

ANTIANAPHYLACTIC AND ANTI-ASTHMATIC PROPERTIES OF NEW PIPERAZINYL 7-(β -HYDROXYPROPYL)- -THEOPHYLLINE DERIVATIVES IN GUINEA PIGS

Ryszard Czarnecki^{1, #}, Tadeusz Librowski¹, Maciej Pawłowski²

¹Department of Pharmacodynamics, ²Department of Pharmaceutical Chemistry, Medical College,
Jagiellonian University, Medyczna 9, PL 30-688 Kraków, Poland

Antianaphylactic and antiasthmatic properties of new piperazinyl 7-(β -hydroxypropyl)-theophylline derivatives in guinea pigs. R. CZARNECKI, T. LIBROWSKI, M. PAWŁOWSKI. Pol. J. Pharmacol., 2001, 53, 131–136.

The present studies have demonstrated that new piperazinyl 7-(β -hydroxypropyl)-theophylline derivatives (R3, R6, R7) possess antihistamine, anti-anaphylactic and antiasthmatic properties. The compound R6 exerted especially pronounced selective protective action in experimental histamine asthma and provided effective prevention against anaphylactic shock in guinea pigs. The evidence was also presented that compound R6 inhibited *in vitro* mast cell degranulation induced by the preparation 48/80 liberating endogenous histamine. It was shown that the compound R6, i.e. 7-(β -hydroxypropyl)-[N1-(N4-benzyl)-piperazinyl]-theophylline efficiently competed with histamine of both endo- and exogenous origin and inhibited the mediator release from the mast cells.

Key words: *theophylline derivatives, experimental histamine asthma, anaphylactic shock*

INTRODUCTION

Theophylline shows the broadest spectrum of actions in comparison with other methylxanthines and this is a reason why this compound and its derivatives have evoked widespread interest among researchers [3, 4, 8, 9]. Specific profile of pharmacological action can be achieved by chemical modification of parent compound which fulfils requirements of pharmacophore system. The results of hitherto conducted pharmacological studies aimed at enhancing bronchospasmolytic, circulatory and diuretic or psychoanaleptic actions substantiate great potential of structural modifications of parent theophylline molecule [6, 11, 12].

Among theophylline derivatives with bronchodilatory activity, the following attracted particular interest which led to the accumulation of most abundant literature data: 7-substituted, 7, 8-disubstituted or 1-alkyl derivatives of 3-cyclopropylxanthine bearing substituents of aldehyde, semiacetal or acetal type at position 7 [5, 10, 13].

The present pharmacological studies investigated bronchospasmolytic activity of three piperaziny derivatives of 7-(*-*hydroxypropyl)-theophylline synthesized at the Department of Pharmaceutical Chemistry, Medical College, Jagiellonian University: 7-*-*hydroxy-*-*[N1-(N4-2-hydroxyethyl)-piperaziny]-theophylline (R3), 7-*-*hydroxy-*-*[N1-(N4-benzyl)-piperaziny]-propyl-theophylline (R6), 7-*-*hydroxy-*-*[N1-(N4-2-hydroxypropyl)-piperaziny]-propyl-theophylline (R7).

MATERIALS and METHODS

Animals

The experiments were carried out on adult male guinea pigs (330–480 g), which had been kept under stable living conditions and on a uniform diet. The animals were not fed for 24 h before the experiments, only drinking water was available *ad libitum*. The animals were kept in cages in a room at a temperature of $20 \pm 4^\circ\text{C}$, under 12/12 h light/dark cycle beginning at 7.00 h. All experiments were carried out between 8.00 and 12.00 h. The experiments were performed in accordance with the ethical requirements.

Drugs

The following substances were used in the tests: histamine (histaminum dihydrochloricum, Polfa),

aminophylline (aminophyllinum, Polfa), tavegyl (clemastin, Polfa), acetylcholine (acetylcholine bromide, BDH Chemicals Ltd.) atropine (atropinum sulfuricum, Polfa) and mepyramine (mepyramine maleate, May-Baker Ltd.).

Statistical analysis

Comparisons between average values determined before and after administration of the compound were made using ANOVA. Data are shown as means of 6–8 determinations \pm (SEM).

Experimental bronchial asthma caused by histamine and acetylcholine

The experiments were carried out on adult male guinea pigs (330–480 g), which had been kept under stable living conditions and on a uniform diet. The animals were not fed for 24 h before the experiments, only drinking water was available *ad libitum*. The groups administered every drug dose as well as control groups always comprised ten animals. The examined compounds were injected subcutaneously, 60 min before a subject was placed in an inhalatory chamber. The appearance of the first evidence of symptoms of asphyxia (the bronchospastic reaction) was monitored. In the control groups, an aerosol of histamine (0.5% histamine dihydrochloride in 0.9% NaCl solution) or acetylcholine aerosol provoked a bronchospastic reaction in 100% of the animals after 40–60 s. The examined compounds delayed the appearance of the bronchospastic reaction by about 400–600 s. The effective dose was determined as that which protected against the bronchial asthma for 200 s ($\text{ED}_{200\text{s}}$) and the ED_{50} for this time point was also determined.

Inhibition of anaphylactic shock in guinea pigs

The examinations were carried out on male guinea pigs, (250–400 g), kept under laboratory conditions for about one month before the experiment. The animals were sensitized twice by intraperitoneal administration of 0.5 ml of a 5% solution of ovalbumin (in 0.9% NaCl) at two-day intervals. On the 21st day after the last administration of a sensitizing ovalbumin dose, an anaphylactic shock was provoked within 20 s by injection of 0.2 ml of 5% albumin into the saphenous vein. Lethal anaphylactic shock developed in all 100% of the animals in the control groups. The animals died within the first 2–4 min from the administration of the causing dose of antigen. Doses of the examined

compounds were injected subcutaneously into animal groups consisting of eight guinea pigs, at two times. Firstly, simultaneously with the antigen and secondly, 1 h before the injection of the second ovalbumin dose. The control groups were injected subcutaneously with the equivalent volume of physiological saline. The criterion used for estimation of drug efficacy was a decrease in the lethality in guinea pigs, which were injected with the antigen at a dose expected to give a lethal outcome in all animals in the control group. The animals which did not die during the critical first 10 min from the antigen administration were regarded as radically (100% protection) preserved from death during the anaphylactic shock.

Degranulation of mastocytes *in vitro*

Experiments were carried out using male Wistar rats, (160–220 g). In a light ether narcosis, fragments of the intestine mesentery were taken and incubated in 5 ml of solution (the incubation solution was Sorrensen buffer pH = 7.4 with a Tyrode solution (composed of 0.16 M NaCl, 0.003 M KCl and 0.0009 M CaCl₂) supplemented with the examined compounds. After the incubation, the mesentery fragments were stretched on a glass base, fixed in methyl alcohol, stained in 0.25% toluidine blue and covered using Canada balsam. Next, the damaged and non-damaged mastocytes were counted. As the damage criterion, the presence of at least 3 grains in the extracellular region was accepted. The experiments aimed to determine the concentrations of the compound R₆, aminophylline and mepyramine

equivalent to dinatriumcromoglycat (DNCG) in inhibiting the mastocyte degranulation, caused by the addition of 0.4 g/ml of histamine liberator (compound 48/80). The composition of the samples incubated for this purpose is given below:

- control sample – incubation solution;
- incubation solution + aminophylline (0.3 mg/ml) + preparation 48/80 (0.4 g/ml);
- incubation solution + DNCG (0.3 mg/ml) + preparation 48/80 (0.4 g/ml);
- incubation solution + compound R₆ (0.3 mg/ml) + preparation 48/80 (0.4 g/ml);
- incubation solution + mepyramine (0.3 mg/ml) + preparation 48/80 (0.4 u g/ml);
- incubation solution + clemastine (0.3 mg/ml) + preparation 48/80 (0.4 g/ml).

The preparation 48/80 was added to the incubation solution after 10 min of incubation with the compounds examined. The incubation was carried out at room temperature. At least three fragments of the mesentery were used for the incubation.

RESULTS

Influence of the compounds on experimental bronchial asthma caused by histamine and acetylcholine in guinea pigs

The compound R₆ at a dose of 0.6 mg/kg *sc* (ED_{200s}) protected 100% of animals against a histaminic asthma attack and assured a ca 24 h survival time after the experiment. The action of the compounds R₃ and R₇ was considerably weaker and,

Table 1. The effect of R₃, R₆, R₇ and clemastine, aminophylline, mepyramine and atropine on the experimental bronchial asthma induced by histamine or acetylcholine aerosol in guinea-pigs

Compound	Histamine-induced asthma				Acetylcholine-induced asthma				ED ₅₀ anti-ACh
	ED _{200s} mg/kg	ED ₅₀ mg/kg	Relative activity	TI (LD ₅₀ / ED ₅₀)	ED _{200s} mg/kg	ED ₅₀ mg/kg	Relative activity	TI (LD ₅₀ / ED ₅₀)	ED ₅₀ anti-Hist
Clemastine	0.11***	0.08	1	2225.0	1.38*	0.97	1	183.5	12.1
R ₃	8.50**	6.85	0.01	310.0	40.00*	31.80	0.03	66.8	4.64
R ₆	0.6***	0.21	0.38	1885.7	> 30.0	> 30.0	> 0.03	> 13.2	> 142.8
R ₇	3.4**	2.60	0.03	769.2	28.00**	19.80	0.04	101.0	7.61
Aminophylline	10.0	7.40	0.01	22.8	19.50	14.70	0.06	13.3	1.98
Mepyramine	0.135*	0.97	0.08	92.7	1.00**	0.81	1.19	111.1	0.83
Atropine	4.1	3.10	0.02	90.3	1.50*	0.80	1.21	350.0	0.25

All investigated compounds were administered *sc* 60 min before histamine or acetylcholine; * p < 0.05, ** p < 0.01, *** p < 0.001

even then, only at higher doses (Tab. 1). Clemastine at a dose of 0.11 mg/kg *sc* protected 100% of guinea pigs against a histaminic asthma attack (ED_{200s}). These values changed only slightly in the experimental acetylcholinic asthma (Tab. 1). Clemastine showed the best, statistically significant ($p < 0.001$) protection against histaminic bronchospasm (as shown by ED_{200s}, ED₅₀ and therapeutic index). Although clemastine acted quantitatively stronger than compound R₆, it did not demonstrate

a selective activity as in these experiments it inhibited the bronchial tree obturation, caused either by histamine or acetylcholine inhalation (Tab. 3). The examined compounds could be ranked in the following order, according to AC/AH coefficient: atropine < mepyramine < aminophylline < R₃ < R₇ < clemastine < R₆. The AC/AH coefficient, which illustrates the anticholinergic to antihistaminic relation, was 0.25 for atropine, 12.1 for clemastine and more than 142.8 for compound R₆ (Tab. 1). How-

Table 2. The effect of aminophylline (AM), R₆, DNCG and mepyramine (MEP) on mast cell degranulation induced by compound 48/80 *in vitro*

Investigated compounds	Dose mg/ml	No. of animals	t	p	Mean % of degranulated mast cells ± SE
Control	–	18			6.76 ± 2.14
AM	0.3	18	0.831	< 0.05	7.67 ± 2.12
AM + 48/80		17	5.8572	< 0.001	28.02 ± 8.22
R ₆	0.3	23	0.7832		9.66 ± 2.87
R ₆ + 48/80		18	14.439	< 0.001	7.97 ± 2.21
DNCG	0.3	24	0.9506		10.67 ± 3.90
DNCG + 48/80		19	4.1061	< 0.001	43.73 ± 7.29
MEP	0.3	23	1.688		13.46 ± 3.58
MEP + 48/80		18	13.523	< 0.001	7.40 ± 3.06
48/80	0.4 g/ml	22			76.81 ± 3.89

Table 3. The effect of R₆, mepyramine and aminophylline on histamine- and acetylcholine-induced experimental asthma in guinea-pigs

Compounds	Dose mg/kg	No. of animals	Mean % of protection after 1 h ± SE	
			Histamine-induced asthma	Acetylcholine-induced asthma
R6	0.5	18	19.87 ± 4.23	–
	1.0	18	61.04 ± 2.85**	4.09 ± 1.54**
	5.0	19	97.21 ± 1.56***	5.12 ± 1.92**
	20.0	23	–	7.36 ± 2.21*
Mepyramine	0.125	23	18.76 ± 3.48	–
	0.25	18	53.42 ± 4.22	29.11 ± 2.18*
	0.5	19	78.94 ± 2.83	36.66 ± 4.08
	1.0	23	98.46 ± 2.48*	41.76 ± 5.34
Aminophylline	5.0	19	19.92 ± 6.12	19.27 ± 3.11
	10.0	18	76.53 ± 3.31	24.49 ± 3.96
	20.0	18	63.89 ± 5.72	35.97 ± 4.34

Compounds given *sc* 60 min before inhalation; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

ever clemastine was the strongest acting compound in histaminic asthma ($ED_{50} = 0.08$ mg/kg *sc*), but its ED_{50} in acetylcholinic asthma was 0.97 mg/kg *sc*. At this dose, the compound exerted a marked depressant activity, expressed by a lack of movement coordination, or even ataxia. The best therapeutic index was that of clemastine (2225.0), next was that of compound R_6 , i.e. 1885.7. The lowest was that of aminophylline averaging 22.8.

Influence of the compounds on the course of an anaphylactic shock

In the control group, after intravenous injection of the antigen (0.5 ml of 5% ovoalbumin) at the shock-producing dose, violent signs of the anaphylactic shock were observed during the first minute with a progressive cyanosis maintained until death within a period of about 4 min. In addition to disturbances of respiration, a dysfunction of the alimentary tract (diarrhoea) and an increase in salivation and urination was observed. The compound R_6 administered 60 min before the injection of the antigen shock-producing dose prevented most efficaciously and for the longest duration an anaphylactic reaction in guinea pigs. At a dose of 1.25 mg/kg *sc* it gave a high protection against respiratory disturbances and a restriction of the progressive cyanosis. Tachypnoea was not observed and, importantly, the apnoea periods occurred later as well. Moreover, there was no appearance of dysfunction of the alimentary tract or involuntary urination in this group. In animals which survived first ten minutes of anaphylactic shock, an improvement in their general condition took place during the following hours.

The compound R_6 at a dose of 5 mg/kg *sc*, administered together with ovalbumin did not inhibit the anaphylactic shock, and death of all animals occurred within the first two–four minutes. The compound R_6 acted a little weaker at a dose of 0.1, 0.5 and 5 mg/kg *sc*. The compound R_3 at doses of 0.1, 0.5 and 5 mg/kg *sc* was not active in the test. Aminophylline showed the weakest inhibitory effect on the anaphylactic shock, and only at a dose of 10 mg/kg *sc* it protected 50% of animals from anaphylactic shock. Only by an increase in the aminophylline dose to 40 mg/kg *sc*, protection of 90% of the animals from the death as a result of anaphylactic shock was afforded. However, after 20–40 min all the animals died, showing increasing symptoms of breathlessness. Mepyramine at a dose

of 0.25 mg/kg *sc* administered 60 min before the antigen decreased the anaphylactic lethality to 40% of guinea pigs and extended the survival times to 30–60 min. However, a dose of 0.5 mg/kg *sc* protected almost 100% animals from death. The animals which survived 10 min died in the next 60 min. Clemastin administered at doses of 0.01 mg/kg *sc* and 0.1 mg/kg *sc* protected 40% and 100% of guinea pigs, respectively, from anaphylactic shock-induced death during 10 min.

Influence of the compounds on the degranulation of mastocytes *in vitro*

The compound R_6 inhibited *in vitro* degranulation of mastocytes caused by the preparation 48/80. The degree of this inhibition, expressed as the number of the morphologically degranulated cells, was higher than the inhibition caused by DNCG and by aminophylline (Tab. 2).

Compound R_6 and also mepyramine, added to the incubation solution *in vitro* at a concentration of 0.4 mg/ml, largely inhibited degranulation of mastocytes exposed to the preparation 48/80 by 92.03% ($p < 0.001$) and 92.6% ($p < 0.001$), respectively. A weaker effect was exerted by aminophylline and by DNCG, which inhibited degranulation of mastocytes by 71.98% ($p < 0.001$) and 56.27% ($p < 0.001$), respectively. It should be added that the inhibitory effect on degranulation was abolished by a single washing of the mastocytes with the incubation solution. This indicates that compound R_6 binds in a reversible manner with cell membrane and this effect is responsible for its protective action.

DISCUSSION

Our results show that the examined dimethylxanthine derivatives, the compounds R_6 , R_7 and R_3 , demonstrate many of the characteristic features of antihistaminic agents. The arguments for the antihistaminic activity of the series of R compounds were obtained from the studies on experimental histaminic asthma and acetylcholinic asthma. Clemastine was shown to be the strongest inhibitor of histaminic asthma. At a dose of 0.11 mg/kg *sc* it protected 100% of the examined animals from all asthmatic symptoms, but at a dose of 1.38 mg/kg *sc* it inhibited also asthma caused by acetylcholine. To achieve a similar 100% protection from histaminic asthma by compound R_6 , it was necessary to

apply a dose of 0.6 mg/kg *sc*. Even, at the highest used dose, i.e. 30 mg/kg *sc*, it had no influence on the bronchospastic reaction caused by acetylcholine. This shows that especially compound R₆ had no spasmolytic activity, but did have selective antihistaminic properties. It is possible that this antihistaminic activity arises from an inhibition of phosphodiesterase and thus from an increase in the cAMP level in smooth muscles, similar to that reported for papaverine [7] and methylxanthines [2]. It has also been shown that the degree of relaxation of smooth muscles caused by methylxanthines and by papaverine is strongly correlated with an inhibitory power for phosphodiesterase activity [1]. It seemed possible that the examined compounds would also have a preventative activity against anaphylactic shock due to antihistaminic activity. In fact, the most effective agent in this group in anaphylactic shock prevention, the compound R₆, at a dose of 1.25 mg/kg *sc* showed an ability, firstly, to inhibit the external symptoms of an anaphylactic shock (dyspnoea, cyanosis, collapsus); secondly, it could transform the irreversible shock into a reversible condition and, thirdly, it prolonged the survival of the animals. The compound R₇ showed much weaker activity. On the other hand, R₃ was almost devoid of any protective influence on anaphylactic shock in guinea pigs. It is possible that the compounds R_n, particularly R₆ with the strongest action comparable with that of clemastine, prevents anaphylactic shock by a complicated mechanism involving: inhibition of degranulation of the target cells, releasing mediators from these cells and a blockage of constitutive activity of histamine, as one of possible patterns. We have also shown that the compound R₆ inhibits the degranulation of mastocytes *in vitro*, which is enhanced by the presence of preparation 48/80, the liberator of endogenous histamine. Therefore, R₆ counteracts some of the pharmacological effects of the endogenous histamine. This activity is reversible, disappearing after rinsing the cells. In conclusion, it seems possible that the structure of 7-hydroxy-N₄-benzylpiperazinopropyl-theophylline is such that it stabilizes the membrane of mastocytes and thus competes efficiently with the endogenous histamine and simultaneously abolishes pharmacological activity of exogenous histamine. From a pharmacological and clinical point of view, the compound R₆ shows the most significant therapeutic action on

the course of the anaphylactic reaction, both inhibiting the releasing process of mediators and also efficaciously competing with endo- and exogenous histamine.

REFERENCES

1. Buckle D.R., Arch J.R.S., Conolly B.J., Fennick A.E., Foster K.A., Murray K.J., Readshaw S.A., Smallridge M., Smith D.G.: Inhibition of cyclic nucleotide phosphodiesterase by derivatives of 1,3-bis(cyclopropylmethyl)xanthine. *J. Med. Chem.*, 1994, 37, 476–485.
2. Butcher R.W., Baird C.E., Sutherland E.W.: Effects of lipolytic and antilipolytic substances on adenosine 3',5'-monophosphate levels in isolated fat cells. *J. Biol. Chem.*, 1968, 243, 1713–1717.
3. Cockcroft D.W.: Pharmacological therapy for asthma: overview and historical perspective. *J. Clin. Pharmacol.*, 1999, 39, 216–222.
4. Corsano S., Scapischi R., Strappaghetti G.: Bronchodilator activity of theophylline derivatives substituted at the 7-position. *Arch. Pharm. (Weinheim)*, 1994, 327, 631–635.
5. Corsano S., Strappaghetti G., Ferrini R., Giglioli N.: Synthesis and pharmacological evaluation of 6-(7-theophylline)-3(2H)-pyridazinone. *Arch. Pharm. (Weinheim)*, 1991, 324, 999–1001.
6. Coward W.R., Sagara H., Church M.K.: Asthma, adenosine, mast cells and theophylline. *Clin. Exp. Allergy*, 1998, 28, Suppl. 3, 42–46.
7. Kukovetz W.R., Pösch G.: The positive inotropic effect of cyclic AMP. In: *Advances in Cyclic Nucleotide Research*, Raven Press, New York, 1972, 261.
8. Minoguchi K., Kohno Y., Oda N., Wada K., Miyamoto M., Yokoe T., Hashimoto T., Akabane T., Kobayashi H., Mita S., Kihara N., Adachi M.: Effect of theophylline withdrawal on airway inflammation in asthma. *Clin. Exp. Allergy*, 1998, 28, 57–63.
9. Mutschler E.: *Arzneimittel-Wirkungen. Lehrbuch der Pharmakologie und Toxikologie*, Wiss. Verl. Ges., Stuttgart, 1991.
10. Negwer M.: *Organic-Chemical Drugs and Their Synonyms*, Akademie Verlag, Berlin, 1996.
11. Page C.P.: Recent advances in our understanding of the use of theophylline in the treatment of asthma. *J. Clin. Pharmacol.*, 1999, 39, 237–240.
12. Sakai R., Konno K., Yamamoto Y., Sanae F., Takagi K., Hasegawa T., Iwasaki N., Kakiuchi M., Kato H., Miyamoto K.: Effects of alkyl substitutions of xanthine skeleton on bronchodilation. *J. Med. Chem.*, 1992, 35, 4039–4044.
13. Zlatkov A.B., Peikov P.T., Danchev N.D., Ivanov D.I., Tsvetkova B.: Synthesis, toxicological, pharmacological assessment, and *in vitro* bronchodilating activity of some 7-theophyllinylacetyloxyglycols. *Arch. Pharm. (Weinheim)*, 1998, 331, 313–318.

Received: March 12, 2001; in revised form: April 20, 2001.