THE EFFECT OF AMBROXOL ON THE VASCULAR REACTIVITY IN THE RABBIT

HOLÉCYOVÁ A., *KRIŠKA M.

OVPLYVNENIE CIEVNEJ REAKTIVITY U KRÁLIKA AMBROXOLOM

Experiments were designed to determine whether ambroxol, a drug used for the treatment of respiratory disorders, affects the basal tension and/or contractions due to adrenergic stimuli in the isolated rabbit portal vein and pulmonary artery. Ambroxol in concentrations of 10^{-6}—10^{-4} mol/l produced a concentration-dependent increase in basal tension, but a decrease in spontaneous mechanical activity of portal vein. The same concentrations of ambroxol failed to influence basal tension of pulmonary artery. However, when the vessel tone was increased by exogenous noradrenaline, ambroxol elicited concentration-dependent contractions also in this vessel. Moreover, ambroxol in the concentration of 10^{-5} mol/l significantly enhanced vascular contractions due to both exo- and endogenous noradrenaline. The results suggest that ambroxol, in biologically relevant concentrations, may influence or even induce vascular contractions to adrenergic stimuli. Their expression, however, depend on the type of vascular smooth muscle. *(Fig. 7, Ref. 25.)*

Key words: ambroxol, vascular smooth muscle, contraction, noradrenaline, transmural nerve stimulation.

Bratisl Lek Listy 1998; 99: 99–103

Cieľom pokusov bolo určiť, či ambroxol, liek používaný na liečbu dýchacích ťažkostí, má vplyv na bazálnu tenziu a/alebo kontrakcie spôsobené adrenergickými stimulmi v izolovanej vrátnici a pľúcnej tepne králika. Ambroxol v koncentrácii 10^{-6}—10^{-4} mol/l vyvolal zvýšenie bazálnej tenzie závisiace od koncentrácie, ale zníženie spontánnej mechanickej aktivity vrátnice. Tie isté koncentrácie ambroxolu neovplyvnili bazálnu tenziu pľúcnej tepny. V prípade, že cievny tonus bol zvýšený exogénnym noradrenalínom, však ambroxol vyvolal takisto v tejto cieve kontrakcie závisiace od koncentrácie. Ambroxol v koncentrácii 10^{-5} mol/l významne ovplyvnil cievne kontrakcie spôsobené endogénnym alebo exogénnym noradrenalínom. Výsledky naznačujú, že ambroxol v biologicky relevantných koncentráciách môže ovplyvniť cievne kontrakcie spôsobené adrenergickými stimulmi. Ich objavenie sa však závisí od typu hladkého svalu cievy. *(Obr. 7, lit. 25.)*

Kľúčové slová: ambroxol, hladký sval cievy, kontrakcie, noradrenalín, transmurálna nervová stimulácia.

Bratisl. lek. Listy, 99, 1998, č. 2, s. 99–103

Ambroxol (ABX) has been proved as a compound with well demonstrated surfactant stimulating as well as mucolytic action (Curti, 1974; Püschmann and Engelhorn, 1978; Elemer and Kapanci, 1981). Both effects predetermine ABX for the treatment of a wide range of respiratory disorders. Among others, for antepartal prophylaxis and postnatal therapy of respiratory distress syndrome (Lorenz et al., 1978; Vauer et al., 1989) as well as for ameliorating bronchial hyperreactivity (Melillo and Cocco, 1986) and chronic bronchitis (Ericsson et al., 1986). Recently also uricosuric effect of ABX has been described (Oosterhuis et al., 1993).

In spite of a current use of ABX in human medicine, its effect on the systems others than respiratory are poorly documented. Particularly, diversity was observed in vascular effects of the drug. Though in animal experiments only slight and transient decrease in blood pressure was described after overdoses of ABX (Püschmann and Engelhorn, 1978; Misawa and Hasegawa, 1989). Kriška (1983) found constriction of isolated rabbit ductus arteriosus in response to ABX. This observation was supported later by the similar finding in ductus arteriosus of guinea pigs and lambs (Kriška et al., 1992). Clinical trial, however, found no effect of ABX on feto-placental circulation in pregnant women at risk of preterm delivery (Kováč et al., 1990). The questions arose of (i) whether vasoconstriction induced by ABX is specific to ductus arteriosus or can be expected generally, and (ii) whether ABX affects vascular smooth muscle directly or by modulating the adrenergic influences. With this aim, the effect of ABX on basal tension and responses to adrenergic stimuli was studied in vessels characterizing different types of smooth muscle, i.e. portal vein and pulmonary artery of adult rabbits.

Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, *Department of Pharmacology, Faculty of Medicine, Comenius University, Bratislava
Ústav normálnej a patologickej fyziológie Slovenskej akadémie vied v Bratislave a Farmakologický ústav Lekárskej fakulty Univerzity Komenského v Bratislave
Address for correspondence: A. Holécyová, MD, PhD, ÚNPF SAV, Sienkiewiczova 1, 813 71 Bratislava, Slovakia.
Phone: +421.7.326 618, Fax: +421.7.368 516

Material and methods

Vessel preparations

Experiments were performed on 18 albino rabbits of both sexes weighing 2.3—3.2 kg. The animals were sacraficed by cervical dislocation and bleeding via carotid artery. The chest and abdomen were opened, pulmonary artery and portal vein were dis-

sected free. Vessels were placed into a cold oxygenated Krebs solution where the final cleaning from fat and connective tissue was performed. From pulmonary artery a 4 mm ring-segment was prepared. From portal vein a longitudinal strip of about 4 mm in width and 12 mm long was cut off.

Isometric tension recoridng

Preparations were suspended in an organ bath containing 20 ml Krebs solution, gassed continuously with 95 % O_2 and 5 % CO_2 at 37 °C. The tension of the preparations was recorded isometrically under a resting tension of about 20 mN for pulmonary artery and 10 mN for portal vein. As preliminary experiments indicated, this was the optimal tension to obtain maximal response in individual vessels. Electromechanical transducer (Sanborn FTA 10) and a potentiometric recorder (Labora TZ 4200) were used. The preparations were allowed to equilibrate for at least 60 min.

As adrenergic stimuli exogenous noradrenaline (NA) and transmural nerve stimulation (TNS) were used. ABX was applied into the bath either cumulatively or in the individual concentration 10^{-5} mol/l.

Exogenous NA-induced responses

In a group of experiments with portal vein, repeated application of exogenous NA in a concentrations $3x10^{-7}$ mol/l was used as a stimulus. Individual stimulus lasted for 2 min, during which time a maximum contraction was attained. Then NA was washed out by overflow. The stimulus was repeated five to six times in 10 min intervals before and after application of ABX (10^{-5} mol/l) and after washing it out. Contractions obtained in the presence of ABX and after washing it out were expressed as a percentage of 3 initial contractions obtained before ABX application.

In a separate group of experiments with pulmonary artery, NA was applied in a cumulative manner from concentration 10^{-9} up to 10^{-5} mol/l to obtain concentration — response curve.

TNS-induced responses

For TNS in portal vein preparations, platinum electrodes positioned on the two sides along the whole lenght of the vessel strip were used. The preparations were stimulated by rectangular pulses of supramaximal voltage, 0.5 ms duration at a frequency of 4 Hz for 20 s, using stimulator ST3 (Medicor). Stimulation was repeated five to six times at 5 min intervals before and after application of ABX (10^{-5} mol/l) as well as after washing ABX out. Neurogenic contractions obtained in the presence of ABX and after washing in out were expressed as a percentage of 3 initial contractions obtained before ABX application.

Solutions and drugs

Krebs solution contained (mmol/l): NaCl 118; $NaHCO_3$ 25; KCl 5; $MgSO_4.H_2O$ 1.2; CaCl 2.5; glucose 11; $CaNa_2$ EDTA 0.03; ascorbic acid 0.55. Freshly prepared (±) — noradrenaline (Spofa) was used. Ambroxol was obtained from Boehringer. Drugs were diluted in distilled water.

Data analysis

All data are expressed as the mean±SEM. Data are compared using Student's t-test for paired or unpaired observations. Probability values <0.05 were considered to be significant.

Results

Effect of ABX on basal tension

Original tracing of the effect of ABX on basal tension in the rabbit portal vein is in Figure 1. Cumulative addition of ABX (10^{-7}—10^{-4} mol/l) induced concentration-dependent increase in basal tension (Fig. 1, left panel). The increase became evident at a concentration of $3x10^{-7}$ mol/l and the mean maximum reached at concentration 10^{-4} mol/l of ABX was 1.06±0.12 mN/mm^2. The increase in basal tension was accompanied by the concentration-dependent decrease in amplitude of spontaneous rhythmic activity of the vessel. In some experiments, spontaneous activity was even abolished at the highest concentration of ABX. After washing ABX out, both parameters i.e. basal tension as well as spontaneous activity returned quickly to their control values, spontaneous activity showing even overshoot.

Addition of NA (10^{-7} mol/l) into the medium caused transient increase in tension of portal vein (Fig. 1, right panel). Pretreatment of the vessel with low concentration of ABX (10^{-7} mol/l) did not influence the amplitude of this transient contraction, however, elicited spontaneous rhythmic contractions superimposed on the NA-induced contraction.

In contrast to portal vein, in the rabbit pulmonary artery, cumulative application of ABX (10^{-7}—10^{-4} mol/l) did not influence the basal tension of the vessel, even at the highest concentration (Fig. 2, left panel). However, when the vessel tension was increased by addition of NA (10^{-7} mol/l), ABX elicited concentration-dependent contractions, superimposed on the plateau of NA contraction (Fig. 2, right panel).

Effect of ABX on exogenous NA-induced responses

Original tracing of the effect of ABX on responses induced by exogenous NA in the rabbit portal vein is in Figure 3. NA in concentration $3\text{-}10^{-7}$ mol/l elicited control contractions, which reached in average 3.97±0.56 mN/mm^2, i.e. about 40 % of maximum NA contraction. Introduction of ABX (10^{-5} mol/l) into the bathing medium caused enhancement of the contractions. The enhancement appeared immediately, persisted throughout the presence of ABX in the bath and its maximum after 50 min was in average 5.53±0.32 mN/mm^2. After washing ABX out, the amplitude of NA-induced contractions gradually decreased to its control value.

Summarized data of these experiments are in Figure 4. Addition of ABX (10^{-5} mol/l) caused significant amplification of NA-induced contractions. The amplification appeared immediately and after 50 min reached its maximum, which was 139.31±2.13 % of the mean control response. After washing ABX out, amplitude of NA-induced contractions decreased gradually and after 50 min did not differ from that of controls.

To study the effect of ABX on NA-induced contractions in pulmonary artery, concentrations-response curves to NA in presence and absence of ABX were obtained (Fig. 5). In control vessels, cumulative addition of NA (10^{-9}—10^{-5} mol/l) produced concentration-dependent contraction. The maximum elicited by the concentration 10^{-5} mol/l was 14.63±1.31 mN/mm^2. Treatment of the vessels with ABX (10^{-5} mol/l) did not influence the contraction to the lower concentrations of NA. However, starting from concentration $3x10^{-7}$ mol/l, contraction became significantly higher. Maximum induced by the concentration of 10^{-5} mol/l was 22.76±0.81 mN/mm^2, i.e. by 55.56±5.55 % higher as compared with control response to NA ($p<0.001$).

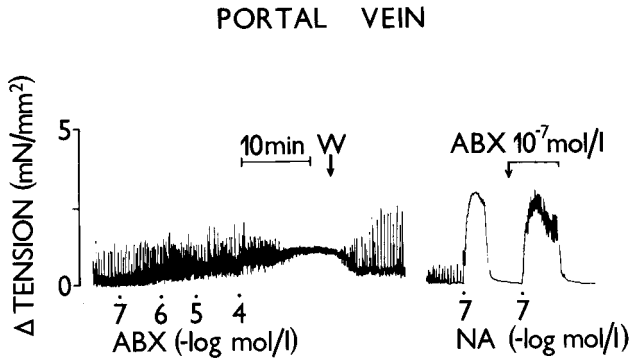

Fig. 1. Typical recording of the effect of ABX on basal tension and NA-induced contraction in the rabbit portal vein. Notice an increase in tension after cumulative addition of ABX (left panel) and induction of spontaneous rhythmic activity after low concentration (10^{-7} mol/l) of ABX (right panel).
Obr. 1. Typický záznam účinku ABX na bazálnu tenziu a kontrakcie vrátnice králika vyvolané NA. Sleduj zvýšenie tenzie po kumulatívnom prídavku ABX (ľavý panel) a vyvolanie spontánnej rytmickej činnosti po nízkej koncentrácii (10^{-7}mol/l) ABX (pravý panel).

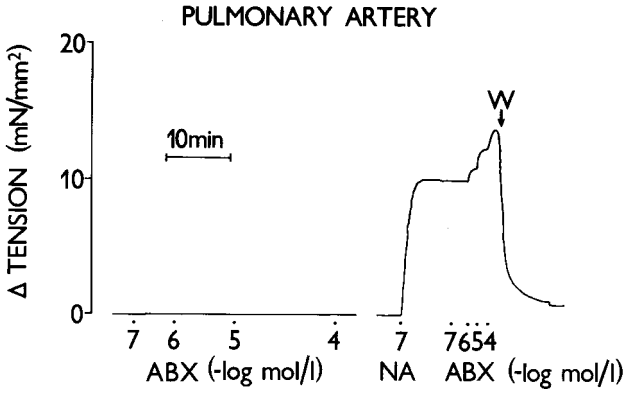

Fig. 2. Typical recording of the effect of ABX on basal tension and NA-induced increas in tension of the rabbit portal vein. Notice no effect after cumulative application of ABX under basal tension (left panel), but concentration dependent contractions under raised tension (right panel).
Obr. 2. Typický záznam účinku ABX na bazálnu tenziu a jej zvýšenie vyvolané NA v králičej pľúcnej tepne. Sleduj neprítomnosť účinku po kumulatívnej aplikácii ABX za podmienok bazálnej tenzie (ľavý panel) na rozdiel od kontrakcií závisiacich od koncentrácie za podmienok bazálnej tenzie (pravý panel).

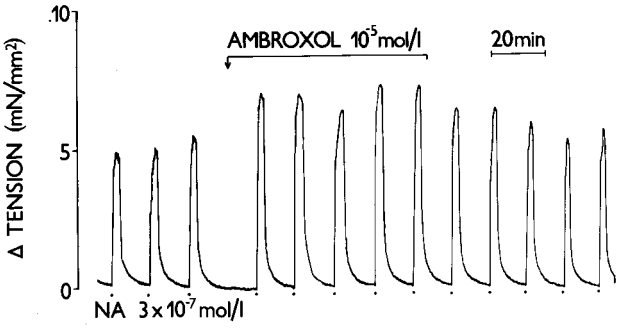

Fig. 3. Typical recording of the potentiation of NA-induced contractions by ABX in the rabbit portal vein.
Obr. 3. Typický záznam potencovania kontrakcií vyvolaných NA po aplikácii ambroxolu vo vrátnici králika.

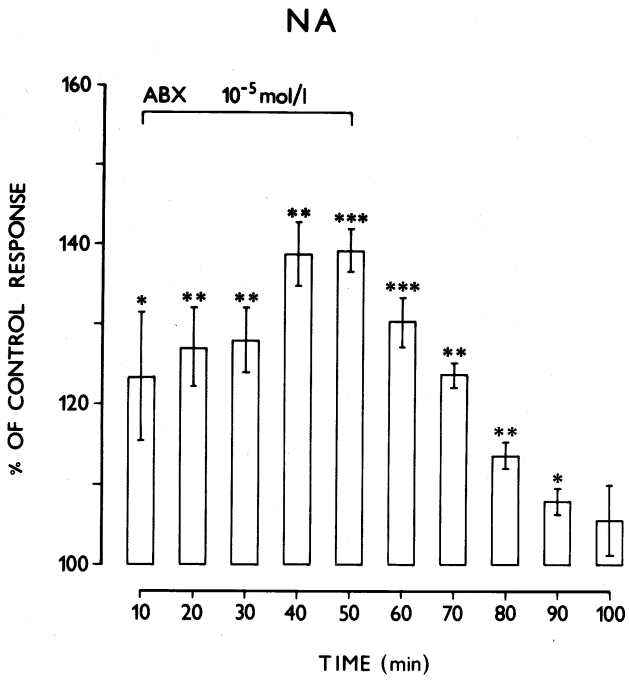

Fig. 4. Time-course of the potentiation by ABX of NA-induced contractions and recovery of the response in the rabbit portal vein. Responses are expressed as a percentage of initial control NA contraction. Data are the mean±SEM (n=6). * p<0.05, ** p<0.01* p<0.001.**
Obr. 4. Časový priebeh potenciácie kontrakcií vyvolaných NA* po aplikácii ambroxolu a úprava reakcie vrátnice králika. Reakcie sú vyjadrené ako percento úvodných kontrolných kontrakcií vyvolaných NA. Údaje vyjadrujú priemer±SEM (n=6). * p>0,05, p<0,01,*** p<0,001.**

Effect of ABX on TNS-induced responses

Original tracing of the effect of ABX on TNS-induced responses in portal vein is in Figure 6. TNS (40 V, 0.5 ms, 4 Hz) induced transient contractions in the rabbit portal vein. In control conditions, the average value of TNS-induced contraction was 4.23±0.39 mN/mm^2. Application of ABX (10^{-5} mol/l) into the bathing medium, caused time-dependent enhancement of the contractions with the mean maximum of 5.38±0.31 mN/mm^2. In addition to the enhancement of contractions, an induction of spontaneous rhythmic activity appeared. After washing ABX out of the medium, TNS-induced contractions returned to their control amplitude and spontaneous activity disappeared. The mean results in the whole group of these experiments are in Figure 7. ABX (10^{-5} mol/l) elicited significant potentiation of TNS-induced contractions. Potentiation occured immendiately after introduction of ABX and persisted during the presence of ABX in the bathing medium. The mean maximum of TNS-induced contractions, reached 20 min after the addition of ABX, was 127.18±5.62 % of the respective control response, i.e. significantly higher ($p<0.01$). Removal of ABX from the medium was followed by a gradual return of the magnitude of contractions to their control value.

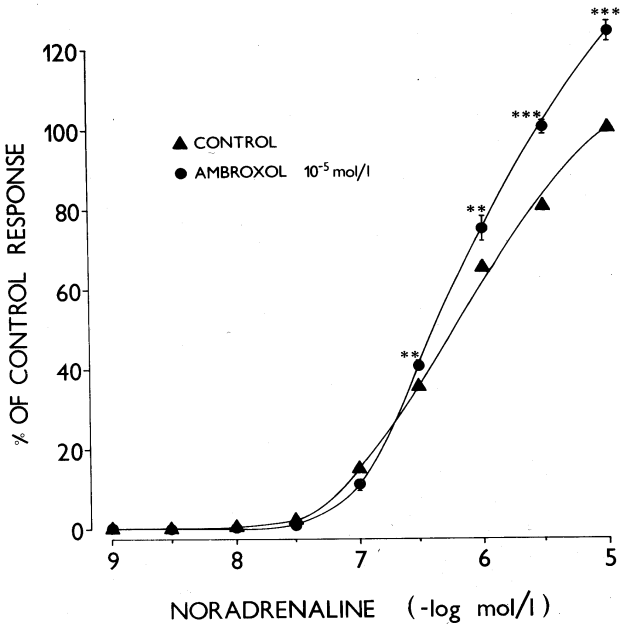

Fig. 5. Cumulative concentration-response curves to NA in the rabbit pulmonary artery during control conditions and after ambroxol treatment. Symbols represent the mean±SEM (n=8). *p<0.05, ***p<0.001.
Obr. 5. Krivky reakcie na kumulatívnu koncentráciu NA v plúcnej tepne králika počas kontroly podmienok a po podaní ambroxolu. Symboly udávajú priemer±SEM (n=8). *p<0,05, ***p<0,001.

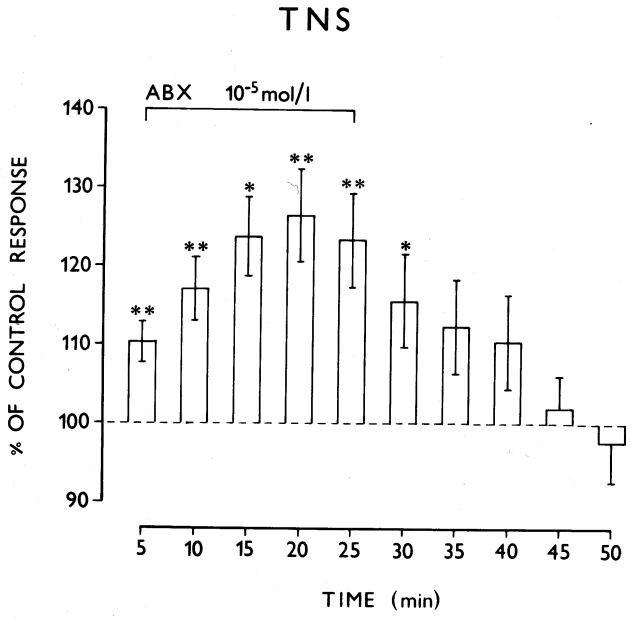

Fig. 7. Time-course of the potentiation by ABX of TNS-induced contractions and recovery of the response in the rabbit portal vein. Responses are expressed as a percentage of initial control TNS-induced contraction. Data are the mean±SEM (n=8). *p<0.05, **p<0.01.
Obr. 7. Časový priebeh potenciácie kontrakcií vyvolaných TNS po aplikácii ambroxolu a úprava reakcie vrátnice králika. Reakcie sú vyjadrené ako percento úvodných kontrolných kontrakcií vyvolaných TNS. Údaje vyjadrujú priemer±SEM (n=8). *p<0,05, **<0,01.

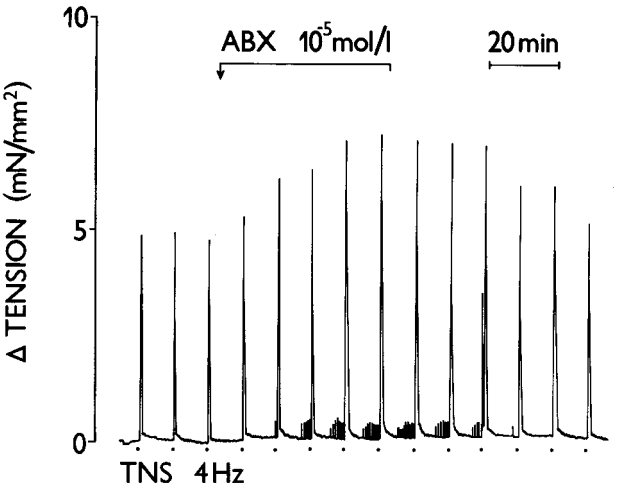

Fig. 6. Typical recording of the potentiation of TNS-induced responses. Notice activation of spontaneous rhythmic activity after ABX treatment.
Obr. 6. Typický záznam potenciácie reakcie vyvolanej TNS. Sleduj aktiváciu spontánnej rytmickej aktivity po podaní ABX.

Discussion

All the results suggest vascular effects of ABX. However, these effects depend on the type of vascular smooth muscle. In smooth muscle, represented by portal vein, ABX induced contraction and activation of spontaneous rhythmic activity. On the other hand, in the type, represented by pulmonary artery, this vasoconstrictor effect appeared only under increased tension. In both vessels, however, ABX potentiated the contractile responses to adrenergic stimuli.

The concentrations of ABX that elicited these vascular effects (10^{-6}—10^{-5} mol/l) were within the range observed in plasma from mini-pigs foetuses after treatment of their mothers with ABX (Rüfer and Busch, 1978) and/or in human plasma during treatment with ABX (Oosterhuis et al., 1993). They may be therefore considered as biologically relevant.

The cellular mechanisms underlying the vasoconstrictor effect of ABX are not known. ABX was found to increase the turnover of phospholipids and particularly phosphatidylcholine in airway cells (Seefeld and Renovanz, 1978; Prevost et al., 1978). Similar effect may be expected in cell membranes of vascular wall. Stoll and Spector (1993) showed that the smooth muscle cells are the primary targets for the effect of lysophosphatidylcholine (LPC) formed within the vascular wall. In these cells, LPC, even at low concentrations, induced an increase in $[Ca^{2+}]_i$ (Locher et al., 1992; Stoll and Spector, 1993). The increase was due to an influx of Ca^{2+} from extracellular space and its amplitude and duration increased as the concentration of LPC increased. The contribution of this Ca^{2+} to modulation of vascular responses was demonstrated by Kugiyama et al. (1990), who reported impaired relaxation of the rabbit aorta to vasodilators after treatment with LPC. This vasoconstrictory effect predominates the initial increase in cGMP and nitric oxide-mediated vasorelaxation observed after LPC by Dudek et al. (1993). The increase in $[Ca^{2+}]_i$ may be also the mechanism for activation of protein kinase C reported by Oishi et al. (1988). Ho-

wever, further studies are needed to explain the molecular basis for the vasoconstriction induced by ABX.

Our finding of activation of spontaneous rhythmic activity in portal vein in presence of ABX supports the suggestion that ABX induces an influx of Ca^{2+} into the vascular smooth muscle cells. In isolated rat aorta, these contractile responses were abolished by a short period of Ca^{2+} withdrawal indicating that they are due to an influx of Ca^{2+} (Godfraind et al., 1986). Moreover, Byron and Taylor (1993) showed in cell culture that entry of extracellular Ca^{2+} into the cytosol is essential for spike generation, while release of Ca^{2+} from sarcoplasmic reticulum may provide an amplification of Ca^{2+} spike that is generated at the sarcolemma.

The contractile effect of ABX in pulmonary artery was shown to be tone-dependent and required higher active tone to be unmasked. In the pulmonary vascular bed, tone-dependent responses have been reported for a number of substances, including acetylcholine (Hyman and Kadowitz, 1989), serotonin (Neely et al., 1992), noradrenaline (Porcelli and Cutaia, 1988). The mechanism of such effect for ABX remains to be elucidate.

The responses induced by TNS in portal vein were due to the activation of intramural nerve fibers, release of NA and its action on postsynaptic sites. In these experiments, the amount of NA released in response to TNS and its postsynaptic concentration was equal to the concentration of exogenously applied NA (as expressed by the magnitude of TNS-induced and exogenous NA-induced control contractions). No significant difference between the potentiation of both responses in presence of ABX therefore suggests the postsynaptic effect of ABX, (i.e. on the vascular smooth muscle).

In conclusion, our data provide consistent evidence of vasoactive effects of ABX in the biologically relevant concentrations. The possibility to induce vasoconstriction increases with the increased concentration of ABX. Fact that the contractile effect was observed in portal vein, the vessel with characteristic features of peripheral circulation, suggests the sensitivity of this part of vascular bed to the effect of ABX. Higher adrenergic tone, as can be observed in some pathological states, could also be a predisposition to the vasoconstrictor action of ABX. This should be taken into account in the clinical use of ABX, especially in those indications where higher doses of the drug are supposed to be used.

References

Byron K.L., Taylor C.W.: Spontaneous Ca^{2+} spiking in a vascular smooth muscle cell line is independent of the release of intracellular Ca^{2+} stores. J. Biol. Chem., 268, 1993, p. 6945—695.

Curti P.C.: Therapeutische Untersuchungen mit Metabolit VII von Bromhexin bei mangelhafter oberflächenaktiver Substanz der Alveolen. Arzneim.-Forsch., 24, 1974, p. 847—849.

Dudek R., Conforto A., Bing R.J.: Lysophosphatidylcholine-induced vascular relaxation and production of cGMP are mediated by endothelium-derived relaxing factor. Proc. Soc. Exp. Biol. Med., 203, 1993, p. 747—479.

Elmer G., Kapanci J.: Morphological approach to surfactant secretion in the lungs with particular reference to ambroxol. Prog. Resp. Res., 15, 1981, p. 234—239.

Ericsson C.H., Juhasz E., Jönsson E., Massberg B.: Ambroxol therapy in simple chronic bronchitis: effect on subjective symptoms and ventilatory function. Europ. J. Resp. Dis., 69, 1986, p. 248—255.

Godfraind T., Miller R., Wibbo M.: Calcium antagonism and calcium entry blockers. Pharmac. Rev., 38, 1986, p. 321—416.

Hyman A.L., Kadowitz P.J.: Influence of tone on responses to acetylcholine in the rabbit pulmonary vascular bed. J. appl. Physiol., 67, 1989, p. 1388—1394.

Kováč R., Kriška M., Kristová V., Caňová R.: Ambroxol-risk assessment in experimental and clinical studies. Abstract. Adverse Drug Reactions in Children, Štrbské Pleso 1990, p. 17.

Kriška K.: Some characteristics of fetal and neonatal circulation with respect to drug actions. Zbl. Pharm., 122, 1983, p. 1383—1386.

Kriška K., Kristová V., Coceani F., Seidlitz E., Kelsey L.: Ambroxol increased contractility of ductus arteriosus. Abstract. V. World Conference on Clinical Pharmacology and Therapeutics, Jokohama 1992, p. 210.

Kugiyama K., Kerns S.A., Morrisett J.D., Roberts R., Henry R.D.: Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins. Nature Lond., 344, 1990, p. 160—162.

Locher R., Weisser B., Mengden T., Brunner C., Velter W.: Lysolecithin actions on vascular smooth muscle cells. Biochim. Biophys. Res. Commun., 183, 1992, p. 156—162.

Lorenz U., Rüttgers H., Wille L., Kuble F.: Klinische Doppelblindstudie zur Frage der Antepartalen Lungenreifung unter Ambroxol. Atemwegs. Lungenkrankh., 4, 1978, p. 30—34.

Melillo G., Cocco G.: Ambroxol decreases bronchial hyperreactivity. Europ. J. Resp. Dis., 69, 1986, p. 316—320.

Misawa M., Hasegawa J.: Effect of inhaled bromhexine on the bronchomotor tone in rats and guinea pigs. Nippon-Yakurigaku-Zasshi, 94, 1989, p. 123—128.

Neely C.F., Haile D., Matot I.: Tone-dependent responses of 5-hydroxytryptamine in the feline pulmonary vascular bed are mediated by two different 5-hydroxytryptamine receptors. J. Pharmacol. Exp. Therap., 264, 1992, p. 1315—1326.

Oishi K., Raynor R.L., Charp P.A., Kuo J.F.: Regulation of protein kinase C by lysophospholipids. Potential role in signal transduction. J. Biol. Chem., 263, 1988, p. 6865—6871.

Oosterhuis B., Storm G., Cornelissen P.J.G., Su C.A.P.F., Sollie F.A.E., Jonkman J.H.G.: Dose-dependent uricosuric effect of ambroxol. Europ. J. clin. Pharmacol., 44, 1993, p. 237—241.

Porcelli R.J., Cutaia M.V.: Pulmonary vascular reactivity to biogenic amines during acute hypoxia. Amer. J. Physiol., 255, 1988, p. H329—H334.

Prevost M.C., Soula G., Douste-Blazy L.: Action d'un derive de la bromhexine sur le phospholipides des macrophages alvéolaires de lapin. Bull. Europ. Physiopathol. Resp., 14, 1978, p. 53—60.

Püschmann S., Engelhorn R.: Pharmakologische Untersuchungen des Bromhexin-Metaboliten Ambroxol. Arzneim.-Forsch., 28, 1978, p. 889—899.

Rüfer R., Busch U.: Pharmakologische Untersuchungen zur Plazentapassage von Ambroxol an trächtigen Zwergschweinen. Atemwegs. Lungenkrankh., 4, 1978, p. 15—21.

Seefeld von H., Renovanz H.D.: Das oberflächenaktive System der Lunge — eine Übersicht. Atemwegs. Lungenkrankh., 4, 1978, p. 1—14.

Stoll L.L., Spector A.A.: Lysophosphatidylcholine causes cGMP-dependent verapamil-sensitive ca^{2+} influx in vascular smooth muscle cells. Amer. J. Physiol., 264, 1993, p. C885—C893.

Wauer R.R., Schmalisch G., Hammer H., Buttenberg S., Weigel H., Huth M.: Ambroxol for prevention and treatment of hyaline membrane disease. Europ. Resp. J., 2, 1989, p. 57S—68S.

Received March 25th, 1996.