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Influence of sympathomimetic drugs (1-phenyl-2-amino-ethan-1-ol derivatives) on the biosynthesis of prostaglandins and thromboxane B_2

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1 The influence of substituted 1-phenyl-2-amino-ethan-1-ols on prostaglandin and thromboxane biosynthesis was studied using ram seminal vesicle microsomes and homogenates of rat lung and of rat stomach fundus.

2 Clear structure-activity relationships were to be seen regarding the effects of sympathomimetic drugs on prostacyclin (PGI_2) biosynthesis in the rat stomach fundus homogenates. 3'- and 4'-Monohydroxy-phenyl derivatives as well as 3',4'-dihydroxy-phenyl derivatives stimulated prostaglandin and thromboxane B_2 (TXB_2) biosynthesis. Contrary to this the 3',5'-dihydroxy-phenyl derivatives orciprenaline, terbutaline and fenoterol were inhibitors of the biosynthesis in rat organ homogenates.

3 All the sympathomimetic drugs tested stimulated cyclo-oxygenase. Orciprenaline suppressed the adrenaline-activated cyclo-oxygenase in a dose-dependent manner.

4 The effects of adrenoceptor antagonists were also studied. Phenoxybenzamine had little effect on cyclo-oxygenase activity whereas phentolamine markedly increased the rate of oxygen uptake. Both the (+)- and (−)-optical isomers of propranolol had no effect on either basal or adrenaline-stimulated oxygen uptake. In contrast, the (+)- and (−)-isomers of pindolol inhibited basal and adrenaline-stimulated uptake.

Introduction

The stimulation of prostaglandin biosynthesis by glutathione (Van Dorp, Beerthuis, Nugteren & Vonkeman, 1964), phenol derivatives (Nugteren, Beerthuis & van Dorp, 1966; Takeguchi, Kohno & Sih, 1971; Smith & Lands, 1972), 5-hydroxytryptamine (5-HT) (Takeguchi *et al.*, 1971), tryptophan (Miyamoto, Ogino, Yamamoto & Hayaishi, 1976), catecholamines (Takeguchi *et al.*, 1971) and by a variety of other compounds has been known for some time. Whereas glutathione is an essential requirement for the isomerase reaction of prostaglandin H_2 (PGH_2) to PGE_2 (Nugteren & Hazelhof, 1973; Ogino, Miyamoto, Yamamoto & Hayaishi, 1977), the other compounds were described as activators for cyclo-oxygenase and prostaglandin peroxidase. In a typical peroxidase reaction, hydrogen donors such as adrenaline, noradrenaline, guajacol and hydroquinone are oxidized along with the conversion of PGG_2 to PGH_2. However, the structural requirements for such cofactors of prostaglandin biosynthesis have not been extensively studied. Also radical scavengers such as phenol and phenylene diamine stimulate the prostaglandin peroxide reaction of bovine seminal vesicle microsomes (Egan, Humes & Kuehl, 1978; Egan, Gale, Beveridge, Marnett & Kuehl, 1980). It has been proposed that the mechanism for this stimulation is the protection of the enzyme system from self-deactivation (Smith & Lands, 1972; Egan, Paxton & Kuehl, 1976) by radicals generated during the metabolism of arachidonic acid to PGH_2.

It was also found that tryptophan, phenol, adrenochrome, adrenaline and various other aromatic compounds markedly protected prostaglandin hydroperoxidase from inactivation caused by its interaction with haem (Ogino, Ohki, Yamamoto & Hayaishi, 1978).

Arising from our studies on the interaction of cardiovascular drugs with arachidonic acid (AA) metabolism (Förster, 1980) we now describe the structure-activity relationships of sympathomimetic drugs of the 1-phenyl-2-amino-ethan-1-ol type on

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prostaglandin and thromboxane B_2 (TXB_2) biosynthesis in rat lung homogenates, PGI_2 biosynthesis in rat stomach fundus homogenates and cyclooxygenase activity in ram seminal vesicle microsomes.

Methods

Preparation of ram seminal vesicle microsomes

Deep-frozen ram seminal vesicles (10 g) were sliced, powdered in liquid air and homogenized for 2 min with an Ultra-Turrax homogenizer in 40 ml NaOH-KH_2PO_4 buffer (0.1 mol/1; pH 8.0) containing 20 mmol/1 disodium edetate. The following procedures were carried out between 0 and 4°C. The homogenate was centrifuged for 10 min at 12,000 *g*. The supernatant was centrifuged for 60 min at 105,000 *g*. The pellet was washed twice with 10 ml acetone and then with 10 ml pentane. The residue was dried under vacuum. These acetone-pentane powders were stored at −24°C and were used within 2 months.

Oxygen incorporation measurements

The incorporation of O_2 into AA was measured with a pO_2-Meter M65 F (VEB Metra Radebeul, GDR) in combination with an electronic differentiator (from this department). We determined the oxygen consumption as well as the velocity of the oxygen uptake (dpO_2/dt) at 37°C.

The 3 ml incubation chamber contained 2.5 ml Tris-HCl buffer (0.1 mmol/l, pH 8.5), 2 μmol/l haemoglobin, ram seminal vesicle microsomes (1.07 mg protein) and the appropriate drug. The volume was made up to 3 ml with NaCl solution (0.154 mmol/l). After preincubation with the drugs at 37°C for 4 min the reaction was started by addition of 0.16 mmol/l AA (sodium salt).

Prostacyclin biosynthesis in rat stomach fundus homogenates

The biosynthesis from tritium-labelled AA was studied by measurement of the metabolite 6-oxo-$PGF_{1\alpha}$ as described earlier (Blass, Block, Förster & Pönicke, 1980).

Prostaglandin/thromboxane biosynthesis in rat lung homogenates

The metabolism of [^{3}H]-AA to $PGF_{2\alpha}$, PGE_2, PGD_2, PGI_2 (measured as 6-oxo-$PGF_{1\alpha}$) and TXA_2 (measured as TXB_2) in rat lung homogenates was analysed by a previously described method (Pönicke & Förster, 1981). The complete thin layer chromatography (t.l.c.) separation of the five AA metabolites was carried out in the organic phase of ethyl acetate/acetic acid/trimethylpentane/water (110/10/20/100) by the use of an unsaturated t.l.c. chamber (relative mobilities: 6-oxo-$PGF_{1\alpha}$ = 0.24, $PGF_{2\alpha}$ = 0.32, TXB_2 = 0.53, PGE_2 = 0.58, PGD_2 = 0.75).

Protein was determined according to Lowry, Rosebrough, Farr & Randall (1951).

Materials

Arachidonic acid was purchased from Serva (Heidelberg, FRG). [5, 6, 8, 9, 11, 12, 14, 15-^{3}H]-(N)-arachidonic acid was obtained from the Institute of Isotopes, Budapest, Hungary (sp.,act. 7.03 TBq/mol). Adrenaline, synephrine, phenylephrine, orciprenaline, fenoterol were obtained from C.H. Boehringer Sohn (Ingelheim, FRG), terbutaline from Astra, Sweden, norfenefrine from Gödicke AG (Berlin-West). Ephedrine was obtained from VEB Fahlberg-List (Magdeburg, GDR). Isoprenaline was from VEB Berlin-Chemie (Berlin, GDR) and noradrenaline was from VEB Jenapharm (Jena, GDR). (−)- and (+)-Pindolol were supplied by Sandoz AG (Basle, Switzerland) and phentolamine by Ciba-Geigy (Basle, Switzerland). (−)- and (+)-Propranolol were obtained from Isis-Chemie (Zwickau, GDR), phenoxybenzamine from Rohm und Haas Pharma AG (Darmstadt, FRG) and indomethacin from Polfa (Cracov, Poland). TLC-Fertigfolien, Kieselgel 60 were purchased from Merck AG (Darmstadt, FRG). Haemoglobin was supplied by Reanal (Budapest, Hungaria).

Authentic $PGF_{2\alpha}$, PGE_2, PGD_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2 were a generous gift from Dr J.E, Pike, Upjohn Co., Kalamazoo, U.S.A. All other reagents were obtained from commercial sources. Significant differences were calculated by means of Wilcoxon's signed-ranks test for paired samples (P) as well as Student's t test (P').

Results

Influence on 6-oxo-$PGF_{1\alpha}$ biosynthesis in rat stomach fundus homogenates

Rat stomach fundus homogenate forms mainly PGI_2 during the biotransformation of [^{3}H]-AA (Pace-Asciak & Rangaraj, 1977) whose hydrolysis product, 6-oxo-$PGF_{1\alpha}$, was detected. This system is easily prepared and therefore is especially suitable for studies of structure–activity relationships. The results of our studies are summarized in Table 1.

Table 1 Influence of substituted 1-phenyl-2-amino-ethan-1-ols R'-CH(OH)-CH_2-N(H)-R" (0.75 mmol/l) on the 6-oxo-$PGF_{1\alpha}$ synthesis in rat stomach fundus homogenates

R'	R"	*% changes of 6-oxo-$PGF_{1\alpha}$ synthesis*	x	$\pm s_x$	*n*	*P*	*P'*
	methyl-	Ephedrine	− 7	5	8	NS	
	methyl-	Synephrine	+ 67	15	8	<0.01	<0.01
	methyl-	Phenylephrine	+119	15	8	<0.01	<0.05
	H-	Norfenefrine	+ 93	10	8	<0.01	NS
	H-	Noradrenaline	+ 94	22	8	<0.01	NS
	methyl-	Adrenaline	+182	31	9	<0.01	<0.05
	isopropyl-	Isoprenaline	+108	24	8	<0.01	NS
	isopropyl-	Orciprenaline	− 40	3	9	<0.01	<0.01
	tert-butyl-	Terbutaline	− 28	6	12	<0.01	NS
		Fenoterol	− 64	4	12	<0.01	<0.01

The average conversion of [^{3}H]-AA to 6-oxo-$PGF_{1\alpha}$ was 6.54 ± 0.46%, $n = 31$

Ephedrine, without hydroxy substitution on the aromatic nucleus, had no effect. Synephrine, with a hydroxy in the 4 position, was less effective than phenylephrine with the hydroxy in the 3 position. Adrenaline, with two hydroxy groups in the 3 and 4 positions, was the most potent. When the methyl group on the nitrogen atom was absent, the stimulant effect was reduced (norfenefrine in comparison with phenylephrine, noradrenaline in comparison with adrenaline). Replacement of the terminal methylamino group by an isopropylamino group also reduced the stimulant effect (isoprenaline in comparison with adrenaline). Arrangement of both hydroxy groups in the 3,5-position caused a statistically significant inhibition of the 6-oxo-$PGF_{1\alpha}$ synthesis (orciprenaline, fenoterol). Also in this case, the substituent at the nitrogen atom was essential for the drug effect. Terbutaline, containing a N-tert.butyl group showed the weakest effect in this series.

Influence on prostaglandin and thromboxane biosynthesis of rat lung homogenate

The studies carried out with selected derivatives did not show the detailed structure-activity relationships that were obtained with rat stomach fundus homogenates. Adrenaline, noradrenaline, phenylephrine and isoprenaline increased $PGF_{2\alpha}$, 6-oxo-$PGF_{1\alpha}$ and TXB_2 synthesis, but had only a weak or no effect on the formation of PGD_2 and PGE_2, respectively. The 3,5-dihydroxy substituted orciprenaline also caused a statistically significant inhibition of synthesis of all five AA metabolites studied (Table 2).

Influence on cyclo-oxygenase activity

Ephedrine was without effect on cyclo-oxygenase activity but the monohydroxy substituted derivatives, synephrine and phenylephrine as well as adrenaline and isoprenaline (substituted in the 3,4 position) caused stimulation. In contrast to the results obtained in rat organ homogenates, the 3,5-dihydroxy substituted drugs, orciprenaline and terbutaline, stimulated cyclo-oxygenase activity (Table 3).

When cyclo-oxygenase activity was stimulated by addition of adrenaline, orciprenaline was a potent inhibitor of the reaction (Figures 1 and 2).

The actions of adrenoceptor antagonists on the cyclo-oxygenase system were very different (Tables 4 and 5). Phenoxybenzamine produced a slight increase in the maximum velocity of O_2-uptake, but in the presence of adrenaline the system was unchanged. Phentolamine markedly enhanced the cyclo-oxygenase activity in the same range as ad-

Table 2 Influence of substituted 1-phenyl-2-amino-ethan-1-ols R'-C(OH)-CH$_2$-N(H)-R'' (0.75 mmol/l) on the prostaglandin and thromboxane biosynthesis of rat lung homogenates

	% changes of synthesis from [^{3}H]-arachidonic acid																	
Substance	$PGF_{2\alpha}$				PGE_2			PGD_2			6-oxo-$PGF_{1\alpha}$			TXB_2				
	n	*x*	$\pm s_x$	*P*	*x*	$\pm s_x$	*P*	*x*	$\pm s_x$	*P*	*x*	$\pm s_x$	*P*	*x*	$\pm s_x$	*P*
Phenylephrine	10	+77	32	<0.05	+42	27	NS	0	10	NS	+100	12	<0.01	+114	21	<0.01
Noradrenaline	10	+126	45	<0.01	+58	26	NS	+30	11	<0.02	+143	17	<0.01	+120	19	<0.01
Adrenaline	10	+124	32	<0.01	+51	21	<0.05	+29	16	NS	+124	20	<0.01	+107	19	<0.01
Isoprenaline	10	+95	27	<0.01	+9	17	NS	+9	9	NS	+108	19	<0.01	+89	18	<0.01
Orciprenaline	10	−49	10	<0.01	−60	11	<0.01	−44	9	<0.01	−34	8	<0.02	−37	7	<0.01

The average conversion of [^{3}H]-AA by rat lung homogenates was $PGF_{2\alpha}$ 0.69 ± 0.14%, PGE_2 0.69 ± 0.16% PGD_2 0.56 ± 0.12%, 6-oxo-$PGF_{1\alpha}$ 2.47 ± 0.32% and TXB_2 0.92 ± 0.16% in the controls. Means ± s.e.

renaline and reduced it in the presence of adrenaline. (+)- and (−)-Propranolol were ineffective under the conditions used. However (+)- and (−)-pindolol inhibited the maximum velocity both in the absence and the presence of adrenaline. On the other hand, O_2-uptake was reduced only in the presence of adrenaline. In the absence of adrenaline, (+)-pindolol caused an increase in O_2-uptake. The differences in the action of the (+)- and (−)-isomers of pindolol were in each case statistically significant. Under the same conditions, indomethacin at a concentration of 0.001 mmol/l, reduced cyclo-oxygenase activity by approx. 50%.

Discussion

Smith & Lands (1972) showed that cyclo-oxygenase is irreversibly self-deactivated during the oxygenation of arachidonic acid. The reduction of the hydroperoxide at carbon 15 of PGG_2 to the hydroxy on PGH_2 forms radicals which were found to deactivate cyclo-oxygenase (Egan *et al.*, 1976; 1980). Therefore phenol and other scavengers of radicals promote the formation of PGH_2. The stimulant action of suitable reducing substances such as hydroquinone could also be explained as a protection of the synthetase against peroxide inactivation during the conversion of PGG_2 to PGH_2 (Van der Oudera, Buytenhek, Nugteren & van Dorp, 1977). Hydroquinone, guajacol or adrenaline are oxidized in stoichiometric quantities in the peroxidase reaction. In addition, *p*-benzoquinone and adrenochrome, oxidized forms of hydroquinone and adrenaline, respectively, stimulate prostaglandin peroxidase (Ohki *et al.*, 1979). However, the stimulant action of these substances cannot be explained by a simple principle such as hydrogen donor or radical scavenger. A direct interaction of such agents with the enzyme must be assumed. Likewise, our results on the structure – activity relationships of sympathomimetic drugs in rat stomach fundus homogenates (Tables 1) could

Table 3 Influence of substituted 1-phenyl-2-amino-ethan-1-ols R'-CH(OH)-CH$_2$-N(H)-R'' (0.2 mmol/l) on the cyclo-2oxygenase activity of ram seminal vesicle microsomes

		% changes of O_2-uptake			% changes of max. velocity		
Substance	*n*	x	±sx	*P*	x	$\pm s_x$	*P*
Ephedrine	9	−1	15	NS	−9	12	NS
Synephrine	9	+65	14	<0.01	+174	29	<0.01
Phenylephrine	9	+136	44	<0.01	+243	56	<0.01
Adrenaline	15	+181	23	<0.01	+207	33	<0.01
Isoprenaline	12	+212	30	<0.01	+192	40	<0.01
Orciprenaline	17	+38	9	<0.01	+11	7	<0.05
Terbutaline	6	+62	9	<0.05	−2	4	NS
Fenoterol	6	+124	35	<0.05	+39	13	<0.05

The O_2-uptake was determined 3 min after the reaction was started by addition of AA. The O_2-uptake in the absence of drugs was 130–140 nmol O_2/mg protein and the maximal velocity dpO_2/dt was 180–240 nmol O_2 min^{-1} mg^{-1} protein. The suspensions of microsomes contained 0.36 mg protein per ml.

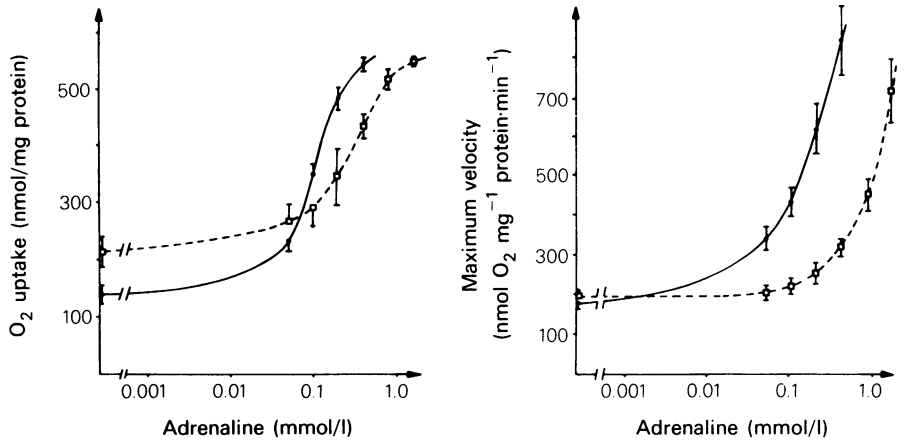

Figure 1 Influence of adrenaline on the cyclo-oxygenase activity in the presence or absence of 0.2 mmol/l orciprenaline. Each point is the mean determined with 6 different enzyme preparations, s.e.means indicated by vertical lines. (●): without orciprenaline; (□) with orciprenaline (0.2 mmol/l).

not be interpreted as changes in the oxidation potentials of the drugs. The clear structure-dependency of the action of phenylethanolamine derivatives on the prostaglandin synthetase system is neither correlated with pharmacological potency nor with the specifity of their effects on α- and β-adrenoceptors. The results obtained with the synthetase system of rat lung homogenates demonstrate also the distinction between the stimulant action of 1-(hydroxyphenyl)-2-amino-ethan-1-ols and 1-(3′, 4′-dihydroxyphenyl)-2-amino-ethan-1-ols on the one hand and the inhibition of the prostaglandin and thromboxane biosynthesis by 3,5-dihydroxy substituted adrenergic drugs on the other hand. The diminished prostaglandin biosynthesis in organ homogenates by orciprenaline is in accordance with findings of other authors (Garg & Sharma, 1977), who showed a decreased production of PGE and PGF from guinea-pig uterine tissue homogenate. The diminished formation of all five AA metabolites studied by orciprenaline in rat lung homogenate suggests an inhibition of the endoperoxide synthetase. In contrast, the cyclo-oxygenase activity of ram seminal vesicle microsomes was enhanced by orciprenaline, fenoterol and terbutaline (only the O_2-uptake was stimulated). Differences in the species and organs cannot be ruled out as the cause of this discrepancy, but the lack of a suitable cofactor also comes into question in the microsomal

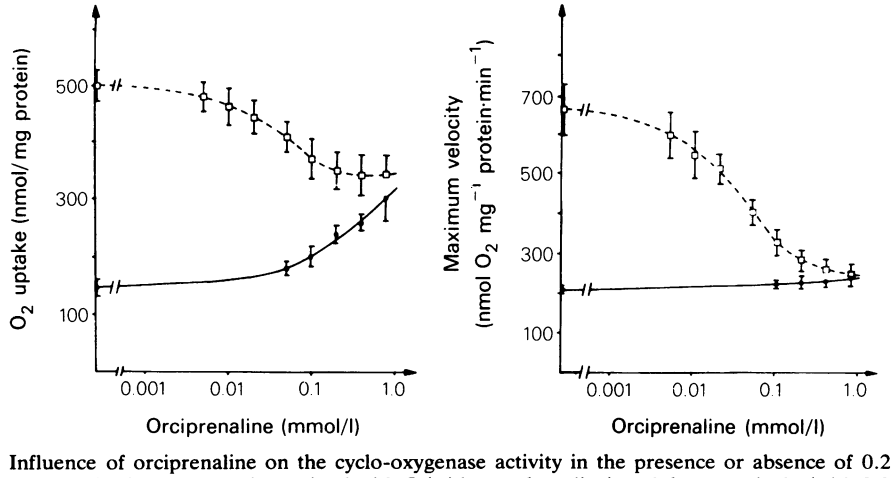

Figure 2 Influence of orciprenaline on the cyclo-oxygenase activity in the presence or absence of 0.2 mmol/l adrenaline. Each point is the mean determined with 5 (without adrenaline) and 6, respectively (with 0.2 mmol/l adrenaline) different enzyme preparations; s.e.means indicated by vertical lines. (●): without adrenaline $n = 5$; (□) with adrenaline (0.2 mmol/l) $n = 6$.

Table 4 Influence of α-adrenoceptor antagonists (0.2 mmol/l) on the cyclo-oxygenase activity of ram seminal vesicle microsomes ($n = 8$)

Substance	*Maximum velocity (nmol O_2 mg^{-1} protein min^{-1})* x	$\pm s_x$	*P*	*P*	*O_2-uptake* (nmol O_2/mg protein) x	$\pm s_x$	*P*
Without adrenaline							
Control	457	17			219	12	
Phenoxybenzamine	587	46	< 0.02		194	15	NS
Phentolamine	1496	115	< 0.01		568	40	< 0.01
In presence of adrenaline (0.2 mmol/l)							
Control	1442	63		< 0.02	620	29	
Phenoxybenzamine	1297	74	NS		587	78	NS
Phentolamine	1137	97	< 0.05		598	57	NS

The suspensions of microsomes contained 0.30 mg protein per ml

preparation. Therefore the influence of orciprenaline on the adrenaline-stimulated cyclo-oxygenase activity was studied. Stimulation of cyclo-oxygenase activity caused by adrenaline was diminished by orciprenaline in a dose-dependent manner (Figures 1 and 2). This effect was obtained whether the drugs were addded to the microsome suspension one after another or simultaneously. These results demonstrate a direct interaction of the drugs with the enzyme.

It seems possible that the tocolytic action of orciprenaline, fenoterol and other β-adrenergic drugs used in the treatment of premature labour are effected by inhibition of the prostaglandin biosynthesis stimulated by natural cofactors. To obtain more information about the mechanism of reduction of the stimulating action of adrenaline we studied the influence of the α-adrenoceptor antagonists, phenoxybenzamine and phentolamine, and the β-adrenoceptor antagonists, pindolol and propranolol, on the cyclo-oxygenase system in absence and presence of adrenaline. Remarkable results were obtained with phentolamine. The cyclo-oxygenase activity was enhanced in the same range as by adrenaline, but the simultaneous presence of adrenaline and phentolamine reduced the cyclo-oxygenase activity. The influence of phenoxybenzamine on the cyclo-oxygenase system was comparatively much weaker.

Both optical isomers of pindolol were potent inhibitors of cyclo-oxygenase activity. But the β-adrenoceptor antagonist (−)-pindolol was slightly

Table 5 Influence of β-adrenoceptor antagonists on the cyclo-oxygenase activity of ram seminal vesicle microsomes

Substance	(mmol/l)	*n*	*Maximum velocity* (nmol O_2 mg^{-1} protein min^{-1}) x	$\pm s_x$	*P*	*P*	*O_2-uptake* (nmol O_2/mg protein) x	$\pm s_x$	*P*	*P*
Without adrenaline										
Control		7	465	14			212	13		
(+)-Pindolol	0.200	7	265	16	< 0.02	< 0.02	299	28	< 0.02	< 0.02
(−)-Pindolol	0.200	7	211	17	< 0.02		233	26	NS	
(+)-Propranolol	0.200	7	474	53	NS		200	17	NS	
(−)-Propranolol	0.200	7	411	25	NS		203	16	NS	
In presence of adrenaline	0.20 mmol/l									
Control			1364	72			614	29		
(+)-Pindolol	0.200	7	296	20	< 0.02	< 0.02	293	28	< 0.02	< 0.02
(+)-Pindolol	0.010	4	801	57			574	43		
(−)-Pindolol	0.200	7	208	14	< 0.02		237	26	< 0.02	
(−)-Pindolol	0.010	4	631	77			499	26		
(+)-Propranolol	0.200	7	1262	58	NS		633	69	NS	
(−)-Propranolol	0.200	7	1286	39	NS		625	45	NS	
Indomethacin	0.001	7	558	33	< 0.02		409	26	< 0.02	

more effective in each case than (+)-pindolol which has almost no β-adrenoceptor blocking activity. The finding that the O_2-uptake in the absence of adrenaline was unchanged by (−)-pindolol but was stimulated by (+)-pindolol could be an indication that both drugs influence self-destruction of the cyclo-oxygenase enzyme.

From these results we may draw the conclusion that the interaction of these substances with adrenoceptors and with the cyclo-oxygenase system are of a different nature.

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