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Nonspecific membrane effects of CH-103: hydrophobicity, surface activity and membrane fluidity studies in comparison with propranolol and practolol

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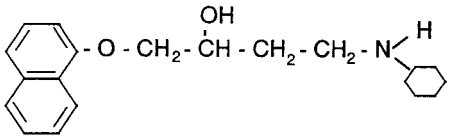
The partition coefficient, surface activity and membrane fluidizing/disordering effects of CH-103, a β-adrenergic receptor antagonist, were compared to those of propranolol and practolol as reference compounds. Changes in membrane fluidity were followed by measuring the steady-state fluorescence anisotropy of bull sperm cells with 1-[4-(trimethylammonium)-phenyl]-6-phenyl-1,3,5-hexatriene(TMA-DPH) as a fluorescence probe. The octanol/buffer (pH 7.0) partition coefficients for CH-103, propranolol and practolol were 32.9, 5.08 and 0.013, respectively; the surface activity of the compounds decreased in the same order. CH-103 and propranolol significantly increased the fluidity of the membrane in a concentration-dependent manner, whereas practolol reduced fluidity. These physicochemical parameters correlated with the effects of these drugs on rat sarcolemmal Ca^{2+}, Mg^{2+}-ATPase, a manifestation of their nonspecific membrane activity. Our results suggest that the physicochemical properties of CH-103, similarly to those of propranolol, are the main determinants of its nonspecific membrane activity.

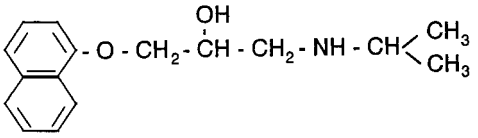
1. Introduction

There is ample evidence that β-adrenoceptor blocking drugs, in addition to their specific interaction with β-adrenoceptors, possess a wide range of nonspecific membrane effects [1]. These membrane actions may be manifested as antiarrhythmic, quinidine-like, local anaesthetic or membrane stabilizing activities [1]. Over the last decades, the list of the nonspecific membrane effects was significantly extended and, in addition to the classical membrane stabilizing activity, it includes e.g. the inhibition of phospholipase A-induced swelling of mitochondria [2], the inhibition of serotonin uptake by human blood platelets [3], the inhibition of human erythrocyte membrane Na^+, K^+-ATPase [4, 5], the relaxation of isolated dog coronary artery [6], the stabilization of lysosomes in anoxic myocardium [7, 8] and the inhibition of synaptosomal noradrenaline uptake [9]. All of these phenomena are, however, thought to be based on similar mechanisms, i.e. physicochemical interactions of the β-blocker with the cell membrane.

CH-103 (4-cyclohexilamino(-1-(1-naphtolenyloxy)-2-butanol maleate) is an amphiphilic molecule with β-blocking, antiarrhythmic and quinidine-like, membrane stabilizing properties [10, 11] developed in Chinoin Pharmaceutical Factory (Budapest, Hungary). It also produces a use-dependent block of fast inward Na^+ currents [12] and a marked inhibition of sarcolemmal Na^+, K^+-ATPase and Ca^{2+}, Mg^{2+}-ATPase in rat myocardium [13, 14]. Because alterations in membrane fluidity have been shown to affect the activity of membrane-bound enzymes [15], some changes in membrane fluidity during the interaction of CH-103 with a biological membrane may be expected.



CH - 103



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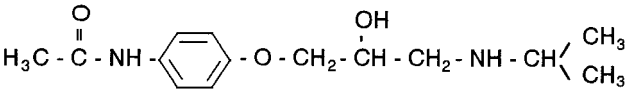

PRACTOLOL

2. Investigations and results

The aim of the present work was to study the physicochemical properties (partition coefficients and surface activity) of CH-103. Changes in membrane fluidity induced by CH-103 were also studied in comparison with two reference compounds, the lipophilic propranolol and the hydrophilic practolol, both of which are well-known competitive antagonists at β-adrenergic receptors. Because the sperm is a spontaneously active cell and has no β-receptors, it was chosen as a simple pharmacological model for studying the effects of β-blockers on membrane fluidity. Finally it was also studied whether there was any relationship between the liposolubility and a manifestation of the nonspecific membrane activity of the β-blockers examined.

2.1. Partition coefficients

Partition coefficients, both as calculated by the atom/fragment contribution method (P_e) [16, 17] and measured as described in the Experimental part (P_a) [18], indicated the same order of lipophilicity: CH-103 was more lipophilic than propranolol, and practolol exhibited a hydrophilic character (Table 1). Our measured partition coefficients show good agreement with the values found by Hellen-

brecht et al. ([18], for propranolol 5.39, for practolol 0.009) as well as by Woods and Robinson ([19], for propranolol 5.4).

Table 1: Partition coefficients (P) of CH-103 and the reference compounds

Compd.	P_a [a] *	log P_a [a]	log P_e [b]
CH-103	32.919 ± 1.058	1.517	4.449
Propranolol	5.079 ± 0.338	0.706	2.597
Practolol	0.013 ± 0.004	−1.886	0.528

[a] P_a, log P_a: apparent octanol/buffer partition coefficient, measured as described under 4.2.2. and its common logarithm. [b] Log P_e, estimated octanol/water partition coefficient, calculated by the atom/fragment contribution method [16, 17]. * mean ±S.E.M., n = 5

2.2. Surface activity

The β-blockers reduced the surface tension in a concentration-dependent manner (Fig. 1). To compare the potencies of the agents, equi-effective concentrations lowering the surface tension by 10 mNm^{-1} were determined graphically according to Hellenbrecht et al. [18] and given in parentheses. CH-103 (1.5×10^{-3} M) was found to be the most potent surface active agent followed by propranolol (5×10^{-3} M), whereas practolol (1.7×10^{-2} M) was at least one order of magnitude less potent than CH-103.

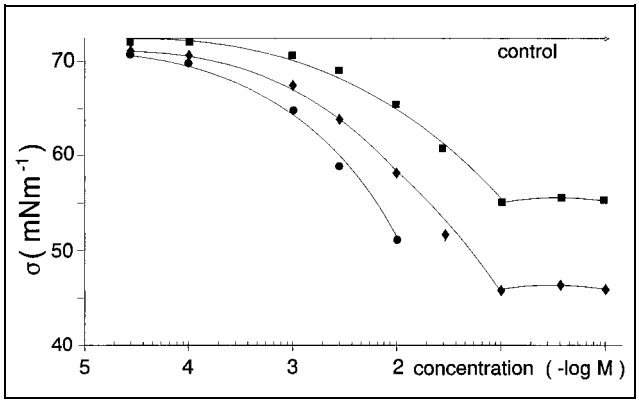

Fig. 1: Effect of CH-103 (●), propranolol (◆) and practolol (■) (n = 4) on the surface tension of water (σ). The surface tension distilled water was taken as control (n = 12)

Comparing these results with those of Hellenbrecht et al. [18], they were similar for propranolol. Practolol, however, was more potent in our experiments than in the hands of these authors since, in their experiments, practolol failed to reduce the surface tension of water by 10 mNm^{-1} even at 1 M, the highest concentration applied. The maximum reduction in the surface tension of water was 27 mNm^{-1} for propranolol and 17 mNm^{-1} for practolol. This was produced by 10^{-1} M of either drug, and higher concentrations up to 1 M failed to reduce further the surface tension (Fig. 1). Unfortunately, because of its limited solubility, it was impossible to study the effect of CH-103 at concentrations higher than 10^{-2} M.

2.3. Effect on membrane fluidity

Fig. 2 shows the effect of β-blockers on steady-state fluorescence anisotropy. CH-103 and propranolol decreased the steady-state anisotropy, i.e. increased the membrane fluidity in a concentration-dependent manner in the 10^{-7}–10^{-4} M concentration range. These changes were statistically highly significant at all concentrations studied. On the other hand, practolol slightly increased the steady-state anisotropy, i.e. reduced the fluidity.

At concentrations of 10^{-7}–10^{-4} M, CH-103 had a higher fluidizing effect than propranolol; both produced their maximum effect at 10^{-5} M. At higher concentrations (10^{-3} M, 3×10^{-3} M) the fluidizing effect of CH-103 and propranolol apparently decreased. At these high concentrations, photobleaching may have caused some error and therefore, to evaluate the membrane fluidizing effect of CH-103 and propranolol, changes in steady-state anisotropy measured at 10^{-7}–10^{-4} M were considered only.

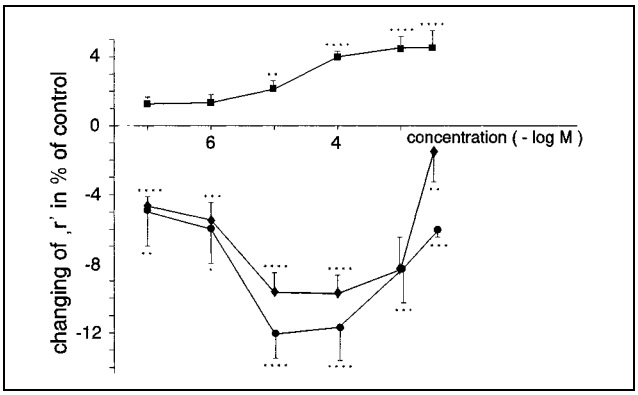

Fig. 2: Effect of CH-103 (●), propranolol (◆) and practolol (■) on steady-state anisotropy (r) (n = 6). Mean values with S.E.M. bars are plotted. Significantly different from control * * ** p < 0.001; * * * p < 0.005; ** p < 0.01; * p < 0.05

2.4. Statistical analysis

Statistical evaluation of the results (Table 2) reveals significant correlations between the physicochemical (log P_a, log P_e, surface activity, maximum effect on steady-state anisotropy) and pharmacological (effect on basic and total Ca^{2+}, Mg^{2+}-ATPase) properties of the β-blockers.

3. Discussion

The chemical structure of β-blockers shows that they have an amphiphilic character, because they posses an aromatic nucleus and a polar amino group, connected by an alkyloxy chain. The marked hydrophobicity of CH-103 and propranolol is conferred by their naphthalene ring. The alkyloxy chain of CH-103, however, is one carbon atom longer than that of propranolol and CH-103 has also a cyclohexyl substituent. The higher lipophilicity of CH-103 as compared to propranolol can be explained by these two differences. The acetamide group connected to the benzene ring gives the practolol molecule a hydrophilic property.

The amphipathic character enables these agents to accumulate at hydrophilic-hydrophobic interfaces and to migrate into the hydrophobic medium. A direct relationship between the surface activity and hydrophobicity, i.e. the respective partition coefficients was demonstrated by Hellenbrecht et al. [18] for a series of β-blockers including propranolol and practolol. CH-103 is, however, more potent in lowering surface tension than the most potent drugs in the experiments of Hellenbrecht et al. [18] (alprenolol, propranolol and KL 225) and, in accordance with this, its partition coefficient (32.9) is higher than those of alprenolol, propranolol an KL 255 (3.27, 5.39 and 3.75, respectively) [18].

It has been reported [20] that the surface tension of water as a function of the concentration of propranolol declines

Table 2: Correlations between the various parameters investigated

Log P_a vs. log P_e	$r = 0.966$	
Log P_a vs. surface activity	$r = -0.999$	$p < 0.05$
Log P_e vs. surface activity	$r = 0.963$	
Log P_a vs. maximum effect on steady-state anisotropy	$r = 0.995$	
Log P_e vs. maximum effect on steady-state anisotropy	$r = -0.919$	
Surface activity vs. maximum effect on steady-state anisotropy	$r = 0.991$	
Log P_a vs. effect on basic Ca^{2+}, Mg^{2+}-ATPase activity[a]	$r = -0.998$	$p < 0.05$
Log P_a vs. effect on total Ca^{2+}, Mg^{2+}-ATPase activity[b]	$r = -0.996$	$p < 0.05$
Log P_e vs. effect on basic Ca^{2+}, Mg^{2+}-ATPase activity[a]	$r = -0.946$	
Log P_e vs. effect on total Ca^{2+}, Mg^{2+}-ATPase activity[b]	$r = -0.936$	
Surface activity vs. effect on basic Ca^{2+}, Mg^{2+}-ATPase activity[a]	$r = 0.999$	$p < 0.05$
Surface activity vs. effect on total Ca^{2+}, Mg^{2+}-ATPase activity[b]	$r = 0.997$	$p < 0.05$
Maximum effect on steady-state anisotropy vs. effect on basic Ca^{2+}, Mg^{2+}-ATPase activity[a]	$r = 1.000$	$p < 0.05$
Maximum effect on steady-state anisotropy vs. effect on total Ca^{2+}, Mg^{2+}-ATPase activity[b]	$r = 1.000$	$p < 0.05$

[a] At 3×10^{-3} M drug concentration in % of control −84.60, −60.72 and +49.75 for CH-103, propranolol and practolol, respectively [14]. [b] At 3×10^{-3} M drug concentration in % of control −74.67, −56.97 and +45.42 die CH-103, propranolol and practolol, respectively [14].

up to 10^{-1} M but gives a plateau above this concentration. The shape of this curve is similar to that seen at the critical micelle forming concentration (CMC) of other surface active agents, which is usually about 10^{-3} M. However, several authors [21, 22] reported that, in the case of propranolol, aggregates of various sizes are formed without the occurrence of real micelles. Attwood and Florence [22] described this self-assoociation process of β-blockers and the role of the amphiphilic character of the molecules in its mechanism. They concluded that the inflection point of the surface tension versus concentration curve of β-blockers is not equivalent to the classical CMC. This aggregation occurs at a concentration much higher than the therapeutic range and, therefore, does not seem to be related to the therapeutic effects of propranolol and particularly of CH-103, which has a limited solubility.

"Membrane fluidity" is usually attributed mostly to the dynamic features of lipid constituents and is often called "lipid fluidity" [15]. For studying the membrane fluidity of intact cells we used one of the most frequently applied methods, the steady-state fluorescence polarization measurements, in which the emission of the fluorophore is measured under continuous illumination. As fluorescence marker TMA-DPH, the positively charged derivative of DPH (1,6-diphenyl-1,3,5-hexatriene) was used. The long carbon chain of TMA-DPH is largely packed prallel to the phospholipid chains and is anchored at the lipid-water interphase due to ionic charges. The β-blockers, as a result of their nonspecific membrane effects, are capable of altering the steady-state fluorescence anisotropy. Levy [23] was the first to show that the nonspecific membrane activity of β-blockers may be related to their physicochemical properties and our results give support to Levy's postulate, since we have found a correlation between surface activity and the maximum effect on steady-state anisotropy (Table 2).

In our experiments the β-blockers – according to their chemical structure – probably perturbed the membrane. Previously Herbette et al. [24, 25] demonstrated that the naphthalene moiety of propranolol partitions into the lipid bilayer, and the charged amine side chain is located within the polar phospholipid head group area. Ondrias et al. [26] supposed that such a spatial incorporation of propranolol into the membrane induces a "free volume" at the hydrocarbon lipid core by increasing the freedom of molecular movement of the lipid chains at depth of the 16th carbon atom. This "free volume" in the hydrophobic membrane core may destabilize the lamellar membrane structure. The rigidization effect of practolol may be associated with its interaction with the polar part of the membrane [26].

The effect of β-blockers on rat sarcolemmal Ca^{+2}, Mg^{2+}-ATPase was also studied in our earlier experiments [14]. As regards the order of potency of β-blockers studied, a parallelism between their effect on membrane anisotropy and Ca^{2+}, Mg^{2+}-ATPase activity could be observed: i.e. CH-103 increased the membrane fluidity and inhibited the ATPase activity more potently than propranolol. Practolol – in both cases – produced inverse effects: decreased the membrane fluidity and increased the ATPase activity [14].

It has been reported that some β-blockers are capable of reducing the size of myocardial infarcts [27]. Marie et al. [28] demonstrated that the membrane-stabilizing activity of β-blockers may significantly contribute to their cardioprotective effects. Moreover, lipid peroxidation mediated by free radicals is supposed to play a major role in the pathogenesis of ischemic/reperfusion injury [29]. Several studies have demonstrated that propranolol pretreatment may provide antiperoxidative protection to myocytes when elevated levels of free radicals are present [30–32].

The *in vivo* relevance of the cytoprotective effects of propranolol observed *in vitro* may seem doubtful if concentrations cytoprotective *in vitro* are compared to therapeutic plasma levels. Calculating with 87% protein binding [33], a free propranolol level of $\approx 9 \times 10^{-9}$ M is necessary to achieve a 50%-reduction in exercise-induced cardioacceleration [33]. To control resistant ventricular arrhythmias (an effect suggested to involve both β-blockade and other mechanisms [34]) free levels as high as 4×10^{-7} M may be needed [33]. This latter concentration is similar to that (5×10^{-7} M) reported to protect the isolated working guinea-pig heart from the consequences of anoxia [28] but much smaller than the level ($> 5 \times 10^{-5}$ M) found to protect myocytes from oxygen radical-mediated injury [31]. However, as observed by Pruett et al. [35] and emphasized by Mak et al. [31], propranolol can accumulate in cardiac tissue during an exposure more prolonged than the short incubation times applied in the above-mentioned *in vitro* studies. Such an accumulation is expected to occur after *in vivo* treatment with propranolol and supports the view that propranolol may mediate a myocellular protective effect *in vivo* [31].

The lipophilic interactions of propranolol with the cell membrane may play a major role in its cytoprotective effect. On the basis of its chemical structure, physicochem-

ical properties and nonspecific membrane activity, we can hypothesize that the cytoprotective effect is even more pronounced in the case of CH-103.

4. Experimental

4.1. Substances

1-[4-(Trimethylammonium)-phenyl]-6-phenyl-1,3,5-hexatriene(TMA-DPH), propranolol hydrochloride, fluorescein diacetate and propidium iodide were purchased from Sigma (St. Louis, USA), practolol was obtained from ICI (Macclesfield, Cheshire, U.K.), CH-103 was a gift from Chinoin Pharmaceutical Factory (Budapest, Hungary). All other chemicals were of analytical grade.

4.2. Methods

4.2.1. Estimation of the partition coefficient

For predicting log P (log octanol-water partition coefficient), the atom/fragment contribution method [16] and its computerized program [17] were used.

4.2.2. Measurement of the apparent partition coefficient

The octanol/buffer partition coefficient was measured at 20 °C according to Hellenbrecht et al. [18]. Solutions of the β-adrenoceptor blocking drugs were prepared in 0.16 M phosphate buffer (pH = 7.0) to give a final concentration of 10^{-4} M. The solutions were shaken vigorously in sealed tubes with buffer-saturated octanol for 30 min at 25 °C. The octanol : buffer volume ratios were 1 : 5 for CH-103 and propranolol, 5 : 1 for practolol. After shaking, phase separation was assisted by centrifugation at 2100 × g for 10 min. The optical density of the aqueous phase at the UV absorbance maxima (E_{max}) of the respective drugs (for CH-103, propranolol and practolol 291, 215 and 245 nm, respectively) was dertermined before and after the procedure with a Specord M40 spectrophotometer (Carl Zeiss, Jena, Germany) allowing the extraction of drug into the organic phase to be estimated. The apparent partition coefficient (P_a) was calculated according to the following equation:

$$P_a = \frac{E_{original} - E_{extracted}}{E_{extracted} xf} \quad (1)$$

where $E_{original}$ is the extinction of the agent in the buffer phase before extraction, $E_{extracted}$ is the extinction of the agent in the buffer after extraction by the octanol phase, and f is the octanol : buffer volume ratio. The measurements were performed five times for each compound.

4.2.3. Measurement of the surface tension

Surface tension was measured with a Du Nouy Tensiometer instrument (Krüss, Hamburg, Germany) at 20 °C. The β-blockers were dissolved in distilled water, the surface tension was measured four times for each solution and each concentration. The technique yielded a mean value of 72.60 ± 0.05 mNm^{-1} (n = 12) for the surface tension of distilled water. In order to compare the respective drug potencies, the concentrations necessary to lower the surface tension by 10 mNm^{-1}, i.e. the equi-effective concentrations, were determined graphically.

4.2.4. Fluorescence studies

4.2.4.1. Cells

Fresh bull semen, obtained from the Institute for Artificial Insemination (Debrecen, Hungary) was diluted with 9 volumes of a special solution containing 0.2 M Tris (Tris(hydroxymethyl)-aminomethane), 0.077 M citric acid, 0.066 M fructose, pH = 6.7 [36]. The cell suspension (5×10^6 cells/ml) was kept at +4 °C and used within 1 h. The ability of the cytoplasmic membrane to retain fluorescein diacetate and exclude propidium iodide served as a measure of viability [37], as judged by fluorescence microscopy. On the average, 65% of cells were alive an 19% were dead in the cell suspensions used in the various experiments. The remainder part of sperm cell-population could be stained by neither of the dyes and thus was supposed to be between the life-dead terminal stages.

4.2.4.2. Fluorescence measurements

Steady-state fluorescence anisotropy was measured with TMA-DPH as a fluorescent probe. TMA-DPH was stored as a 5×10^{-4} M stock solution in *N*,*N*-dimethylformamide [38]. This solution was added in a ratio of 1 : 200 to a suspension of 5×10^6 sperm cells per ml. The cells were incubated with the probe for 5 min at 37 °C. The final cell concentration during anisotropy measurements was 1×10^6 cells/ml. The β-blockers were dissolved in distilled water. Steady-state fluorescence anisotropy was measured in the absence (controls) or in the presence of β-blockers at 20 °C, six times for each concentration. Gentle stirring was necessary to prevent the cells from sedimenting. Measurements were made by using $\lambda_{(excitation)} = 355$ nm and $\lambda_{(emission)} = 430$ nm. Fluorescence spectra were recorded on a Perkin-Elmer MPF-44B fluorescence spectrofluorimeter (Perkin-Elmer Corp., Norwalk, CT) with 8 nm excitation and 6 nm emission band pass.

4.2.4.3. Evaluation of membrane lipid dynamics

Steady-state fluorescence anisotropy values were obtained by simultaneous measurements of $I_\parallel$ and $I_\perp$, where $I_\parallel$ and $I_\perp$ are the fluorescence intensities polarized parallel and perpendicular to the direction of polarization of the excitation beam. The error coming from the light scattering phenomenon should be corrected by means of measurements in unlabeled cell suspensions. The corrected fluorescence anisotropy is defined as

$$r = \frac{(I_\parallel - I_\parallel^S) - (I_\perp - I_\perp^S)}{(I_\parallel - I_\parallel^S) + 2(I_\perp - I_\perp^S)} \quad (2)$$

where the upper index S refers to the corresponding values of an unlabeled cell suspension [39]. The steady-state fluorescence anisotropy of the light emitted by the fluorophore incorporated into the lipid membrane provides information about the dynamic characteristics of its surroundings as expressed by the Perrin equation [39]:

$$\frac{1}{r} = \frac{1}{r_0}\left[1 + \frac{C_r \tau T}{\eta}\right] \quad (3)$$

where r and r_0 are the measured and the limiting anisotropies, C_r is characteristic of the volume of the fluorophore, T is the absolute temperature, τ is the excited state lifetime and η is the average microviscosity of the lipid region around the fluorophore.

4.2.5. Statistical analysis

The apparent partition coefficients and changes in anisotropy in per cent of control are expressed as mean ±S.E.M. In the case of steady-state anisotropy, the significance of differences from the control was estimated by Student's t test. A *p* value of less than 0.05 was considered statistically significant. Correlation coefficients between the various parameters were also calculated.

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References

1 Smith, H. J.: J. Mol. Cell. Cardiol. **14**, 495 (1982)
2 Seppala, A. J.; Saris, N.-E. L.; Gauffin, M. L.: Biochem. Pharmacol. **20**, 305 (1971)
3 Lemmer, B.; Wienhold, G.; Hellenbrecht, D.; Bak, I. J.; Grobecker, H.: Naunyn-Schmiedeberg's Arch. Pharmacol. **275**, 299 (1972)
4 Godin, D. V.; Wan N G, T.; Tuchek, J. M.: Biochim. Biophys. Acta **436**, 757 (1976)
5 Whittaker, M.; Wicks, R. J.; Britten, J. J.: Clin. Chim. Acta **119**, 107 (1982)
6 Sakanashi, M.; Nishi, K.: Eur. J. Pharmacol. **70**, 83 (1981)
7 Welman, E.: Br. J. Pharmacol. **65**, 479 (1979)
8 Hieda, N.; Toki, Y.; Sugiyama, S.; Ito, T.; Satake, T.; Ozawa, T.: Cardiovasc. Res. **22**, 219 (1988)
9 Street, J.; Walsh, A.: Eur. J. Pharmacol. **102**, 315 (1984)
10 Szegi, J.; Szabó, J.; Szentmiklósi, J.; Harsányi, K.; Korbonits, D.: Abstr. 6th International Congress of Pharmacology, p. 376, Helsinki 1975
11 Mészáros, J.; Kelemen, K.; Marcia, R.; Kecskeméti, V.; Korbonis, D.; Kovács, G.; Szegi, J.: Arch. Int. Pharmacodyn. **262**, 250 (1983)
12 Mészáros, J.; Korbonits, D.; Kovács, G.; Szegi, J.: Arch. Int. Pharmacodyn. **279**, 268 (1986)
13 Szabó, J.; Nosztray, K.; Szegi, J.: Pharmacol. Res. Commun. **20**, 173 (1988)
14 Szabó, J.; Nosztray, K.; Varga, E.; Szegi, J.: Acta Physiol. Hung. **75**, 261 (1990).
15 Szöllősi, J.: in: Damjanovich, S.; Edidin, M.; Szöllősi, J.; Trón, L. (Eds): Mobility and proximity in biological membranes, p. 138, CRC Press, Boca Raton 1994
16 Meylan, W. M.; Howard, P. H.: J. Pharm. Sci. **84**, 83 (1995)
17 KOWWIN, Octanol-Water Partition Coefficient Program for Microsoft Windows 3.1 Version 1.55, Syracuse Research Corporation, Merillane, Syracuse, NY 13210, 1997
18 Hellenbrecht, D.; Lemmer, B.; Wiethold, G.; Grobecker, H.: Naunyn-Schmiedeberg's Arch. Pharmacol. **277**, 211 (1973)

19 Woods, P. B.; Robinson, M. L.: J. Pharm. Pharmacol. **33**, 172 (1981)
20 Elliott, D. H.; Elworthy, P. H.; Attwood, D.: J. Pharm. Pharmacol. **25**, 188P (1973)
21 Attwood, D.; Agarwal, S. P.: J. Pharm. Pharmacol. **31**, 392 (1979)
22 Attwood, D.; Florence, A. T.: Surfactant systems, Chapman and Hall, London 1983
23 Levy, J. V.: Eur. J. Pharmacol. **2**, 250 (1968)
24 Herbette, L.; Napolitano, C. A.; Messineo, F. C.; Katz, A. M.: Adv. Myocardiol. **5**, 333 (1985)
25 Herbette, L. G.; Trumbore, M.; Chester, D. W.; Katy, A. M.: J. Mol. Cell. Cardiol. **20**, 373 (1988)
26 Ondrias, K.; Stasko, A.; Jancinova, V.; Balgavy, P.: Mol. Pharmacol. **31**, 97 (1987)
27 Frishman, W. H.: Am. J. Cardiol. **70**, 21 (1992)
28 Marie, P. Y.; Zannad, F.; Parisot, M.; Royer, R. J.: Eur. J. Pharmacol. **163**, 337 (1989)
29 Flaherty, J. T.; Weisfeldt, M. L.: Free Radical Biol. Med. **5**, 409 (1988)
30 Mak, I. T.; Weglicki, W. B.: Circ. Res. **63**, 262 (1988)
31 Mak, I. T.; Kramer, J. H.; Freedman, A. M.; Tse, S. Y. H.; Weglicki, W.: J. Mol. Cell. Cardiol. **22**, 687 (1990)
32 Mak, I. T.; Weglicki, W.: Circ. Res. **66**, 1449 (1990)
33 Benet, L. Z.; Øie, S.; Schwartz, J. B.; in: Hardman, J. G.; Limbird, L. E.; Molinoff, P. B.; Ruddon, R. W.; Goodman Gilman, A. (Eds): Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., p. 1707, McGraw-Hill, New York 1996
34 Murray, K. T.; Reilly, C.; Koshakji, R. P.; Roden, D. M.; Lineberry, M. D.; Wood, A. J. J.; Siddoway, L. A.; Barbey, J. T.; Woosley, R. L.: J. Clin. Invest. **85**, 836 (1990)
35 Pruett, J. K.; Walle, T.; Walle, U. K.: J. Pharmacol. Exp. Therap. **215**, 539 (1980)
36 Szöllősi, J.; Takács, T.; Balázs, M.; Gáspár, R.; Mátyus, L.; Szabó, G.; Trón, L.; Resli, I.: Magyar Állatorvosok Lapja **41**, 459 (1986) (Abstract in English)
37 Resli, I.; Gáspár, R.; Szabó, G.; Mátyus, L.; Damjanovich, S.: Magyar Állatorvosok Lapja **38**, 38 (1983) (Abstract in English)
38 Kuhry, J. G.; Duportail, G.; Bronner, C.; Laustriat, G.: Biochim. Biophys. Acta **845**, 60 (1985)
39 Shinitzky, M.; Dianoux, A. C.; Gitler, C.; Weber, G.: Biochemistry **10**, 2106 (1971)

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