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Modulation of the Airway Tone by the Epithelium: Effects of Carbocisteine

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Short title:
Tube/ring tracheal preparation

Summary

We improved our previous model of tracheal tube preparation and examined the effects of oral treatment of rats with carbocisteine (CCS) and its interaction with bronchial epithelium. The model permitted isometric or isotonic measurements of smooth muscle contraction or relaxation in cannulated or tracheal ring preparations, with or without epithelium. We found that oral treatment with carbocisteine and not preincubation of preparations in vitro, diminished sensitivity of preparations without epithelium to carbachol (EC_{50}, -log(M) values: IN – luminary perfusion, -EP, controls vs. CCS: 5.8±0.06 vs. 5.5±0.09, $p<0.005$; OUT - serosal perfusion, -EP, controls vs. CCS: 5.9±0.06 vs. 5.6±0.05, $p<0.005$), while the sensitivity to aminophylline, degree of shortening, and the velocity of contraction of rat tracheal rings stimulated by 10^{-6}M carbachol was not affected. Normal sensitivity to carbachol stimulation was re-established if preparations were preincubated with capsaicin. We conclude that carbocisteine has small

inhibitory effects on the sensitivity to carbachol of the rat tracheal smooth muscle denuded of epithelium. Described model is valuable for examining the effects of bronchial epithelium on bronchial smooth muscle contraction.

Keywords: Smooth muscle models; tracheal epithelium; carbocisteine

Introduction

In this study we demonstrated the use of an improved model for testing bronchial smooth muscle contraction in either tube (cannulated tracheal segments) or tracheal rings preparations. Various airway tube preparations (cannulated trachea or bronchus) have been used in the past in an attempt to better mimic in vivo responsiveness of airways and dissociate pure smooth muscle responses from airway epithelium modulated smooth muscle responses (10). That interest is justified since there is increased evidence that airway epithelium is in close relation with underlying smooth muscle not only structurally but also functionally. Indeed, airway epithelium, which separates inhaled gas from underlying airway tissue, forms an important barrier between living structures and the environment. The preservation of its normal (secretory) function is a precondition for maintenance of an effective air - tissue barrier. In inflammation and particularly in asthma, the mucus gel becomes thicker, stagnant and could, together with oedema and bronchial smooth muscle

spasm, produce narrowing and plugging of the airways, seriously compromising air flow and ventilation. In addition, it seams that normal functioning of the bronchial epithelium is important for the maintenance of normal bronchial reactivity to various bronchoconstricting or relaxing agents. To further test the hypothesis that bronchial epithelium, either as a diffusion barrier or by actively secreting yet not well defined mediators (prostaglandins, leukotrienes or other mediators) could modulate bronchial smooth muscle sensitivity to various stimulating agents (see 12, 20 and 25 for references) we used our newly adapted system of perfused tracheal tubes.

The subject of our study was mucolytic agent carbocisteine (S-carboxymethyl-L-cysteine) (CCS) that was found to favour production of sialomucins at the expense of fucomucines (5, 11) which could result in the improvement of mucociliary transport. Improving mucociliary transport is one of the therapeutic goals in asthma therapy, where in addition to anti-inflammatory agents (14, 26) mucolytic agents are frequently used (2, 17). Existing controversy about the mechanisms of action of carbocisteine (4, 15), finding that it could produce a reduction

(6), or an increase in sputum viscosity (24), or even to have an anti-inflammatory action by promoting sialoglycoprotein secretion which could have kinin-inhibitory effects (19) - further justified our aim: to examine the role of bronchial epithelium as a specific target tissue of that mucolytic agent and its influence on bronchial smooth muscle isometric (cannulated) or isotonic (rings) contraction. The preliminary results that were done in the earlier described model (20) already showed that carbocisteine could influence sensitivity of tracheal smooth muscle of the rat (3, 23). Particular facility of the proposed model, i.e. the possibility to easily switch from a tube to a ring preparation, permitted us to examine selectively effects of inner (epithelial) or outer (serosal) perfusion with an agent which influences the bronchial smooth muscle tone.

Methods

Experiments were performed on tracheas taken from male Sprague - Dawley rats weighing 390 - 420 g. All animals were housed in individual cages and received water and food ad libitum. The group of pretreated animals (CCSpr) received carbocisteine mixed with water (200 mg/kg/ day) for two weeks. During the third week they were sacrificed for the experiments. They were stunned by a blow on the head and quickly exsanguinated. The tracheas were immersed in Krebs solution (in mM: 137 NaCl, 4 KCl, 1 $MgCl_2$ x $6H_2O$, 1 KH_2PO_4, 12 $NaHCO_3$, 2 $CaCl_2$, 6.5 glucose) and cleaned from surrounding tissue.

Perfusion studies. Proximal tracheal ends (10 tracheal rings long) were used for the experiments. In one half of the preparations the epithelium was removed (-EP) by gently rubbing with a cotton - wrapped metal stick; in the other half of the preparations the epithelium was left intact (+EP).

Under microscopic control two stainless steel hooks were

passed through the tracheal wall around two adjacent cartilagineous rings as close as possible to the tracheal muscle insertions. The tracheal segment was then longitudinally connected to steel tubes built in the "carrier block" of the apparatus (in-out system) and firmly tightened with silk thread. The apparatus used (Fig. 1A, EMKA Technologies, Paris, France) was an improved version of the cannulated tracheal system described previously (20). Improvements consisted of the fact that the lower hook, which served as a "fixed point", was attached with the silk thread to the micrometric screw serving to adjust the hook tension on the tracheal wall. This allowed precise adjustment of the resting tension. The upper hook was connected to a force transducer (IT1-25, EMKA Technologies), the latter being attached to micromanipulator which permitted displacement of the upper hook along a strict vertical axis. Any change in tension at the level of the tracheal muscle was registered by the recorder (Gould AT 550) to which the amplified signal (EMKA technologies 4 ways Amplifier) from transducer was connected. The "carrier block" for cannulated trachea could be easily removed and replaced by one suitable for tracheal rings mounting (Fig. 1B, ring

system, below). Described system accommodates for use of electrical field stimulation, which was not used in this experiment.

The Krebs solution (at 37°C, pH 7.4, gassed with 95% O_2/5% CO_2) was perfused at a constant flow rate (2ml/min) through the organ bath (outer perfusion - OUT) and through the lumen of the tracheal segment (inner perfusion - IN) by using peristaltic pumps (Watson Marlow 5025, Falmouth, Cornwall, UK).

Fluid tightness of preparation. Methylene blue test. To ensure that the hooks did not induce fluid leak through the tracheal wall, in separate experiments (n = 4) a solution of methylene blue was perfused into the tracheal lumen or into the bath. No cross - staining was observed. Bio-assay. In addition to the methylene blue test a bio-assay was performed (n = 5) in the following way. In the same organ bath in which the cannulated tracheal segment was mounted a tracheal ring consisting of two tracheal cartilages was suspended between the stainless steal hooks, one served as a fix point and the other was attached to the isometric force transducer. Following a stabilisation period of the one

hour the luminary (epithelial side) of the cannulated tracheal segment was perfused with carbachol 10^{-3}M. The tension of both preparations (cannulated tracheal segment and the ring preparation) were recorded. Carbachol 10^{-3}M fully contracted the cannulated tracheal segment whereas no change in tension was observed in the tracheal ring preparation. This demonstrated that there was no significant leakage of solution from the luminary side of the cannulated tracheal segment towards outside bath solution.

Tracheal rings. To determine the effects of carbocisteine on the degree of shortening and the velocity of contraction, tracheal ring preparations was mounted in the customary manner using ring system of the apparatus connected to the isotonic force transducer (Harvard Apparatus, South Natik, MA, USA), linked to the paper recorder (GOULD AT 550, Cleveland, OH). The tracheal muscle was stretched to its optimal length which was established, in the preliminary experiments, to correspond to a counter - weight of 1.0g.

Procedure.

Perfusion studies. In this experimental model studies were realised on preparations with and/or without epithelium taken from animals pretreated with carbocisteine (CCSpr) or from the control preparations (contr).

After a period of stabilisation (45-60 min.) the tracheal muscle was stretched to its optimal length. Preliminary essays were performed to determine optimal stretch of the muscle as previously described (22). The length - tension relationship was not found to differ significantly between preparations (CCSpr) and the controls (contr).

In the set of preliminary experiments cumulative concentrations of carbachol (10^{-7} to 10^{-3}M) were perfused IN (+EP or -EP) or OUT (+EP or -EP) in (CCSpr) or from the controls (contr) (n = 8 for each). Since the accent of the work was to test the new improved model, we restricted control experiments to only those which were mandatory. Accordingly, we concluded from preliminary experiments that responses to IN and OUT stimulation in -EP preparations were identical (on the basis of EC_{50} and maximal responses), and in order to further examine observed effects in preparations -EP on (IN) stimulation with carbachol,

we designed another 3 sets of experiments.

First, to examine effect of preincubation, preparations taken from untreated animals were incubated for 60 min. with carbocisteine 10^{-3}M (CCSinc; -EP, n=8) and cumulative concentrations of carbachol were perfused luminary the preparations (IN).

In the second set of experiments preparations CCSpr (-EP) were preincubated for 60 minutes in indomethacin 10^{-6}M (INDinc; n =8) or capsaicin 10^{-5}M (CAPSinc, n = 6) and cumulative concentrations of carbachol (IN) were perfused. Effects of carbocisteine on relaxant effects of aminophylline were examined in preparations CCSpr (-EP) which were first pre-contracted with medium concentration (10^{-6}M) of carbachol (OUT) and then perfused with aminophylline (IN) 10^{-8} to 10^{-3}M (n =10) and compared with responses to aminophylline in preparations (contr) (n = 6). In the third set of experiments, to examine direct relaxant effects of carbocisteine, preparations taken from the control animals were pre-contracted (OUT) with medium concentration of carbachol (10^{-6}M) and then perfused (IN) with cumulative concentrations of carbocisteine 10^{-7} to 10^{-3}M (+EP, n = 7; -EP, n =

7).

Tracheal rings. Degree of shortening (maximal shortening) and relative velocity of contraction (time to 50, 80 and 100% of maximal shortening) elicited with carbachol of preparations taken from animals pretreated (n=11) and not pretreated (n=11) with carbocisteine (+ or - EP) were compared. Relative degree of shortening was used because measuring the small size change of the rat tracheal muscle required extremely precise instrumentation not available in the laboratory. After the equilibration period, the preparations were stimulated with carbachol 10^{-6}M. When maximal shortening of the tracheal muscle was achieved, they were washed with Krebs solution and allowed to relax completely. Then, the preparations were preincubated for 60 min. in carbocisteine (10^{-3}M) and stimulated again with carbachol (10^{-6}M).

Substances. The following substances were used: carbachol (carbamylcholine chloride, Sigma), indomethacin (Sigma), aminophylline (theophylline - ethylene diamine, Pharmacie Centrale des Hopitaux,

Paris, France), capsaicin (Sigma), and carbocisteine (Park-Davis, 45071 Orleans, France). The carbocisteine was diluted in 10% NaOH and final dilutions made in Krebs solution.

Analysis of results. The data are expressed in percent of maximal response and in absolute values (g and second) and given as means ± s. e. means. Half - maximal concentration (EC_{50}) values were calculated by means of regression analysis of probit - transformed data and the results are given as means of -log EC_{50} values obtained. Statistical analysis was conducted by use of analysis of variance and the Student's t-test for paired or unpaired data adjusted for multiple comparisons, as appropriate. $p<0.05$ was regarded as being statistically significant.

Results

The animals pretreated with oral carbocisteine did not show any clinical sign of disease or metabolic disturbances.

Contractility. In preparations taken from animals pretreated with oral carbocisteine for 7 days (CCSPr) we found diminished sensitivity to carbachol in preparations (-EP), but not in preparations (+EP) (Table 1 and 2; Fig. 2). Contrary to the oral pretreatment effect, preincubation for 90 minutes in carbocisteine (CCSinc) did not affect sensitivity of the preparations (-EP) to carbachol (IN) stimulation [-log EC_{50}: 5.71 ± 0.07 (x ±s.e.m.)] (Table. 3).

Removal of the epithelium increased sensitivity to carbachol in all preparations except in the control preparations stimulated with carbachol (OUT) (Table 1 and 2). All preparations (+EP) were more sensitive to carbachol (OUT) stimulation then to carbachol (IN) stimulation. Interestingly, diminished sensitivity to carbachol (IN) stimulation of preparations CCSpr(-EP) was absent in preparations

preincubated with capsaicin 10^{-5}M (CAPSinc) [-log EC_{50}: 5.99 ±0.25 (x ±s.e.m.)] but was maintained in preparations pre-incubated with indomethacin 10^{-6}M (INDinc) [-log EC_{50}: 5.59 ± 0.09 (x ±s.e.m.)] (Table 3; Fig. 3). In preparations (+EP or - EP) pre-contracted with (10^{-6}M) carbachol (OUT) carbocisteine alone in the concentration range from 10^{-7} to 10^{-3}M (IN) had neither contracting nor relaxant properties. Also the maximal tension (Tmax) developed following stimulation with carbachol did not depend on the side of stimulation (OUT or IN), and was not affected by the removal of epithelium, pretreatment with carbocisteine, preincubation in carbocisteine 10^{-3}M, indomethacin 10^{-6}M or capsaicin 10^{-5}M (results not shown). Sensitivity to aminophylline (IN) in the preparations (-EP) precontracted with carbachol 10^{-6} (OUT) was not affected by pretreatment with carbocisteine (-log EC_{50}: 3.23 ±0.1 / 3.66 ±0.11; CCS / Controls; n. s.). The degree of maximal shortening and the velocity of contraction of the rat tracheal ring (+ and - EP) stimulated by 10^{-6}M carbachol was not affected either by pretreatment or preincubation with carbocisteine (Fig. 4).

Discussion

We used an improved in vitro model which permitted independent perfusion of the epithelial (luminary) and serosal (outside) aspects of the airway (20, 21, 22). Important feature of this preparation was that each surface can be stimulated independently and two potentially different responses distinguished. Furthermore, tracheal epithelium could be mechanically removed and responses compared, permitting not only comparison of the responses to luminar and outside pharmacological stimulation, but also to better appreciate the influence of tracheal epithelium removal on tracheal muscle contraction. The in vitro model used this time was essentially the same as the model previously described except (i.) the pull of the hook on the tracheal wall which could be adjusted with precision even during the experiment (Fig 1). Before attempting to adjust the smooth muscle resting tension, the lower hook was pulled until minimal tension on the higher hook was perceived, and at that position the micrometric screw was blocked. This allowed more

precise adjustment of the tension on the tracheal muscle and optimal determination of the stretch. (ii.) The system (now ready to be used) incorporates interchangeable carrier for tracheal or bronchial ring preparation. This facilitates comparative experiments and does not impose significant financial burden when doubling equipment and use of both techniques is necessary.

In our experimental study we found that removal of epithelium rendered preparations more sensitive to carbachol stimulation. On the other hand, pretreatment with carbocisteine rendered preparations without epithelium less sensitive to carbachol stimulation as compared to controls (-EP). These changes, although small, were significant and require an explanation. The results of a number of experimental studies, including ours, have clearly demonstrated that tracheal epithelium can modulate tracheal smooth muscle contraction (7, 12, 20, 25). However, it seems that not only the contracting but also the relaxing effects of some pharmacological agents are dependent on the presence of intact epithelium (7). This may be relevant for better understanding different airway pathologies. It has been shown that bronchial epithelium is damaged in

patients with severe asthma, illustrating that bronchial epithelial damage and bronchial hyperreactivity could be linked (13).

There are studies which have demonstrated that bronchial epithelium could be a powerful diffusion barrier (18, 20) and could attenuate the effects of pharmacological agents applied luminary. Our present finding of diminished sensitivity to carbachol (IN) as compared to carbachol (OUT) in preparations with intact epithelium further supports this hypothesis. In our earlier study we found difference of the rate of tension development but we did not find difference in sensitivity in response to IN and OUT stimulation (20). However previously we used younger animals (300g) than in this study (400g). We believe that the capacity of the epithelium to act as a diffusion barrier depends on the tightness of intercellular junctions, epithelial cell functional properties, and epithelial cell geometry. All of these could be species or/and age dependent.

In the present study, diminished sensitivity of tracheal smooth muscle denuded of epithelium was observed following pretreatment of animals for 2 weeks with oral carbocisteine and not in tracheal preparations

preincubated with the agent in vitro. Our findings confirm our earlier preliminary experimental results (3, 23) obtained in a preceding in vitro model (20) and suggest that, following carbocisteine pretreatment, (i) epithelial diffusion barrier increases (very low sensitivity to carbachol in preparations +EP, IN), (ii) epithelium presence increases relative sensitivity of preparations, and (iii) epithelium removal decreases sensitivity of preparations; resultant effects of (i) and (ii) being visible in preparations CCSpr, +EP, IN) and that of (iii) in preparations CCSpr, -EP, IN or OUT.

There is evidence that blocking enkephalinase, which degrades kinins by phosphoramidon, could increase bronchoconstriction similarly as epithelium removal (9). Indeed, mechanical removal of the epithelium could produce liberation of different mediators from mast cells, which could be responsible, at least in part, for bronchial hyperresponsivnes observed experimentally (8). It has been shown also that carbocisteine could increase production of sialomucines (5, 11) and suggested that sialomucines could have an anti kinin action (19), what could explain diminished sensitivity of the preparations denuded of the epithelium to

carbachol stimulation.

However, capsaicin sensitive nerve terminals could secrete various mediators (16). It is conceivable that some as yet unidentified mediators originating from capsaicin nerve terminals could have inhibitory effects on tracheal smooth muscle contraction. Nerve terminal destruction by capsaicin and the disappearance of the putative inhibitory agent could have in turn increased the sensitivity of tracheal smooth muscle to carbachol as compared - in our experiments - to the preparations (CCSpr) not pre-incubated with capsaicin. On the other hand, pretreatment with carbocisteine could have promoted production of some epithelium derived contracting factor (EpDCF). Removal of the epithelium and removal of an excitatory agent secreted by the epithelium could have rendered tracheal smooth muscle less sensitive to carbachol, which would explain our finding of diminished sensitivity in CCSpr(-EP) preparations. The prostaglandins, products of arachidonic acid metabolism, do not seem to be involved since preincubation of the preparations in Indomethacin did not affect the sensitivity of preparations. The fact that carbocisteine did not have a direct and immediate effect on

tracheal smooth muscle reactivity suggests a complex indirect mechanism. It is intriguing that one very recent study, that is available only in Japanese, so we are not going to list it as a reference, suggests that carbocisteine could modulate cAMP level - what would offer quite comfortable explanation for its effects on bronchial smooth muscle contractility (Ishibashi Y, Okamura T, Masumoto Y, Tachiiri T, Momo K. Effects of carbocisteine on airway inflammation and related events in SO_2-exposed rat. Nihon Kokyuki Gakkai Zasshi, 39: 17-23, 2001).

In conclusion, we have presented an improved model of bronchial preparation for use in cannulated preparations and, with little adjustment, for bronchial rings, which makes the system easily accessible to the investigators. Availability of this system could encourage further investigation of the role of the bronchial epithelium in bronchial smooth muscle contraction. In this improved model of cannulated isolated trachea we have demonstrated that pretreatment of animals with carbocisteine induces relatively small decrease of reactivity of rat tracheal smooth muscle denuded of epithelium. As carbocisteine is widely used as supplementary therapeutics in various pathologies

characterised by airway obstruction, further explanation of its mechanisms of action would be valuable for better understanding possible beneficiary effects.

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Table 1 **EC_{50}, -log (M), for carbachol (IN)**

	+EP	-EP	p
contr	5.40 ±0.11	5.85 ± 0.06	<0.005
CCSpr	5.19 ±0.08	5.52 ± 0.09	<0.005
p	n.s.	<0.005	

Means (± s. e. mean) of -log EC_{50}, -log (M) values, obtained in preparations with (+EP) and without epithelium (-EP), taken from controls (contr) or animals pretreated for 2 weeks with oral carbocisteine 200mg/kg/day (CCSpr), following stimulation from the epithelial side (IN) with cumulative concentrations of carbachol (n. s. = non significant).

Table 2 **EC_{50} for carbachol (OUT)**

	+EP	-EP	p
contr	6.01 ± 0.09	5.92 ± 0.06	n.s.
CCSpr	6.17 ± 0.08	5.61 ± 0.05	<0.005
p	n.s.	<0.005	

Means (± s. e. mean) of -log EC_{50}, -log (M) values, obtained in preparations with (+EP) and without epithelium (-EP), taken from controls (contr) or animals pretreated for 2 weeks with oral carbocisteine 200mg/kg/day (CCSpr), following stimulation from the serosal side (OUT) with cumulative concentrations of carbachol (n. s. = non significant).

Table 3 EC_{50} for carbachol (-EP, IN) following preincubation

	-EP
contr	5.85 ± 0.06
CCSinc	5.71 ± 0.07
CCSpr	5.52 ± 0.09
CCSpr (INDinc)	5.59 ± 0.09
CCSpr (CAPSinc)	5.99 ± 0.25

Means (± s. e. mean) of -log EC_{50}, -log (M) values, obtained in preparations without epithelium (-EP) following stimulation from the epithelial side (IN) with cumulative concentrations of carbachol. Preparations were taken from controls (contr) and pre-incubated in carbocisteine 10^{-3}M (CCSinc) or from animals pretreated for 2 weeks with oral carbocisteine 200mg/kg/day (CCSpr) and pre-incubated in

indomethacin 10^{-6}M (INDinc) or capsaicin 10^{-5}M (CAPSinc) (contr/ CCSpr $p<0.005$, values presented also in the table 1, CCSpr/CCSpr (CAPSinc) $p<0.03$).

Figure legends

Figure 1

Schematic representation of the experimental apparatus. (A) The new tracheal tube preparation with the "carrier" block. Inner, luminary (IN) and outer (OUT) perfusion solutions are maintained at 37° C, bubbled with 95% O_2 - 5% CO_2 and a constant flow rate of 2 ml/min is maintained. Under microscopic control two stainless steel hooks were passed through the tracheal wall around two adjacent cartilagineous rings as close as possible to the tracheal muscle insertions. The lower hook, which served as a "fixed point", was attached with the silk thread to the micrometric screw serving to adjust the hook tension on the tracheal wall. This allowed precise adjustment of the resting tension. The upper hook was connected to a force transducer. (B) Tracheal ring preparation. The "carrier block" for cannulated trachea could be easily removed and replaced by one suitable for tracheal rings mounting.

Figure 2

Cumulative concentration - responses curves constructed after administration of carbachol epithelialy (IN, a and c) or serosaly (OUT, b and d) in rat isolated trachea with (+EP, a and b) and without epithelium (-EP, c and d). Preparations were taken from control animals or animals pretreated for two weeks orally with carbocisteine 200mg/ kg/day (? controls, ? CCSpr). Tension is expressed as percentage of the maximal tension (Tmax) obtained and presented as mean data ± s. e. mean.

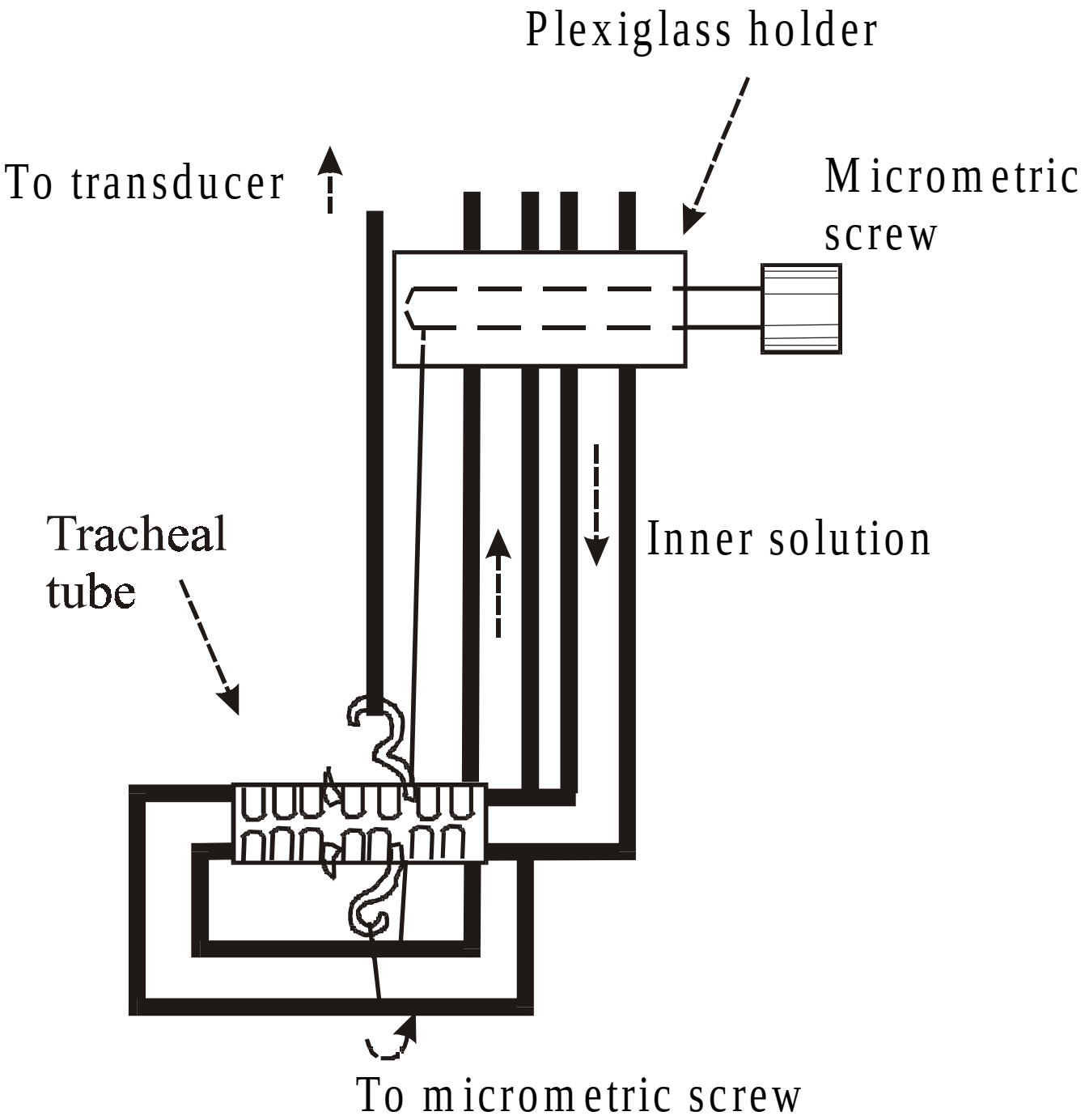
Figure 3

Cumulative concentration - responses curves constructed after administration of carbachol epithelialy (IN) in rat isolated trachea without epithelium (-EP). Preparations were taken from animals pretreated for 2 weeks with carbocisteine 200mg/kg/day and not incubated (CCSpr, ?) or incubated for 60 min. in capsaicin 10^{-6}M (CCSpr + capsaicin, ?). Tension is expressed as percentage of the maximal tension (Tmax) obtained and presented as mean data ± s. e. mean.

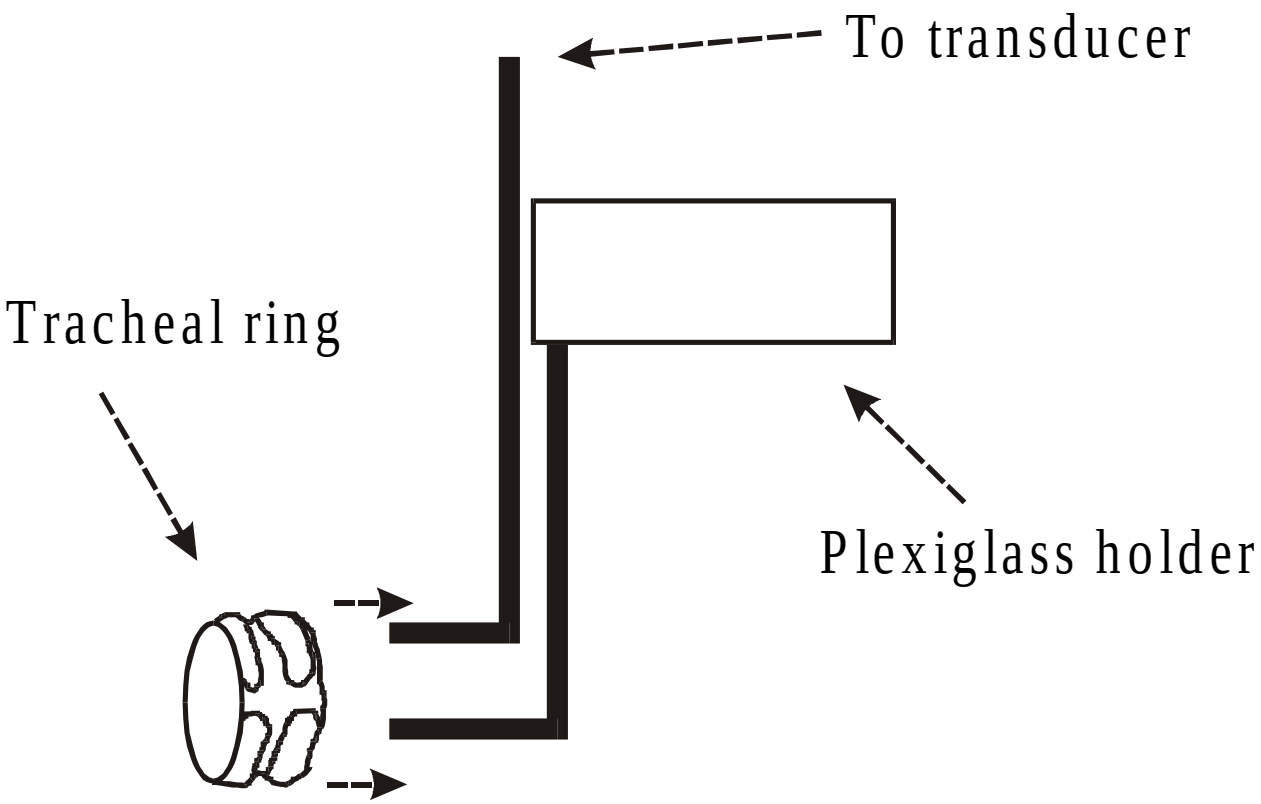
Figure 4

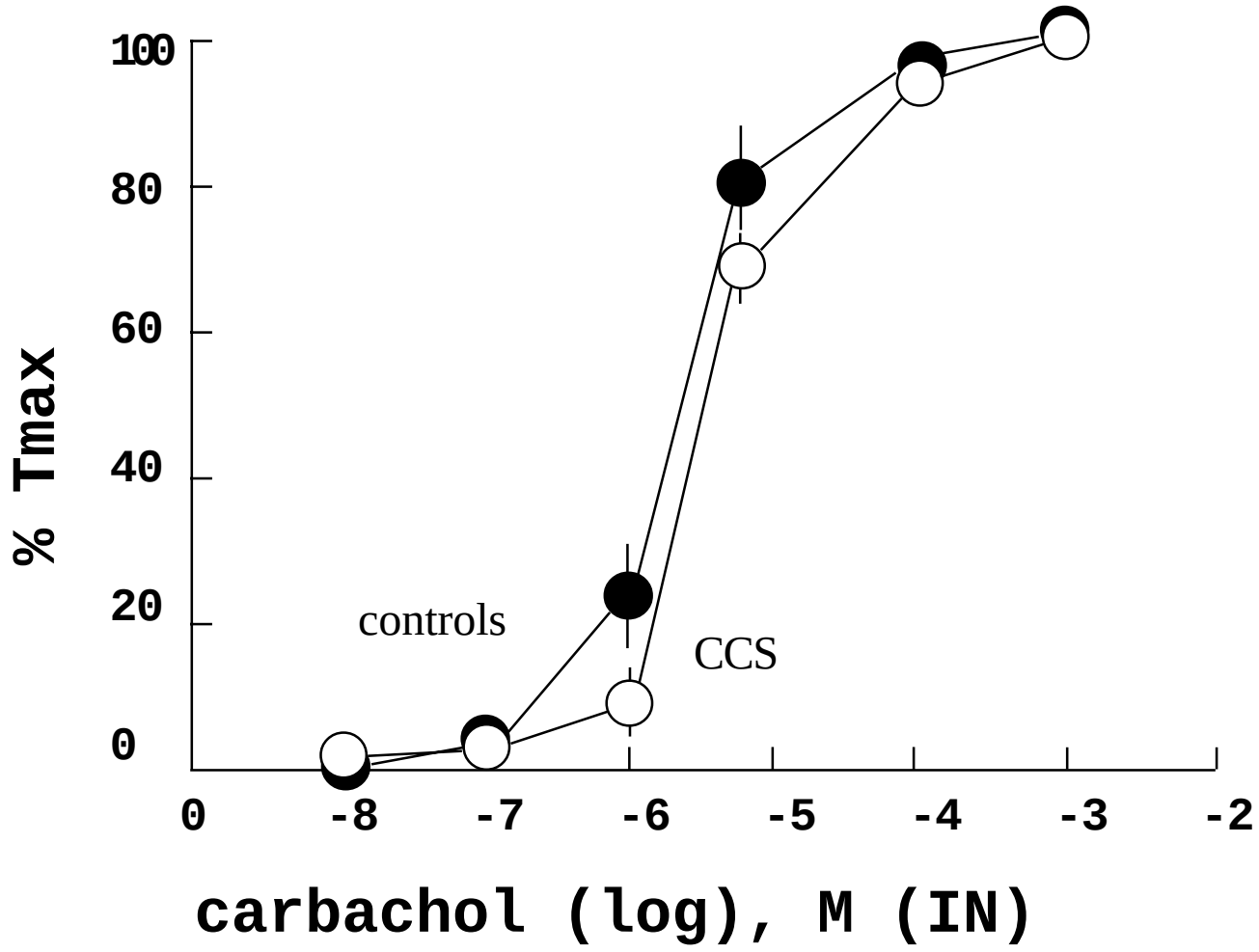
Relative velocity of contraction (time to 50, 80 and 100% of maximal shortening) elicited with carbachol 10^{-6}M in tracheal rings preparations taken from animal pretreated with oral carbocisteine (abscissa: time in seconds; ordinate: degree of isothonic shortening in percents of maximum (mean ± s. e. mean); ?, CCSpr (+EP); ?, controls (+EP) ?, CCSpr (-EP), and ?, controls (-EP); (differences not significant).

A.


Plexiglass holder
To transducer
Micrometric screw
Tracheal tube
Inner solution
To micrometric screw

B.

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carbachol (log), M (IN)

Fig 2a

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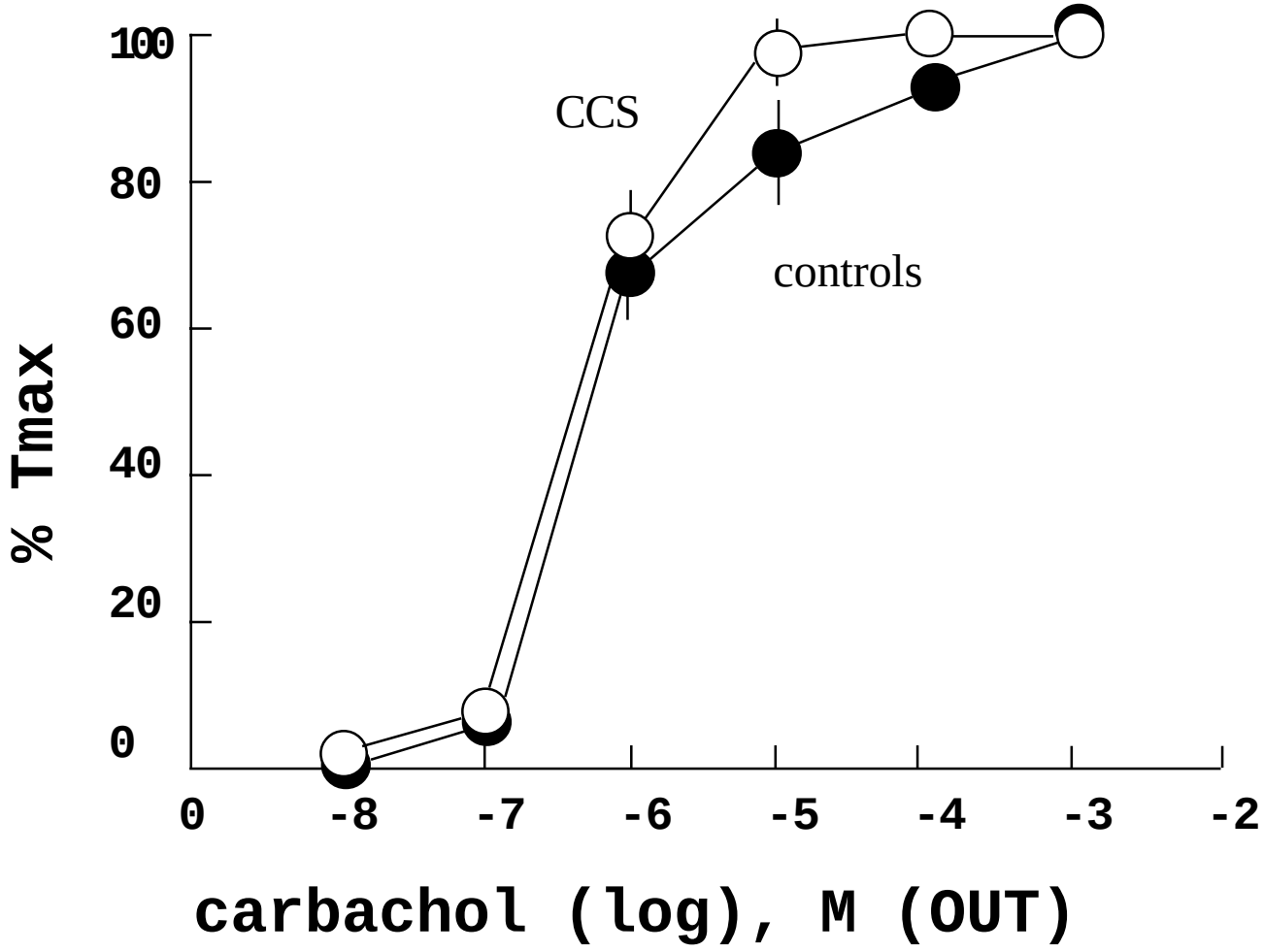
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Fig 2b

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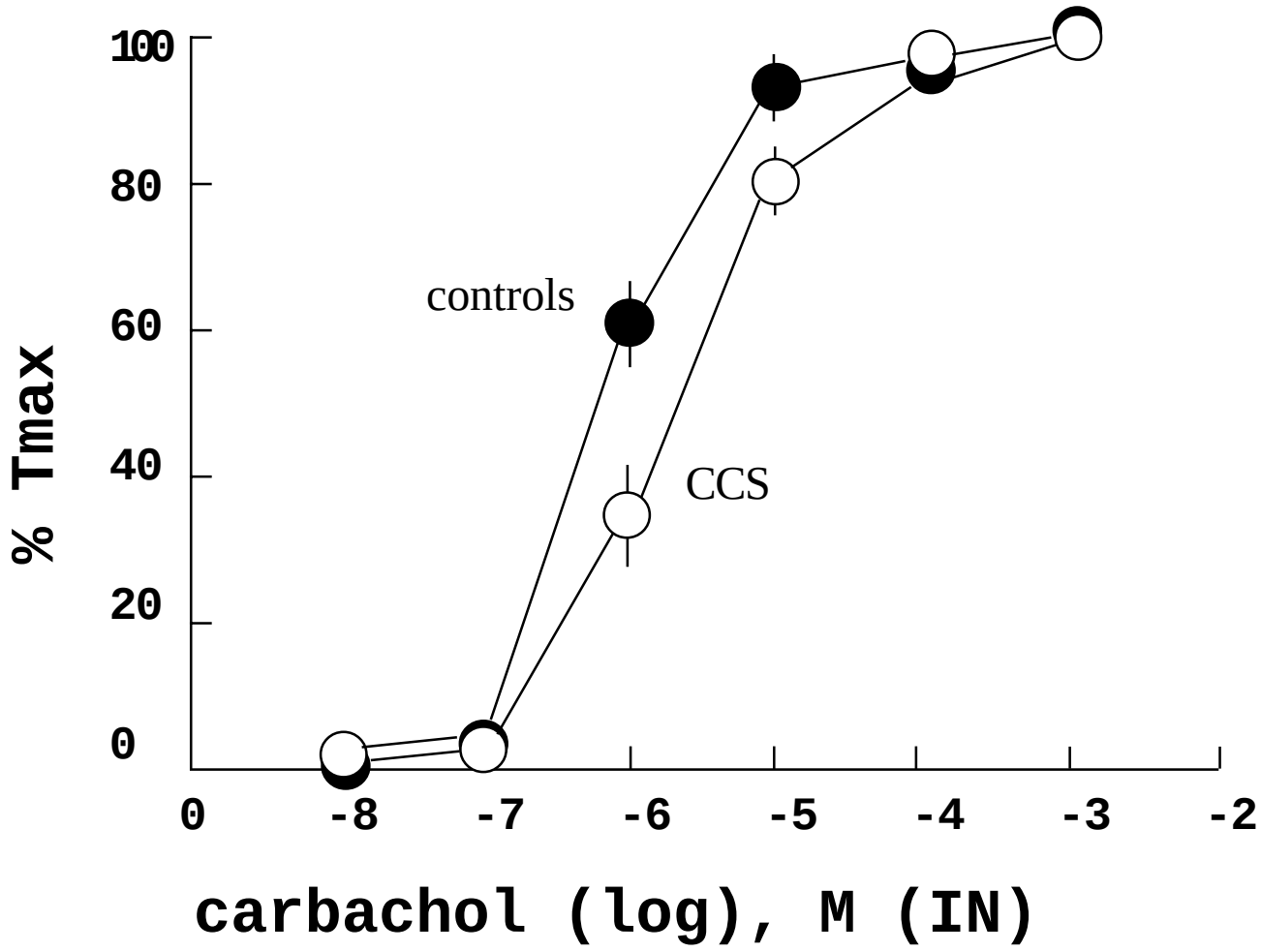
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Fig 2c

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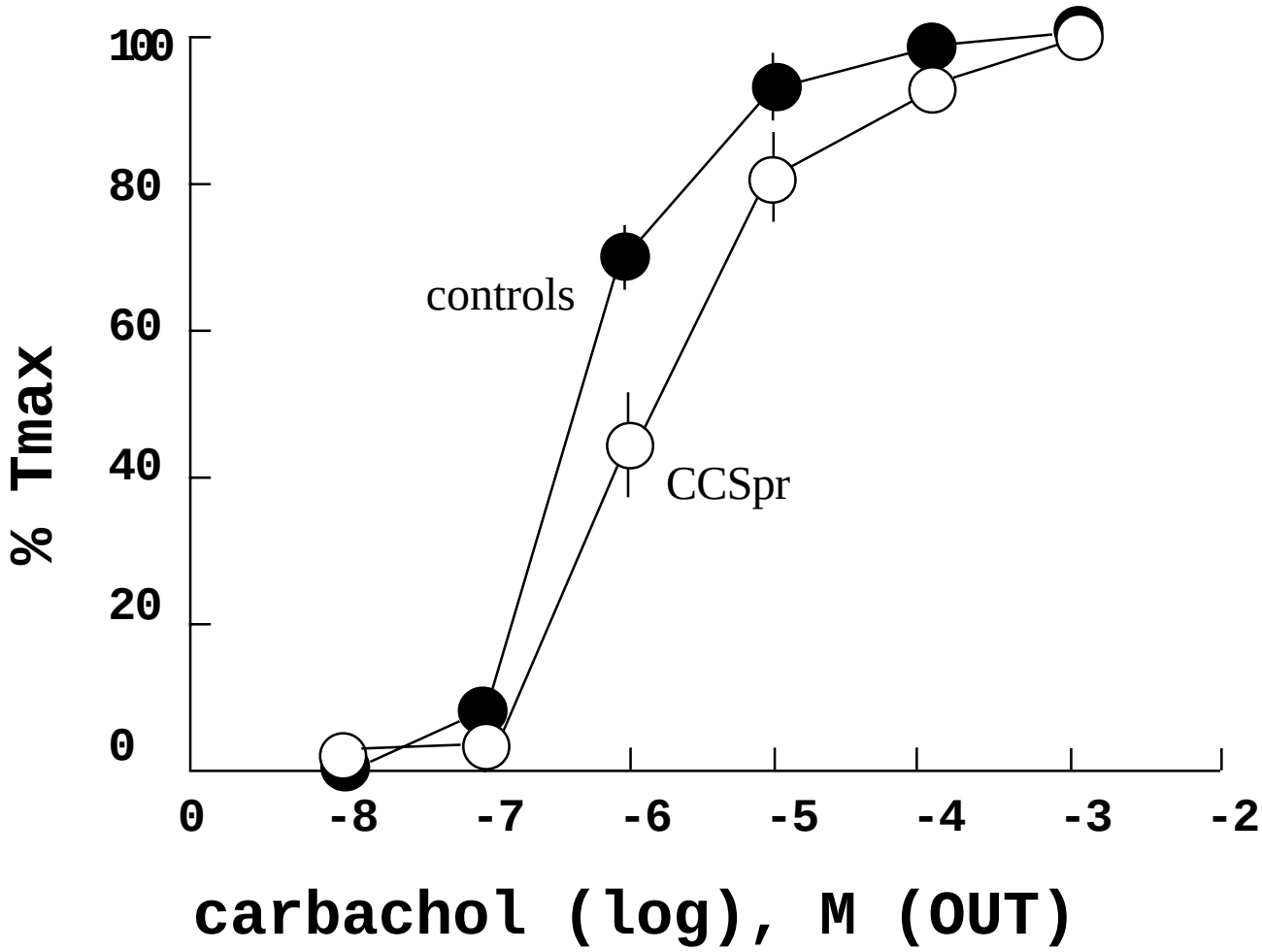
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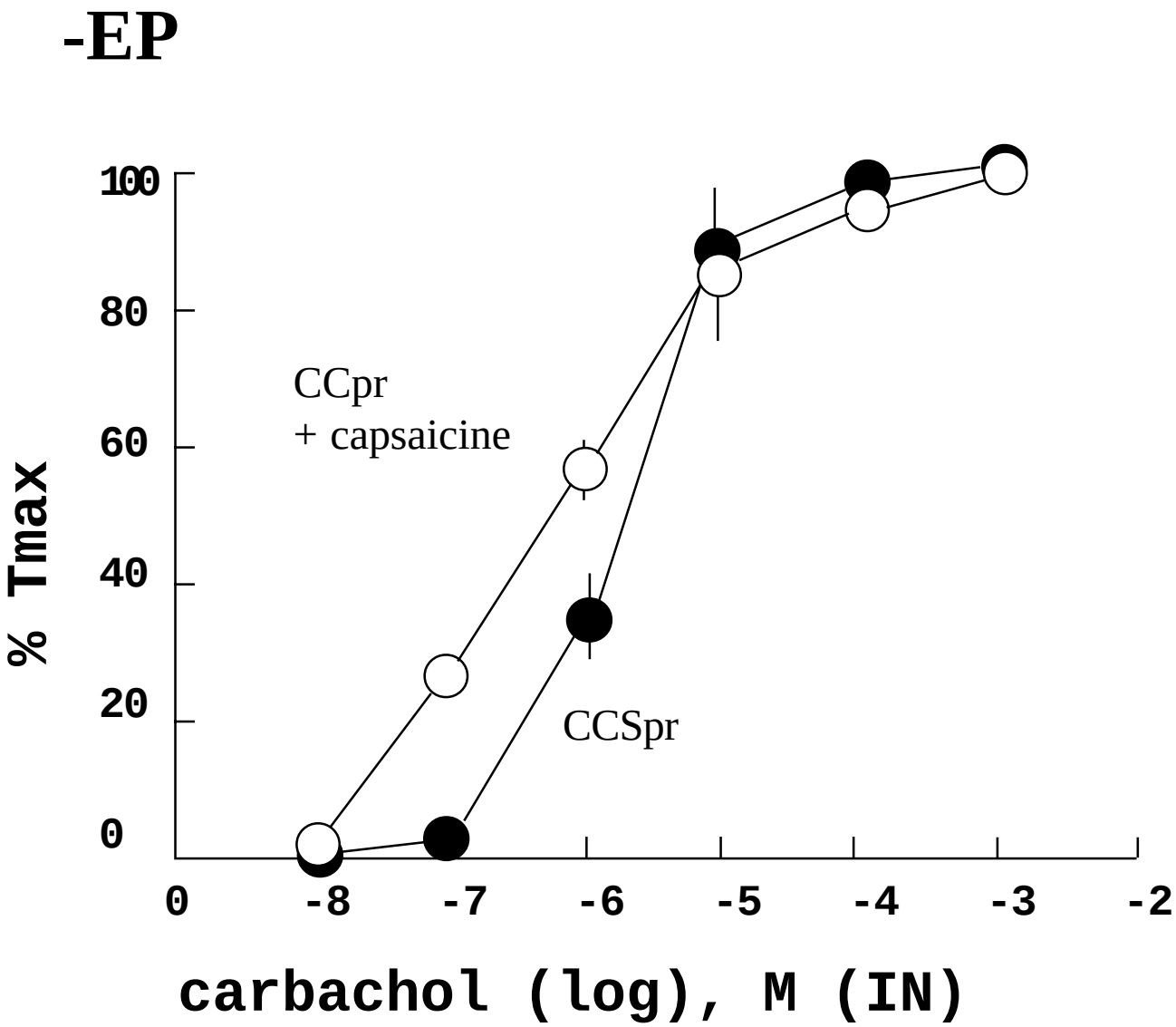
Fig 2c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carbachol (log), M (IN)

Fig 3

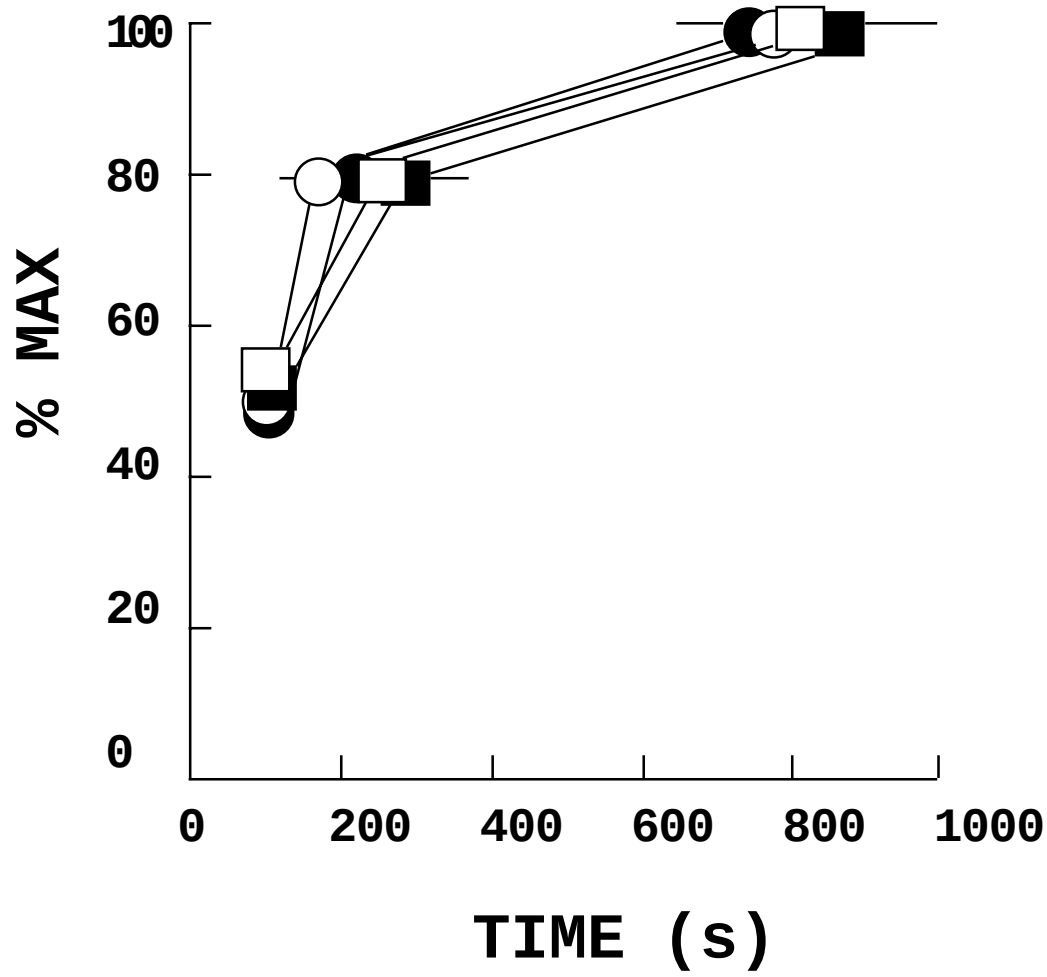

Fig 4

9. Appendix: Works related to the presented model

A. Own works

Pavlovic, D., M.Fournier, M.Aubier, and R.Pariente.

Modulation of the tracheal smooth muscle response to carbachol stimulation by the epithelium in the rats.

Am.Rev.Resp.Dis. 137 (suppl):243, 1988.

Pavlovic, D., M.Fournier, M.Aubier, and R.Pariente.

Epithelial versus serosal stimulation of tracheal muscle: role of epithelium.

J.Appl.Physiol. 67: 2522-2526, 1989. (*)

Pavlovic Dragan, Evelin Brione, Michel Fournier, et Michel Aubier.

L'epithelium inhibe la relaxation du muscle lisse tracheal de rat induit par un activateur des canaux potassiques (BRL 38227).

Proceedings of Journee de la Recherche (UFR Xavier Bichat), Paris, Sept. 1990.

Pavlovic, Dragan., Evelin Brione, Michel Fournier, and Michel Aubier:

Epithelium partialy inhibits tracheal smooth muscle relaxation induced by potassium channel activator BRL 38227.

Br. J. Pharmacol., 110: 139-144, 1993.

Pavlovic, Dragan., Naima Viires, Christine Zedda, Michel Fournier, and Michel Aubier:

Effects of corticosteroids on epithelial structure and smooth muscle function of rat trachea

Am. J. Respir. Crit. Care Med., 4: A905, 1994**.**

Pavlovic, D., Cheik-Zeinedinne, T., Fournier, M., and Aubier, M.

Carbocisteine diminishes rat tracheal smooth muscle reactivity in epithelium denuded preparations.

Eur. Resp. J. Vol 7,suppl 18: 76s, 1994.

Pavlovic, D., Moldovan, F., and Aubier, M.

Selective perfusion of rat tracheal preparation: no evidence for EpDRF secretion

Eur. Resp. J. Vol 8, suppl 19: 43s, 1995

D. Pavlovic, N. Viires, F. Roux, and M. Aubier

Chronic Hypoxia Diminishes Sensitivity of Rat Tracheal Smooth Muscle to Carbachol Applied Epithelialy

Am. J. Resp. Crit. Care. Med., Vol. 153, N8 4 (suppl., part 2): A840, 1996.

Ouksel, H., D. Pavlovic, N. Viires, Y. Amrani, N. Seta, and M. Aubier

Modifications in MLCK Expression Induced by Repeated Bronchoconstriction

EMC, XXV European Muscle Congress, Montpellier, France, 14 - 17 Sept., 1996.

Ouksel, H., N. Viires, D. Pavlovic, C. Peiffer, N. Seta, Y. Amrani, and M. Aubier

Modifications in MLCK Expression in Guinea-pig Airway Smooth Muscle: Role of Repeated Bronchoconstrictions and Role of Inflamation

Am. J. Resp. Crit. Care. Med. Vol 155, N8 4 (suppl, part 2): A370, 1997.

Ouksel, H., N. Viires, D. Pavlovic, C. Peiffer, C. Zedda, and M. Aubier

MLCK and SERKA Expression in Tracheal Smooth Muscle From Sensitized Guinea-pigs

Eur. Resp. J. vol 10, 291s (suppl), 1997.

Ouksel, H., N. Viires, D. Pavlovic, C. Peiffer, C. Zedda, M. Pretolani, C. RuffiÈ, and M. Aubier

Effects of Inflamation on Myosin Light Chain Konase (MLCK) Expression in Guinea-Pig Model of Bronchial Hyperreactivity

Am. J. Crit. Care Med., Vol 157, 3 (Suppl): A519, 1998

Pavlovic, Dragan., Naima Viires, Christine Zedda, Michel Fournier, and Michel Aubier:

Effects of a high dose corticosteroids on smooth muscle function in rat trachea.

Eur. Respir. J., 11: 575-582, 1998

H. Ouksel, N.Viires, D. Pavlovic, C. Ruffie, C. Zedda, C. Peiffer, C. Vizzuzaine, M. Pretolani, M. Aubier

Allergic Bronchial Hyperreactivity In The Guinea-Pig Is Associated With An Inflammation-Dependent Increase In The Expression Of MLCK In the Tracheal Smooth Muscle

Am. J. Crit. Care. Med. vol 161 (3) (Suppl): A840, 2000.

A. Samb, C. Lisdero, J. Callebert, D. Pavlovic, M. Pretolani, M.

Aubier, J. Boczkowski

Decreased Lung Expression And Activity Of Type I Nitric Oxide Synthase (nNOS) After Ovalbumin Immunisation And Aerosol Challenge In Guinea Pigs.

Am. J. Crit. Care. Med. vol 161 (3) (Suppl): A919, 2000.

Submitted for publication

Pavlovic, D., Cheik-Zeinedinne, T. N., Nedeljkov, V., Wendt, M., and Aubier, M. **A**

New Interchangeable Tube/Ring Tracheal Preparation: Effects of Carbocisteine.

(J. Appl. Physiol.)

Ouksel, H., N. Viires, D. Pavlovic, C. Peiffer, C. Ruffie, C. Vissuzaine, M. Pretolani, and M. Aubier,

Quantitative Modifications of Myosin Light Chain Kinase Associated with Airway Inflammation in a Guinea-pig Model of Bronchial Hyperreactivity

(J Clin. Invest.)

B. Works of other investigators using our model

*The model has been taken up by a group from Utrecht that produced at least one PhD (**Dr. Sadeghi-Hashjin G**) and number of publications. We give a short list of some of them as appear in Medline.*

Folkerts G, Kloek J, Geppetti P, Van der Linde HJ, Nijkamp FP.

Factors that determine acetylcholine responsiveness of guinea pig tracheal tubes.

Eur J Pharmacol. 2001 May 25;420(2-3):151-7.

Sadeghi-Hashjin G, Folkerts G, Henricks PA, Van de Loo PG, Van der Linde HJ, Dik IE, Nijkamp FP**.**

Induction of guinea pig airway hyperresponsiveness by inactivation of guanylate cyclase.

Eur J Pharmacol. 1996 Apr 29;302(1-3):109-15.

Figini M, Ricciardolo FL, Javdan P, Nijkamp FP, Emanueli C, Pradelles P, Folkerts G, Geppetti P.

Evidence that epithelium-derived relaxing factor released by

bradykinin in the guinea pig trachea is nitric oxide.

Am J Respir Crit Care Med. 1996 Mar;153(3):918-23.

Folkerts G, van der Linde H, Verheyen AK, Nijkamp FP.

Endogenous nitric oxide modulation of potassium-induced changes in guinea-pig airway tone.

Br J Pharmacol. 1995 Aug;115(7):1194-8.

Folkerts G, van der Linde HJ, Nijkamp FP.

Virus-induced airway hyperresponsiveness in guinea pigs is related to a deficiency in nitric oxide.

J Clin Invest. 1995 Jan;95(1):26-30.

Nijkamp FP, van der Linde HJ, Folkerts G.

Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Role of the epithelium.

Am Rev Respir Dis. 1993 Sep;148(3):727-34.

Eidesstattliche Erklärung

Hiermit erkläre ich, daß ich die vorliegende Dissertation selbständig verfaßt und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät vorgelegt worden.

Ich erkläre, daß ich bisher kein Promotionsverfahren erfolglos beendet habe und daß eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Datum

7. 3. 2002

Unterschrift

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Dragan Pavlovic

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1976-1977 Ärztlicher Vorbereitungsdienst (AiP), Medizinische Fakultät Belgrad Universität, Jugoslawien (4. 7. 1976 - 17. 7. 1977) und Sportmedizin Praxis, "Medicinar", Belgrade, Jugoslawien

1977-197 Allgemeiner Arzt, Ambulanz Porec und Koper, Jugoslawien. (18. 7. 1977 - 20. 8 1978)

1978-1979 Assistenzarzt, Abteilung für Anästhesie und Intensivmedizin, Parazelsus Klinik, Marl, W. Deutschland. (1. 9. 1978 - 31. 7. 1979)

1979-1981 Sports Medizin Praxis (Sportmedizin Ärztlicher Vorbereitungsdienst), "Medicinar" Karate Club, Belgrade, Jugoslawien. (1. 8. 1979 – 30. 7. 1981)

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2000 - ... Assistenzarzt und Wissenschaftlicher Mitarbeiter , Klinik und Poliklinik für Anästhesiologie und Intensivmedizin, Ernst-Moritz-Arndt Universität, Greifswald, B. R. Deutschland (seit 1. 1. 2000 - ...)

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1983 Kursus in British Medical Practice auf Postgraduate Medical School, University of Exeter, Groß Britannien
(April 1983 - Juni 1983).

1985-86 Kursus in Physiologie und Pharmakologie des

Atemweges, auf Universität
Paris V., Frankreich. (September 1985-Juni 1986)

Sommer 1989, Kursus in *patch-clamp*, auf Faculte des Sciences (Pr. M. Lazdunski), Nice, Frankreich, und auf Faculte de Pharmacie, Strassburg, Frankreich (Pr. Stockle).

Sommer 1991, Kursus in "*Optical techniques* of membrane potential recording and intracellular ion concentration recording", auf Faculte de Medicine , Marseille (Pr. Bongrand), Marseille, Frankreich.

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1985 - 1999 Wissenschaftlicher Mitarbeiter, *UnitÈ INSERM U408*, FacultÈ Xavier - Bichat,
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(1. 1. 2000 -....)

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