992). K_{ATP} openers were originally of interest because of their ability to relax smooth muscle, thus making them potentially useful for treating hypertension, asthma, and urinary incontinence (Robertson and Steinberg, 1990). K_{ATP} opening relaxes smooth muscle by hyperpolarising the tissue, thereby inhibiting inward calcium current through voltage operated channels (Ito *et al.*, 1992). A number of studies demonstrated that K_{ATP} openers such as cromakalim and levcromakalim (lemakalim), have antispasmogenic activity on smooth muscles including rat aorta and guinea pig trachea (Allen *et al.*, 1986; Buckingham *et al.*, 1989).

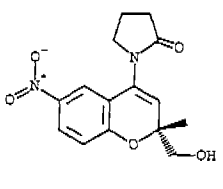
Previous work in our laboratory has demonstrated that benzopyran analogue KR-30450 and its metabolite KR-30818 (chemical structure shown in Fig. 1) are not only potent antihypertensives but potent relaxant of isolated canine coronary artery. These compounds were shown to be selective activator of cardiac K_{ATP} channel in patch clamp studies (Kwak *et al.*, 1995). In the present study,



Lemakalim



KR-30450



KR-30818

Fig. 1. Structures of benzopyran derivatives.

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the effects of KR-30450 and its metabolite KR-30818 have been investigated against responses induced by several spasmogens in the guinea pig bronchi. Relative potencies have also been evaluated by comparing with lemakalim.

MATERIALS AND METHODS

Tissue preparation

Male Hartley-outbred guinea pigs (Samyook Laboratory Animal Inc., Osan, Korea) weighing 400-600 g were stunned and bled. The trachea and main bronchi were rapidly removed and the main bronchi were dissected free from surrounding tissue. After removal of excess fat and connective tissues, guinea pig bronchi were cut into rings approximately 3 mm long (four bronchial rings from each guinea pig). The preparations were mounted vertically in organ baths containing 20 ml of Krebs solution of the following composition (mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, and Glucose 11.1) at 37 ℃, gassed with 95% O_2 and 5% CO_2, which produced a pH of 7.4. Rings were suspended between two L-shaped stainless steel hooks and a thread attached to one end of the hook was fixed to a transducer. The preparations were adjusted to give resting tensions of 0.5 g, which were found to be optimal for measuring changes in tension. Isometric tension changes were recorded through a force-displacement transducer (Grass FT03) connected to a Grass 7D polygraph. Tissues were allowed to equilibrate for 60 min before experiments were begun. During equilibration, tissues were washed with fresh buffer at 15 min intervals.

Effect of KR-30450 and KR-30818 on guinea pig bronchi

When stable baseline tone was achieved after the equilibration period, tissues were pre-contracted with 10^{-5} M histamine or 3×10^{-6} M prostaglandin $F_{2\alpha}$ or 10^{-6} M acetylcholine, and then cumulative concentration-relaxant response curves for lemakalim (10^{-8}-10^{-5} M), KR-30450 (10^{-8}-10^{-5} M) or KR-30818 (10^{-8}-10^{-5} M) were obtained. The relaxant responses were expressed as a percentage of the maximum relaxation obtained with 10^{-5} M papaverine.

In separate experiments, the effects of K_{ATP} antagonist glibenclamide were investigated on cumulative concentration-response curves produced by the addition of lemakalim, KR-30450 or KR-30818. Exposure time of the rings to glibenclamide was 20 min.

Statistical analysis of the data was performed by means of the Student's t-test, linear regression and one-way analysis of variance. The level of significance was taken at $p<0.05$. All data were expressed as mean±S.E.M.

Drugs used were lemakalim (KRICT), KR-30450 (KRICT), KR-30818 (KRICT), histamine (Sigma), prostaglandin $F_{2\alpha}$ (Sigma), acetylcholine (Sigma), glibenclamide (Sigma) and papaverine (Sigma). Stock solution (10^{-2} M) of lemakalim, KR-30450, KR-30818, and 10^{-4} M prostaglandin $F_{2\alpha}$ were made up in 100% dimethyl sulfoxide (DMSO), then diluted with warm saline to give a finally desired concentration. Histamine and acetylcholine were dissolved in distilled water. The vehicles used to dissolve drugs (0.1% DMSO) had no significant effect on the preparations.

RESULTS

In guinea pig isolated bronchial rings pre-contracted with histamine (10^{-5} M), KR-30450 (10^{-8}-10^{-5} M) and KR-30818 (10^{-8}-10^{-5} M) produced concentration-related relaxation (Fig. 2). The potency of KR-30450 was greater than those of KR-30818 and lemakalim. The EC_{50} values for KR-30450, KR-30818 and lemakalim were 0.108± 0.007, 0.403±0.023 and 0.968±0.036 μM, respectively (Table I). The order of potency was KR-30450>KR-30818>lemakalim. Maximal relaxation was 80 to 95% of that induced by a maximal concentration of papaverine (10^{-5} M). Glibenclamide, a selective blocker of K_{ATP}, antagonized responses to KR-30450 and KR-30818 shifting the response curves to the right. Against 3×10^{-6} M

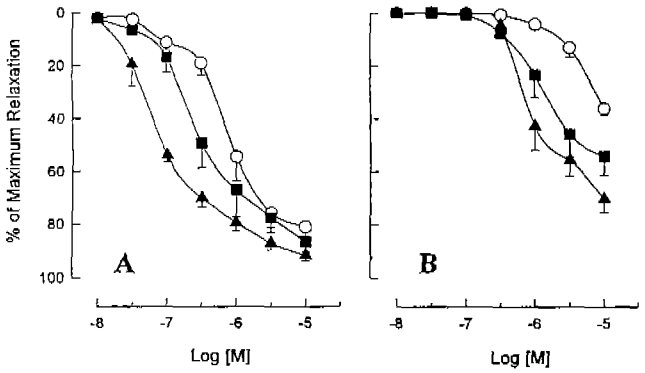

Fig. 2. Effect of lemakalim (○), KR-30450 (▲) and KR-30818 (■) in guinea pig bronchial rings against 10^{-5} M histamine-induced tone in the absence (pannel A) or presence (pannel B) of 10^{-6} M glibenclamide. Maximum relaxation was produced by 10^{-5} M papaverine. Each value represent mean± S.E.M. of at least 5 experiments.

Table I. Anti-spasmogenic effects of lemakalim, KR-30450 and KR-30818 on guinea pig isolated bronchi precontracted with histamine (10^{-5} M) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 3×10^{-6} M)

	Histamine-precontraction		$PGF_{2\alpha}$-precontraction	
	$^{b}EC_{50}$ (μM)	^{a}RP	$^{b}EC_{50}$ (μM)	^{a}RP
Lemakalim	0.968±0.036	1.0	0.138±0.019	1.0
KR-30450	0.108±0.007*	9.8	0.018±0.001*	7.7
KR-30818	0.403±0.023*	2.4	0.028±0.003*	4.9

^{a}RP represents relative potency. $^{b}EC_{50}$ is the concentration of each compound producing 50% relaxation of the pre-contracted tissues. *p<0.01 vs. lemakalim.

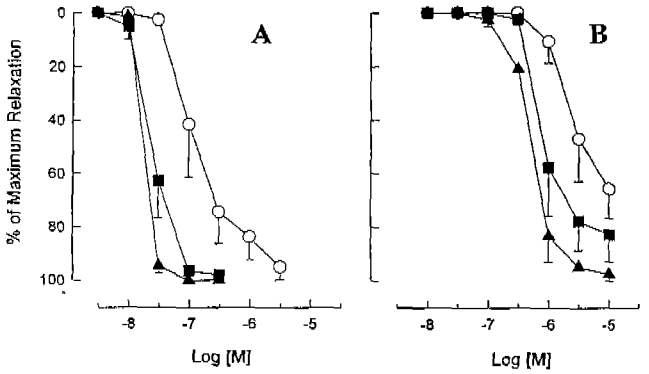

Fig. 3. Effect of lemakalim (○), KR-30450 (▲) and KR-30818 (■) in guinea pig bronchial rings against 3×10^{-6} M prostaglandin $F_{2\alpha}$-induced tone in the absence (pannel A) or presence (pannel B) of 10^{-6} M glibenclamide. Maximum relaxation was produced by 10^{-5} M papaverine. Each value represent mean±S.E.M. of at least 4 experiments.

prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced tone, KR-30450 had more potent relaxant effect than KR-30818 and lemakalim (EC_{50}, μM: KR-30450, 0.018±0.001; KR-30818, 0.028 ±0.003; lemakalim, 0.138±0.019), with order of potency of KR-30450>KR-30818>lemakalim. The slopes of the concentration-response curves were greater than that against histamine-induced pre-contraction (Fig. 3). This effect was also significantly inhibited by 20 min pretreatment with glibenclamide (10^{-6} M). As shown in Fig. 4, however, KR-30450, KR-30818 and lemakalim had little effect against acetylcholine-induced pre-contraction.

DISCUSSION

Potassium (K^+) channels are present on a variety of cell types including smooth muscle (Cook, 1988), and are important in repolarizing or hyperpolarizing excitable cells. Several studies have provided evidence of their existence on airway smooth muscle because drugs that

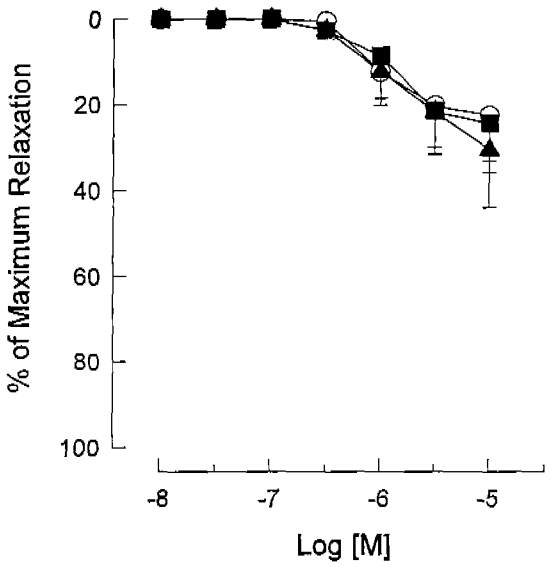

Fig. 4. Effect of lemakalim (○), KR-30450 (▲) and KR-30818 (■) in guinea pig bronchial rings against 10^{-6} M acetylcholine-induced tone in the absence (pannel A) or presence (pannel B) of 10^{-6} M glibenclamide. Maximum relaxation was produced by 10^{-5} M papaverine. Each value represent mean±S.E.M. of at least 4 experiments.

block K^+ channels, such as tetraethylammonium (TEA) and 4-aminopyridine, produce spontaneous action potentials and increased excitability (Kannan *et al.*, 1983; Marthan *et al.*, 1989). Conversely, drugs that activate K^+ channels result in relaxation of airway smooth muscle (Allen *et al.*, 1986; Murray *et al.*, 1989), both *in vitro* and *in vivo*. Furthermore, electrophysiologic studies have demonstrated the presence of K^+ channels in isolated airway smooth muscle cells (McCann and Welsh, 1986; Kotlikoff, 1989).

Activation of potassium channel has been proposed as the mechanism by which several structurally dissimilar compounds cause relaxation of smooth muscle (Cook, 1988; Hamilton and Weston, 1989; Black and Barnes, 1990). These compounds have been considered mainly as vasodilators for the treatment of hypertension. It is, however, evident that potassium channel activators, cromakalim in particular, have been shown to inhibit spontaneous tone in the guinea pig isolated trachea (Allen *et al.*, 1986; Arch *et al.*, 1988) and humans (Baird *et al.*, 1988). As a result of these actions, cromakalim may be of value as a bronchodilator in the treatment of asthma (Williams *et al.*, 1990). Because it is generally recognized that asthma is a multimediator disease, a prerequisite for an effective bronchodilator is that it should be able to prevent and reverse bronchoconstriction induced

by a variety of spasmogenic agents. Early work by Arch *et al.* (1988) showed that cromakalim and pinacidil, when administered before challenge, inhibited 5-hydroxytryptamine (5-HT)-induced bronchoconstriction as well as histamine-induced contraction in conscious guinea pigs. Dyspnea due to histamine is in fact a constriction of small rather than large airways (Fujiwara *et al.*, 1988), therefore by using the guinea pig bronchi rather than trachea in this study, we have examined the effects of the novel potassium channel activators, KR-30450 and KR-30818 against several spasmogens. In this work, we have shown that both KR-30450 and KR-30818 are capable of inducing significant rightward shifts of the concentration-response curves in the guinea pig bronchi precontracted with histamine or $PGF_{2\alpha}$, and the potency of these compounds are greater than that of lemakalim. The potency of lemakalim was comparable to that reported by Arch and colleagues (Arch *et al.*, 1988), who found that the racemic mixture cromakalin inhibited tone induced by histamine in guinea pig isolated bronchi. These relaxations are likely to be the result of effect on K_{ATP} channels by KR-compounds. This is based on the findings that a selective blocker of K_{ATP}, glibenclamide significantly inhibited the relaxant response to KR-30450 and KR-30818.

In the present study, constrictions induced by acetylcholine stand out as the one spasmogen whose contractions of guinea pig bronchi were poorly relaxed by the potassium channel openers indicating that small as well as large airways are not relaxed by potassium channel activators when challenged with muscarinic agonists. Similar results have recently been reported (Taylor *et al.*, 1992) showing that acetylcholine-induced bronchoconstriction in anesthetized guinea pigs was unaffected by cromakalim, although other workers (Martini and Black, 1990) have reported that cromakalim can relax carbachol-induced tone in guinea pig bronchi.

One possible explanation for the relative lack of effect of the potassium channel activators against muscarinic-induced responses is that the postreceptor mechanisms differ between muscarinic responses and other agonists. It has been reported that in bovine and guinea pig trachea, differences exist in inositol phosphate-3 mobilization between carbachol and histamine and the effect of inhibitory agents (Hall and Hill, 1989; Langlands *et al.*, 1989). Another explanation is still possible. It has been reported that in gastric smooth muscle cells, muscarinic agonists antagonize potassium currents activated, by beta-adrenoceptor agonists (Sims *et al.*, 1989). If they also block potassium currents opened by potassium channel activators, this would account for potassium channel activators not inhibiting muscarinic responses.

Even though acetylcholine is an important spasmogen mediating some reflex bronchoconstriction mechanisms, the failure of the potassium channel activators to antagonize muscarinic-mediated bronchoconstriction in guinea pigs may be of little relevance to their therapeutic potential in humans. Hall and Maclagan (1988) have reported that cromakalim inhibits vagally induced contractions of guinea pig trachea. These workers have also shown no effect of cromakalim on exogenous acetylcholine and have suggested that cromakalim may inhibit vagal transmission. Furthermore, Ichinose and Barnes (1990) have shown cromakalim to inhibit both acetylcholine-induced and, especially, vigally induced bronchoconstrictions *in vivo* in capsaicin-treated guinea pigs.

In conclusion, this study demonstrated that KR-30450 and KR-30818 are effective relaxant of guinea pig bronchial muscle against tone induced by histamine and $PGF_{2\alpha}$ via the action mechanism of K_{ATP} opening. This result provides the potential of these agents as therapeutic treatments for asthma.

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