R. NOSÁL

PHARMACOLOGY OF HISTAMINE LIBERATION. CATIONIC AMPHIPHILIC DRUGS AND MAST CELLS

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia

The effect of betaadrenoceptor blocking drugs atenolol and propranolol was studied in both nonstimulated and stimulated isolated rat mast cells. Atenolol did not liberate histamine from non-stimulated mast cells, decreased spontaneous secretion, inhibited 48/80 stimulated histamine release, increased ^{32}P incorporation into membrane phospholipids, decreased membrane fluidisation and decreased arachidonic acid liberation from membrane phospholipids of stimulated mast cells. Propranolol dose-dependently liberated histamine from nonstimulated mast cells and inhibited histamine liberation, it nonsignificantly increased membrane phospholipid turnover but significantly increased membrane fluidisation and inhibited stimulated arachidonic acid liberation in stimulated mast cells. The results indicated the interaction of atenolol and propranolol with mast cell membranes, particularly with the phospholipid bilayer, resulting in a possible inhibition of phospholipase A_2 activation. Histamine liberation suggested its displacement from granule binding sites after intracellular propranolol accumulation in mast cells.

K e y   w o r d s: *histamine, phospholipids, phospholipase A_2, mast cells, beta-adrenoceptor*

INTRODUCTION

Isolated mast cells liberate histamine after stimulation in a process of exocytosis or non-specifically as demonstrated with bacterial and yeast toxins as well as with cationic amphiphilic drugs (CAD: 1—5). Histamine liberation with the CAD exaprolol a beta-adrenoceptor blocking (BAB) drug appeared intracellularly as the result of histamine exchange in isolated mast cells (6). On the other hand, CAD were demonstrated to inhibit stimulated histamine liberation from isolated mast cells dose-dependently in a non-receptor mediated way (7, 8). The interaction of BAB drugs with mast cells is of special interest. First, there is clinical evidence that BAB drug-induced bronchocon-

striction may be at least in part mediated by histamine liberated from lung mast cells by the action of these drugs (9—11). Second, BAB drugs, as typical representatives of CAD, altered stimulated blood platelets and mast cells according to their physico-chemical properties particularly at the plasma membrane level and intracellularly. This interaction resulted from the alteration of CAD-induced changes in membrane fluidisation and phospholipid turnover of mast cells and blood platelets, as well as from the inhibition of the stimulated arachidonic acid cascade in these cells (12—14). To learn more about the mechanisms of nonreceptor CAD-cell interaction it was of interest to investigate and compare the effect of two BAB drugs, i.e. hydrophilic selective atenolol and lipophilic nonselective propranolol, on isolated nonstimulated and stimulated mast cells at extracellular and plasma membrane levels, as well as intracellularly.

MATERIAL AND METHODS

Ficoll: Pharmacia Uppsala, histamine-2-HCl: Koch Light, human serum albumin: Imuna Š. Michalany, atenolol (ATE), propranolol (PRO): ICI, 48/80: Sigma, concanavaline A and phosphatidylserine: Serva, o-phthaldialdehyde: Merck, stearic acid with dimethyloxazolidine group at the 5th and 16th carbon position: Syva-Palo Alto: USA, ^{32}P-sodium orthophosphate (400 MBq/ml) and $N_2^{34}SO_4$: ROTOP Germany, (^{3}H-5, 6, 8, 9, 11, 12, 14, 15)-arachidonic acid (7 TBq/mmol/L): Institute of Isotopes Budapest.
All other chemicals were from commercial sources of analytical grade.

Animals and mast cell isolation

Male albino Wistar rats (SPF unit Dobra Voda, 300—350 g) were used in all experiments. After decapitation in light ether anesthesia, pleural and peritoneal washes in buffer salt solution (130 mmol/1NaCl, adjusted to pH 7 with Sörensen phosphate buffer) were layered over 30 and 40% Ficoll and mast cells were separated by differential centrifugation as described earlier (15). Samples of mast cells for incubation with BAB drugs were treated as published previously (6). Histamine was determined in aliquots of supernatant and sediment spectrofluorometrically omitting the extraction procedure (16, 17).

Labelling of mast cell granules with ^{35}S

Rats were injected 14 to 16 days before mast cell isolation with $Na_2{}^{35}SO_4$ subcutaneously (9.25×10^7 Bq/rat). The ^{35}S is evenly distributed in the granular heparin within 12 days after $Na_2{}^{35}SO_4$ injection (1). Labelled mast cells were isolated and washed as described above.

For ^{35}S activity determination the aliquots from the supernatant and sediment were taken. The ^{35}S activity was determined by means of Bray's scintillation fluid in Packard Tri-Carb scintillation counter No. 300.

[32]P incorporation into mast cell membrane phospholipids

Isolated mast cells (10^6 cells in each 2 ml sample) were incubated at 37°C with atenolol or propranolol (both 1 mmol/L) and 15 MBq ^{32}P for 15 min. Incubation was terminated at 0°C. After centrifugation mast cells membrane phospholipids were extracted with chloroform: methanol and the radioactivity was measured as described earlier (18).

Membrane fluidisation in isolated mast cells

Isolated mast cells (1.5—2×10^6/ml) were incubated with atenolol and propranolol for 5 min or with compound 48/80 for 3 min at 37°C. After cooling to 0°C and centrifugation (15 min, $35000 \times g$, 4°C) sedimented cells were resuspended in 50 µl of supernatant and mixed with 10 µl of stearic acid with the dimethyloxazolidine group at the 5th and 16th carbon (spin label). After mixing the cell suspension was filled into a glass capillary and before measurement samples were heated for 4 min at temperatures of 27, 30, 34, 37 and 40°C. ESR spectra were measured in the spectrometer ESR-230-X band and the parameter A was evaluated as described previously (12, 19).

[3]H-arachidionic acid liberation from membrane phospholipids

Purified mast cells (10^6 cells/ml) were prelabelled by incubation with ^{3}H-arachidonic acid (^{3}H-AA, 2.08×10^{-2} MBq) for 60 min at 37°C. They were then stimulated with compound 48/80 (1 µg/ml) or concanavalin A (Con A — 100 µg/ml) plus phosphatidyl-serine (PS — 2.5 µg/ml) for 1 and 15 min, respectively. Phospholipid extraction and radioactivity measurements were processed as described earlier (20).

RESULTS

Fig. 1 shows dose-dependent histamine liberation from IRMC with propranolol and atenolol. It is evident from the figure that PRO in the concentration of 10^{-4}/L liberated 7 times more histamine than did atenolol.
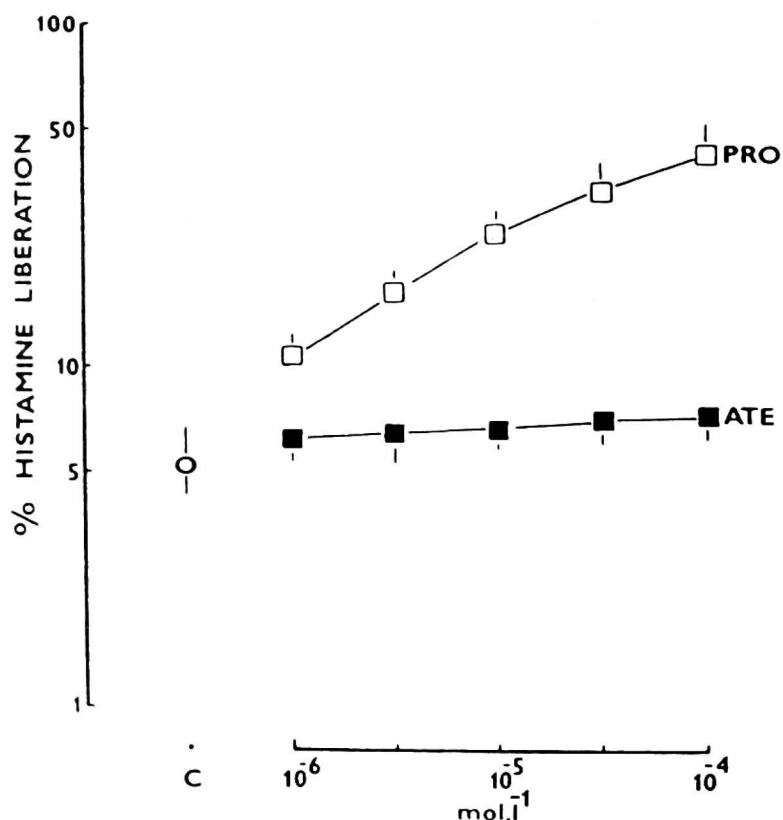
Fig. 1. Dose-dependent liberation of histamine from isolated rat mast cells with propranolol and atenolol (3 min at 37°C). $n = 6$ to 8, $x \pm$ S.E.M.

Table 1. Effect of atenolol and propranolol (100 μmol/L) on secretory index in mast cells. The secretory index represents the ratio between the percentage of histamine liberation and the percentage of degranulation as measured by ^{35}S liberation. Compound 48/80 (1 μg/ml), c = control (untreated sample), n = 4 to 6, mean $\pm$ SEM.

Sample	Secretory index S_i % histamine release % degranulation (^{35}S)
Control	0.85 ± 0.05
Compound 48/80	1.5 ± 0.07
Atenolol	0.6 ± 0.05
Propranolol	3.2 ± 0.1

The secretory index calculated from the ratio between the percentage amount of histamine liberation and the percentage of degranulation as measured by means of ^{35}S labelled granules is demonstrated in *Table 1.* Control cells liberated spontaneously an equal amount of granules and histamine with a ratio close to 1. Compound 48/80 liberated 1.5 times more histamine than the granules. The ratio of 0.6 indicates that atenolol slightly decreased histamine liberation compared to degranulation. Propranolol, on the other hand, liberated 3.2 times more histamine as compared to degranulation.

Fig. 2 demonstrates the dose-dependent inhibitory effect of atenolol and propranolol on histamine liberation from 48/80-stimulated IRMC. In the concentration range of 10^{-5}, 10^{-4} and 10^{-3} mol/L, atenolol and propranolol decreased the 48/80 stimulated histamine liberation by 6 and 11%, 14 and 21% and 44 and 58%, respectively.

Fig. 2. Dose-dependent effect of atenolol and propranolol on compound 48/80 stimulated isolated rat mast cells. Cells were preincubated for 3 min with beta-blockers, followed by 1 min incubation with 48/80, both at 37°C. $n = 6$ to 7, $x \pm$ S.E.M. $*p \leqslant 0.05$, $**p < 0.01$
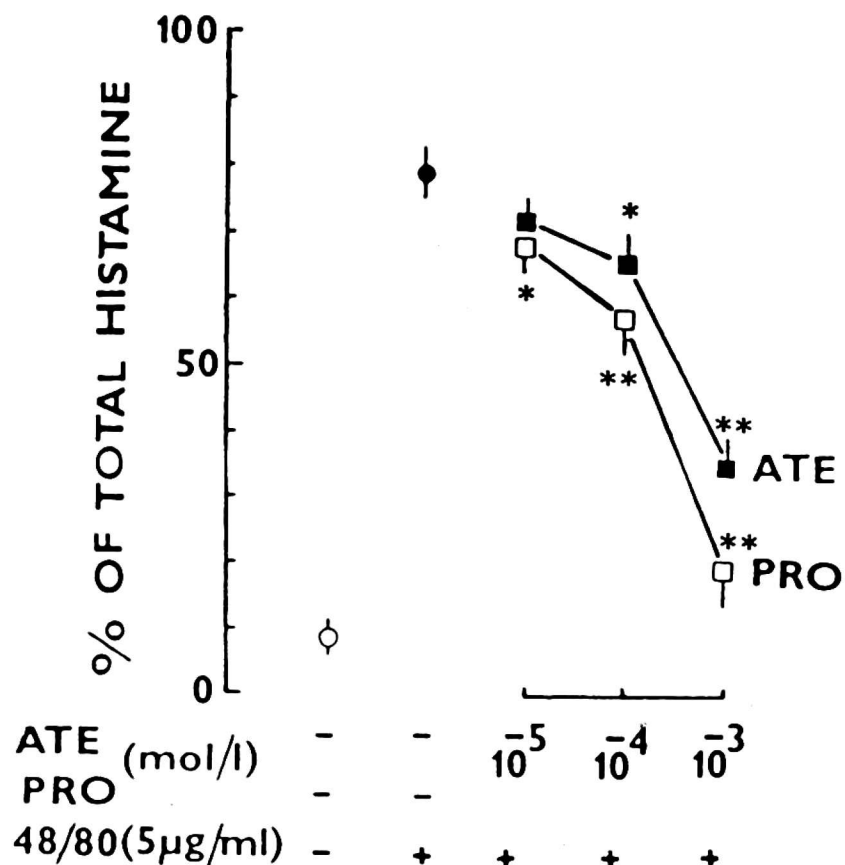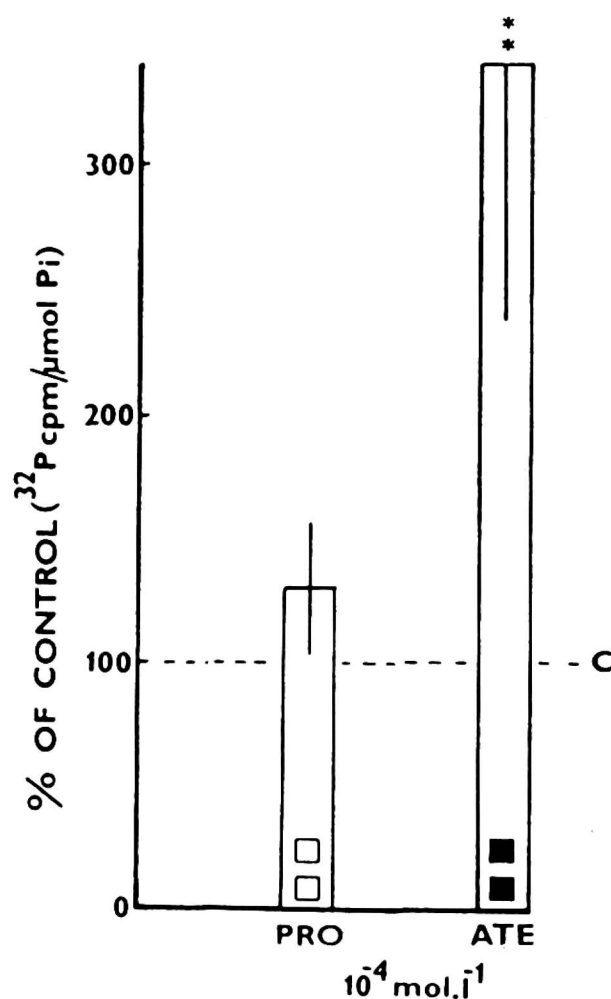
Fig. 3. Effect of atenolol and propranolol (10^{-4} mol/L) on ^{32}P incorporation into total mast cell membrane phospholipids at 37°C. Per cent values are related to the controls representing the cpm of ^{32}P in the sample. $n = 4$ to 6, $x \pm$ S.E.M. $**p \leqslant 0.01$

In our experiments compound 48/80 time-dependently increased ^{32}P incorporation into the mast cell mebrane phospholipids (21). *Fig. 3* shows that lipophilic propranolol in the concentration of 10^{-4} mol/l nonsignificantly

increased ^{32}P incorporation into total membrane phospholipids. Hydrophilic atenolol in the same concentration increased ^{32}P to 340% of the control value.

Fig. 4 demonstrates the temperature-dependent effect of atenolol and propranolol on the membrane order parameter $A'_\perp$ of ESR spectra in isolated mast cells in comparison with the control value and 48/80-stimulated cells. By increasing the incubation temperature from 27°C to 40°C, the parameter $A'_\perp$
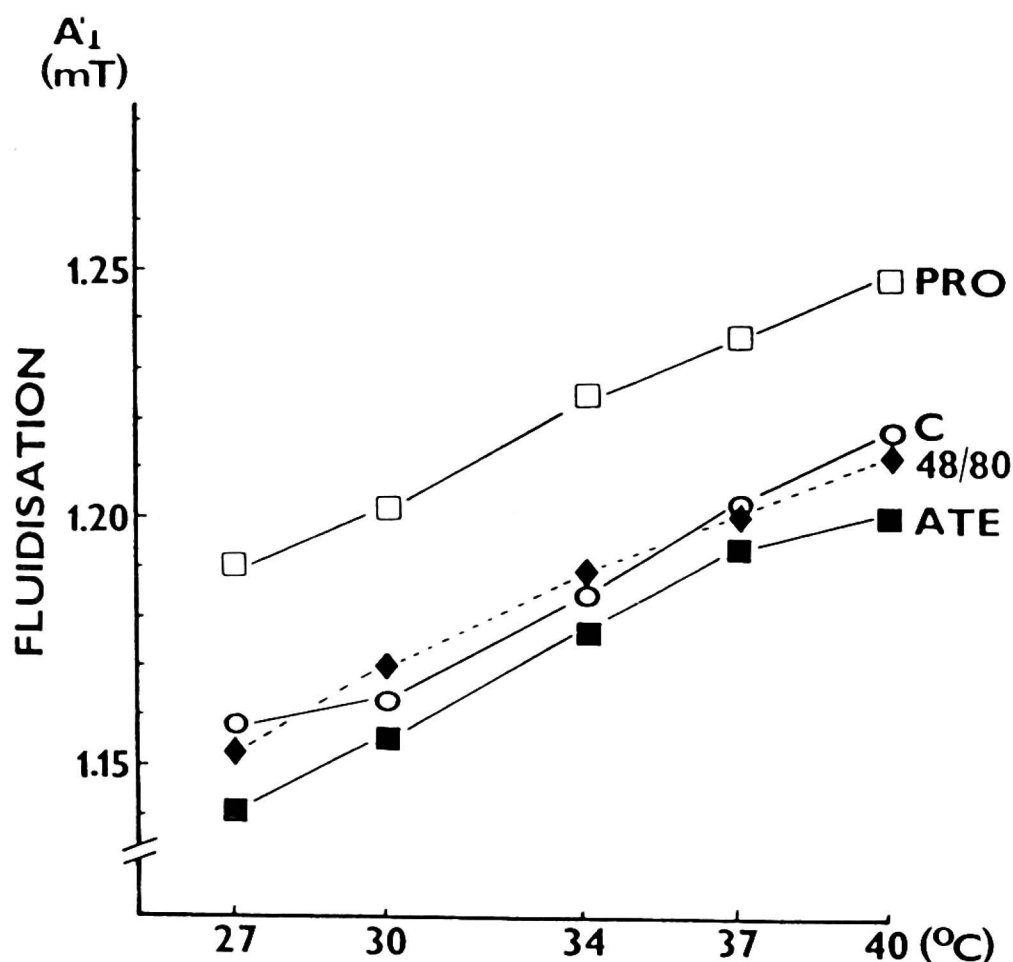
Fig. 4. Effect of atenolol, propranolol (in 1 mmol/L concentration and compound 48/80—5 µg/ml) on membrane order parameter $A'_\perp$ calculated from the ESR spectra of whole mast cells in a temperature — dependent relationship. Each point represents the mean from 2 to 4 measurements. Calculated maximal deviations are within the symbols.

increased from 1.1588 to 1.216. The values for 48/80 stimulated cells did not change significantly. These data confirmed that with increasing the incubation temperature the membrane fluidisation increased proportionally. Propranolol increased the parameter $A'_\perp$ from 1.191 (27°C) to 1.248 (40°C). Membrane fluidisation by propranolol at 30°C corresponded to the heating of the mast cell to 37°C. Atenolol decreased the parameter $A'_\perp$ as compared with controls to 1.141 at 27°C and to 1.199 at 40°C. The effect of atenolol and propranolol on ^{3}H-arachidonic acid liberation from total membrane phospholipids of 48/80 and conA + PS stimulated mast cells is shown in *Fig. 5.* Compound 48/80 and conA + PS increased ^{3}H-AA liberation from 10.4 ± 0.5 to 15.3 ± 1.9 and $20.5 \pm 0.8\%$, respectively. Atenolol decreased the 48/80 and conA + PS
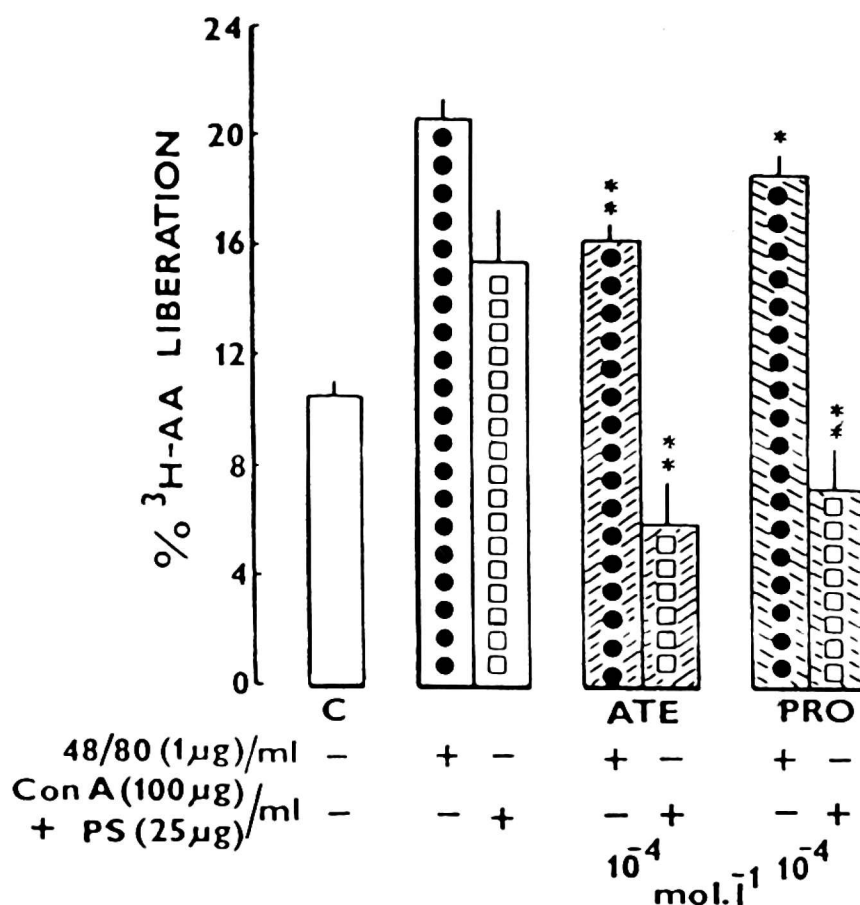
Fig. 5. Effect of atenolol and propranolol (100 µmol/L) on ^{3}H-arachidonic acid liberation from total membrane phospholipids of isolated rat mast cells stimulated with compound 48/80 and concanavalin A+phosphatidylserine (Con A+PS). C = control, n = 4 to 6, $x \pm$ S.E.M. $*p \leqslant 0.05$, $**p \leqslant 0.01$

stimulated ^{3}H-AA release to 16.2 ± 0.5 and $5.8 \pm 1.5\%$, respectively. Propranolol decreased the 48/80 and ConA+PS stimulated ^{3}H-AA release to 17.8 ± 0.7 and $6.2 \pm 1.6\%$, respectively.

DISCUSSION

Propranolol, but not atenolol, liberated histamine dose-dependently from isolated rat mast cells. The ratio of histamine liberation to degranulation indicated that propranolol, in the concentration of 100 µmol/l, liberated 3.2 times more histamine than granules. A wide variation among BAB drugs was demonstrated for their histamine and serotonin liberating activity from mast cells and platelets, respectively (7, 22, 23). This effect of BAB drugs was shown to be closely related with physio-chemical properties of BAB drugs, particularly with their liposolubility (24, 25). Moreover, histamine liberation by highly lipophilic drugs occurred intracellularly, indicating that CAD drugs, after passing the plasma membrane of mast cells, displaced histamine from granule binding sites (6). This may explain why propranolol, which is 1000 times more liposoluble than atenolol, liberated histamine from mast cells (24).

On the other hand, both propranolol and atenolol inhibited 48/80-induced histamine liberation from isolated mast cells in a concentration-dependent manner. Propranolol was more potent than atenolol. A similar inhibitory effect was demonstrated for trimepranol (26) in 48/80 stimulated isolated rat mast cells. The inhibition of stimulated histamine secretion with BAB drugs seems to be the result of nonspecific rather than specific inhibition at the plasma membrane site. Atenolol, but not propranolol, significantly increase membrane phospholipid turnover in isolated mast cells as measured by ^{32}P incorporation. A similar effect was described in chlorpromazine, desmethylimipramine and propranolol treated C6 glioma cells and was associated with a shift of precursor incorporation toward acidic phospholipids (27). On the contrary, propranolol significantly increased plasma membrane fluidisation in the deeper phospholipid bilayer (C_{16}). Atenolol, on the other hand, tended to make the membrane more „rigid" by decreasing its fluidity. A comparable difference in membrane fluidisation was observed in blood platelets treated with atenolol or propranolol (12). Since both atenolol and propranolol bind to mast cells (18) these CAD can enter the mast cell plasmic membrane, eliciting however, different responses.

Propranolol, the more lipophilic CAD, has been suggested to pass through the membrane increasing the phospholipid fluidity and slightly potentiating membrane phospholipid turnover. By entering the intracellular environment, propranolol becomes protonated and may displace histamine from intracellular granules, as demonstrated also on isolated mast cells granules (15). Atenolol, which is evidently hydrophilic (24), most probably accumulated only in the superficial part of the mast cell plasma membrane. As a result of this incorporation atenolol decreased membrane fluidisation and increased membrane phospholipid turnover as measured by ^{32}P incorporation.

Both atenolol and propranolol significantly decreased stimulated arachidonic acid liberation from mast cell membrane phospholipids. This is most probably the result of membrane phospholipase A_2 inhibition induced by both drugs. CAD were demonstrated to inhibit PLA_2 activation in cells and tissues (28). These drugs have been suggested to inactivate PLA_2 either by occupying the active centre of the enzyme or by binding to membrane phospholipids thus preventing arachidonic acid liberation (27, 29). Stimulation of isolated mast cells with 48/80 is associated with PLA_2 activation and arachidonic acid liberation (30). The inhibition of 48/80 stimulated arachidonic acid liberation and secretion by atenolol and propranolol resulted most probably from the inhibition of PLA_2 in mast cells. This suggestion, however, requires further direct experimental evidence on the interaction of atenolol and propranolol with the AA pathway in isolated mast cells.

REFERENCES

1. Nosál R, Slorach SA, Uvnäs B. Quantitative correlation between degranulation and histamine release following exposure of rat mast cells to compound 48/80 in vitro. *Acta Physiol Scand* 1970; 80: 215—221.

2. Lau HJA, Pearce FL. Effect of antihistamine on isolated rat peritoneal mast cells and model membrane systems. *Agents Actions* 1990; 29: 151—161.

3. Nosál R, Rýc M, Mašek K, Rašková H. Effect of staphylococcal alpha toxin on isolated rat mast cells. *Toxicon* 1972; 10: 329—333.

4. Nosál R, Novotný J, Šikl D. The effect of glycoprotein from Candida albicans on isolated rat mast cells. *Toxicon* 1974; 12: 103—108.

5. Drábiková K, Nosál R. On the interaction of local anesthetics with mast cells. *Agents Actions* 1987; 20: 191—193.

6. Nosál R, Pečivová J, Drábiková J. Evidence for intracellular histamine liberation in isolated rat mast cells. *Agents Actions* 1982; 12: 601—607.

7. Nosál R, Menyhardtová Z. The effect of beta-adrenergic blocking drugs and inhibitors of phosphodiesterase on histamine release from isolated mast cells. *Agents Actions* 7; 1977: 231—236.

8. Drábiková K, Pečivová J, Nosál R. Inhibitory effect of chloroquine on rat mast cell activation. *Agents Actions*. Spec Conf Issue 1992: C318—320.

9. Terpstra KG, Raaijmakers JAM, Nassink G. Propranolol induced bronchoconstriction: a non-specific side-effect of beta-adrenergic blocking therapy. *Eur J Pharmacol* 1981; 73: 107—108.

10. Gerritsen J, Kalter GH, Van Der Weele LT, Knol T. Propranolol inhalation challenge in relation to histamine response in children with asthma. *Thorax* 1988; 43: 451—455.

11. Vanda B, Montano LM, Segura P, Rubio H, Seleman M, Vargas MH. Propranolol induced hyperreactivity in guinea pig lung parenchyma strips. Possible role of histamine. *Arch Int Pharmacodyn* 1992; 319: 101—103.

12. Nosál R, Jančinová V, Ondriaš K, Jakubovský J, Balgavý P. The interaction of beta-adrenoceptor blocking drugs with platelet aggregation, calcium displacement and fluidisation of the membrane. *Biochim Biophys Acta* 1985; 821: 217—228.

13. Jančinová V, Nosál R. Differences among betaadrenoceptor blocking drugs in modifying platelet aggregation and arachidonic acid liberation under thrombin stimulation. *Thromb Res* 54; 1989: 687—698.

14. Nosál R, Pečivová J, Drábiková K. Alterations in membrane phospholipid turnover, arachidonic acid liberation and thromboxane formation in isolated mast cells treated with propranolol and atenolol. *Agents Actions*, Spec Conf Issue 1992: C253—C255.

15. Nosál R, Pečivová J, Drábiková K. On the interaction of beta-adrenoceptor blocking drugs with isolated mast cells. *Agents Actions* 1985; 16: 478—484.

16. Shore PA, Burkhalter A, Cohn VH Jr. A method for the fluorometric assay of histamine in tissues. *J Pharmac Exp Therap* 1959; 127: 182—186.

17. Bergendorff A, Uvnäs B. Storage of 5-hydroxytryptamine in rat mast cells. Evidence for an ionic binding to carboxyl groups in a granule protein complex. *Acta Physiol Scand* 1972; 84: 320—331.

18. Pečivová J, Drábiková K, Jančinová V, Nosál R. On the relationship between incorporation of ^{32}P into phospholipids and binding of beta-adrenoceptor blocking drugs to isolated mast cells. *Agents Actions* 1988; 23: 146—148.

19. Nosál R, Ondiaš K, Pečivová J, Drábiková K. Histamine liberation and membrane fluidisation of mast cells exposed to the betaadrenoceptor blocking drug propranolol. *Agents Actions* 1988; 23: 143—145.

20. Pečivová J, Prábiková K, Jančinová V, Petríková M, Nosál R. Atenolol, exaprolol and mast cell membranes. *Agents Actions* 1991; 33: 41—43.

21. Pečivová J, Drábiková K, Nosál R. Effect of beta-adrenoceptor blocking drugs on ^{32}P incorporation into and arachidonic acid liberation from phospholipids in stimulated rat mast cells. *Agents Actions* 1989; 27: 29—32.

22. Lemmer B, Wiethold G, Hellenbrecht D, Bak IJ, Grobecker H. Human blood platelets as cellular model for investigation of membrane active drugs: Beta-adrenoceptor blocking drugs. *Naun Schmied Arch Pharmacol* 1972; 275: 299—313.

23. Nosál R, Drábiková K, Jančinová V, Pečivová J. Cellular dose-dependent relationship for evaluation of side and adverse reactions of beta-adrenoceptor blocking drugs. In Dose-Response Relastionship of Drugs. Wingender W. (ed). 1990, W. Zuckschwerdt Verlag Munchen, pp. 61—66.

24. Nosál R, Drábiková K, Pečivová J, Ondriaš K. Membrane perturbing activity of beta-adrenoceptor blocking drugs in isolated rat mast cells. *Agents Actions* 1989; 27: 36—38.

25. Nosál R, Jančinová V, Petriková M. Antiaggregatory properties of beta-adrenoceptor blocking drugs are related to their physico-chemical properties. *Platelets* 1992; 3: 29—33.

26. Nosál R, Menyhardtová Z. The effect of trimepranol on thrombocyte function and histamine release in the rat. *Agents Actions* 1975; 5: 9—14.

27. Leli U, Hauser G. Modifications of phospholipid metabolsim induced by chlorpromazine, desmethylimipramine and propranolol in C6 glioma cells. *Biochem Pharmacol* 1987; 36: 31—37.

28. Pappu AS, Izaki PI, Hostetler KY. Inhibition of purified lysosomal phospholipase A$_2$ by beta-adrenoceptor blockers. *Biochem Pharmacol* 1985; 34: 521—524.

29. Leli U, Hauser G. Mechanism of modification of rat brain lysophospholipase A activity by cationic amphiphilic drugs. *Biochim Biophys Acta* 1987; 918: 126—135.

30. Okano Y, Ishizuka Y, Nakashima Y, S, Tohmatsu T, Takagi H, Nosawa Y. Arachidonic acid release in rat peritoneal mast cells stimulated with antigen, ionophore A 23187 and compound 48/80. *Biochem Biophys Res Commun* 1985; 127: 726—732.

Author's address: R. Nosál, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská 2, 842 156 Bratislava, Slovakia.