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EXPERIMENTAL STUDY

The influence of animal species on the relationship between ATP-sensitive potassium ion channels and defense reflexes of the airways

Sutovska M[1], Franova S[1], Sutovsky J[2]

Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia.
sutovska@uniba.sk

Abstract: *Background:* The ATP-sensitive potassium ion channels (K^+_{ATP}) are widely expressed on airway sensory nerves that mediate cough and other protective reflexes; dependent on airways smooth muscles (ASM) reactivity.
Objective: The study was conducted to determine the involvement of K^+_{ATP} in airway sensory nerves activity of different animal species.
Methods: In this study we have evaluated the cough reflex inhibiting potential and modulation of ASM reactivity in vitro, by K^+_{ATP} opener-pinacidil in guinea pig and cats.
The cough in guinea pig was induced by administration in cats by mechanical stimulation of the airways. ASM reactivity was tested by organ tissue both method of 0.3 M citric acid aerosol at 3 min interval.
Results: 1) Pinacidil inhibits cough reflex in guinea pigs which was antagonized when pre-treated with K^+_{ATP} blocker glibenclamide. 2) Pinacidil exhibited antitussive activity in cats comparable to codeine and was noticeably higher than dropropizine effects. 3) ASM reactivity was significantly abolished by pinacidil and almost completely antagonized by glibenclamide in guinea pigs. 4) Pinacidil significantly attenuated ASM contraction in cats only in highest concentrations of contractile mediators.
Conclusion: K^+_{ATP} may be involved in mechanisms of cough reflex, likewise in antitussive activity of several more agents and also on defence reflexes dependent on ASM reactivity in different animal species such as guinea pigs and cats *(Fig. 8, Ref. 20)*. Full Text (Free, PDF) www.bmj.sk.
Key words: pinacidil, conscious cats, guinea pigs, antitussive activity, airways.

The sensory nerves playing the main pathophysiological as well as regulatory role in defence reflexes of the airways, express a wide scale of receptors and ion channels, which modulate their activity.

The stimulation of potassium ion channels prevents the development of action potential. In this way potassium ion channels exhibit important physiological roles in cellular signalling processes that regulate heart rate, insulin secretion, as well as smooth muscle contraction and neurotransmitter release. Several K^+ channels with heterogeneous molecular basis contribute to regulatory basal K^+ conductance in nerves or smooth muscle cells (1): Kv (voltage-gated); BK^+_{Ca} (large conductance calcium-activated); SK^+_{Ca} (small conductance calcium-activated); Kir (inward rectifier); stretch-dependent and K^+_{ATP} (ATP-sensitive). There is increased evidence that K^+_{ATP} ion channels play a role in ASM reactivity and both, afferent and efferent airway sensory nerves activity.

K^+_{ATP} ion channels are heteromeric complexes of poreforming inwardly rectifying potassium channel subunits and regulatory sulfonylurea receptor subunits regulated by cell metabolism and provides a means of linking the electrical activity of a cell to its metabolic state (2). The several endogenous agonists (e.g. intracellular nucleotides and calcitonin-gene related peptide; CGRP) activate K^+_{ATP} resulting in hyperpolarization and relaxation, a response that is similar to the effect of K^+_{ATP} openers pinacidil or mogusteine (3). Nielsen-Kudsk (4) showed that pinacidil relaxed guinea pig isolated trachea either tone was spontaneous or induced by a range of airway contractile mediators (histamine, PGF_2, leucotrienes or carbachol) enforcing in asthma pathogenesis. The ASM relaxation produced by pinacidil was selectively blocked by the antidiabetic glibenclamide. Morita and Kamei (5) demonstrated suppression of capsaicin-induced cough in guinea pigs on mogusteine administration prevented by glibenclamide. Hence, it is presumable that K^+_{ATP} ion channels may be involved in the mechanisms of cough, antitussive activity of more agents as well as airways defense reflexes based on ASM reactivity in guinea pigs. The potential use of these ion channels openers as antitussives and in therapy of inflammatory airway diseases such as asthma requires exploration of the effect in more animal species. For that reason the presented study was aimed to

[1]Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, and [2]Department of Neurosurgery, Jessenius Faculty of Medicine Comenius University and Faculty Hospital in Martin, Martin, Slovakia

Address for correspondence: M. Sutovska, MD, PhD, Dept of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Sklabinska 26, SK-037 53 Martin, Slovakia.
Phone: +43.4132535, Fax: +43.4134807

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ascertain the role of ATP-sensitive potassium ion channels in the airways function in guinea pigs and cats.

Materials and methods

Animals

All experiments were approved by the Ethics Committee of the Jessenius Faculty of Medicine in accordance with the revised Declaration of Helsinki from 1983 and follow the criteria of experimental animal's well fare.

The adult male TRIK strain guinea pigs, weighing 150–350 g (8 in each group) used in the first part of experiments, were obtained from Department of Experimental Pharmacology, Slovak Academy of Sciences, Dobra Voda, Slovakia.

Adult cats of both sexes weighing 1500–2500 g (6–8 in each group) were used in the second part of experiments.

The animals were housed in approved animal holding facility. The experiments were carried out after prescribed adapting period and after adaptation of animals to laboratory conditions.

Chemicals

The modulators of potassium ion channels activity, citric acid and contractive mediators' acetylcholine and histamine were obtained from Sigma-Aldrich (Germany). Codeine and dropropizine were purchased from Lachema (Czech Republic). Pinacidil and glibenclamide were dissolved in 10 % DMSO; codeine and dropropizine in water for injection and all other drugs in 0.9 % saline solution.

The course of experiments

Experimental design in guinea pigs

1) the cough reflex induced by citric acid aerosol followed in groups (n=8):

– medicated by agonist of K^+_{ATP} ion channels (dose 1 mg kg^{-1} b.w.),
– medicated by antagonist/agonist combination of K^+_{ATP} ion channels consisting of pretreatment by antagonist glibenclamide in dose 3 $mg.kg^{-1}$ b.w. followed by pinacidil medication in dose 3 $mg.kg^{-1}$ b.w. after 20 min interval,
– medicated by codeine (dose 10 $mg.kg^{-1}$ b.w.).

2) the airways smooth muscle reactivity *in vitro* condition registered in both above-mentioned groups (n=8) treated by modulators of K^+_{ATP} ion channels activity and control group (n=8) medicated by solvent (water for injections; dose 1 $ml.kg^{-1}$ b.w. intraperitoneally).

Experimental design in cats

1) the cough reflex induced mechanically followed in groups (n=8):

– medicated by agonist of K^+_{ATP} ion channels (dose 1 $mg.kg^{-1}$ b.w.),
– medicated by codeine (dose 10 $mg.kg^{-1}$1 b.w.),
– medicated by dropropizine (dose 30 mg. kg^{-1} b.w.).

2) the airways smooth muscle reactivity *in vitro* condition registered in group (n=6) treated by selective agonist of K^+_{ATP} ion channels pinacidil (dose 1 $mg.kg^{-1}$ b.w.) and control group (n=6) medicated by solvent (water for injections; dose 1 $ml.kg^{-1}$ b.w. intraperitoneally).

The method of citric acid-induced cough reflex

Awaken guinea pigs were individually placed in a bodypletysmograph box (HSE type 855, Hugo Sachs Elektronik, Germany) and were exposed to citric acid aerosol in concentration 0.3 M for 3 min. The citric acid aerosol was generated by a jet nebulizer (PARI jet nebuliser, Paul Ritzau, Pari-Werk GmbH, Germany, output 5 $l.s^{-1}$, particles mass median diameter 1.2 μm) and delivered to the head chamber of the body plethysmograph.

The following two methods for detection of cough were used to distinguish the cough effort from sneezing and movements:

A) The changes of the expiratory airflow interrupting the basic respiratory pattern during cough were measured by pneumotachograph connected to the head chamber of bodypletysmograph.

B) The typical cough reflex movement and sound recognized by trained observer.

The number of coughs evaluated on the basis of sudden enhancement of expiratory flow accompanied by a typical cough movement and sound during 3 min inhalations of the tussigen was counted. The cough response was measured in all groups of animals before administration of codeine, ion channels agonists or medication by antagonists/agonists (N) and than after their application in confirmed time intervals (0.5, 1, 2 and 5 h). The solution of the K^+_{ATP} ion channels modulators was applied parenterally: agonist pinacidil via subcutaneous route of administration and glibenclamide intraperitoneally. The positive control agent codeine was used intraperitoneally.

Minimal time difference between two measurements of cough response to prevent cough receptors adaptation on that kind of irritation was 2 h.

Student-t test was used for the statistical analysis of the obtained results. Data are presented as mean±standard error of the mean (SEM) $p<0.05$ was considered statistically significant. Significance of $p<0.05$ and $p<0.01$ is shown by one and two symbols, respectively.

The method of cough reflex induced by mechanical stimulation

The details of mechanically-induced cough reflex in cats were previously described (6). Briefly, a chronic endotracheal cannula was surgically implanted under general anesthesia, followed by standard surgical care of the wound during a period of seven days. The cannula enabled mechanical stimulation of tracheobronchial (TB) and laryngopharyngeal (LP) airways areas and the recording of the side tracheal pressure on a Biograph 12-03 electromanometer in conscious animals. The cough-related parameters, i.e. the number of efforts (NE), intensity of cough attack in expiration (IA^+) and inspiration (IA^-), cough frequency ($NE.min^{-1}$), and intensity of maximum efforts in expiration (IME^+) and inspiration (IME^-) were recorded and statistically evaluated. The parameters marked on figures as N (control) represented the

cough values obtained after stimulation of the airways in the absence of the tested compounds. The solution of the K^+_{ATP} ion channels agonist pinacidil was applied via subcutaneous route of administration. For comparative purpose most active antitussive drugs used in clinical practice to treat cough were tested along with the potassium ion channels modulators under the same conditions: centrally acting codeine (labeled as C in graphs) and agent influencing the peripheral parts of cough reflex arc dropropizine (D). Both control substances were administered intraperitoneally. The selected dose of antitussives used in clinical practice represented the amounts, which in our earlier experiments exhibited the highest antitussive activity (7). The effect of drugs was monitored in time intervals 0.5, 1, 2, and 5 h.

Statistical evaluation of the results was carried out by the method of Wilcoxon and Wilcox (8). Data are presented as mean±standard error of the mean (SEM). $P<0.05$ was considered statistically significant. Significance of $p<0.05$ and $p<0.01$ is shown by one and two symbols, respectively.

The airways smooth muscle reactivity in vitro condition

The animals were killed (cats by overdose of thiopental, guinea pigs by cutting the spinal cord in cervical region) 1 h after parenterally applied K^+_{ATP} ion channels agonist, antagonist/agonist medication or water for injections; trachea and lungs were immediately excided. Tracheal smooth muscle strips (two tracheal rings cut on the opposite side of the smooth muscle) and lung tissue strips (2x2x15 mm) from the right lungs were mounted between two hooks and placed into 30-ml organ chambers containing Krebs-Henseleit buffer of the following composition: NaCl 110 mmol.l^{-1}, KCl 4.80 mmol.l^{-1}, $CaCl_2$ 2.35 mmol.l^{-1}, $MgSO_4$ 1.20 mmol.l^{-1}, $KHPO_4$ 1.20 mmol.l^{-1}, $NaHCO_3$ 25 mmol.l^{-1}1, and glucose 10 mmol.l^{-1} in glass-distilled water. The chambers were maintained at 36.5±0.58 °C and aerated continuously with a mixture of 95 % O_2 and 5 % CO_2 to maintain pH 7.5±0.1. One of the hooks was connected to a force transducer (TSR 10 G, Vyvoj, Slovakia) and an amplifier (M1101 SUPR, Mikrotechna, Czech Republic), and tension recordings were made on Line Recorder TZ 4620 (Laboratorni Pristroje, Czech Republic). Tissue strips were initially set to 4 g of tension for 30 min (loading phase). Then the tension in each strip was readjusted to a baseline of 2 g for another 30 min (adaptation phase). During both periods, the tissue strips were washed at 10-min intervals. Thereafter, cumulative doses of acetylcholine (10^{-8}–10^{-3} mol.l^{-1}) and histamine (10^{-8}–10^{-3} mol.l^{-1}) were added, and a continual recording of contractions was made.

Student-t test was used for the statistical analysis of the obtained results. Data are presented as mean±standard error of the mean (SEM). $p<0.05$ was considered statistically significant.

Results

Antitussive activity of potassium ion channels modulators in guinea pigs

Evaluation of registered data showed that pinacidil, K^+_{ATP} ion channels opener, administered in dose 1 mg.kg^{-1} b.w. subcutaneously lead to statistically significant decline of citric acid-induced cough values in all measured time intervals in comparison with baseline cough efforts number (Fig. 1). The character and intensity of decrease was comparable to codeine effect. Pretreatment by selective inhibitor of K^+_{ATP} ion channels activity – glibenclamide applied in the dose 3 mg.kg^{-1} b.w. intraperitoneally almost reduced pinacidil influence on number of coughs except for measurement in 2 h time interval.

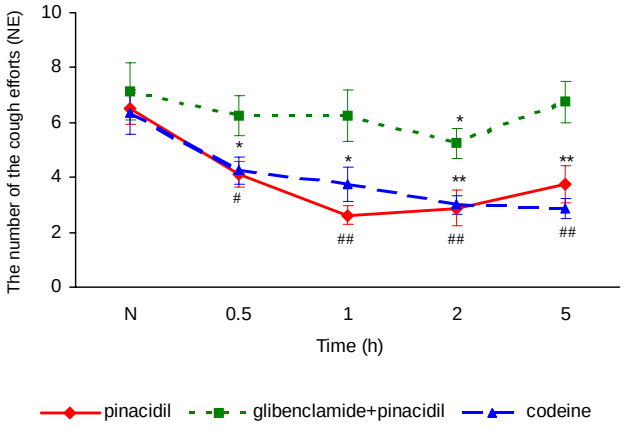

Fig. 1. The influence of pinacidil on the number of citric acid- induced cough (NE). The antitussive effects was assessed 0.5, 1, 2 and 5 h after s.c. administration of pinacidil (1 mg.kg^{-1} b.w.) compared with activity of codeine (10 mg.kg^{-1} b.w. intraperitoneally). The effect of the selective blocker s glibenclamide (dose 3 mg.kg^{-1} b.w. intraperitoneally) on antitussive effects of ATP-sensitive potassium ion channels opener followed in the same time intervals. The values labelled as N represents baseline measurements result before any agents application. p<0.05; p<0.01 codeine vs N values, # p<0.05; ## p<0.01 pinacidil vs N values.

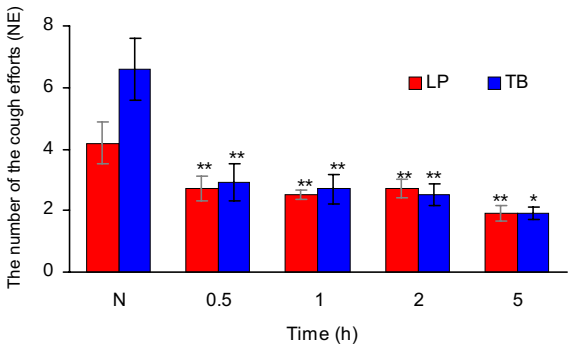

Fig. 2. Effect of pinacidil, agonist of K^+_{ATP} ion channels administered subcutaneously in 1 mg.kg^{-1} b.w. dose to conscious cats on number of cough efforts (NE) from laryngopharyngeal (LP) and tracheobronchial (TB) areas of airways. N is the control value of cough parameter, error bars indicate ±SEM, p<0.05 is marked with * and p<0.01 with **. The antitussive effect of pinacidil was assessed in 0.5, 1, 2 and 5 h intervals.

Antitussive activity of potassium ion channels modulators in cats

The agonist of K+ATP ion channels pinacidil applied by subcutaneous route of administration in the dose 1 mg.kg^{-1} b.w. in-

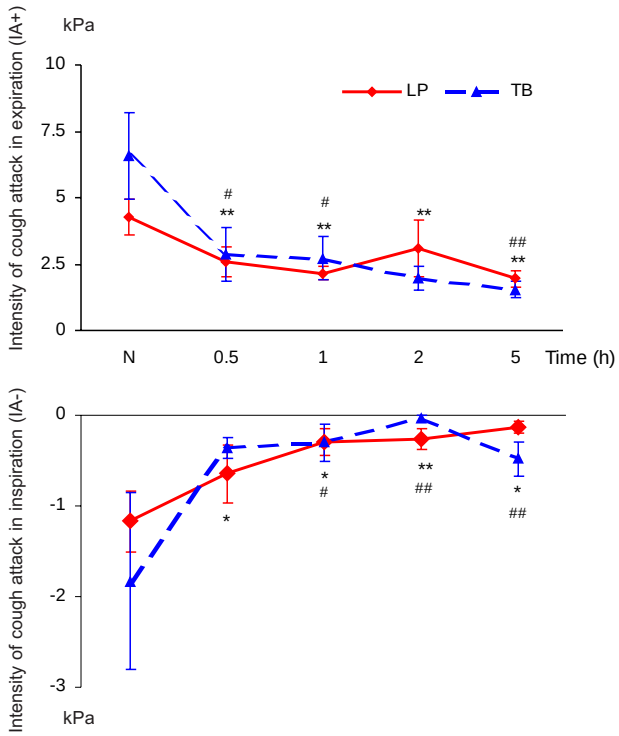

Fig. 3. Effect of pinacidil on intensity of cough attacks during expiration (IA^+) and inspiration (IA^-) from laryngopharyngeal (LP) and tracheobronchial (TB) areas in awaken cats. For other symbols see also Fig. 1. p<0.05; p<0.01 TB values vs N, # p<0.05; ## p<0.01 LP vs N values.

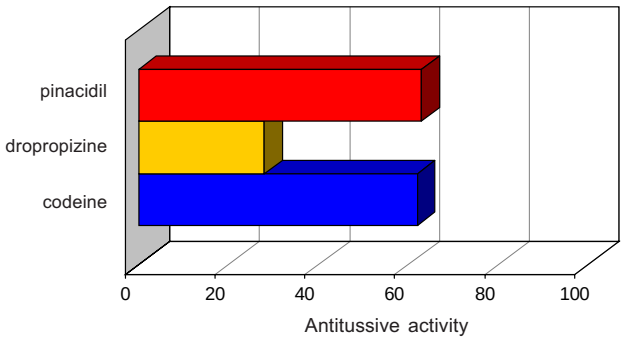

Fig. 4. Antitussive activity (in %) of pinacidil (expressed according to NE, IA^+ and IA^-) in comparison to reference drugs (codeine, dose 10 mg.kg^{-1} b.w. intraperitoneally and dropropizine, dose 30 mg.kg^{-1} b.w. intraperitoneally) on mechanically induced cough in conscious cats.

fluenced all followed parameters of mechanically induced cough reflex, especially main predictors of antitussive activity (NE, IA^- and IA^+). Their changes are presented in graphs. Pinacidil significantly decreased the number of cough efforts (NE) from both tracheobronchial (TB) and laryngopharyngeal (LP) irritated areas of the airways (Fig. 2). The first statistically significant drop of NE 30 min after administration of pinacidil indicated prompt onset of the effect. The suppressive effect lasted throughout all measured time intervals of the experiment. The intensity of cough attacks during inspiration (IA^-) and during expiration (IA^+) from both TB and LP airways regions remained also significantly reduced in all time intervals after administration of the compound except for two values from LP area: IA^+ 120 min and IA^- 30 min after applied K^+_{ATP} agonist (Fig. 3). The comparison of calculated antitussive activity (NE, IA^- and IA^+) showed that agonist of K^+_{ATP} ion channels pinacidil mildly exceeded same ability of centrally acting codeine. Furthermore, efficacy of this agent was impressively higher than effectiveness of antitussive with peripheral mechanism of action dropropizine (Fig. 4).

The modulators of potassium ion channels and ASM reactivity in guinea pigs (in vitro measurement)

We found that administration of K^+_{ATP} agonist pinacidil resulted in statistically significant decrease of tracheal smooth muscle response on cumulative doses of contractile mediator histamine and acetylcholine (Fig. 5). The reactivity was maximally inhibited ($p<0.01$) in concentrations 10^{-5}–10^{-3} mol.l^{-1} of acetylcholine and in the histamine concentration 10^{-4}–10^{-3} mol.l^{-1}.

The decrease of pulmonary smooth muscle reactivity on pinacidil application was less prominent than tracheal smooth muscle responsiveness. The statistically significant changes ($p<0.05$) of pulmonary smooth muscle strips were registered only oin higher concentrations of both used mediators: 10^{-5}–10^{-3} mol.l^{-1} of acetylcholine and 10^{-4}–10^{-3} mol.l^{-1} of histamine (Fig. 6).

Observed influence of potassium ion channels agonist pinacidil on airways smooth muscle reactivity was completely inhibited by antagonist glibenclamide pretreatment.

The modulators of potassium ion channels and ASM reactivity in cats (in vitro measurement)

Unlike guinea pigs, the reactivity of airways smooth muscle in cats was influenced by pinacidil only mildly. The contraction of tracheal smooth muscle strips had significantly lower amplitude in the acetylcholine concentration 10^{-3} mol.l^{-1} and in concentrations 10^{-7}, 10^{-4}–10^{-3} mol.l^{-1} of histamine (Fig. 7).

The reactivity of pulmonary smooth muscle stayed statistically unchanged despite the medication by pinacidil. There wereas observed a none statistically significant influence of K^+_{ATP} agonist pinacidil in comparison to of water for injection observed (Fig. 8).

Discussion

The cough and other airway's reflexes based on ASM reactivity are considered as essential physiological defense mechanisms preventing the airways obstruction. However, many airway diseases (e.g. asthma) are defined by changed ASM reactivity as well as excessive and somewhat deleterious coughing due to a lowering of the activation threshold for initiation of this reflex. As a result, cough may be evoked by stimuli that are normally considered innocuous. Except for centrally acting anti-

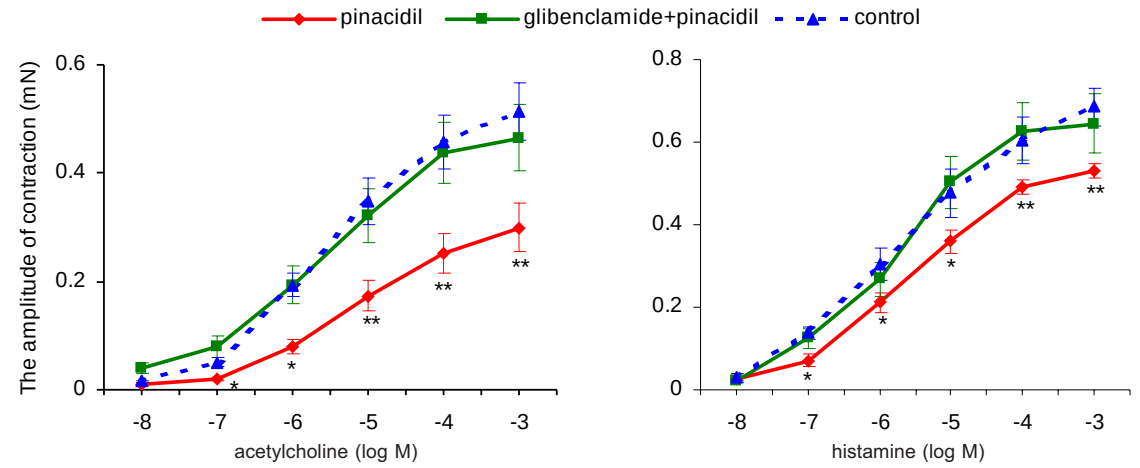

Fig. 5. The pinacidil induced changes of tracheal smooth muscle contractile response expressed as amplitude of contraction (mN) on cumulative doses of acetylcholine and histamine compared with control group of animals (received water for injection in the dose 1 ml.kg^{-1} b.w. intraperitoneally). The influence of glibenclamide on pinacidil effect measured under the same conditions. p<0.05; p<0.01 vs. control values.

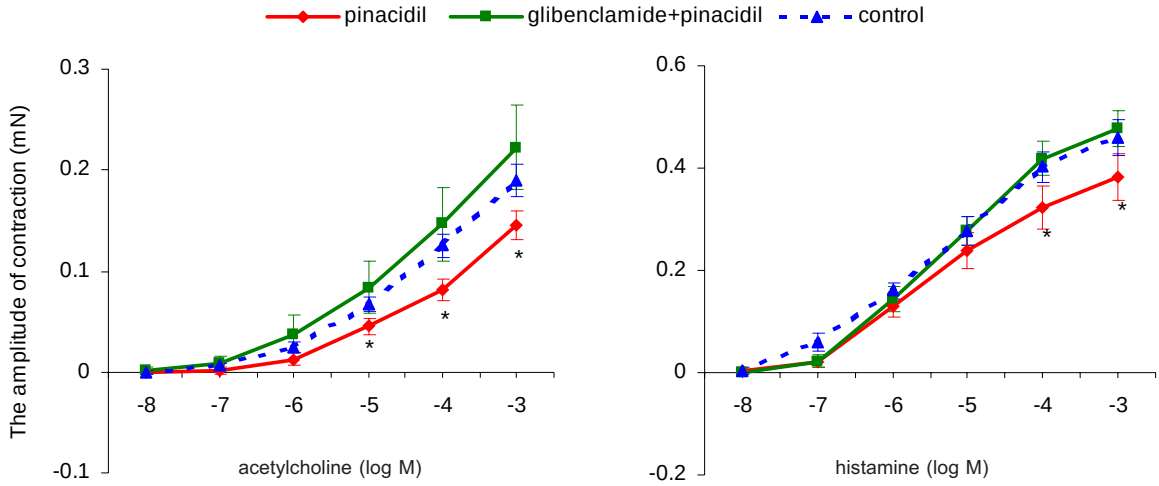

Fig. 6. The influence of pinacidil on the changes of pulmonary smooth muscle contractile response expressed as amplitude of contraction (mN) on cumulative doses of acetylcholine and histamine. The influence of glibenclamide on pinacidil effect followed under the same conditions. p<0.05; p<0.01 vs control values.

tussives, e.g. codeine (with generally known serious side effects), current antitussive therapies have proven to be inadequate for relieving persistent cough, providing only moderate relief for patients with cough disorders. This, in part, reflects our limited understanding the neuroanatomical and neurophysiological mechanisms that compose the sensory cough pathway, including participation of certain subcellular structures (9).

In the present study, we studied the role of K^+_{ATP} ion channels in cough and other airways defence reflexes based on ASM reactivity in addition with potential use of agents interacting with this structures as new drugs against the cough as well as agents reduced ASM responsiveness.

We found that administration of K^+_{ATP} agonist pinacidil produced marked decline of citric acid-induced coughs number in guinea pigs. Furthermore, antitussive activity of pinacidil was almost antagonized by pretreatment of selective blocker, glibenclamide. The significant effect of pinacidil in one s.c. applied dose (1 mg.kg^{-1} b.w.) lasted 5 h and intensity of cough suppression is very close to effects of centrally acting codeine. Similarly, pinacidil applied to cats resulted in distinct decrease of all mechanically-induced cough parameters. The antitussive activity of K^+_{ATP} agonist was comparable with codeine and significantly higher than the efficacy of peripheral antitussive agent dropropizine. Results of experiments indicated that the antitussive effect of pinacidil is mediated via activation of K^+_{ATP} ion channels. We confirmed Morita et al (10) findings, which demonstrated that K^+_{ATP} openers significantly and dose-dependently suppressed the capsaicin-induced cough in guinea pigs.

Although cough and bronchoconstriction are believed to have separate afferent neural pathways, they often occur simulta-

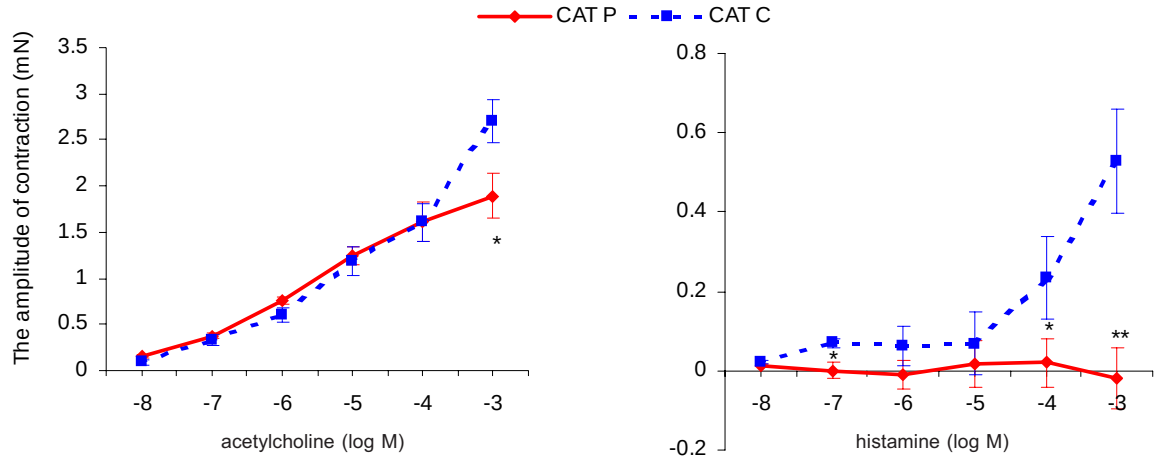

Fig. 7. The changes of tracheal smooth muscle contraction (mN) on cumulative doses of both mediators, acetylcholine and histamine, recorded on pinacidil s.c. administration in cats (curve labelled as CAT P). Measurements in vitro compared with control group (CAT C) of animals (received water for injection).

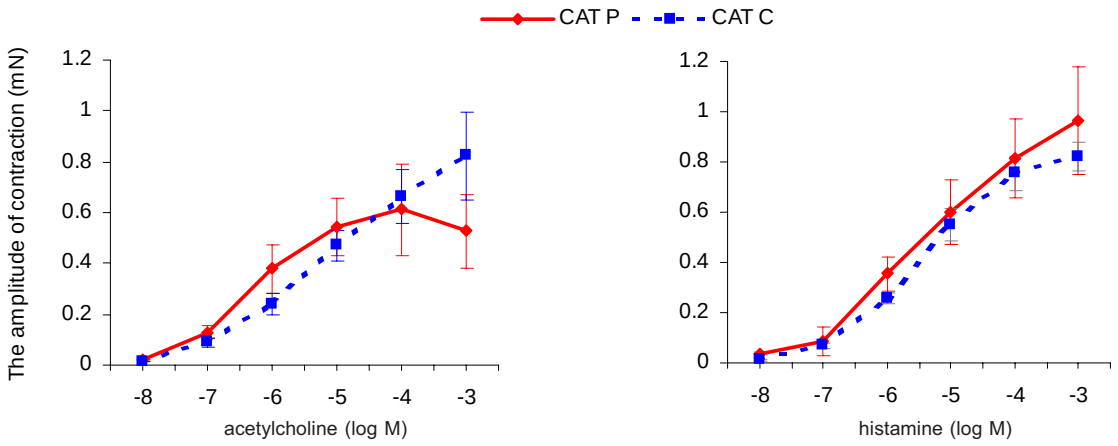

Fig. 8. The pulmonary smooth muscle reactivity on cumulative doses of contractile mediators, acetylcholine and histamine, influenced by s.c. applied pinacidil (CAT P) compared with control group (CAT C) in cats. Measurements in vitro, $p<0.05$; $p<0.01$ vs control values.

neously and have been considered to be closely related (11). Several literature data informed that promoting K^+ efflux through potassium ion channels inhibits bronchoconstriction as well as cough suppressive effect of bronchodilatory drugs had been also reported (12, 13, 14). The experiments *in vitro* with guinea pigs showed that K^+_{ATP} ion channels are involved in ASM reactivity. Therefore it is possible that antitussive effect of K^+_{ATP} agonist is partially a consequence of bronchodilation. Even though significant inhibition of ASM reactivity observed on pinacidil in guinea pigs, it is distinct that certain mechanisms independent from bronchodilatory activity are involved in antitussive effect of K^+_{ATP} agonist. While influence of pinacidil on ASM reactivity completely antagonized pretreatment by glibenclamide, its antitussive effect was abolished only partially.

Unlike guinea pigs, the pinacidil impressively suppressed experimentally induced cough in cats, but produced mild attenuation of ASM reactivity *in vitro* with significant decrease of contraction on higher concentrations of acetylcholine and histamine used. This result supported supposition regarded to relative independence of bronchoconstriction from cough reflex. In spite of the recorded ASM reactivity results, the participation of bronchodilation on pinacidil cough suppression cannot be strictly excluded. Recently, Poggioli at al (15) reported the antitussive effect of pinacidil in doses, which had no influence on stimuli-induced bronchospasms.

Experiments *in vitro* in guinea pigs showed that ASM reactivity, both tracheal and pulmonary, on cumulative doses of acetylcholine was reduced more significantly after s.c. administered pinacidil in comparison with contractile response on histamine. Except for K^+_{ATP} opening, other mechanism may participate on pinacidil bronchodilatory effect. There is increasing evidence that K^+_{ATP} activity plays an important role in NO-mediated, cGMP-independent, response to acetylcholine in more animal species (16, 17).

The relationship of K^+_{ATP} agonist pinacidil with airways defence reflexes was explored in a very suitable animal model-guinea pigs, in which the receptors distribution isin the airways is most similar to human respiratory system (18).

The efficacy of pinacidil on cough reflex in cats was tested by a method of mechanical stimulation of laryngopharyngeal (LP) and tracheobronchial (TB) mucous areas of airways. The mechanical impulse simulates the natural conditions of cough, induced by foreign solids or saliva. Moreover, it represents point stimulation; the intensity of irritation is constant and the possibility of receptor adaptation to this kind of irritation is unlikely (19). According to Korpáš and Nosáľová (20), the cats are the most suitable animals for the cough modeling and the testing of various substances for their effects on the cough reflex.

In conclusion we can summarize that ATP-sensitive potassium ion channels play an important role in cough and other defence reflexes of the airways coupled with reactivity of ASM in guinea pigs as well as in cats. These experiments also provide a basis for potential use of these ion channels openers as antitussives and in human therapy of airway diseases characterized by cough and hyperresponsiveness of ASM, such as asthma.

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